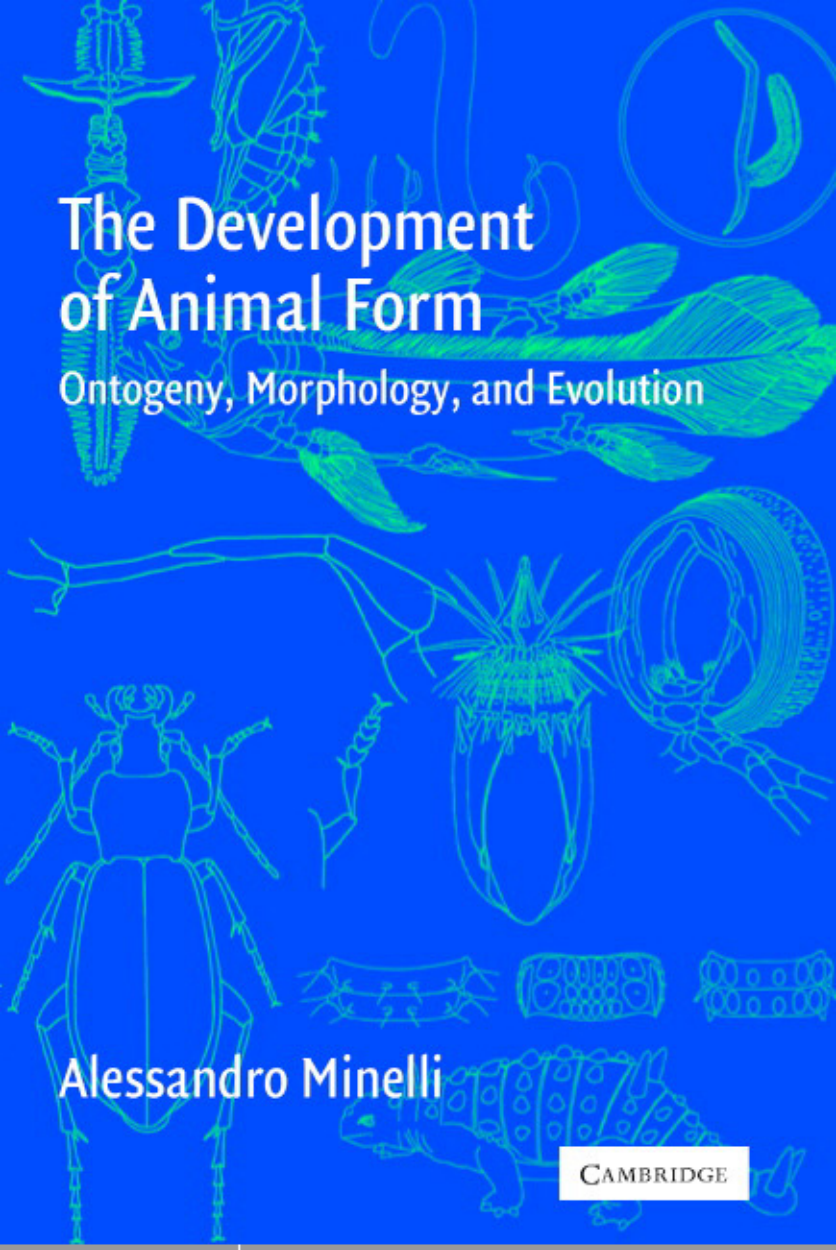

The Development of Animal Form

Ontogeny, Morphology, and Evolution

Alessandro Minelli

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Contemporary research in the field of evolutionary developmental biology, or 'evo-devo', have to date been predominantly devoted to interpreting basic features of animal architecture in molecular genetics terms. Considerably less time has been spent on the exploitation of the wealth of facts and concepts available from traditional disciplines, such as comparative morphology, even though these traditional approaches can continue to offer a fresh insight into evolutionary developmental questions. *The Development of Animal Form* aims to integrate traditional morphological and contemporary molecular genetic approaches and to deal with postembryonic development as well. This approach leads to unconventional views on the basic features of animal organisation, such as body axes, symmetry, segments, body regions, appendages, and related concepts. This book will be of particular interest to graduate students and researchers in evolutionary and developmental biology, as well as to those in related areas of cell biology, genetics, and zoology.

Alessandro Minelli is a Professor of Zoology at the University of Padova, Italy. An honorary fellow of the Royal Entomological Society, he was a founding member and vice-president of the European Society for Evolutionary Biology. From 1995 to 2001, he served as president of the International Commission on Zoological Nomenclature. He has served on the editorial board of multiple learned journals, including *Evolution & Development*.

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Ontogeny, Morphology, and Evolution

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For Pia

Contents

Preface

> Contemporary studies into the development and evolution of the head largely comprise two parallel approaches or research strategies: the model systems approach and the comparative approach. The two strategies share the same general goal – greater understanding of cranial development and evolution – but typically emphasize different problems, ask different questions, and employ different methods, reflecting the contrasting backgrounds and biases of each group of investigators; there has been relatively little true synthesis. Each strategy is making important and valid contributions, but both have limitations. Resolution of many fundamental and long-standing problems in cranial development and evolution will require a combined approach that incorporates the technical and conceptual strengths of each discipline.
>
> J. Hanken 1993: 448

Until recently, evolutionary biology and developmental biology have proceeded along separate pathways. Evolutionary biology is mainly a science of remote causes, investigating genotypic and phenotypic changes in species and populations, the origin of adaptations, and the diversity of life. Developmental biology, instead, is a science of proximate causes, grounded on experimental investigation of the cellular and biochemical mechanisms responsible for organ and tissue differentiation. The evolutionary biologist's interest in developmental biology was mainly limited to a little amount of descriptive embryology used to reconstruct phylogeny, but this loan has been steadily decreasing, along with growing dissatisfaction with Haeckel's recapitulationist views. Nevertheless, if the number of facts and concepts transferred from developmental biology to evolutionary biology was limited, the contribution of evolutionary biology to developmental biology was zero. With the exhaustion of the nineteenth

century's tradition in descriptive embryology and the deployment of an experimental approach to the study of development, attention became increasingly focussed on a limited number of model species. Thus research lost sight of the comparative method.

This sweeping historical opening is but a broad generalisation. It would be impossible, for example, to ignore the insightful contributions of authors such as Goodrich, de Beer, and Severtzoff, whose papers and books still make profitable reading today (not to mention their writing style, incomparably more enjoyable than the dull prose of most of today's scientific literature). Those authors, however, did not succeed immediately in establishing a tradition and a research agenda in evolutionary developmental biology. With hindsight, we regard them as precursors, as Geoffroy Saint-Hilaire and Bateson have been before them.

Evo-devo biology – the marriage of evolutionary biology and developmental biology – has been met with enthusiasm from many different sectors of the biological community. However, the most sensible researchers in this cross-disciplinary field (e.g., Wagner 2000, Robert, Hall, and Olson 2001, Arthur 2002) feel that a lot more theoretical work is still required before we can really greet evo-devo biology as an established field of enquiry. Problems are conceptual, methodological, and factual. This book will provide some examples of these problems and some suggestions on how to deal with them.

Today's evo-devo biology is thus fostering the intertwining of two distinct threads, respectively grown within the two research traditions as distinguished by Mayr (1982): the biology of remote causes and the biology of proximate causes.

During the last decade, the usual signs of academic success have marked the advent of the new discipline, including the publication of handbooks and monographs on the subject; the launch of new, specialized journals; and the growth of a number of evo-devo meetings and symposia held in the framework of prestigious international congresses. Some universities filling the first chairs of evo-devo biology have finally crowned this trend.

One could expect evolutionary biologists to take the lead in these conceptual and operational efforts. The Darwinian view of life explains evolution as the effect of the differential fitness of different phenotypes, but it tells us little about their origin. To say that the adaptive traits of the winner in the struggle for life are passed on to its progeny does not help explain the origin of those traits in the first place. This is exactly the point in

which evolutionary biology needs to be complemented by developmental biology (Arthur 1997).

Historically, however, most of the steam needed to push the new engine is coming from developmental biology rather than from evolutionary biology. Developmental biology is rapidly transferring to evolutionary biology a wealth of precious data and concepts, which are revolutionizing our current views on homology, body plans, the origin of evolutionary novelties, and many other pithy topics. This message is particularly clear not only in the recent books of Hall (1992, 1998a), Raff (1996), Gerhart and Kirschner (1997), Carroll, Grenier, and Weatherbee (2001), Davidson (2001), but also in those of Gehring (1998), Wolpert et al. (1998), and Coen (1999).

Of special importance, in this process, is the role of developmental genetics, especially because this discipline has expanded its field of enquiry beyond the first handful of model animals: a nematode (*Caenorhabditis elegans*), an insect (*Drosophila melanogaster*), a fish (*Danio rerio*), and a frog (*Xenopus laevis*), as well as a chicken and mouse.

This largely one-sided origin of evo-devo biology explains its focus on genes and cell–cell interactions, and its very limited attention to some of the key components of traditional evolutionary biology, such as population genetics (Gilbert et al. 1996).

There are, however, many other threads of investigation, whose contribution to both evolutionary biology and developmental biology has been large in the past. Today, however, they are out of focus in either branch of research and virtually untouched in the bridging field of evo-devo biology. This is particularly true of disciplines such as comparative morphology and the study of postembryonic development.

In this book, I will try to inject from these traditional branches of biology into the lively arena of evo-devo biology a number of facts, concepts, and problems, which have failed, until now, to find the place they deserve in today's debates and research agenda.

My aim is not so much to shift focus from the gene to the phenotype or from the embryo to the larva and the metamorphosis. The choice of the questions to be discussed within these pages does not simply stem from the wish to fill some obvious gaps in the current programme of evo-devo biology. Nor does it simply mirror the light and dark facets of my own background, obviously biased in favour of comparative morphology rather than genetics, of larvae rather than embryos and of arthropods rather than vertebrates.

The basic philosophy underlying my approach to evo-devo biology is that we need to redress the balance between the metaphysics of evolutionary biology and the metaphysics of developmental biology. The latter, in my view, is still heavily biased by a finalism whose equivalent in evolutionary biology has been long since removed by Darwinian revolution. I think that a more sober approach to evo-devo biology is worth pursuing. In this book, I will try to argue why and how.

Padova, Italy
March 28, 2002

Acknowledgements

I have been thinking for at least two decades about writing a book on the evolutionary developmental biology of animal form. But this project first begun to take shape during a 1993 sabbatical leave I spent at the Zoological Institute of Munich University. There I enjoyed stimulating exchanges of viewpoints with Diethard Tautz and his students, Markus Friedrich especially.

In a proper sense, however, the first event in the long history of this book was a lunchtime meeting with Ward Cooper in August 1999. This happened in Barcelona, during the seventh congress of the European Society for Evolutionary Biology. Ward encouraged me strongly to submit to Cambridge University Press a proposal for a book on evo-devo matters. Eventually, this proposal went into the hands of Ellen Carlin, who very sensibly cared for the development of my book, until she left CUP–the very week my manuscript reached her office. No harm done, as my work went into the equally competent hands of Katrina Holliday, validly assisted by Michael Shelley. At last, during the hectic production phase I have enjoyed the very careful assistance of Veronica Mauro Precup (of TechBooks, Fairfax, Virginia) and the painstaking copy editing of Vivian Mason. Thanks a lot, Ward, Ellen, Katrina, Michael, Veronica, and Vivian. Without your encouragement and help, my book would simply not exist.

In later years, I had the opportunity of talking about many of the topics discussed within these pages with various colleagues and friends. It is impossible to give a full list of all those from whose advice I benefitted over the years. To most of them, I will give intellectual credit by citing their illuminating books or papers. I will only mention those people whose assistance has been technically coupled to this book project.

I am sincerely grateful to Wallace Arthur, James Courtright, Giuseppe Fusco, Nigel Hughes, Tomaso Patarnello, Ester Piccinni, and Michael

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My final words are for Pia, who has been patiently respecting my intellectual adventures since the first time I confessed to her my love for evo-devo biology – some 25 years ago.

CHAPTER ONE

The Nature of Development

> Ontogeny is the unfolding of coupled developmental mechanisms whose parameters are largely specified by the genome. We hardly understand when and whether such mechanisms give rise to a few forms robustly or a plethora of forms each requiring the most delicate genetic balance among the control parameters. If robust flow into one or a few morphologies, governed by parameters easily held in vast volumes of parameter space, is the norm when many mechanisms are coupled, then robust morphogenesis could be the norm as well. Robustness may flow from complexity itself.
>
> B.C. Goodwin, S.A. Kauffman and J.D. Murray 1993: 143

> The evolution of the cell can be regarded as the 'big bang' of biological evolution even though it took a very long time. The origin of embryonic development from cells can be regarded as the 'little bang' since the cell was already there.
>
> L. Wolpert 1994: 79

Development for the Sake of Development

The shapes of things are temporarily stable configurations compatible with the underlying dynamics. This is obviously true of a flame, a river or a water drop. But this is also true of life in all its manifestations. The origin of life is the origin of a peculiar set of processes rather than the origin of peculiar things. Development is the sum of the never-ending changes of multicellular organisms, a set of processes that transcends the conventional limits of one generation, from egg to adult.

With many examples often drawn from organisms made of a small number of cells, Bonner (2000) has shown that development is the direct consequence of multicellularity. In other words, development is simply the sum of the changes multicellular systems undergo through time. This might

seem like a trivial rephrasing of the conventional notion of development, but it is not. It is the gateway to abandoning the traditional adultocentric view of development. Development, we are accustomed to saying, is the way an egg (or a seed or a spore) turns into an adult, a 'complete' organism. Residuals of finalism are even present in Striedter's (1998) otherwise attractive definition of development as the trajectory of a complex physical system with multiple stable states. What is at stake is the prospect of moving at last toward a *scientific theory of development* – a target, to be sure, far beyond my most ambitious aims with this book.

Finalism has been largely expunged from evolutionary biology, but it is still widely entrenched in developmental biology. Even to those like myself, who refrain from taking Gould and Lewontin's (1979) paper too literally, the lesson of San Marco's spandrels seems to have put an end to that naive adaptationism which looks after purpose in anything less than the most trivial evolutionary change. Things are very different in developmental biology. Take, for example, Davidson's (1991: 11; 1) statements that "development is the execution of the genetic program for the construction of a given species of organism", or "an embryo is not simply equivalent to a set of differentiating cells, even a spatially organized set. A particular function of embryonic cells is to interact in specific ways, in order to generate morphological structure". It is true that function is not a strong word as is purpose (Amundson and Lauder 1994), but to say that embryonic cells are there "in order to generate morphological structure" smells of finalism nevertheless. This finality may seem more tangible, in respect to the putative finality of evolutionary adaptations, as the ontogenetic game is played in a much shorter time dimension than the evolutionary game. One could say: You have simply to watch a hen's egg turning into a chick, and the latter growing into a cock or a hen, or an oak seed turning into an oak seedling, slowly growing into a mature tree, to convince yourself of the purposefulness of development. Consistent with this viewpoint is the current metaphor of the developmental programme inscribed in an organism's genome. Programme for what? For building an adult, of course.

I admit that life would not continue were it not for the fitness of the adult animal, but the same can be said of *any* developmental stage. Van Valen (1970) rightly remarked that a critical examination of some adult structures would help us find restrictive boundary conditions on developmental processes. But this is only true in terms of an objective analysis of the development of a given species, not as a general prescription of how development must run to build the adult.

It seems more sensible to me to follow Oyama (2000a: 161), who describes a developmental stage as "a kind of temporal slice through the life cycle. It carries the evidence of past gene transcriptions, mechanical influences inside and outside the organism, results of past activities, nutrition or lack of it, and so on, and it has certain prospects for change".

Many criticisms have been levelled at the metaphor of the genetic programme (e.g., Oyama 1985, Nijhout 1990, Müller and Wagner 1991, Bolker and Raff 1996, Neumann-Held 1999, Laubichler and Wagner 2001). Oyama (2000b: 62–63) dares to say "that whenever a program is invoked, a developmental question is being ignored, or worse, being given a spurious answer". More explicit is Keller (2000), who suggests that to speak in terms of genetic programme is to commit a basic error in categorisation: genetic is equated to programme at the same time as epigenetic is equated to data. But development depends not only on genetic memory, but also on the machinery of the cellular structures, which in turn are set in place by cellular memory rather than by genetic information (see chapter 3).

Even among those who accept the metaphor of the genetic programme, indeed, there are critics of the widespread notion of development as a single control cascade initiated by a first-moving gene. The genomic regulatory system does not constitute a serial-processing algorithm, because at any time many genes are found to act in parallel (Kauffman 1993).

But the very concept of developmental processes initiated by a single gene expression oversimplifies reality by ignoring the load of the system's past history (Minelli 1971, Oyama 2000a), not to speak of the external influences to which it is steadily exposed. A gene 'initiates' a sequence of events only if our investigation starts at that point (Oyama 2000a).

I believe that we can replace this finalistic view with a more sober notion of development as quasi-cyclical process, of which the egg (if any) and the adult (if any) are generally the most conspicuous and well-characterized phases rather than the beginning and the end of a non-return way. There is little scope for objecting that the way an egg (or a juvenile, or a larva) gives rise to an adult is quite different from the way an adult gives rise to the next generation's eggs. This is not necessarily true. Consider the different ways a cnidarian polyp may become a medusa. Cubozoan polyps metamorphose into medusae; that is, the whole polyp is changed into a medusa, much as juveniles (but only a fraction of what we call larvae) change into the corresponding adult. In hydrozoans and scyphozoans, however, the medusa buds off from the polyp, or detaches itself from it, much as gametes are released from the adult animal. These rough comparisons only

invest the hard mechanics of the processes, but this seems enough for embracing the concept of a cyclical, rather than goal-directed, nature of development.

The reader will be ready with the next objection: where is the difference between this cyclical notion of development and the common notion of life cycle? Is it not true that this cyclical notion of development simply makes development synonymous with multicellular life?

To some extent, it does. Adopting Griesemer's (2000) suggestion, we can regard development as the set of processes that must occur before a multicellular biological system is capable of reproduction. To study development is thus to study multicellularity (Bonner 2000).

This means that the basic unit of development is the cell. This may seem another truism, perfectly in line with the current perspectives on animal development, in which each chapter of the story begins with that unique cell, the egg, fertilized or not. But to reduce development to the deployment of an egg's potentialities is, at the same time, to give too much emphasis to the egg and, more important, to underrate a basic fact in development. Every cell starts its own version of life business anew, a version differing from those of the other cells, egg included, only because of the constraints provided by local circumstances, both informational and trophic, that result from a more or less long segment of history of the cell lineage to which this cell belongs. Sooner or later, however, fate and metabolic performance of a given cell cluster or sheet become fixed, the only possible alternative being starvation or death. Some cluster of cells, however, may be saved from this irreversible fate, ready to start new ventures at a later stage. Such are some clusters of set-aside cells (e.g., the imaginal discs of the insects or the adult primordium in a sea urchin larva). Also, such are the stem cells, as well as the cells of the germ line, the only survivors, generally, from the final defeat of the whole multicellular company.

From this perspective, there is nothing like a developmental programme. In a sense, there is nothing special in the mechanisms of development and, in particular, nothing corresponding to final causes.

On the other hand, development is much more than a simple sum of cellular behaviours or mechanisms. This also implies that development is much more than the sum of the expression patterns of an arbitrarily long list of genes. Development, even in its simplest forms – those that give rise to the simple multicellular organism so dear to John Tyler Bonner – is the complex *networking* of cellular behaviours and mechanisms influenced by the expression of all these genes.

In particular, it is impossible to understand development if we do not pay enough attention to all those feedback mechanisms whose existence is one of the main conditions explaining the predictability of course and outcome of developmental processes. The very existence of a feedback, however, does not imply the existence of a programme.

All these behaviours, mechanisms and genes are not there *to ensure* the deployment of the wonderfully complex shapes of living beings. Much more modestly, they are simply there and consequently affect other cellular behaviours, mechanisms, or genes and set in place those forms of self-regulation that are the key to avoid developmental bankruptcy.

From this perspective, development is deprived of the mysterious finalistic overtones which have thus far constrained our ability to understand it. On the other hand, development becomes an even more pervasive dimension of biology than we are accustomed to accept. Everything important in the biology of multicellular organisms belongs to development. In Bonner's (1993) words, organisms are not just adults – they are life cycles and life consists of a succession of life cycles. Development is thus a key aspect of the unending continuity of life. We are accustomed to cutting life's thread into generations, but even this periodisation is debatable (Griesemer 1996), especially when we are dealing with haplodiplobiont or agamic organisms.

If we are ready to abandon a finalistic view of development, as the deployment of a programme inscribed in an egg's nuclear genes, we should be also ready to accept Berrill's (1961) view (see also Goodwin 2000) that the simplest and more direct type of development is to be found in the meristematic development of buds or in units of colonial organisms rather than in the eggs with their highly specialised mechanisms of embryogenesis. The *Hydra*, in this sense, is a sort of permanent embryo (Lohmann and Bosch 2000), because even adult polyps have a striking capacity to regenerate, suggesting that molecular mechanisms underlying pattern formation are permanently active and self-regulatory. In terms of phylogeny, the *Hydra* is not basal within the Hydrozoa, or the Cnidaria generally, but this polyp may well work as a model of a primitive metazoan condition, in which morphogenetic potentials were still diffuse within the multicellular assembly, rather than reduced and restricted, as in modern animals generally. A good indicator of this primitive condition in the *Hydra* is its permanent availability to axis formation.

In so far as its cytoplasm preserves the heavy imprint of maternal gene transcription, the egg is more constrained, in terms of morphogenesis,

than a naive cell could be. But this naivety is not a consequence of being, in terms of gene expression, the equivalent of a *tabula rasa*. On the contrary, we should expect the transcriptome of an average hydra cell to be very rich and less biased toward some transcripts than may be an egg, under the belated effect of maternal gene transcription.

An argument in favour of this view of development is the presence of organisms (admittedly, not metazoans) which do not have a 'basic', or 'default' morphology. An example is *Candida albicans*, which can switch among forms so diverse as single budding cells, multicellular threadlike hyphae and strings of yeastlike cells plus long septate filaments, known as pseudohyphae (Braun and Johnson 1997, Ishii et al. 1997, Magee 1997). The pervasive character of plasticity and polymorphism suggested to Newman and Müller (2000) that the correspondence of a genotype to one morphological phenotype, as typically seen in higher animals, should be considered exceptional. In other terms, this tight correspondence is a highly derived condition in which an overdetermining genetic circuitry filters out or buffers the impact of extrinsic or intrinsic variables on the organism's morphology. In Newman and Müller's view, the beginning of multicellular era on our planet was a 'pre-Mendelian' world, in which the connection between genotypes and morphological phenotypes was very loose; that is, any given genotype would have mapped onto many phenotypes. A closer linkage between genetic change and phenotypic change would have emerged later, with the evolution of what may now appear as genetic redundancy (but see page 231) and other mechanisms supporting reliability of developmental outcome.

The non-adultocentric notion of development I am advocating here is perfectly compatible with most current concepts of both developmental and evolutionary biology – for example, with the concept of the developmental module (see page 234), a local cell population with its own developmental dynamics, but also interacting with the other modules in a kind of metapopulation of cells (the biological individual or colony).

Moreover, it gives better sense to phenomena, such as dissogony and paedogenesis. Dissogony is a peculiarity of some comb-jellies (Ctenophora) that reproduce twice in their life, the first time at a very early developmental stage, the second when they have reached the conventional adult stage. Paedogenesis, known from several arthropods and flatworms, means the production of mature eggs when the animal is still in a stage comparable with the larva, or juvenile, of its closest relatives.

A finalistic, adultocentric view of development requires every stage to be compatible with the following ones. The alternative view defended here seems more sober, in that it simply requires every stage to be compatible with the previous one. Natural selection will then select and stabilise developmental sequences compatible with the continuity of life.

Developmental Competition between Body Parts

If development is simply the network of dynamics going on in multicellular systems, there is no reason to regard development as a global property of an organism as such. Cells and multicellular units within it are equally involved in these dynamics and will be expected to compete with other units for access to metabolic or informational resources. Wagner's (1996) concept of the developmental module (see page 234) comes close to this idea, as do Buss's (1987) theory of the evolution of individuality or Edelman's (1987) model of neural Darwinism. The fractal geometry of many biological structures (so widespread among trees, inflorescences, corals and branching systems of vessels and tracheae) also speaks in favour of a multicentric view of development.

Apoptosis, in its many manifestations, is also an expression of this differential success of different cell lineages within a developing organism. During the ontogeny of the hermaphrodite individuals of *Caenorhabditis elegans*, 131 of the 1,090 somatic cells normally die by apoptosis, and more than 80% of the ganglion cells in the cat retina die shortly after they are born. In the latter case, differential cell survival depends on competition for limiting amounts of neurotrophic factors secreted by the target cells these ganglion cells 'try' to innervate (Meier, Finch, and Evan 2000). Martin Raff suggested that cell death is the default fate of all metazoan cells. (This would be the same as saying that the lemming voles of the Arctic are programmed to suicide.) Survival would be obtained through the sustained supply of environmental survival signals, including soluble cytokines and hormones, synaptic connections, and direct physical interactions with heterotypic cell neighbours and extracellular matrix (Raff 1992, Raff et al. 1993, Raff, Durand, and Gao 1998, Meier et al. 2000). I do not underrate the importance of these data, but Raff's interpretation is, in my view, one more expression of an adultocentric view of development. I would describe these in more plain terms of Darwinian competition, as Moreno, Basler, and Morata (2002) also do. Every cell simply does all

it is able to do, given its history, its metabolic state, and the influences it receives from outside. Before choosing as prototype of metazoan cells those that die from apoptosis, one should pay attention to the extraordinary potential of individual blastomeres [e.g., in frogs (Spemann 1938) and sea urchins (Driesch 1892)] that are capable of generating a fully formed embryo if isolated during an early cleavage stage.

Competition between broadly equivalent cells may be instrumental in refining early embryonic patterns, as in the case of invertebrate synapses known to change during development through competition between axons (Lnenicka and Murphey 1989).

Competition at the cell level may translate into visible effects of competition between organs (cf. Rensch 1959). In tetrapod vertebrates, there is a fairly consistent inverse relationship between limb reduction and vertebral elongation or, as in the Palaeozoic lepospondyls, an increased number of vertebrae (Carroll 1999). According to Gluesenkamp (1997), limb reduction in lizards is possibly determined by spatial constraints due to vertebral elongation, causing a decrease in the contribution of somites to the limb anlagen.

In scarab beetles, the production of horns reduces the size of neighbouring body parts: antennae, eyes, or wings, depending on the cephalic or thoracic location of the horns (Emlen 2001). Nijhout and Wheeler (1996) have remarked on the unique conditions under which adult structures grow in holometabolous insects. The metamorphosing insect does not feed during the pupal stage. Therefore, at variance with the large majority of growing systems, the imaginal structures grow within a virtually closed system in which, by consequence, body parts are in direct and strict competition for metabolic resources (Roth and Mercer 2000). As noted by Nijhout and Emlen (1998), this is an old notion, familiar to both Darwin and Geoffroy Saint-Hilaire, but it is difficult to demonstrate by experiments. Smith and French (1991), however, obtained relevant results experimenting with the flesh fly *Sarcophaga*. By destroying selected histoblast nests (groups of cells from which a part of an adult segment forms during metamorphosis), they obtained the corresponding deletion of adult structures accompanied by enlargement of adjacent structures within the same segment and in neighbouring segments (Smith and French 1991). Nijhout and Emlen (1998) studied organ competition in two different insects. The butterfly *Precis coenia* was one of them. Nijhout and Emlen removed one or two hind wing imaginal discs from several larvae of this species at the beginning of the final larval instar. After metamorphosis, the relative size of

the adult fore wings showed a compensatory response proportional to the number of hind wing discs removed. Comparable results were obtained by hormonal manipulation of male scarab beetles of the genus *Onthophagus*, in which a reduction in the size of the cephalic horns was accompanied by an increase in the size of the eyes. A spin-off of these studies is the suggestion (Klingenberg and Nijhout 1998) that fluctuating asymmetry may be controlled by competition among growing organs from a limiting resource.

Genes with specific effects on the control of cell competition are known. In *Drosophila*, the *warts* gene is required for cell proliferation to occur in the correct amount and direction, thus allowing a normal course of morphogenesis. Absence of its normal expression leads to the formation of fragmented and overgrown cell clones with hypertrophy of the epithelial cells in the imaginal discs (Justice et al. 1995).

Developmental biology has traditionally emphasised integration and regulation to such an extent that the 'default' independent activity of multiple local foci of growth and differentiation has been often overlooked. This emphasis on the holistic aspects of development is a characteristic expression of the current adultocentric views. However, even in those animals whose development appears to be more sophisticated and subject to a complex network of regulatory interactions, there is still a large scope for local autonomy, possibly culminating in competition between cells or cell lineages. Local autonomy is even compatible with syncytial organisation, in which one would not expect the slightest degree of compartmentalisation to occur. Brentrup and Wolf (1993) experimented on eggs of different developmental stages of the hymenopteran *Pimpla turionella* fused in parabiotic tandem. The interactions between the two partners were limited to the exchange of a few nuclei, but each of them followed its own temporal schedule of development, although all their nuclei were still contained in a single syncytium.

The Robustness of Morphogenesis

Goodwin, Kauffman and Murray (1993) asked: is morphogenesis an intrinsically robust process? Robust means that it would not be disrupted by temporary disturbances of reasonably modest intensity. Goodwin et al. suggested that some dynamic principles arising from a coupling of different developmental mechanisms (molecular synthesis, gene activation, spatial patterning of substances, cell interactions, cell sorting, and morphogenetic movements) result in significant reduction in the degrees of

freedom available to the whole developmental system. As a consequence, morphogenesis is intrinsically robust.

The amount of external disturbance a developing system may tolerate is often larger than the development of *Drosophila*, *Caenorhabditis* or *Xenopus* would suggest. Think of what cell sorting may achieve in a reaggregating mass of dissociated cells.

Robustness of development may depend on the number of developmental processes going on concurrently in the same system. Goodwin et al. (1993) imagined a developmental system, in which a cell sorting mechanism based on differential cohesion and surface adhesion forces (cf. Steinberg 1970), is coupled to a patterning process based on a Turing mechanism (cf. Turing 1952). In this system, two different cell types, generated as a consequence of the operating Turing mechanism, would start sorting out according to their surface properties. They would thus change position, and in these displacements they would carry with them the morphogen concentrations on which the Turing process depends. Coupling of the two processes will eventually determine the production of a stable form. Generalizing from this example, Goodwin et al. (1993) stated that the plurality of developmental mechanisms acting concurrently in developmental systems could explain the observed robustness of the latter, despite opposite predictions from a consideration of their structural complexity. This would be true, in particular, for the robustness of the so-called phylotypic stage (cf. page 123), a point also made by Galis (1999).

Azevedo and Leroi (2001) have recently criticized the current deterministic trend prevailing in developmental biology, in which due attention is not paid to the considerable level of stochasticity that has been demonstrated in most cellular properties, including gene expression patterns, mitotic rates, and migration routes. It is important to realize that development is much more flexible, *at the individual level*, than textbook schemes usually suggest. More interestingly, this flexibility is not just a property of advanced or terminal developmental stages, but is also widespread in the earliest ones. It is the sheer morphological simplicity of early developmental stages that limits our chances of spotting this variability. Modern technical tools, however, can provide the support we need. With the aid of a 4D-microscope system (multifocal, time-lapse video recording system), Schnabel et al. (1997) revealed, in the normal embryogenesis of *Caenorhabditis elegans*, variability in cell division timing, cell positioning, and cell–cell contacts not seen previously with more traditional techniques. In their analysis of the distributions of the descendants of the early founder blastomeres at

the premorphogenetic stage, they demonstrated that founder blastomeres establish discrete regions in the embryo through a considerable amount of cell movements, with different patterns in different embryos. Cell fate assignment is nevertheless conserved; This is not due to an autonomous invariant specification of cell fates, but to cell–cell interactions occurring at very early stages when the topology of blastomeres in the embryo is sufficiently precise, thus ensuing reproducible patterns of induction. Apparently, the role of cell lineage, despite its strict reproducibility, is not really responsible, per se, for subsequent cell fate. If so, the embryonic development of *C. elegans* would follow the same basic principles seen in the embryos of other animals, in which body regions are more obviously established by cell–cell interactions (Gurdon 1992, Schnabel et al. 1997). Comparative evidence from other nematodes, on the other hand, demonstrates that there has been exaggeration in the traditional view of a precise cell lineage as a universal attribute of nematode development (Voronov and Panchin 1998).

It has been shown recently that the robustness of a developmental system may have something to do with the peculiar topology of the network of interactions existing between cells or other subsystems within the developing organism. Interestingly, robustness is a characteristic of the so-called scale-free networks (other examples being social networks or the Internet), a class of networks with inhomogeneous distribution of wiring. These networks are very sensitive to selected attacks on a limited number of key nodes, but otherwise robust in front of even high degrees of failure at all remaining nodes in the network (Albert, Jeong, and Barabási 2000), which therefore demonstrate their considerable degree of autonomy from the rest of the network.

CHAPTER TWO

Everything Begun to the Service of Development: Cellular Darwinism and the Origin of Animal Form

> Recent progress in developmental genetics [..] has given us remarkable insights into the molecular mechanisms of morphogenesis but has at the same time blurred the clear divide between structure and function. At the genetic or molecular level, it is difficult to tell where one ends and the other begins. One scientist's 'cause' is another scientist's 'phenotype.'
>
> S.F. Gilbert and J.A. Bolker 2001: 1

Without its robust calcified exoskeleton, a crab would be very vulnerable prey. Its pincers would be harmless; its whole locomotory apparatus would be at loss. The same applies to a mammal, or a bird, without its internal skeleton. If the adult is to be endowed with a complete, functional skeleton, the business of constructing one must start early in development. But how and why were skeletons invented?

If an explanation of what happens in development is to be searched for in development itself, as argued in chapter 1, then the reasons for the first appearance of a skeleton must be sought for in development rather than in mechanics. I am not speaking of the heavy armour of an adult crab nor the sophisticated architecture of a vertebra. I am speaking, instead, of the reasons why some early multicellular organisms found it profitable to produce a cuticle, or to adventure into the previously unexplored paths of biomineralisation. To place this question in a plausible context, we need to digress some.

Cilia, Cell Division, and Morphogenesis

Cell division is one of the basic prerequisites for building a multicellular organism and, at the same time, one of the most dangerous threats to its viability. In multicellular organisms, cell division cannot proceed

uncontrolled. Buss (1987) suggested that the earliest metazoans found a way out of this difficulty by exploiting what we could otherwise regard as a weak point of their protist ancestors: their inability to divide once they had differentiated cilia. This is still true of the cells of modern metazoans (but not of all ciliated cells; think, for example, of the ciliates, which never lose their cilia). A metazoan ciliated cell may lose the cilia and thus regain the ability to divide. According to Buss (see also Gilbert 2000 for a summary), the early metazoans blocked cell proliferation by differentiating into a ball of ciliated cells, something comparable to a conventional blastula. As these ciliated cells did not divide, and could not differentiate into other cell types, the future of these organisms remained with a few non-ciliated cells which stayed in (or migrated into) the ball's inner cavity (or blastocoel), in which they could eventually proliferate and differentiate. In this way, a two-germ layer organism (something like a gastrula) was produced as a result of a compromise between the contrasting needs of movement, differentiation, and control of cell division within a 'federation' of genetically identical cells.

This evolutionary scenario does not negate, of course, the obvious functional value of cilia in locomotion and food gathering, but it suggests an additional *developmental role* of cilia. We could call this role a morphostatic one, because ciliated cells are removed from the proliferating cell lineage(s) and thus help maintain the little animal's shape. This is especially true because these ciliated cells represent the animal's external cell layer.

Epithelia without Cilia

Cilia were not invented with the origin of multicellularity, but were already available and thus ready to take a new role in development. There is a major animal lineage, however, from which true cilia have apparently disappeared since time immemorial. This lineage is the Ecdysozoa, the superphylum of the moulting animals (i.e., arthropods, nematodes, and their relatives). This higher taxon has been recently defined (Aguinaldo et al. 1997) based on molecular evidence, but the most obvious feature uniting such diverse animals as a nematode and a fruit fly is the presence of a cuticle, which is periodically shed and replaced with a new one.

Vertebrates, on the other hand, have retained cilia, but their ciliated epithelia have disappeared from their body cover. Cilia are still present in the epidermis of a close relative of the vertebrates, the amphioxus, but only in the very young larva. In the adult amphioxus, the outer border of

the epidermal cells is highly cuticularised (Young 1981). In vertebrates, the epidermis is multilayered and nearly always devoid of cilia; these are only present in localised regions of the skin in the early developmental stages of some amphibians.

Thus, ecdysozoans and vertebrates are two major animal groups in which the differentiation of cilia was not available as an option to restrain cell division and to help preserve body shape. In these two groups, the requirement of a generalised control of cell division and body shape had to be obtained by a completely new means.

Now we are in a position to return to the chapter's opening question.

My argument is that the first cuticle and the first experiments in biomineralisation were useful to development per se. That is, they represented ways to make development more stable, more predictable, and more robust. This is so not only because of the mechanical advantages eventually offered to a 'final' privileged stage (the adult), but also because of the advantages conferred by the cuticle (or biomineralisation) to the developing animal as such, independent of any advantage with which a cuticle (or biomineralisation) might eventually provide the same animal in a still unwritten ontogenetic and phylogenetic future.

Origin of the Ecdysozoan Cuticle

The cuticle can be soft and pliable as in spiders and earthworms, or tough but elastic as in nematodes, or rigid and stony as in crabs. It is all too easy to point to the mechanical properties of these animals' cuticles and the functional advantages with which these cuticles provide their possessors in antagonising muscles in locomotion or opposing a valuable defence to a predator's attack.

It is quite possible, however, that a cuticle first evolved as a means to stabilise shape and only later became a mechanical or protective device.

An argument in favour of this putative primacy of a developmental role of the cuticle is that arthropods develop cuticles and undergo moults while still in the egg. At that stage, a protective or mechanical function of the cuticle can be excluded (without mention of the very delicate nature of these embryonic cuticles). In many insects, three successive embryonic cuticles are shed (e.g., Louvet 1974, Dorn and Hoffmann 1981), although some authors (e.g., Sbrenna Micciarelli and Sbrenna 1976) do not interpret the first of them as a true cuticle. Three embryonic moults are also the rule in the wingless hexapod collembolans or springtails, but in *Neanura* there are as many as four moults before hatching (Claypole 1898, Schaller 1970).

Things are not that different in nematodes. In *Caenorhabditis elegans*, as soon as embryonic elongation is complete, epidermal cells make a cuticle that stabilises their final shape (Chin-Sang and Chisholm 2000). At that stage, any mechanical (locomotory) or protective function is obviously excluded. The morphogenetic (or morphostatic) role of embryonic cuticles in nematodes is confirmed by mutations in cuticular collagens that cause gross morphological abnormalities (Kramer et al. 1990).

Therefore, it is clear that a cuticle – independent of any additional advantage it may provide – stabilises a little animal's shape by providing a three-dimensional reliable frame and, possibly, by controlling cell proliferation. The morphogenetic importance of a cuticle, however, is probably not limited to its citostatic and morphostatic roles. In *Drosophila melanogaster*, the *dumpy* gene seems to be a component of the process by which epidermal cells control the properties of the overlying cuticle and vice versa. In *dumpy* mutants, both growth and morphogenesis are affected, as well as cuticle composition and function. In some larval lethal mutants of this gene, tracheae and mouthparts grow out of proportion to the remainder of the body, showing that *dumpy* normally restricts growth in these tissues (Wilkin et al. 2000).

A question then arises: How far is growth compatible with the presence of a cuticle? Detaching the epidermis from the overlying cuticle, thus allowing epidermal cells to divide before a new cuticle is formed, is obviously costly, but perhaps not much more difficult than obtaining the same result by reversibly losing the cilia.

There may be other means to escape from the size (and shape) constraints of a cuticle. The most spectacular example is possibly that of *Sphaerularia bombi*, a parasitic nematode whose cuticle could not allow the rapid increase in volume required by the enormously growing female genital apparatus. Consequently, the latter (which as an internal organ is not covered by cuticle) literally explodes out of the animal's body which, in the end, appears as a tiny appendage of its genital structures (Figure 2.1). There is an interesting, although less dramatic, equivalent outside the ecdysozoans: the larval cuticle of the acanthocephalans is shed off and not replaced with a new one. As soon as these worms enter the host, that is, as soon as they get in contact with a food source, they demonstrate very rapid growth.

It must be noted, in addition, that a few ecdysozoans may be able to grow even in the absence of moults. This phenomenon is especially conspicuous in some parasitic nematodes, such as *Ascaris*, which attain an adult body size much larger than the last larval stage. In Arthropoda, growth without

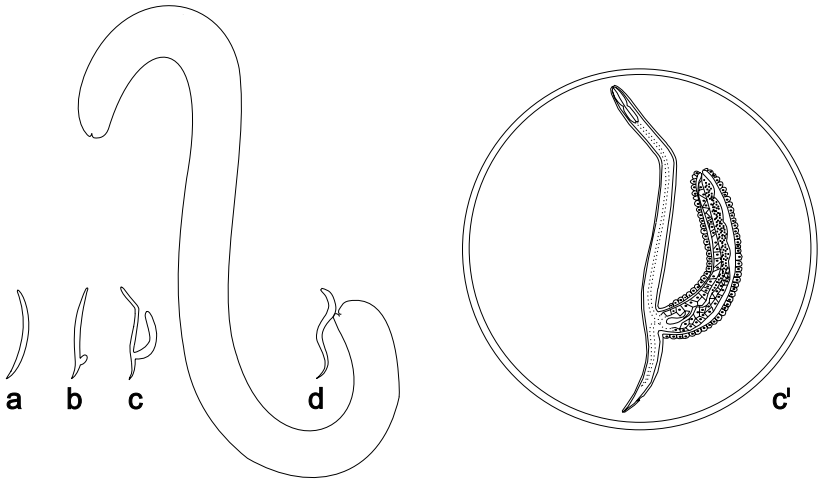

Figure 2.1. In the absence of moults, a cuticle does not allow for a rapid increase in size of the internal organs. This would be too limiting for the female genital apparatus of the parasitic nematode *Sphaerularia bombi*. Following the final (adult) moult, the genital apparatus is everted from the worm's body and grows to enormous size (a–c), until the worm itself – whose size does not change – appears as a little appendage of its genitalia (d). (c′) A highly magnified view of (c).

moults seems to be much less widespread. Examples are known in pycnogonids (*Pycnogonum littorale*; Lotz and Bückmann 1968), in parasitic representatives of the copepods (Snodgrass 1956, Kabata 1979), and in parasitic mites (e.g., *Vatacarus ipoides*, which lives in the lung of the sea snake *Laticauda laticauda*: the six-legged larva hatches from eggs 250–300 μm in diameter and grows without moulting up to 4.5 mm final length; Audy, Nadchatram, and Vercammen-Grandjean 1972). It would be very interesting to know how far the spectacular growth of these animals actually depends on cell proliferation in their epidermis.

Cuticle, Body Size, and Internal Fertilisation

Ecdysozoa seem to have adopted internal fertilisation well before they invaded habitats (freshwater, terrestrial) in which external fertilisation would be forbidden.

Among the Ecdysozoa, external fertilisation is only found in the horseshoe crabs and in the largest, macrobenthonic representatives of the Priapulida. Internal fertilisation is found in the smallest, meiobenthic

Priapulida and in all remaining groups (Nematoda, Nematomorpha, Kinorhyncha, Loricifera, Tardigrada, Onychophora, and nearly all Arthropoda; external fertilisation found in the tiny meiobenthic mystacocarids is certainly derived within the crustacean lineage). The shift to this kind of fertilisation may have been positively selected as an efficient resource allocation for a free-living, active metazoan. A comparison to internal fertilisers may help clarify the concept. External fertilisers are easily driven by natural selection towards a large size (leading to higher production of gametes) and lesser mobility (with most resources being allocated to reproduction rather than to somatic structures, including an efficient locomotory system). On the contrary, internal fertilisers are selected for a less variable, species-specific size (permitting a better fit between the two partners' copulatory structures) and higher motility (facilitating finding a partner). Considering that a precisely controlled adult size was important, a strict control of growth becomes much more important than in external fertilisers. A critical step in achieving this result may be tight control of the postembryonic increase in the epidermal body cover. An external cuticle may help, because it constrains epidermal growth, thus allowing for periodic bursts of mitosis only in coincidence with moults. In arthropods, in the time span between two moults, epidermal cells are tightly attached to the overlying cuticle by means of hemidesmosomes.

Palaeontologists continue to dispute whether early bilaterians, and early arthropods in particular, were very small animals (Cooper and Fortey 1998) or not (Budd and Jensen 2000). If they were, this circumstance may help explain the first appearance of the ecdysozoan cuticle in putatively tiny metazoans at the dawn of the Palaeozoic era. A cuticle covering the body could hardly be of any protective significance for a tiny animal that could be easily sucked in, or filter-collected, by a microphagous predator.

In my opinion, the first adaptive significance of this cuticle was in the control of mitosis in the epidermis, hence in the control of growth and, by consequence, body size. Only when representatives of the Ecdysozoa acquired larger body size, later in evolution, this cuticle acquired additional value in providing protection and reliable sites of muscle attachment.

Less easy to understand is the case of the nematodes. In this group, the final (adult) stage is always obtained after four moults, irrespective of the final size. However, whereas free-living nematodes, such as *Caenorhabditis elegans*, do not increase much in size and do not undergo mitoses after the final moult, things are different in some large parasitic forms. In the latter, there may be a very conspicuous increase in body size (up to 10 times

in body length and at least 100 times in body volume in genera such as *Ascaris*, *Ascaridia* and *Syngamus*; Malakhov 1994), sustained by mitotic activity in the epidermis (which, in these large nematodes, is syncytial), the musculature and the intestine.

Segmentation (see chapter 9) is another way to control morphogenesis, because it brings about a uniform distribution of cell clusters and organ anlagen.

Origin of Mineralised Skeletons

A parallel scenario can be suggested for the origin of the biomineralised and especially the phosphatised skeletons. Clearly, the ability to control mineralisation was acquired before minerals find their first use in skeletons. In the case of phosphates, the primary significance of mineral deposits was probably as a form of storage of the generally scarce, but metabolically important, phosphate anion. Among living animals, both the calcitic mollusc shells and the phosphatic vertebrate bones may act, in addition to their skeletal function, as stores of mobilisable calcium ions.

Phosphates are generally stored in amorphous rather than crystalline granules. Interestingly, amorphous granules are also the structural phosphatic compounds used by the large majority of the animal phyla, the exceptions (with the use of crystallised phosphates) being the skeletons of vertebrates and certain cnidarians (the conulariids; Hughes, Gunderson, and Weedon 2000), the cuticle of some basal arachnates (aglaspidids; Briggs and Fortey 1982), and the shells of some inarticulate brachiopods (Lowenstam and Weiner 1989). At variance with its limited diffusion in the hard parts of present-day animals, calcium phosphate was a favourite of the earliest animals with skeletons. Calcium as a cation and phosphate as an anion were obvious choices, because animals had already evolved the ability to manipulate calcium due to its importance in muscular activity and other aspects of cell functioning; phosphate was available in the form of store granules. Phosphatised skeletons developed when biomineralisation became associated with the production of extracellular glycoproteins which, in turn, were involved since their first appearance in the control of developmental processes, such as directional cell movement.

Yet in many protist lineages, biomineralisation followed alternative routes, involving different materials which provided advantages of another type than those suggested herein for metazoans with phosphatised skeletons.

Organic Matrices

Forcing cells to multiply in monolayers (i.e., in epithelial sheets) is possibly a way to avoid uncontrolled cell proliferation. The production of a basement membrane is a way to achieve this result. It is well known that adhesion to a substrate is a force in development (reviewed in McNeill 2000). Thus we can speculate that a cell matrix also originated for development. One might speculate that, in Precambrian times, in early multicellular organisms living in contact with a solid (mostly inorganic) substrate, the latter could influence cell proliferation and patterning. The production of an organic extracellular matrix was a way through which cell lineages could achieve better control of their own growth and patterning in respect to the corresponding behaviour of their neighbours – something that provided a selective advantage that increased the chances of survival of these lineages and their way to development. In 'lower' metazoans, this control is sometimes still reversible. For example, in *Podocoryne carnea* (a hydromedusa), maintenance of the differentiated state of the striated muscle depends on carbohydrate-mediated interactions between the cells and the extracellular matrix. If these interactions are disturbed, the striated muscle cells undergo DNA replication and transdifferentiate to smooth muscle and nerve cells (Reber-Müller et al. 1994).

Coda

When stepping away from an adultocentric view of development and emphasising the high degree of local autonomy that cells and other subsystems within a developing organism may enjoy, despite all forms of internal control, one may wonder why the specific shapes of organisms – both as adults and during earlier developmental phases – are actually important *right as shapes of developing organisms*. In my opinion, these shapes are, simultaneously, the cause and effect of a control of cellular Darwinism. I do not see any need to subscribe to the widespread belief that developmental processes exhibit specific adaptations to the putative function of creating a complete organism (Chipman 2001).

Animals have invested a lot in controlling their shape, since the earliest times of their evolutionary history. But why is shape so important? The question is not about the adaptive significance of a well-designed wing or fin or foot. The question is whether a control of form is necessary *for development to proceed*. Development, I contend, has its own logic, besides and before being the means to produce a larva, a juvenile, or an

adult. If development is the way by which the continuity of life is guaranteed, then it is possible to explain developmental features in terms of their role in producing *further* developmental features. Thus, shape emerges in developmental importance for two reasons: (1) because it helps control cell proliferation within the growing 'cell federation' and (2) because shape helps organise shape.

Cuticles and skeletons, as well as the basement membrane, may have also been important, beyond their probable role in the control of cell proliferation and in the preservation of specific shapes – a role comparable with that of the ciliary complexes in ciliates (Frankel 1989). The lack of corresponding features is possibly the reason for the extreme morphological plasticity of multicellular organisms such as mushrooms and sponges.

CHAPTER THREE

Development: Generic to Genetic

> Genes are not 'determinants' representing the one or the other part of the body; rather, they are modifiers of the developmental processes, intervening in this or that cellular functioning, hence in these or those morphological outcomes.
>
> E. Guyénot 1929: 40 (my transl.)

> Shouldn't we do well at this stage to be flexible, rather than succumb to the current tendency for each aspect or event in an organism's life that attract our interest promptly to become the express responsibility of a gene.
>
> J. Cooke 1980: 217

Developmental Genes

Are there true 'developmental genes'? Yes and no. Yes, in the sense that patterns of expressions of many genes are strictly limited to and correlated with specific times and events in development. Yes, in so far as mutations in these genes may critically and conspicuously alter the normal course of development. No, however, if we take any of them as directly responsible for the origin of an organ or the shaping of the body. At least we need to consider genes in context, not just with other genes, but with the whole cellular environment (Maclean and Hall 1987, Keller 2000, Nijhout 2000, Hall 2001).

I subscribe fully to Gabriel Dover's (2000: 45) text that, "There is a naivety about genetic determinism in both evolution and development that signifies intellectual laziness at best and shameless ignorance at worst when confronted with issues of massive complexity." Genes encoding transcription factors and the components of intercellular signalling pathways, such as ligands and receptors, are very similar in animals with very different body plans (Davidson 2001). These genes have been probably conserved

by natural selection because of their generic ability to stabilise form, not because of any specific role in generating a particular body pattern. Let's keep some distance from the well-entrenched habit of evaluating an animal's form in terms of adult fitness only. Overall shape and detailed architecture of a developing organism are important per se, at any given stage, as expression of a consistent and self-supporting system of cells and cell lineages involved in multifarious interactions, including competition among the system's parts. Genes may help in developing or maintaining a given spatial arrangement. Genes are canalisers of life along the quasi-cyclical process of development. But, in a sense, it is form that captured genes more than it is genes that created form (cf. Budd 1999).

The spatially and temporally restricted patterns of expression of transcription factors and batteries of genes they control do not obscure the fact that a large fraction of all genes in a genome are expressed virtually everywhere in the organism. Neither should we discount this fact as developmentally irrelevant, by qualifying this silent majority as an indifferent background of housekeeping genes and the like, with no consequence for morphogenesis. Differentiation and patterning of any given tissue or body part require thousands of genes, whose expression is controlled by extensive regulatory networks (Davidson 2001). The number of genes involved in controlling the expression of other genes is impressive. It has been estimated to be about 12% of the total in *Arabidopsis* and up to 18% in the yeast (Finnegan 2001). Without this control, gene expression would obviously be much more uniform across the different parts of the organism, and body patterning would be very limited and less stable. Evolution has clearly favoured spatial repression, especially along the evolutionary history of the bilaterians (Davidson 2001).

Many genes are expressed in different cell types and body parts, but – in addition to the expression of a limited number of tissue- or organ-specific genes – differences in the temporal expression patterns and quantitative profiles of the shared expressed genes will probably make the difference. If so, Wagner, Chiu, and Laubichler (2000) are correct when noting that the evidence at hand does not support the conclusion that any particular gene is instrumental in the origin of major characters, such as insect wings.

In his recent book, Davidson (2001) has convincingly argued that there are no genes specific to a given body plan. There are no insect genes, no sea urchin genes, and no vertebrate genes. Differences between a beetle and a frog are not a matter of building stones, nor are they a matter of the kind of paper and ink used to draw a blueprint of their respective body plans.

To reduce developmental processes and the complex body structures to which they give shape to the exclusive action of one or few genes can lead us astray. Observing that, in vertebrates, *Brachyury (T)* is required for the specification of the notochord, whereas its *Drosophila* homologue *T-related gene* is required for specification of the hindgut, Kispert et al. (1994) raised the question of a common evolutionary origin of the hindgut of insects and the notochord of vertebrates. On the other hand, Technau and Bode (1999) have simply described this fact as a lack of conservation of the function of the *Brachyury* homologues in the different phyla. This difference of attitude reminds me of what a comparative biologist would call a willingness to compare two objects (Eldredge and Cracraft 1980, Rieppel 1988, Minelli 1993). Why are we interested in discussing the possible homology between the wing of a bird and the forearm of a man, whereas we never seriously try to compare the wing of a bird with the caudal fin of a fish? In the absence of specific arguments to the contrary, shared patterns of gene expression should not lead us, per se, to homologise organs that a comparative morphologist would never try to compare.

The biological literature of the last few decades has been distinctly dominated by a gene-centred view of development. Here's an example: "Recent results indicate that for several well-studied organs there is a single gene or a small set of genes that specifies the basic form of the organ. These genes are expressed early in development in wonderfully complex patterns that prefigure the complex form the organs will take. They are not passive markers of organ structure, but rather are key morphogenetic regulators that drive the early developmental events that shape the organs. [..] I suggest the name 'genomorphen' for this class of genes, to emphasize their key roles in generating organic form" (Krasnov 1997: 235, 237).

It is fair to say that less extreme positions are emerging. Rather than seeing developmental decisions as the effect of single 'developmental genes', many researchers regard them as 'logical operations' allowing the effects of many signals, both short and long range, to be integrated through the action of multiple transcription factors. See, for example, Ghazi and VijayRaghavan's (2000) commentary on Halfon et al.'s (2000) demonstration that integration of several transcription factors (Pointer, dTCF, Mad, Twist, and Tinman) determines the specificity of the Ras inductive signalling towards muscle and heart development in *Drosophila*.

There is much more in the genome than a simple catalogue of genes. Alternative splicing can create more different transcripts from a single gene than there are different genes in the animal's genome. This mechanism

may have an extremely important role in raising the diversity in the proteomes. It may help understand why the number of genes in an organism's genome does not correlate with its morphological complexity (Graveley 2001), irrespective of the metrics we use to estimate the latter.

Statements to the contrary notwithstanding, the role of genes in morphogenesis is likely *always* to be an indirect one. Thus, there is nothing different from the non-specific effects observed in transgenic animals; for example, in the cohn salmon where the insertion of a gene construct caused significant changes at once in the shape of the cranium, abdomen, and caudal peduncle (Ostenfeld, Mclean and Devlin 1998).

There are many reasons to reject a reductionist view of development as the mere expression of the genotype or, as it is often presented, as the outcome of a genetic programme.

Genes, as Guyénot wrote in 1929, are not 'determinants' of any part of the body, but modifiers of the developmental processes involved in specific morphogenetic activities only as far as they control a specific aspect of cellular functioning. The most recent advances of developmental genetics cannot but confirm the validity of Guyénot's farsighted view.

A common reductionist approach is to distinguish between housekeeping genes responsible for basic metabolism and 'luxury' (in principle, disposable) genes responsible for generating patterns. In this context, there is an interest in determining size and content of the minimum genome required by a living being (e.g., Hutchison et al. 1999). But the distinction between housekeeping and luxury genes does not seem to be warranted. Think of a cake. You can make it square, round, or heart-shaped. The cake's surface may be plain or decorated; the outside may be more or less distinguishable from the inside. But are these differences the outcome of postbaking patterning or repatterning (cf. the product of luxury developmental genes), or simple by-products of the very baking event (cf. the product of housekeeping genes)? The surface pattern is possibly the result of your geometrical skill, but a regularly spaced pattern of bubbles may simply result from the action of the yeast or, better still, of the inanimate baking powder working inside the dough. Note the origin of a beautifully cracked surface whose morphogenesis would defy any deliberate attempt to do 'by hand' what happens 'quite naturally' in the oven.

This is not to say that all genes are the same and, particularly, that all genes perform just housekeeping jobs. But we must dispose of the idea that housekeeping genes evolved once and forever, in a remote aeon, and are now continuing to perform their job 'at the service' of a separate and

still evolving company of 'higher level' developmental genes. The whole system and all its components are evolving without rest.

According to Keller (2000), the notion of a genetic programme depends on the common mistake of identifying the distinction between 'genetic' and 'epigenetic' with the distinction between 'programme' and 'data'. The genetic information present in a zygote is usually equated to a computer programme, but it might be regarded otherwise as a set of data to be processed by a programme already embodied in the structure of the cell. The programme, for example, might reside in the transcription and translation machinery. Keller's argument does not reject the programme metaphor. However, it shows clearly how arbitrary is the current identification of the genome with a programme and how unjustified is the widespread disregard for the role of the cytoplasm (or, for the sake of the argument, of anything in the cell other than the genome) in carrying and transmitting "effective traces of intergenerational memory" (p. 287).

In a single cell – but not in its genome alone – there is enough of complexity, morphological and metabolic, to account for the variety of behaviours cells offer when interacting in multicellular systems. Interestingly, this point has been made by a microbiologist (Harold 1995), whose problem was not so much how multicellular systems acquire their organization during development, but, more basically, how to bridge the gap between the genes, which specify the structure and control the synthesis of molecular components, and the spatial (I would add: and temporal) order these components enjoy in the cell. In other words, cellular forms are dependent on the collective expression of many gene products, but only through a network of epigenetic processes by which the actual forms are generated. I ask the reader for indulgence if he or she finds here and there, in the following pages, commonplace expressions such as 'genetic control' and the like. It is not easy to refurbish the language systematically according to premises other than those prevailing in the literature.

Master Control Genes?

The fashionable concept of master control gene was introduced by Lewis (1992) for the homeotic genes of the *Bithorax* complex in *Drosophila*, but was mostly championed by Gehring (for an historical perspective, see Gehring 1998). A master control gene would be responsible for a major switch in the expression of a large number of downstream genes.

Induction of ectopic eyes by targeted expression of the *eyeless* (*ey*) gene in *Drosophila* has been used to support the proposition that *ey* is the master

control gene for eye morphogenesis. The presence of homologous genes in a variety of metazoans, such as vertebrates, ascidians, insects, cephalopods and nemerteans, led to the suggestion that the *Pax6* genes – of which *ey* is an homologue – may function as master control genes in the production of eyes throughout the Metazoa (Halder, Callaerts, and Gehring 1995). Corresponding experiments with *Pax6* gene expression in *Xenopus* (Chow et al. 1999) led to the same results. The involvement of *Pax6* homologues in eye morphogenesis has been also ascertained in squids (Tomarev et al. 1997), amphioxus (Glardon et al. 1998), nemerteans (Kmita-Cunisse et al. 1998) and planarians (Pineda et al. 2000). The purported conclusion was that the origin of the eye is monophyletic throughout the animal kingdom (Gehring and Ikeo 1999, Gehring 2000). More cautiously, however, Wagner (2001) identifies the most likely ancestral role of *Pax6* homologues in initiating the development of light-sensitive epithelia, thus providing what in the end was the phylogenetic precursor of both the camera eye of vertebrates and squids and the compound eye of arthropods.

This is an old story. As early as 1897, Minot (p. 939) wrote that, "A morphologist [..] must [..] feel grave hesitation in assuming that main lateral eyes have been evolved in the Articulates and Vertebrates without any genetic relationship. It is natural, therefore, to test the assumption that the articulate eyes and the vertebrate eyes are phylogenetically homologous, it being, of course, understood that the comparison excludes ocelli, accessory eyes in Annelids and the pineal eye of Vertebrates. We note at once that the visual sensory apparatus in both cases is epithelial in type, and is derived immediately from the ectoderm, and further that in both eyes the sensory apparatus is directly connected with nervous substance [..] and finally that in both cases it runs from the optic apparatus a fibre tract, which is [..] a part of the central nervous system itself." Is modern molecular genetic evidence more convincing than this?

Harris (1997) and Meyer-Rochow (2000) have strongly opposed the idea that eyes were invented only once. As poignantly argued by Harris, there are good reasons for taking seriously the hypothesis that there has been an evolutionary reason to conserve the role of *Pax6* in eye development. But this does not necessarily imply that one subscribes to this hypothesis' bold version, [i.e., that *Pax6* is a (the) master regulator of eye development]. More reasonable is a weaker version of this hypothesis, namely, that *Pax6* is a patterning gene, expressed in the head, which has been repeatedly coopted in the regulation of eye development. *Pax6* expression in vertebrates is not limited to the eye, but extends to nasal placodes, diencephalon and

latero-ventral hindbrain, and spinal chord (Li et al. 1994, Amirthalingam et al. 1995). In *Drosophila*, the homologous gene *ey* is expressed in the brain and ventral nerve cord, in addition to its expression in the eye disc. In squid, *Pax6* expression in the eyes is accompanied by further expression in the brain and in the arms (Tomarev et al. 1997). Homologues of *Pax6* are known in eyeless animals such as the nematodes, in which these genes are involved in differentiation of the cephalic body end (*vab-3*), or peripheral sense organs of the tail (*mab-18*; Chisholm and Horvitz 1995, Harris 1997), and in sea urchins, in which the *Pax6* homologue is expressed in the tube feet (Czerny and Busslinger 1995).

The very existence of master control genes, however, is questionable. Casci (2001) remarks that, over the past few years, seven *Drosophila* genes (*eyeless*, *twin of eyeless*, *eyes absent*, *sine oculis*, *dachshund*, *eye gone*, and *optix*) have been assigned the master role in initiating eye cell fate. None of them passed the criterion of producing homeosis if not expressed at its usual site. Davidson (2001: 27) rejects the very concept of master control gene as a "fantasy of earlier days". Evidence suggests that morphogenetic functions are controlled by complex networks of signal systems and transcriptional regulators, rather than by a linear hierarchical control sequence beginning with a hypothetical master gene (Davidson 1993).

Self-Assembly or Cytotaxis?

Contrary views notwithstanding, self-assembly is not enough to build a cell. No cell will form following Lederberg's (1966) recipe: make the polypeptide sequences at the right time and in the right amounts, and the organisation will take care of itself.

This is not to deny the role of self-organisation in complex systems as are biological systems (e.g., Kaufman 1993). It is probable that, in primordial biological systems, self-organisation played a more important role than it plays in our times.

Nevertheless, today's cells do not form by aggregation of their constituent parts, but only by growth and division of pre-existing cells. A new cell needs a pre-existing cell as a template. With development as the self-perpetuating dynamic of multicellular organisms as suggested in chapter 1, we expect transmission of structure and form not to be exclusively dependent on the transmission of genetic information. This property places life in that widespread category of natural phenomena that are reproducible without being encoded by a programme. Such are

ecological successions or the daily ebb and flow of city traffic (Harold 1995). (Interestingly, as noted by Gould (2002), a similar metaphor was offered by Darwin's cousin Francis Galton (1884) to emphasise the recurrence in evolution of discontinuous patterns basically depending on an organism's internal structure, with the role of natural selection limited to pushing the system from a stable configuration to another within a limited number of available alternatives.) All these phenomena are *historical* in nature. Each event is at once the effect of earlier events and the cause of subsequent ones. It is only because like causes elicit like effects, not because the whole sequence is run by a programme that the sequence recurs time after time. I wholeheartedly subscribe to Harold's (1995) conclusion that we urgently need a conception of the organism in which our preoccupation with genes would be placed at the service of the pivotal concept of the persistence of structural order.

Ciliate protists have long offered a wonderful opportunity to demonstrate the importance of cytoplasmic memory in determining the most complex structural details of generation after generation of dividing cells. The extraordinarily sophisticated, species-specific architecture of their cellular cortex, with its patterned rows of cilia and its morphologically and functionally specialised membranelles, is built after the template offered by the rows of cilia and the membranelles of the dividing cells, without any direct intervention from the genome (e.g., Tartar 1961). In his important monograph on pattern formation in ciliates, Frankel (1989) stresses the general significance, for the study of both heredity and morphogenesis, of the demonstration that inheritance is not limited to nucleotides, but extends to whole complex structures independent of either DNA or RNA. From the point of view of animal evo-devo biology, the question is whether these aspects of cytoplasmic information (cytotaxis sensu, Sonneborn 1964) are limited to ciliates or are a general property of cells. In eukaryotes other than ciliates, a molecular marker of cell polarisation has been described in diploid yeast cells, which repeatedly polarise and bud from their poles. A membrane protein called Rax2 behaves as a cell polarisation mark. This protein is cytoplasmatically inherited, for several generations, in its fixed position at the cell cortex (Chen et al. 2000). Convincing comparative evidence is not abundant, but good examples of cytotaxis are provided by other unicellular organisms, such as *Trypanosoma brucei*, in which a specific connection develops during cell division between the old flagellum and the new flagellum. This connective structure is only present

during duplication and seems to be responsible for the correct replication of the helical cell pattern and polarity (Moreira-Leite et al. 2001). Another example of cytotaxis is the multicellular freshwater green alga *Hydrodyction*, whose structure is a three-dimensional lattice made of many cells which also serve as a template for the three-dimensional pattern of the next generation (Bonner 1993).

I am not aware of any demonstration of the inheritance of intracellular patterns in animals, but it is certain that very few biologists have thought of it as a rewarding topic to be investigated. This is a pity, however, due to the acknowledged importance of pre-existing structures, such as microtubule organizing centres and selected membrane domains, in determining the structural organization of animal cells.

There are two reasons why animals are generally unlikely to transmit major morphological traits by non-genetic means. First, their life cycle usually includes a unicellular stage (the egg), which is only a tiny part of its parent's body, hence it cannot be expected to possess and transmit a sizeable and useful part of the body pattern of the multicellular parent (Frankel 1989). Second, animals are gametogamic rather than gamontogamic as ciliates (and other protists) are. That is, their basic sexual event is the fusion – and morphological modification – of two gametes, rather than the exchange of gametic nuclei between two gamonts (conjugants) without disruption of their cellular integrity, the complex cortical pattern in particular. Nevertheless, there are situations in which conservation and transmission of cellular and supracellular structural patterns may be expected in animals as well. This is the case with asexual reproduction, regeneration and the morphogenetic events accompanying arthropod moults.

Frankel mentions Sonneborn's (1930) experiments with the flatworm *Stenostomum*, a freshwater animal with asexual reproduction. Sonneborn obtained somatically modified doublet worms. Similar to ciliates with surgically modified ciliature, these worms gave rise to a progeny that perfectly reproduced their doublet configuration.

Locke (1990) suggested a somatic inheritance of intracellular patterns for the so-called Siamese twin cells in the epidermis of caterpillars. I wonder whether human fingerprints, with their lifelong invariance, or the polygonal patterns of the arthropod cuticle (Figure 3.1), which are basically conserved from one stage to the next (e.g., Bennet-Clark 1971), are also largely dependent on the continuity of extragenetic structural information (cf. Fusco, Brena & Minelli 2000).

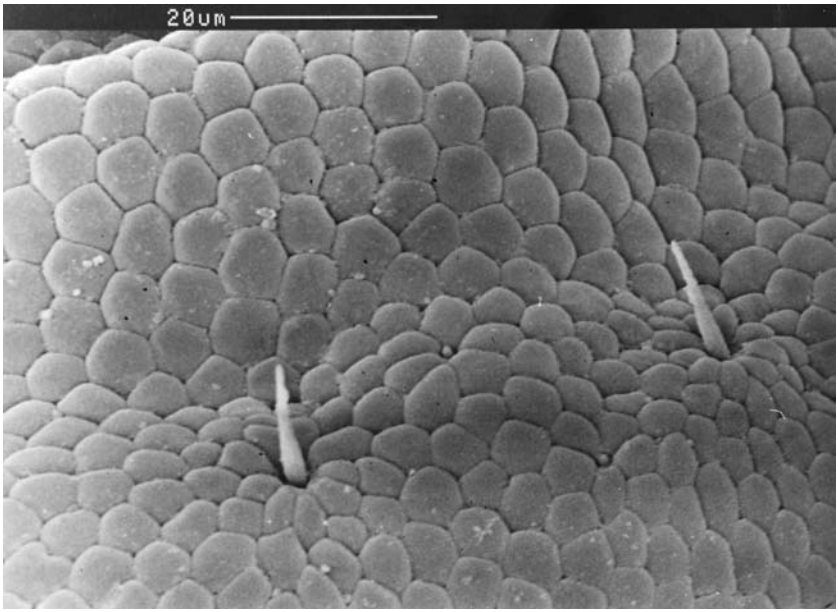

Figure 3.1. Scanning electron micrograph of part of an antennal article of the centipede *Himantarium gabrielis*. In the cuticle covering the epidermal cells of the arthropods, a polygonal pattern is commonly observed, which mirrors the outline of the external surface of the individual cells.

Default Morphology

Developmental genetics is discovering more and more examples of 'default morphology' manifested when some gene activity is lacking. One could ideally push gene silencing further and further, until a minimum gene number is found, by which a given level of morphological complexity is still generated or, better, saved and transmitted. At present, we must content ourselves with experiments with a few silenced genes and a few selected structures.

Carroll, Weatherbee, and Langeland (1995) have demonstrated that insect wing formation, although subject to control by *Hox* genes, is not promoted by any of them. The potential for wing production extends to most trunk segments, but is repressed in different body segments by different *Hox* genes (*abdominal-A*, *Ultrabithorax*). This is possibly a derived feature, evolved later than the first origin of insect wings.

Insect antenna has been often regarded as the default appendage, representing a state towards which other appendages revert in the absence

of the specific input required for their determination (Stuart et al. 1991, Hughes and Kaufman 2000). This has been interpreted as proof that all the gnathal, thoracic, and abdominal segments (and their appendages, where present) are built from a ground plan comparable with that of the antennal segment (Rogers and Kaufman 1997).

In *Drosophila*, for example, the expression of both *proboscipedia* and *sex combs reduced* is required for the specification of the sucking mouthparts. In the absence of these two activities, an ectopic antenna forms in place of the 'proboscis' (Percival Smith et al. 1997). Other evidence points in the opposite direction. In the absence of *spineless* activity, the distal part of a *Drosophila* antenna is transformed into leg (Duncan, Burgess, and Duncan 1998). Specification of an appendage as antenna, on the other hand, requires specific input from *extradenticle* and *homothorax*. By removing the function of either of these genes, an antenna-to-leg transformation is observed (Casares and Mann 1998). One reason for suggesting that the antenna is more primitive than the leg is the presence, in the latter, of a distinct intermediate domain between the proximal and the distal one, a feature without parallel in any arthropod antenna (Dong, Chu, and Panganiban 2001). The argument is not too strong, because the phylogenetic concept of primitive feature is clearly not the same as the putative 'default morphology', which is perhaps nothing more than pure abstraction. In their recent review of this issue, Casares and Mann (2001) suggest that the ground state of the ventral appendages in *Drosophila* (antennae, legs, genitalia and analia included) is a leg-like appendage consisting of a proximal segment and a distal tarsus.

One wonders in the end what 'default morphology' may really mean. All together, from this contradictory evidence, one main conclusion seems to emerge – that is, the identification of a given body part with the specific activity of one or a few genes is unwarranted. In Lawrence's (2001) words, it is incorrect to conclude that a gene must be a major or pivotal one if a particular process does not work in its absence. The gene is ignorant of its role in development (Dover 2000).

As early as 1990, Nijhout wrote one of the most perceptive pages on the inadequacy of a strictly gene-centred view of development. First, he rejected the view that genes control development, stressing the endless continuity of development and its manifold causality, which includes, besides gene expression, structural, chemical, and physicochemical components. Rather than as direct controllers of developmental processes, genes act as selectors, steering development among alternative options. Moreover,

their action is far from showing the linearity of the hierarchical cascades all too often offered as a model of gene action in development. Second, Nijhout rejected the notion that the genome contains a developmental programme. He choose the example of *bicoid*, whose expression in the early *Drosophila* embryo is currently described as essential to the establishment of the antero-posterior body pattern. What is generally overlooked is the fact that the correct gradient of the *bicoid* product is only established if many other specific gene products are also produced and distributed at the correct time, thus allowing a number of specific interactions with structural elements in the cytoplasm to take place. The only sensible question to ask about the way genes may be said to control development is whether a *difference* in a given character between two individuals is due to genetic factors more than environmental factors.

Generic Forms

"It is possible that current evolutionary theory, with its emphasis on parsimony, may not be well equipped to deal with the numerous findings of parallelism (and convergence) in morphological evolution" (Hodin 2000: 10). Convergence, indeed, is much more widespread that was traditionally believed (a point recently made by Conway Morris 1998). This conclusion is now commonplace in molecular phylogenetic papers. The phenomenon is not circumscribed to organisms with very simple organisation, where morphology cannot help too much unraveling phylogenetic relationships. It is also rampant in animals and plants with very complex organisation, from orchids (Chase and Palmer 1988) to amphibians (Wake 1991). In the latter case, Wake commented that the extraordinary amount of homoplasy found in plethodontid salamanders is very likely to be the rule, rather than an exception. This is confirmed by Moore and Willmer's (1997) extensive survey of the main animal phyla. Occasionally, parallel evolution may simply be the result of chance, with the use of aldehyde dehydrogenase as a lens crystallin in squid and elephant shrews (Hodin 2000), but it is likely to owe a lot to the widespread occurrence of generic mechanisms in development.

A ciliate protozoan and an insect embryo have very little in common, whatever unit of measure we might adopt in the comparison. Neither are the two organisms closely related in terms of phylogeny, one good billion years having elapsed since their phyletic lines separated from their most recent common ancestor. Nevertheless, these two biological systems have several features in common that Frankel (1989) regards as the product of quasi-universal generative rules governing global pattern formation in

living organisms: separation between polarity pattern and differentiation pattern, global control, morphallactic reorganisation, positional continuity, spacing of positional values, and generative rules. How to explain these extensively similar behaviours? It may be simply that similar generative rules are probable in biological systems of similar size (both the germ band of insects and many large ciliates are in the range of 0.2–1.5 mm).

Jeong et al. (2000) analyzed the metabolic networks of 43 organisms – not just animals and plants, but also prokaryotes (eubacteria and archaebacteria). They found that, despite significant differences in the individual constituents and pathways in these networks, the metabolic organisation is not only identical for all living organisms, but also universally endowed with the design principles of robust and error-tolerant, scale-free networks.

Universality of mechanisms does not imply that exactly the same molecules are involved. This makes sense from a mechanistic point of view, but note the evolutionary implications. If similar but not identical molecules actually perform the same job in different organisms, this means that the same result was probably obtained independently more than once. In other words, we are facing convergence rather than common descent. This may be true, for instance, for the mechanisms establishing positional information in developing systems (Kauffman 1993).

Newman and Comper (1990) suggested that many morphogenetic and patterning effects in living systems are the inevitable outcome of the physical properties of cells and tissues. This does not negate the relevance of genetic mechanisms, but their action is simply complementary to that of generic physical mechanisms. Newman and Comper (1990: 1) define generic mechanisms "as those physical processes, that are broadly applicable to living and non-living systems, such as adhesion, surface tension and gravitational effects, viscosity, phase separation and phase-diffusion coupling", and may generate morphogenetic rearrangements of cytoplasmic tissue and extracellular matrix components.

According to Newman (1994), two of the major types of gastrulation (epiboly and involution) and possibly a third (delamination) could have originated as simple consequences of differential adhesion, even if nowadays these processes are triggered and influenced by specific patterns of gene expression. Observation of cell behaviour during epiboly in zebrafish seems to support a similar reduction of gastrulation to physical forces. The morphogenetic changes that accompany epiboly may result from the passive response of cells to the reproducible forces that change the overall shape of the blastoderm prior to gastrulation (Wilson, Cretekos, and Helde 1995).

Van Essen (1997) suggests that some morphogenetic mechanisms shaping the central nervous system of mammals may reduce to mechanical tensions involving axons, dendrites, and glial processes. For example, tension along axons in the white matter might explain the species-specific patterns into which the cortex folds.

The morphogenetic potential of simple physicochemical processes must not be underrated. Jiang et al. (1999), for example, have shown how the periodic patterning process in feather morphogenesis in birds is probably dependent on the self-organising properties of the feather-producing cells. In their model, cells that become competent to form feather primordia have, initially, equivalent probabilities of becoming primordia or interprimordia. When a given threshold of cell density is reached, their adhesive properties lead to the random formation of many unstable microaggregates. The latter process leads to increased concentrations of adhesion molecules, so that some microaggregates increase in size or merge by collision. Aggregates that reach a certain threshold exercise a long-range lateral inhibition that halts the neighbouring regions from becoming feather primordia. Because the growth of these cell microaggregates is based on competition, this leads to their even spacing.

Drosophila mutants are known to develop symmetric patterns on successively shorter length scales (half embryo, a quarter of embryo, etc.), suggesting the existence of 'generic' wave-like phenomena by which the embryo might be actually subdivided into increasingly shorter longitudinal domains, down to single segments (Lacalli, Wilkinson, and Harrison 1988, Goodwin and Kauffman 1990, Kauffman 1993). Unfortunately, in the study of arthropod segmentation, very little attention has been focused on the four-segment periodicity observed in the early expression of some pair-rule genes in the tobacco hornworm *Manduca sexta* (Carr and Taghert 1989), later followed by bisegmental patterns and finally by segmental patterns, possibly proof of the action of generic mechanisms in segmentation.

In this context, the main role for pair-rule gene expression may reside in generic properties of cell adhesion, as manifested during germ-band extension in *Drosophila*. After onset of gastrulation, the length of the germ band increases over two-and-a-half fold. This change of shape corresponds to an increase in the number of cells along the antero-posterior axis and a decrease along the dorso-ventral axis. Mutations affecting the segmental subdivision of the embryo along the antero-posterior axis, such as those in pair-rule genes, have negative effects on cell intercalation along the

longitudinal axis, thus reducing germ-band extension. According to Irvine and Wieschaus (1994), cell intercalation is thus dependent on the establishment, through pair-rule gene expression, of adhesive differences between stripes of cells.

Recently, Gordon (1999) has devoted a massive two-volume work to his concept of differentiation waves. When a wave of a process, such as cell contraction or expansion, cytoskeletal changes, ionic currents, concentration changes, chemical and stereochemical reactions, mechanical effects, and changes in gene transcription, traverses a tissue in an embryo, the tissue is split into two new tissues. As Papageorgiou (2001) remarks in reviewing this book, Gordon's theory smells of heresy from the viewpoint of traditional embryology, but there is no reason why it should be dismissed without adequate testing. In the meantime, one may speculate whether the morphostatic role I suggested in the previous chapter for the cuticle of ecdysozoans might have something to do with blocking differentiation waves.

A Bestiary of Generic Forms

Segments In a paper provocatively entitled, *Is segmentation generic?*, Newman (1993) defended the thesis that the capacity of undergoing segmentation may be 'generic' to tissues, that is, an outcome of their most general physical and chemical properties, without regard for any difference in their specific molecular makeup. This property, if true, would make segmentation a generic property likely to evolve independently in several phyla, with the possible involvement of different molecular mechanisms in different taxa. Newman's concept of segmentation is close to that of compartmentalisation (for a definition, see page 242). In his view, the segmental organisation is frequently based, at least in part, on the inability of otherwise similar tissues to exchange cells at their interface. There is no shortage of evidence in support of this view. Immiscibility of cells from neighbouring segmental units is found, for example, between *Drosophila* compartments (Lawrence and Johnston 1986), vertebrate rhombomeres (Guthrie and Lumsden 1991), and half-somites in the chick embryo (Stern and Keynes 1987).

Tubes An experimental proof that generic processes may generate tubes has been provided by Coucouvanis and Martin (1995), with the conversion of a solid primordium into a hollow tube as in the early mouse embryo, in which the solid embryonic ectoderm changes into a columnar epithelium

surrounding a cavity. Cavitation in this case is the result of the interplay of a signal from an outer layer of endodermal cells that induces apoptosis of the inner ectodermal cells and a rescue signal. It is mediated by contact with the basement membrane that provides for the survival of the columnar cells lining the cavity.

Hollow Spheres Both theoretical models and experiments with reaggregating dissociated cells have shown that simple self-sorting mechanisms due to differential surface adhesion properties of the cells may produce hollow spheres, provided these cells are non-adhesive over portions of their surfaces (e.g., Keller and Segel 1970, Newman and Müller 2000).

Layers, Sheaths, Tubes, Rods, Spheres, Etc., as Aggregates of Cells More generally, simple biomechanical forces are sufficient for moulding groups of cells into layers, sheaths, tubes, rods, spheres, and so forth. As noted by Newman and Müller (2000), the morphogenetic role of these structures is not limited to their importance as geometric templates for more advanced patterns or as barriers allowing the partitioning of the organism into functional modules, but may extend to some form of control of gene activity (see also Ingber 1993, Chen et al. 1997).

The Earthworm and the Ankylosaurus

Many animals belonging to different phyla are provided with dorsal or ventral appendages, sensory organs or simple colour spots, which form a kind of regular, segmental checkerboard. Although the number of segments is widely different from case to case (and the developmental origin of these segments may also be different; cf. chapter 9), the number of elements per transversal row seems to follow strict, nearly universal rules.

In most earthworm species, there are four pairs of setae per segment. Their mutual spacing is different in the various genera, but distribution is highly stereotyped, so that the whole array is simply described by specifying the pattern of setae in a segment and the total number of segments in the body. (I am disregarding minor alteration of the pattern in the terminal segments and in the clitellum, a short range of segments involved in the production of the egg cocoon, which are usually marked by a different colour and become thicker at maturity.) This set of regularly patterned setae repeated regularly from segment to segment gives rise to a checkerboard pattern which has its equivalent in a lot of different animals. Many insects, centipedes, millipedes, mites, etc., have one or two rows of

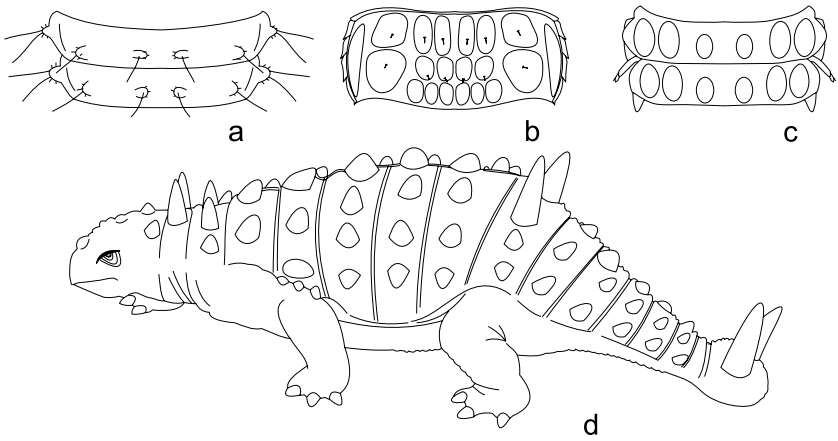

Figure 3.2. Details of dorsal checkerboard patterns from different animals: a, two abdominal segments of the larva of the ladybird *Rodolia cardinalis*; b, trunk segment X of the millipede *Polydesmus coriaceus*; c, segments VI and VII of the anterior trunk region (pereion) of the isopod crustacean *Buddelundiella cataractae*; d, habitus of *Scolecosaurus*, an armoured dinosaur from the Upper Cretaceous period. (All redrawn– a, from Stehr 1991; b, from Schubart 1934; c, from Oliver and Meechan 1993; and d, from Charig 1979.)

big setae per segment, with a generally small and fixed number of setae per row. The pattern is more or less extensively repeated along the chain of segments in the trunk. The large setae distributed in such regular patterns are called idionymic setae.

Checkerboard patterns are not limited to setae, however. Similar, for instance, is the pattern of dorsal plates of the big extinct ankylosaurs, or the dorsal bumps of several small Recent arthropods, including the woodlice genus *Buddelundiella*, the pill millipede *Trachysphaera*, and the springtail *Morulina*. Similar, again, are segmentally periodic colour patterns such as those of the European blood leech *Hirudo medicinalis* and the typical pill millipede *Glomeris* (Figure 3.2).

I would argue that the structure of all these checkerboard patterns, despite the wild diversity of the animals in which they appear, is not serendipitously the same. Each of these patterns likely evolved independently from most of the others, but it is possible that all these patterns represent a generic form; that is, they are the product of the same generic mechanism (e.g., Goodwin, Kauffman, and Murray 1993).

A common background to these checkerboard patterns might reside in some common feature of the patterning of the nervous system. In

vertebrates, for example, the neural ectoderm of the embryo is subdivided into a checkerboard of domains by the intersection of longitudinal and transverse compartmentalisation (Rubenstein and Puelles 1994). Similarly, in the embryonic central nervous system of *Drosophila*, the combinatorial expression of the gene products of antero-posterior and proximo-distal axis-patterning genes defines a checkerboard of equivalent groups (Skeath and Carroll 1994) defined by homologous cell lineages (Stent 1998). Number and spatial arrangement of these equivalence groups are the same as the basic pattern of sensory setae in the segments of several arthropods.

These correspondences are not limited to the nervous system and the associated sensory organs. In *Drosophila*, each adult segment originates, during metamorphosis, from four pairs of histoblasts containing between six and thirteen cells each. These eight histoblasts are arranged in two transverse rows (Milner and Bleasby 1985), similar to the arrangement of the sensory setae on the dorsal and ventral sclerites of so many arthropods.

Deceptive Numbers

Simple patterns and numerical identity may be positively deceptive. In insects, there are eight cells in the photoreceptor of each ommatidium, the morphofunctional unit of the compound eye. One could expect these eight cells to be the product of three successive mitoses, starting with a founder cell, but this is not the case; the eight cells of each ommatidium are not related by lineage (Gurdon 1992, Lawrence 1992). Even more attractive for arithmetical speculations is the number (32) of 'true' segments in leeches. This number would be easily obtained through five binary divisions of a founding element ($2^5 = 32$), but nothing of this kind actually happens in leech development. Segments are founded by cells derived from seven blast cells (m, n_f, n_s, o, p, q_f, q_s), each of which, through a stereotyped series of divisions (Zackson 1984, Bissen and Weisblat 1989), generates cells which will contribute to the initial cell pool of each segment. Nothing in this developmental sequence exhibits anything of the regularity expected from arithmetical considerations. First, the divisions of the blast cells are sequential, rather than hierarchical. Their progeny is produced in a regular antero-posterior sequence, one cell after the other, rather than by a short series of equivalent (if not also synchronous) binary divisions leading to an exponentially growing number of progeny cells. Second, the progeny of each blast cell is not strictly limited to the 32 cells that would suffice, according to an hypothetical principle of economy, with

one offspring per blast cell allocated to each segment, without any waste cell to be discarded. Blast cells produce about 100 cells each (Weisblat and Shankland 1985, Weisblat and Huang 2001). Supernumerary cells will die without contributing to the definitive segments (Zackson 1982). It is not yet clear how the astoundingly constant segment number of leeches is actually determined.

Another example of deceptive numerical correspondence has been discovered by Huys and Boxshall (1991) through their analysis of the homology of individual segments of the antennule in different genera of copepods. For example, the same number of antennular articles is present in *Macrocyclops* and in *Cyclopinoides*, but there is no one-to-one homology between the 17 segments of the former and those of the latter. Distribution of sensilla along the appendage allows tracing the correspondence of the articles to the basic segmental architecture of the copepod antennule, thus the first segment of *Macrocyclops* represents segments I–VI of the hypothetical ground-plan copepod antennule, followed by a free VII, whereas in *Cyclopinoides* I and II are free, but III–V are collapsed together, as are VI–VII.

But there are also many other numerical relationships that suggest the existence of underlying common rules, even where our current understanding of development fails to offer advice. Some examples are briefly discussed elsewhere in this book (in particular, in respect to arthropod segmentation, see pages 206–209). Another numerical pattern emerges from comparison of the vertebral position of the hindlimbs with respect to the forelimbs in three species of Palaeozoic amphibians. The two limb pairs are about sixteen vertebrae apart in *Urocordylus wandersfordii* (Nectridea), about twenty-four in *Utaherpeton franklini* (Microsauria), and about sixty-four in *Brachydectes newberryi* (Lysorophia; Carroll 1999). A possibility exists that this octonary pattern has something to do with the segmentation clock that seems to control the pace at which vertebrate somites are formed (Pourquié 2000, 2001). The common occurrence of octonary segment patterns in annelids (see page 140), arthropods (see page 209), and vertebrates (this paragraph) is very likely to be serendipitous.

Genetic Assimilation

According to Wolpert (in Wolpert, Ghysen, and García-Bellido 1998), the development of the earliest animals was messy and very susceptible to environmental perturbations. Newman and Müller (2000) imagine a late

Precambrian 'pre-Mendelian world', where the earliest multicellular aggregates were subject to morphogenesis, as dictated by their properties of chemically excitable, viscoelastic soft matter. Without involvement of 'genetic programmes', master genes, and developmental control cascades, those properties allowed the production of a profusion of multilayered, hollow and even segmented forms. With the subsequent transition to a 'Mendelian world', cellular specificities and gene control acquired an increasingly larger role because of the presence of metabolically differentiated cell subpopulations produced by compartmentalisation. Biochemical differences between the different tissues provided components of one another's environment, thus eventually bringing embryonic induction into existence. Early in metazoan evolution, cell–cell communication was perhaps mediated by small molecules such as the peptides that in 'lower' animals such as *Hydra* control biological processes as diverse as muscle contraction and neuron differentiation and establish gradients of positional values (Bosch and Fujisawa 2001).

The complexity and specificity of the new interactions within these developing living systems did not cancel the physicochemical generic properties that were responsible for the whole limited morphogenetic activity of the oldest multicellulars. Newman and Müller believe that the vertebrate skeleton may have arisen in a similar way. Connective cells and other cells arrange themselves along stress fields without requiring any additional (say, genetic) input (Harris, Stopak, and Wild 1980, Bard 1990). Secretion of a cartilage matrix is an autonomous property of mesenchymal cells, dependent on generic features, such as cell number and density (Cottrill, Archer, and Wolpert 1987) and compression (Vogel and Koob 1989, Robbins, Evanko, and Vogel 1997). Therefore, any mesenchymal tissue mass above a certain threshold size may have begun to produce, without any specific input, arrays of matrix-secreting cells along stress fields generated by passive and active movements. This view of the origin of skeletons is perfectly compatible with my suggestion that skeletogenesis originated as a way to stabilise development (cf. page 18).

We can suppose that in the course of evolution the role of genes in canalising developmental events became increasingly larger. Reading against the arrow of time this means that, in the earliest animals (or, at any rate, in the earliest multicellulars organisms), this canalising effect of genes was limited, with development being mainly driven by generic processes. In this sense, the similarity between some features of biological developing systems and those of non-biological systems may represent something

more than a simple analogy. Meinhardt (1996) cites high sand dunes or sharply contoured rivers as examples of patterns developing from almost homogeneous initial conditions in non-living systems. In these systems, as (putatively) in the living ones, the continuous growing of patterns is caused by the strong feedback of initially small deviations from a homogeneous distribution. In the case of primary embryonic pattern formation, Gierer and Meinhardt (1972) and Meinhardt (1982, 1992) suggested coupling of a short-range autocatalytic process with a long-range reaction that opposes it.

Actual examples of developmental canalisation evolving through geological time are difficult to obtain. For a comparatively well-studied group like trilobites, results are mixed. There is some evidence that early trilobites were more developmental plastic than later representatives of the group (Hughes 1991), but the role of external selection in promoting segment-rich, more variable morphotypes in Cambrian times, but not so much in later times, must also be taken into account (Hughes, Chapman, and Adrain 1999).

Genes and Phenotype

The relationship between genotypic and phenotypic changes is far from linear (Jablonski 2000). Dramatic morphological changes are sometimes associated with point mutations. Phenotypes can be remarkably stable despite extensive genetic differences. Interestingly, this may be true even when typical 'developmental genes' are involved. For example, no phenotypic variation was found in the three-spine stickleback, despite intraspecific variation in the expression domains of *Hox* genes along the main body axis (Ahn and Gibson 1999a, 1999b). Differences in the developmental mechanisms by which the vulva is produced in different strains of the nematode *Pristionchus pacificus* do not correlate with the genetic distance between those strains. In particular, peculiar developmental mechanisms were found in a strain from California, genetically identical to another strain from Poland, which lacks them (Srinivasan et al. 2001).

A related issue is phenotypic plasticity (Schlichting and Pigliucci 1998). One speaks of phenotypic plasticity when, in response to different external conditions, different phenotypes are produced that are adaptive, each in its own environment. If you rear the grass-feeding caterpillar of the moth *Pseudaletia unipuncta* on hard grass, its head grows much bigger than if you rear it on soft wheat seedlings. This difference in the size of the head

capsule, with the correlated differences in mandibular power, has a direct effect on the insect's ability to exploit hard food: larger heads are obviously better when dealing with hard grass (Bernays 1986). Closely related species may have different, even opposite, responses to the same environmental cues. For example, salmon growing in fast flowing waters become more robust than their conspecific growing in slow waters, whereas brown trouts growing in fast flowing waters become slightly more streamlined (Pakkasmaa and Piironen 2001). A nice example of environmental regulation of morphogenesis is offered by the hermit crab *Clibanarius vittatus*, whose asymmetry, although not dependent on the initial possession of a shell, can be lost within a few moults, if shells are no longer available (Harvey 1998).

No less interesting, however, are stories in which phenotypic plasticity is evoked by different competitive frameworks. Pfennig and Murphy (2000) compared the tadpole development of two closely related species of the spade-foot toad (*Spea bombifrons* and *Spea multiplicata*), when reared alone or together. Depending on the diet available, individuals of both species can develop into either an omnivore morph, mostly feeding on detritus, or a carnivore morph, mostly feeding on small crustaceans. The head in the omnivore morph is much smaller than in the carnivore morph. When the two species are reared in the same tank, the presence of *S. multiplicata* enhances the production of the carnivore morph in *S. bombifrons*, and the latter, in return, suppresses the production of the carnivore morph in *S. multiplicata*. In this way, competition pushes either species towards the feeding habit for which it has advantage over the other species. As in this experiment the environment eliciting the response of either tadpole species is tadpoles of the same age of a closely related species, one is tempted to compare this system with a developing embryo, in which different cell lineages are pushed towards one of their potentially open developmental pathways by the influence from neighbouring cell lineages. Between the two tadpole species, there are genetic differences, whereas confronting cell lineages within an embryo only differ because of the history of their gene expression and former inductive interactions with other cells. However, the overall difference is one of degree rather than one of kind.

Evolutionary Dissociation between Genes and Phenotypes

Homology of sequence is not a guarantee of conserved function. This is clearly what we expect to find in the case of gene duplication, when two paralogues may have functionally diverged to a more or less great extent.

But evolutionary dissociation is also common between vertically homologous (orthologous) genes and homologous morphological features (Wray 1999, Wagner 2000). Shigetani et al. (2002) have shown that the expression in the chicken of 'developmental genes' involved in the specification of the mandibular arch is different from the corresponding expression of apparently orthologous genes in the lamprey, thus suggesting heterotopic shift of tissue interactions in the evolution of vertebrate jaws. In vertebrates, the 'functions' of *Pax9* genes have sequentially expanded through new expression domains accompanying the emergence of more complicated body plans. In hemichordates and non-vertebrate chordates, the *Pax9* gene is only expressed in the pharyngeal slits; but, in the 'lower' vertebrates, it is also expressed in the nasal placode and in the cranial ectomesenchyme. In the amniotes, finally, its expression extends to the somites (Ogasawara et al. 2000). In *Drosophila*, *dorsal* and *snail* specify the mesoderm and the dorso-ventral polarity of the main body axis. But in the leech *Helobdella robusta*, the same genes probably play a role in the diversification of cell types within segment primordia (Goldstein, Leviten, and Weisblat 2001). In insects, the Ultrabithorax protein has properties not present in its equivalent from onychophorans, possibly due to acquisition of new cofactors or activity modifiers since the divergence of the two lineages (Grenier and Carroll 2000). In *Drosophila*, *even-skipped* has a typical pair-rule (bisegmental) expression, but in the grasshopper *Schistocerca*, its homologue does not serve a pair-rule function in early development. The same gene has a conserved role in neurogenesis in both grasshopper and fruitfly (Patel, Ball, and Goodman 1992). The *engrailed* gene has multiple expression domains and many ascertained developmental roles in arthropods and chordates, but few if any of these domains and roles are homologous across phyla (Davis et al. 1991, Duboule 1994b, Rogers and Kaufman 1996, Bely and Wray 2001).

In dipterans, the class 3 *Hox* gene evolved into *zen*, which is not expressed along the main body axis as the *Hox* gene from which it derives, but specifies instead extraembryonic tissue (Falciani et al. 1996). A recent duplication of this *Hox3/zen* gave rise to *bicoid*, which in *Drosophila* is required for normal development of the head and thorax; but in another fly, the phorid *Megaselia abdita*, it is also required for the development of four abdominal segments (Stauber, Taubert and Schmidt-Ott 2000). Extensive changes in spatial and temporal patterns of expression and in developmental roles of the homeobox genes have occurred in the different echinoderm classes (Lowe and Wray 1997). Homoplasies, or parallel changes of gene structure and function in different phyla, are also known. For example, *Antennapedia*

class genes are involved in the specification of trunk structures in both protostomes and deuterostomes, but have duplicated independently in the two phyletic lines (Holland 1992, Schubert, Nieselt-Struwe and Gruss 1993, Zhang and Nei 1996).

Gene function may be maintained despite a genome structure in flux, if compensatory changes evolve in the genome (Dover 1992).

A Role for the 'Developmental Genes'

By now it is clear that the specific role of most of the so-called developmental genes is rather mundane (Shubin and Marshall 2000). Most of them regulate the rate of cell proliferation or determine cell adhesion properties or act as transcription factors, binding to specific DNA sequences. The most advanced 'morphogenetic' roles of these genes may lay in demarcating regions or groups of cells fated to specific differentiation routes. The developmental role of all these genes is the result of their involvement in more or less complex and pervasive genetic circuitries.

The scientific literature of the last decade is full of examples of genes whose temporally and spatially correct expression is critical for the production of complex, specific features, but at the same time are known to encode for proteins whose mechanism of action is quite unspecific (e.g., in regulating changes of the cytoskeleton; Schejter and Wieschaus 1993). Another important aspect is the apparent lack of specificity of action, in that different transcription factors can apparently be substituted for one another. This is due to the combinatorial nature of genes, whereas what is really relevant is the structure of promoter modules. The historical continuity of function rests with these elements rather than with the whole gene (Akam in the discussion of Abouheif 1999).

The *Hox* Code

In a fascinating reconstruction of the evolution of animal body plans from the point of view of the genetic control of development, Davidson (2001) affirms that the secret of the bilaterians, with respect to building body parts, is their use of abstract patterning mechanisms. These mechanisms are derived from pre-existing regulatory pathways, which, instead of running differentiation gene batteries, as their role was in more primitive multicellular organisms, acquired a new function in the regional specification of transcription.

Current evidence suggests that some gene families were more suitable than others in providing 'abstract patterning mechanisms' – none,

apparently, better than the *Hox* genes, whose organization in an ordered cluster is probably the best key to their success in body patterning (García-Fernàndez and Holland 1994). The long history of association between the evolution of the *Hox* gene family and the evolution of patterning along the main body axis of bilaterians is apparent from the fact that the terminal genes (*labial*/*Hox1* and *Abdominal B*/*Hox13*), which are expressed in body regions close to (but not coincident with) the two body ends, are probably the oldest, in that they contain the most divergent homeodomains (Gehring 1998).

Hox genes are responsible for major differences in the body architecture of different arthropod groups. One example is the difference between flies and butterflies in the number of larval legs and adult wings: transformation of the posterior wings into a pair of halteres in the flies is correlated with divergent regulation of *Ultrabithorax* (Warren et al. 1994). In the echinoderms, Lowe and Wray (1997) relate reorganisation of body architecture to extensive changes in the deployment and roles of homeobox genes, including the evolution of new developmental roles and modifications in the symmetry of the expression domains. In this phylum, genes such as *Distal-less*, *engrailed* and *orthodenticle* show unique radial expression domains which correspond to structures that are also unique to echinoderms. For example, *orthodenticle* is expressed in the podia of brittle stars and sea urchins, and *Distal-less* is expressed in the podia of sea urchins and sea stars, as well as in the buccal tentacles of sea cucumbers.

The role of *Hox* genes in determining major changes in body architecture has been extensively debated. For example, Irvine and Martindale (2000) have put the distinct posterior boundaries of expression of *CH-Hox1* and *CH-Hox2*, both in the ninth body segment of *Chaetopterus variopedatus*, in relation with the unusually complex regionalisation and segment specialisation of this annelid, in which the ninth segment marks a major morphological boundary (Figure 3.3).

Arthropods, with their extensive diversity in the specialisation of segments and body regions, have been the focus of the debate. A key role of changes in *Hox* gene regulation in the origin of major morphological changes is suggested by the good correlation found in different crustaceans between changes in the expression patterns of *Ultrabithorax* and *abdominal-A* and modification of the anterior thoracic limbs into maxillipedes (Averof and Patel 1997). Similar suggestions derive from comparisons between the expression domains of *Hox* genes and the morphological boundaries of body regions in insects and isopod crustaceans (Popadić et al. 1998, Abzhanov and Kaufman 1999, 2000a). The same applies to

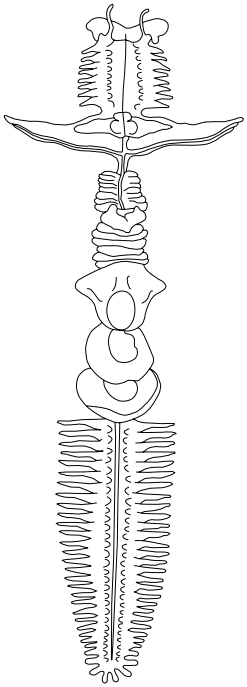

Figure 3.3. The two long 'wings' of the polychaete *Chaetopterus* correspond to the posterior boundaries of expression of the *Hox* genes *CH-Hox1* and *CH-Hox2*.

comparisons of the expression patterns of the trunk *Hox* genes *Sex combs reduced*, *Antennapedia*, *Ultrabithorax*, *abdominal-A*, and *caudal* in the crayfish *Procambarus clarkii* and the woodlouse *Porcellio scaber* (Abzhanov and Kaufman 2000a, 2000c). There is no phylogenetic evidence for putative ancestors of Recent arthropod clades directly comparable with the phenotypes corresponding to mutants defective in the expression of these *Hox* genes (Akam 1998, Kettle et al. 1999, 2000).

Our better familiarity with vertebrate and arthropod model animals has often obscured the fact that the *Hox* genes do not play any role in patterning the embryos or larvae of many bilaterians, such as sea urchins and polychaete annelids. In *Caenorhabditis elegans*, an essentially complete embryo is produced in the absence of any *Hox* gene expression (Salser and Kenyon 1994, Van Auken et al. 2000). According to Davidson (2001), this is the ancestral developmental condition, over which the bilaterians have superimposed a completely new mechanism through which the adult body plan will emerge. In fact, in both the sea urchin *Strongylocentrotus*

purpuratus (Arenas-Mena et al. 1998) and the polychaete *Chaetopterus* (Peterson et al. 2000), *Hox* genes are expressed only in the tissues that will contribute to the adult body, not in the larval ones, which will be discarded at metamorphosis.

Some important events in animal evolution seem to be associated with major duplications of the *Hox* gene cluster. It has been proposed that one of the conditions that permitted the Cambrian explosion of animal body plans was the duplication by which a proto*Hox* gene cluster gave rise to two distinct paralogous sets, the *ParaHox* and the *Hox* (proper) genes (Brooke, García-Fernàndez and Holland 1998). More popular is the hypothesis that the success of the vertebrate lineage owes much to a wholesale quadruplication of the *Hox* gene cluster (Bailey et al. 1997, Holland 1997), perhaps following the polyploidisation of the ancestor's set of chromosomes (Spring 1997).

There is no need to use a lot of words to explain the concept of the *Hox* code (for an early review, see Lawrence and Morata 1994), or to discuss the colinearity between the chromosomal order of the *Hox* genes and the antero-posterior order, along the main body axis, of the anterior boundary of their expression domains (e.g., Krumlauf 1992, 1994). These concepts, based on an impressive amount of experimental evidence accumulated during the 1980s and early 1990s, are illustrated at length in standard works such as Gerhart and Kirschner (1997), Wolpert et al. (1998), Hall (1999) and Carroll, Grenier, and Weatherbee (2001). Therefore, I will only mention a recent paper suggesting caution in generalising. The *Hox* code is probably not so simple, and colinearity is not so widely conserved as generally believed.

It is well known that, in *Drosophila*, the *Hox* gene complex is split into two separate clusters, less known is that, in the different fruitfly species, the complex is split at different places. In *Drosophila melanogaster*, the two clusters are the *Antennapedia* complex and the *Bithorax* complex, respectively, whereas in *Drosophila virilis*, the split occurs within the latter complex. In other terms, *Antennapedia* and *Ultrabithorax* are in two different clusters in *D. melanogaster*, but in the same cluster in *D. virilis*, whereas *Ultrabithorax*, *abdominal-A*, and *Abdominal-B* are all in the same cluster in *D. melanogaster*, but not in *D. virilis* (von Allmen et al. 1996). The two independent events by which these two different splits were generated occurred with the separation of *D. melanogaster* from *D. virilis*, possibly in the order of 60 million years ago (Beverly and Wilson 1984). These events suggest that, in this group at least, the physical arrangement of these genes

is not strictly constrained. One may wonder whether this has anything to do with the near-synchronisation of morphogenesis and patterning along the longitudinal axis of the quickly developing *Drosophila* embryo.

In the mouse, for example, the *Hox1* group genes do not respect the rule of spatial colinearity; this is relevant, even if this deviation from colinearity is to be interpreted as a secondary phenomenon (Wada et al. 1998, Wada, García-Fernàndez and Holland 1999, Holland 2000). Nogi and Watanabe (2001) dare to suggest that the lack of colinearity they find in the planarian *Dugesia japonica* (the anterior expression boundary of *Dj-AbdB-a* being anterior to those of two central-class genes *Plox4-Dj* and *Plox5-Dj*) could be ancestral. I suspect, that this suggestion derives from the traditional view of flatworms as basal bilaterians, a concept that many recent assessments of animal phylogeny (e.g., Aguinaldo et al. 1997, Jenner and Schram 1999, Adoutte et al. 2000, Jenner 2001, Peterson and Eernisse 2001, Nielsen 2001) have largely shaken.

Two possible ways a *Hox* code could work were distinguished by Holland and García-Fernàndez (1996). One theoretical possibility is for the *Hox* genes to mark fixed (absolute) axial positions (in vertebrates, somite numbers), thus controlling the development of the specific traits appropriate to that position in a given species. The other possibility is for *Hox* genes to mark presumptive phenotypic characters. In this case, the axial level of their expression patterns would be expected to shift between divergent taxa. Burke et al. (1995) found strong support for the latter model, what is comfortable for the recent widespread use of the *Hox* gene expression patterns as indicators of homology. This means that the first thoracic vertebra, as specified in both animals by the anterior limit of expression of *Hoxc6*, may be regarded as homologous, despite the somite affected is number 12 or 13 in the mouse, but number 19 or 20 in the chick. In the comparative anatomy of arthropods, a very strong tradition acknowledges homology to equally numbered segments irrespective of their actual specialisation. This is probably unwarranted, as argued in chapter 9.

The temporal and spatial expression patterns of several *Hox* genes shows that the classical *Hox* code model is unrealistic. Castelli-Gair (1998) has backed this objection through evidence from the expression of the *Sex combs reduced*, *Ultrabithorax*, and *Abdominal-B* genes in *Drosophila*. The expression of these genes is dynamic, involving fewer segments at early stages of development than at more advanced stages. At any instant in time, within a segment, there is a mosaic of cells expressing a given *Hox* gene and cells not expressing it.

In the bee, the anterior expression border of *Deformed* changes during gastrulation from segmental to parasegmental (see page 220 for an explanation of the term) in the ventral part of the maxillary segment, not as a result of cell movement, but because of the intrinsic dynamic character of the gene expression itself (Fleig et al. 1992). This implies that the *Hox* code identity of a cell changes during development. In this case, which is its 'correct' segment identity, if any?

Castelli-Gair (1998) examines the fate of a *Drosophila* embryo in which the expression of *Abdominal-B* is prevented in what would normally be the seventh abdominal segment (A7). The question is, whether this segment would assume a more anterior identity, as conventionally described for a loss-of-function *Hox* gene mutant. Interestingly, the answer is yes and no. The consequences of preventing *Abdominal-B* expression in A7 are manifest in the corresponding segment of the adult; but, in the embryo, many A7 structures, such as the local differentiation of the central and peripheral nervous system, are normal (Heuer and Kaufman 1992). Only some larval structures of A7, such as the segment-specific denticle belt in the larva and the cells fated to form the adult epidermis, do actually require *Abdominal-B* expression. Still more importantly, in the context of the question about the segmental identity of a cell whose *Hox* code changes during developmental time, is the fact that, in A7, the organisation of the peripheral nervous system is the same as in the preceding segments A2–A6 (Ghysen et al. 1986) despite the fact that A2–A4 do not express *Abdominal-B*, whereas A5–A7 do. This uniform patterning of the peripheral nervous system irrespective of the expression of this gene does not mean that *Abdominal-B* is dispensable for the development of the peripheral nervous system. This is demonstrated by how *Abdominal-B* mutants affect the peripheral nervous system of A8. The different responses of A7 and A8 to *Abdominal-B* expression is likely due to a difference in the temporal expression of this *Hox* gene in the two segments. This shows that the outcome of *Hox* gene expression may change with time; what is obviously contrary to what we would expect according to the conventional *Hox* code hypothesis (Castelli-Gair 1998). Different developmental effects following expression at different developmental times are not limited to *Abdominal-B*.

The role of *Hox* genes in patterning the body of diploblastic animals is not known, and it may be unwarranted to expect it to be the same as in triploblastic animals such as insects and vertebrates. Nevertheless, the expression of the *Hox* gene *Cnox-2* in *Hydractinia symbiolongicarpus* is interesting in the context of this discussion of the developmental changes

in a cell's *Hox* gene identity. *Cnox-2* is expressed in different aboral-to-oral patterns in different kinds of polyps of this polymorphic cnidarian, and the experimental conversion of one polyp type to another is accompanied by concordant alteration in *Cnox-2* expression (Cartwright, Bowsher, and Buss 1999).

During the 1990s, the idea of a *Hox* code has been also fashionable in plant developmental biology. Differentiation of the four basic kinds of floral elements (sepals, petals, stamens, and carpels) has been interpreted as deriving from the homeotic control of three classes of genes: A, B, and C. Of these, A would specify as sepals the elements of the most external whorl in the flowers; those of the following whorl would instead develop as petals, stamens and carpels, under A + B, B + C, and C function, respectively. It has been soon realized, however, that things are more complex. At least two A-function and two B-function genes have been discovered in the model plant *Arabidopsis thaliana*. The ABC genes, if expressed in a part of the plant other than the flower, do not transform leaves into flower elements: expression of genes of the SEPALLATA class is additionally required for the specification of a flower. In front of this increasingly complex picture, Theißen (2001) wonders whether the ABC combinatorial model will still be of any use in our future attempts to understand the linkage between molecular genetic events and floral phenotypes.

A simplistic and generalised application of the *Hox* code concept is also impossible in the case of group 11, 12, and 13 *Hox* genes, which control size and number of digits in tetrapods. In the mouse, it has been shown that this control is dose-dependent, rather than the effect of a qualitatively differentiated *Hox* code. The same response was found in morphogenesis of the baculum (Zákány et al. 1997): digits and external genitalia are, in part, under the same genetic control (see page 176).

Organic Codes

Major evolutionary transitions are marked by the appearance of new organic codes [i.e., non-deterministic, historically frozen rules of correspondence between patterns (Barbieri 2003) as in the genetic code or in human language]. All this means is a kind of 'evolution by natural conventions'. When a new code appears, the old ones are not necessarily cancelled. Therefore, with every new code appearing, there is a net increase in global complexity.

We have just seen that the so-called *Hox* code should not be taken too literally. Whatever consistency this 'code' may have, it is quite probable

that in this case, too, as with the genetic code, the relationship between the molecular 'symbol' and its phenotypic translation is little more than a frozen accident. Its lability during the short span of one embryonic development seems to confirm this suspicion. Look, for example, at the relationship between the temporal and lineage restrictions of the expression of *mab-5*, the homologue of the *Drosophila Hox* gene *Antennapedia* in *Caenorhabditis elegans*. In a given body region, the start of this gene's expression stimulates proliferation in a cell lineage; but shortly thereafter, a temporary suspension of its expression is required to specify epidermal structures. But this is just the first half of the story. In just one branch of the epidermal cell lineage specified following the silencing of the *mab-5* expression, reactivation of the latter promotes neuroblast formation, but a new silencing of this gene is required again for the establishment of a correctly formed sense organ (Salser and Kenyon 1996).

Universal Genetic Tools

A conserved genetic machinery seems to control morphogenetic events such as branching and anastomosing of tubes in widely diverging animal clades (e.g., vertebrates and insects). But these features, most likely, have been reinvented many times. The fact that they are now controlled by orthologous genes in different phyla is probably due to an independent (homoplasious, convergent) co-option, rather than derivation from a common ancestor with similar structures already patterned under the control of those genes.

Think, for example, of the branching morphologies in the lungs, kidneys and mammary glands of many vertebrates. Common to all these structures is contact between an epithelium and a mesenchyme, the former branching more or less extensively into the latter (Hogan et al. 1997). Fleury (2001) has recently demonstrated how all these branching organs may develop following a simple physical process called viscous fingering. This process occurs whenever a less viscous fluid is pushed to penetrate into a more viscous fluid. Simple physical forces may generate branched fingers, but do not stabilise them. Neither can they provide regularity in the spatial and temporal patternings of the sites in which similar morphologies will originate. This is where the generic-to-genetic transition is found again, with the intervention of genes, such as those actually involved in these processes.

One could expect that the self-similarity or fractal structure of these branching patterns would be a bonus obtained at no extra genetic cost, but this is not necessarily the case. In *Drosophila*, each of the ten segments from the mesothoracic to the eight abdominal is provided, at each side, with a unit of the tracheal system, including first-, second-, and third-order branches. First-order tracheal branches are multicellular tubes, whereas the second-order tubes are the product of individual cells and the terminal, third-order branches (tracheoles) are tiny intracellular tubes hosted within cytoplasmic protrusions. Different genes are involved at each level of branching. In particular, *breathless* is needed for first-order branching, but its expression regulates, in addition, the activation of other genes involved in second-order branching, such as *pointed*, which, in turn, is also required for activation of the gene(s) responsible for the development of the tracheoles (Samakovlis et al. 1996a). The same happens in mammals. In a mouse lung, there may be six to eight orders of branching. This number raises to ca. 20 in a human lung, leading to the formation of ca. 17 million branches. Here again, as in *Drosophila*, the pattern and structure of branching are specified at each branching level under specific genetic control (Sutherland, Samakovlis, and Krasnow 1996, Metzger and Krasnow 1999).

In the development of these respiratory structures, a complex genetic control has been superimposed on the simple iterativity of a self-similar process that could produce per se the fractal geometry of the organ. We can only speculate about the adaptive significance of this genetic control or about the tiers of frozen developmental history, of which the current developmental schedules may retain the memory. It is clear that the simple iterativity of a fingering process which adequately accounts for the beautiful patterning of mineral dendritic forms is also adequate to explain many patterns found in living beings, such as vein branching in the leaves or the fractal geometry of the liver parenchyma (Ng and Iannaccone 1992). The lesson we learn from the mouse lungs and the fruitfly tracheae is a clear warning of the often complex and sometimes circuitous ways adopted by nature in producing what seems to be a geometrically simple pattern. This is also the case of the regular pattern of pair-rule gene expression in *Drosophila*, in which seven or fourteen serial stripes of expression of genes, such as *even-skipped*, *hairy*, and *runt*, do not appear to mirror the peaks of a regular wave-like distribution of a signal along the longitudinal axis of the embryo, as a parsimonious hypothesis would suggest, because the individual stripes appear to be under independent genetic control (e.g., Howard, Ingham, and Rushlow 1988, Klinger et al. 1996).

Conserved mechanisms of epithelial tube fusion are suggested by research on the *escargot* homologues known from both insects and vertebrates. Networks of tubular structure are widespread throughout the whole animal kingdom. These include the vascular system of a great number of animals, vertebrates included, as well as the vertebrate kidney and the tracheal system of most groups of terrestrial arthropods. In the tracheal system of *Drosophila*, a cell located at the prospective point of fusion along a tube expresses a sequence of specific markers and contacts a corresponding cell from another tube. An intercellular junction forms between the two cells, which acquire a doughnut shape, like the sponge porocytes. Samakovlis et al. (1996b) have identified one of the earliest expressed fusion markers as the *escargot* gene, whose ectopic expression suppresses branching throughout the tracheal system, activates the fusion process and has a homologue in vertebrates (Nieto et al. 1992).

Genetic Networks and Morphogenesis

Mastick et al. (1995) estimated the number of target genes downstream of *Ultrabithorax* as about 170, but Liang and Biggin (1998) have later claimed that *most* genes in *Drosophila* are regulated by homeotic proteins, whether directly or indirectly, thus suggesting very extensive feedback and cross-regulation. The complexity of these interactions would be responsible for phenotypic stability (Kauffman 1993), despite the perpetual state of flux of the underlying genetic architecture (Gibson 1999).

Focussing on the operation of genetic networks rather than on simple linear control cascades means that "molecular synarchies are more relevant than hierarchies in the governance of genetic operation" (García-Bellido 1994b). García-Bellido (1994a, 1994b) introduced the term syntagma to define an ensemble of genes at the service of a complex developmental operation, as a conceptual tool to attack the modular nature of embryonic development. "Organisms seem to develop as articulated, multicellular modules such as polyclonal compartments or segments within which patterned cell differentiation occurs" (García-Bellido 1994b: 18). García-Bellido (1994a) believes that these functional complexes are conserved in evolution since the early times of eukaryote evolution. Accordingly, specific cellular behaviours, such as mitosis and cell proliferation, cell recognition and selective adhesion, axonal guiding, folding of epithelia, segregation of cell types (e.g., epithelial from neural), and differentiation of the main cell type, would be brought about, in all animals, by the same syntagmata and the same genes. With development and cell

proliferation progressing, territories in which specific syntagmata are acting grow to a larger size and eventually split into smaller territories governed by different syntagmata. A similar concept is part of Davidson's (2001) recent model of the origin and evolution of the bilaterian body plan.

In this perspective, it is interesting to note to which extent metabolism may be affected by a single-locus mutation. In *Arabidopsis thaliana*, the lack of a single enzyme causes significant differences in 153 of 326 metabolites quantified using gas chromatography/mass spectrometry (Fiehn et al. 2000).

Recently, Akam (in the discussion of Wray 1999) has shifted our attention from genetic networks (in the sense of complexes of genes encoding proteins that interact with a DNA site that regulates another gene) to protein networks (i.e., proteins physically working together). I subscribe to his view that protein networks are evolutionarily more stable than genetic network, because DNA–protein interactions are likely to re-evolve while that is not so easily expected for protein–protein interactions.

CHAPTER FOUR

Periodisation

> Stages exist in the mind of the biologist, not in the larva. All that is needed in order to recognize a stage is a fixed starting and finishing point.
>
> C.S. Hickman 1999: 27

For centuries, philosophers have been discussing continuity versus discontinuity. I embrace the view that these questions depend on the tools we adopt in measuring and describing phenomena: "we need not suppose that the material world is fundamentally discontinuous, it does appear that some continuities involve steeper gradients than other" (Ahl and Allen 1996: 166). This applies to the spatial (structural, morphological), as well as the temporal (ontogenetic) dimension. It was previously described how arbitrary it is to cut the fundamental continuity of life into individual life cycles. In this chapter, I will identify additional problems with the periodisation of a life cycle, that is articulating it into meaningful and comparable temporal units. Corresponding problems with morphological units, such as segments or teeth, will be discussed in the last two chapters of this book.

A study of the spatial aspects of development – such as segmentation, tagmosis or the positioning of the appendages – makes little sense if not coupled with research into the temporal dimension of ontogeny. A distinction between temporal and spatial aspects of the molecular control of development is often artificial. This is true, in particular, in the context of the colinearity between the spatial organization of the *Hox* genes along the chromosome, the temporal sequence of their activation and the spatial order of the regions along the animal's main body axis, in which each of these genes is expressed (Freeman 2000). Therefore, it is wise to identify a search for correspondence between spatial (morphological) and

temporal (developmental) units and patterns as a primary target of developmental (and evo-devo) biology (Minelli 1996a). How to develop such a research programme, however, is far from obvious. A concern for homology is granted in morphology, as exemplified by the recent debate about the single versus multiple origin of the eyes, heart, appendages and segments in bilaterians (see page 193), but similarly heated discussions have seldom arisen, if ever, about the single or multiple origin of temporal slices of development, such as the gastrula or larva. Curiously enough, developmental evidence has been one of the major sources of insight in establishing the homology of morphological features, but this tradition has failed thus far to generate a comparable concern for the homology of the developmental stages.

Therefore, there are good reasons for questioning the traditional ways of establishing a periodisation of developmental time.

The Primacy of Time

Kenyon et al. (1997) asked an interesting question: whether the early metazoans first set up their *Hox* gene expression pattern by controlling cell lineage, or through the expression patterns of other genes that could provide cells with positional information or through a tight temporal control of the *Hox* gene expression itself. In various modern representatives of the bilaterians, different mechanisms distinctly prevail: cell lineage in *Caenorhabditis*, positional information in *Drosophila* and temporal control in the leeches. It is possible that these differences only reflect secondary specialisations. Time, position and lineage are, in principle, three different facets of the same process. In plants, movement (change in position with time) is nothing more than differential growth. The directionality of locomotion may also become the cue for polarising growth and differentiation, as with the cilia at Hensen's node whose beating seems to be causally involved in establishing the left-right asymmetry of the vertebrate *situs viscerum* (Nonaka et al. 1998). It is possible that cellular clocks provided animals with the ability to make 'time segments' that later in evolution supplied a blueprint for organising 'spatial segments' (i.e., segments in the conventional sense). At least in the case of vertebrates, this seems to be the case (e.g., Cooke and Zeeman 1976, Cooke 1981, Palmeirim et al. 1997). I wonder if animals might be able to develop spatial patterning without any reference to temporal patterning. One example from non-segmented structures is in the *Drosophila* eye; here, the planar polarity of

the epithelial cells is inverted if one induces the morphogenetic wave sweeping through the presumptive retinal epithelium to move in the reverse direction. According to Wehrli and Tomlinson (1995), the planar polarity of this epithelium may be determined effectively by the direction of the morphogenetic wave.

Time Schedule: Synchronous Versus Metachronous

According to the theoretical models, more stable patterns are generated sequentially rather than simultaneously (Oster et al. 1988). This is what happens, in fact, in most developmental systems, with the quasi-synchronism of germ-band segmentation in dipterans (e.g., *Drosophila*) as a conspicuous exception. However, as soon as a given pattern (or prepattern) is revealed, further differentiation may proceed in parallel at many serially homologous spots. Thus, even the basic antero-posterior polarity of growth and development can be safely ignored without any danger of giving rise to irregular or unstable patterns. Closely related species may differentiate their segmentally repeated appendages following different developmental schedules. Of the four species of *Euphausia* (the shrimp-like krill so important as whale food) studied by Menshenina (1990), three (*E. frigida*, *E. triacantha*, and *E. crystallorophias*) show an antero-posterior progression in the degree of differentiation of the thoracic appendages; but in *E. superba*, the differentiation of all thoracopods is synchronous.

Units in Time

In principle, two different approaches can be taken in the periodisation of development. We can select one process or feature whose progression provides reference points for distinguishing temporal units. Otherwise, we can take the whole developing system into account, thus dividing its ontogeny according to whatever set of features or developmental processes may mark the beginning of a new stage. In this case, the size of the character set to which we refer determines the temporal extension of the stages into which we partition the developmental time (Wheeler 1990). In any case, the basic continuity of development makes all such distinctions arbitrary. As seen in chapter 1, the very starting point of the individual life cycle is all but uncontroversial (not simply within the confines of bioethics). To avoid misunderstanding, operational definitions are thus required. For example: “For the purposes of this review, post-embryonic development in mammals is taken to cover the period between the implantation of the

embryo till just after birth. In non-mammalian vertebrates it encompasses development from the stage when the organism begins feeding until it acquires the adult phenotype upon completion of metamorphosis but not a full-grown adult" (Tata 1993: 239).

Homology of Developmental Stages or Events

Richardson (1999: 609) defines "developmental stages [as] temporal clusters of morphologic character states." I like this definition, because it shows the tight connection, historical as well as operational, between the temporal (causal, dynamic) and spatial (morphological, descriptive) components of developmental biology. However, two questions arise. First, are these temporal clusters discrete enough to justify formal periodisation? Second, can homology actually be predicated of developmental stages or events, in the same way as we predicate homology of morphological features?

Problems with the homology of developmental stages have been often discussed in the past. One difficulty derives from the diversity of developmental processes by which a given stage may be attained. This is particularly true of the earliest stages in ontogeny, the gastrula for example (Hall 1995). But homology may be less than obvious, even in the presence of equivalent developmental processes, if we are facing a case of heterochrony. If the temporal progression of different developmental processes is not the same for two different animals, can we nevertheless say that they develop through homologous stages? My answer is yes and no. The fact that we have identified a case of heterochrony presupposes that we were able to see the temporal progress of a given developmental process against the background of other components of the animal's development. The process, or processes, on which we are focussing are thus reasonable candidates to become our developmental homologues, irrespective of the way they might be intertwined in an animal's overall developmental schedule. Chances that these individual components of development actually rely on common mechanisms are obviously much larger than in the case of whole putatively homologous stages. As development progresses through the combined effect of a plurality of individual developmental processes, developmental stages may appear as mosaics of homologous and non-homologous components. This may be disturbing from a traditional all-or-nothing view of homology, but not necessarily so from a different perspective, such as combinatorial homology (see page 224).

We do not need to consider heterochrony to realize that different parts of the same animals can have different ages.

Sehnal (1985) remarked that in the cockroach, metamorphosis concerns wings but not legs. In the pupa of *Tenebrio*, legs start differentiating earlier than the antennae (Quennedey and Quennedey 1993). I have seen two adult male specimens of earwigs (*Forficula auricularia* and *Apterygida albipennis*) with asymmetrical cerci (the branches of the posterior forceps) – one in preadult form and the other in adult form. In these insects, the cerci of the preadult male are similar to those of the adult female, but the two specimens were not gynandromorphs (half male, half female), as sometimes found in insects, but 'temporal mosaics'. Similar heterochronies are well known from human pathology, and their equivalent is known from other insects as well. Sometimes in the tobacco hornworm *Manduca sexta*, not all larval neurones degenerate during metamorphosis to be replaced by adult neurones derived from embryonic neuroblasts. A set of larval neurones are retained and perform new functions in the adult, following a morphological and synaptic reorganisation. The problem, for the moth, is that these larval neurons, despite the cellular rearrangement, continue to display pupal-like behaviour even in the adult (Levine and Truman 1982). By treating the different-aged larvae of the butterfly, *Precis coenia*, with a juvenile hormone mimic, Kremen (1989) demonstrated that pupal commitment of the epidermis occurs in a strict temporal and spatial progression from the anterior to the posterior border of each segment. In this way, there is a critical stage in which the animal is a larva and a pupa at the same time. It is important to note that this desynchronisation does not impair the final achievement of a fully co-ordinated adult. A correct final structure will be obtained even if different body parts proceed at a different pace with their developmental schedule and this is beautifully illustrated by the following experiment. Niemuth and Wolf (1995) applied a linear temperature gradient of about 10°C/mm in either direction along the main body axis of the egg of the hymenopteran *Pimpla turionella*. This treatment, applied for up to 5 hours, resulted in a dramatic desynchronisation of development, up to 9.3 hours between the egg poles. Within the same egg, up to seven mitotic waves were observed at the same time, and the cellularisation process was extremely asynchronous. Consequently, developmental processes which in normal development occur successively, now took place simultaneously, and vice versa. This strong disturbance notwithstanding, the developmental processes resumed normal pace after the temperature gradient was switched off. Thus, this egg's development demonstrated

robustness, with extreme desynchronisation during early development causing no problems on the segment pattern of the resulting embryos.

Comparing Stages

A comparison of the developmental stages of different animal species presupposes the existence of some objective, if arbitrary, criteria for establishing a convenient degree of equivalence between the stages to be compared. In other words, a comparison between the gastrula of a sea urchin and the gastrula of a squid is not that different from a comparison between the guts or the nervous systems of the same pair of animals. In both cases, some degree of homology is assumed between the two items to be compared. In the case of the developmental stages, there is an additional difficulty, besides those we met in the comparison of morphological features. The difficulty is that the boundaries separating developmental stages along the temporal axis are generally less clear-cut than the boundaries between organs in an animal's body architecture. This difficulty appears very clearly upon first inspection of any of the so-called standard tables of development that have been drawn for a few dozen animal species, vertebrates especially. Those tables make frequent reference to external parameters (e.g., standardised temperature) and quite often partition an animal's embryonic development into hours or days, rather than into developmental segments punctuated by precise events. From a practical point of view, those tables allow standardisation of experimental protocols and comparison of results, as long as the same model species is used. They do not solve the problems of comparison between more or less distantly related species.

When moving from the standardisation of the developmental schedule of a given species to comparison of the developmental schedules of two species, the main problem is heterochrony. A given developmental stage of species A might be more advanced than a given developmental stage of species B, if the differentiation of organ X is used as reference. The reverse may be true regarding organ Y. For example, the first postembryonic stages of two pill millipede genera (*Glomeris* and *Spelaeoglomeris*) are equivalent with respect to the rate of addition of dorsal plates at each moult, but not with respect to the rate of addition of new pairs of legs. Within this perspective, stadium II of *Glomeris* (8 leg-pairs) would correspond to stadium III in *Spelaeoglomeris*, and stadium IV of *Glomeris* (13 leg-pairs mostly) to stadium V in *Spelaeoglomeris* (Enghoff, Dohle, and Blower 1993).

Twenty-five years ago, Gould's book on *Ontogeny and Phylogeny* (1977) spawned an interest in heterochrony that previous literature, including

de Beer's lucid treatment on *Embryos and Ancestors* (1958), had failed to obtain. The enthusiasm for heterochrony, in palaeontology especially, is embodied in the already classic papers and collections of McNamara (1986, 1990, 1995), McKinney (1988) and McKinney and McNamara (1991). It also 'infected' developmental genetics, in which the concept of the heterochronic gene was established – first for putative examples from *Caenorhabditis elegans* (Ambros 1989, Ruvkun and Giusto 1989, Ambros and Moss 1994, Slack and Ruvkun 1997) and later for other animals, such as sea urchins (Ferkowicz and Raff 2001). In *C. elegans*, for example, loss of function *lin-14* causes the precocious appearance of cell lineages normally observed in descendant cells one or two larval stages later (Ruvkun and Giusto 1989). Interestingly, heterochronic mutants in *C. elegans* affect a variety of tissues differentiating during larval development, whereas the embryonic stages are not affected (Ambros and Moss 1994). In a recent review paper, Gould (2000) lamented that, during the last decade, terminology of heterochrony has become internally incoherent because of an extension of terms properly devised to describe shifts in developmental timing of shapes and features, and the rates and timings that cause these shifts. It is clear that what is enough for a palaeontologist cannot easily satisfy a developmental biologist. But this is not the major reason for concern.

Smith (2001) is correct in lamenting that the overwhelming majority of papers on heterochrony have focussed, until now, on growth heterochrony, whereas too little attention has been focussed on sequence heterochrony. Growth heterochrony deals with differences between two animals in the time a given developmental process starts or ends, or in the rate at which it proceeds. There are two problems, however, with this approach. The first problem is one of periodisation, that is of establishing a sound frame of reference against which to determine when the developmental process we are studying begins or ends. The second problem is the exclusive attention to one aspect of development. If we apply the concept of heterochrony to a wide range of developmental processes at the same time, we must standardise developmental schedules independent of measures of absolute time, either direct or indirect (e.g., by measuring size) and provide a means to determine timing shifts of many developmental events or processes simultaneously. Smith (2001) thus stresses the importance of a 'developmental sequence analysis', a method which has been adopted, although in a less explicit and systematic way, by authors such as Richardson (1995), Cubbage and Mabee (1996), Mabee and Trendler (1996), Velhagen (1997), and Nunn and Smith (1998). A good example of sequence heterochrony

approach was provided by Smith (1997), who assessed the timing of each of the 28 developmental events in nine species of mammals by comparing the relative timing of every event with every other event. In the subsequent analysis, each pair of developmental events was regarded as a character (in this example, 378 characters in all).

Interestingly, some pivotal points of the developmental schedule may resist major changes in life-style, including the transition from indirect to direct development. *Eleutherodactylus coqui* is a small tree frog without a conventional tadpole (larval) phase, but it is possible to determine with accuracy the developmental stages from which the aquatic larval stage has been deleted (Callery, Fang, and Elinson 2001). A structure homologous with the larval operculum (a skin fold covering the gills and the presumptive limb buds) has been identified in *E. coqui*. In the frogs with a tadpole stage, the operculum is ruptured at the beginning of the metamorphosis, allowing the forelimbs to emerge. Formation and perforation of the operculum are thus convenient markers of the larval life of the anurans. In *E. coqui*, these events are not separated by weeks or months, but are condensed into a short interval lasting only a few hours. This occurs at about two-thirds of the way through *E. coqui* embryogenesis. Callery et al. believe that there has been time condensation, but not an heterochronic pre- or postdisplacement of the operculum perforation with respect to the other morphogenetic events. Therefore, it is possible to determine the precise point at which it was deleted from ancestral ontogeny.

In the case of developmental sequences punctuated by clear-cut events, such as moulting in the postembryonic life of arthropods or nematodes, it may seem easy to identify the temporal units to be compared (e.g., the last larval stages of two nematode species or the larvae of two beetle species freshly hatched from the egg). But things are much less straightforward than this would imply.

In animals with discontinuous growth punctuated by moults, especially arthropods, the number of developmental instars may be different between the species we wish to compare, sometimes even within one species. Let's consider a few cases.

In some arthropods, the number of postembryonic stages is constant within the species and often uniform across large groups. Most beetles have three larval stages. Among arachnids, there are five moults in the Ricinulei, but six in uropygids, (most) mites, schizomids, and pseudoscorpions. Therefore, there seems to be no problem in treating as equivalent the

second larval stages of two different beetle species, or the fourth postembryonic stages of two different pseudoscorpions. There are problems when the number of stages varies, either between members of the same group or within a species. If butterfly A has four larval instars but butterfly B has five, is there a meaningful way to compare a given larval instar of A with a given larval instar of B, or are these instars individually indiscernible?

In some instances, there is no way to identify a one-to-one correspondence between stages, thus allowing a distinction between 'normal' and 'supernumerary' or 'missing' stages. Apparently, the whole length of the postembryonic development may be partitioned in a variable number of instars, with equivalence only existing between the whole of them, irrespective of the number of instars into which the postembryonic development is partitioned. This is perhaps the case of anostracan (10–22 stages in *Eubranchipus serratus*; Schram 1986) and notostracan crustaceans (*Triops cancriformis*), reported by Kaestner (1970) to moult up to 40 times, that is, two to three times a day). Another example is collembolans, with as much as 50 moults reported in *Orchesella villosa* (Schaller 1970).

In other cases, one or more major events punctuate the postembryonic life, so that the instars, whatever their number, are distributed among these few major developmental segments. Within each of these, the identification and comparison of individual instars may be meaningless. In mites, for example, the developmental schedules are generally quite conservative. Ticks are an exception, in that the number of their nymphal instars varies among different species and sometimes even varies intraspecifically. The beginning of the nymphal stage series (with a moult from the six-legged larva) and the end of the same series (with the moult to adult) are, nevertheless, unquestionable. The lack of one-to-one correspondence of individual instars between ticks with a different number of postembryonic moults is restricted to the nymphal segment of life. The number of nymphal stages may be as small as two, but it is generally three to four, although some species have as many as five to eight nymphs. In many species, males reach adulthood in one less nymphal instar than do their conspecific females. Moreover, these numbers are not necessarily constant for a given species (Oliver 1989).

In Orthoptera, the number of postembryonic stages varies extensively, and comparisons are not always obvious, for example when the females have more instars than the conspecific males (Dirsh 1967), or when comparing locusts or crickets with a widely different number of instars. But

there are more subtle problems and less trivial terms of comparison. In two populations of *Chorthippus brunneus* from East Anglia (UK), nearly all females have one instar more than usual. According to Hassall and Grayson (1987), this is due to an additional instar (IIa) between the normal instars II and III. The IIa nymph is morphologically intermediate between nymph II and III. Its wing buds are similar to those of nymph II, whereas genitalia are more like those of nymph III. The fact that the additional instar fits neatly between nymph II and nymph III suggests that the moult between these latter stages marks a more important transition (with a characteristic reversal of the developing wing; Uvarov 1966) than the other moults between nymphal stages. An equivalent 'intercalation' of a postembryonic stage at a fixed, 'critical' time along the schedule of postembryonic development has been recorded in *Chorthippus mollis* (Thorens 1991) and in the earwig *Labidura riparia* (Caussanel 1966).

More intriguing, in suggesting the non-equivalence of the larval moults a caterpillar undergoes before pupation, is Franzl, Locke, and Huie's (1984) study of lenticles – innervated sensory structures found in the larvae of lycaenid and hesperiid butterflies. Each abdominal segment is generally provided with a set of five lenticles on each side; but, in the first larval instar, the positions corresponding to the future lenticles are occupied by setae. After a moult, lenticles are expressed at three of the five positions. After one more moult (i.e., in the third larval instar), lenticles appear at the two positions in which no lenticle had been expressed in the previous instar, whereas the three lenticles of the previous instar are not there. These changes are repeated cyclically when the larva moults to the fourth and then to the fifth (final) instar.

What is clear, from all these arthropod examples, is the unequal meaning of moults along an arthropod's postembryonic development. Some moults are associated with major developmental events, as in the case of the last instar larva of holometabolous insects turning into a pupa. But most moults are only associated with a substantial increase in size, with minor associated change in body surface or appendages. Sometimes no difference is found between the animal's conditions before and after the moult. This is especially true of arthropods with a very high and variable number of moults, such as the springtails and crustaceans described previously. Postembryonic development seems to be punctuated by a few momentous moults separating 'hard developmental segments' within which the number of 'soft' moults may vary. One would thus suggest that the larva, the pupa and the adult are such 'hard developmental segments' of

holometabolous insects, irrespective of the number of larval instars eventually present in a given species' life cycle.

This way to analyze postembryonic development has been followed by some acarologists, starting with Grandjean (1938, 1951), who introduced a complex nomenclature based on his concept of *stase* (see also André 1988, 1989, 1992). A stase is defined as a major segment of an arthropod's postembryonic life. It spans between two moults, provided that these bring about discontinuous changes in external characters, what does not necessarily happen with every moult. The number of stases, according to these acarologists, is fixed for any major taxon, with some exceptions due to secondary simplification of the developmental schedule. There is abundant evidence that this periodisation of postembryonic development is adequate for mites and, perhaps, for some other groups, but the few efforts thus far produced to generalise this concept to all arthropods have not been successful.

What Is a Larva?

Terms such as larva and metamorphosis are used in different animal groups for unrelated stages and developmental phenomena (Sehnal, Švácha and Zrzavý 1996).

I recommend restricting the meaning of the term 'larva' to those cases in which a real metamorphosis occurs. This means excluding the use of the term in the case of nematodes and many arthropods. 'True' larvae are those of the holometabolous insects, those of many marine invertebrates and those of many amphibians. It is clear that the use of the term 'larva' does not imply, per se, any degree of homology between the corresponding developmental stages of different animal groups. The homology between larvae is problematic even within lower taxa. For example, whether the three amphibian orders have evolved their larvae only once or in more independent instances is still an unresolved question (Hanken 1999).

What is a larva? Criteria for delimiting this stage, other than habitus differences between early and more advanced postembryonic stages, include the lack of structures in the larva strictly related to reproduction, as well as dramatic changes in life-style, morphallaxis, and growth of subsequent stages from set-aside cells or at least the presence in early stages of parts that will be discarded later. It is typical of the way the meaning of technical terms evolve that this latter trait (i.e., the presence in the larva of parts that will not be present in the adult) is totally at variance with Aristotle's

concept of a larva. In his *Generation of Animals*, we read (Aristotle 1942: 732a29-32) that, "The difference between an egg and a larva is this: an egg is something from *part* of which the new creature is formed, while the remainder is nourishment for it; whereas in the case of the larva, the *whole* of it is used to form the whole of the offspring" (italics in A.P. Peck's translation).

Any definition of 'larva' is bound to be somehow arbitrary (Strathmann 1993), and a general definition may be even impossible (McEdward and Janies 1993) for three reasons. The first reason is the continuous nature of development; hence any starting and ending point of the larval stage will be always determined arbitrarily. The second reason is the evolutionary diversification of development. The third reason is the lack of a necessary overlap between morphological, ecological, and morphogenetic definitions so that a form may be a larva by one definition and not by others (Hickman 1999).

One could suggest using discontinuities in ontogeny as a help to delimit the larval phase. This may work with the arthropods, or the Ecdysozoa generally, whose postembryonic development is punctuated by moults. It will hardly work with the essentially continuous development of other animals, including those whose life history includes major changes, such as those of a tadpole turning into a frog, or a bilaterally symmetrical bipinnaria turning into a radially symmetrical starfish. In these life histories, there is no major event by which one can unambiguously fix the end of larval life and the start of postlarval life. Moreover, because of the heterochronic trends so common in many phyla, any trait we might arbitrarily select as typically larval in the life cycle of a given animal group may be found in the adults of a few species, as much as 'typically adult' traits have been anticipated instead to a stage that we would otherwise call larval (Jägersten 1972).

Metamorphosis offers a more or less clearly defined final term of the larval stage (Webb 1999) – a term quite prolonged in time and not necessarily synchronous for all organs and features of the animal. Less obvious, perhaps, is the problem that hatching does not necessarily offer a clear-cut watershed between embryonic and larval life. This is true, at least, of animals such as sea stars, in which hatching may occur at different developmental stages (comparable to a blastula, a gastrula, or some later stage), according to the species. For this reason, McEdward and Janies (1993) would best restrict the term 'embryo' to the stages of development that are of universal occurrence (from the cleavage stages to the gastrula),

irrespective of the fact that any of these occurs before or after hatching (i.e., as free-living stage or not).

Metamorphosis as Metagenesis

Metamorphosis is thus another ill-defined term in which two important aspects are associated: First, a nontrivial amount of change; second, and more important, the presence in the premetamorphic stage (the larva) of transitory features that we cannot simply explain as a scaffold for the morphogenesis of the postmetamorphic stage. In other words, a 'true' metamorphosis is a kind of rudimentary alternation of generations (metagenesis), with the new 'generation' forming from less than the whole premetamorphic stage. What of the larva is discarded at metamorphosis, however, may differ enormously from trivially small parts to most of it.

In the case of marine invertebrates with free-living larvae, the abrupt change in body organisation brought about by metamorphosis does sometimes separate (and associate) two very different body plans: the pre- and the postmetamorphic ones, respectively. Larvae belonging to different phyla may be quite similar to one another. This explains why Williamson (1992, 2001) may have suggested an origin of free-living larvae through cross-phylum hybridisation, an hypothesis experimentally refuted by Hart (1996) and simply ignored by the large majority of invertebrate zoologists. Metagenesis explains the nature and meaning of metamorphosis. Metagenesis fits very well within the cyclical, non-adultocentric view of development suggested in chapter 1. One could ask how many different 'lives' an animal may fit into its cycle. The insects with hypermetamorphosis whose life cycle is briefly described on page 89 (see also Figure 5.4), together with some other insects and many tapeworms, have probably reached the top of metamorphic complexity available to animals.

Postembryonic Development

One Life throughout the Metamorphosis

The metamorphoses of many animals – from frogs to barnacles, from sea urchins to butterflies – are often described as catastrophic. Such events do not simply imply profound changes in the form and habits of the metamorphosing animal, but are often accomplished through the actual destruction of extensive parts of the larva, with the adult forming in a sense anew, starting from a limited number of set-aside cells. The case of the frog tadpole, whose tail and gills disappear while lungs develop (which

allow the adult animal to live its terrestrial life), is comparably much less momentous. More extensive changes are undergone by a sea urchin or fruit fly, whose adult body derives, in large part, from a tiny imaginal primordium in the first case and from a localised set of imaginal discs in the latter.

Closer investigations of even the most intensively studied animals are revealing that some of these metamorphoses, conspicuous as they often are, are nevertheless less catastrophic than described until now. For example, the larval musculature of *Drosophila* does not get totally lost at metamorphosis, as long believed, whereas the longitudinal visceral musculature of the midgut is perfectly preserved into the adult stage (Klapper 2000). Generally, in holometabolous insects, the larva-to-adult continuity is mainly provided by the nervous system. In the beetle *Tenebrio molitor*, for example, a set of motor neurons and interneurons persist during metamorphosis in the thoracic nervous system. Essentially only quantitative changes of the neuronal shape were observed during the pupal stage. No pupa-specific degeneration of certain axodendritic structures of these neurons was found (Breidbach 1987). In other cases, changes in the neural architecture during the pupal stage may be more extensive, as in the case of lepidopterans, in which several separate larval ganglia fuse (in patterns typical of different clades within the order) to form a lesser number of compound adult ganglia (Amos and Mesce 1994). No neuronal scaffold is available for adult organs without a larval precursor. In the pupal wing of *Drosophila*, there are no larval nerves that act as pioneers for the later developing adult neurons. Axon bundles are first observed 15–18 hours before the pupal moult, that is, before the formation of the wing veins (Anderson 1984).

Developmental Stages as Units of Competition?

A useful classification introduced by Davidson (1991; see also Cameron, Peterson, and Davidson 1998) distinguishes three main types of embryos.

In type 1 embryos, blastomeres form from invariant cleavage, and their fate is generally also invariant. But under experimental conditions, they show regulative capacities. Only one embryonic axis is prespecified during oogenesis, the second being specified after fertilisation. Cell-type specification occurs in situ: Founder cells arise during cleavage and precede any large-scale embryonic cell migration. Embryogenesis is completed in 10 ± 2 cell divisions and gives rise to larvae that will undergo a 'maximum metamorphosis' postembryonic development. This type of early

development is widespread among marine invertebrates, such as sea urchins and nemerteans.

In type 2 embryos, cleavage is variable and no canonical cell fate assignment is possible; regulative capacities are high. At most one future body axis is prespecified during oogenesis. Cell specification is preceded or accompanied by extensive cell movements. The embryonic body plan is largely specified by early regional inductions, such as in vertebrates.

In type 3 embryos, a blastoderm forms through a cleavage occurring in syncytial conditions. Cellularisation follows blastoderm formation. Antero-posterior and dorso-ventral axes of the future embryos are both prespecified during oogenesis. Regional specification of the body plan is largely accomplished before cellularisation, as in *Drosophila* and other arthropods.

With his concept of maximum metamorphosing marine invertebrates, Davidson has aptly called attention to the dramatic events occurring in the postembryonic life of these animals. By dramatic I do not refer so much to the obvious changes in body shape, as to the fact that a sizeable and often major part of the larval body is literally discarded at metamorphosis. At the same time, the whole machinery of transcription and protein synthesis shifts largely from a set of embryonic or larval genes to a distinct set of 'adult genes'. This circumstance invites comments.

First, what happens to the marine invertebrates with type 1 development has much in common with the development of the holometabolous insects. In both groups, most of the adult body derives from set-aside cells: those of a small primordium in the case of a sea urchin, those of the histoblasts and the imaginal discs in the case of a fruit fly. From a grossly mechanistic point of view, the distance between the two systems is probably not so large as the independent research traditions in insects and echinoderms with corresponding different terminologies would suggest. The comparison between the two systems provides one more example of the plasticity of development and the power of attraction of a few successful solutions, which have independently evolved in taxa without any close phylogenetic relatedness.

Extending the comparison to the holometabolous insects invites caution before an otherwise attractive hypothesis formulated by Davidson (2001). In his view, type 1 larvae represent the original body plan of the bilaterian ancestor. Differentiation of many cell types which is completed, in modern bilaterians, after the evolutionary novel features of the adult body plan have been laid down, was already part of the ancestor's developmental

schedule. It has been progressively delayed by intercalation of increasingly complex morphogenetic events, those by which the adult primordium set aside in the larva is finally allowed to deploy. Thus, the larva is phylogenetically old, the adult is novel, and the final cell differentiation is largely old. This evolutionary scenario is recapitulative in so far as the larva, ontogenetically younger than the adult, is phylogenetically more conservative than the latter. It is not recapitulative, however, at the level of cell differentiation. In the case of the holometabolous insects, the scenario is much less recapitulative. The adult is broadly conservative, not just at the level of the cell types forming its organs, but also in its general body architecture. What is novel, in this case, is the larva. The question then arises, whether comparable cases may have evolved in other phyla, despite their seemingly primitive larvae. Detailed information from more than a few model species is obviously wanted.

Davidson's (2001) elegant model also invites a comment on the coexistence in the same individual of two partly independent sets (better, networks) of developmental genes: those expressed in early life (embryonic or larval genes) and those only expressed at metamorphosis or later (adult genes). Could competition develop between the two (or more, in the case of more complex metamorphoses) networks of genes? In other words, could competition exist among developmental stages, as it exists, potentially at least, among cell lineages within an individual? Competition, if any, could produce either a sharpening of boundaries or the disappearance of one of the two competitors. Abrupt metamorphoses are thus perhaps the consequence of a sharpening of the contrast between two temporal segments of postembryonic development, due to a large degree of independence of the corresponding genetic networks. The disappearance of a developmental stage may be, instead, a consequence of the assimilation into a larger genetic network of a specific set of genes under whose control that stage has been until then. This scenario may help understand why the number of major transitions during one life cycle is never large. The size of the genome (or the total amount of 'developmental genes') does not allow building and maintaining more than a handful of networks corresponding to as many developmental stages and, at the same time, to coexist in a balanced state. The degrees of freedom available to the evolution of the complexity of a developmental schedule may not only (or even primarily) depend on the adaptive value of the corresponding phenotype, but also on the possibility to set up and maintain a correspondingly complex architecture of gene networking.

The Evolution of Moulting Schedules in the Ecdysozoa

One of the most conspicuous advances in metazoan phylogenetics during the last decade of the twentieth century has been the recognition of the Ecdysozoa as a monophyletic group. As described in chapter 2, the Ecdysozoa are the animals, such as arthropods and nematodes, whose postembryonic development is punctuated by moults. Moulting is required because the animal is covered with a cuticle that must be periodically cast away to permit growth. Thus, these animals' metric growth is discontinuous. There are two kinds of moults. Some moults are simple growth accidents, with the newly moulted animal strictly resembling the last premoult stage, except for increased size. Other moults, on the contrary, separate widely different stages, such as caterpillar and pupa, or pupa and butterfly. We call these events growth moults and metamorphic moults, respectively.

In many Ecdysozoa, there are no true metamorphic moults; therefore, their preadult stages are usually called juveniles, rather than larvae. This is true, for example, of spiders, scorpions, or woodlice. Juvenile stages separated from the adult by a more or less conspicuous metamorphosis are primarily called larvae or nymphs (if mobile) and pupae (if motionless), but the nomenclature is far from standardised. This is due, in part, to the difficulty in identifying reliable criteria for homologising developmental stages of different animals. Early in the twentieth century, Berlese (1913) suggested that the larval stages of holometabolous insects, such as butterflies, beetles, bees, and flies, are not homologous to the preadult (nymphal) stages of dragonflies, crickets, and true bugs, but rather correspond to late embryonic stages of the latter. Evidence concerning the hormonal control of development has recently suggested (Truman and Riddiford 1999) that the putative three stages of the first insects (pronymph, nymph, and adult), still retained in modern heterometabolous insects, are indeed equivalent to the larva, pupa, and adult stages of holometabolous insects, respectively. If so, there is no meaning in comparing the number of nymphal stages in heterometabolous insects to the number of larval stages in holometabolous insects. But it is interesting to note that, in both series, a similar number of instars and similar evolutionary trends in reducing this number can be observed.

Homology between postembryonic stages of different arthropods is difficult to assess (Švácha 1992, Sehnal, Švácha, and Zrzavý 1996), even between closely related species differing in the number of preimaginal stages, sometimes also in groups in which the total number of larval or nymphal stages is not that high.

The number of larval instars in Coleoptera is mainly three to five, but it is up to nearly 30 in some Dermestidae, only two in some Histeridae and Leiodidae and only one in some ultraspecialised cavernicolous Bathysciinae. Most lady beetles (Coccinellidae) have four larval instars, but there are only three in *Hyperaspis lateralis* (McKenzie 1932).

To take an example from butterflies, there are five larval stages in *Parnassius apollo*, but only four in *Parnassius mnemosyne* (Ebert and Rennwald 1991).

Intrageneric differences are also common in the number of nymphal stages in grasshoppers; for example, there are six stages in *Conocephalus discolor*, but only five in *C. dorsalis*; there are seven stages in *Tettigonia viridissima*, but six in *T. cantans*; and there are seven stages in *Metrioptera roeselii* and *M. bicolor*, but six in *M. brachyptera* (Detzel 1998). Differences are increased if we take species from different genera and families, but still within the same order, as in the following examples taken from European species of crickets and grasshoppers in which, additionally, intraspecific differences have been recorded: four to five stages in *Chorthippus albomarginatus*, five to six stages in *Ephippiger ephippiger*, eight to ten stages in *Gryllotalpa gryllotalpa*, and nine to sixteen stages in *Acheta domesticus* (Detzel 1998).

Sometimes, males undergo one moult less than their conspecific females. This is a common occurrence in Orthoptera, in which such a difference has been recorded, for example, in *Tetrix*, *Oedipoda*, *Stethophyma*, *Omocestus*, *Stenobothrus*, and *Chorthippus*. In the same insect order, sexual dimorphism is often compounded with individual variability. For example, whereas all males of *Chorthippus brunneus* and *Ch. apricarius* have four nymphal stages, their females may have either four or five stages. In *Ch. mollis*, both males and females may have either five or six nymphal stages, but the higher number is much more frequent in the females than in the males (Detzel 1998). In the jumping spider *Evarcha jucunda*, most females reach maturity as instar VII, most males as instar VI (Hansen 2000), whereas in the orb-web spider *Argiope argentata*, there are only five or six moults in the males but as many as eleven to thirteen in the females (Kaestner 1970).

That moults do not necessarily separate obviously different stages is also clear from the presence of adult-to-adult moults in many arthropod groups, such as Xiphosura, Amblypygi, spiders (but only in the females of the Mygalomorphae, Filistatidae, and possibly Mesothelae), Diplopoda (but not in the short-lived Chordeumatida), Chilopoda, Symphyla, many

crustaceans (e.g., Isopoda and Decapoda, but not Copepoda), Collembola, Diplura (but not Protura), Archaeognatha, Zygaentoma, and, within the winged insects, in Ephemeroptera. Adults, however, do not moult in Uropygi, Acari (except in some Prostigmata and, possibly, the Notostigmata; Evans 1992), Ricinulei, Pseudoscorpionida, Scorpiones, Protura, and in the large majority of insects, nor in nematodes. In Loricifera, a moult separates a subadult from the true adult stage, as in the mayflies.

Number of Moults, Dyar's Coefficient, and Targeted Growth

There is little evidence for the number of moults in arthropods to be genetically fixed, even in the groups in which this number is low and fixed within the species and the adult does not moult anymore. It is much more probable, instead, that the moult giving rise to the adult is determined indirectly, through cues that in many cases are nevertheless likely to produce a fixed result. Illuminating is the following evidence provided by Nijhout and Williams (1974) and Nijhout (1975, 1999). The larva of the tobacco hornworm *Manduca sexta* undergoes metamorphosis only if it has reached a precise threshold size. This includes the independent 'assessment' of two different measures, the size of the hard parts of the caterpillar's exoskeleton and the caterpillar's biomass. The first measure is constant during the whole length of a larval stage, but the other measure increases continually as a consequence of feeding. There is a correlation between the two measures, in that the biomass attained before a given moult affects the size of the exoskeleton of the following instar and, in particular, the size of its mouthparts. The size of the mouthparts, in turn, affect the rate at which a larva feeds and hence grows between two moults. For example, the weight of a *Manduca* larva is about 1 g at the beginning of its last larval stage and 7–9 g at the end of it. But this final size, which is required for the animal to moult to adult, is only attained if the mouthparts of the larva are large enough; that is, if the width of the head capsule is at least 5 mm. Therefore, final instar larvae are those (and only those) with head capsules wider than 5 mm, irrespective of the number of times they may have moulted previously. Under normal conditions, this species has five larval instars, but it may have a sixth if the larva has been growing under starving conditions.

A similar story of targeted growth is that of the cockroach *Blattella germanica*, whose adults are of the same size, irrespective of the number of instars (either five or six) through which they have grown. Among individuals attaining adulthood following the same number of moults, the coefficient of variation in body size increases until the third instar, then it

decreases towards the adult stage, with smaller individuals compensating with larger growth (Tanaka 1981).

More generally, the following factors are likely to determine the number of moults an arthropod (or ecdysozoan) will undergo before attaining maturity, as well as the constancy of this number: the initial size i (itself possibly constrained by some size requirement of the germ-band stage), the final (adult) size f (which is likely to be under a complex selective regime), and the geometric mean of linear postmoult to premoult size ratios r (when constant, known as Dyar's coefficient, which, in addition to its environmental control, may also depend on a more or less strictly controlled mitotic rate per moult).

The number N of moults before adulthood is therefore

$$\mathrm{N} = \log(f/i)/\log r.$$

Within arthropods, ratios of postmoult to premoult size vary within a large range (from less than 1 up to 2.37), but it has been contended (e.g., Cole 1980) that there are Dyar's coefficient values typical for some lineages, such as holometabolous and heterometabolous insects.

Little attention has been focussed on the fact that, for many rigid body parts (e.g., the length of the femur of the third leg in a grasshopper or the width of the cephalic capsule in a caterpillar), f/i does not vary much, being frequently in the range of four to five. For example, this is true for the length of several homologous parts measured from hatching to maturity in 16 species of orthopterans studied by Ingrisch (1976). Consequently, a fairly strict inverse relationship exists between N and r. But r (adequate feeding permitting) is directly related to the mitotic rate of the external epithelium (i.e., to the cellular process which is more likely to be under direct, and largely genetic, control). This, in turn, will determine N, without any need for this number to be actually 'counted' by the animal.

One might speculate whether the number of moults undergone by the earliest ecdysozoans was low, as in modern nematodes, or high, as in several groups of arthropods, trilobites (e.g., Chatterton and Speyer 1997), and Cambrian crustaceans, such as *Rehbachiella* (Waloßek 1993). As in many arthropod lineages there is a trend for the number of moults to become smaller and fixed, one is tempted to speculate that the earliest ecdysozoans also underwent many moults. But this would be probably true only if those animals were incapable of any sizeable intermoult growth. If, on the contrary, they could grow even between moults or after the last moult, as nematodes do (at least those of the *Ascaris* type), then the alternative

possibility might also be true. I suggest that in the earliest ecdysozoans, the number of moults was not fixed and was possibly high. This number, however, became progressively stabilised, and eventually reduced, as the original significance of the cuticle as a means to control proliferation of epidermal cells became coupled to more specific morphogenetic events, such as the serial production of segments, or, more generally, to the control of overall body shape.

Lazarus Developmental Features

Palaeontologists define Lazarus taxa (Flessa and Jablonski 1983, Smith 1994) as taxa with a significant gap in the fossil record: they seem to become extinct, but suddenly reappear after a while. By analogy, I suggest calling Lazarus developmental features those features that disappear from an animal's body architecture at a given developmental stage, only to reappear at a later stage.

An example of embryonic features temporarily disappearing from early postembryonic stages, but found again in later stages, is the fourth pair of legs in the mites: present in the embryo and lacking in the larval instar, these legs are nevertheless present in the nymphal and adult stages of most mites. Moreover, several actinotrichid mites present a phenomenon termed *hysteromorphosis* by van der Hammen (1980, 1989), in which the posterior segments of the embryo temporarily 'disappear', but one to three segments differentiate again in the course of postembryonic development. When as much as three Lazarus segments are involved, 13 segments are recognisable in the newly hatched larva, 14 in the protonymph, 15 in the deutonymph, and 16 in the tritonymph. The actual fate of the Lazarus segments during their eclipse is not known. However, on the basis of what is known about segmentation in other arachnids (and arthropods generally), they disappear as external morphologically distinct units, but still retain their individuality, at least as clusters of segmentally specified cells.

Several examples of Lazarus appendages have been recorded from decapod crustaceans (Balss et al. 1940-61, Schram 1986). In the shrimp *Sergestes*, the appendages of the pereion (the maxillipedes as well as the locomotory legs), which are present in the previous mysis stage, loose their exopodites when reaching the mastigopus stage and the last two pairs of locomotory legs are completely regressed. All these structures will reappear during later stages. The same was observed for the maxillae and maxillipedes of a larva referred to the decapod genus *Petalidium*. In scyllarids,

maxillae, first maxillipedes and pereiopods IV–V, already formed in the embryo, are partially reduced in late-embryonic and early postembryonic stages, but in later stages are fully redeveloped.

Examples of temporary regression of appendages are common in the postembryonic development of several pycnogonids (Dogiel 1913). In stadium V–VI, the three first pairs of appendages undergo reduction and, finally, histolysis, so that in stadium VII no trace of these appendages is left. Second and third pairs of larval appendages regress temporarily and later grow again, thus acquiring new form and function. Dogiel compared this transdifferentiation with the change of abdominal to thoracic identity of a segment during regeneration in the polychaete *Spirographis* (currently *Sabella*).

Lazarus appendages are also present in some parasitic copepods as the monstrillid *Cymbasoma rigidum*, whose larval life is primarily spent in the blood vessels of the serpulid worm *Salmacina dysteri*, whereas the adult reverts to free life. This copepod begins its postembryonic life as a free-swimming larva, a nauplius with the usual three pairs of anterior appendages. Once the nauplius has found its host and punctured it with its mandibular hooks, it casts off its cuticle and appendages and enters the host, becoming an oval mass of undifferentiated cells. The parasite makes its way into the ventral blood vessel of the annelid, where it secretes a new cuticle, while two arm-like processes grow out from its ventral side. Adult organs develop within the larval cuticle, the first antennae are among the first features to be regenerated (Snodgrass 1956).

In some millipedes of the order Julida, the whole complex of sexual secondary characters may behave as a Lazarus feature. This phenomenon, discovered by Verhoeff (1923), is called periodomorphosis. Many millipedes (but by no means all of them; see page 95) are long-living arthropods which do not stop moulting with sexual maturity. As a rule, this means that a series of two or more sexually mature stages are thus obtained. In the males of some Julidae and Blaniulidae, the first adult stage may moult into an 'intercalary' male with fully regressed secondary sexual characters, including one or two pairs of conspicuous specialised appendages (gonopods). After one, but sometimes two or even three intercalaries, the male moults to a new reproductive stage, in which the sexual secondary characters are fully fledged anew (Sahli 1990).

Completely distinct from the Lazarus developmental features are the atavisms that one would be tempted to call 'evolutionary Lazarus features'. A nice molecular example is provided by the expression of *Distal-less* in

the abdominal segments, which has been suppressed at an early stage of insect evolution, thus determining the generalised lack of limbs on these segments, but has been de-repressed in butterflies, whose caterpillars are thus provided with larval legs or prolegs (Panganiban, Nagy, and Carroll 1994).

Recapitulation

Cameron, Peterson and Davidson (1998) equate the cellular organisation of the marine larvae of modern indirectly developing invertebrates to the putative organisation of the earliest bilaterians, which are supposed to have been of minuscule size. The modern adult bilaterian would thus represent an evolutionary innovation 'grafted' onto the previously existing organisation of the earliest metazoans, an organisation now surviving in the larval stage only. In my view, this phylogenetic scenario is unnecessarily biased by Haeckelian recapitulationism. Why should the larva be primitive and the adult derivate? The fact that, in the conventional (adultocentric) description of ontogeny, the larva precedes the adult is not necessarily proof that the latter's features have been phylogenetically added to those of its larva-like ancestor. Neither is the fact that the larva, in most cases, is structurally simpler than the corresponding adult. The holometabolous insects offer a good case in point. There is little doubt as to the fact that their larvae are an evolutionarily innovation rather than a recapitulative ontogenetic stage.

This is also seen at the level of cells. Insect epidermis is normally composed of diploid dividing cells. What has deviated from the standard and represents an evolutionary novelty in the cyclorrhaphous Diptera or some Hymenoptera is the layer of polyploid larval epidermal cells, *not* the disc cells from which a large part of the adult will originate.

The case of holometabolous insects is also particularly illuminating with respect to time and mechanisms of the separation between larval and adult components of the developing animal, in *Drosophila* at least. The cells that will form the imaginal discs are set aside very early in development. Following the last embryonic wave of mitosis, the larval cells polyploidise, cease to divide and differentiate into the different tissues and organs of the larva. The imaginal cells, to the contrary, remain diploid, continue to proliferate, and do not differentiate until metamorphosis. At which stage exactly the larval and imaginal developmental pathways become separated is not definitively clear. Harbecke et al. (1996) have demonstrated

that this does not happen very early, say at the blastoderm stage, as it was previously believed.

The main objection to recapitulation, from an evo-devo perspective, is that the processes of pattern formation are phylogenetically more recent than many or most pathways of cell differentiation, but cell differentiation, in ontogeny, is largely delayed until most of body patterning had been accomplished (Davidson 2001). This is, in essence, the result of what Gehring and Ikeo (1999) call intercalary evolution, a process by which genes originally involved in differentiation pathways have been increasingly co-opted, by duplication and functional divergence, or by fusion with new enhancers or promoters, to their current functions in body patterning.

Isolated examples of recapitulation can be found even at the levels of cells, molecules and developmental mechanisms.

It has been observed, for example, that phylogenetically older cell types are expressed earlier in development than phylogenetically younger types (Flickinger 1994). In *Caenorhabditis elegans*, a shift in expression over the course of development has been reported from evolutionarily conserved genes to worm-specific genes (Hill et al. 2000).

In *Drosophila*, phylogenetically deeper and ontogenetically earlier developmental events, such as formation of the pharynx, take place almost normally in embryos deficient for *reaper* (*rpr*). This is a gene whose expression is required by cells undergoing apoptosis, but later events which are mostly associated with head involution – such as retraction of the clypeolabrum, formation of the dorsal pouch and fusion of lateral gnathal lobes – are evolutionarily more recent and fail to occur normally in *rpr*-deficient embryos (Nassif et al. 1998).

CHAPTER FIVE

Body Regions: Their Boundaries and Complexity

> Comparative molecular genetics is nothing but comparative anatomy by other means.
>
> J. Deutsch 2001: 48

Tagmosis

The bodies of many animals are obviously divided into regions: the head, thorax, and abdomen in insects; the prosoma and opisthosoma in spiders; and the head, trunk, and tail in vertebrates. Comparable distinctions apply to most other bilaterians, segmented and not segmented alike.

Is this distinction just a subjective description, or does it correspond to an intrinsic pattern? If the external regionalisation corresponds to a regionalised 'internal description', how far is this pattern comparable across lower and higher taxa? In other terms, is a head always a head? Is the trunk of a mollusc meaningfully comparable with that of a vertebrate?

An interesting feature of body regions, or tagmata, is their number. No zoology textbook assigns more than three to five regions to the animals with the most extensively patterned main body axis. Does this number correspond to some intrinsic constraint of body design?

When approaching the study of body tagmosis, we face the usual problems of definition. What is 'really' a tagma? How are its boundaries defined? Things are generally simple, but the more interesting questions are found in the exceptions.

Take, for example, the insect thorax. This is the second body region, between the head and abdomen. It comprises three segments, each of which bears a pair of legs. No 'true' leg is found on the head or abdomen segments. In the vast majority of cases, we do not have any difficulty recognising the anterior and posterior limit of the thorax, even in insects (or insect

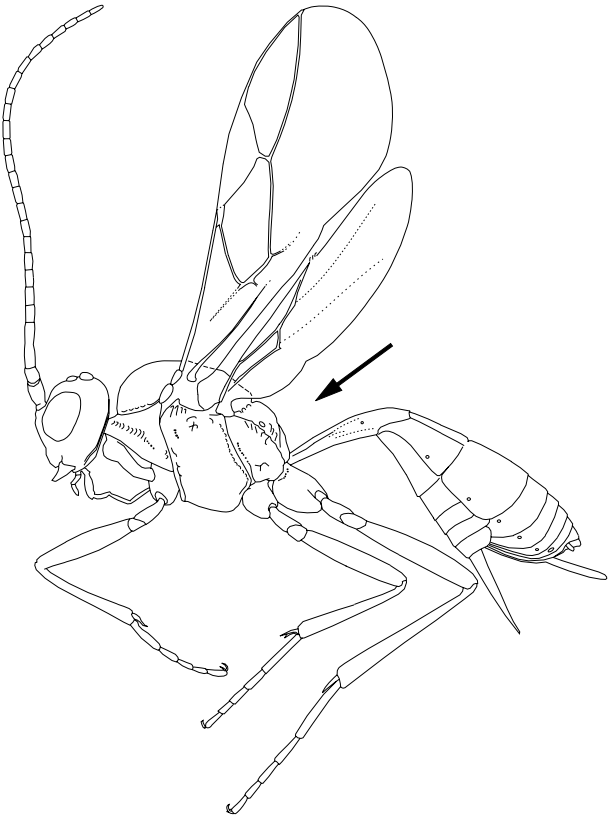

Figure 5.1. In parasitic and aculeate hymenopterans, four segments corresponding to the three segments of the conventional insect thorax plus a reduced first abdominal segment (the propodeum; arrow) form a morphofunctional unit called the mesosoma. The example in the figure is a euphorine braconid wasp. (Redrawn after Goulet and Huber 1993.)

developmental stages) where legs are missing, as in the larvae of flies and mosquitoes. Problems, however, arise with hymenopterans, such as ants, wasps, and bees in which the morphological postcephalic tagma in front of the typical wasp waist corresponds to four segmental units, despite the fact that it bears no more than the usual three pairs of legs. The standard interpretation of this body region is that the first segment of the abdomen, the propodeum, has been added to the thoracic segments to form the so-called mesosoma (Figure 5.1). No one says that the thorax of these insects comprises four segments, but this is simply a matter of tradition, not the consequence of a well-argued assessment of homology.

Figure 5.2. Praying mantis (*Mantis religiosa*). (Courtesy of P. Fontana.)

Uncertainty in the delimitation of tagmata may even extend to the distinction between head and trunk (or thorax, or pereion, according to the different groups and the anatomical nomenclature in use). This is the case of the numerous arthropods in which one or more pairs of appendages, belonging to segments posterior to those that have conventional mouthparts, are morphologically and functionally transformed into feeding tools called maxillipedes. These are either poisonous fangs (centipedes) or toothed Swiss army knives (mantids, mantid shrimps, and mantis-like lacewings or Mantispidae) used to capture prey (Figure 5.2), or simpler maxilla-like appendages used to select, clean, tear apart, or otherwise manipulate the food (many crustaceans). Thus, from the point of view of the specialisation of the appendages, one says that the head of these arthropods comprises one or a few segments more than the head of their relatives lacking maxillipedes. On the other hand, segments with maxillipedes may retain their autonomy, and their non-cephalic structure, despite the feeding specialisation of their appendages. This is the case, for example, of the forcipular segment of the centipedes, which is not that different from the leg-bearing segments next to it, despite specialisation of its two

appendages as poison-claws, and the prothorax of the mantis, which maintains its thoracic identity despite its unusual elongation and mobility and the raptorial specialisation of its legs. In many crustaceans, integration within the cephalic tagma of one or more segments with maxillipedes is much more advanced. This process has been regarded as a prolongation of a phylogenetically deeper event that led to the cephalisation of maxillar segments, a process regarded as still (primitively) incomplete in the lower crustacean lineage of the Cephalocarida (Dahl 1991). Integration of further segments into the anterior tagma does not imply an unambiguous extension of the head to embrace a number of segments higher than usual, because the number of conventionally postcephalic segments fused to the head does not necessarily match the number of segments with maxillipedes. In Leptostraca, the first thoracic segment is fused to the cephalon, but its appendages are not specialised as maxillipedes and are not different from the other thoracic appendages. In the Euphausiacea, there is a cephalothorax comprising the head and all eight segments of the pereion (as in crabs, lobsters, and shrimps), but none of these segments has masticatory maxillipedes.

Standard comparisons are still more uncertain when the transition between two regions (say, thorax and abdomen) occurs at positions that are otherwise regarded as different (e.g., at different segment number).

A compromise solution adopted by some arthropod comparative anatomists has been the use of the term *pseudotagmata* for body units such as mesosoma and metasoma in scorpions, gnathosoma and idiosoma in mites and ricinuleans, and proterosoma and hysterosoma in actinotrichid mites (van der Hammen 1989). There is no harm if these terms are simply used for descriptive purposes, but it is clear that this is simply a way to avoid problems in tracing homologies – not a way to solve them.

Boundaries between conventional tagmata are often less clear than anatomists would hope. Fuzzy boundaries between tagmata are quite common in polychaetes. In capitellids, a change in the orientation of the parapodia marks the transition from thorax to abdomen; but, in some genera, this change is diluted over several segments. In goniadids, the anterior body region is characterised by uniramous parapodia, the posterior region by biramous parapodia, but the transition between the two types, which is abrupt in some genera, is spread over 40 segments in others (Edmonds et al. 2000).

Further difficulties are caused by a mismatch between the external and internal organisations. In the internal anatomy of insects, for example, the

thorax/abdomen boundary is often less than clear; the first abdominal ganglion is often fused to the third thoracic ganglion, even if the latter is distinct from those of the first and second thoracic segments (Hanström 1928). Problems are sometimes caused by regressive evolutionary trends that cause an usually integrated region to be 'decomposed' into its original components. This is the case of the pontellid copepods, in which the antennary region is not incorporated into the cephalic tagma, as it is in all other copepods and in arthropods generally (Kabata 1979). Much more common, however, is the opposite trend, with an increasing number of elements (segments) incorporated into a given tagma. This is observed in the caligiform copepods, in which an increasingly larger anterior tagma evolves, starting with the conventional head (cephalon) of their relatives. In *Dissonus*, head plus one maxillipedal segment fuse to form a cephalosome, in *Trebius* one more trunk segment is incorporated in this anterior tagma to form a cephalothorax, whereas in *Caligus* this region extends to the third thoracic segment (Kabata 1979).

Instability of Tagmatic Boundaries

A principle illustrated by Bateson (1894) in his *Materials for the Study of Variation* is that variation in the elements of a series is very often concentrated at the end of the series. Variability at the end of series is manifested not only in the frequency of cases of absence of terminal members, but also in the frequency of extra members in their neighbourhood. Bateson gave many examples for mammal teeth and digits. This principle is virtually identical to one of the 'general laws of arthropod metamerism' formulated by Lankester (1904), stating that the most anterior and most posterior segments of a tagma are particularly liable to regressive evolution. This is often true of the first abdominal segment of insects, the first abdominal segment of the arachnid opisthosoma and, in general, one or more segments at the posterior body end.

The anterior end of a tagma behaves very often as a 'subduction zone' (in geological parlance). In many myriapod groups (Diplopoda, Pauropoda and Symphyla), the first postcephalic segmental unit is a legless collum. A reduction of the first postcephalic segment is also clear in the Collembola (Figure 5.3). Many arachnid groups lack the dorsal plate (tergum) of the first segment of the opisthosoma, and the reduction may extend to the next two segments (scorpions, pseudoscorpions, and harvestmen). In an orthopteran suborder (Caelifera), the ventral plate (sternum) of the last thoracic segment is largely fused to the corresponding sclerite of the first

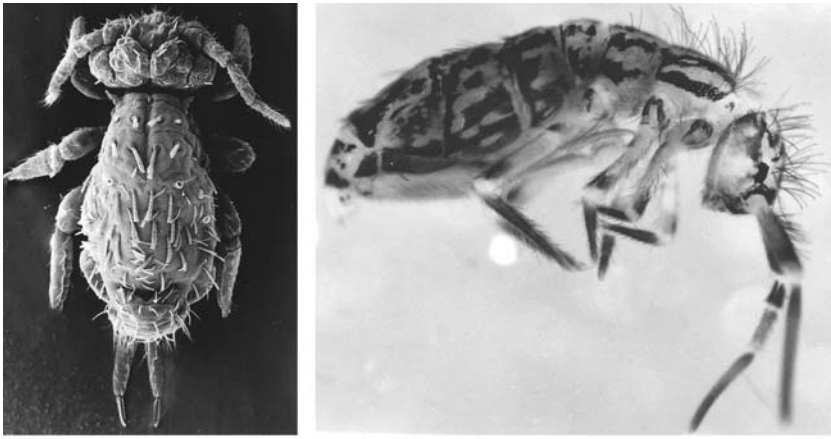

Figure 5.3. In collembolans (a, *Lipothrix lubbocki*, Sminthuridae; b, *Orchesella villosa*, Entomobryidae), the pronotum (i.e., the dorsal sclerite of the first thoracic segment) is more or less extensively reduced. (Courtesy of R. Dallai, Siena.)

abdominal segment (Beier 1972). In the stick insects (Phasmodea), the dorsal plates of the same two segments are fused together (Beier 1968). In bees, wasps, and ants, as previously described, the first segment of the abdomen becomes the propodeum. In beetles, a reduction of the first abdominal segments is commonplace.

A similar phenomenon was reported by Bateson (1894) for the teeth of mammals, in which variability at the end of the series is manifested not only in the frequency of cases of absence of terminal members, but also in the frequency of instances in which an extra teeth is present in their neighbourhood.

Nowadays, it is easy to explain this 'law' in terms of *Hox* gene expression. As the transition from one tagma to the next corresponds to the anterior boundary of expression of one or more *Hox* genes, we must be prepared to regard this transition as a morphogenetic 'hot spot', in which diverse specialisations may be expected, with segmental reduction included.

Lankester's principle does not apply to arthropods only; in the mouse, the skeletal defects caused by systematically knocking out the *Hox* genes appear to be concentrated at certain 'hot spots', notably the boundaries between different skeletal types (i.e., the boundaries between the anatomist's vertebral column regions; Chen and Capecchi 1997).

In the woodlouse *Porcellio scaber*, the expression domains defined by the *Hox* genes *Antennapedia*, *Ultrabithorax*, and *abdominal-A* coincide with

morphological boundaries between tagmata (Abzhanov and Kaufman 1999, 2000b). However, these borders do not coincide with those of insects, and similar differences were observed for the *Hox* genes expressed in the posterior cephalic segments. For example, *Sex combs reduced* is expressed in the maxillae of *Porcellio*, whereas the *Hox* gene *proboscipedia* is expressed in the corresponding maxillary and labial segments of insects.

Homology of Tagmata

Terms such as head, thorax and abdomen have been used for the body regions of the most diverse animals, from vertebrates to polychaetes, from insects to non-malacostracan crustaceans. Is there any degree of homology between the body regions of all these animals, or of bilaterians generally, to justify the generalised use of the same terms for their body regions? As often happens in respect to homology problems, the answer is yes and no.

There are problems even within a limited group, such as crustaceans (Kaestner 1970). Three main regions are primarily recognisable in these arthropods – a head and two postcephalic tagmata – but the nature of the latter ones is probably not the same in malacostracans and in the other crustaceans. In malacostracans, both regions are typically provided with appendages; but, in most non-malacostracan crustaceans, appendages are present in the first postcephalic region only. The latter condition has been long regarded as equivalent to that of the insects, which have a thorax with three pairs of legs and an abdomen practically devoid of appendages. Accordingly, the two terms used for the two tagmata of the insect trunk – thorax and abdomen – are used for copepods and other non-malacostracan crustaceans, but not for the malacostracans, where the two postcephalic regions are known instead as the pereion and the pleon. According to Gruner (1997), pereion and pleon together would be equivalent to the thorax of the basal crustaceans, whereas the abdomen would have disappeared or nearly so.

For non-malacostracan crustaceans, some evidence is available about molecular markers of the body regions and their boundaries. Averof and Akam (1995) compared the expression patterns of several *Hox* genes in *Artemia franciscana* (a non-malacostracan, branchiopod crustacean) with those of the homologous genes in insects. They found that *Antennapedia*, *Ultrabithorax*, and *abdominal-A* are expressed, in *Artemia*, in largely overlapping domains in the uniform thoracic (pregenital) region, whereas their non-overlapping expression leads, in insects, to the specification of distinct segment types in the thorax and abdomen. Accordingly, Averof

and Akam tentatively suggested that the pregenital 'thorax' of *Artemia* would correspond to the sum of the thoracic and the pregenital abdominal segments of insects. We shall also consider that non-malacostracan crustaceans with a limbless 'abdomen' (e.g., copepods and anostracans) do not have abdominal ganglia. Segments of their abdomen receive nerves from the thoracic part of the central nervous system. From this point of view, this 'abdomen' would better compare with an appendage like the vertebrate tail, rather than with a part of the main body axis. In these crustaceans, however, the anus opens at the end of these 'abdominal' segments, whereas it is anterior to the tail in vertebrates. It is therefore not advisable to call these segments a 'tail', as tentatively suggested by Averof and Akam (1995). Lack of individual neuromeres in this region invites enquiry as to whether this depends on a regional inhibition of the differentiation of segmentally distributed neural precursors or, what I regard as more likely, is the whole of this 'abdomen' derived from secondary segmentation of just one primary segment (one eosegment, cf. pages 200–203).

Thus, we shall not expect a given region to comprise a fixed number of segments. This is true for vertebrates as well as for annelids and arthropods. It does not depend on the precise mechanism of segmentation adopted by a particular animal group. I will return briefly to this phenomenon of 'transposition' when discussing homology in the last chapter.

Number of Tagmata and Convergence

The precise correspondence between the anterior (or posterior) expression boundary of a given gene along the main body axis and the anterior (or posterior) end of a given body region should not be construed as an argument in favour of the thesis that gene expression patterns are an infallible guide to the homology of body regions across the most diverse taxa. Tagmosis is probably constrained to a very limited number of alternative possibilities, and the genomic organisation of gene families, such as the *Hox* genes, offers a convenient and easily available patterning tool, so that we cannot rule out convergence.

The main constraint to tagmosis is perhaps the number of body regions an animal may differentiate. Admittedly, the concept of tagma is to a large extent arbitrary. This is clearly seen in the uncertainty of the morphological nomenclature applied to certain animal groups, in which some zoologists recognise a smaller number of body regions and others recognise a larger number of body regions. For example, how many postcephalic regions do we recognise in a millipede? Some zoologists separate the first few

segments as 'thorax', with zero to one pairs of legs each, from the remaining set of 'double segments' with two pairs of legs each. Yet other zoologists regard the millipede trunk as a single tagma. In scorpions, one might use the basic arachnidan blueprint, thus recognising a prosoma and an opisthosoma, but the latter is clearly articulated into a broader mesosoma and a narrower metasoma, and so on.

However, even in the hands of the splitters, regional specialisation along an animal's main body axis does not reasonably allow for the distinction of more than three to five regions. Thus, this question arises: is there any reason why animals with a main body axis articulated into a dozen regions do not exist?

There is a reason for that. It might be found in a issue of complexity discussed by Kauffman (1993) as an intrinsic limit of adaptive systems. Let's consider a system with many parts (in our case, body regions). As the number of those parts and the richness of interactions among the parts increase, there will also be a rapid increase in the number of conflicting constraints of design among the parts. Those conflicting constraints imply that optimisation can only attain increasingly poorer compromises. This limitation is intrinsic to the system's design, that is, it cannot be overcome by stronger adaptive selection. If Kauffman (1993) is correct, no patterning system, even the richest and most versatile, could further increase the number of differentiated parts; the upper limit depends instead on intrinsic structural rules. This may be indeed the case. In hypothetical, strictly combinatorial terms, a *Hox* code would permit differentiation of many body regions, but this is not what we observe. Interestingly, strict limits to the number of kinds of parts apply also to the longitudinal axis of the appendages and the other differentiated series of body units, such as tetrapod digits and mammal teeth. Many examples include just three elements, such as the trimerous coelom (proto-, meso-, and metacoel; or axo-, hydro-, and somatocoel) of bryozoans, echinoderms and other groups. No more than four kinds of elements – to borrow an example from botany – are present in the flowers, with their sepals, petals, stamens, and carpels (the latter being the elements of the ovary). There are four kinds of teeth in the mouth of a mammal (incisors, canines, premolars, and molars). There are four to five main transversal bands of the basic wing pattern in butterflies (Nijhout 1991), or the kinds of segments in the polychaete *Chaetopterus* (Figure 3.3). There are five kinds of fingers in tetrapod vertebrates. And, there are up to six – and this seems to be an unbridgeable upper level – main veins in the insect wing.

Interestingly, five, or something close to five, is also the upper number of 'mental boxes' into which perceptual schemes and naive taxonomies are organised. We have no difficulty organising a mental image of the relative position of three to five objects distributed along a horizontal or vertical axis. Of three objects, one is in the middle, one is on the left and one is on the right. Or, there is one in front, one below and one above. Things are less complicated, but still manageable, if two more objects are added (e.g., one more to the left and one more to the right). Additional elements, however, would easily disrupt our whole perceptual and mental constructs. A counterpart to these perceptual constraints is provided by our extremely frequent use of three to five categories in the most diverse kind of taxonomies we may adopt, including periodisation of time (e.g., past, present, future; or, at most, remote past, recent past, present, near future, and far future; cf. also the number and names of the geological eras). Five is also the highest number of taxonomic levels (or categories) in the most elaborate of the main folk taxonomies of animals or plants examined thus far around the world (Berlin, Breedlove, and Raven 1973, Berlin 1992).

One might speculate whether this upper limit around number five of our perceptual schemes and basic mental constructs has anything to do with Kauffman's (1993) principle of "optimized compromise" among differentiated units at a given structural level. I suspect it has, but I do not know how far the limit in complexity we see at the perceptual/mental level depends on the limit in complexity of the underlying biological systems (our five fingers included) rather than on its own intrinsic structural constraints. It is tempting to imagine a causal cascade, beginning with the structural constraints of genetic networking, moving to the morphological level with the determination of no more than five kinds of fingers and ending with rules governing the way we count or describe our perceptual universe. It is more likely, however, that common structural rules apply to all these levels, so that the agreement between levels is more the effect of a canalised drift than the expression of a historically determined causal cascade.

The Complexity of Postembryonic Development

A final speculation on this theme is that the putative limit in the complexity of body patterning along a given structural dimension descending from Kauffman's principle might also apply to the complexity of the temporal dimension of animal development. That is, the same principle might possibly explain why the number of basically different kinds of body

organisation displayed by an animal along its whole life cycle is always very low, seldom higher than two or three (e.g., tadpole and frog, pluteus larva and sea urchin, polyp and medusa, caterpillar chrysalis and butterfly). Simple growth stages, even when separated by moults, as in the case of arthropods or nematodes, or the mostly smooth contrast between a non-reproductive juvenile and a fully reproductive adult should not be taken into account, because of the fundamental continuity of these life processes. Things are quite different, instead, when a life history is punctuated by major metamorphic events, such as in many cnidarians, echinoderms and holometabolous insects. The most complex life cycles are probably those of the so-called hypermetabolous insects.

Hypermetaboly occurs in Strepsiptera, in several beetles (all Meloidae, Rhipiphoridae, Micromalthidae, and a few Carabidae and Staphylinidae; Figure 5.4) and in some members of Neuroptera (Mantispidae), Lepidoptera (Gracillariidae), Diptera (Bombyliidae, Acroceridae, Nemestrinidae), and Hymenoptera (Chalcidoidea). A basic feature of the life cycle of all these insects is the occurrence of two to four different kinds of larvae. In the case of the blister beetles (Meloidae), for example, only the first larval instar (called a triungulin because its legs seem to end with three claws; in fact, between the two true claws there is a robust seta of similar size) is active, long-legged and very mobile. Its mobility is instrumental in finding either a wild bee that will bring the tiny triungulin to its nest, or the egg mass laid by a grasshopper in the sandy soil of a hot dry region. As soon as it makes contact with its future foodstuff, the triungulin moults into a short-limb feeding larva that rapidly grows by feeding on the grasshopper's egg or on the egg (or the young larva) of the bee, together with the pollen the latter had stored in the nest for its offspring's benefit. What follows varies according to the genera; but, for our purposes, it is enough to say that in *Meloe* the feeding larva moults into a non-feeding, resting prepupa or hypnotheca. The latter, however, does not give rise to the adult, as one might expect, but to another active but not feeding larval instar. It will eventually moult into a true pupa, which in due time, at last, will give rise to the adult. An extra resting stage, such as the prepupa of the blister beetles, does not occur in the majority of the life cycles of the other hypermetamorphic insects. In all of them, the larval stages preceding the pupa are not structurally uniform, as in the conventional caterpillars or in the larvae of the majority of beetles, flies, and wasps, because the first-instar larva (generally, the most mobile segment of the insect's preadult

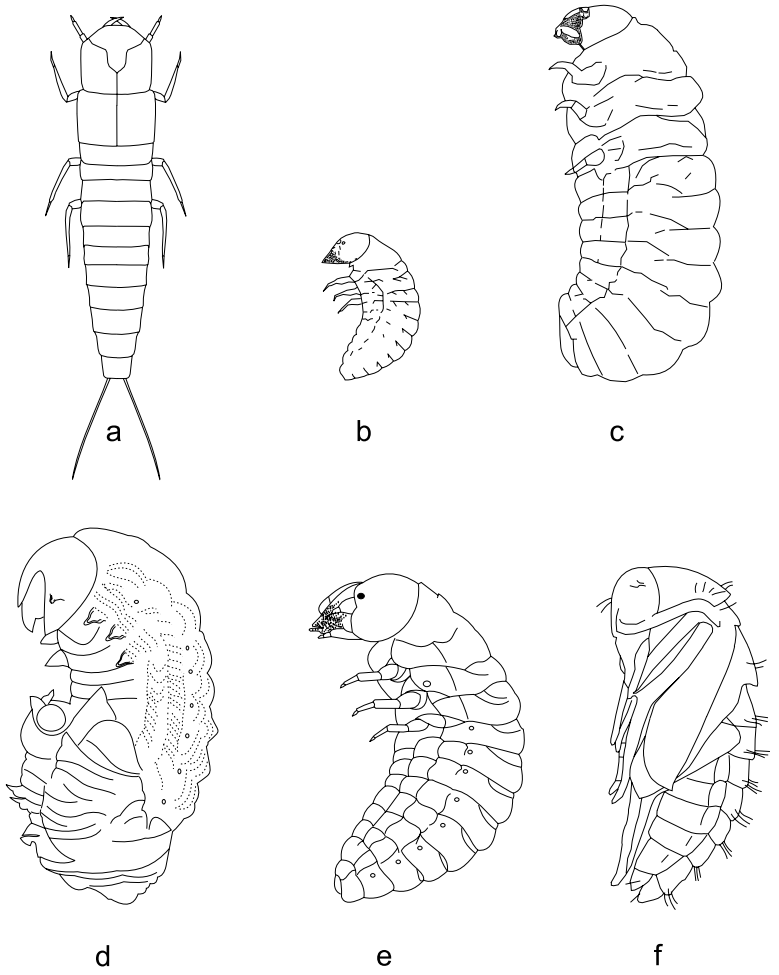

Figure 5.4. Larval and pupal stages of *Mylabris variabilis*, a representative of the hypermetamorphic blister beetles (Meloidae). a, larva I (triungulin); b, larva II; c, larva III; d, hypnotheca or pseudopupa; e, last larval stage; f, pupa. (Redrawn after Bologna 1991 and other sources.)

life) is followed by a larva of a very different kind. Further complications may occur, as in the beetle *Micromalthus debilis*, in which as much as four different larval kinds have been described.

Life cycles of a complexity comparable with that of hypermetabolous insects are known from flatworms (flukes and tapeworms) and from the recently described phylum Cycliophora. Interestingly, all flukes and

tapeworms are parasites, as are most of the hypermetabolous insects previously described. The only cycliophoran thus far described, *Symbion pandora*, is probably a commensal, living on the mouthparts of the Norway lobster (Funch and Kristensen 1995). The sporozoans or Apicomplexa (the protist phylum to which the malaria agent *Plasmodium* belongs) are also parasitic, and it is directly in this phylum that the most complex life cycles among unicellular protists are encountered, with up to three different kinds of reproduction and four or five different kinds of cells.

One might speculate whether Dawkins's (1982) concept of extended phenotype might help explain this generalised (but far from universal) co-occurrence of very complex life cycles and parasitic habits; but this is not a point to be discussed in this book. What matters, for the argument I am developing in this chapter, is the very low limit (around four or five) of the number of different kinds of stages that might occur, even in the most complex life cycles. In my opinion, it is not by chance that this upper limit is of the same order of magnitude as the upper limit of differentiated parts in any given series or body dimension, be it the number of tagmata or the number of kinds of fingers in a tetrapod limb.

Number of kinds, of course, is not the same as number of individual parts. There is no clear upper limit, for example, of the number of body segments. Among the polychaetes, the Phyllodocidae may have up to 600 segments, the Oenonidae up to more than 1000, and the Eunicidae up to about 1500 (Edmonds et al. 2000). Among the oligochaetes, more than 600 segments are present in *Rhinodrilus* (Glossoscolecidae; Hartmann-Schröder 1982). The arthropods with the highest number of segments are some centipedes (*Gonibregmatus plurimipes* with up to 191 pairs of legs, *Himantarium gabrielis* with up to 189 pairs of legs) and millipedes (*Illacme plenipes* with up to 375 pairs of legs (Hoffman 1982, Minelli, Pasqual, and Etonti 1984, Enghoff 1990, Minelli and Golovatch 2001). Large numbers of vertebrae are present in some sharks (up to ca. 400; Fiedler 1991), bony fishes (at least ca.750 in the eel relative *Nemichthys*; Nelson 1984), amphibians (more than 200 in some caecilians; Noble 1931), and snakes (more than 300 in some typhlopids; Gans, Laurent, and Pandit 1965). In all these cases, however, the tagmosis of these longest of animals is never more complex than in their relatives with fewer segments. Indeed, if a trend in complexity is present at all, it runs the other way. Thus, an increase in the number of body segments is often accompanied by a *reduction* in the complexity of the patterning along the main body axis, something that snakes, eels, centipedes, and millipedes exemplify at will.

Williston's Rule

In the literature on macroevolution (e.g., Saunders and Ho 1984), 'Williston's rule' is sometimes cited, according to which there should be a general trend from polymerous and homonomous to oligomerous and heteronomous organisation (e.g., towards a reduction in the number of serial parts, such as segments or teeth and a corresponding increase in their specialisation). This 'rule', however, is far from universal. Opposite trends are found, for example, in many fish groups (Lindsey 1988) and in myriapods (Berto, Fusco, and Minelli 1997, Fusco & Minelli 2000a). There is also a curious historical point, i.e., that the attribution of this principle to Williston is incorrect. Williston (1914) observed that no 'new' bone ever appeared in the skulls of reptiles, birds, and mammals, whereas, at the same time, many bones of the skulls of the so-called lower vertebrates have apparently got lost in evolution. But in another work he wrote: "We may hardly venture to guess as to the primitive number of vertebrae in reptiles. We are quite sure that there has been an increase in number in some, a decrease in others" (Williston 1925:94). A trend towards the reduction in number and the increrase in specialisation of serial parts had been formulated long before Williston by naturalists-philosophers like Treviranus (1820–22), Meckel (1821), von Baer (1828), and Bronn (1858).

Developmental Time and Body Axes

Correspondence between the temporal sequence of morphogenetic events and the growth and patterning of the main body axis is easily observed. In many, if not most, of the metazoans, differentiation proceeds in antero-posterior order. This progression begins in the embryonic segment of development, but it may extend through postembryonic development to the attainment of the adult condition. The best example of antero-posterior progression of growth and differentiation during postembryonic life is possibly the anamorphic arthropods, in which new segments differentiate, moult after moult, at the posterior end of the trunk. Many crustaceans, for example, begin their postembryonic life as nauplius larvae, with only a few anterior segments fully differentiated and just three pairs of appendages. The remaining segments and their appendages will progressively appear with later moults. Behind a fully differentiated head, most newly hatched millipedes have a short trunk, with less than the final number of segments, and just three (rarely four or more) pairs of legs. The remaining segments and leg pairs will appear progressively at later stages.

Developmental time is also mirrored by the proximo-distal axis of the appendages. Here, differentiation of the proximal structures often precedes differentiation of more distal ones; but no simple rule applies. In arthropods, the joint between the femur and tibia appears before the more proximal joint between the trochanter and femur; the latter joint, in turn, precedes differentiation of more distal elements: the tibia and the tarsus (Norbeck and Denburg 1991).

There is no need to look for special mechanisms as an explanation of the correspondence between the temporal and spatial dimension of body patterning and differentiation. Animals have developed ways of controlling the timing of cell activities (mitosis, cell migration, etc.), and the orderly progression of differentiation along the main body axis, or the axis of the appendages, is simply a 'mechanical' by-product of the timing of cellular processes. Nevertheless, correspondence between the complexity of developmental schedules and the patterning of the main body axis is often far from trivial.

Recent developments in the study of complex systems have revealed that all definitions of complexity are bound to be partial and subjective. This limit notwithstanding, complexity can be often defined and – what matters more – measured in a way suitable for attacking a defined set of problems. This has been shown, for example, by Daniel McShea in a series of important papers on the structural (morphological) complexity of multicellular animals (e.g., McShea 1991, 1992, 1996a, 1996b, 2000, 2001). The epistemological background to these questions about complexity has been discussed by Ahl and Allen (1996), moving from the observation that complexity is a function of the relationships between the units and levels we identify in a system; but these units and levels depend on the observer's criteria and do not exist independent of them. The concept of level is relative to the particular point of view taken by the observer. Complexity is not a feature of the external world, but strictly depends on the way we ask questions about the world.

Studying the origin and evolution of animal form confronts us with different kinds of complexity. On the one hand, we are interested in comparing the complexity of the body organisation of animals belonging to different species, or the complexity of different developmental stages of the same animal. On the other hand, we would like to measure the complexity of the developmental process itself.

Let's start with morphological complexity. Comparisons of closely related species seem to be amenable to simple metrics, such as those

proposed by McShea (1993) and Fusco and Minelli (2000a) to measure the morphological complexity of serial structures (e.g., the vertebral column of mammals or the segments of centipedes). Difficulties, however, grow enormously when we look for criteria for comparing the architectural complexity of more distantly related species. A rough criterion that applies, in principle, to all living beings is the number of cell types (Bonner 1988), but this criterion suffers from several shortcomings – the most obvious being the subjectivity in classifying an organism's cells into distinct types worthy of a name. For example, there are just 302 neurons in the nervous system of *Caenorhabditis elegans*, but these few neurons can be classified into not less than 118 structural classes (Thomas 1994). Furthermore, the diversity of specialised cell types increases with the animal's size, independent of phylogeny (Bell and Mooers 1997). Whatever metrics we might devise to describe an animal's morphological complexity, it will hardly reveal differences in complexity between closely related animals only different in total size, irrespective of size-dependent differences in cell-type diversity. Therefore, we cannot expect to learn too much from this criterion for estimating complexity. It seems more meaningful, even if limiting, to restrict our comparisons within the range of a class or an order, as I will do in the next few paragraphs. Criteria for measuring morphological complexity will remain subjective. I will take a very simple, semiqualitative approach and follow some largely intuitive principles (Minelli 1996a): (1) complexity is not dependent on the number of parts (strictly homonomous sequences of segments have the same complexity, irrespective of differences in the number of units); (2) there is an increase in complexity when changing from unsegmented to segmented organisation; (3) there is little difference in complexity between a strictly homonomous pattern and a gradient-like pattern, such as in those insect antennae in which the size of the articles regularly decreases from the base to the tip; and (4) there is, instead, a high increase in complexity when a strong structural boundary occurs, such as between head and thorax, or thorax and abdomen.

These rules may be used to obtain a comparative estimate of complexity along an animal's main body axis, as well as along the proximo-distal axis of an appendage. Interestingly, there is no difficulty in applying an identical set of rules to the temporal rather than to the spatial dimension, thus obtaining a comparative estimate of the complexity of a developmental schedule. Subjectivity of periodisation notwithstanding, there seems to be no problem in saying that cnidarians with both polyp and medusa have a more complex life cycle than those with polyp only or medusa only;

that the postembryonic development of holometabolous insects – such as flies, butterflies, beetles, and bees – is more complex than the postembryonic development of heterometabolous insects, such as grasshoppers or cockroaches. This is little more than a truism. But we will see soon that this approach may reveal unexpected correspondence among the relative degrees of complexity found along different dimensions.

To begin with, in many arthropods, the complexity of postembryonic development and the morphological complexity of the main body axis of the adult appear to be coupled.

The Cambrian crustacean *Rehbachiella kinnekullensis* developed through a very high number of moults, perhaps more than 30, but the morphological changes following each moult were minimal. The high number of moults is distinctly mirrored by the high number of body segments, whereas the smooth changes in body organisation along the whole postembryonic development had its equivalent in the animal's faint tagmosis (Waloßek 1993).

In crustaceans, we can identify an ideal (but not strictly phylogenetic) series of taxa with an increasingly smaller number of moults, proceeding from *Rehbachiella* to Anostraca (e.g., *Artemia*) to Conchostraca to Ostracoda. At the same time, the amount of change (posterior addition of new segments, differentiation of appendages) brought about by each moulting event increases in the same order. That is, the moulting sequence changes from merely quantitative control of growth to qualitative control of body patterns. This is expressed, along the main body axis, by a progressive reduction in the number of body segments and appendages, together with increasing morphological and functional specialisations. Were such a sequence actually supported by phylogeny (what is not at stake in this context, however), we would have an example of evolution according to Williston's law.

In millipedes (Diplopoda), postembryonic development follows a more or less complex and tightly fixed schedule. Enghoff, Dohle, and Blower (1993) distinguish three developmental modi. The first is euanamorphosis. Here the number of postembryonic moults is not strictly fixed; there is also some individual variation in the number of segments added after a given moult, and the number of body segments continues to increase from moult to moult, even after the animal has reached sexual maturity. The number of segments in the adult is also variable within a species. More canalised is teloanamorphosis, in which there is a fixed number of postembryonic moults, with a regular increase in the number of trunk

segments after each moult. The last moult coincides with the attainment of sexual maturity and a fixed number of segments. In the third kind of anamorphosis, called *hemianamorphosis*, postembryonic development is divided into two parts: a strictly programmed series of early larval stages, through which, as in teloanamorphosis, a fixed number of segments is achieved, followed by some more postlarval stages which do not acquire additional segments or appendages, but just increase in size and finally reach sexual maturity. These differences in the complexity of postembryonic development correspond to the different degrees of complexity in adult morphology: millipedes with euanamorphosis have a worm-like trunk, with the lowest level of regional differentiation, whereas those with hemianamorphosis have the most advanced regionalisation. This does not imply, however, that hemianamorphic millipedes are phylogenetically more advanced than those with euanamorphosis. Cladistic analyses show that the most elongate, worm-like forms of millipedes, with a trunk made of many similar segments, are more derived than those with shorter and more extensively regionalised bodies. The same trend is observed in centipedes (Enghoff 1990, Berto, Fusco, and Minelli 1997, Foddai and Minelli 2000, Regier and Shultz 2001). In this case, Williston's law is clearly not supported.

In Hymenoptera, the most primitive forms (Symphyta) have many morphologically similar larval stages, whereas adults possess a more or less primitive multisegmented abdomen. In the more advanced Apocrita, the complexity of postembryonic development is higher, and the number of postembryonic stages is reduced. In some Parasitica, the younger and older larva of the same animal are completely different. At the same time, there are major changes in the abdomen. The dorsal plate (tergum) of the first abdominal segment becomes incorporated into the thorax, the following segment(s) may form one or two knot(s), as in the ants, and some segments of the abdomen may more or less extensively fuse together, as the tergal plates of some abdominal segments do in Braconidae (Figure 5.5).

A trend towards the reduction of the number of moults is also seen in Diptera. There are six moults in Simuliidae, four in the remaining Nematocera, five to eight in Orthorrhapha, but only three in Cyclorrhapha. This is broadly matched by a corresponding reduction of the number of free abdominal segments in the adult.

Among the arachnids, Acari and Ricinulei are those with a more complex developmental schedule (in particular, these are the only arachnid groups in which the newly hatched animal – currently termed a larva – has less than

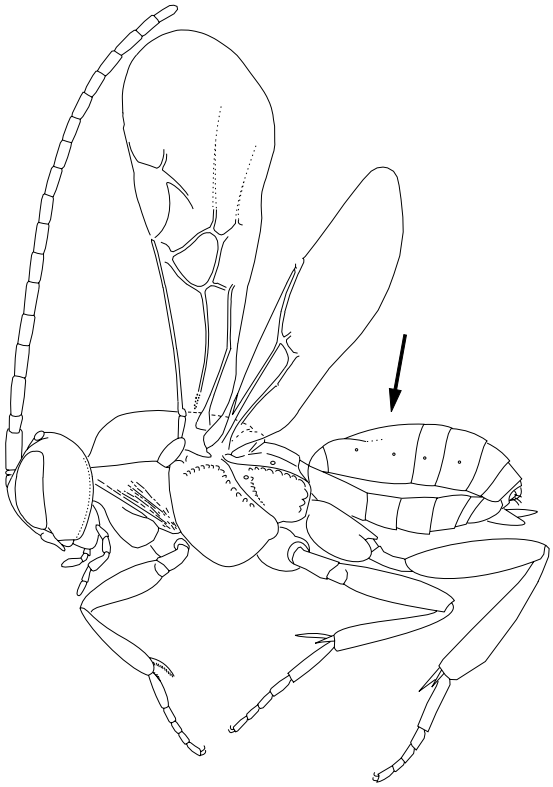

Figure 5.5. Mismatch between dorsal and ventral segmentation in an adeline braconid wasp. (Redrawn after Goulet & Huber 1993.)

the final number of legs; i.e., three pairs rather than four). At the same time, these are also the arachnids with the most complex tagmatisation. This is reflected in anatomical nomenclature: instead of the usual terms (prosoma and opisthosoma) used for the body regions of most of the remaining arachnids (e.g., the spiders), no less than six different terms (gnathosoma, hysterosoma, idiosoma, metapodosoma, propodosoma, and podosoma) are used to identify the different body regions recognisable in the mites.

The Time Axis of Development and the Patterning of the Proximo-Distal Axis of the Appendages

There are several examples of agreement between the complexity of postembryonic development and the morphological complexity of the axis of adult appendages (Minelli 1996a).

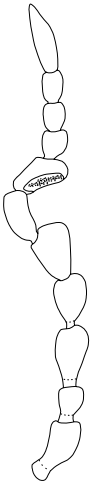

Figure 5.6. Antenna of a male *Meloe proscarabaeus*. (Redrawn after Bologna 1991.)

In a previous section, the hypermetabolous blister beetles, with their different kinds of larvae developing in regular succession along the life cycle, were described. This developmental complexity is exceptional among the insects, the beetles in particular. Some blister beetles also have some of the most complex antennae of all Coleoptera (Figure 5.6). A superficial observer will be struck by the lamellate antennae of the Scarabaeoidea or by the comb-like antennae of other beetles (e.g., some click beetles or Elateridae) more than by the basically moniliform antennae of *Meloe*. Upon closer inspection, however, the antennae of several male *Meloe* reveal a nearly unique trait: modification of a few mid-length articles which interrupt the otherwise uniform series of antennomeres. This is very unusual for arthropod antennae. These are not necessarily made of identical articles, but departures from a basic uniformity along the proximo-distal axis of the appendage are nearly universally limited to the most proximal and/or the most distal articles. The latter ones, for example, may form a more or less distinct club, as in many beetles and ants. The peculiarity (indeed, the complexity) of the antennae of some male *Meloe* is due to the presence of a 'local anomaly' occurring at mid-length, whereas the articles preceding this 'anomaly' and those following it present the basic default shape. How and when is the 'mid-antennal anomaly' of *Meloe* determined? I think that it is determined very early, probably before the antennal anlage is completely segmented, whereas the usual patterning of the most proximal or distal articles of other insect antennae is perhaps specified later,

through molecular signalling from either end of the appendage. We know very little about the way a beetle antenna is segmented. For now, we can only record the coexistence, in *Meloe*, of an unusually complex life history (hypermetamorphosis) with an unusual degree of morphological complexity in the antenna.

Some arachnids show a corresponding agreement between the complexity of the postembryonic development and the morphological complexity of the appendages. Upon hatching, most arachnids already have their definitive organisation, with a full complement of body segments and appendages. There are two exceptions, however: the Ricinulei and the mites (Acari). In both groups, as previously described, the first postembryonic stage is a six-legged larva, morphologically distinct (in some mites, to a dramatic degree) from the following stages. In the Acari, up to three nymphal stages are usually interposed between the larva and the adult. Interestingly enough, segmentation and patterning of the legs in Ricinulei are more complicated than in the other arachnids. There is no parallel in the mites, but these (most species are less than 1 mm long) are probably too small for a more complex patterning of the leg segments; however, mite legs are frequently provided with apical structures of unusual complexity.

Topology

Morphology and Developmental Topology

"Whatever sense may exist to the phrase 'neighboring developmental program,' distant regions on the fate map can have neighboring programs" (Kauffman 1993: 511). A developmental analysis of form often reveals an intrinsic geometry which is very different from that of the resulting animal form. Topographically close body parts can be topologically far away, and vice versa, in the network of developmental interactions that link them together. For example, why not speak of ontogenetic proximity of body structures found at opposite body ends when reading that, in *Drosophila*, the gene *head involution defective* is required for embryonic head involution, as well as for the rotation of male terminalia in the pupal stage (Abbott and Lengyel 1991)? The same is true for *Drosophila* lacking both maternal and zygotic *hunchback* functions (Lehmann and Nüsslein-Volhard 1987) or *headless* mutant embryos of the parasitic wasp *Nasonia*, with anterior and posterior gap defects (Pultz, Pitt, and Alto 1999).

Metaplastic transformations, or conversions of a cell or group of cells from an expected developmental fate to another, suggest the proximity of the specific developmental programs of the two cell types (Kauffman 1993).

Slack (1985) summarized 21 types of homeotic transformations (epithelial heterotopia and metaplasia) in humans, concentrated in the gastrointestinal, urinary and female reproductive systems. Most, but not all, of these transformations are between tissues whose precursors are neighbouring regions of a common cell sheet during early embryogenesis, which are therefore likely to have neighbouring patterns of gene expression or developmental fates. Three rules were identified by Kauffman (1993) in terms of the possible metaplastic transitions from one cell type to another: (1) *limitation* – no cell type can reach all cell types; (2) *connectedness* – there is a large, strongly connected set of cell types which can mutually reach one another; and (3) *asymmetry* – some cell types can join this strongly connected set from outside, but can not be joined from inside.

In the tiny parasitic wasp *Nasonia vitripennis*, the *minus stripes* mutation causes small gaps in two thoracic segments (T1 and T2, pro- and mesothorax), as well as in the sixth and seventh abdominal segment (A6 and A7), but not in those in between (Pultz et al. 2000). Thus, in a sense, T2 is closer to A6 than it is to T3. In this question of topology, a factorial approach (see chapter 10 for a corresponding approach to homology) is the best way to analyze the facts. We can recognise topological proximity between T2 and A6 from the point of view of the *minus stripes* gene expression, but other genes would certainly suggest otherwise. There is no *one* functional topology of body parts; there are, instead, many topologies cross-linked by the functional relationships of genetic networking.

Maps of the phenotypic effects of a pleiotropic gene are possibly the best way to demonstrate the intricacies of the topology of body morphogenesis. More accessible, but also more limited, evidence is obtained from the very often discontinuous expression patterns of some genes (e.g., the pair-rule genes). Another way to 'materialise' the internal topology of developmental events is the mapping of mitotic domains. These are developmental units found at early developmental stages (before gastrulation), characterised by the synchronisation of mitotic activity of a cluster of cells embedded in a context of cells which start dividing at an earlier or later time. In the blue-bottle *Calliphora*, in which mitotic domains have been accurately studied, there are several simple domains formed by one area each or by two bilaterally symmetrical areas of synchronously dividing cells. But a few domains, such as those labelled as $\delta_{14}16$, $\delta_{14}17$, $\delta_{14}21$ and $\delta_{14}25$, comprise many serially repeated cell clusters. Besides dividing at the same time, all the cells in these domains occupy homologous positions in the presumptive segments of the embryo (Foe and Odell 1989).

Developmental proximity is often a matter of communication. The vertebrate hindbrain (rhombencephalon) is divided in a series of rhombomeres whose persistence as independent units depends on the recognition properties of their constituent cells. If reciprocally put in contact, cells from even-numbered rhombomeres would easily mix together, as would cells from different, odd-numbered rhombomeres. But cells from odd- and even-numbered rhombomeres sort out spontaneously (Guthrie and Lumsden 1991, Guthrie, Prince, and Lumsden 1993). This is enough to keep the cell populations of the different compartments separate. But this is also an example of the contrast between spatial and functional proximities. Similarly, sorting-out experiments with cells from dissociated imaginal discs of *Drosophila* demonstrated that differences between the different legs (which belong to the three thoracic segments) were not as great as the differences between wing cells and leg cells from the same segment (mesothorax). Interestingly, whereas the cells destined to form the three pairs of legs were capable of sorting out regardless, the wing cells were not able to sort out from those destined to form the wing-homologous structure, the haltere, despite the much more obvious morphological differences existing between a wing and a haltere, with respect to the differences between the fore-, mid- and hind legs (Fehon, Gauger, and Schubiger 1987).

Among serially repeated body parts, the topological closest relatives are sometimes arranged in a selectively discontinuous series rather than in the obvious series of contiguous segments which defines a tagma such as the insect thorax or abdomen. One example is the ten pairs of tracheal placodes which form in those (para)segments in which the expression of the *spalt* gene does not prevent their differentiation (Kühnlein and Schuh 1996).

Ghysen et al. (1993) have shown that specification of cell fates within a given tissue is not the result of a progressive subdivision of the tissue into increasingly smaller developmental units down to the level of individual cells. For example, the formation of sense organs in *Drosophila* is not the result of a subdivision of the organ-forming material into compartments within compartments within compartments, but is accomplished by instructing cells at particular discontinuous locations within the tissue to adopt a particular fate.

Thinking in terms of topology of developmental processes may help establish a conceptual frame for discussing the possible independence of different sets of characters – not only developmental, but also morphological. This issue is of primary concern to systematists, in their efforts to fill in and analyze data matrices in which mutual independence of the

characters recorded and coded represents one of the single best qualities of the work.

Topology of Coaptations

A peculiar problem of topology is offered by those body parts which must mutually fit together to perform their function (coaptations). These parts may belong to the same animal or to two sexual partners. The vertebrate heart originates as two separate halves, left and right, at either side of the embryo, which are destined to fuse together eventually following migration towards the midline. Another example is the dorsal closure of the embryo in arthropods such as *Drosophila*, which also requires a correct fitting together of the two halves. Coaptations are also common, and more easy to observe, in postembryonic stages. Conspicuous examples are those of animals able to roll up into a sphere, such as the pill millipedes (Figure 5.7) and many woodlice and armadillos, or those of the bivalve shells, in which the two valves are generally provided with the precisely interlocking teeth and dental sockets that help them fit together at closure. Many insects can accommodate some of their appendages, when at rest, within perfectly shaped ventral or latero-ventral grooves, such as those accommodating the rostrum of many true bugs, or those used by weevils for protecting the antennae.

The coaptations most extensively studied to date are those that involve sexual appendages and sexual openings (e.g., Eberhard 1985). Attention, however, has been focussed primarily on the complementary structure of male and female parts, according to the traditional (and a bit naively adaptationist) key-and-lock model. Much less attention has been aimed at the positions these parts occupy in the body architecture of each partner. Many pairs of sexual structures are located at equivalent positions in both the male and female. This seems to be easily explained with parallel determination of this position through equivalent expression of genes, such as *Abdominal-B* (see page 181). There are many exceptions, however. Spider males and dragonfly males have secondary penises (cf. page 177) whose position does not correspond in any obvious sense to the position of the female (and male!) genital opening in the same animals. Close interactions between mating dragonflies are not limited to those between the secondary penis of the male and the genital opening of the female, but extend to the male's caudal appendages which interlock with the female's prothorax during the whole nuptial flight. Thus, the question is whether there is any correlation between the relative positions of these interlocking

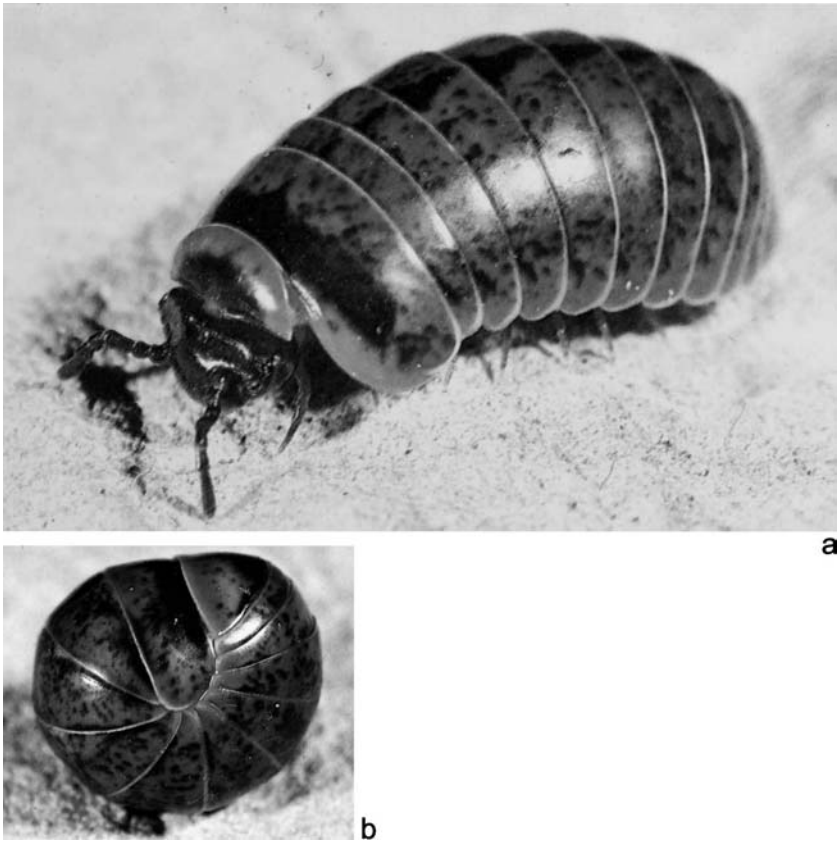

Figure 5.7. A pill millipede (*Glomeris*), uncoiled (a) and coiled (b). (Courtesy of P. Rigoni, Asiago.)

parts. To the best of my knowledge, no study has been devoted to date to this question, but it might be worth consideration in future work as a possible correlate of some conserved feature of the *Hox* gene expression patterns.

The questions we may ask with respect to body parts involved in coaptations could also apply to aspects of synorganisation, or joint (integrated) organisation of parts (Endress 1990), such as observed in the complex floating colonies of the Siphonophora, in which the parts that the comparative morphologist describes as specialised individuals (polyps and medusae) look more like organs of conventional animals than typical biological individuals. Similarly, the many florets associated to form an inflorescence, such as a daisy or a sunflower, look like parts of a typical flower rather than

whole flowers. The rules according to which these synorganised parts fit together are still to be approached from an evo-devo perspective.

Topological Breakdown

The body architecture of most animals is based on the simple topology of linearly arranged and/or branching elements, however extensive their external or internal pattern. Sometimes this simple topology is disrupted during development.

An example is provided by dragonflies, in which the usual antero-posterior sequence of dorsal plates (terga) is interrupted by the lateral plates of the second thoracic segment (the left and right mesopleura), which join together dorsally, pushing the tergum of the mesothorax back, apart from the corresponding dorsal plate of the prothorax (see page 198 and Figure 9.3).

Casanova (1991, 1993) suggested a similar or, rather, inverse interpretation for the carapace of malacostracan crustaceans. The terga of the gnathal segments (i.e., those of the posterior half of the head, bearing the mandibles and two pairs of maxillae) would be longitudinally split into halves that are pushed to occupy a medio-lateral position, whereas the dorsal surface of the carapace would be derived from the antennal segments anteriorly and from the terga of the first segments of the pereion posteriorly.

Another curious disruption of the basic topology of body parts is provided by the anastomosing tracheae of the geophilomorphs (Füller 1960, Minelli 1985). The leg-bearing segments of these centipedes have one pair of spiracles each. Two dorsal tracheae originating from the close proximity of each spiracle anastomose with the corresponding tracheae of the other body side, thus producing a network that may help the animal remain provided with oxygen over its whole length even if some segments – due to compression when burrowing in the soil – may be impaired in their ventilatory function. The topological consequences of these tracheal anastomoses emerge when the animal moults. The tracheae, as all ectodermal derivatives, are covered by a cuticle, which can be cast off only following breakage at the points in which the right and left branches are anastomosed. In these places, concentric fragments of cuticle similar to the annual rings of a tree accumulate, moult after moult, thus leaving a permanent record of the number of moults the animal has undergone.

Some important developmental processes require real topological breakdown. This happens not so much in the cavitation of a previously

solid mass of cells (e.g., in the formation of a typical blastula), as with the transformation of the animal into a veritable tube, when the digestive tract obtains a second opening in addition to the mouth.

The most obvious way to obtain a topological breakdown is by programmed cell death. The morphogenetic consequences of this mechanism are less disruptive. Think of its role in separating the digits of a vertebrate hand or feet (a process recently investigated at the molecular level by Gañan et al. 1998) or in destroying a tadpole's tail. Programmed cell death is also involved in very subtle refinements of form, as in the hindbrain of mammals, in which segmental boundaries between rhombomeres are established in part by selective removal of misplaced cells (Rusconi, Hays, and Cagan 2000) and during segmentation of the *Drosophila* embryo (Pazdera et al. 1998).

CHAPTER SIX

Differentiation and Patterning

> Arguably the cell is the most fundamental biological unit. The cell is also the unit of ontological primacy in molecular biology.
>
> G.P. Wagner and M.D. Laublicher 2001: 144–5

> The complexity of development is really about the complexity of the cell. Cells are much more complicated than embryos – what's going on in a cell is much more complicated than what's going on between cells in an embryo. And morphogenesis, which is about changes in form, is largely about cell mechanics, and the types of forces that cells generate are very few: tensions, occasionally extensions, and changes of neighbors.
>
> L. Wolpert, in L. Wolpert, A. Ghysen and A. Garcia-Bellido 1998: 515

> Although morphogenesis appears to be quite deterministic on a macroscopic scale, on a microscopic scale cellular activities during the formation of the limb appear to be nearly random. Order emerges only as an average outcome, a biasing of many individual motions, each of which has a large stochastic component. Therefore, we can only speak of the probability of a certain developmental process occurring in a given setting.
>
> G.F. Oster et al. 1988: 862–3

Cells as Units of Differentiation

Differentiation is how the different cell types in a developing animal are established. I will not discuss whether there is a finite and possibly small number of discrete cell types, as virtually everyone seems to tacitly assume. This is not strictly so for Kauffman (1993), who maintains that cell types are discretely different only because he restricts his definition ("*A cell type corresponds to a state cycle attractor* in the dynamical behavior of

the genomic system"; italics as in the original) to "only those patterns [of gene expression] which are recurrent asymptotic behaviors of the genome" (page 467).

In Rosine Chandebois's (1977) metaphor, pattern formation and development in general, is the product of the rules of cell sociology. One might refrain from using such an anthropomorphic language in respect to cells; nevertheless, Chandebois's image captures a basic concept that forcefully emerges from our current awareness of developing systems. That is, that the cell is the basic unit of development, and that understanding cell properties and functions is the main key to understanding the emergence of multicellular systems (Bonner 2000).

Akam (in the discussion of Wray 1999) observed that to jump straight from a gene network to a morphological structure, as is common practice today, amounts to missing a critical step (i.e., analysis at the level of the cell). I would push the argument further by saying that we should start with cells, as the main actors in the developmental play. Genes and their transcription and translation products are the words and sentences in the dialogues among the cells on the developmental scene. Words and sentences are continually updated along the unending series or performances offered by life, one generation after another.

Many questions arise. Is there a list of basic cellular properties that exhaustively account for the emerging properties of multicellular organisms? If so, to what extent are these properties cell-autonomous rather than interactive?

I will review a few basic properties and functions of cells which are conspicuously involved in the generation of animal form. We will see that these properties and functions are general properties of cells, but, at the same time, we will see that their details have been fine-tuned in individual systems. Fine-tuned, but still at the level of cell properties and behaviours (i.e., cell cycle) or cell–cell communication. Look at the incredible subtlety of detail in the single joints of a fly's leg. How is all this detail specified and controlled? "There's not such a level of control, it is a fallacy. There is nobody controlling the things in this detail. The actual shape of a bone, or of a fly leg, is the result of a series of genes doing vulgar things. As Lewis [Wolpert] said, it's a combination of genes working in many places in the fly, and mutations in any element of the combination will perturb the whole process." (García-Bellido 1998: 515, in Wolpert, Ghysen, and García-Bellido 1998).

How can vulgar genes or vulgar processes account for the emergence of exquisite patterns?

Cell Cycle Length

Let's start with cell cycle length. At first sight, this might appear as a trivially technical affair. To the extent in which cell cycle length can be adjusted, we might expect this length to be shorter in early embryogenesis than later in development, but these differences can only be expected to affect cell number and cell size, without any obvious morphogenetic effect. But this is not so. The length of the cell cycle, as such, can be critical for morphogenesis.

Without a minimum number of mitoses, morphogenesis could not proceed. A proliferative phase, beginning with the egg's first cleavage division, does precede most important morphogenetic events. The two things – cell proliferation and differentiation – are, to a large extent, mutually exclusive. In many, if not most, embryos, the first divisions occur at such a pace as to not allow differentiation to occur at the same time. This is simply due to competition between DNA replication and transcription, but also perhaps to the total reorganisation of the cytoskeleton that occurs during mitosis, probably precluding a mitotic cell from contributing to morphogenetic mechanics (Foe 1989).

In the earliest phases of embryonic development, during cleavage in particular, cell cycles are unusually short. Ten minutes is the average length of a cleavage nuclear division in *Drosophila*, what is obviously a very short interval for a eukaryotic cell. Some animals can go even quicker: In some appendicularians, tiny marine planktonic invertebrates closely related to the ascidians, the length of a cell cycle during cleavage is just four to five minutes (at 22°C; Fenaux 1976). Very short mitotic cycles do not give the cell the time to be engaged in transcription, but even somewhat longer cell cycles may be quite selective in this respect. The time required for a gene to be transcribed is proportional to its length; and for some genes containing very long introns, transcription may be simply impossible unless the mitotic cycle reaches a suitable length. Some examples have been summarized by O'Farrell (1992): working at a rate of about 3 kilobases per minute (at 37°C), the mammalian RNA polymerase requires 11 hours to copy the 2,000 kilobases of the dystrophin gene, whereas the *Drosophila* polymerase, progressing at about 1.4 kilobases per minute, may require 55 minutes (at 25°C) for transcription of the *Ultrabithorax* gene. Timely transcription, therefore, requires coordination of cell cycle length and gene size (Rothe et al. 1992). This, in turn, can obviously have far-reaching consequences on differentiation and patterning. In fact, it has been shown (Ohsugi, Gardiner, and Bryant 1997) that growth factors may affect pattern

formation by acting on the cell cycle. The effects of the length of the cell cycle on pattern formation in developing chick limb buds were studied by locally slowing the process. When the cell cycle of anterior cells was lengthened (e.g., by reversibly inhibiting DNA replication), digit duplication was observed, following the local expression of genes characteristic of the posterior polarising region.

Cell Types

Classification of cell types is possibly one of the most arbitrary taxonomic exercises in biology, nevertheless it may be safe to say that there are many stable states that cells can enter and in which they can proliferate (Gurdon 1992). Once a cell has differentiated, it and its daughters never normally change to another cell type; that is, transdifferentiation is rare. A muscle cell does not change into a nerve or a gut cell, but the nucleus of a muscle cell, if transplanted into the cytoplasm of an egg, will generate all major cell types of a *Xenopus* embryo (Gurdon 1986, 1999).

Cell Autonomy, Induction, and Repatterning

There is little reason to maintain the old distinction between cell-autonomous and inductive development. Even at the level of gene action, a distinction between inductive and cell-autonomous events is often unwarranted, the same for the functions of Notch in the development of embryonic muscle and epidermis in *Drosophila* (Baker and Schubiger 1996).

Nematodes have been described as typical metazoans with strictly lineage-dependent development, but a few decades of close investigation on the development of *Caenorhabditis elegans* have demonstrated how poorly grounded was that belief. Schnabel (1996) identifies at least ten inductive events during the blastula stage of this nematode, more numerous than the inductive events observed before gastrulation in amphibians (i.e., in animals whose development has always been described as heavily dependent on inductions). According to Schnabel, the only difference between determinate and indeterminate embryogenesis is perhaps, in the end, the number of cells involved in determination events. The difference between the stereotyped cell lineage in the nematodes (or, at least, in many species of this group; see page 11) and the much less precise cell lineage in other animals probably does depend on the very limited degree of topological freedom due to the fact that regions of the early embryo are not represented by groups of cells, but by single blastomeres.

Stereotyped cell lineage is a common feature of developmental systems. Often it provides only raw material that is later patterned independently of its genealogical origin. One example is provided by leeches, in which it is impossible to draw segment boundaries that include all the progeny of just one set of primary blast cells (Weisblat, Price, and Wedeen 1988). Another example is peracarid crustacean embryos, in which the intersegmental furrows do not correspond to genealogical limits, and the anlagen of the appendages are composed of parts of different clones (Dohle and Scholtz 1988, 1997).

Further examples of ontogenetic repatterning are provided by Rieppel's (1992, 1993a, 1993b) studies on ossification patterns in fossil and extant reptiles. Here, the pattern of chondrogenetic condensations is sometimes modified at the later stage of ossification, with the secondary fusion of some originally separate primary elements or the lack of ossification in others. More complex are the co-ordinate ontogenetic repatternings in the feeding system of plethodontid salamanders illustrated by Wake in a series of papers (reviewed in Wake and Hanken 1996). The phenomenon affects the reduction or remodelling of gill archs and associated epibranchial cartilages, as well as the reorganisation of the visual system and the motor nuclei of the brainstem.

Cell Contacts and Cell Communication

Cell contacts can orient cell division axes (Goldstein 2000), influence the duration of the cell cycle or even determine its arrest (Serras, Dictus and Van den Biggelaar 1990).

In early embryonic stages, one can often identify communication compartments, as in the ectoderm of *Patella* and *Lymnaea* (Serras et al. 1989, Serras, Notenboom, and Van den Biggelaar 1990). In *Caenorhabditis elegans*, the original pair of germ line cells is completely uncoupled from the soma since the beginning of morphogenesis; further subdivision of the soma into communication compartments is delayed until the later stages (Bossinger and Schierenberg 1992). Morphogenetic specificity of these early cell–cell contacts is enhanced by the fact that topology, temporal sequence and way of functioning of these communication compartments are little conserved phylogenetically, as shown by the differences existing between two nematode genera (*Caenorhabditis* and *Cephalolobus*) studied by Bossinger and Schierenberg (1996). The mechanisms through which cell–cell interactions may influence development are diverse, but the signalling molecules involved in the specification of positional

information across cell membranes are basically the same across the metazoans (Livingston and Wilt 1993).

Asymmetric Cell Divisions

One of the major processes leading to differentiation is asymmetric cell division (for a review, see Horvitz and Herskowitz 1992).

In principle, two distinct types of mechanisms may generate asymmetric cell division (Posakony 1994). In the first type, the mother cell is intrinsically polarised with respect to the plane of division (e.g., because of segregation of a particular molecule to one side of the mother cell) so that it would be distributed asymmetrically to the daughters. Consequently, the two daughter cells are different since their birth. Remember Rax2, the molecular marker of cell polarisation recently described in yeast cells (Chen et al. 2000; see page 28). In the second type of asymmetric division, the dividing cell is asymmetrically influenced by some external cue, either from the substrate or from interactions with other cells. For example, intercellular contacts generate cortical differences leading to asymmetric cell division in the embryo of the freshwater annelid *Tubifex* (Takahashi and Shimizu 1997). In *Caenorhabditis elegans*, the asymmetry of cell division during embryogenesis is controlled by the product of the *lit-1* gene which, for up to six consecutive divisions, causes one of the two cells produced at each cleavage to assume a posterior fate (Kaletta, Schnabel, and Schnabel 1997).

Positional Homology and the Hot Spots of Differentiation

Positional Information or Informational Position?

One of the most important concepts that has dominated developmental biology over the last three decades is Lewis Wolpert's concept of positional information, first proposed in 1969 (see also Wolpert 1989). According to this model, each cell is defined by its positional value, a parameter related to its position in the developing system. Cells interpret their positional value by differentiating into a particular cell type or by exhibiting changes in their state, growth, or migratory behaviour. One could argue that Wolpert's positional information would be better described as informational position (Minelli 1975, García-Bellido in Wolpert, Ghysen, and García-Bellido 1998). Cells do not need to know their position as such, but their different positions provide them with different cues that the cells will translate into different behaviours or states.

The main question is whether cells are really mapping in such great detail their position within the developing organism. There is evidence that, in a dynamic context, such as provided by a morphogen gradient, cells make a direct and continuous assessment of their position (Gurdon, Mitchell, and Mahony 1995). Such an assessment of axial identity is also important in regeneration, because the blastema only gives rise to structures distal to its level of origin (Brockes 1997).

The organism's self-description is much less precise and thus more economical than a mapping of individual cell positions. There is little reason (Gubb 1998) why adult cells with the same developmental fate should have different positional information.

Zootype and the Patterning of the Nervous System

When introducing the concept of positional information, Wolpert (1969) hypothesised the possible existence of a 'universal positional field' shared by all developing animals. Years later, following the growing knowledge of expression patterns and developmental functions of the *Hox* genes, Akam (1989) and Awgulewitsch and Jacobs (1992) came close to formulating what Slack, Holland, and Graham (1993) later defined as the zootype, the basic antero-posterior patterning of the body common to all animals. It is defined by the phylogenetically conserved and ontogenetically early expression of a restricted set of genes. It is doubtful if and how the zootype concept could apply to cnidarians (but also to some bilaterians, such as ascidians and sea urchins; see Hodin 2000). A 'perennial embryo' such as *Hydra* may have the equivalent of the anterior and posterior regions of a bilaterian, but no central or trunk region. If so, the *Hox* genes may not have the same role in cnidarians as they have in bilaterians. Their roles may be mixed, as suggested by the joint expression of *Cnox-3*, which seems to play a role in anterior patterning, whereas *Cnox-2* antagonises it in preventing anterior patterning (Bode 2001). Some authors (Deutsch and Le Guyader 1998, Martínez et al. 1998) explicitly restrict the zootype concept to the bilaterians or even regard the antero-posterior patterning system based on *Hox* gene expression as a developmental synapomorphy of bilaterians (Davidson 2001).

The zootype concept stands despite changing views on the value and significance of a *Hox* code and the apparently counterintuitive results of increasingly extensive interphylum and intraphylum comparisons. If a general increase in the number of *Hox* genes accompanied the main events

in animal phylogeny (Kourakis and Martindale 2000, Ferrier and Holland 2001b), culminating in the putative quadruplication of the whole set at the very root of vertebrate radiation (Spring 1997), there is little doubt about a reduction in the number of *Hox* genes in the phyletic line of the nematodes or a loss of the original role in antero-posterior body patterning in what are now the genes *zen*, *bicoid*, and *fushi tarazu* of *Drosophila* and other dipterans. In addition, in arthropods with very little patterned trunks (such as centipedes), there is no shortage of *Hox* genes. All 10 *Hox* genes to be expected in an arthropod were found in *Lithobius* (Hughes and Kaufman 2002) and even in the worm-like geophilomorph *Pachymerium* (Bastianello et al. 2001).

The main question concerning the zootype concept is thus not so much whether this generalisation is worthwhile or not, but rather how this generalised and evolutionarily conserved antero-posterior body patterning first arose and how it evolved. Deutsch and Le Guyader (1998) introduced the interesting hypothesis of the neuronal zootype, according to which the major function of the zootype genes is to ensure a correct wiring of the neuronal network. Deutsch and Le Guyader postulate that the primordial function of the zootype genes is to specify the neuronal network in bilaterian animals. A colinearity of the neuraxis and the genome organisation of the *Hox* genes would establish a link between the molecular colinearity of the genomic organisation and expression of the *Hox* genes with the arrow of developmental time and further anatomical colinearity which exists between various parts of the central nervous system and the parts of the body they innervate. Some *Hox* genes such as the leech *Lox2* and *Lox4* genes and the amphioxus *Hox3* are only expressed in neuronal tissues. This would have been the primary expression and thus function of the *Hox* genes of the zootype which only in a second step would have been recruited to control other morphological features. That the original role of *Hox* genes was the patterning of the nervous system is also suggested by the generalised expression of these genes in the developing neural axis, whereas their expression and involvement in the specification of other features seemed to have evolved secondarily, in different phyla, in divergent ways (Wada, García-Fernàndez, and Holland 1999).

The central role of the nervous system in the origin and evolution of body patterning is shown by the daily discoveries that genes that have an important role in patterning other structures – not only ectodermal, but also often meso- or endodermal ones – also have expression in the nervous system.

After closer scrutiny, they appear to have been involved originally in the patterning of the nervous system, whereas all other roles they might have in different tissues are likely to be secondary. Among the many examples, there are the *Lox6* gene of the leech *Hirudo medicinalis*, which is an orthologue of the *Drosophila Deformed* gene (Kourakis et al. 1997, Wong and Macagno 1998) and the vertebrate *Otx*, originarily expressed in mesendoderm and the central nervous system (Williams and Holland 1998). van der Hoeven et al. (1996) believe that, in vertebrates, a few *Hox* genes were originally involved in the determination of the oral-aboral polarity in the digestive tract and were later recruited in the patterning of other axial structures, including the nervous system and the somitic mesoderm.

It has been said that about seventy 'essential' genes are required for proper development of the peripheral nervous system in the *Drosophila* embryo (Salzberg et al. 1994), and that much of a vertebrate's neural structure depends on the dynamic and differential expression of tens to hundreds of different adhesion molecules responsible for neuron-neuron and neuron-glia adhesion, neurite fasciculation and nerve guidance (Takeichi 1990, Edelmann and Crossin 1991, Crossin 1994). But these figures are likely to underestimate the complexity of the genetic control in nerve growth and patterning, because more than 50% of an animal's genes are expressed in its central nervous system (Tata 1993). The sense organs have their substantial share in this massive gene expression. Halder, Callaerts, and Gehring (1995) estimate that perhaps 2,500 genes are involved in building and maintaining the *Drosophila* eye. This would amount to ca. 18% of the fly's whole genome (Adams et al. 2000).

This behaviour is possibly typical of bilaterians, whereas in cnidarians such as *Hydra*, the nervous system does not seem to have a correspondingly important patterning role. Schaller, Rau, and Bode (1980) found that *Hydra* specimens consisting almost exclusively of epithelial cells had normal morphogenetic behaviour. Despite the lack of nerves, these polyps were able to reproduce asexually by budding and regenerate a head or foot without troubling the original polarity.

It is possible that, in some lower bilaterians, the muscle pattern is established independent of the positional information provided by the nervous system. In the acoel flatworm *Convoluta pulchra*, embryonic musculature is developed in two steps (Ladurner and Rieger 2000). A primary orthogonal grid of short, isolated, circular muscle fibres is established, which eventually elongates to encircle the embryo completely. The first longitudinal fibres are formed later, along with some new primary circular fibres.

This primary orthogonal muscle grid serves as a template for myoblast differentiation. The whole process runs without using positional information from the nervous system.

Cellularity and Positional Information

In the early 1980s, when the expression patterns of *Drosophila* segmentation genes were revealed and the syncytial nature of the early blastoderm stages was definitively ascertained, it was soon realised that the transcription factors encoded by those genes can rapidly diffuse through the blastoderm and reach their target nuclei, all because of the syncytial nature of the early blastoderm. Soon thereafter, cellularisation of the blastoderm dramatically reduces mobility of the macromolecules across the embryo, thus mechanically contributing to consolidation of the precise pattern of molecular markers (or, equivalently, of locally differentiated cellular states) dynamically achieved during the first few hours of embryonic development.

However, subsequent study of the expression patterns of segmentation genes in the embryos of other insects such as the little flour beetle *Tribolium* – in which the blastoderm is cellular since its first appearance – required a revisitation of this initial speculation. There is little doubt that macromolecules will move much more freely across a syncytium than across a cellularised cell layer, but the syncytial condition of the early *Drosophila* embryo should not be regarded as an adaptation to a particular way of patterning. The latter interpretation would be one more concession to the fashionable adultocentric view of development. Much more likely, the syncytial organisation found in Diptera, such as *Drosophila*, can be explained as an adaptation for speedy development. An enormous number of dipteran larvae exploit rapidly decaying (and rapidly disappearing) food sources, such as carrion, dung, soft mushrooms and the like. It is therefore advantageous to them to have an embryonic life as short as possible, so that the newly hatched larvae can successfully compete for food soon after the mother has discovered the food source and laid her eggs on or in it.

In fact, the seven-stripe expression pattern of the *fushi tarazu* (*ftz*) gene develops in *Drosophila*, even if cellularisation is prevented (although, in a syncytial context, the pattern is stable only if *ftz* RNA is rapidly degraded; Edgar, Odell, and Schubiger 1987).

That a syncytium is not required for segment formation and patterning in insects is also clear from the development of the tiny wasp *Copidosoma floridanum*, which is polyembryonic, with up to 2,000 individuals forming

from one egg. Following partitioning of the egg material into so many units, the antero-posterior axis is (re-)established within each embryo, in a cellularised environment. Segmentation and segment patterning genes, such as *engrailed*, *Ultrabithorax/abdominal A*, and *even-skipped*, are not expressed during the early proliferative phase. But the transcription factors for which they code are present later, in each embryo, with basically conserved patterns of expression (Grbič et al. 1996).

A broad comparison of those insects for which we know enough of both embryo organisation (cellular vs. syncytial) and expression pattern of segmentation genes and *Hox* genes does not seem to suggest any qualitative difference between the two kinds of organisation as for the opportunities they offer in the patterning of the embryo.

Any suggestion about the specific advantages or disadvantages of the syncytial organisation with respect to body patterning is derived from broader comparisons, with nothing known about 'developmental gene' expression in largely syncytial animals, such as the silicosponges and the acoel flatworms. Little insight comes from other systems, such as nematodes, in which the advantages of syncytial organisation in organising the body plan have been suggested with reference to the tail tip in the male *C. elegans* (Nguyen et al. 1999).

Transpatterning

Provisional Scaffolding

In the standing dynamics of multicellular systems, pattern generates pattern generates pattern. Ontogeny is full of processes that the current adultocentric view of development would describe as ways to make provisional scaffolds for some definitive adult feature.

During limb development in vertebrates, future long bones are first laid down as continuous condensations of cartilage precursor cells. The earliest condensations appear in the proximal region of the future limb, with tissue that will eventually differentiate into the humerus (or femur). Mesenchymal condensations then expand and branch, giving rise to the primordia of the radius and ulna (tibia and fibula). Further branching of these bones will give rise to the carpals (tarsals), metacarpals (metatarsals), and phalanxes. This branching process is strictly sequential, in that the formation of one joint is necessary to determine the position of the next (Spitz and Duboule 2001). The whole cartilage scaffold, in turn, is necessary for subsequent ossification.

During neuronal development, transient glial boundaries are found around functional groups of neurons and their outgrowths (dendrites and axons), in vertebrates and insects alike. The specialised glial cells forming this early scaffolding for neural growth express both inhibitors of neurite growth and attractant molecules that guide growing neurites to regions where they fasciculate, thus providing identity and positional information to migratory cells and their growing processes. These glial boundaries, although fated to disappear with progressing development, reappear following brain lesions in the adult (Steindler 1993).

Segments are obvious scaffolds for morphogenesis. One of the roles of the segment border cells is guiding migrating muscle fibres to their attachment sites (Volk and VijayRaghavan 1994). In vertebrates, a transient segmental organisation is used as provisional scaffolding for the orderly growth of many ultimately unsegmented features. In the adult, only the spinal nerves and the vertebral column retain the original segmental organisation. But in the early embryo, a segmental arrangement is evident (e.g., in the distribution of motor axons growing out from the central nervous system in the rhombomeres) in the branchial arches and in the somitic organisation of the mesoderm (Ingham and Martínez Arias 1992). Recent investigations of the expression of 'developmental genes' have shown that loss of segmentation of the vertebral column is prevented by the persisting expression of *Pax1* at the level of the intervertebral discs (Christ et al. 1998). Fading out of the expression of the same gene in other parts of the skeleton (e.g., at the level of the basioccipital bone or between the dens axis and the body of the axis) precedes and possibly prepares the fusion of the corresponding bones (Wilting et al. 1995).

A recent discovery in cnidarians suggests that we should look for the possible occurrence of transient segmentation, even in animals where the adult organisation is farthest away from that of conventional segmented metazoans. In the planula larva of the hydrozoan *Podocoryne carnea*, Gröger and Schmid (2001) found a set of cytochemically defined nerve cells arranged into a serially repeated nerve net along the antero-posterior body axis. This pattern disappears when the planula metamorphoses into a polyp.

In the development of adult muscles during the metamorphosis of *Drosophila*, nearly all larval muscles degenerate and are replaced by new adult muscles. In the larval thorax, there is a morphologically recognisable class of myoblasts (the imaginal pioneers) associated with the imaginal discs. By attaching to the epidermis at sites corresponding to the future muscle

insertions, these myoblasts serve as foci for myoblast fusion and thus act as a scaffold for the developing adult muscles (Rivlin, Schneiderman, and Booker 2000).

Unavowed residues of Haeckelian recapitulationism will probably suggest that temporary scaffolding used in development may represent a phylogenetically old feature, by now reduced to a simple 'preparatory role', but the true history is sometimes the other way. This is the case of the holometabolous insects, whose larva is evolutionarily younger than the corresponding adult. In *Drosophila*, some larval muscles actually escape from the generalised destruction at metamorphosis and form the core of the dorsal longitudinal flight muscles of the adult, used as scaffolding for the correct placement of the new adult fibres. What is phylogenetically new, in this case, is not the production of the flight muscles, but the use of phylogenetically younger larval scaffolding during adult myogenesis (Fernandes and Keshishian 1996).

The fact that provisional scaffolding is often provided by cells destined to disappear at a later stage of development is likely to be per se an evolutionarily derived feature. In some instances, we still see what is probably the original version of provisional scaffolding, that is the presence of embryonic cells that acquire transient states influencing the specification of other cells in the embryo, but are not reflected in the developmental potentials of the cells themselves.

None of these examples of 'provisional scaffolding' provided by embryonic or larval features towards the construction of adult structures represents an obstacle for a non-adultocentric view of development. In the case of the flight muscles of *Drosophila*, phylogeny shows that we shall actually read the relationship the other way. As the fly's larva is evolutionarily younger than the adult, it is the larval muscles that originated from the adult muscles and found a way to develop a divergent morphology, transiently used during preimaginal life. No wonder larval and adult morphologies are compatible to the extent that the latter builds on what is not destroyed of the first. More generally, in all cases discussed in this section, one could advance an explanation for the 'scaffold' structures in terms of congruence with the developmental dynamics of the ontogenetic stages at which these structures appear, rather than simply seeing them in the light, or to the service, of the adult structures that will later develop. This reversal of perspective applies even to the commonly acknowledged role of the nervous system as providing a prepattern for the later development of musculature or other features. This 'nerves first' principle is far from

universal. In *Drosophila*, the development of somatic musculature is independent of nerve supply. In *prospero* mutants, in which pioneering of peripheral motor nerves is delayed, before differentiation of the motor nerves there is normal fusion of myoblasts to form syncytial myotubes which form normal attachments to the epidermis. In this way, a larval musculature comparable to the wild-type pattern is eventually produced (Broadie and Bate 1993).

Segments, Vertebrae, and Scales

Segmentation is primarily a mesodermal (annelids, vertebrates) or ectodermal (arthropods) trait. Nevertheless, evidence of segmentation of the endoderm is present in several groups. The question is if and how this segmentation of the endoderm is related to segmentation of the other germ layers.

In the leech *Helobdella*, the homeobox gene *Lox3* is expressed in a segmentally iterated pattern within the endoderm. Wide stripes of *Lox3* expression mark the caecal outpouchings of the intestine, whereas the constrictions between subsequent pairs of caeca are marked by thin *Lox3* stripes. If the segmental mesoderm is ablated at an early embryonic stage, the definitive endoderm fails to appear and an abnormal gut tube is formed. The defect extends precisely and exclusively to the segment(s) where no mesoderm is left. This suggests that the mesoderm normally promotes patterning of the endoderm via local cell–cell interactions. In portions of the endoderm surrounding such deficits, the segmental pattern of *Lox3* expression is more or less untouched. This confirms that segmentation of the endoderm is not established by lateral interactions within that germ layer, but is likely imprinted by vertical interactions with the segmental mesoderm (Wedeen and Shankland 1997).

Both germ-layer autonomy and influence of one germ layer on another have been shown to coexist in *Drosophila*, in which the visceral mesoderm, like the ectoderm, acquires segmental periodicity. The progenitors of the visceral mesoderm are cells from only the anterior half of each mesodermal parasegment, which merge to form a continuous band running along the main axis of the embryo. In this continuous band, however, *connectin* is expressed in eleven metameric patches, suggesting an iterative organisation comparable to ectodermal compartments. Segmental subdivisions of the visceral mesoderm are independent of *Hox* gene activity and form in response to ectodermal signals encoded by the segment polarity genes *hedgehog* and *wingless*. As connectin patches align with

ectodermal *engrailed* stripes, subdivisions of the visceral mesoderm correspond to parasegmental boundaries in the ectoderm. Induction from the mesoderm to the endoderm will finally subdivide the gut along the antero-posterior axis (Bilder and Scott 1998).

In vertebrates, numerical correspondence between segmental derivatives of two distinct germ layers are sometimes suggestive of mutual relationships, but some degree of mismatch actually invites closer inspection. In the 'shell' of turtles, ectodermal and mesodermal components do not overlap perfectly, despite the identical number of elements contributed by each of the two sets (Gilbert et al. 2001). The mesoderm contributes with 38 paired and 12 unpaired bones dorsally and 8 paired and 1 unpaired bones ventrally. These bony scutes are then covered with epidermal scutes, whose number corresponds with the number of paired bones involved in the shell, but the shield and bone patterns are not in register. Overlapping of the pattern of the sulci between neighbouring epidermal scutes with the sutures between neighbouring bones is minimal.

A comparable example is offered by the correspondence between vertebrae and rows of transverse ventral scales in other reptiles – snakes and the amphisbaenians or worm lizards. In these groups, arrangement of the scales reflects, more or less closely as the vertebral column itself does, the primary pattern of mesodermal segmentation (Alexander and Gans 1966). Most snakes have a 1:1 ratio between the number of ventral shields and the number of vertebrae, whereas approximately two (from 1.5:1 to 2.3:1) ventral scale rows to each vertebra are present in the vast majority of the amphisbaenians and in the primitive snakes of the Typhlopidae, Leptotyphlopidae and Uropeltidae. The number of vertebrae is far more constant than the number of scales (Gans and Taub 1965, Gans, Laurent, and Pandit 1965). Dorso-ventral mismatch in external segmentation is common, especially in boid snakes. In some colubrids, a 1:1 scales to vertebrae ratio is observed ventrally, whereas the dorsal shields are more numerous. For example, in *Thrasops flavigularis* there is a 1:1 ratio between vertebrae and ventral scutes, but 0.59:1 and 0.78:1 ratios were found, in two different specimens, between vertebrae and dorsal scutes (Alexander and Gans 1966). Dinosaurs, too, would offer interesting patterns for consideration: in ankylosaurs such as *Euhoplocephalus*, each dorsal row of plates correspond to two vertebrae in the axial skeleton.

Similar to the correspondence between vertebrae and scale rows in reptiles is the correspondence between vertebrae and scale rows in bony fishes. In this case, however, correspondence is much less precise. As a

rule, the number of vertebrae and the number of scales are both stabilised early in development. But at least in the case of the European minnow (*Phoxinus phoxinus*), an increase in scale count throughout life has been suspected (Repa 1974). Apparently the spacing of the scales rows, which become visible well after the pattern of myomeres has been established, is dictated by the spacing of the latter. In some cases, the number is secondarily doubled by intercalation of new elements between two scale initials of the first run (Lindsey 1988).

Guidelines to Follow

Of all bilaterians, vertebrates are, by far, those in which cell migration has the most important role in morphogenesis. Extensive migrations, such as those of the neural crest cells (Hall 1999), are virtually absent in invertebrates. In the latter animals, most cells are formed in their final position. But there are exceptions. In *Caenorhabditis elegans*, migrations of some neuroblasts during larval life span the animal's whole body length and are influenced by *Hox* gene expression (Salser and Kenyon 1994).

Cell migration may occur through different mechanisms (e.g., Levi, Duband and Thiery 1990, Hynes and Lander 1992): as migration of a whole cell sheet, as migration of isolated cells through an extracellular matrix or as migration of isolated cells over the cellular processes of another cell. The latter mechanism is especially relevant in the case of the developing nervous system. This is far from a trivial problem, as the growing axons are seeking a target that also moves in the meantime (Jacob, Hacker, and Guthrie 2001). Growth cones are guided by four different mechanisms: contact attraction, chemoattraction, contact repulsion and chemorepulsion (Tessier-Lavigne and Goodman 1996).

What guides motor neurons in their migration – extrinsic cues or the different genes they express? The question is not that different from another question often raised in biology, whether the shape of a gall depends on (the genome of) the plant where it develops or on (the genome of) the insect inducing its production. Both questions are actually ill-founded, as both agents are jointly responsible for the outcome of their interaction, despite the contrary views of some authors.

As for motor neuron migrations, Sharma et al.'s (2000) experiments point to a major role of the genes expressed by the neurons themselves, rather than the matrix. As distinct motor neuron subtypes normally express unique combinations of LIM-type homeodomain factors that are likely involved in cell migration, axon navigation, or both, Sharma et al. forced

all motor neurons, irrespective of their potential subtype, to express ectopically a LIM gene combination appropriate for the subgroup that normally innervates axial muscles. This genetic alteration was sufficient to convert all motor neurons to the cell migration behaviour of the axial subtype. Concordance with this type was also indicated by gene expression profile and the pattern of their axonal projections.

Genetic control of cell migration has been demonstrated in many cases. In *Hydra*, for instance, an *Otx* homologue plays a role in cell movements leading to the establishment of a new body axis (Smith et al. 1999). In the *Drosophila* embryo, *even-skipped* and *islet* constitute a bimodal switch regulating axonal growth in such a way that motor axons are specifically directed to ventral or dorsal regions of a muscle field (Landgraf et al. 1999). In *Caenorhabditis elegans,* the *unc-129* and *unc-6* netrin genes are required to guide pioneer motor axons along the dorso-ventral axis (Wadsworth and Hedgecock 1996, Codavita et al. 1998). Genes controlling cell migration may exercise their effects by modifying the properties of the environment through which cells will migrate. The product of the *mig-17* gene in *C. elegans*, the metalloprotein MIG-17, directs migration of the distal tip cells of the two U-shaped arms of the gonad by remodelling the basement membrane (Nishiwaki, Hisamoto, and Matsumoto 2000).

To alter cell behaviour, the influence of the matrix does not need to be molecularly specific. Chen et al. (1997) cultured human and bovine capillary endothelial cells on micropatterned substrates that contained adhesive islands coated with extracellular matrix. Larger islands allowed cells to grow and expand, whereas smaller islands negatively influenced cell growth, and a very small island of extracellular matrix forced cell apoptosis. Thus, cell shape decided whether individual cells grew or died. But this influence was the same, regardless of the type of matrix protein or antibody to integrin used to mediate adhesion.

In *Drosophila*, establishment of the final somatic muscle pattern depends on reciprocal signalling between the epidermal muscle attachment cells and the approaching myotube. The first signals come from the epidermal muscle attachment cells, inducing myotube attraction and adhesion to their target cells. As soon as they are attached to their target cell, the muscle cells send back a signal to the muscle attachment cells; this signal induces their terminal differentiation into tendon-like cells (Becker et al. 1997).

If, in most cases, the behaviour of migrating cells is the result of their intrinsic specificities, mediated by interactions with the substrate and

cellular environment in which they move, then the case of tracheolar cells migrating from the base to the tip of the wing disc in moths and butterflies is apparently a passive process whose motive force resides in adjacent epithelial cells (Nardi 1984).

Quite often, patterning by cell migration is apparently controlled by multiple cues. The routes followed by sensory axons in the *Drosophila* embryo, for example, are influenced by the pre-existing patterns of the somatic muscles and the tracheae, but are not strictly dependent on them. Nevertheless, in the presence of both muscles and tracheae, the sensory nerves grow more rapidly, and the number of misrouted axons is sensibly lower (Younossi-Hartenstein and Hartenstein 1993). Blair and Palka (1989) induced the formation of single sensory neurons in a variety of abnormal locations in the developing wing of *Drosophila*, that is, along the longitudinal wing veins L2, L4, and L5 rather than along L1 and L3 as in the normal wing. Ectopic neurons located in the distal part of vein L2 have nearly a 100% tendency to grow in a normal proximal direction. The percentage is reduced to 70% in distal vein L4 and falls to a 50% chance along vein L5. This shows that axons growing out of neurons forming in ectopic regions of the wing, but near the normal axon pathways (veins L1 and L3), have a high probability of migrating in the correct direction.

One is probably tempted to say that these migrating cells, or axons, follow redundant cues, but using this language would be one more concession to the adultocentric view of development. If a message is redundant, it is redundant from the point of view of a given (potential, expected or actual) receiver. If a developmental process is considered redundant, it is so from the point of view of its expected outcome (i.e., the adult). But the fact that a given developmental process happens because of several independent circumstances, any of which would per se suffice to determine it, would be better studied and described in terms of the generic character rather than genetic character of its causes – by treating it as a developmental attractor.

Phylotypic Stage and Phylotypic Period

The study of cell lineage in the earliest stages of embryonic development was one of the favourite activities of embryologists between the end of the nineteenth century and the beginning of the twentieth century. The precise regularity and constancy of cell lineage found in many species reinforced a naively attractive hypothesis: that the earliest decisions in development are the most important and, therefore, most likely to be under

tight control, whereas increasingly higher degrees of freedom are likely to be accorded to increasingly more advanced stages. This view was discarded when it was realised that closely related species differ widely in the ways their earliest developmental stages proceed, only to converge, later in development, towards a shared stage (the phylotypic stage). After this stage, their ontogenies may diverge again, culminating in two different adults. The best example of such a divergent-convergent-divergent pattern of development is provided by two sea urchin species (*Heliocidaris erythrogramma*, with relatively large eggs and direct development, and its close relative *H. tuberculata*, with small eggs and indirect development) which have been the subject of an extensive study by Rudy Raff and his group (e.g., Raff and Wray 1989, Raff et al. 1990, Wray and Raff 1991, Kissinger and Raff 1998, Raff 1999a, 1999b, Nielsen et al. 2000, Fercowicz and Raff 2001).

The term 'phylotypic stage' was introduced by Sander (1983) to denote the ontogenetic stage in which the main traits of body architecture are laid down. Such are the germ-band stage of arthropods and the pharyngula of vertebrates. Duboule (1994a) introduced the metaphor of the egg-timer to visually depict this convergence of ontogenetic trajectories towards a phylotypic stage, irrespective of the extent of the possible divergence of the initial conditions and later divergence of ontogeny towards the adult stage.

Divergence of early embryonic stages is strong even in those phyla that were traditionally considered to have a very stereotyped development, such as nematodes (Schierenberg 2001). In *Acrobeloides nanus*, for instance, early cleavage requires zygotic gene activity, whereas in *Caenorhabditis elegans*, transcription does not begin before the embryo is comprised of 100 cells. In *A. nanus*, early blastomeres take the developmental role of lost neighbouring cells, thus showing some degree of regulative behaviour of which there is no evidence in *C. elegans*. In the latter species, the asymmetric position of sperm entry plays an essential role in the establishment of antero-posterior polarity; but, in other species, this function of the sperm is apparently absent. Despite these initial variations, in all nematodes studied thus far, gastrulation begins before the 30-cell stage with the immigration of the primordial gut cells. From this phylotypic stage onward, development proceeds for a while in a very similar way in the different species.

Phylotypic stages are probably limited to bilaterian animals. In plants, the flower is perhaps 'phylotypic', despite its late occurrence in ontogeny, according to the traditional adultocentric periodisation of plant

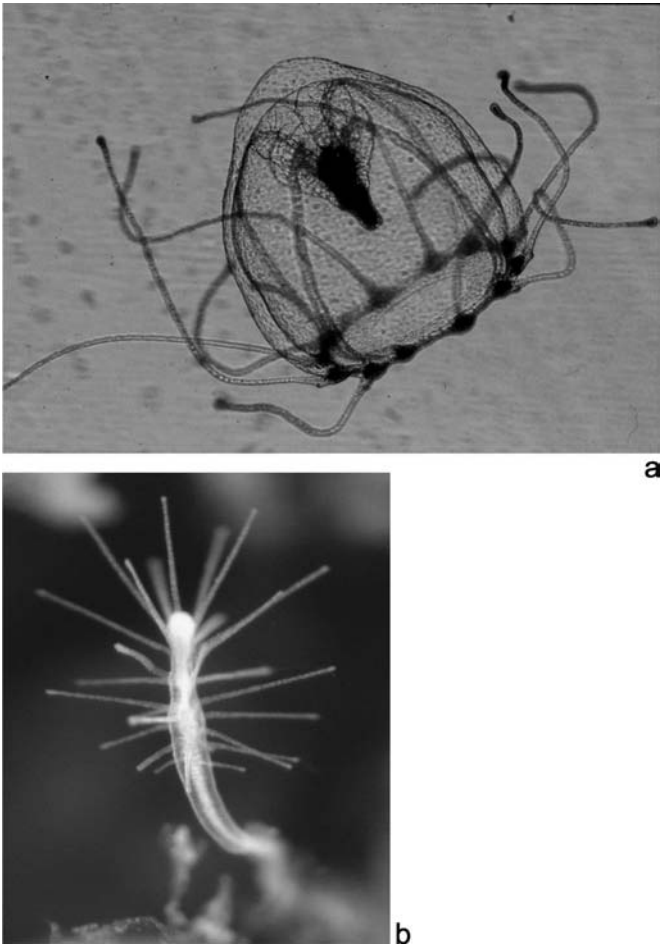

Figure 6.1. Polyps (a) and medusa (b) of the hydrozoan *Turritopsis nutricula*. Under certain conditions, the medusa of this species may revert to polyp. (Courtesy of S. Piraino, Lecce.)

development from the germinating seed to the maturing fruit; but for the vegetative structures, there is probably no phylotypic stage. In sponges, the lack of a phylotypic stage is suggested by the fact that a complete sponge can be reconstituted from dissociated cells. In cnidarians, by the phenomenon of transdifferentiation – illustrated by Bavestrello, Sommer, and Sarà (1992) and Piraino et al. (1996) in *Turritopsis nutricula* – the medusa, under certain conditions, can literally reverse its developmental history, by transforming back into a polyp (Figure 6.1). Two groups of bilaterians,

however, should be investigated closer from this point of view. One is bryozoans, in which freshwater forms rebuild their complex architecture from very simple multicellular resting stages, the statoblasts. The other is nemerteans, in which large forms, such as *Lineus*, when severely starved, may regress to a microscopic cluster of cells from which a new worm is later produced. The integration of developmental processes that should characterise the phylotypic stage (Galis 1999) is a kind of morphogenetic ratchet from which these animals seem to escape. Different is the case of some tunicates (e.g. the ascidian genus *Botryllus* and the thaliacean genus *Doliolum*) in which perfectly comparable 'adults' are built by embryogenesis or vegetative reproduction (blastogenesis; Manni et al. 1999). In this case, the question is not so much if a phylotypic stage exists, but rather how 'generic' this stage is, as it is not simply achieved through different early embryogenetic processes, but also from developmental processes other than embryogenesis.

This last case offers an opportunity to introduce regeneration. It has long been speculated that vegetative reproduction is not that different from regeneration (e.g., Dehorne 1916, Berrill 1952, Herlant-Meewis 1953). In modern terms, this view has been reformulated as the hypothesis that fission can be derived from regeneration processes recruited for a new role in reproduction. This amounts to imagining (Bely and Wray 2001) that paratomic fission in annelids is accomplished by initiating regeneration in the middle of an undamaged worm. Similarities between the two processes have been demonstrated in developmental genetic terms. In the annelid *Pristina leidyi*, for example, the role of *Otx*-class genes in patterning anterior body structures is largely the same in both contexts. In *Hydra*, *HyBra1*, a homologue of the *Brachyury* gene, is associated with the formation of the hypostome (the apical region of the polyp) in different developmental situations, such as embryogenesis, the budding process, and the very earliest phases of regeneration (Technau and Bode 1999).

Sánchez Alvarado (2000) speculates that the genetic organisation operating in the blastemas of simpler organisms during regeneration or asexual reproduction may have been co-opted in sexually reproductive animals to produce novel embryonic structures, such as the limb bud.

An equivalence between limbs and buds is also implicit in Dewel's (2000) suggestion that colonial cnidarians, such as the sea pens (Pennatulacea), may provide a blueprint for reconstructing the organisation of the first bilaterians, in which growth would have occurred along the primary axis (cf. a sea pen's primary polyp), as well as along lateral branches perpendicular to

it (cf. a sea pen's secondary polyps). In the end, I might add, the former axis might be consolidated into the bilaterian's main body axis, the latter into its appendages. This comparison is attractive, but we cannot underscore the fact that cnidarians lack a mesoderm. The availability of the latter would have helped to exclude the endoderm from becoming involved in formation of those lateral axes which would have become stabilised as limbs rather than growing as true buds destined to form new zooids. Bud/limb equivalence is discussed later in chapter 8.

Regeneration and fission are not necessarily correlated with each other or with embryogenesis. For example, in the freshwater oligochaete annelid *Paranais litoralis*, there is fission but not full anterior regeneration (Bely 1999). The same is true of some ascidians. Probably, in the vegetative reproduction complex, interactive networks comparable with those active in embryogenesis around the phylotypic stage may be set in motion; that is not true for local regeneration.

Heterochrony can affect the phylotypic stage, diluting it into a 'phylotypic period', as demonstrated by Richardson (1995) in the case of vertebrates. At the tail bud stage, generally regarded as a conserved embryonic stage, vertebrate embryos show extensive variations in form, due to allometry, heterochrony, and differences in body plan and somite number. These differences foreshadow important differences in the body form of corresponding adults (Richardson et al. 1997). In particular, somite number at this stage does not show the constancy we should expect according to the 'developmental hourglass' model. This is due to dissociation of the process of somitogenesis from the conserved positional field encoded by genes of the zootype (Richardson et al. 1998; cf. also Tabin and Johnson 2001). Temporal dissociation from the establishment of the positional framework (zootype) is also shown by differentiation of the limbs and pharyngeal arch, so that it is virtually impossible to identify a common stage of pharyngula as a zootype common to all vertebrate classes.

Morphological Assimilation in Ontogeny and Phylogeny

The zootype is far from being the only example of target developmental stage reached from diverse or less regular beginnings.

Held (1979) analyzed the lineages of cells on the first tarsal article of the second pair of legs in *Drosophila melanogaster* and found that the bristle pattern of this leg segment does not originate in this final form. The

bristle cells of each row are arranged at first in a jagged line, which is later straightened by cell movements.

Remodelling of 'draft organs' during embryonic development is common. An example is vasculature in vertebrates (Roman and Weinstein 2000). What actually deserves our attention are those organs which are apparently built by bringing together pieces originally grown as physically separated units. Cell biology has accustomed us to self-assembly, both intracellular (microtubuli, cytoskeleton) and intercellular (as extensively demonstrated by cell self-sorting within reaggregating mixtures of different kinds of cells). But a large leap separates these phenomena from the actual formation of organs from previously isolated precursors. This is what the individual components of the neural crest actually do (Hall 1999).

Another example is offered by the leech *Helobdella*. I have already described (page 38) the founding contribution of the progeny of seven pairs of different blast cells to the cell population of the leech's 32 segments. This requires an alignment of the different teloblast lineages, but this is not provided by a co-ordinated progression of the blast cells in generating the corresponding longitudinal bandelets. The latter acquires axial patterns independently and is later brought into alignment along the anteroposterior axis through a process of morphogenetic assembly (Nardelli-Haefliger, Bruce, and Shankland 1994).

Organs forming from bilaterally symmetrical anlagen, which for a while develop independently of one another, are known from different phyla. In *Drosophila*, for example, the brain originates from two bilaterally symmetrical neurogenic regions, which are initially separated from each other and from the ventral nerve cord (Therianos et al. 1995). In *Drosophila*, again, the dorsal tracheal trunk is generated by fusion of adjacent tracheal metameres, following migration of a distinct subset of tracheal cells (the so-called dorsal trunk cells), under the influence of a transcription factor encoded by the gene *spalt* (Kühnlein and Schuh 1996). In vertebrates, as previously described, heart precursors originating at either side of the embryo migrate towards the midline where they eventually fuse. In zebrafish, this migration is controlled by the *miles apart* gene (Kupperman et al. 2000).

If these morphogenetic events compare favourably with the phenomena of self-assembly better known at a lower level of organisation, there are others for which the most obvious term of comparison is instead the semiconservative duplication of DNA. This is what happens during blastogenesis (multiplication by budding) in the ascidian family Didemnidae. In these

sea-squirts, the body consists of a neatly distinct thorax and abdomen. The thorax can produce a second abdomen, and the abdomen can produce a second thorax. The process gives rise to two complete zooids, one consisting of the old thorax and the new abdomen, and the other formed by the new thorax and the old abdomen. Brien (1968) suggested that one of the two members of a *Didemnum* pair, following production of the two buds, might even be derived from the assemblage of the new thorax with the new abdomen, but this has never been proved (P. Mather, personal communication). If true, this would be a unique example of an animal formed by the assemblage of units originally formed in total independence of one another, such as the fruiting body of a cellular slime mould.

Another group worthy of discussion in this context is the so-called annual fish. In these minuscule vertebrates, immediately following gastrulation, the blastomeres disperse and then reaggregate, thus disrupting any pattern they might have obtained until then. Only following reaggregation does morphogenesis actually begin. In the genus *Cynolebias*, in particular, eggs are often diblastodermic; that is, two separate blastoderms are formed, which develop independently from the one-cell stage until the advanced blastula. But this is not a beginning of polyembryony. When these two blastoderms begin to gastrulate, the blastomeres forming both of them reaggregate, giving rise to one fusion embryo. No structural or functional evidence of the original duplicity has been recorded in the young fish after hatching (Carter and Wourms 1993).

Multiradiate starfish (i.e., those with more than five rays) represent one of the most astounding examples of morphological assimilation. Starfish with more than five rays are much less familiar than their five-ray counterpart, but are nothing of a rarity, having evolved independently in fourteen living families. According to Hotchkiss's (2000) 'five-plus' hypothesis, supernumerary rays develop separately from the five primary rays. Hotchkiss postulates that the ontogeny of the primary rays is highly integrated, synchronic and developmentally constrained. That is, the five primary rays develop as a unit through a pathway operating briefly during the time of metamorphosis. A pause precedes development of the supernumerary rays. There seems to be no heritable variation in the number of rays formed in the first instance that could be co-opted for the production of supernumerary rays. The latter develop in a variety of ways, all independent of the mechanism by which the primary set of five rays is produced. Despite this difference in their origin, supernumerary rays are hardly different, morphologically and functionally, from those of the primary set.

This is not that different from the mechanism by which some amniotes (e.g., moles and panda) generate supernumerary digits. Galis, van Alphen, and Metz (2001) prefer to call these appendages digit-like structures rather than extra digits, but it is also possible that early tetrapods used more than one mode of digit development at the same time (Wagner, Chiu, and Laubichler 2000). This is a suggestion that the polydactylous appendages of *Acanthostega*, with a set of larger and smaller digits (Coates and Clack 1990), could support.

The homonomous vertebral column of snakes, the homodont teeth of whales and the distal part of the ichthyosaur fins – in which any distinction between carpals, metacarpals and phalanxes is lost – are the result of a phylogenetic reduction in the degree of differentiation among the elements of a series, that is, a loss of modularity (Wagner 1996). Therefore, these are examples of phylogenetic, rather than ontogenetic, assimilation. The same is also seen in the secondary homonomy of body segments in some centipedes (the scolopendromorph *Plutonium* and the geophilomorphs generally) and in the vermiform larvae of several insects [e.g., many biting midges (Ceratopogonidae) and click beetles (Elateridae)].

A striking example of phylogenetic assimilation, quite likely dependent on homeotic co-option, is provided by the coelacanth (*Latimeria chalumnae*), in which the dorsal and anal fins have secondarily acquired the skeletal pattern previously evolved in the paired fins (Ahlberg 1992).

Patterning in Regeneration

Embryonic Patterning Versus Patterning in Regeneration

Much of the experimental work on animal regeneration has little or nothing to do with the animal's real life. A recent review on regeneration in insects (Marsh and Theisen 1999), for example, only deals with the regeneration power of the imaginal discs in *Drosophila*, a hidden virtue that the fruit fly will never use to its own benefit. The fact that the power of regeneration extends beyond the repair routines that the animal will likely use during its lifetime seems to justify Goss's (1992) view of regeneration as an epiphenomenon of development – that is, something that is not a special, adaptively evolved phenomenon. Cells multiply, move or differentiate at their best, in the context of the cycling system of which they are part. Regeneration, like the basic cellular properties from which it results, may proceed irrespective of its adaptive value.

The problem is that regeneration, much like illness and many other physiological or pathological conditions and processes (and what about

to be a male or female?), is *not* homologous across the different groups (a view defended by Sánchez Alvarado 2000). It is simply a convenient term for a class of events involving multicellular organisms. That regeneration is mainly 'basic cell biology' is shown by experiments demonstrating that regeneration does not necessarily imply any memory of the original form. Experiments with reaggregated cells in the *Hydra*, which sorted out eventually to form a new polyp, failed to show any preferential incorporation of cells deriving from the apical region ('head' with tentacles) into the corresponding region of the newly formed polyp (Sato et al. 1992, Technau and Holstein 1992). In *Botrylloides*, a colonial sea squirt, any minute fragment of a peripheral blood vessel containing a few blood cells isolated from an adult zooid may give rise to a complete organism with (the equivalent of) all three embryonic layers (Rinkevich, Shlemberg, and Fishelson 1995). In the flatworm *Dugesia tigrina*, the same set of patterning *Hox* genes is activated in embryonic development and in regeneration, but with a different time schedule (Bayascas et al. 1998). In more complex organisms, such as vertebrates, in which the regenerative power is primarily limited to the appendages (limbs and tail), regeneration may recapitulate normal development. In newts, for example, the regenerating limb blastema produces a zone of polarising activity, that is a signalling centre of antero-posterior patterning, as a normal developing limb would do (Imokawa and Yoshizato 1997).

Terminal or Apical Control Versus Regeneration

Arthropods, nematodes and the other moulting animals do not regenerate any missing part of the main body axis. Itow's (1986) claim to have obtained supernumerary segments in the horseshoe crab *Tachypleus* by applying inhibitors of DNA synthesis cannot be used as an argument to disprove the universality of this rule. The observed behaviour may simply be derived from induced secondary subdivision of the animal's primary segments.

That ecdysozoans do not regenerate parts of the main body axis does not mean that they lack regenerative powers completely. Collembolans, for example, regenerate all appendages without trouble and renovate the whole midgut epithelium at each of their numerous moults (Schaller 1970).

In many cases, the simplest explanation for the lack of regeneration is the lack of mitotic activity in the tissues, regions or stages in which the amputation occurred (Rockett and Woodring 1972). Ticks, for example, regenerate limbs completely; but in all other mite families, there is little regeneration power or none at all. No regeneration, for example, is possible in the tetranychids where, interestingly, there seems to be no mitotic

activity during the whole postlarval life (Woodring 1969), whereas extensive mitosis occurs in the postlarval instars of the ticks (Balashov 1963).

This explanation, however, does not hold true for most ecdysozoans, where mitotic activity, although mainly confined to the (pre)moulting phase, may last over most of postembryonic life. The principal cause for the lack of regeneration of portions of the main body axis is possibly another, namely, very early determination in embryonic development of both termini of the body, anterior and posterior, through expression of molecular markers that the animal is not able to re-express later in life, what would be necessary for regeneration of missing parts of the main body axis.

Interestingly, the gastrotrich *Turbanella*, if cut into two parts, can regenerate (Manylov 1995). In the traditional classifications, gastrotrichs were classified, together with nematodes and several other groups, in a superphylum of aschelminths or pseudocoelomates. In the new classifications contrasting ecdysozoans and lophotrochozoans (e.g., Aguinaldo et al. 1997, Adoutte et al. 2000, Jenner 2001, Peterson and Eernisse 2001), gastrotrichs are widely separated from the moulting aschelminths such as nematodes, which are placed, together with arthropods, in the newly recognised superphylum Ecdysozoa. One of the characters that seems to define this clade (a character, however, never mentioned in the recent literature) is the lack of regenerative power, or its limitation to the appendages. *Turbanella*, which regenerates, confirms the lack of close affinities between gastrotrichs and their former putative relatives now classified with Ecdysozoa.

CHAPTER SEVEN

Size Factors

> A causal explanation for the striking correlation between miniaturization and novelty may lie in part in the effect of size reduction on the morphogenetic mechanisms of pattern formation, many of which are size dependent.
>
> J. Hanken and D.B. Wake 1993: 507

Cell Size Critical for Morphogenesis

Cell size can be deadly critical for morphogenesis. In *Drosophila*, embryos lacking the product of the *string* gene do not undergo cell divisions following the thirteenth mitotic cycle. For a while, this defect does not cause any problem. Following cellularisation of the originally syncytial blastoderm, *string* embryos undergo a noticeable degree of morphogenesis and tissue differentiation. However, at a later stage, they fail to gastrulate, due to the excessive size of their cells which cannot undergo the normal movements necessary for gastrulation (Foe 1989).

A correct cell size, however, is not simply a mechanical requirement for normal development. In a more subtle sense, cell size is often the grain of organic form. Organs or organisms composed of cells of smaller size may attain higher complexity than comparable organs or organisms whose cells are larger. A beautiful example is provided by the variation in cell size and overall complexity of the tectum mesencephali, the main visual centre in amphibians. Frogs with small cells have a tectum with more complex morphology than those with large cells; this is independent of body and brain size. Circumstances are different in salamanders, in which the morphological complexity of the brain is correlated in addition to the brain size–body size relationship. The simplest tecta are found in small salamanders with large cells, whereas the most complex tectal morphologies are those of large salamanders with small cells (Roth, Blanke, and Wake 1994).

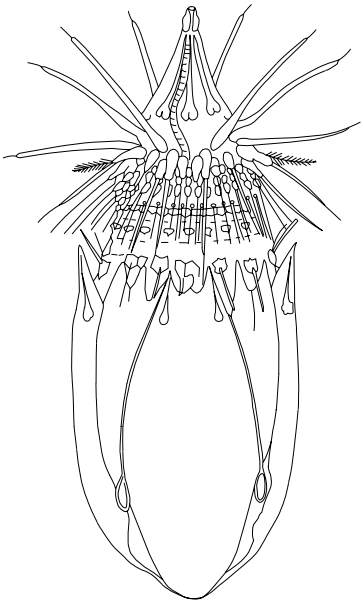

Figure 7.1. Habitus of a loricipheran. (Redrawn after Kristensen 1991.)

The extremely small cell size typical of the loriciferans – miniscule marine invertebrates first described just twenty years ago (Kristensen 1983), which measure as adults at 300 μm, but are made of more than 10,000 cells (Kristensen 1991) – is a precondition for an exquisitely fine-grained morphology. The complexity these animals achieve despite their very small size is clearly seen in Figure 7.1. One is tempted to suggest that small cell size has been critical in this respect; not so much that it allows carving the finest details of structure, but rather because of the strict limits a tiny size might impose on the total complexity of mRNA sequence in each cell, as found by Ernst et al. (1980) in sea urchin micromeres. Another group of marine invertebrates of very small size, the appendicularians, have followed a completely different strategy. Their body is made of a very small number of cells, some of which are of enormous size. Less than a dozen cells, for example, are enough for lining their relatively robust stomach (Carlo Brena and Paolo Burighel, personal communication; Figure 7.2).

An inverse correlation between cell number and cell size, is far from being a universal rule. From the viewpoint of morphogenesis, there are cases such as the *chico* mutant in *Drosophila*, which is less than half the size of a wild-type fly, owing to fewer but also smaller cells. For example, in the case of the wing area, the reduction in cell number accounts for 68%,

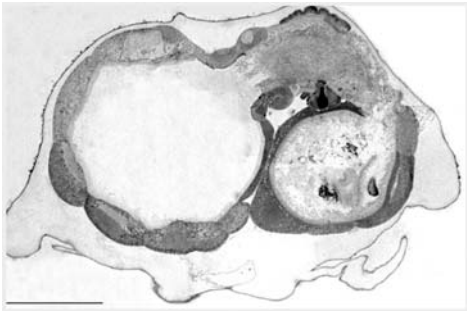

Figure 7.2. A very small number of large cells form the stomach and rectum of the appendicularian *Fritillaria pellucida* (longitudinal section). Scale bar = 80 μm. (Courtesy of C. Brena and P. Burighel, Padova.)

and the reduction of the average cell size accounts for the remaining 32%; similar results were recorded for the eye (Böhni et al. 1999).

An inverse relationship between cell number and cell size has been observed in contexts where such behaviour would hardly be expected (Oldham et al. 2000). A great example has been provided by Neufeld et al. (1998), again in *Drosophila*. These authors induced genetically marked cell clones characterised by the expression (or overexpression) of genes whose products either accelerate or slow down cell-cycle progression. In either case, changes in the mitotic rate affected the number of cells thus produced, but failed to have an effect on overall growth. Cells expressing Rb produced fewer but larger cells; those expressing E2F and Dp produced numerous, but smaller cells, with the total area of the clone remaining in either case unaffected.

These facts raise a wealth of questions for future comparative cytology. What is the real minimum size for a eukaryotic cell? Why are animals with such tiny cells as those of loriciferans so rare? Is there any metabolic or developmental trade-off compensating for their unusually small average cell size? What is the minimum number of cells necessary to achieve a given degree of anatomical complexity?

Size and Cell Number in Embryonic Anlagen

Early Because Small or Small Because Early?

Most fundamental aspects of body patterning are established during early developmental stages, within small or very small anlagen, with most of the remaining story being mainly one of differential growth. Much depends on overall size, however. In the tiny freshwater gastrotrichs (whose adults

are between 70 and 1,500 μm long), all mitoses are apparently completed by the time the juvenile hatches from the egg (but some mitoses happen postembryonically in the marine forms of the same group).

The segmental organisation of the *Drosophila* embryo is generated the first five hours after fertilisation (Akam 1987). Dipterans go through embryonic development at an unusually speedy pace, but the very early completion of the basic body architecture of the *Drosophila* embryo is but an example of the very general rule that the most important decisions in shaping an animal are made very early in development.

Even apparently trifling details, such as distribution of sensory setae on the body surface of mites, are apparently made very early in embryonic development (van der Hammen 1988).

It has been believed (e.g., Cooke 1980) that, in vertebrate embryos, many important decisions about body architecture are taken not so early as in other animals (e.g., nematodes or insects); but some recent studies suggest otherwise. In the mouse, anterior identity is probably established before gastrulation, as it is first manifested in extraembryonic tissue which is essential for normal development of anterior structures, such as the forebrain (Beddington and Robertson 1998). Izpisúa-Belmonte et al. (1993) found that the chick homeobox gene *goosecoid* (*gsc*) is expressed in a small cell population near the posterior margin (Koller's sickle) of the unincubated egg, long before its expression can be detected in Hensen's node (the so-called chick organiser). Transplantation experiments suggest that *gsc*-expressing cells have inducing activity even before Hensen's node is differentiated; therefore, the development of the chick organiser starts earlier than previously thought.

Still, in vertebrates, the proximo-distal pattern of the limb is also likely set up very early in development (Hartmann and Tabin 2001). What has been traditionally described as the developmental production of this proximo-distal pattern is simply a later elaboration of an already existing pattern, brought about by cell proliferation. This is demonstrated by the behaviour of the lipophilic dye in which cells at the limb bud tip (the so-called progress zone) were labelled at the early stages. The dye remained in the labelled cellular population rather than becoming distributed among cells with a different fate, as would have occurred if cells at the distal tip of the limb were continually respecified as limb outgrowth proceeds, as previously thought.

Further results supporting an early determination of the whole limb pattern in tetrapods – possibly within a cell layer only a few hundred μm thick – have been recently published by Sun, Mariani, and Martin (2002) and by Dudley, Ross, and Tabin (2002).

It is no surprise that directional asymmetry is often established at the very beginning of embryonic development, no matter if the situs viscerum of a vertebrate or the direction of shell coiling in a mollusc is involved (Palmer 1996). But this extends to many other fundamental events, such as those establishing polarity of the main body axis or differentiation of the organiser.

A question arises as to what the critical factor is: either the size of the anlage, and the number of cells in it, or the relative developmental time per se. The question is similar to one of the basic issues in the analysis of allometry (e.g., Fairbairn 1992): is selection acting on shape as such, or through its allometric dependence on a selectively affected size parameter?

Davidson (2001) believes that the basic developmental strategy of most bilaterians is to divide the egg into polyclonal lineages of differentiating cells at the earliest possible time. This fits well within the traditional adultocentric view of development. I think, however, that size and cell number are the relevant factors. There are cases in which important decisions are delayed, relative to embryogenesis, but this still happens within small circumscribed clusters of set-aside cells, such as the adult primordium in the sea urchin larva or the imaginal discs in the larva of the holometabolous insects. There are obvious mechanistic reasons for limiting decisions about patterning within small cell clusters. One of the reasons is the limited range of action of the morphogens, which is in the order of 10 (Gurdon et al. 1994) or at most 30 cell diameters or less than 1 mm in the maximum linear dimension (Wolpert 1989). In sea urchin larvae, *Hox* gene expression is limited to the somatocoel, when this is only 2–300 μm across (Arenas-Mena, Cameron, and Davidson 2000). Consequently, I think that the widespread occurrence of most patterning events at early stages in development is a simple by-product of the fact that early developmental stages are small and rapidly get to comprise a suitable number of cells; not the other way around. In the same vein one could suggest that the segmentation of very large eggs is partial not because of mechanical problems but mainly because a dialogue between very large blastomeres would be difficult, due to the limited range of action of morphogens.

Critical Number of Cells in Embryonic Anlagen

Too low a cell number would clearly limit the chance of establishing a sufficient number of subunits with different patterns of gene expression and different developmental fate. In appendicularians, in which the whole digestive tract is made of very few cells, the stomach and rectum are morphologically distinct, but the histochemical properties of their cells

are unusually similar (Carlo Brena and Paolo Burighel, personal communication).

In the imaginal discs of *Drosophila*, *Distal-less* expression (Beerman et al. 2001) and competence to differentiate adult structures (Minder and Nöthiger 1973) require a minimum of previous cell divisions in the disc. Following Minder and Nöthiger's suggestion, Kurushima and Ohtari (1975) investigated the behaviour of wing discs in the silkworm (*Bombyx*) and found that these acquire competence to pupal-type behaviour when the number of cells is four times as large as after the third ecdysis. In their interpretation, the acquisition of competence to develop into the pupal type may parallel the increase in cell number.

In vertebrates, adult long bone size are somehow dependent on the number of mitotically active cells in the original condensation. In turn, this number can often be related to the timing of the condensation process (Moss-Salentijn 1974, Kember 1978). If condensation is reduced below a critical threshold, skeletogenesis may not even begin (Hall and Miyake 2000). Goodwin and Trainor (1983) developed a physico-mathematical model for the possible dependence on the size (i.e., cell number) of the limb primordium of the number of cartilage condensations which will give rise to carpal/tarsal, metacarpal/metatarsal and finger/toe bone elements. This model aimed to prove the global nature of the limb field, hence the inapplicability of one-to-one correspondences between tetrapods limbs with different number of digits. Subsequent studies of limb development have rejected applicability of Goodwin and Trainor's model to the generality of tetrapods. In some cases it works. This is suggested, for example, by the fact that extra digits are known to occur occasionally in St. Bernards and other big dogs, but not in small size poodles (Alberch 1985).

Cell number at a critical stage may influence overall adult size. Björklund (1996) analysed growth in two closely related species of birds: the great tit (*Parus major*) and the blue tit (*P. caeruleus*). The former species is larger than the latter in all external traits, but this difference is not due to differences in growth rate and neither to differences in the time at which the offset of growth occurs. Size differences are already manifested upon hatching and remain so throughout ontogeny.

In principle, intraspecific and interspecific differences in organ or body size depend on differences in the number of cells, their size or both. Mammal data summarised by Stevenson, Hill, and Bryant (1995) point to a major role of cell number rather than cell size differences. These data include measurement of cells in the liver, thyroid, and renal epithelia and

red blood cells in mammal species spanning a big range in body size. Things, however, are different in *Drosophila*. Stevenson et al. (1995) – by comparing organ and cell allometry in the wing, eye and basitarsus of several Hawaiian *Drosophila* – found that cell size may contribute between one-third and two-thirds to evolutionary changes in organ and body size. This problem was also investigated experimentally. Partridge et al. (1994) studied replicated lines of *Drosophila* that had been maintained for five years at 25°C or at 16.5°C. Those kept at the lower temperature showed higher thorax length and wing area. The evolutionary effect of temperature on wing area was entirely a consequence of an increase in cell area, with a small effect on cell number in males only.

A minimum cell number is also required for regeneration. A square flat sheet excised from a *Hydra magnopapillata* polyp gradually rounds up, turning into a hollow spherical 'shell' with a continuous ectodermal layer outside and a continuous endodermal layer inside. This occurs, however, provided that the fragment is large enough. The smallest spherical shell that could be produced contained 300 epithelial cells (Shimizu, Sawada, and Sugiyama 1993).

Miniaturisation

Miniaturisation and Body Patterning

Miniaturisation may affect the expression or even the presence of a given body feature. Bateson (1894) wondered what the least size might be in which a given tooth can be present in a species which sometimes has it and sometimes not. His data were detailed enough to show that the least size of a tooth is different for different teeth and for different animals. Some minimum seems to exist for any body feature.

Before causing the total disappearance of a given feature, miniaturisation sometimes causes a loss of regularity. An example is the asymmetry in the distribution of the usually symmetrically arranged slit sensory organs in the tiny spider *Comaroma bertkaui*, just 1.6 mm long (Kropf 1998). A similar loss of symmetry does not occur in the dorsal bristle patterns of mites, even in those species, which are thousands, that are much smaller than *Comaroma*. One wonders if this difference only correlates with the recent miniaturisation of the spider, as opposed to an extremely long history of the mite lineage as one of very small arthropods. In other words, mites might have long since adjusted their bristle patterning mechanisms to their tiny size, whereas miniaturisation of *Comaroma* is probably a recent

event whose developmental consequences have not yet been assimilated well enough.

Similarly, the very small body size (1 mm total length) may explain the peculiar distribution of the setae on the body surface (the terga especially) of the larvae of some tiny scydmaenid beetles (e.g., *Cephennium* and its relatives). Instead of the setal pattern usually found in beetle larvae, with a few pairs of regularly spaced setae per segment (cf. page 36), in these scydmaenids the entire dorsal surface is nearly uniformly covered by a large number of very coarsely patterned microscopic setae. This is counterintuitive – one of the tiniest beetles having larvae with much more abundant setae than its larger relatives – but this may depend on the impossibility of the usual spacing signals between setae-producing cells (lateral inhibition effects?) to take place within too small a field.

Miniaturisation may have far-reaching consequences on an animal's biology (cf. Rensch 1959). Some body features (e.g., the nervous system) do not seem to be amenable to any arbitrary reduction in size. Consequently, there is less relative (not just absolute!) space for the reproductive organs than in a larger animal. In the female, this effect may be hard to accept, unless a suitable reproductive strategy is adopted. If there is no chance of producing a large number of eggs, as the total amount of cell material available for reproduction is very limited and the individual eggs cannot be arbitrarily small as other cell types could be, miniaturised females may adopt a *k* reproductive strategy – that is to produce very few but very large eggs, thus maximising the chance of individual survival of the offspring. This explains one more apparent paradox: why many miniaturised animals produce larger eggs than their non-miniaturised relatives.

Miniaturisation, Segments, and Cells

In both annelids and arthropods, miniaturisation is often (but not universally) coupled to a reduction in the number of body segments (oligomerisation). Examples among the annelids include the enchytraeid oligochaete *Marionina eleonorae* (16 segments, the lowest number in the family) and some 'polychaetes', such as *Parergodrilus* (8–9 segments), *Dinodrilus* (0.4 mm total length, ca. 9 indistinct body segments) and the Nerillidae (0.3–2 mm total length, 7–9 segments). It may not be by chance that the segment number 8, or its double, is 'nodal' in these miniaturised annelids.

In centipedes, segment number is invariable in the Lithobiomorpha, irrespective of size (up to 45 mm total length in *Eupolybothrus*, but only 3 mm in *Catanopsobius*), but it is often prone to oligomerisation in the

smallest representatives of the Geophilomorpha. A good example is provided by the genus *Schendylops*, which includes species in the 6–70 mm range of total length and 27–87 leg-bearing segments. The lowest segment numbers (27 or 29 in the males, 29 or 31 in the females) are found in the tiny *Schendylops oligopus*, which is also the smallest member of the genus. Interestingly, oligomerisation of very small geophilomorphs occurs in clades in which the number of segments is intraspecifically and interspecifically variable; but it does not occur in a clade (family Mecistocephalidae) in which intraspecific variability is virtually absent and interspecific differences are also very limited. In this group, a very small species, such as *Nannarrup hoffmani* (10 mm in length), has the same number (41) of leg-bearing segments as its closest relatives that are up to 75 mm long (*Anarrup* sp.; Foddai et al. 2002).

One may wonder if originally segmented animals undergo such a degree of miniaturisation that segmentation is actually impossible or useless. This is possibly true of the pseudosegmentation of many collembolans and some groups of mites, such as the eriophyids, whose total length may be no more than 100 μm. A similar consequence of small body size is perhaps the incomplete articulation (pseudoarthrosis) of some appendages: the tarsus in the *Monotarsobius* centipedes (Verhoeff 1902–25) and the labial palp in the tiny rove beetle *Atlantostiba franzi* (Pace 1994).

This indiscernibility in miniaturised animals of otherwise clearly articulated features raises a question of comparative method. In some of them, it may be impossible to identify the homologues of individual features that are distinct in their non-miniaturised relatives. In these dwarfs, there might not be an amount of tissue, or a number of cells, large enough to support specification of some of the developmental (and morphological) units.

The number of cells available in specific anlagen may explain the reduction or even the complete atrophy of some organs in miniaturised animals (Hanken and Wake 1993). The cells of the minute marine snail *Caecum glabrum*, only 1 mm long, are of approximately the same size as those of large marine snails such as the edible winkle *Littorina littorea* (Goetze 1938). We do not expect that the reduction in body size be accompanied by a corresponding reduction in average cell size, at least when miniaturisation is a phylogenetically recent event involving one or a few species in a lineage. Circumstances may be different in taxa with a long history of miniaturisation, as in the exceptional case of Loricifera, described previously, in which a dramatic reduction in cell size is the most obvious correlate of a very reduced body size.

CHAPTER EIGHT

Axes and Symmetries

> The essential unity of the phenomenon of Repetition of Parts and of its companion-phenomenon, symmetry, wherever met with, has been too little recognized, and needless difficulty has thus been introduced into morphology.
>
> W. Bateson 1894: 21

> [Co-option] is the fundamental process by which evolutionary change in bilaterian form has occurred.
>
> E.H. Davidson 2001:164

The Animal's Main Body Axis

Zoologists and lay persons alike would not hesitate in identifying the main body axis in animals such as an earthworm, a leech, a grasshopper, a fish, or a bird. However, difficulties will appear if one is asked to point to the main body axis of a sea star, a sea squirt, or a hydra.

What is an animal's main body axis? It is the longitudinal axis uniting the animal's fore and rear ends. The fore end coincides with the animal's head, where the mouth, brain, eyes, and other important sensory organs are located. The rear end, to the contrary, is reasonably identified with the site of the animal's anus and, perhaps, excretory and genital openings. In turn, the anterior position of both mouth and eyes, and the posterior position of the anus, correspond to the animal's polarity with respect to locomotion. The animal explores the environment with the aid of the anterior sensory organs and is ready to use its anterior mouth to exploit any food items its sensory organs might discover. As for the faeces, it is convenient to leave them behind in such a way as to minimise further contact with them. Corresponding advantages may be derived from a posterior location

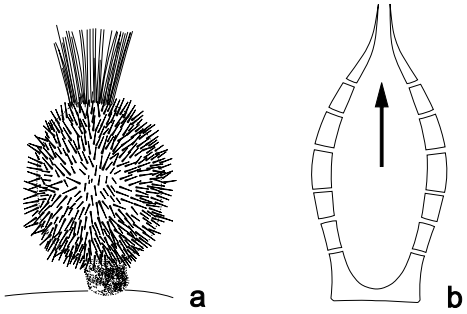

Figure 8.1. Habitus (a) and organisation (b) of the small sponge *Sycon*. The arrows marks the water flow towards the upper opening (osculum).

of both excretory and genital openings. Then where is the question concerning identification of the main body axis?

Polyps, Sipunculans, and Squids

Indeed, there are many questions. Some animals do not move at all. A sedentary life is a sponge's destiny, the same for a polyp or an ascidian. A few small sponges with only one osculum, such as *Sycon* (Figure 8.1), have a distinct polarity, with the osculum (the opening through which water leaves the sponge's internal cavity) being opposite the site of attachment to the substrate. It would be difficult to identify in the body plan of other animals an equivalent of this attachment site-osculum polarity of the sponge. Comparisons would be difficult even with a simple polyp, such as a *Hydra*, which lives attached to the substrate like a sponge, because *Hydra*'s only opening is a mouth, rather than an outlet for water and waste.

As for the body axes of polypoid animals, one must clearly distinguish between cnidarians, such as a *Hydra* and sea anemones, and sessile bilaterians, such as bryozoans and ascidians. In the case of cnidarian polyps, identification of a main body axis, one that goes straight through the oral and aboral poles, is quite obvious. The problem is, whether this axis may be meaningfully equated with the main body axis of a bilaterian or not (Henry and Martindale 1998, Gauchat et al. 2000). It may be equated, in so far as it goes through the mouth, which is not simply (anatomically and functionally) the opening through which food is introduced into the digestive cavity, but also (developmentally) a point (in cnidarians, *the* point) where ectoderm and endoderm meet – a boundary characteristically marked by the expression of a *Brachyury* homologue (Arendt, Technau, and Wittbrodt

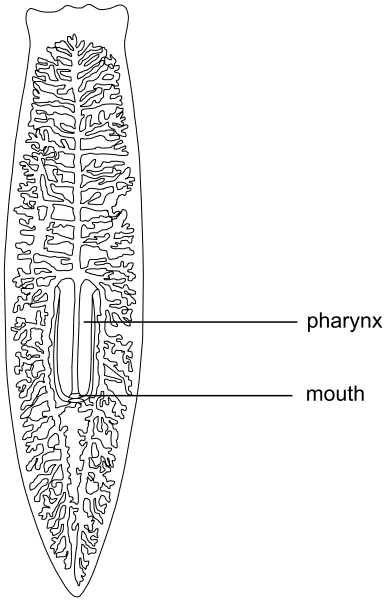

Figure 8.2. Schematic drawing of a planarian, with the outline of its extensively branched gut. The mouth opens on the ventral side, far from the anterior end of the body.

2001). But the similarity between cnidarians and bilaterians ends here, because the main body axis of most cnidarians supports a radial symmetry, whereas the main body axis of the vast majority of the bilaterians lays within a single plan of bilateral symmetry.

Ironically, there are many bilaterians in which is it difficult to recognise the equivalent of that main body axis which seems so obvious a prominent feature in the body plan of the average bilaterian. It is more difficult than comparing the average bilaterian to a cnidarian.

In planarians, for example, the ventral mouth is very far from what is called the animal's fore end, and no anus exists (Figure 8.2).

Things may seem easier in sipunculans (Figure 8.3), whose vermiform, broadly cylindrical shape invites comparison with other vermiform, cylindrical bilaterians such as earthworms or caterpillars. At one end of the sipunculan's body, there is the mouth, as expected. The anus, however, is not at the other end of the body, or close to it. It opens, instead, quite close to the mouth. The first half of the fairly long gut of the sipunculan goes from the mouth to near the opposite end of the sac-like body, but then, rather than finding its way out, it coils back onto itself until it reaches the oddly

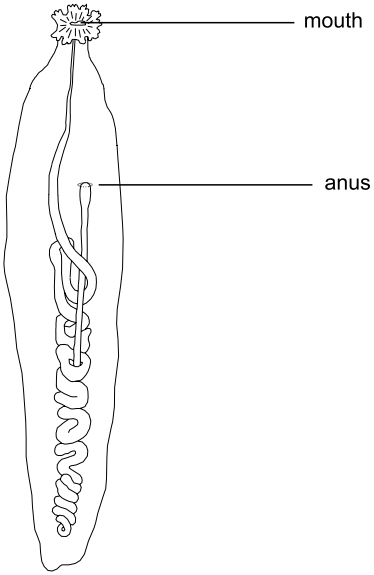

Figure 8.3. A sipunculan and its coiled digestive tract.

placed anus. The question then is how to identify the sipunculan's main body axis? In regard to its external shape, the main body axis would be the longitudinal axis of the body cylinder, as in the earthworm. The posterior end of the sipunculan's body does not mark the position of the anus. Yet by identifying the main body axis of a sipunculan with the segment spanning between the mouth and anus, we would get something else from what is suggested by the animal's external shape.

Basically, the same happens in all polypoid bilaterians. In these organisms, the aboral pole (i.e., the pole opposite the mouth) marks the site of attachment to the substrate, whereas the anus – following the U-shaped or otherwise coiled development of the gut – is in the vicinity of the mouth.

True polyps are sessile, but something in their gross anatomy is shared by some of the most active and speedy-moving invertebrates (i.e., squids and allies). In cephalopods, the digestive tract is also U-shaped and the anus, together with the genital and excretory openings, is placed close to the mouth. These unusual spatial relationships did not go unnoticed in the early heroic times of comparative anatomy, those of the great debate between Georges Cuvier and Étienne Geoffroy Saint-Hilaire (Appel 1987). Two pupils of Geoffroy, Meyranx and Laurencet, had hopes, like their teacher, of reducing the body plans of the most diverse animals to a

common scheme in which the dorsal side of the vertebrates was equated with the ventral side of the arthropods. Briefly, Meyranx and Laurencet suggested that to compare squids with vertebrates, you just have to imagine the straight body of the latter bent onto itself by dorsally turning the posterior half forward. In this way, the anus is brought to the same level as the mouth, a comparison that Cuvier (1830), not surprisingly, outright rejected. The comparison suggested by Meyranx and Laurencet still deserves attention – not literally, of course. But the standard textbook scenario we offer to our students to explain the evolution of cephalopods from a monoplacophoran-like ancestor retains, from their courageous speculations, much more than we could readily admit. We are accustomed to say that the functional (external) antero-posterior body axis of a squid is derived from the original dorso-ventral axis of primitive molluscs, whereas the former antero-posterior axis is now reduced to the short span between the mouth and the opening of the mantle cavity, where the anus is placed. This is a correct or, at least sensible, suggestion. This suggestion implies uncoupling of the *functional* polarity of the body, corresponding to the long axis of the squid's cartilaginous pen and the *anatomical* polarity suggested by the positions occupied by the mouth and anus. I will argue that this dichotomy, no less than the inverted dorsal/ventral relationships in vertebrates and arthropods, is probably intrinsic to the animal's developmental rules, rather than to our descriptive frames.

Less extreme positional relationships, but still somewhat embarrassing when we try to define the animal's main body axis, are much more frequent than one might suppose – take for example the leeches (Figure 8.4). Following the external shape, the rear end of the body is given by the posterior sucker, because the anus opens dorsally, just before the sucker (an obviously good position, in functional terms). Determining where the main body axis actually terminates is complicated by the fact that the posterior sucker is formed by segments XXVII–XXXII, whereas the anus, despite its actual position, would originally occur at the end of the last (XXXII) body segment.

There are problems for the vertebrates as well. Not so much in the case of a bird, whose external 'tail', made of long feathers, does not obviously belong to the anatomical main body axis, but in the case of all vertebrates provided with a true tail, be it neatly separated from the trunk as in all tailed mammals, or less so as in fishes and snakes. The gut does not run through a vertebrate tail, which is therefore wholly posterior to the anus. An obvious alternative to regarding the tail as a part of a vertebrate's main body axis is to regard it as an appendage (see page 156).

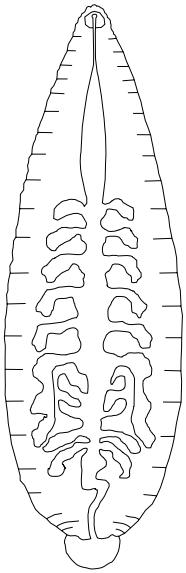

Figure 8.4. Schematic drawing of a glossiphoniid leech, with an outline of the digestive tract. In this example, there are 6 + 4 pairs of segmentally arranged side branches.

The Dual Animal

This long discussion of body axes should not be taken as a futile exercise in abstract morphology (promorphology), such as those in some zoological literature of the late nineteenth and early twentieth centuries. It serves instead as an introduction to one of the basic concepts that may help us understand the origin and nature of animal body patterning.

This concept is that of 'dual animal'. I am borrowing the term from Romer, who introduced it in a slightly narrower context (Romer 1972).

Romer's focus was on vertebrates. He speculated about the possible coexistence, in the trunk of these animals, of two largely independent segmental systems: one basically ectomesodermal ('somatic') and the other mainly endodermal ('visceral'). In other words, he contrasted skeletal segmentation (with the associated serial arrangement of spinal nerves, basically related to the seriality of the myotomes), with the segmentation of the gill slits (together with associated serial features such as the visceral arches, the muscles that move them and the nerves that supply this region). Romer further speculated about a possible relationship between this double segmentation and the neural dualism, with the autonomic 'visceral' system contrasting with the central 'somatic' nervous system. Romer's concept of

dual animal went practically unnoticed in mainstream zoological literature, except for Jefferies (1986).

I will generalise this notion of 'dual animal' to contrast the somatic ectomesodermal system with the visceral endodermal system of triploblastic animals generally. The reason for stressing this contrast is the large independence these two systems show in patterning. Mutual relationships between the two 'animals' are obviously expected and are positively known, but the dialogue between the two does not obscure the large independence of which either benefits in development and patterning.

The cases of cephalopods, sipunculans, and polypoid bilaterians are remarkable because they are conspicuous and far from exceptional. Think of flatworms such as the common freshwater planarians, with their mouth opening on the ventral side, about mid-length (with respect to the 'somatic animal'), and one of the three main intestinal branches extending forwards in front of the mouth. Think of gastropods, which are worthy of a critical reappraisal of the relative contributions of each of the two 'animals' to the torsion of the visceral sac.

The functional independence of which an earthworm's gut takes advantage in respect to the external epithelio-muscular sheath is an expression of the visceral/somatic dualism of bilaterians. It is not by chance, that in leeches, where the coelomic cavities are reduced and the gut is surrounded by a solid mesodermal mass, rather than by a thin peritoneal cell layer, the dialogue between the somatic and visceral animal is much more intense than in the case of earthworms. This has been documented experimentally (Wedeen 1995, Wedeen and Shankland 1997), but we do not need to look for gene expression patterns. The presence in many leech families of metamerically arranged gut coeca (Figure 8.4) is macroscopic proof of the patterning influence exercised by the segmentally arranged mesoderm on a 'segmentally naive' endoderm. Among the possible effects of a strict dialogue between ectomesoderm and endoderm are also the so-called gastropore (a secondary external opening of the digestive tract) of *Gastrostomobdella* and other leeches (Sawyer 1986), the genito-intestinal channel of some flatworms (Hyman 1951) and the branchial pharynx of enteropneusts and chordates.

Buss (1987) was probably correct when suggesting that gastrulation (process by which a first separation of germ layers is accomplished) first developed in early metazoans as a consequence of the divergence between an external lineage of ciliated undividing cells and an internal lineage of nonciliated cells that retained the potential to undergo mitosis. No wonder

that such a primary distinction, one of survival value for a multicellular organism otherwise at risk of mutiny from any of its own cell lineages, fell under the control of maternal determinants to the point that the two primary cell layers (the ectoderm and the endoderm) can be said to be predetermined in the egg (Hall 1998b). What matters here, in the perspective of the developing body architecture, is that a large degree of independence in the morphogenetic role of ectodermal versus endodermal derivatives continued to be advantageous, even to animals whose embryos became very different from those early multicellular systems in which something comparable with gastrulation first evolved. One wonders, in this context, whether one of the advantages provided by the third germ layer (the mesoderm), when it first appeared in a developing animal, was perhaps that of furthering and supporting the morphogenetic independence of ectodermal and mesodermal derivatives, even if, in due course, it becomes a third, largely autonomous player or, otherwise, a mediator of ectodermal–endodermal interactions. But let us return to our dual animal.

Many animals, especially those living on plant food, need a long digestive tract, but there might be constraints on their external shape (in particular, on their overall length), such that the gut cannot simply run straight from the mouth to the anus (if the anus lies opposite the mouth), parallel to the main body axis of the 'somatic animal'. A coiled gut is the obvious solution – one adopted by animals as diverse as vertebrates, insects, and molluscs. This is a solution that does not easily suggest the fundamental independence of the two systems, visceral and somatic, as shown instead by sipunculans and polypoid animals. But the difference between these latter animals and a vertebrate, insect or mollusc with a coiled gut is just matter of degree. We must accept that the functional interdependence of endodermal and ectomesodermal derivatives does not imply a strict interdependence in morphogenesis.

Sipunculans and *ParaHox* Genes

One might speculate whether this duality is mirrored by the phylogenetic old splitting of a proto-*Hox* gene complex into two paralogous complexes: the *Para-Hox* genes and the *Hox* genes in the strict sense (Kourakis and Martindale 2000). Whereas the *Hox* complex seems to have evolved in association with the patterning of some component of the ectomesodermal 'somatic animal', there are suggestions as to a primary role of the *ParaHox* complex in the patterning of the endodermal 'visceral animal'

(Brooke, García-Fernàndez, and Holland 1998),based on experimental evidence from leeches, arthropods, amphioxus and vertebrates (Duprey et al. 1988, Wright, Schnegelsberg and De Robertis 1989, Calleja et al. 1996, Offield et al. 1996, Brooke, García-Fernàndez, and Holland 1998). This suggestion is perhaps supported by the reduction or 'degeneration' of the *ParaHox* complex in some animal groups in which the influence of the *Hox* gene-patterned 'somatic animal' onto the 'visceral animal' is particularly evident. In this sense, I think, we shall interpret the different degree of conservation or diversification of the *ParaHox* gene complex in different phyla. Phylogenetic parsimony suggests that the common ancestor of protostomes and deuterostomes had three *ParaHox* genes (*Gsx*, *Xlox*, and *Cdx*). Three *ParaHox* genes have been cloned from a variety of deuterostomes, but (with one exception) all those protostomes for which information is available have either one or the other, or two at most, of the three genes. This might be expected at least in animals such as the leeches, in which we observe a strong patterning influence on the endoderm of the *Hox* gene-patterned ectomesoderm. But what about sipunculans, with their long gut freely coiling onto itself in the tissue vacuum of the large body cavity? We should expect these worms to have conserved all three *ParaHox* genes. They have, indeed. Ferrier and Holland (2001a) found that sipunculans are the only protostomes known to date to possess a full complement of *ParaHox* genes. This puts them on similar footing with deuterostomes such as vertebrates and echinoderms, where the patterning of the gut [see, e.g., sea urchins and holothurians; Figure 8.5 (but also think of our own viscera)] is obviously independent of the patterning of the body surface. In insects, the digestive tract comprises three parts, of which the first (foregut or stomodaeum) and the last one (hindgut or proctodaeum) are of ectodermal origin, whereas the intermediate part (the midgut or mesenteron) is of endodermal origin. Interestingly, for an interpretation of the insect organisation in terms of the dual animal hypothesis, mutations are known in *Drosophila* which affects left-right polarity of the gut with separate effects on the fore-, mid-, and hindgut (Hayashi and Murakami 2001). Data on the embryonic expression patterns of these genes is clearly needed to check the value of my hypothesis.

Morphological Versus Functional Polarity

To return to the opening question of this chapter (What is an animal's main body axis?), it seems sensible to me to identify it with the main longitudinal axis of the 'somatic animal', irrespective of the concordant

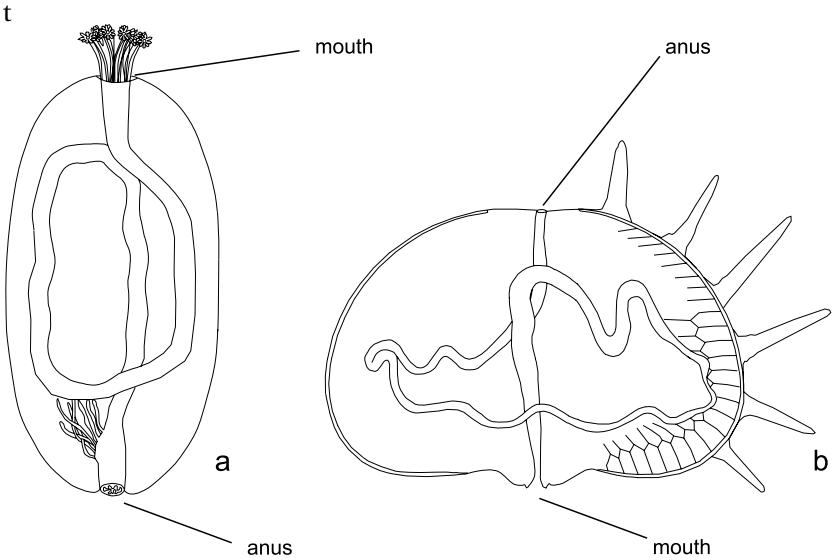

Figure 8.5. Simplified drawings of holothurian (a) and sea urchin (b) anatomy, highlighting their digestive tracts.

(e.g., earthworm) or discordant (e.g., sipunculan) behaviour of the 'visceral animal'. This is what we do in practice when we say that the mouth of a planarian, or the anus of a sipunculan, is not placed at the anterior or posterior end of the body, but somewhere 'at mid-length'. The polarity of the somatic animal will be defined, in turn, in terms of the direction of locomotion, if the animal is mobile. If it is sessile, it is sensible to define its polarity with respect to its relation to the substrate; but this polarity, especially in the case of polypoid bilaterians, is clearly a secondary one. Specification of the antero-posterior polarity of the 'somatic animal' in a polypoid bilaterian, such as *Phoronis*, seems to be quite labile (Freeman 1991).

The functional polarity of an animal's main body axis, be it the axis of the somatic animal or that of the visceral one, is defined by the relationship between the animal and its immediate environment. This is most obvious for the polarity of the somatic animal, but the same is also true for the visceral one, with the polarity determined in this case by movement of the progressively digested food, or its faecal remains, along the intestinal tube.

A question thus arises: Is this functional polarity, defined in relational terms, underpinned by an *intrinsic* polarity of the animal?

The answer is yes, at least in some cases. Think, for example, of a vertebrate's somites or an earthworm's segments. These serial units are

produced one after the other, in a progression that strictly follows the conventional antero-posterior axis of the 'somatic animal'. In this case, the polarity-specifying reference does not reside in the interactions with the external world, but flows directly from the progression of the animal's own developmental time. Time's arrow, in a sense, directly translates into spatial polarity.

There are many other ways in which time factors may determine spatial polarity. Developmentally 'higher' regions (i.e., regions from which signals are sent to developmentally 'lower' regions) may be those with longer cell cycles, in which the longest primary transcripts of genes full of long introns can be produced – something forbidden to cells with too short cell cycles (Rothe et al. 1992).

Cartesian Axes, or Not

Cartesian co-ordinates dominate animal morphology and, to a large extent, developmental biology as well. This attitude is quite naive and unsatisfactory, even for bilaterians, not to speak of radially symmetrical cnidarians.

One of the major problems with the conventional descriptions is represented by the third axis that would complement the two less controversial ones (i.e., the antero-posterior and the dorso-ventral ones). Most people would recognise, as a third element, a left-right axis; but it is easy to show how this is incorrect. Antero-posterior and dorso-ventral axes are unidirectional, whereas the so-called left-right axis is bidirectional. The first two axes identify the single plan of symmetry, which divides the animal into two mirror halves. If we wish to single out a third Cartesian axis, we need to consider only one-half of the animal without its mirror duplicate. In this way, we discover the 'inside-outside axis', which proceeds out of the mirror symmetry plan perpendicular to it (Coen 1999). In no case is this axis strictly equivalent to others. However, along this axis, there is no need to specify positional values as it is along the other two axes. Eventual specification of the two semi-axes (midline-to-left and midline-to-right), giving rise to directional asymmetry, is something other than axis specification or patterning. I will return to this point later on in this chapter.

My unease with the traditional Cartesian descriptions in terms of the three axes of the body (or of an appendage) is not restricted to the lack of equivalence between these axes. More generally, I believe that these are much more figments of our imagination than biological reality. In some

instances, these axes are not functionally separate in their roles, and cellular addresses and their interpretations cannot be so easily schematised as usually assumed (Newman 1996).

In the proximo-distal patterning of the vertebrate limb, several *HoxA* and *HoxD* genes are involved jointly. However, the progressively restricted expression domains of progressively more 5′ genes of the two classes translate into progressively posterior-distal regions of the developing limbs (Gardiner et al. 1998), rather than into 'pure' antero-posterior or proximo-distal values. This continues into later limb bud stages, where skeletal elements are differentiating proximally and limb differentiation is about to commence distally; the expression of *Hoxd11–13* moves towards more proximal *and* anterior positions at the same time (Nelson et al. 1996). A general multiaxial patterning system was suggested by Dollé et al. (1993), following the observation that mice mutants for the last gene in the *HoxD* complex (*Hoxd13*) display skeletal alteration affecting all 'Cartesian' body axes.

In *Drosophila*, we have proof of the simultaneous involvement of one gene in the patterning of the antero-posterior axis and the dorso-ventral axis (Munn and Steward 1995). This depends on events during mid-oogenesis, when the nucleus of the oocyte, then in a posterior position within the ovariole, sends a chemical signal (the product of the *gurken* gene) to the nearest follicle cells causing them to assume a posterior identity. At a later stage, the oocyte nucleus, now positioned at the anterior end of the oocyte, sends a second Gurken signal to the follicle cells, which then acquire a dorsal identity. These inductive events are mediated by the product of another gene, *spindle*. The final polarity of the oocyte and the embryo is thus influenced by the asymmetries in the surrounding follicle cells (Anderson 1995, Gonzalez-Reyes, Elliott, and St. Johnston 1995, 1997). It is possible that *gurken* is not directly responsible for the establishment of cell fates along a body axis, but restricts and orients a later axis-forming process (Roth and Schüpbach 1994).

A role in the regulation of growth or patterning along more than one axis has also been postulated, in *Drosophila*, for genes involved in signal integration, such as *vestigial* (Kim et al. 1996).

Similar to what happens along the main body axis, a multiaxial patterning system is also at work along the appendages of *Drosophila* (Lecuit and Cohen 1997).

Living organisms, or their parts, are sometimes described with reference to co-ordinate systems other than Cartesian. Polar co-ordinate systems, in

particular, have often been applied in describing the organisation of the ciliate cell (e.g., Frankel 1989) or the fate map of insect imaginal discs (e.g., French, Bryant, and Bryant 1976, Bryant 1993, Couso, Bate, and Martínez-Arias 1993). Readers may be reassured about the meaningfulness of polar co-ordinate systems when reading about genes that might be involved in their specification. In *Drosophila*, *wingless* is initially associated in the embryo with a pattern of stripes along the antero-posterior axis that fits well into a Cartesian co-ordinate system; but later, during development of the imaginal discs, expression of the same gene is associated with a pattern of sectors establishing a polar co-ordinate system (Couso, Bate, and Martínez-Arias 1993). Again, in *Drosophila*, Wilkins and Gubb (1991) proposed that, in the imaginal discs, segment polarity genes might specify the angular component of the polar co-ordinate system. Held (1993) tested this hypothesis by predicting that mutations in segment-polarity genes should cause abnormal patterning within precise sectors of the imaginal discs. This was accurately confirmed by the defects (deletion of specific rows of chemosensory bristles or an abnormal increase in their number) found in specific sectors of the first tarsal segment of the second pair of legs in segment-polarity mutants.

Cartesian geometry still dominates animal morphology, despite the fact that a straight antero-posterior axis is a poor reference when describing the anatomy of snails, squids or bryozoans. One may be skeptical before the deformed reference grids used by D'Arcy Thompson (1942) or by modern morphometricians (e.g., Bookstein 1991) to help comparisons between body outlines of more or less strictly related animals; but the capricious curves in these grids may be less arbitrary than straight lines crossing at right angles, with the precision worthy of a geometry textbook.

Straight-jacketing different animals into a universal geometrical framework may suggest non-existing correspondences. For example, Hidalgo (1998) compared the embryonic expression of *engrailed* in *Drosophila* to that of *En-1*, one of his vertebrate homologues. Whereas the former gene is expressed in transversal stripes marking the anterior/posterior compartment boundary, the latter is expressed along a longitudinal stripe running the length of the embryo. In Cartesian terms, this can be described as if the expression of *En-1* in vertebrates is shifted by 90°, compared with its equivalent in *Drosophila*. But what is really meant by this?

Completely different from these geometrical tricks is Geoffroy Saint-Hilaire's (1822) old hypothesis, according to which the dorsal side of a vertebrate is equivalent to the ventral side of an arthropod. Soon after

Arendt and Nübler-Jung (1994) revived this hypothesis in terms of molecular developmental genetics, extensive experimental data (e.g., Holley et al. 1995, Biehs, François and Bier 1996, De Robertis and Sasai 1996) provided support for this view by demonstrating the phylogenetic conserved function of the *Drosophila short gastrulation* and *decapentaplegic* genes and the homologous *Xenopus chordin* and *Bone Morphogenetic Protein-4* (*BMP-4*) genes in subdividing the primitive embryonic ectoderm into neural versus non-neural domains – but at opposite sides (dorsal and ventral) in the two kinds of animals. In most bilaterians, the initial dorso-ventral polarity of the body is established in the ectodermal derivatives only, through the interaction of proteins from the neural side with morphogens (BMP-like) diffusing from the opposite side; this is as true of *Drosophila* as it is of amphioxus (Holland and Holland 1999). In the vertebrates, a parallel BMP-based system has evolved in the mesoderm (Graff 1997, Hemmati-Brivanlou and Melton 1997), so that dorso-ventral polarity is established in both germ layers simultaneously.

The Syntax of the Body

Despite the sheer diversity of body plans evolved in the different animal lineages, there are some invariant features, in their body architecture, which go far beyond the obvious requirements of functional design. For example, why did no fish species evolve paired fins at a trunk level posterior to the anus (Coates and Cohn 1999)?

Another generally overlooked feature of animal body syntax is that, in hermaphrodites and in gonochoric animals, where male and female genital openings do not occur in identical position, the female gonopore or the female gonad is nearly always anterior to the male gonopore or gonad (clitellate annelids seem to be an exception to this rule). In this context is the organisation of the genital imaginal disc of *Drosophila*, which gives rise to the genital and anal structures of the adult fly. This disc is multisegmental, in that it comprises cells from three abdominal segments: A8, corresponding to the primordia of the female genitalia; A9, corresponding to the primordia of the male genitalia; and A10, corresponding to the primordia of anal structures (Casares et al. 1997). This multisegmental organization preserves the anterior position of the female with respect to the male genitalia.

There is no key to explain all these hard-wired points of animal anatomy. I hope that reviewing them in the context of this book may attract attention

to these overlooked features, which have perhaps a developmental, rather than an adaptive, explanation.

To answer these kinds of puzzles, we need not only the practical tools of molecular developmental genetics, but also the theoretical tools of updated comparative morphology. Some of these concepts have already been discussed in these pages (e.g., the distinction between a somatic and a visceral animal), and other concepts (such as axis paramorphism and double segmentation) will be introduced later.

Most important in the analysis of body syntax is distinguishing, as much as possible, between appendages and main body axis.

How this distinction applies to echinoderms is not an easy question (e.g., Hotchkiss 1998, Popodi and Raff 2001). The problem of identifying the antero-posterior axis of these animals has been recently attacked by Peterson, Arenas-Mena, and Davidson (2000), using different lines of evidence, including palaeontology, comparative skeletal anatomy and the expression pattern of a posterior class *Hox* gene in the coelomic cavities of the adult primordium. In their interpretation, the antero-posterior axis runs from the mouth through the adult coelomic compartments. This and other considerations lead these authors to the conclusion that the five rays of these animals are not primary (equivalent) body axes as some zoologists had speculated, but outgrowths of the one main body axis. But what about the tail of a vertebrate? Is it an appendage or part of the main body axis?

What Is a Tail?

In vertebrate comparative anatomy, there has been a long controversy on whether the tail forms by a developmental mechanism distinct from gastrulation, which is responsible for the formation of the trunk. The verdict seems to be still out (Kanki and Ho 1997). Nevertheless, the large majority of vertebrate zoologists would not hesitate to regard the tail as the posterior part of the animal's main body axis. I am not ready to subscribe to this traditional view.

A first argument is, that the tail, like the paired limbs of vertebrates, is only made of ectodermal and mesodermal derivatives. No endodermal component extends into it.

A second argument is that, in frogs, treatment with retinoic acid can switch the identity of a tail blastema so that it gives rise to limbs rather than to a new tail (Mohanty-Hejmadi, Dutta, and Mahapatra 1992, Maden 1993, Brockes 1997). This suggests that the tail, like the limbs, is an appendage rather than a part of the main body axis.

Extrapolating from this experiment straight to the deep phylogenetic history of the chordates is perhaps too hazardous, but there was a segmented tail prolonging a short unsegmented body in Romer's (1972) putative vertebrate ancestor. This hypothetical animal (Romer's 'somatico-visceral animal') is not that different from the tadpole larva of a sea squirt, or from one of those Palaeozoic calcichordates that Jefferies (1986) interpretes as ancestors of the vertebrates.

The most conspicuous component of a segmented tail is, of course, a segmented musculature, whose functional advantages do not need be stressed. One might easily conceive that the segmental musculature of Romer's hypothetical vertebrate ancestor was accompanied by segmentally arranged nerves. This might have been the case. In the tadpole larva stage of the ascidian *Halocynthia roretzi*, Wada, Holland and Satoh (1996) found a spatially iterative expression (15 'segmental' spots) of a gene (*HrPax-37*) homologous to two vertebrate genes (*Pax-3* and *Pax-7*) which function in the differentiation of the dorsal neural tube. Also relevant in this context is Crowther and Whittaker's (1994) finding of serial repetition of pairs of cilia along the epidermis of the tail of another ascidian larva (*Ciona intestinalis*).

In metazoans with dorsal neural cord, such as the chordates, it would be easy to extend the neural axis of the trunk straight into the tail (i.e., into a posterior appendage obviously dorsal to the anal opening). This topographical circumstance places the tail in a privileged condition, relative to both the lateral appendages and the posterior appendage of animals with ventral neural cord, such as the telson of arthropods.

It is easy to think that a neural continuity between trunk and tail facilitated morphological assimilation between trunk and tail. If vertebrates are derived from a tunicate-like ancestor (perhaps from neotenous forms comparable with the tadpole larva of present-day ascidians), then this assimilation implied the forward extension of the notochord from the tail to the trunk. This event paved the way to the development of the skeletal (vertebral) axis which evolved to replace the notochord, both morphologically and functionally. On the other hand, the tail-to-trunk assimilation did not proceed so far as to cancel the tail's appendicular nature, as the tail did not acquire any endodermal derivative, including coelomic pouches (chordates are enterocoelic) and the viscera associated with them. But there was, apparently, no difficulty in extending to the trunk the segmental character originally associated with the musculature and the nervous system of the tail.

An indirect argument from developmental genetics potentially supporting this hypothesis of morphological assimilation is that no *Hox* gene has an anterior boundary of expression in the tail (Prince et al. 1998).

One more argument in favour of the appendicular nature of the vertebrate tail is the structural plasticity it may exhibit during postembryonic life. Salamanders may add tail vertebrae continuously through life, as observed by Noble (1931) in the plethodontid *Batrachoseps attenuatus*, in which a young, but completely formed, specimen with a snout-to-vent length of 2.3 cm had 28 caudals and an adult with a snout-to-vent length of 4.75 cm had 61 caudals; the number of dorsal vertebrae was 22 in both. The reverse phenomenon (reduction and fusion of tail vertebrae) is typical of metamorphosing frogs.

It is thus possible to answer Coates and Cohn's question: no fish evolved fins caudal to the anus because the posterior body region, the tail, is simply (or mainly!) an appendage, like the fins themselves.

The Time Arrow of Growth and Differentiation

Along the main body axis, morphogenesis usually proceeds in an anteroposterior direction, along the appendages in a proximo-distal direction. This is the way vertebrates produce somites and annelids produce segments. This is also the way teloblasts produce their rows of progeny cells. We could even say, following Dollé et al.'s (1993) reinterpretation of Wolpert's (1969, 1989) concept of positional information, that the anteroposterior axis of the trunk and the proximo-distal axis of the appendages are spatiotemporal axes defined by the progression of growth and patterning. The notion of the *Hox* code (Kessel and Gruss 1990) has clearly played a major role in the recent interpretations of this space-time correspondence. As all broad generalizations, however, this time-space correlation in morphogenesis has its exceptions.

First, the posterior area, or group of cells, from which new body parts are progressively formed, is often not strictly terminal, but subterminal. The posterior terminus is often one of the first body features to be specified, and this is important, especially if the animal will engage in active life even before its main body axis is completed. This is the case, for example with crustacean nauplii and the early postembryonic stages of millipedes and anamorphic centipedes (*Lithobius*, *Scutigera*).

Second, embryologists have long since identified, in some animal groups, nonterminal differentiation centres from which differentiation proceeds

both ways (i.e., in a rostral, as well as in a caudal, direction). This has been also seen, more recently, in the temporal changes of the expression patterns of some 'developmental genes', such as *engrailed* in the grasshopper *Schistocerca* (Patel, Kornberg, and Goodman 1989).

The Beginnings of Animal Polarity

Symmetry-breaking events that establish the antero-posterior and/or dorso-ventral polarity of a developing animal may occur during oogenesis, during fertilization or later.

In *Drosophila*, as described previously, the two main axes of the body are established during oogenesis, when the oocyte comes to lie posterior to the nurse cells and signals through the Gurken/Egfr pathway to induce the adjacent follicle cells to adopt a posterior fate. This directs the movement of the egg nucleus and associated *gurken* mRNA from a posterior to an anterior corner of the oocyte, where Gurken signals for a second time induce the dorsal follicle cells, thereby polarising the dorso-ventral axis (Gonzalez-Reyes, Elliott, and St. Johnston 1997, Micklem et al. 1997).

In *Caenorhabditis elegans*, as in many other animals, the primary spatial cue for antero-posterior polarity is derived from microtubules emanating from the sperm asters (Wallenfang and Seydoux 2000).

Early specifications are not necessarily definitive. In the nemertean *Cerebratulus lacteus*, the dorso-ventral axis is set up prior to the first cleavage division, but blastomeres isolated at the two-cell stage will regulate to form normal, although miniature, pilidium larvae (Henry and Martindale 1997). In this case, polarity is obviously established through extensive cellular interactions. One is reminded of the complete repatterning of the egg material in polyembryonic wasps (page 115) and diblastodermic annual fish (page 129).

For a long time it has been believed (see Gurdon 1992) that, in the mammalian embryo, establishment of the primary body axes is much delayed with respect to the other animals and may even happen after implantation. However, a correlation has been demonstrated recently between the asymmetry of the fertilised oocyte, as defined by the position of the second polar body and the entry point of the sperm, and the orientation of the animal-vegetal and embryonic-abembryonic axes in the preimplantation blastocyst. In turn, the animal-vegetal axis of the blastocyst does possibly correspond to the antero-posterior axis of the future gastrula (Tam et al. 2001).

Conventional descriptions following a Cartesian frame of mind acknowledge that the first axis to be formed is not always the same, more

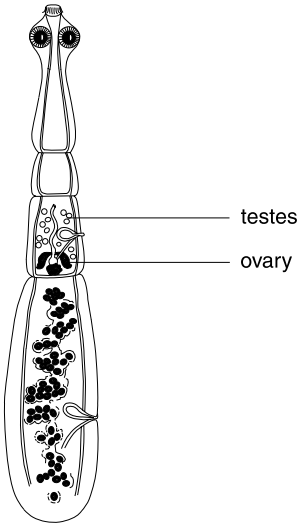

Figure 8.6. A tiny adult tapeworm (*Echinococcus granulosus*). In the conventional interpretation of tapeworm polarity, with the scolex as the worm's fore end, the testes – within each proglottis – are anterior to the ovaries.

frequently the antero-posterior one, but sometimes (as in mammals) the dorso-ventral one. In any case, the first axis of the embryo is formed in very different ways in different species (e.g., Gurdon 1992, L.Z. Holland 2000), following what in a first instance are probably generic cues, such as the sperm entrance point, or gravity, as in the chicken (Eyal-Giladi 1969).

Tapeworm Polarity

Growth and differentiation are sometimes intercalary rather than (sub) terminal. In some instances, this circumstance may even obscure the 'real' antero-posterior polarity of the animal, as in the case of the tapeworms. All modern textbooks describe these animals as provided with an anterior scolex and a posterior chain of proglottids (Figure 8.6), but is this description really correct? Tapeworm polarity was debated in several papers at the beginning of the twentieth century. Directionality in locomotion – the most obvious test of antero-posterior polarity for a bilaterian – does not apply here, due to the sessile habit of tapeworms. It is the same for the polarity of the 'inner animal', as tapeworms lack a mouth and digestive tract. More useful is the position of the growth zone. Comparative arguments would suggest that this is posterior (subterminal) rather than anterior (immediately following a 'head'), thus rejecting the conventional interpretation of

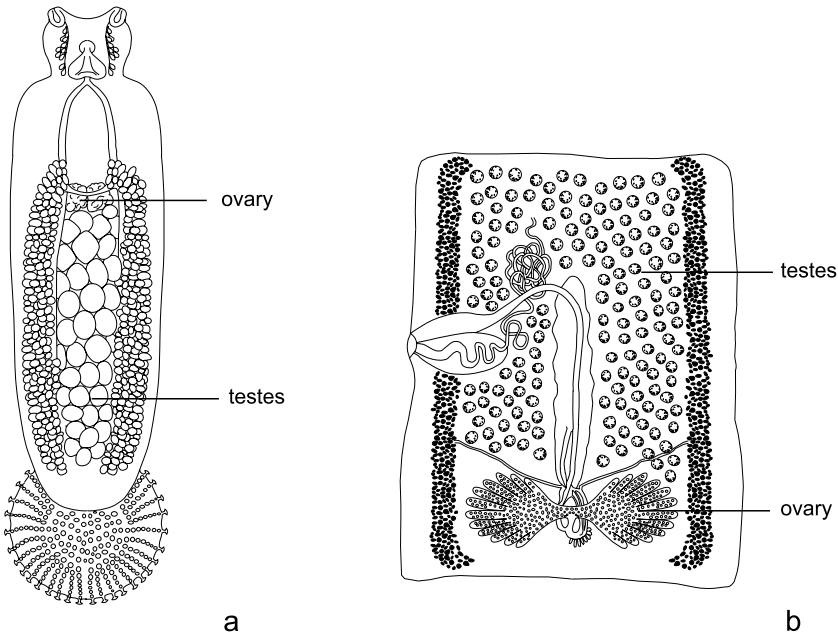

Figure 8.7. In monogeneans, a group of flatworms closely related to the tapeworms, ovaries are anterior to the testes (a, the monogenean *Acanthocotyle*; reproductive organs are highlighted). In tapeworms, the arrangement would be the same, if the conventional polarity (b, a proglottis of a tetraphyllid tapeworm) is reversed back-to-front. (Redrawn after Hyman 1951.)

tapeworm polarity. Thus, the scolex would be the posterior, rather than the anterior, end of the body. This was the main argument of Cohn (1908) and Watson (1911), whose views have been long forgotten. One would object to having an animal with reproductive organs at the fore end, but this is not that different from the anatomy of free-living flatworms, such as planarians (reasonably close relatives of the tapeworms), in which the ovaries are very close to the body's anterior end (much more rostral than the mouth). Arguments from body syntax may go further. *In each tapeworm proglottid*, the testes are in front of the ovary (if we take the scolex as the anterior end of the worm). This is contrary (e.g., Hyman 1951) to what happens in free-living flatworms and, more important, in the monogeneans, which are the sister group of the tapeworms. This contrast disappears as soon as we regard the scolex as the posterior end of the tapeworm (Figure 8.7).

Differentiating Back to Front

In some organs, differentiation progresses against the basic antero-posterior trend. In others, progression is bipolar, as in *Hydractinia echinata*. In this colonial hydroid, during the embryonic and larval stages of development, a distinctly decreasing spatio-temporal pattern of cell proliferation is observed. Cells at the posterior pole of the elongating embryo are the first to show arrest in S-phase, followed by those at the anterior pole. The quiescent cells at the two body extremities will give rise to the terminal structures of the polyp: hypostome and tentacles from the posterior part of the larva, basal plate and stolon tips from its anterior part (Kroiher, Plickert, and Müller 1990). In most cichlid fishes, the lateral line's trunk canal grows bidirectionally: the anterior part in the antero-posterior direction and the posterior part in the opposite direction (Webb 1990).

In the decapod crustacean *Penaeus setiferus*, the antennular anlagen appear after the antennal and mandibular ones (Heldt 1938). In caridean decapods (shrimps), legs typically appear in antero-posterior succession; but in some families (Alpheidae, Hippolytidae, Palaemonidae), leg V may appear before legs III and IV (Schram 1986). In some stomatopod crustaceans, the appendages of the posterior body region (pleon) differentiate before those of the middle body region (pereion; Schram 1986). In a dozen cases of 250 copepod species covered by his survey, Ferrari (1993) found that the rami of some anterior trunk appendages have a segment that is formed later in development than homologous segments on legs located on posterior body segments. These exceptions to the general rule – that anterior appendages are found in a developmentally more advanced stage than those of posterior segments – appear to have originated more than once during copepod evolution. It is found in 10 different families of the Calanoida (Diaptomidae, Acartiidae, Euchaetidae, Aetideidae, Calanidae) and the Harpacticoida (Parastenocaridae, Cristacoxidae, Thalestridae, Diosaccidae, Tisbidae).

The sequence of ossification in the limb skeleton of *Alligator mississipiensis* provides another example of back-to-front direction of differentiation. Whereas most of the postcranial axis ossifies along an antero-posterior gradient, the neurocentral suture is closed in a caudo-cranial sequence. The alligator's limbs also provide an example of a distal-to-proximal pattern of differentiation. During the initial phases of ossification, humerus and femur lag behind radius/tibia and ulna/fibula respectively (Rieppel 1993a).

Something comparable with these examples of back-to-front progressing development occurs in the morphogenesis of the double-combed

antenna of male moths, as illustrated in *Antheraea polyphemus* (Steiner and Keil 1993). In this large species, the antennal flagellum consists of about 30 segments, each of which bears two pairs of side branches. These branches do not split off the stem as second-order appendages, but are literally sculpted by segmental and intersegmental incisions proceeding from the margin towards the stem of a leaf-shaped antennal anlage. This happens during the pupal stage, starting with primary incisions which form double branches, which are then split into single branches by parallel-running secondary incisions.

Polarity Reversal

The lernaeopodids are a family of copepods whose adults are profoundly modified with respect to the copepod ground plan in accordance to their parasitic habits. No trace is left of the original segmentation of the cephalothorax. During the process of fusion of the corresponding segments, the appendages of the cephalothorax remained in their original sequence, with the exception of the maxillipeds and the second maxillae. In genera such as *Tracheliastes* and *Vanbenedenia*, these two pairs of appendages are at the same level; but in other lernaeopodids, they have reversed their positions, so that the maxillipeds are now anterior to the second maxillae (Kabata 1979).

A unique case of polarity reversal has been recently discovered in the development of the nasal appendages of the star-nosed mole *Condylura cristata*. The nose of this small mammal is surrounded by 22 long appendages whose first embryonic evidence is a series of waves in the superficial epidermal layers, followed by the production, in a deep layer of epidermis, of 22 epidermal cylinders embedded in the side of the mole's face. Later, the caudal end of each cylinder erupts from the face and rotates forward to project rostrally, remaining attached only to the tip of the snout. Strictly speaking, these appendages do not originate as outgrowths of the body surface. The rostral end of each appendage is derived from caudal embryonic facial tissue, and the caudal end of each appendage is derived from rostral facial tissue (Catania, Northcutt, and Kaas 1999).

Axis Paramorphism and Origin of the Appendages

Animals with appendages are more complex than animals without them. This seems to be a truism, the same as the obvious corollary that animals with appendages are derived from animals without them. The fact that snakes and whales are derived from ancestors with well-developed limbs

does not represent a serious objection to this generalisation, as embryological and anatomical evidence of the secondary loss of the appendages is clearly present in these limbless vertebrates.

I will argue that animals provided with appendages were not necessarily derived from a common ancestor already endowed with appendages. The appendages of the vertebrates are not the same as those of the arthropods, any suggestion from developmental genetics notwithstanding. In my view, developmental and structural similarities between the appendages of distantly related phyla are the transitive result of two different kinds of relationships: those between an animal's main body axis and the axis of its appendages, and those between the main body axes of animals belonging to different phyla, such as arthropods and vertebrates.

Axis Paramorphism

The core of this hypothesis is the notion of *axis paramorphism*, according to which the appendages are evolutionary duplicates of the main body axis (Minelli 2000b).

The existence of some degree of homology between the main body axis and the appendages of the same animal was advanced by a few authors [e.g., Dollé et al. (1993) and Held (1995)], but this idea was not explored in depth; instead, evo-devo biologists began to reason in terms of the evolutionary co-option of individual genes or whole genetic cassettes (e.g., Raff 1996, 1999b, Abouheif et al. 1997, Arthur, Jowett, and Panchen 1999). To say that genes otherwise used by the same animal (e.g., in the patterning of its main body axis) were co-opted to specify features along an appendage implies that the animal already possessed that appendage, and this raises the question of how the animal first acquired it. This question, to which the co-option hypothesis does not offer a solution, is perhaps explained through the hypothesis of paramorphism.

Let's begin with some evidence from comparative morphology.

An important first generalisation is that no segmented appendage occurs in non-segmented animals. The reciprocal correlation is a bit less strict, but we can safely state that most segmented animals have segmented appendages.

In arthropods, segmentation is not limited to the paired limbs (antennae, mouthparts, legs), but extends to posterior unpaired appendages, such as the filum terminale of silverfish and the caudal appendage of several arachnids (thelyphonids, schizomids, and palpigrads; Figure 8.8). In onychophorans (Figure 8.9) and tardigrades, the less distinct segmentation

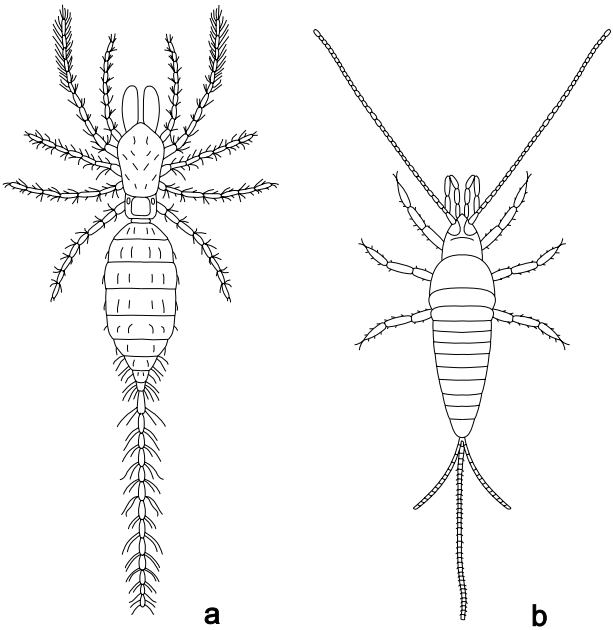

Figure 8.8. Posterior segmented appendages of segmented animals: a, palpigrad; b, silverfish.

of the main body axis is adequately mirrored by the faint segmentation of their appendages. In vertebrates, segmentation of the trunk has its counterpart in segmentation of the paired fins or legs, not to mention the posterior dorsal and anal fins of *Latimeria* (Figure 8.10). The argument also applies to the tail if the latter, as I have argued previously, is to be regarded as an appendage rather than as the posterior part of the trunk.

Of the three main phyla of segmented animals, some difficulties arise, from this point of view, with the annelids only. Segmented appendages are rare in this phylum and strictly limited to a few polychaete families, such as Nereidae and Hesionidae (two-segmented palps; some hesionids also with

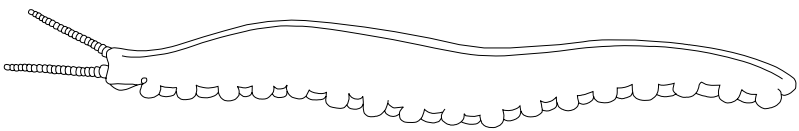

Figure 8.9. Unsegmented appendages (lobopods) of an animal with faint segmentation (onychophoran).

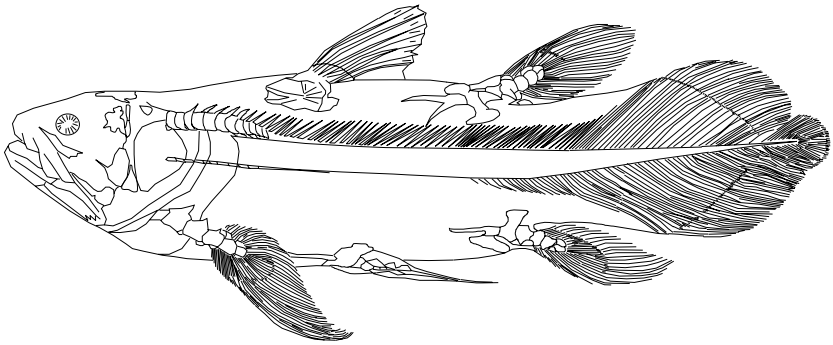

Figure 8.10. Segmented appendages of vertebrates. In the coelacanth (*Latimeria chalumnae*), segmentation is not limited to the paired fins (the pectorals especially), but extends to two unpaired appendages: the posterior dorsal and the anal fin.

multisegmented cirri), Dorvilleidae (multisegmented palps), and Syllidae (multisegmented cirri) (Figure 8.11). The polychaete parapodia and the large majority of their anterior appendages (antennae, cirri, palps) are unsegmented, and the branchial filaments of some leeches (*Ozobranchus*, *Croatobranchus*) and oligochaetes (*Branchiura*) are also unsegmented.

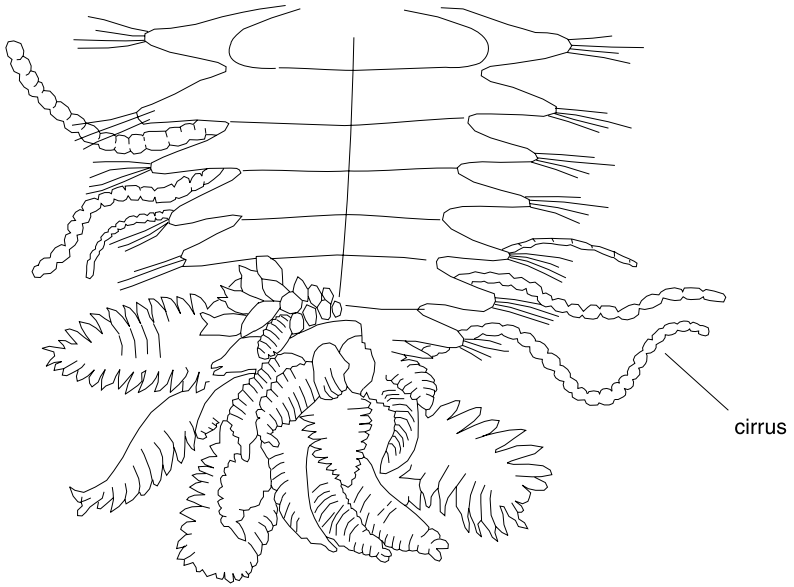

Figure 8.11. Segmented cirri in a syllid polychaete. The drawing illustrates the posterior segments of the animal, with a cluster of offspring worms produced by vegetative reproduction. (Redrawn after Edmonds et al. 2000.)

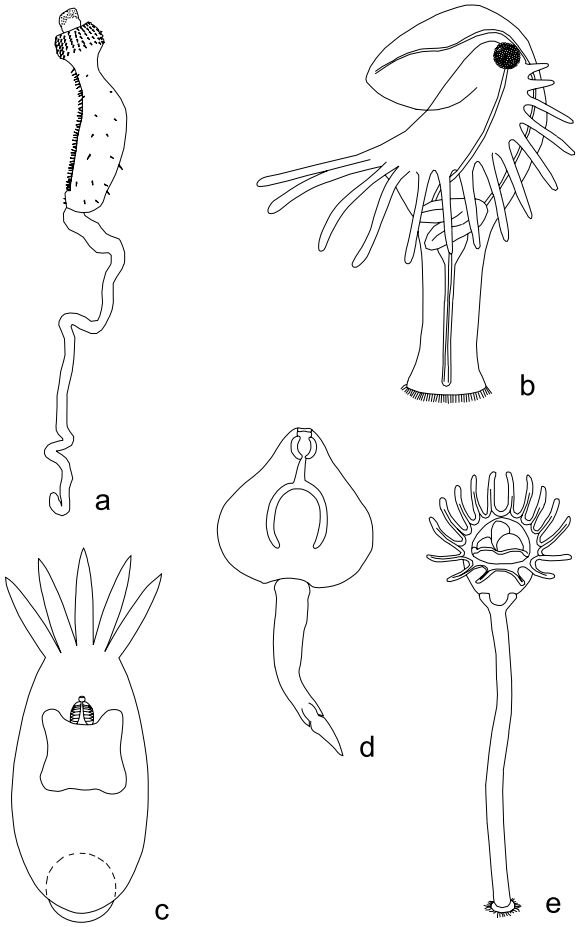

Figure 8.12. Unsegmented appendages of unsegmented animals: a, the priapulan *Tubiluchus*; b, the actinotrocha larva of *Phoronis*; c, a temnocephalid flatworm; d, the cercaria larva of the digenean fluke *Cotylophoron*; e, an entoproct. (d, Redrawn after Hyman 1951.)

There is no problem, of course, in contrasting the segmented appendages of segmented animals with the unsegmented appendages of unsegmented animals. A list of the latter may include the tentacles of phoronids, entoprocts, ectoprocts, brachiopods, sipunculans, molluscs and pterobranchs, the tail of priapulans, the proboscis of nemerteans, and the different kinds of appendages (most commonly posterior) of flatworms, such as the tail of the cercariae, the caudal appendage of *Stenostomum* (Catenulida) and the anterior lobes of the temnocephalids (Figure 8.12).

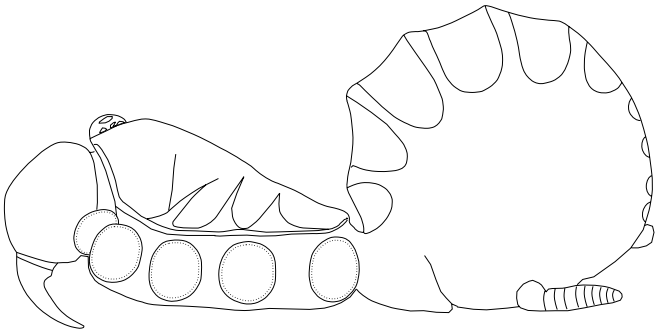

Figure 8.13. Segmented opisthosoma and segmented spinnerets in a liphistiid spider (all legs removed). (Redrawn after Millot 1949.)

Structural correspondence between the main body axis and its appendages are not limited to the presence, or not, of segmentation.

Arthropods, with their virtually unending diversity of appendages, offer many examples of features or trends affecting the antero-posterior body axis and the proximo-distal axis of the appendages at the same time (Minelli, 1996a, 2000b).

Within Diptera, the vast majority of the species belonging to the suborder Nematocera both have a slender and nondescript abdomen with up to 10 distinct segments and a slender antenna, basically with 14 segments (Hennig 1973). In the Cyclorrhapha, the abdomen is shortened, with frequent specialisation and reduction in the number of free segments; the antenna is uniformly reduced to the short four-segmented appendage found, for example, in *Drosophila* and the housefly. The Orthorrhapha, traditionally placed within the Brachycera, together with the Cyclorrhapha, but probably paraphyletic with respect to the latter, are largely intermediate between the Nematocera and the Cyclorrhapha, both in abdominal morphology and in the segmentation of antennae.

The large majority of living spiders lack overt segmentation in the opisthosoma as in the spinnerets, but in the primitive spider family Liphistiidae, both the opisthosoma and spinnerets are segmented (Figure 8.13).

An example from the crustaceans is provided by copepods, in which geniculation of the antennules (a feature found in the males of most species) is somehow mirrored by the body flexure (i.e., a 'mid-tagma accident') superimposed on the basic tagmosis, in that it articulates the pleon in an anterior and a posterior section (Huys and Boxshall 1991; Figure 8.14).

In the different orders of the Chilopoda (centipedes), correspondence between the main body axis and the appendages is found in the way these

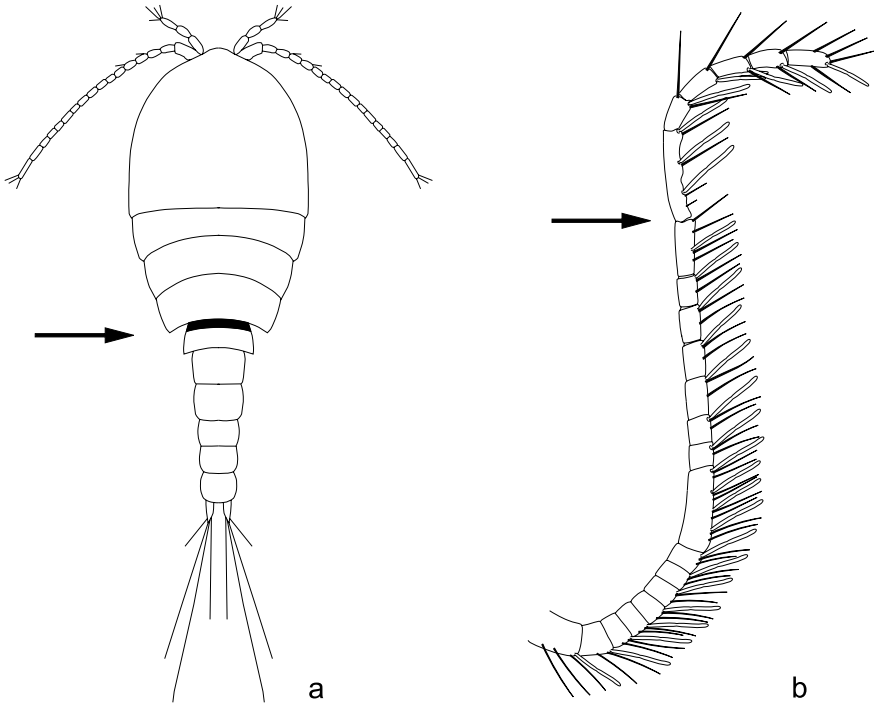

Figure 8.14. Mid-tagma accident (a, flexure in the posterior thorax) and mid-appendage accident (b, geniculation in a male antennule) in copepods. (b, Redrawn after Huys and Boxshall 1991.)

axes develop during postembryonic life (Minelli et al. 2000). Scutigeromorpha, Lithobiomorpha and Craterostigmomorpha are hemianamorphic; that is, the number of body segments increases postembryonically during the early (so-called larval) stages, whereas the Scolopendromorpha and Geophilomorpha are epimorphic, that is, the full number of body segments is already present at birth. This contrast is mirrored by the postembryonic increase in the number of segments of the antennae in the hemianamorphic and the corresponding lack of postembryonic increase in the epimorphic centipedes.

In the primitive Upper Cambrian crustacean *Rehbachiella kinnekullensis*, the high number of body segments and the nondescript tagmosis were matched by an unusually high number of joints per appendage and by a minimal differentiation of the articles. In this case as well, the spatial pattern of differentiation was in accordance with the pace of postembryonic development, as *Rehbachiella* seems to have undergone a huge number

of growth moults, more than thirty perhaps, accompanied by very limited additions of new elements (segments, appendages or appendage articles) from one stage to the next (Waloßek 1993).

In vertebrates, van der Hoeven et al. (1996) observe an interesting similarity between the main body axis and the axis of the appendages, in that all these axes obey a principle of proximal stability versus distal variability. This posteriorly (terminally) increased tolerance for functional variability, dubbed by van der Hoeven et al. as *laxitas terminalis* (terminal laxism), is paralleled by higher divergence in the Hox protein sequence.

There are several features in common, including WNT signalling, between the pathways involved in the growth and patterning of the main body axis, and the growth and patterning of a limb axis (Moon, Brown, and Torres 1997, Shubin, Tabin, and Carroll 1997, Tabin, Carroll and Panganibam 1999). In mice, the existence of a common multiaxial patterning system is suggested by *HoxD* mutants, with skeletal alterations affecting all body axes (Dollé et al. 1993). In *Drosophila*, *wingless* is involved in patterning both the antero-posterior body axis and the proximo-distal axis of the leg (Campbell and Tomlinson 1995, Nagy and Williams 2001), and the wing disc is subdivided into alternating sectors comparable with the stripes into which the expression of pair-rule genes partitions the antero-posterior axis of the embryo (Sturtevant et al. 1997). Held, Duarte, and Derakhshanian (1986) described the tarsi of the *Drosophila* mutant *spiny legs*, which may contain up to eight joints, of which four correspond to those of the wild-type flies, whereas the others are extra joints dividing every tarsal article (the fifth excluded) into two subarticles. The most interesting feature is that nearly all extra joints have inverted polarity. By consequence, in the tarsus of *spiny legs* mutants, there is an alternation of normal and inverted joints, perfectly comparable with the alternation of normal and extra segment boundaries found along the main body axis in the embryonic lethal mutant *patched*, thus suggesting that the appendage and the main body axis may share some patterning mechanism.

Tabin et al. (1999) suggested four different classes of explanations for the similarities between arthropod and vertebrate limb development: (1) the common ancestor of arthropods and vertebrates was already provided with appendages whose growth and patterning was controlled by the same genes now acting in both arthropod and vertebrate limbs; (2) these genes, originally involved in the formation of body outgrowths other than true limbs, were independently co-opted to pattern fins, legs, and wings; (3) genes originally linked together in a stable cassette before being used,

in arthropods and vertebrates alike to pattern their appendages, become involved eventually in the latter process; and (4) the genes now involved in limb patterning were individually recruited from other roles so that their joint operations in arthropod and vertebrate limb development is simply coincidental.

According to Tabin et al. (1999), the last common ancestor of arthropods and vertebrates was not provided with appendages from which those later evolved in the two phyla might have derived as historical homologues. How then do we explain the large use of the same genetic information in the developmental processes by which animals belonging to different phyla form their body appendages?

According to the hypothesis of axis paramorphism, this may be explained by regarding body appendages, such as arthropod and vertebrate limbs, as duplicates of the main body axis, although devoid of any endodermal component – that is, without involvement of the 'visceral animal.' In my concept, *a secondary axis is an axial paramorph of the main body axis if it originated as a duplicate expression of genes already involved in the growth and patterning of the latter* (Minelli 2000b). Later, divergence of the paramorphs with increasing specialisation of the appendages is obviously expected in time, through the evolution of new regulatory interactions (Shubin and Marshall 2000).

The only serious alternative to axis paramorphism would be gene co-option: the animal 'invented' its appendages independent of the genes now involved in its growth and patterning. These genes, already present in the animal but involved in some other cellular machinery and developmental process, later became associated with the development of the appendages, as in Tabin et al.'s (1999) second alternative.

There are problems with the co-option hypothesis. The main problem is not the amount of co-option implied to have occurred during the evolution of appendages, but how the animal 'invented' its body appendages in the first place. Where did the animal find the genetic tools needed to produce its secondary axes, if the latter had nothing to do, at the outset, with the main body axis? Still harder, what prevented the new tools now producing the secondary axes from interfering with the growth and patterning of the main body axis?

The axis paramorphism hypothesis answers the first question. As to the second question, it is important to note that – with very minor exceptions, such as pycnogonids – the paired limbs of arthropods and vertebrates (as well as the vertebrate tail) do not include the endodermal component

present in the main body axis. It is difficult to imagine an animal with lateral appendages similar to the main body axis in possession of endodermal derivatives (i.e., lateral branches of the gut). Rather than a solution for more effective interactions with water or substrate (e.g., locomotion or food manipulation), this could be, at most, a way to reproduce asexually. There are a few annelids whose organization is not too far from this hypothetical monster. One example is the polychaete *Syllis ramosa*, a sedentary parasite of sponges whose lateral body axes will eventually give rise to new individuals (Beklemishev 1969). It is interesting to note that *Syllis* belongs to the annelids, a phylum where the dialogue between ectomesoderm (Romer's 'somatic animal') and endoderm (Romer's 'visceral animal') is probably more extensive than in arthropods (or ecdysozoans generally). This may explain why some annelids (and flatworms, such as catenulids) produce buds with endodermal derivatives, something that ecdysozoans never do. As undetached buds may change a conventional individual into a colony, this circumstance may help explain why, in an enormous clade as the Ecdysozoa, there is no instance of colonial organization, not even in a sessile group as the barnacles.

The presence or absence of endodermal derivatives may well be the criterion separating asexual reproduction from the production of body appendages. I have recently suggested (Minelli 2000b) that differentiation of a body outgrowth as a bud destined for asexual reproduction or as an appendage may depend on the presence or absence of markers similar to those in the *Hydra*, where the position of the mouth remains fixed, although it literally disappears, morphologically, when the animal is digesting freshly engulfed prey (Technau and Holstein 1995).

This is the scenario against which we can discuss the phylogenetic relationships between vertebrate and arthropod limbs. The discovery that *Distal-less* is involved in initiating the outgrowth of limbs in vertebrates (Beauchemin and Savard 1992, Dollé, Price, and Duboule 1992) and insect alike (Cohen et al. 1989, Cohen 1990) seemed to support a common origin of these appendages, but *Distal-less* expression was soon found to mark the prospective tip of many kinds of appendages in different phyla (Popadić et al. 1996). Panganiban et al. (1997) suggested that the earliest bilaterians already possessed some component of the genetic machinery for the production of appendages and even ventured to hypothesise (see also De Robertis 1997) that Urbilateria, the putative common ancestor of vertebrates and arthropods, already possessed some kind of 'humble'

appendage. Dong, Chu and Panganiban (2001) even suggest the existence, in the appendage of the common ancestor of arthropods and vertebrates, of proximal and distal domains under distinct genetic control.

What arthropods and vertebrates share in terms of organisation and genetic control of their appendages is likely to be the secondary consequence of three distinct relationships: (1) phylogenetic conservation, between arthropods and vertebrates, of genes and control pathways involved in laying down the basic features of their main body axis; (2) paramorphism of arthropod limbs in respect to the main body axis of arthropods; and (3) paramorphism of vertebrate limbs with respect to the main body axis of the vertebrates. Vertebrate limbs and arthropod limbs are not true historical homologues, but homoplastic paramorphs of historical homologues (Minelli 2000b). This is not the same as calling them 'developmental paralogues' (Shubin, Tabin, and Carroll 1997), because the latter concept only captures the homoplasy of the appendages without linking them to the origin, patterning and evolution of the main body axis.

In any case, the dorso-ventral axis of the appendage is probably not the same in arthropods and vertebrates (Minelli 2000b). In arthropods (primitively at least), the dorso-ventral polarity of the limbs is largely comparable with the dorso-ventral polarity of the trunk, but things seem to be different in vertebrates. In particular, the genes involved in patterning the antero-posterior and proximo-distal axes of vertebrate and arthropod limbs are somewhat the same (Lawrence and Struhl 1996, Shubin, Tabin, and Carroll 1997), but this is not true for the dorso-ventral axis.

Terminal Control and Axis Paramorphism

Many, but not all, bilaterians have strong morphological markers at both body ends and both body ends, not just the anterior one, are defined very early in development. This is shown well by *Drosophila*, in which both body termini are defined by gradients of maternally encoded gene products.

Animals with early and clearly defined termini are also provided with appendages with a strongly and early defined tip. This allows patterning of the axis of the appendage, between its proximal and apical end, somehow comparable with the patterning of the main body axis. This is what seems to be excluded in the case of appendages, such as polychaete cirri, found in metazoans with a less defined posterior body end. A strongly marked posterior body end, translated by paramorphism into a strongly marked tip of the appendages, is perhaps one of the keys to the evolutionary success

Figure 8.15. Eyes on the tip of the long tentacle of *Helix lucorum* (Photo by L. Gamberucci, courtesy of F. Giusti, Siena.)

of both arthropods and vertebrates – humans, are with their wonderfully multifunctional hands, are obviously included.

Another phylum with very clear control of the posterior body end is the molluscs; but the large majority of these animals have invested much less in appendages than arthropods or vertebrates. Nevertheless, the arms of cephalopods, with their rows of suckers (and occasionally hooks), show that molluscs may also be able to develop a sizeable degree of patterning of their appendages. A major clade within the pulmonate gastropods – the Stylommatophora, to which our common terrestrial snails and slugs belong – have been able to place their eyes at the apex of a pair of cephalic outgrowths (Figure 8.15).

Regressive trends may obscure body patterns as well as phylogenetic relationships. In this respect, a closer study of *Xenoturbella* would be rewarding. This is a marine worm-like animal of extremely simple body architecture, virtually devoid of organs except for a large digestive cavity opening in the middle of the ventral side (Westblad 1949). A superficial inspection of its morphology would invite a tentative assignment of this animal to the flatworms; but newly acquired molecular and developmental evidence places it firmly with the bivalve molluscs, in the vicinity of the

nuculids (Israelsson 1997, 1999, Norén and Jondelius 1997). Of all the molluscs, bivalves are probably those in which the anterior and posterior body ends are less strongly marked by complex features, but they still preserve quite distinct body ends which should be defined by molecular markers expressed early in ontogeny. It would be interesting to know whether anything of these putative markers' expression is left in *Xenoturbella*.

Gene Co-option

Somehow intermediate between the notion of axis paramorphism and the more fashionable explanations in terms of gene co-option is Arthur, Jowett and Panchen's (1999) suggesion that any outgrowth, whether external or internal, can be ectopically copied to produce lateral limbs. The cellular basis of outgrowing, in terms of polarised mitoses and/or cell migration, could be the same, irrespective of the germ layer in which outgrowing occurs. If so, one might speculate whether there is any relationship between the faculty of outpouching by which deuterostomes form their coelomic cavities and the ease with which these animals produce appendages, either simple as the podia of the echinoderms, or much more elaborate as the limbs and tail of the vertebrates. One might even read the argument the other way around and ask whether systems originally used for producing external appendages (i.e., outgrowths involving the ectoderm and the mesoderm) have been ever co-opted to give rise to endodermal outgrowths. A case in point is possibly offered by the leeches. In most species of this group, there is no external appendage comparable with the parapodia of the polychaetes, the exceptions being represented by the (putative) respiratory appendages of some representatives of Piscicolidae and Ozobranchidae, and the exceptional erpobdellid *Croatobranchus* (Sket et al. 2001). Leeches are often provided with segmental gut coeca, whose patterning is controlled by the *Hox* gene *Lox3* (Wedeen and Shankland 1997).

If the gene co-option hypothesis is too weak to explain the correspondence between the main body axis and its appendages, it is obviously attractive in other simpler contexts.

Morphogenetic signals do not need to be specific for the structures they induce and may even be phylogenetically older than the anatomical characters they specify (Müller and Wagner 1996).

The role of *Hox* genes in specifying regional identities of the body axis is more primitive than its role in the appendages, in which these genes have been secondarily co-opted (Shubin, Tabin, and Carroll 1997, Cohn and Coates 1999, Shubin and Marshall 2000). Cohn and Coates (1999),

in particular, propose that localised patterns of 5′ *Hox* expression first evolved to pattern the mesoderm of the gut. The problem remains whether the co-option of *Hox* genes into limb patterning occurred at the same time or not in the forelimb and hind limb. Tabin and Laufer (1993) suggest that *Hox* genes were originally expressed only in the hind limb bud. Their further expression in the forelimb would be the result of a further co-option, resulting in a homeotic assimilation of the forelimb to the pattern already present in the hind limb.

Co-option is probably responsible for the multiple use of the same molecules in different aspects of neural patterning (Goodman, Davis and Zito 1997). For example, control of synaptic remodelling involves some of the molecular mechanisms, often involving specific cell-adhesion molecules, that control selective growth and guidance during early stages of axon path-finding and synapse formation. Another example is provided by Fasciclin II, which controls selective axon fasciculation (the patterning and stabilization of synapses) and the growth of synapses during synaptic remodelling. Its putative co-option into different morphogenetic processes has placed the function of Fasciclin II under three different regulatory networks, each operating during one of the three developmental stages in which this molecule is involved.

Co-option, again, has been advocated by Arthur, Jowett and Panchen (1999) to account for the origin of segmentation in the mesoderm and ectoderm as a newly acquired function of genes previously used to produce serial repetition in the nerve cord (observed in nonsegmented animals like flatworms) or the cuticular rings of kinorhynchs.

Limbs and Genitalia

Early in the nineteenth century, Johann Wolfgang Goethe speculated about the possible origin of petals and other flower parts from conventional green leaves, and the origin of the braincase from modified vertebrae (Goethe 1790, 1817–1820). It took a little less than two centuries to gather convincing evidence for accepting the first and rejecting the second of those speculations. Besides the definitive verdict on either proposition, what matters more is that Goethe's ideas, like Etienne Geoffroy Saint-Hilaire's quests for a unity of plan in animal organisation, were not empty, fruitless speculations, but propositions for which experimental tests could be eventually developed.

From the vantage point of current awareness of homeotic mutants, we are now ready to add more and more terms to a list of *prima facie*

improbable, but experimentally supported, serial homologues. This list already includes petals and leaves, legs and antennae, and halteres and wings. The latest addition to this list is limbs and genitalia (Minelli 2002).

About fifteen years ago, Shearn, Hersperger, and Hersperger (1987) reported that mutations at two loci on the third chromosome of *Drosophila melanogaster* caused a lot of homeotic transformations, not just those of the more usual kinds (antenna to leg, proboscis to leg and/or antenna, first and third legs to second leg, haltere to wing), but also genitalia to leg and/or antenna. Similar reports became more frequent in subsequent years, thus enlarging the series of homologous ventrally paired appendages of insects to include genitalia, in addition to antennae, mouthparts, and legs. Comparable evidence is now available for vertebrates. The question then arises as to what extent animal genitalia may fit into this scheme of homology.

Some preliminary observations are required, as animals with internal fertilisation have evolved a great diversity of genital appendages, either paired (such as the copulatory appendages found on the parapodia of some pisionid polychaetes, or the hemipenes of many snakes and the pelvic claspers of chondrichthyes) or unpaired, as those of mammals and most insects. These genital appendages are not necessarily located in close proximity to the genital opening, but sometimes occur close to the opposite end of the body. This sheer diversity of genital appendages suggests multiple origins; nevertheless, some degree of homology can still be traced among them.

Three main classes of genital appendages can be distinguished: (1) appendages, mostly unpaired, developed from the genital opening's rim (example: the genital papilla of many flatworms; Figure 8.16); (2) appendages derived from specialisation of non-genital appendages already existing in the proximity of the genital opening (example: the pelvic claspers of chondrichthyes; Figure 8.17); and (3) appendage(s) derived from specialisation of non-genital appendages topographically unrelated to the genital opening [examples: the palps of male spiders (Figure 8.18), the antennae of sminthurid springtails (Figure 8.19), and the secondary penis of dragonflies (Figure 8.20)].

At first sight, there seems to be little hope of regarding these appendages as other than analogous, but perspective changes if we adopt a combinatorial approach to homology (see page 224). To this aim, we need to consider, alongside the appendages (both genital and non-genital), another component of the animal's architecture: the main body axis. Problems are easier to solve if we accept the principle of axis paramorphism and are ready to

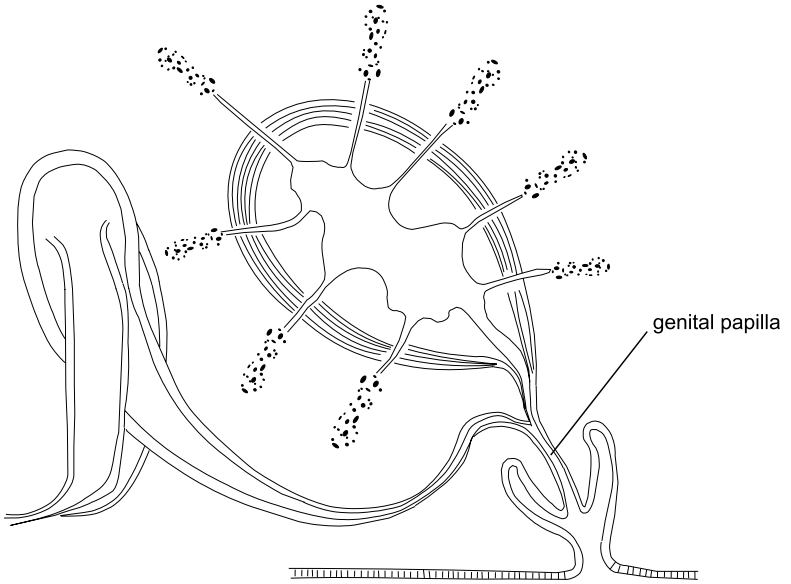

Figure 8.16. Part of the sagittal section of the polyclad flatworm *Stylochus*, with genital papilla and other reproductive structures. (Redrawn after Hyman 1951.)

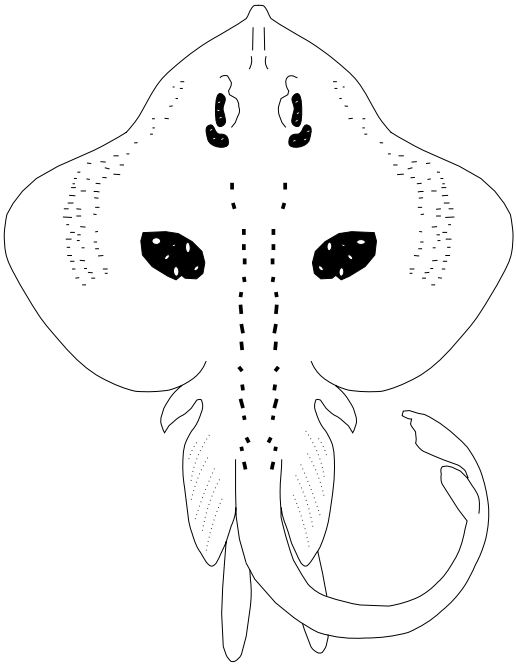

Figure 8.17. A male skate *Raja naevus*, with paired rod-like pelvic claspers. (Redrawn after Tortonese 1956.)

Figure 8.18. Male and female spiders, *Theridion melanostictum*, from Yemen. The male is easily recognisable because of its conspicuous palps. (Courtesy of B. Knoflach and K. Thaler, Innsbruck.)

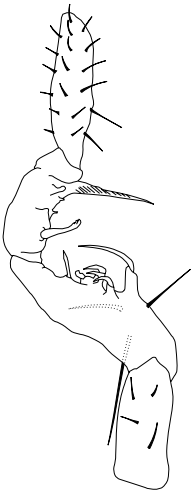

Figure 8.19. Antenna of a male springtail (*Sminthurides aquaticus*). (Redrawn after Massoud and Betsch 1972.)

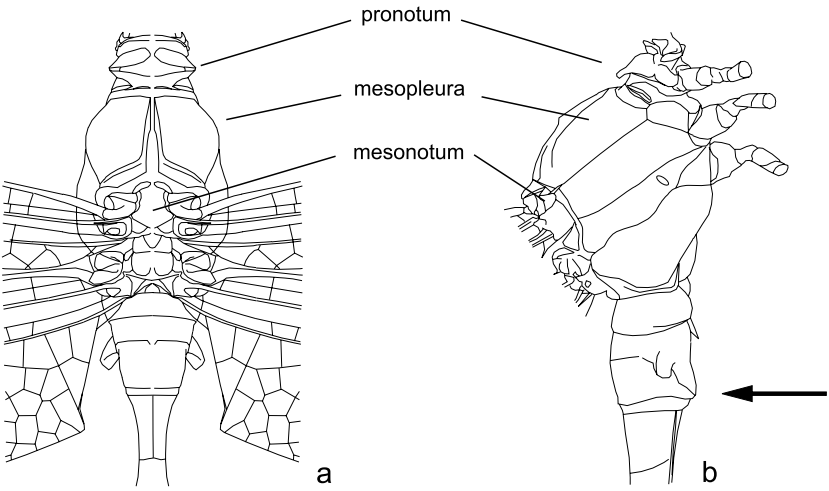

Figure 8.20. Thorax and anterior abdomen of a male dragonfly (a, top view; b, lateral view). The arrow in (b) marks the position of the secondary penis. In this insect order, two originally lateral sclerites (the mesopleurae) expand towards the dorsal midline, where they come in contact, thus separating the pronotum (the dorsal sclerite of the first thoracic segment) from the mesonotum (the dorsal sclerite of the second thoracic segment). (Redrawn after Watson and O'Farrell 1991.)

acknowledge that two features may share only one major component of homology: either positional or special homology.

For example, the secondary penis on the ventral side of the second abdominal segment of male dragonflies has very little to do, morphologically as well as functionally, with the gonopods, one or two pairs of specialised legs (between the eight and tenth pairs) in the male juliform millipedes. Gonopods are claspers used by the male when embracing the female during copulation, but these appendages have nothing to do with sperm transfer. To the contrary, the latter function is performed by male dragonflies through their secondary penis. As for the origin (more or less clearly reflected in the shape of the appendage), gonopods are derived (in ontogeny as well as in phylogeny) from normal locomotory legs, whereas the dragonfly's penis lacks a limb-like ontogenetic precursor (although in phylogenetic perspective, it might be serially homologous to a limb pair). Despite these big differences, the two kinds of genital appendages (Figures 8.20 and 8.21) seem to share positional homology (Minelli and Schram 1994, Minelli 2001), because they are located on the ventral side of probably equivalent segments (for segment equivalence in arthropods, see pages 204–208).

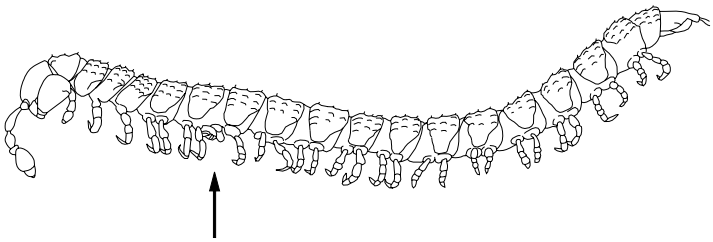

Figure 8.21. A male millipede (*Macrosternodesmus palicola*). Arrow marks the modified sexual appendages or gonopods. (Redrawn after Blower 1985.)

The opposite relationship, of morphologically equivalent genitalia developing in two animal groups in different positions, is harder to find. Something approaching the case is found in nematodes, in which the relative position of the gonopores is different in the two sexes. In the males, it is subterminal, as in the majority of metazoans, whereas the female gonopore occurs at mid-body length, in a site that possibly corresponds, in terms of relative position, to the site occupied by millipede gonopods and the dragonfly's penis.

More generally, but still in terms of positional homology, along the main body axis there seem to be just two 'hot spots' where genital structures may develop.

If genital openings are mainly at the level of the posterior hot spot, the anterior one is often the site of sexual appendages. Neither spot seems to be a genital hot spot *per se*. Rather, it is as if the animal was provided with two 'morphogenetic hooks' to fix the position of different, important features of its body architecture. The phylogenetic conservation of these two hot spots suggests a very ancient origin of the mechanisms by which these positions are specified. Recent data on the differentiation of external genital structures in mice (Kondo et al. 1997), spiders (Damen and Tautz 1999a, 1999b) and nematodes (Kagoshima, Cassata, and Bürglin 1999) suggest that differentiation of external genital structures is controlled by *Hox* genes of the posterior (*Abdominal-B*) class. In *Drosophila*, the absence of *Abdominal-B* in the genital disc leads to the replacement of genitalia with legs or, less frequently, with antennae (Estrada and Sánchez-Herrero 2001). This brings us straight to the topic of homology of limbs and genitalia. In the case of the replacement of genitalia by legs or antennae in the absence of *Abdominal-B* expression in the genital disc, these transformations are accompanied by the ectopic expression of genes, such as *Distal-less* or *dachshund*, which are normally required in these appendages.

In humans, a hand-foot-genital syndrome is known to depend on a *Hox* gene mutation. Del Campo et al. (1999) reported about two unrelated children with reduction in the number of all fingers and toes, accompanied by hypomorphic development of the external genitalia. Both children were heterozygous for a deletion that eliminates many members (including all posterior ones) of the *HoxD* gene cluster.

These concordant reports from different phyla suggest that the differentiation of external genital structures is under the control of *Hox* genes of the posterior (*Abdominal-B*) class, but we know very little about the mechanisms involved. The concept of axis paramorphism may help us understand some aspects of the localisation of genital or sexually related structures in many animals.

In terms of axis paramorphism, a distal position along an arthropod limb (or a distal/posterior position in a vertebrate limb) corresponds to a posterior position along the main body axis. Therefore, as genital structures mostly evolve in a posterior position along the main body axis, we could expect some genital or, at least, sexually related feature to evolve, along a limb axis, in a subapical position in arthropods and in a subapical/posterior position in vertebrates. On the other hand, there is no reason why these structures should be restricted to the *posterior* legs. Examples of anterior appendages bearing specialised genital/sexual features close to their distal end are all but rare. Examples include the swelling thumb (a distal element of the *fore*limbs) of many male anurans (Figure 8.22), the terminal article of the palps (*anterior* appendages) of male spiders (Figure 8.18), and the enlarged tarsi of the *anterior* legs of many male beetles (Figure 8.23).

Common genetic control of patterning in limbs and genitalia may even extend to the analia. In mice, *Hox13* and *Evx-2* are expressed in the genitalia and the internal anal sphincter (Hérault et al. 1996, Kondo et al. 1996). In *Caenorhabditis elegans*, the *Evx-2* homologue *vab-7* is required for generation of rectal muscle cells, where its expression largely overlaps the expression of *egl-5*, a nematode cognate of the posterior *HoxD* genes of vertebrates (Ahringer 1996).

Symmetry and Asymmetry

If development is nothing but the temporal deployment of cellular dynamics occurring in multicellular systems – and these systems do not necessarily coincide with whole embryos, but with developmental units of different size, as suggested in chapter 1 – then a symmetrical pattern can

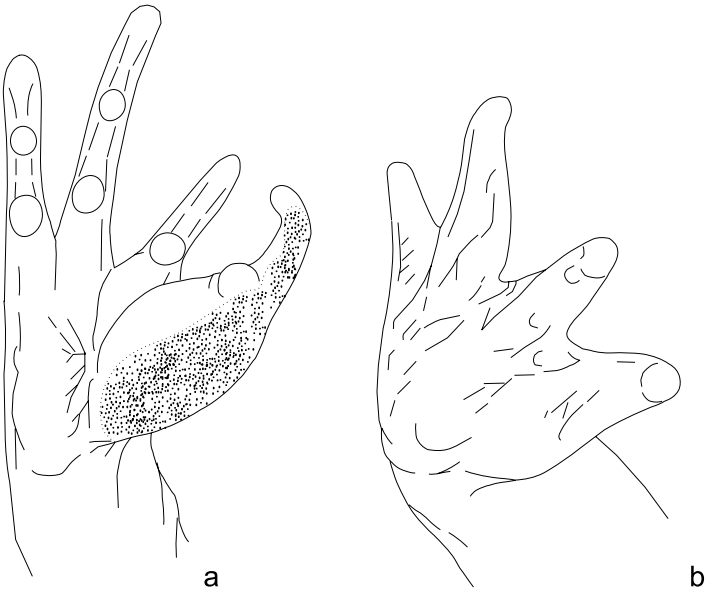

Figure 8.22. Swelling thumb (a distal element of the *fore*limbs) of two male anurans: a, *Rana maculata*; b, *Bufo bufo*. (Redrawn after Duellman and Trueb 1994.)

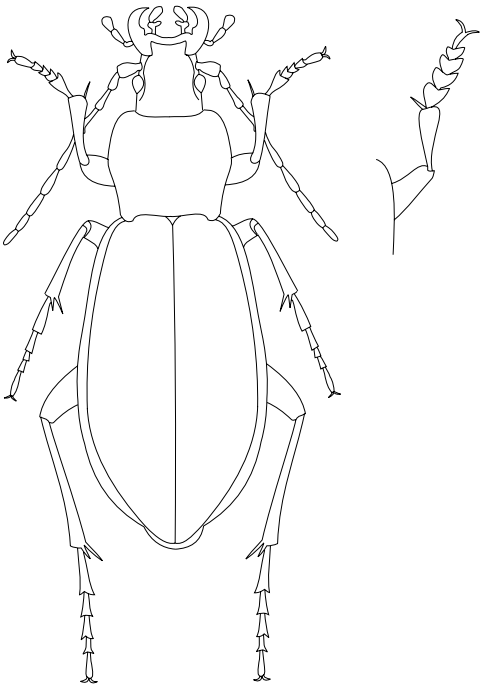

Figure 8.23. Enlarged tarsi of the *anterior* legs of a male ground beetle (*Carabus olympiae*, detail only). Compare with those of the female (habitus drawing).

often be expected as the 'default' product of largely independent developmental trajectories going on in parallel within separate units (modules, see page 234). It is a pity that morphologists and developmental biologists failed to pay attention to Bateson's (1894: 21) remark about "The essential unity of the phenomenon of Repetition of Parts and of its companion-phenomenon, symmetry." That symmetrically growing structures, despite their more or less extended developmental independence, do not have individuality (if by individuality, we mean the possession of traits by which we can distinguish one trait from the other; Nijhout 2001) is clearly beyond the point I am making here.

In birds, fluctuating asymmetry in feather length increases rapidly when feathers have just begun to grow, but it decreases again when the feathers' development approaches completion. Aparicio (1998) induced one of the seven primary feathers in the left wing of house sparrows to moult two days apart from the corresponding feather in the right wing. Feathers of both wings thus grew in a similar way, independent of the growth on the other body side. Asymmetries in feather length were less and less conspicuous with the progress in growth, but differences in growing time persisted throughout development. This experiment disproves the existence of a mechanism of compensatory growth. The more likely explanation of the convergence of the two sides towards a similar feather length is targeted growth: the feathers on the two sides follow a similar but independent trajectory, which tends towards a fixed maximum potential size.

This brings us to one of the most interesting, but difficult, questions in developmental biology: To which extent is the main body axis produced by an integrated behaviour of the whole embryo, rather than by the concurrent but largely independent development of its two halves? An overlapping of autonomy and interactions is revealed by Khaner's (1996) study on the formation of separate body axes in separated left and right halves of a chick embryo. That a complete embryo is not necessary to the formation of the main body axis is an argument in favour of local morphogenetic autonomy. Interactions are nevertheless obvious. If the two embryo halves are separated at an early stage, then shifted along the midline and finally reunited in staggered fashion, a single embryonic axis develops if in the shifted-paired halves the posterior end of one half is adjoining the posterior area pellucida region of the other half. Two embryonic axes develop if the shift is so large that the posterior end of one half is fused to the central area pellucida of the other half.

Directional Asymmetry

Fluctuating asymmetry notwithstanding, symmetry is the default state, although evolution can be expected to bring about reductions in the degree of symmetry of cells and multicellular systems. Ciliates with their elegantly skewed cortex, and gastropods with their beautifully coiled shells, are masterworks of specialisation. Hermit crabs are clearly derived from crustaceans with bilaterally symmetrical pleon.

Living beings do not escape the general rule that the most highly symmetrical systems are also the most random (Ball 1999). What we need to explain is asymmetry rather than symmetry. But this does not rule out phylogenetic reversals, such as the symmetrisation of the pleon by which an hermit crab lineage (the king crabs) resumed the symmetrical habitus of ordinary crabs (Cunningham, Blackstone, and Buss 1992). Contrary to common belief, the radial symmetry of hydrozoans, cubozoans, and scyphozoans is probably secondary, as suggested by the phylogenetically basal position of Anthozoa, whose polyps are biradial to bilateral (Martindale and Henry 1998).

Directional asymmetry may affect the whole body, as in some parasitic isopods (Bopyridae), but this is rare. More often, it is limited to one tagma, as in the corixid water bugs (asymmetric abdomen) and hermit crabs (asymmetric pleon). There is little need to recall the asymmetry of the *situs viscerum* in vertebrates (see Ryan and Izpisúa-Belmonte 2000 for a good review), or the visceral mass of gastropods, which becomes asymmetrical following the torsion of the visceral mass with respect to the foot which maintains its original symmetry. I will only call attention to that strange opisthobranch, the 'bivalve limpet' *Tamanovalva limax*, which has dextrally arranged viscera, but a small sinistral spiral at the apex of the shell.

More frequently, directional asymmetry affects the appendages, generally one pair only, including the antennae of some springtails, the antennules of many male copepods, the mandibles of several termites and beetles, and the chelipeds of many decapod crustaceans (Figure 8.24). In the cross-bills (the bird genus *Loxia*), the upper and lower halves of the bill are asymmetric and turned into opposite directions (which helps them a lot in removing seeds from larch or spruce cones).

Directional asymmetry does sometimes affect either the male or the female sex. Male-only asymmetries include terminalia of several insects [e.g., embiopterans and dipterans, the copulatory organ (aedeagus) of many beetles], and several features (vas deferens, gonopore, fifth foot)

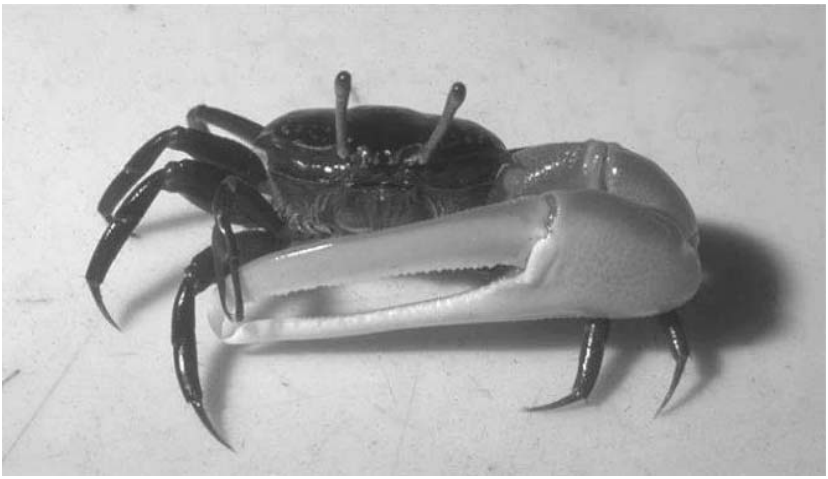

Figure 8.24. Strongly asymmetric claws in the crab *Uca annulipes* (Ocypodidae) from Inhaca Island (Mozambique). (Photo by R. Innocenti, courtesy of M. Vannini, Firenze.)

of many copepods. Female-only asymmetries include the genital apparatus of many insects (e.g., viviparous leaf beetles, aphids, dipterans) and birds, in which only one ovary is developed; in the scarabaeine beetles, this condition seems to be shared by all members of the taxon (Halffter and Matthews 1966).

In a few interesting cases, phenotypic directional asymmetry is a consequence of the late unilateral loss of one appendage. Male spiders of the genus *Tidarren* amputate one of their palps a few hours after the penultimate moult, hence adult males have only one palp (Knoflach and Van Harten 2000). The 'racemic' result (the surviving palp being either the left or the right, at random, in different individuals) has its equivalent in many cases of developmental (non-traumatic) directional asymmetry. For example, in the male of the blood-sucking fly *Phlebotomus garnhami*, the postabdominal inversion (hypandrium inversum) can occur in either direction (Just 1973).

The presence or absence of directional asymmetry is not a problem of size. Very complex patterns of directional asymmetry are typical of unicellular organisms such as ciliates (Frankel 1989). In animals, we may compare the tiny thrips or thysanopterans (insects whose adults may be just 0.5 mm long) with their uniquely asymmetrical mouthparts, with the mites, where

virtually no case of asymmetry is found, not even in the largest representatives of the group – the ticks.

The phylogenetic distribution of directional asymmetry (Palmer 1996) reveals some systematic bias, something we should perhaps investigate more closely as the possible expression of some hitherto unrecognised developmental principle (cf. Arthur 2002). In two large arthropod clades (Chelicerata, Myriapoda), directional asymmetry is virtually absent. The only case of which I am aware is *Freyanella plataleae* (male only?), a mite which clings on feather barbs of the host thanks to an enormous scapular articulated seta, only present on the right side, close to the posterior border of the propodosoma (Dubinin 1953). Directional asymmetry is common in Crustacea and Insecta. Curiously, no snail-eating beetle has evolved asymmetrical cephalic structures. Alternative morphological strategies, as far as we can see, were more readily available: cychrisation in particular, that is an exteme narrowing of the anterior half of the body, thus allowing the beetle to prosecute its prey well into the shell.

In their controversial book on *Asymmetry, Developmental Stability, and Evolution*, Møller and Swaddle (1997) discussed the ontogenetic plasticity of directional asymmetry in some crustaceans, such as the robber crab *Birgus*. At the beginning of its postlarval life, *Birgus* has a symmetrical abdomen that develops asymmetry once the crab starts living in a gastropod shell, but it reverts to symmetry when the animal changes its life-style again by abandoning the shell. Govind (1992) suggested that, in lobsters, the more active claw became the crusher and its less active counterpart the cutter; bilateral differences in predominantly mechanoreceptive input to the paired claws would somehow lateralise claw ganglion and, through it, the musculature of the appendage and its whole structure.

CHAPTER NINE

Segments

> Segment identity is a subjective concept that originates from the observation that in a particular species, a number of cell characteristics are always associated in a given segment.
>
> J. Castelli-Gair 1998: 441

What Is a Segment?

From a developmental point of view, segments are something different from the modular building blocks we are used to seeing in millipedes, earthworms, and caterpillars. Generally, we regard segments as modules of body architecture advantageous to, and perhaps evolved 'in view of', some advanced mechanisms of locomotion. In this chapter, I will suggest a very different perspective. Basic to reformulation of our views about segments is the appreciation that this term has been and is still currently applied to many (perhaps too many) different kinds of units (Minelli and Fusco 1995).

Segments are often skeletomuscular units used in locomotory mechanics. Segments are also those units, often quite well circumscribed, that behave (or seem to behave) as convenient homologues in comparative anatomy, even if the traditionally held principle – that segments should correspond to (pairs of) coelomic pouches – suffers too many exceptions. Segments are also developmental units, whose nature, origin, and evolutionary potential are the subject of this chapter.

There is nothing wrong with identifying segments with anatomical or functional units, or in describing segmentation as a kind of symmetry (Beklemishev 1969) or, more precisely, as translational symmetry (Coen 1999, Fusco and Minelli 2000b). There is a danger, however, that these purely descriptive or abstract approaches bring us to the point that we regard all segments as basically the same, were it not for the specialisations

that make the difference (e.g., between thoracic and abdominal segments in an insect). To say that these segments are different because different sets of genes are expressed in each of them does not help with understanding the nature of segments as developmental units, or whether all segments are basically the same or not.

According to Chaudonneret (1979), a typical insect metamere implies the coexistence of an ectodermic segment delimited by intersegmental furrows, a vascular segment or angiomere, a muscular segment or myomere, a nephridial segment or nephromere, a nervous segment or neuromere, and a unit bearing one pair of appendages. But do all these elements necessarily come in perfect register? That is, are there true 'eusegments' in the animal kingdom? Even restricting our attention to morphology (developmental aspects of segmentation will be discussed later), segment boundaries are more similar to hybrid zones between closely related species than to the neat borders of national states: between neighbouring segments we can expect 'introgression' of muscles, tracheae, blood vessels, and other. This explains the historical contrast between the Gratiolet-Whitman school (Gratiolet 1862, Whitman 1884, 1892) and the Moore-Castle school (Castle 1900, Moore 1900) in delimiting segments in the leeches. According to Castle and Moore, the ventral ganglia occupy the longitudinal middle of the segment, whereas Gratiolet and Whitman regarded the ganglia as located at the anterior limit of the segment.

Budd (2001) has recently argued against the very idea of 'eusegmentation' as a typological hindrance to understanding animal segments and correctly stressed the fact that individual parts or organ systems may have developed segmentation independently.

Students of arthropod morphology are often in doubt as to the 'true' segmental nature of some serial units of an animal's main body axis. This is why Kabata (1979), for example, remarks that segmentation of the copepod *Synergasilus* is sometimes obscured by the presence of 'false-rings', whereas Huys and Boxshall (1991), in describing the structural modifications of some other copepods (e.g., *Paramesochra mielkei*, *Intermedopsyllus intermedius*, and *Psammocyclopine hindleyi*), with respect to their more generalised relatives, recognise the presence of a secondary 'pseudosomite' separated from the anterior end of the genital 'double somite'.

In other cases, the difference between 'true' and 'false' segments seems to be obvious, as in the case of the leeches, with their annulated segments, but probably – as we will see – too much has been made of this distinction.

Similar problems are sometimes found in interpreting the segments of arthropod appendages, paramorphs of the main body axis. Not too rare, for example, are instances of pseudoarthrosis, in which a suture in the cuticle suggests subdivision into two segments. But no articular membrane between the two parts is found, for example, in the tarsi of legs I–XIII of several lithobiid centipedes (see page 141).

The basic problem is, what do we mean by segment?

I will follow Alberch and Kollar's (1988) suggestion in defining *segmentation as the subdivision of an embryonic field into sharply defined populations of cells linearly arranged along the antero-posterior axis*. What this subdivision actually means, in terms of the future development of the embryo, is not necessarily the same in all instances. In the case of insects, segmentation is a system of mutual activation of cell states that locally exclude each other (Meinhardt and Gierer 2000). In the case of rhombomeres, the segmental units of the vertebrate hindbrain, it is a way to sequester cells, limiting their subsequent migration as neural crest cells to neighbouring areas (Lumsden, Sprawson, and Graham 1991, Takeichi et al. 1997). The same is possibly true for the diencephalic neuromere boundaries, which act as a scaffold for early axon pathways in the neuroepithelium of this vertebrate brain region (Boncinelli 1994).

From an evo-devo point of view, of particular value is the fact that Alberch and Kollar's definition of segmentation also applies to the regularly patterned arrangement of selected groups of cells in animals that lack any morphologically overt segmentation. Wood and Edgar (1994) describe the organisation of the first larva of *Caenorhabditis elegans* as partly metameric, in that blast cells with the same or similar developmental potential are regularly spaced along the antero-posterior axis. During subsequent development, these cells will give rise to homologous cell lineages. In particular, dorsal, lateral, and ventral ectoderm cells are each repeated six times along the animal's length (Salser and Kenyon 1994). The six pairs of ventral cells (P cells) interdigitate at the midline, forming a single row which contributes neurons to the ventral nerve cord.

Virtual Versus Physical Segmental Boundaries

What is primary: the segment or the limit between segments? In terms of morphology, the boundaries between compartments are virtual boundaries, with nothing likely to be seen through the lenses of the microscope and nothing to be seen in fossils. But compartment boundaries may

be secondarily elaborated into physical boundaries, something one can actually see in the animal. At this stage, what in fact exists is an alternating pattern of longer and shorter units, with the longer units corresponding to conventional morphological segments and the shorter units corresponding to the 'walls' between them. Physical boundaries are not required for functional integration within each segment and/or for functionally isolating its global metabolism. Local integration, if elicited by a specific local trigger, may rest on a higher frequency of interactions within the segment with respect to the interactions between segments. A conversation does not require partners to be physically isolated within a room, but people involved in a lively conversation, even if in a crowded room, are nevertheless functionally isolated from all the other people around them. Physical boundaries may offer an opportunity for stabilising a segmental pattern which may be later translated, as prepattern, into more advanced serial architectures. In *Drosophila*, for example, the parasegmental boundary has been regarded as an organising centre since it is used as a reference to pattern neighbouring cells (Ingham 1991, Ingham and Martínez-Arias 1992, Dahl, Koseki, and Balling 1997).

Rather than as a frozen structure conserved throughout development, boundaries should be thought of as dynamic features. In the *Drosophila* embryo, parasegmental boundaries are maintained by the pair-rule gene expression that established them in the first place, for the first three hours only, when many of the pair-rule genes switch off and their products disappear. These boundaries are dynamically maintained by segment polarity genes. This persistence of parasegmental boundaries is critical to avoid mixing of neighbouring cell populations that are still dividing, to set limits to gene expression, and to establish gradients of positional information (Lawrence and Sampedro 1993). The basis of insect segmentation is thus a juxtaposition of distinct cells to form a boundary that is self-perpetuating (Akam 1994).

The boundary between the histoblasts and the surrounding larval cells and segment boundaries is also a self-perpetuating unit. Old experiments with *Drosophila* demonstrated that a histoblast nest grafted in a larval segment in inverted antero-posterior polarity could not affect the polarity of the host abdomen; but this effect was obtained if the inverted histoblasts were accompanied by the polyploid larval epidermal cells which surround them and which will be destroyed during metamorphosis. This was interpreted as proof that the positional information responsible for a normal pattern resides in the polyploid epidermal cells (Milner and

Bleasby 1985). It is quite clear that it resided instead in the boundary between histoblast and polyploid cells: When polyploid epidermal cells alone are grafted into the wrong position, histoblast differentiation is unaffected.

How Many Times Did Metazoans Evolve Segmentation?

There might well be more than one way to subdivide an embryonic field into sharply defined populations of cells linearly arranged along the antero-posterior axis. If so, there is little reason to expect that the segments of all segmented animals will be strictly comparable from a developmental point of view. I will briefly review evidence suggesting that segmentation mechanisms are so diverse as to imply a multiple origin of segments along the evolutionary history of metazoans. The common features undeniably shared by all segmented animals will be explained as due to two different causes: the 'generic' character of segmentation (see page 35) and the multiple, independent involvement of similar genes, or gene batteries, in the segmentation processes.

As soon as we realize that all segments are not necessarily the product of a single developmental process, we may also ask whether all segments within one and the same animal – be it an annelid, an arthropod or a vertebrate – are strictly equivalent from a developmental point of view. This point will be discussed later.

During the last two decades, our knowledge of expression patterns and developmental roles of the so-called segmentation genes have grown quite considerably, at least for some 'model' animals. It is unfortunate that we still know very little about the role these genes play in animals that a comparative morphologist would suggest as representative of peculiar kinds or degrees of segmentation, as are the myriapods. The first evidence, at last, is being published for the expression of the segment-polarity gene *engrailed* in the geophilomorph centipede *Strigamia maritima* (Kettle et al., in press) (Figure 9.1) and for the *Hox* genes – which are marginally involved, in this case, in segmentation – of the lithobiomorph centipede *Lithobius* (Hughes and Kaufman 2002).

One of the most important generalisations obtained thus far regarding the identity and roles of segmentation genes in different metazoan groups is the extensive similarity of genes and control cascades found in the different phyla of segmented animals. This has been often construed as proof that, contrary to the traditional phylogenetic views, segmentation might

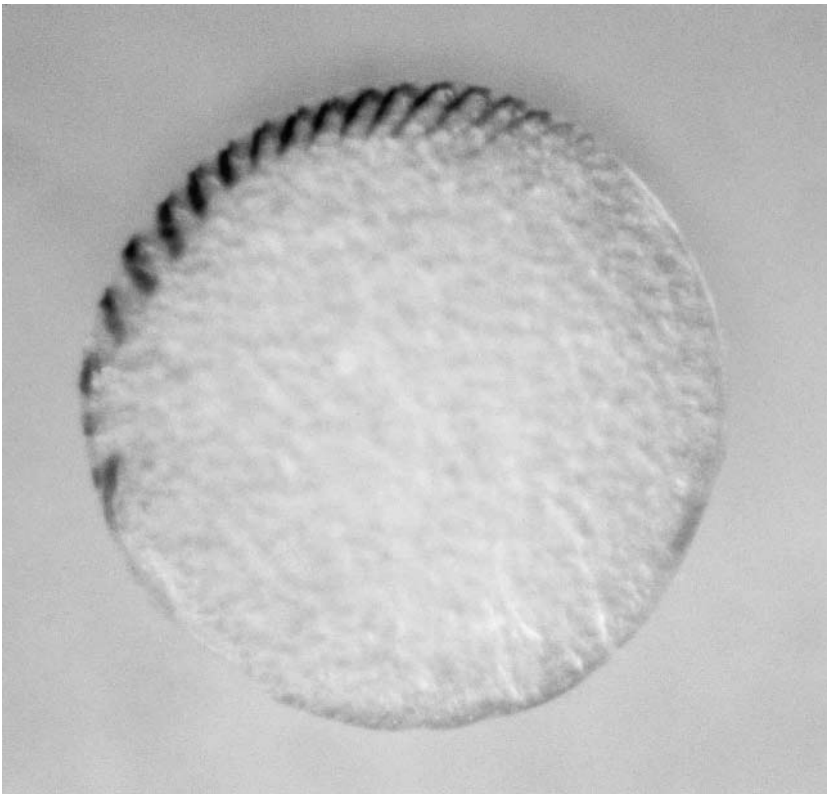

Figure 9.1. Expression pattern of the segment-polarity gene *engrailed* in the growing germ band of the geophilomorph centipede *Strigamia maritima*. (Courtesy of C. Kettle and W. Arthur, Sunderland, UK.)

have arisen only once in the history of metazoans. This view has developed into a key component of the concept of Urbilateria, the putative common ancestor of all bilaterian (triploblastic) animals (De Robertis and Sasai 1996).

Reconstructing Urbilateria

A segmented Urbilateria has been suggested, more or less explicitly, by several recent authors (Kimmel 1996, De Robertis 1997, L.Z. Holland et al. 1997, Palmeirim et al. 1997, Christ et al. 1998, Holland and Holland 1998). Less explicit are Valentine, Erwin, and Jablonski (1996), when crediting this bilaterian ancestor with 'some form of serial structure', whereas Dewel (2000) goes so far as to speak, more specifically, of segments ontogenetically

expressed as blocks of mesoderm, somites and probably adjoining fields of ectoderm or neuroectoderm.

In addition to segmentation, the following set of traits has been attributed to Urbilateria:

- a 'humble appendage or antenna-like outgrowth' (De Robertis 1997, based on the presence of *fringe*, *serrate* and other genes; Panganiban et al. 1997, with doubt; Morata and Sánchez-Herrero 1999) or even a patterned outgrowth (Dewel 2000, Dong, Chu, and Panganiban 2001); but this is rejected by Mittman and Scholtz (2001)
- a hemocoel (Valentine, Erwin and Jablonski 1996), or a more articulate series of internal cavities, including at least two large undivided lateral or dorso-lateral coeloms and serially repeated lateral gonocoels, each with a gonoduct and gonopore to the exterior; perhaps also serially repeated coelomic cavities with connections (gill slits and pores) to both the gut and the exterior (Dewel 2000)
- a contractile blood vessel or heart (De Robertis 1997, based on the presence of genes such as *Tinman/Nkx2.5* and *DMEF2*, which are involved in the specification of a heart)
- a skeleton (Jacobs et al. 2000)
- a primitive photoreceptor (Bolker and Raff 1996, De Robertis 1997, Gehring and Ikeo 1999, Neumann and Nüsslein-Volhard 2000, Kumar 2001), based on the presence of some homologue of *Pax6/eyeless* (see pages 25–26)
- brain and brain areas (Arendt and Nübler-Jung 1996)
- development through a primary ciliated larva with a tube-shaped gut divided into anterior, middle and posterior portions (Arendt, Technau, and Wittbrodt 2001)
- cephalisation (Finkelstein and Boncinelli 1994, Dewel 2000)
- antero-posterior polarity (De Robertis 1997, based on the presence of *Hox* gene complexes with antero-posterior colinear expression; cf. page 47)
- dorso-ventral patterning (De Robertis 1997, based on the presence of pairs of dorso-ventral patterning genes homologous to *sonic hedgehog/chordin* and *decapentaplegic/Bone Morphogenetic Protein-4*; cf. page 155)

I have listed these features in approximate order of increasing likelihood, starting with segmentation and the presence of appendages [two features

that no student of phylogeny (e.g., Ax 1995) would probably ascribe to the common ancestor of all bilaterians] and finishing with features such as antero-posterior and dorso-ventral patterning, which are, in a sense, defining features of the bilaterian organisation. However, several other authors (e.g., Akam 1989, Abouheif 1997, Laufer et al. 1997, Shubin, Tabin, and Carroll 1997, Arthur, Jowett, and Panchen 1999) have regarded most of these putative features of Urbilateria as independently acquired by several phyletic lines, through independent co-option of the same, or similar genes and control pathways. Axis paramorphism (Minelli 2000b) may have contributed to the spread of these convergent features.

It must be noted that none of the authors who have expressed their views on the putative organisation of Urbilateria used any of the standard methods currently available for phylogeny reconstruction. Therefore, from a methodological point of view, none of these Urbilaterias qualify as reliable ground-plan reconstruction. The more features are added to the picture, the more Urbilateria becomes similar to the Urpflanze (the archetypal plant) drawn by P.J.F. Turpin for a French edition of Goethe's natural history work (1837). Turpin's plant is dramatically overloaded by samples of all kinds of leaves, roots, flowers, and inflorescences (Figure 9.2). Typological thinking (cf. Richardson, Minelli, and Coates 1999) collapses features over features onto one archetypal form, but it does not help understanding their actual origin or their changes in evolution.

Davidson (2001) has forcefully argued against the fashionable reconstructions of the Urbilateria, which sum up to produce "an impossible and illogical image of the bilaterian common ancestor" (p. 189). It is clear that these reconstructions underestimate (and sometimes simply ignore) how much animal phyla may differ in important details of the developmental processes by which they obtain segments, eyes, hearts, and so on. What is conserved across the whole of bilaterians are the differentiation gene batteries involved in specification of equivalent body parts in different phyla, but the actual ways they develop may differ no less than their final morphologies.

Segments in Annelids, Arthropods, and Vertebrates

This is not the place for reviewing in detail the mechanisms by which segments are made in annelids, arthropods, and vertebrates. A few notes may suffice for the purpose of later discussion. In annelids and in vertebrates, segmentation is basically a mesodermal affair, whereas in arthropods it is primarily ectodermal (Minelli and Bortoletto 1988, Dewel 2000).

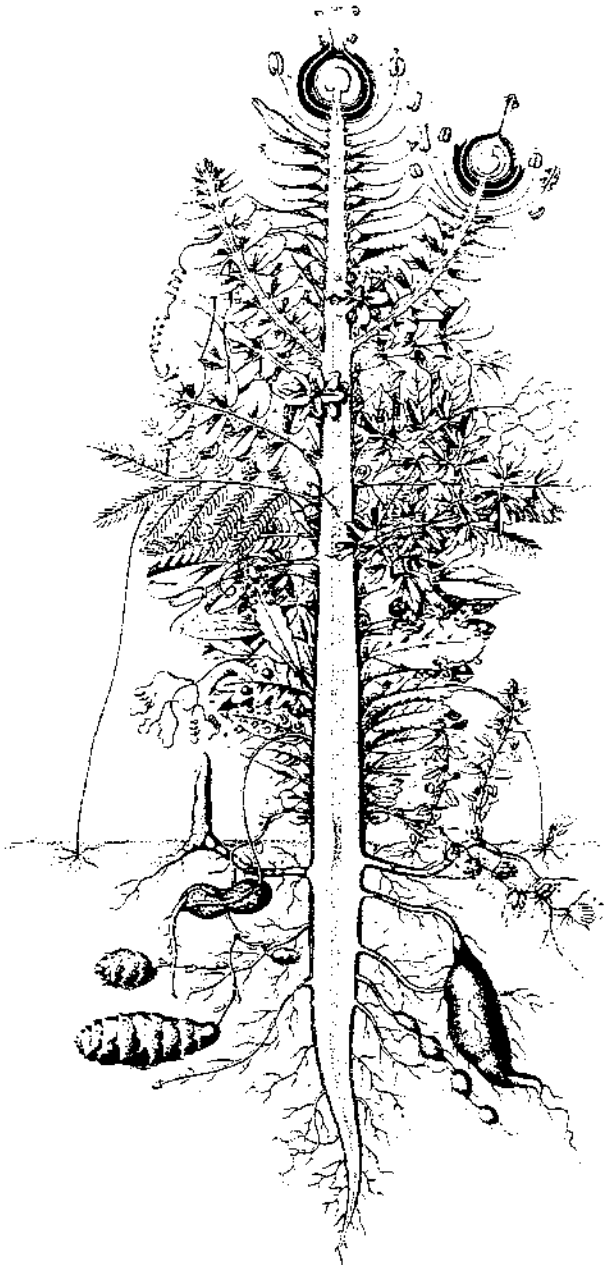

Figure 9.2. Goethe's Urpflanze (archetypal plant) as depicted by P.-J.-F. Turpin to illustrate a French edition of Goethe's natural history works (1837).

In the trunk of vertebrates, the primordial segmentation (somitogenesis) of the paraxial mesoderm (Jacobson 1993) is associated with a molecular oscillator, the segmentation clock, whose periodicity matches that of somitogenesis (Pourquié 2001). The existence of such a clock in the cells of the presomitic mesoderm was originally proposed in theoretical terms (Cooke and Zeeman 1976, Cooke 1981). Its existence was demonstrated later (e.g., Palmeirim et al. 1997) on the basis of the periodic expression of several genes appearing in a dynamic sequence as a wave sweeping caudo-rostrally along the whole presomitic mesoderm once during each somite's formation (reviewed in Cooke 1998, Pourquié 2001). Kerszberg and Wolpert (2000) have rephrased Cooke and Zeeman's model in terms of a temporal prepattern translated into a spatial (segmental) pattern. The prepattern would be a stable sinusoidal wave originating from the oscillator in the proliferative ('progress') zone. When leaving it, the cells keep a permanent record of the state of oscillation in which they are found at that time. More recently, a molecular link has been suggested between *Hox* gene activation and the somitic segmentation clock (Tabin and Johnson 2001, Zákány et al. 2001). The clock ticks at the rate of one somite every 30 minutes in zebrafish (Stickney, Barresi, and Devoto 2000) and one somite every 90 minutes in the chick (Stockdale, Nikovitz, and Christ 2000).

To date, no annelid has been studied in comparable detail. In leeches, we know that segments are 'founded' by cells serially produced by ecto- and mesoteloblasts. There is segmental expression of *engrailed* in the nervous system of the developing embryo, but no evidence suggests that in annelids this gene is involved in segmentation, in striking contrast to this gene's role in arthropod segmentation (Wedeen and Shankland 1997, Seaver et al. 2000, Bely and Wray 2001).

As for arthropods, it is clear by now that the mechanisms of segmentation first studied in *Drosophila* (for reviews see Lawrence 1992, Carroll, Grenier, and Weatherbee 2001), involving the expression of pair-rule genes and segment polarity genes, are largely shared by other members of the phylum, but differences between the different lineages are increasingly emerging. In some groups, for example, there is a distinct role for some *Hox* genes in segmentation, at odds with what happens in *Drosophila*.

Limits of a Typological View of Segments

We should avoid taking segmental identity too literally. In insects, appendages originally belonging to a given segment may associate secondarily with a contiguous segment. Thus, the cerci (i.e., the appendages of the vanishing abdominal segment XI) are more or less closely attached to

segment X. Even more conspicuous is the fusion of mandible and maxilla, two appendages belonging to different segments, in the larvae of some orthorrhaphous Diptera (Chaudonneret 1979, Teskey 1981). In silverfish and dragonflies, some tergal muscles are transsegmental, crossing the segmental border to become attached to the tergum of the next segment (Matsuda 1970). During embryonic or postembryonic development, some body segments seem literally to disappear, at least on their dorsal (or ventral) aspect. This is frequent in the posterior body segments (genital region) of many insect orders. The most conspicuous case is probably that of the entomobryomorph Collembola, in which the dorsal sclerite of the first thoracic segment (pronotum) disappears completely during embryonic development, therefore the first dorsal sclerite behind the head is the mesonotum (Matsuda 1979).

The anatomical proximity of originally neighbouring segments sometimes gets lost following rearrangement of some segmental components. In the thorax of dragonflies, the two wing-bearing segments (meso- and metathorax) are strongly associated to form a complex called the pterothorax. The 'true' dorsal sclerites (terga) of these two segments are quite small and do not cover the whole dorsal surface of the pterothorax. The anterior half of this complex is covered instead by the pleurae (originally lateral) of the mesothorax, which are joined along the midline, in front of what remains of the mesotergum. In this way, the dorsal sclerites of the first thoracic segment (prothorax) are widely separated from the corresponding mesothoracic sclerite (Figure 8.20). In some sphecid wasps, the usual wasp waist becomes a long peduncle, entirely formed by the second segment of the abdomen, despite the fact that it seems to comprise two segmental units, rather than one. This is because the dorsal and ventral sclerites of this segment have shifted longitudinally apart in such a way that the anterior part of the peduncle is covered by the sternal plate only and the posterior part is covered by the tergal plate only (Figure 9.3).

In other cases, unexpected segment composition is the result of profound metamorphoses, such as those undergone by *Drosophila*, in which three pairs of imaginal discs (clypeo-labral, eye-antennal and labial) produce the whole imaginal head. Therefore, only three of the theoretical six head segments (labral, antennal and labial) do actually contribute to the imaginal head, whereas the other three segments (intercalary, mandibular and maxillary) do not seem to be involved. The contribution of each imaginal disc goes beyond its expected segmental domain. For instance, the eye-antennal disc gives rise, in addition to the eye and the antenna,

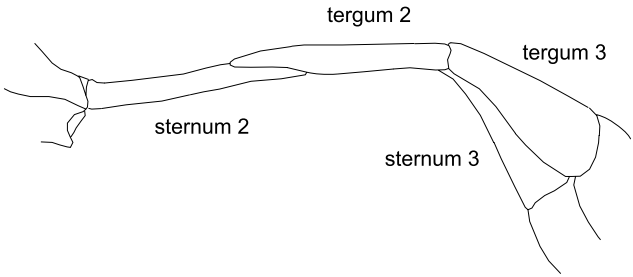

Figure 9.3. Anterior abdominal segments of the sphecid wasp *Ammophila sabulosa*. Tergum and sternum of the second abdominal segment are dissociated and define a segment-like unit each; those of the third segment are in normal register. Head and mesosoma of the wasp (cf. Figure 5.1) are to the left.

to most of the head capsule and also to the maxillary palp. In this respect, it takes the role of several separate anlagen in other insects (e.g., the cabbage butterfly *Pieris*). This unexpected origin of the maxillary palp raises the question of its 'real' nature, as suggested by traditional comparative morphology. Cell lineage and position of the anlage suggested instead that the palp may be antennal in origin (Morata and Lawrence 1979, Struhl 1981), even if it belongs, morphologically, to a segment three units more caudal with respect to the antennal segment. The problem is, whether the eye-antennal disc does correspond exactly to one segment, as suggested in terms of cell lineage (Lawrence 1981), or not. The apparently odd origin of the maxillary palp of *Drosophila* could be explained by the fact that the antennal and maxillary anlagen of the larva occupy nearly adjacent positions on the blastoderm map and might have been incorporated into the same disc simply due to their proximity (Jürgens et al. 1986).

Segmentation may get lost during metamorphosis, often in relation to sessile life. This is common in parasitic copepods, especially in the females, which may grow to a truly enormous size, 20 or 30 cm, whereas most free-living species are 0.5–2 mm long. In *Lernaeascus*, which is externally unsegmented, the retention of the swimming legs offers good morphological landmarks that permit identification of the original body segments, but this is next to impossible in the female of *Leposphilus*, in which the only segmental reference left is the position of the genital orifice (Kabata 1979). In a group of tiny parasitic crustaceans close to the copepods, the tantulocarids, the adult trunk is sac-like and lacks any evidence of segmentation. The terga and the appendages of the thorax are lost at the time of the final moult (Schram 1986). In the females of the scale insects, which

are completely sessile following the first active larval instar, the abdominal segments are more or less completely fused (Strümpel 1983). The most dramatic loss of segmentation during development is offered by rhizocephalans such as *Sacculina*. When the female cypris larva of these crustaceans fixes itself on a crab, the thoracic appendages and the abdomen are literally amputated and cast off, and the rhizocephalan changes into a rhizoid branching body that expands through the victim's tissues, only to emerge from it later in the form of a bag into which a male larva will at last deliver its equally shapeless content.

Segmentation: One Animal, More than One Mechanism

To acknowledge that segmentation processes are not the same in animals as different as insects and vertebrates should not come as a surprise, whereas the notion that not all the segments of a segmented animal are the product of the same mechanism may meet more resistance. There are good reasons, however, to acknowledge the widespread coexistence of two different kinds of segments within the same animal.

Double Segmentation: Eosegments and Merosegments

Leeches, with their secondarily annulated segments, may help in introducing the notion of double segmentation (Minelli 2000a).

A mid-body segment (those closer to the two body ends have reduced annulation or no annulation at all) is basically split into three first-order annuli ($a_1 a_2 a_3$), as in most species of the family Glossiphoniidae. In the medicinal leech and in many other leech species, the first and the last of these three primary annuli split into secondary annuli, giving rise to a five-annulated segment ($b_1 b_2 a_2 b_5 b_6$). More complex patterns are also known, as in the six-annulated $b_1 b_2 a_2 b_5 c_{11} c_{12}$ segments of *Dina lineata* or the seven-annulated $c_1 c_2 b_2 a_2 b_5 c_{11} c_{12}$ segments of *Trocheta subviridis dalmatina*, with two third-order *c* annuli replacing one or more secondary *b* annuli.

Zoology textbooks are clear in pointing out the difference between the 'true' segments – identified by the repetition of nephridia, nervous ganglia, and so on – and the superficial annuli. This is correct, but it obscures the fact that many kinds of 'segments' found in other animals are much more similar to leech annuli than to leech segments.

An example is the rhombomeres, the segmental units of the vertebrate hindbrain. An interesting feature of rhombomeres is that they do not form in regular antero-posterior progression (or synchronously), but through a

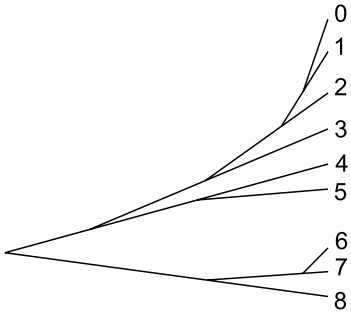

Figure 9.4. Order of splitting of chicken rhombomeres (0–8, rhombomeres r0 through r8). (After data in Vaage 1969.)

form of intercalary splitting. In the chick embryo, a first transversal fission separates the anterior precursor of r1–r5 from the posterior precursor of r6–r8 (Vaage 1969). These two units behave as first-order segments, which split into second-order segments following a characteristic schedule (Figure 9.4). This peculiar generation order depends on surface properties of the rhombomeres. As described previously, all odd-numbered rhombomeres are formed by cells with the same surface properties, as are all even-numbered rhombomeres, whereas the cells from an odd- and an even-numbered rhombomere, if mixed together, sort out the mix with those of their kind. Let us call A and B the two kinds of cells and let A be the kind of cells in the founding rhombomere. From this first unit, a B cell population may segregate either anteriorly or posteriorly; say posteriorly, thus giving rise to an A**B** pattern. (I will use **boldface** for the newly formed unit.) The next step will be the production of a new B unit in front, or an A unit at the rear, thus producing respectively a **B**AB or an AB**A** triplet. Either (or both) of the terminal units of the triplet can now contribute to expanding the set. Thus, BAB may generate either **A**BAB or BAB**A** (or **A**BAB**A**); ABA may generate either **B**ABA or ABA**B** (or **B**ABA**B**). The middle unit of the triplet cannot contribute any more to the formation of new units, because a B middle unit, for example, would produce an A offspring unit, whose cells would soon merge with the A cells of the segmental unit immediately in front or to the rear of the dividing unit. The argument would be obviously the same if starting with an A middle unit; just exchange A for B and vice versa). In this kind of string, only the terminal units can produce new units; all the others remain 'frozen' in the state in which they were produced.

The way rhombomeres are produced does in fact agree with this model, as apparently does (an experimental proof would be welcome) annulation

of leech segments. Along another axis, the same process seems to be at work in the multiplication of the 'segments' of the flagellar part of the antenna, in arthropods as diverse as a dragonfly larva (Aguesse 1965) or an isopod crustacean (Ronco and Minelli, unpublished results).

Clearly, these segmentation rules do not apply to the production of the 'true' segments of the leech or the somites of vertebrates. In a sense, leech annuli and vertebrate rhombomeres are 'lower level segments' with respect to leech metameres and vertebrate somites.

Therefore, it seems sensible to distinguish between *holomeric segmentation*, involving the whole body axis (or the whole axis of an appendage) and producing 'true' segments (*eosegments*), and *meromeric segmentation* producing *merosegments* within a previously defined eosegment (Minelli 2000a).

In developmental terms, both kinds of units deserve the name of segments, because they subdivide an embryonic field into distinct cell populations linearly arranged along the animal's main body axis. How this subdivision is achieved, however, is different. Meromeric segmentation, in particular, is probably nothing more than a sustained and stabilised form of compartmentalisation, a cellular process whose relationship to segmentation has been occasionally suggested (Newmann 1993, Newmann and Müller 2000; cf. also Boncinelli 1994).

Meromeric segmentation is not limited to the few examples given previously. More instances are provided by polychaetes (cf. Edmonds et al. 2000). In the Arenicolidae, for example, the genus *Branchiomaldane* has two annuli per segment, and the other genera have five annuli per segment in the trunk region bearing external gills, fewer on the anterior gill-less segments. In Scalibregmatidae, the anterior segments are two- or three-annulate, but annulation extends to the dorsal surface only. In the syllid *Procerastea halleziana*, strong contractions of the longitudinal segmental muscle cause fragmentation of the worm which breaks at predetermined points (megasepta) corresponding to deep constrictions of the gut (Okada 1929). One wonders whether these intestinal constrictions – possibly expressions of a patterning influence of the mesoderm on the endoderm, as we have seen occur in the corresponding feature in some leeches – correspond to limits between eosegments, each of them subdivided into several merosegments. In many syllids, regularly spaced pigment bands divide the worm in what seem to be, once more, eosegments, which are regularly (but not uniformly) subdivided into merosegments.

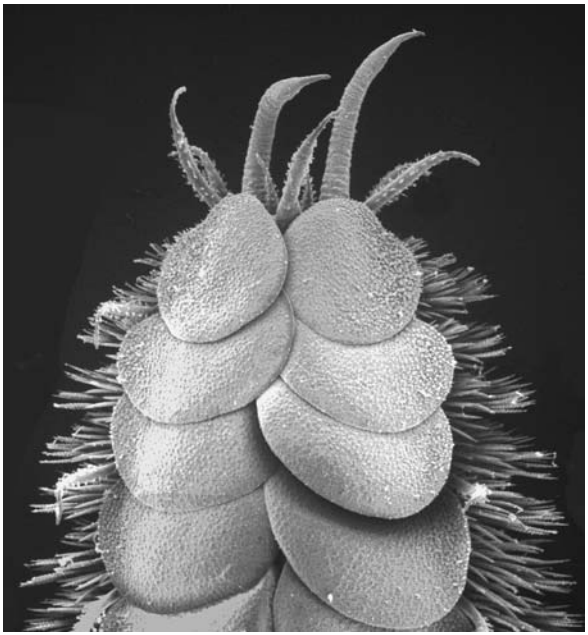

Figure 9.5. *Harmothoe* sp. (Polynoidae) from Massachusetts. (Courtesy of F. Pleijel, Paris.)

In other polychaetes (aphroditids, eulepethids, polynoids, and sigalionids), segments with and without large dorsal appendages (elytra; Figure 9.5) alternate regularly, but with one 'accident' (elytra on both segments 4 and 5) and a change of pace at a critical spot. In these families, elytra are generally present on segments 2, 4, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23, and sometimes on segments 25 and 27. Posterior to this segment, the pattern changes and is less universal, with elytra occurring, for instance, on all further segments in sigalionids, but on *every third segment* in aphroditids (Edmonds et al. 2000, Fauchald 2000). Once more, this periodic pattern is probably the consequence of double segmentation. Comparable examples are found in the oligochaetes. In *Pontoscolex* and some other glossoscolecid earthworms, the setae are not arranged in continuous longitudinal rows, as in the majority of the oligochaetes; but pairs of setae are alternatively closely and widely paired in successive segments (Sims 1982). 'Double segments' that might exemplify merosegmentation are also found in some tardigrades [e.g., in some species of *Diphascon* and *Milnesium* (Maucci 1986; Figure 9.6)].

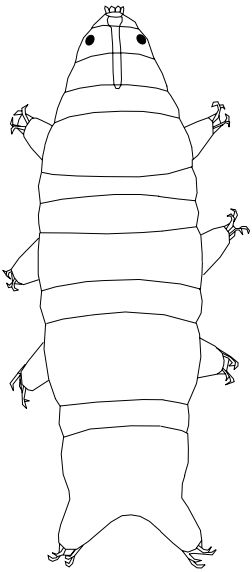

Figure 9.6. Habitus of a tardigrade (*Macrobiotus*) with secondarily subdivided body segments.

The Naupliar-Postnaupliar-Meromeric Model of Arthropod Segmentation

Eosegments and merosegments are two different kinds of segmental units that may coexist in one animal. In addition, there are good reasons to argue that not all eosegments in a given animal are necessarily the same. They may have a different origin, in phylogeny as well as in ontogeny.

In the case of annelids, there have been many speculations about a possible contrast between a small set of anterior larval segments and a longer series of postlarval segments. This concept's champion was the Russian zoologist Ivanov (= Iwanoff), who claimed he was able to read this distinction in the events accompanying the metamorphosis of a trochophora larva into an adult polychaete (Iwanoff, 1928). This interpretation was later extended to other animals, the arthropods in particular, which were then regarded, according to tradition, as the annelids' closest relatives (Ivanov 1940, Remane 1950). The main arguments putatively supporting the distinction of larval and postlarval segments (Siewing 1967, Korn 1982) were the different origin and prospective value of the mesoderm in either part of the trunk and the different timing of segment formation, simultaneous in the larval segments but successive in the postlarval segments. The

factual basis of these arguments has been seriously undermined by Dohle's (1979) criticism, but there is some ground for reopening the question, whether the segments in one given segmented animal are all necessarily determined the same way. I will examine mainly the phylum with which I am more familiar – the arthropods.

That the process of segmentation of the anterior head (the so-called prosocephalon) of insects is basically different from the process responsible for segmentation of the posterior head and the whole trunk (the thorax and abdomen) is by now beyond any reasonable doubt (Grossniklaus, Cadigan, and Gehring 1994, Vincent, Blankenship, and Wieschaus 1997, Rogers and Kaufman 1997, Wimmer et al. 1997). Therefore, despite prevailing contrary views (e.g., Scholtz 1997), segments of the anterior head are not strictly homologous to those posterior to them.

In terms of genes whose early expression is involved in determining the insect's segmental architecture, the anterior head lacks any expression of the pair-rule genes, whose role in segmentation has been widely studied in *Drosophila* (reviewed, e.g., in Lawrence 1992, Carroll, Grenier, and Weatherbee 2001). This evidence (cf. Jürgens and Hartenstein 1993, Rogers and Kaufman 1996, 1997, Wimmer et al. 1997) contributed to the development of a five-tagma model of insect architecture, in which the head proper or prosocephalon was considered as basically distinct from the posteriorly adjoining gnathocephalon, and the traditional abdomen of insect morphologists was split into 'true abdomen' and postabdomen (Akam, Dawson, and Tear 1988).

If the anterior head lacks any expression of the pair-rule genes, it corresponds to the domains of expression of other genes, such as *orthodenticle*, *empty spiracles* and *buttonhead*. It is probably not without significance that homologues of these genes are also expressed in the forehead of vertebrates. The boundary between the anterior head and the rest of the body could be homologous in insects and vertebrates (Holland, Ingham, and Krauss 1992, Finkelstein and Boncinelli 1994). This correspondence is possibly ancestral; but in my view, it has only to do with axial patterning, not with segmentation per se, due to the basic difference in the segmentation process in the two phyla.

But a distinction between anterior head and the rest of the body is not just suggested by gene expression domains. The independent segmentation of the first few body segments is also suggested, in arthropods, by the organisation and development of the nauplius, the typical larva of many crustacean groups whose very short body bears just three pairs of

appendages (antennules, antennae and mandibles) corresponding to as many body segments. Caudal to the mandibular segment is a growth zone from which the remaining segments will form later.

The fact that the anterior half lacks the pair-rule gene expression which marks the differentiation of segments in the remainder of the body is likely to be the cause of major developmental differences between the two sets of segments: the naupliar and postnaupliar ones (Minelli 2001).

Transcriptional control and expression patterns of the pair-rule genes are adequately known from one model species only (*Drosophila melanogaster*), but there is evidence enough to suggest that the spatial and temporal expression of these genes may be different in other insects and in arthropods at large. However, despite the limited evidence available to date, there seem to be good reasons to expect some pair-rule effect in arthropods generally. The role of pair-rule genes in the segmentation of the germ band has been demonstrated, for example, in the beetle *Tribolium* (Sommer and Tautz 1993), in spiders (Damen, Weller, and Tautz 2000) and also in the grasshopper embryo (Davis, Jaramillo, and Patel 2001), in which segmentally smooth progression in the expression of *engrailed* stripes had previously induced doubt regarding the presence of any pair-rule pattern. To introduce the argument, it is better to put the fruit fly temporarily aside to focus instead on multisegmented arthropods, such as millipedes and centipedes.

Segment number in these arthropods is extremely diverse. Centipedes, for example, range from the 15 leg-bearing segments of the house centipede (*Scutigera*) and the brown centipede (*Lithobius*) up to 191 segments in a geophilomorph species, whereas millipedes may have just 13 (some penicillates or pin-cushion millipedes) or as many as 375 pairs of legs, as described in a previous chapter. What is most puzzling, however, is not this numerical diversity. More puzzling is *the lack of diversity* in segment number in the other arthropod groups, were it not modest and obviously secondary reduction at either extremity of the abdomen (insects) or the opisthosoma (arachnids). Why should a scorpion, a spider and a tick have the same number of body segments? Why should we find the same number of segments in a caterpillar, a mayfly, and a cockroach? Numerical diversity rules nearly all serial structures, from the vertebral column to the teeth arcades in mammals, from the segments of the annelids to the petals and stamens of flowers. But let's go back to myriapods. These arthropods, finally, *are* diverse in the number of body segments. But this is right where something unexpected awaits us.

First, many myriapods, despite their fairly large number of segments, do not have any individual variation in segment number. For example, *Dicellophilus carniolensis* is a centipede with 43 pairs of legs: no exception has ever been recorded, despite the thousands of specimens collected across its extensive geographic range. In the family (Mecistocephalidae) to which *Dicellophilus* belongs, most species have either 41 or 45 or 49 pairs of legs, but this number is rigorously fixed for each individual species; there are one or two exceptions among the few species with more than 49 segments (Bonato, Foddai and Minelli in press). The same is true for many millipede families, within which the number of leg pairs is rigorously 60 or 64, for all individuals of all species in the family (Hoffman 1982). How can these animals develop their full complement of segments without introducing the slightest error? In vertebrates, for comparison, the vertebral number is mostly quite stable but, as expected, it becomes increasingly variable as its mean value increases, behaving as a typical meristic character under genetic control. The heritability of vertebral number in vertebrates has been recently shown by Lindell (1996) for the European common viper (*Vipera berus*), by Jockusch (1997) for the plethodontid newts of the genus *Batrachoseps* and by Billerbeck, Orti, and Conover (1997) for a small fish, the Atlantic silverside (*Menidia menidia*).

The puzzling question of the possible origin of the astonishing stability in segment number shown by some myriapods was first addressed by Maynard Smith (1960), who suggested this precision could be only expected if the animal generates segments through a two-step process. The first step would be the production of a small number of primary units, the second the splitting of these primary units into a fixed number of secondary units corresponding to the animal's conventional (morphological) segments. It seems to be much easier to control the production of a few primary units and their subsequent duplication than to get a rigorously unerring output from a single source producing a few dozen elements serially. Maynard Smith's suggestion looks obviously attractive, as a possible explanation for the lack of variation in the number of body segments in several myriapod lineages, but there are further arguments to support it.

Look, for instance, at centipedes. Oddly enough, there is no adult centipede with an even number of segments. Moreover, ca. 1,000 centipede species with 15 pairs of legs are known, but none with 17 or 19. The next higher number recorded in nature is 21 (as in most scolopendromorphs), then 23 (as in the remaining scolopendromorphs). Another gap (no centipede species with 25 pairs of legs is known) must be bridged before we

reach the lowest extreme of the very large range (27–191), over which the number of leg-bearing segments of the worm-like geophilomorph centipedes is distributed. Of the numbers in this range (27, 29, 31, ... , 191), some are distinctly preferred over those immediately lower or higher. We have already seen that, in the Mecistocephalidae, the large majority of the species have either 41 or 45 or 49 pairs of legs – those with either 43 or 47 being very few. In all centipedes with 15, 21, or 23 pairs of legs and in nearly all Mecistocephalidae, segment number is common to all individuals within the species. In the remaining geophilomorphs, there is intraspecific variability in segment number and this variability is generally accompanied by differences between the sexes, with the segment number distribution for the females shifted towards higher numbers (often 2 or 4 units higher than in the conspecific males). This picture clearly points to the existence of developmental constraints (Arthur and Farrow 1999): more precisely, of multiplicative mechanisms based on the production of a common set of primary segmental units (*eosegments*) subsequently subdivided, by a stereotyped process of subdivision, into secondary units (*merosegments*: Maynard Smith 1960, Minelli and Bortoletto 1988, Minelli 2000a).

The expression patterns of pair-rule genes are suggestive of molecular and cellular mechanisms that could materialise this multiplicative segmentation process. In *Drosophila*, an earlier 7-stripe pattern sets the blueprint for a later 14-stripe pattern; in *Tribolium*, 8 and 16 stripes are respectively seen at earlier and later stages of pair-rule gene expression. However, a few comments are required.

First, pair-rule gene expression, suggesting early determination of primary segments and their subsequent multiplication, does not extend to the anterior head. Merosegmentation would only affect the postnaupliar eosegments, but not the anterior naupliar ones.

Second, the degree of merosegmentation is possibly the same in all species within very large groups where adult segment number does not show interspecific diversity, as in insects and malacostracan crustaceans. But it may vary, even between closely related species, as in the centipede genus *Mecistocephalus*, in which most species have 49 pairs of legs, many have only 45 and others have more than 49.

The problem is whether there is any factual evidence as to the production of arthropod segments in other than a plain anterior-to-posterior progression. Admittedly, evidence is vanishingly scanty, but this may be due, in addition to the expected technical difficulty, to the fact that this question has never been seriously addressed. Nevertheless, the expression patterns of segmentation genes in the tobacco hornworm *Manduca sexta* suggests

(Kraft and Jäckle 1994) that segment formation in this insect may *not* be due to sequential addition from a posterior budding zone.

There are several reasons to believe that the number of eosegments is much more strictly controlled than the pattern of merosegmentation. My strongest argument is derived from centipedes. It is reasonably easy to fit the recorded pattern of diversity in segment number in this class to a model where the leg-bearing segments (plus the first trunk segment whose appendages are transformed into poison claws) is derived from eight postnaupliar eosegments. The degree of merosegmentation would be two for the eosegments of Scutigeromorpha, Lithobiomorpha, and Craterostigmomorpha (15 leg-bearing segments); three (but probably two for a few anterior eosegments) for the Scolopendromorpha (21 or 23 leg-bearing segments); and 4 to 24 for the Geophilomorpha (27–191 leg-bearing segments; Minelli et al. 2000). To account for the segments forming the posterior head and the legless terminal segments, we may hypothesise the involvement of two more eosegments. This makes a possibly fixed total of 10 eosegments, giving rise to a variable number of postnaupliar merosegments. Segmental organisation of the crustacean nauplius and of the fore end of arthropods generally suggests that three more (eo)segments form the naupliar part of the body (Minelli 2001). Thus, we have a grand total of 13 eosegments, possibly basic to the body architecture of all arthropods. If so, we will be forced to reject (at the level of eosegments at least) the current view that individual segments of arthropods can be gained or lost from the body plan within particular lineages (Brusca and Brusca 1990, Nagy and Williams 2001).

These 13 eosegments might even be derived from an equal number of serially repeated elements already present in a primitive ecdysozoan, before the development of overt segmentation in the arthropod lineage. Suggestions come from two different groups of non-arthropod ecdysozoans: nematodes and kinorhynchs. Thirteen is the number of pioneer motoneurons which migrate in the developing ventral nerve cord of *Caenorhabditis elegans* (Sulston and Horvitz 1977, Walthall 1995). Thirteen, again, is the number of body units (imperfect segments or 'zonites') forming the body of the adult kinorhynchs (Kristensen and Higgins 1991; Figure 9. 7).

Reliable Patterning of Eosegments and the Variable Schedule of Merosegmentation

In centipedes, the basically uniform series of trunk segments is generally punctuated by a 'mid-body anomaly' (Minelli et al. 2000) which only affects one or a few segments. In lithobiomorphs, craterostigmomorphs

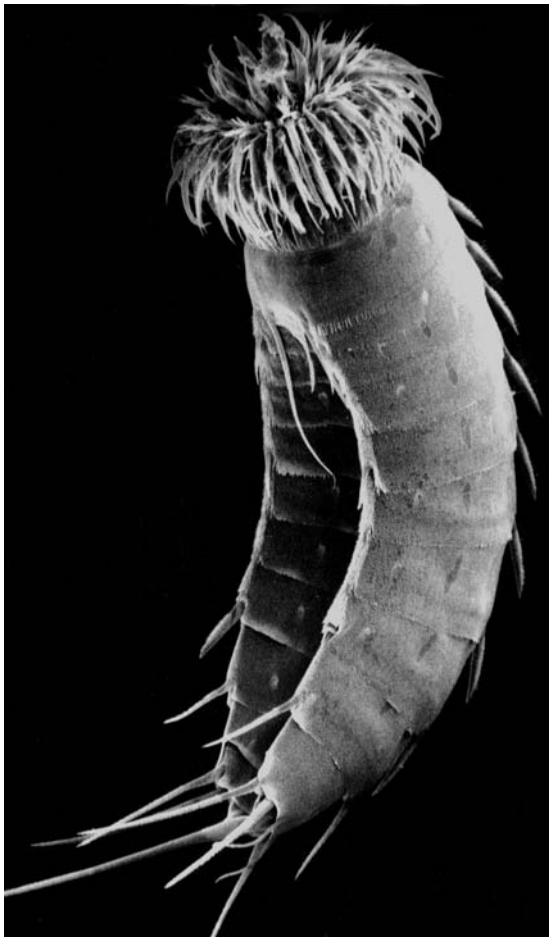

Figure 9.7. A kinorhynch (*Campyloderes* sp.). (Courtesy of B. Neuhaus, Berlin.)

and scolopendromorphs, the basic alternation of segments with long dorsal plate (tergum) and lateral spiracles and segments with short tergum and no lateral spiracles is 'disturbed' by what seems to be the lack of a short tergum segment. In scutigeromorphs, a corresponding 'anomaly' is given by one long tergum covering three leg-bearing segments rather than two, as the other terga do. In most geophilomorphs, the mid-body anomaly is generally less conspicuous and sometimes hardly visible at all, but it is very visible in some species, as in *Stigmatogaster gracilis*, in which a short range of segments are ventrally marked by conspicuous ventral grooves. I demonstrated (Minelli 1992) that the range of segments with sternal grooves expands with age, from a virtual source at about 0.43 relative

segment position along the trunk. This *relative* position is the same as the position of the mid-body anomaly in all remaining centipedes, irrespective of the actual ordinal number of the segment(s) involved. This position is approximately the same as the position of the genital opening in other arthropods, whereas the genital opening in centipedes is at the posterior body end and the mid-body anomaly could correspond to the original expression of *Abdominal-B* (a *Hox* gene whose relation to genital/sexual structures has been described on page 181; Minelli et al. 2000). Hughes and Kaufman (2002) have demonstrated that in the centipede genus *Lithobius*, *Abdominal-B* is expressed twice, first at a mid-trunk level (i.e., in a region corresponding to the future mid-body anomaly) and later at the posterior end of the trunk, where the genital opening and the sexual appendages will differentiate. My interpretation is that the anterior spot of expression of *Abdominal-B* is specified very early in development, before merosegmentation. The anterior spot of expression, marking the position of the mid-body anomaly, does apparently coincide with the same eosegment in all centipedes; the different segmental position of the anomaly in the different taxa will only depend on the different degree of merosegmentation of their eosegments (Minelli 2001).

It is important to stress that, in my interpretation, eosegmentation is achieved very early in development, whereas merosegmentation – as the actualisation of a developmental fate already assigned to each eosegment – may be extensively delayed and occur in an antero-posterior progression over long spans of embryonic and also possibly postembryonic life. In the growth zone of a tiny nauplius, there should be space enough for all postnaupliar eosegments, but we cannot expect to see all full-fledged merosegments from very early on. The same is true for anamorphic myriapods. One may wonder, in the latter case, whether all myriapods actually exploit their full potential of merosegmentation. Doubts are warranted at least in the case of juloid millipedes, with their cylindrical body mostly terminating with a variable number of apodous segments and with a very large intraspecific variability in the number of adult segments. In this group, adult specimens without apodous rings are documented in a few species (Sahli 1969), but this condition is quite unusual (Enghoff, Dohle, and Blower 1993).

There is a relationship, but not necessarily a simple one, between merosegmentation and the pace at which postembryonic development proceeds. Enghoff, Dohle, and Blower (1993) believe that, in millipede evolution, a regular addition of sets of two segments (or multiples thereof) with the completion of each moult is a later acquisition rather than an early feature.

But we need to consider that this acquisition paralleled the change from primitive millipedes with a relatively low number of segments, as depicted in Enghoff's (1990) millipede (chilognathan) ground plan, to clades with much larger segment numbers, such as polyzoniids and julids. No specimen with an odd number of segments was found by David and Couret (1984) in a vast material of *Polyzonium germanicum*. The same occurs in the Chordeumatida, in which only even numbers of leg-bearing sternites have been recorded, starting from the third postembryonic stadium onwards. In all Juliformia, except for a few secondarily modified cases, only odd leg-pair numbers are found.

In arthropods, merosegmentation does not necessarily end with the determination of as many units as are the conventional segments the morphologist recognises in their adults. In many cases, the process continues with further division of most, or some, of the trunk segments (never, however, those corresponding to the naupliar part of the body) in subsegmental units. Things are a bit equivocal in the millipedes. Equivocal, at least, in terms of the conventional standard, according to which a segment should bear just one pair of appendages. The majority of the segmental units in a millipede's trunk are provided with two pairs of legs each, hence the term diplosegments currently given them. Closer inspection reveals that the duplicity so evident in the appendages – and mirrored by internal features such as ganglia, spiracles, tracheae, and heart ostia – is also present, in some way, in the dorsolateral aspect of the body, with more or less evident distinction, within each diplosegment, of an anterior prozonite and a posterior metazonite. Dorsal praeterga and ventral praesterna are also present in some centipedes, in front of the main dorsal and ventral plates of each segment respectively. This duplication of segments also affects the insects. This is often seen in the thoracic segments (dorsally divided into the scutum and scutellum, laterally in the episternum and epimeron) and sometimes also in one or more segments of the abdomen, as in many neuropterans (Achtelig 1975). Among the crustaceans, a few copepods appear to have five to eight abdominal somites, rather than the usual four. This is regarded as the consequence of secondary annulation (= merosegmentation) of the basic number of abdominal somites (Huys and Boxshall 1991).

Heterogeneous Segments in Vertebrates and Annelids

Quite probably, there are 'two heads' in vertebrates (Duboule 1995) as there are two in arthropods. The distinction between an anterior and a posterior head is clearly marked in the central nervous system, in which the

expression domains of the homeobox genes of the *Otx* and *Emx* families, homologous to the *Drosophila orthodenticle* and *empty spiracle* genes, are restricted to the developing rostral brain. Patterning of the developing hindbrain and the corresponding branchial region and spinal chord are under the influence of *Hox* gene expression (e.g., Bally-Cuif and Boncinelli 1997, P.W.H. Holland 2000).

Xenopus provides a wonderful example of how serially homologous elements, such as the trunk somites of a vertebrate, may be produced by different mechanisms within the same animal (Cooke 1980). Embryos which have started somite formation as artificially small neurulae with less than the usual number of cells manage to adapt the size of their anterior somites appropriately, but this regulation is forgotten in later axial morphogenesis. Cell number is the same in somites numbered from 20 onwards in initially small embryos and in their normal siblings. This experiment demonstrates that two different modes of pattern control operate, in different times, in the generation of one series of somites.

In mice, the formation of the second pharyngeal arch involves a distinct developmental process from that responsible for the production of the third and fourth arches (Wendling et al. 2000).

The vertebrate peripheral nervous system consists of two groups of nerves which have a metameric series of proximal roots along the body axis: the branchial and the spinal nerves. Spinal nerve metamerism is patterned after the somites, whereas segmental distribution of the branchial nerves is in part intrinsic to the rhombomeres, the segmental compartments of the hindbrain.

Still more impressive is the change in segmentation modes in the polychaete *Spirorbis moerchi*, in which larval segments develop simultaneously in the ectoderm, followed by segmentation of the mesoderm. Postlarval segments are initiated in the mesoderm, followed by segmentation of the ectoderm (Potswald 1981).

The few cases described in this chapter show that, in vertebrates and annelids alike, different sets of segments originate through different mechanisms in the same animals, as with naupliar and postnaupliar eosegments in arthropods; but the similarities between the three animal groups also extend to double segmentation (eo- vs. merosegmentation). Even if double segmentation seems to account adequately for the numerical idiosyncrasies of centipedes and millipedes, as well as for many other aspects of arthropod segmentation, there is no reason to regard it as a unique trait of the arthropodan clade.

I have already mentioned leeches, with their annulated segments. At variance with the outcome of merosegmentation of arthropod eosegments, the secondary units of the leeches are not 'promoted' to the status of full segments, probably because the main segmentally arranged structures, such as nephridia or the ganglia of the ventral nervous chain, are already specified when merosegmentation begins.

In other annelids, however, things are possibly different and more similar to what we have seen in arthropods. In the Oligochaeta, the clitellum generally includes segment 16; but in the few exceptions to this rule, it usually includes segment 32. This looks like the effects of early merosegmentation (2 merosegments per eosegment), followed by the development of the merosegments as full segments. Also interesting, although less convincing, is the comparison of some of the shortest annelids in terms of total number of setigerous segments: there are 8 segments in the female of *Parergodrilus* (but 9 in the male) and 16 in *Hrabeiella* and *Marionina eleonorae*, the shortest of the Enchytraeidae (Rota 1997).

Polychaetes could be the best group in which to study the different layers of segmentation potentially existing within the same animal. In these annelids, besides the examples of merosegmentation previously described (see page 202), there are genera in which a seemingly opposite pattern has been reported [i.e., two or three pairs of ganglia per morphological segment (*Pectinaria*; Nilsson 1912)].

In vertebrates, the first two halves of the rhombencephalon (anterior = r1–r5 and posterior = r6–r8) behave like eosegments, within which merosegments (rhombomeres) are progressively established in a characteristic sequence.

Comparable with the cases thus far discussed is the origin of dentition in mammals. In most mammals (but not, for example, in mice), the permanent dentition is of mixed developmental origin (McCollum and Sharpe 2001), because incisors, canines, and premolars develop from successional laminae that differentiate from the remnant of the primary dental lamina of their deciduous precursors, and molars develop directly from extensions of the primary dental lamina.

As a footnote, if homologous parts, such as segments or teeth, are thus frequently generated by more than one mechanism within the same animal, we shall expect that the same will be true of cell types. Morphologically and functionally equivalent cells may have different origins within the same animal. Smith, Kachinsky, and Miller (1994) found that myogenic

cells in the different regions of a mouse somite (dermatome, myotome, and sclerotome, as well as the dorsal versus ventral halves of somites) have different patterns of expression of four muscle regulatory factor proteins; therefore, they suggest that myogenic cells in the somites may have multiple sites of origin and form through multiple molecular pathways.

Germ Layers and Segmentation

In different animals, segmentation affects body architecture more or less extensively, generally involving both ectodermal and mesodermal, but not endodermal, derivatives. Tissues derived from the different germ layers are sometimes segmented independently. When segmentation of one germ layer's derivatives is in register with segmentation of another germ layer's derivatives, we expect this to be due to transpatterning, but there seems to be no general rule (Holland 1988).

Segmentation of the endoderm is admittedly rare, the most conspicuous example being the segmental gut caeca of rhynchobdellid and many gnathobdellid leeches. These are in register with as many ectomesodermal segments. This endodermal segmentation is in part autonomous, in so far as a periodic pattern of expression of the *Lox3* gene precedes and coincides spatially with the segmental (intercoecal) constrictions of the gut. But this endodermal segmentation is also dependent on the mesoderm, as the ablation of segmental mesoderm prevents differentiation of the definitive endoderm and expression of *Lox3* RNA (Wedeen 1995, Wedeen and Shankland 1997; cf. page 119).

Joint involvement of ectodermal (epidermis, nervous system) and mesodermal derivatives (musculature, coelomic cavities, skeletons) is the rule. Ectoderm and mesoderm, in turn, follow largely independent routes in acquiring segmentation, with ectoderm leading the process in arthropods and mesoderm leading the process in annelids (mostly) and vertebrates. An exception to this generalisation is the rhombomeres in vertebrates. Another exception is polychaetes such as Nereidae, Eunicidae, Tomopteridae, and Serpulidae (Korn 1982). The problem thus arises as to how the ectodermal and mesodermal (and, when involved, also endodermal) derivatives are put in register during the development of segmented animals.

In the leech *Helobdella robusta*, normal development of the segmentally repeated cell clones derived from one of the blast cells does not depend on signals from adjacent clones derived from the other blast cells (Seaver

and Shankland 2000). As previously described (page 128), the longitudinal bandelets generated by the individual blast cells are brought into alignment during a later process of morphogenetic assembly.

In vertebrates, the most conspicuous evidence of segmentation is found in the derivatives of the mesodermal somites – in the vertebral column especially, also often in the arrangement of the trunk musculature. Head segmentation is less obvious, but segmental interpretation of cephalic structures has been a recurrent theme since Spix's (1815) and Goethe's (1820) speculations about a vertebral origin of the skull bones. Segmentation is now clearly demonstrated in articulation of the hindbrain in neuromeres, but this has nothing to do with patterning of the skull bones. In the trunk, segmentation is first expressed in the mesodermic somites, which impart segmentation to the spinal cord. The latter is not primarily organised in neuromeric segments analogous to the rhombomeres. Roles are reversed in the posterior (branchial) region of the head, where bone and connective tissue are generated by the neural crest, which is derived from the hindbrain rhombomeres. Experiments demonstrate that the cranial neural crest is prepatterned or imprinted with positional information before migration (Lumsden, Sprawson, and Graham 1991, Krumlauf 1993).

Segmental Mismatches and Resegmentation

If we still need arguments in favour of a less typological view of segmentation than has been fashionable up to now, we can find some in the widespread mismatch between the dorsal and ventral expressions of segmentation in the same animal and in the phenomenon of resegmentation.

Dorso-Ventral Mismatches

The archetypal or, better, text-book arthropod segment is covered by a tergum dorsally and a sternum ventrally. Elaborations upon this basic scheme are very common. These may include additional (pleural) sclerites placed between the tergum and the sternum, or the more or less distinct subdivision of these primary sclerites into an anterior and a posterior half each. These additional features notwithstanding, the basic architecture of the segment remains nevertheless recognisable. But often, things are not that simple. I do not discuss body regions, such as the head, which are usually described as resulting from the 'fusion' of segments. Instead, I will note some examples of dorso-ventral mismatch in the number, arrangement or patterning of segments.

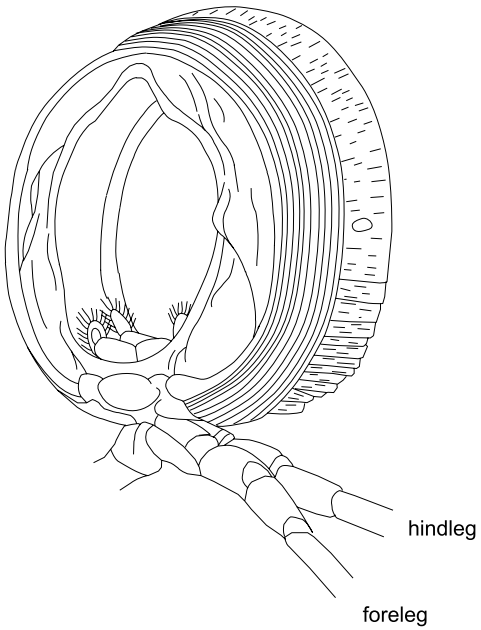

Figure 9.8. A millipede ring. (Redrawn after Demange 1981.)

The most extreme instance of dorso-ventral mismatch is possibly found in the parasitic copepods *Aethon* and *Norion*, in which there is no evidence at all of segmentation left on the ventral side, whereas traces of segmentation persist on the dorsal side (Kabata 1979). One could disregard this example as the result of a morphological deconstruction related to parasitism, but we do not need to look for such 'degenerate' forms to find arthropods in which the number of dorsal segmental units does not correspond to the number of the ventral ones.

In notostracan crustacean, the mismatch between body rings and pairs of appendages is so great and unpredictable that the conventional concept of segment does not seem to capture the actual organisation of the trunk (Linder 1947, 1952).

In an entire class, the millipedes (Diplopoda), most of the trunk segments, as defined on the dorsal aspect, are clearly double in their lateral and ventral aspects, with the most conspicuous feature being the presence of two pairs of legs per 'diplosomite' (Figure 8.22 and Figure 9.8). Correspondence between the dorsal and ventral aspects is still worse in the millipedes with free sternites, in which the correlation between terga

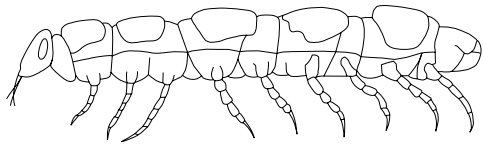

Figure 9.9. Habitus of a pauropod.

and sterna does not follow a consistent rule; that is, the number of leg pairs cannot be inferred from the number of terga and vice versa (Enghoff, Dohle and Blower 1993).

Terga corresponding to more than one latero-ventral unit (two units each, generally) are also present in two other myriapod groups: the tiny pauropods (Figure 9.9) and the scutigeromorph centipedes. Similar organisation is found in the extinct euthycarcinoids. Some branchiopod crustaceans have more than one pair of limbs per 'segment'; and more than one pair of legs was also present on each thoracic segment in the Early Cambrian arthropod *Fuxianhuia* (Bergström and Hou 1998).

In all these animals, the degree of segmentation is higher on the dorsal side than on the ventral side. The opposite is found in symphylans, where two (sometimes more) tergal units correspond to each segment as defined by the ventral aspect and the number of leg pairs (Figure 9.10).

In a previous section, I mentioned other arthropods (sphecid wasps) in which the number of dorsal sclerites corresponds to the number of ventral sclerites, but a few of these units are shifted longitudinally apart to generate a 'dorsal only' and a 'ventral only' segment.

More widespread are dorso-ventral differences in patterning. The most conspicuous example is provided by centipedes (Chilopoda), in which the ventral side is basically homonomous (all segments alike), and the dorsal side is basically heteronomous, with alternating shorter and longer terga, most conspicuously in Lithobiomorpha.

Not unexpectedly, striking differences between the dorsal and ventral aspects of segmentation have been recorded for some gene expression patterns. During larval segmentation of the posterior part of the trunk of the notostracan crustacean *Triops longicaudatus*, *Tlwnt-1* is only expressed in

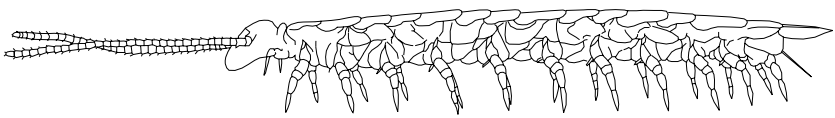

Figure 9.10. Habitus of a symphylan.

the ventral portion of the epidermis (Nulsen and Nagy 1999). In insects, the anterior boundary of expression of *Antennapedia* resides in the posterior labial segment ventrally, but in the anterior prothoracic segment and appendages laterally (Peterson et al. 1999).

Resegmentation

In all segmented animals, the first signs of serial patterning are manifest since early or very early developmental stages. The segments seen in the body architecture of many adult metazoans do not necessarily have the same anterior and posterior boundaries as the first segmental units which are formed in the same animals. The problem is not simply one of merosegmentation (see page 202), but also one of *resegmentation*.

The concept of resegmentation was introduced by Remak (1855), who regarded the adult vertebrae of amniotes as shifted by one half unit with respect to the sclerotomal (bone-forming) portion of the somite. In other words, the posterior half of the sclerotome of one segment would fuse to the anterior half of the sclerotome of the next segment, so that the definitive centrum (the body of the vertebra) will be intersegmental rather than segmental. According to Williams (1959), such a resegmentation does not affect the whole of the vertebra, as it would apply to the bulk of the neural arch and the pleurocentrum, but not to the prezygapophysial region of the neural arch and neither to the ventral arch and to the intervertebral disc. The latter structures would be strictly segmental; that is, they would retain the primary segmentation. In this sense, a modern amniote vertebra would be a mosaic of pieces derived from primary segmentation, together with pieces subjected to resegmentation.

The resegmentation hypothesis has been alternatively accepted (e.g., Carroll 1989, O'Higgins et al 1997), based partly on new embryological evidence (e.g., Bagnall, Higgins, and Sanders 1988, Ewan and Everett 1992, Huang et al. 1995), and doubted (e.g., Keynes and Stern 1988) or outright refuted (e.g., Verbout 1985). It is also possible that differences exist between different amniote lineages. Convincing evidence for resegmentation is available, for example, for the dorsal vertebrae of the turtles (Rieppel 2001), in which the neural arches shift forward by half a segment carrying the ribs with them (Goette 1899).

According to Verbout (1985), an alternative explanation to the changing pattern of segmentation in the vertebral column of amniotes could be a simple realignment of the relative positions of the sclerotome and myotome, rather than actual fusion of half sclerotomes from adjacent somites.

There is no clear experimental evidence in favour of his hypothesis (Tam and Trainor 1994).

The final verdict on this question does not seem to have been formulated yet, but molecular developmental genetics seems to come in support to Remak's hypothesis. In the mouse, starting 10 days postcoitum, the serially repeated expression of the homeobox gene *Hlx* is out of register with the intersomitic fissures (the original segmental boundaries), suggesting a molecular blueprint for resegmentation (Lints et al. 1996).

In arthropods, the issue of resegmentation has been introduced by Martínez-Arias and Lawrence (1985). They noticed that the segments of *Drosophila*, as marked by the intersegmental membranes in both larva and adult, do not correspond to the serial units defined by the segmentation genes, but are shifted by one half unit in respect to them. Martínez-Arias and Lawrence introduced the term parasegment for the original units of segmentation to distinguish them from the 'true' segments of larva and adult. As each parasegment comprises two compartments (anterior and posterior), resegmentation in *Drosophila* means the association of the posterior compartment of parasegment i with the anterior compartment of parasegment $i + 1$ to produce a definitive (morphological) segment. Lawrence (1992) regards parasegments as the only fundamental unit of segmentation, in developmental terms. Segments, conspicuous as these units may be in morphological and functional terms, "are largely a figment of our imagination" when considered from a developmental point of view (Lawrence in discussion to Riechmann et al. 1997: 2922). This is certainly true for the abdomen of *Drosophila*, but it is not clear how pervasive resegmentation occurs in insects or in arthropods at large. Parasegments have been found in malacostracan crustaceans (Scholtz and Dohle 1996). In the centipede *Lithobius*, Cynthia Hughes (personal communication) found both segmental and parasegmental patterns, but the latter seem to come out later than the first, thus making resegmentation unlikely. Even in *Drosophila*, where resegmentation is obvious in the abdomen, this is not necessarily true for the thorax and the head (Rogers and Kaufman 1996, 1997). As in many other aspects of development, however, deciding whether an animal undergoes resegmentation or not may critically depend on evidence from the correct temporal layer. Parasegmental patterns may appear during a restricted temporal window only to be swamped soon by resegmentation. A quick transition from a parasegmental pattern to a segmental pattern may invest the expression of the same class of genes. In *Drosophila*, early expression of *fushi tarazu* and *even-skipped*

helps allocate the cells to the 14 parasegments, with the anterior boundaries of anterior *fushi tarazu* and *even-skipped* stripes delimiting the sets of cells that will eventually establish the parasegment. Parasegmental is also the expression of *abdominal-A* that is transcribed from parasegment 7 to parasegment 12 in the somatic mesoderm and from parasegment 8 to parasegment 12 in the visceral mesoderm. The homeotic genes expressed a bit later, such as *Antennapedia* and *Sex combs reduced*, are not expressed in parasegmental pace, but with the periodicity of the compartments or even that of the definitive segments. Apparently, the larva is initially built in parasegments, but the adult body plan is specified in compartments or segments (Lawrence and Morata 1994).

There are aspects of insect morphology that could be explained either as a retention of parasegmental architecture or as a (re)resegmentation with respect to the conventional segmental pattern. The collum of insects, according to Matsuda (1979), is part labial and part prothoracic origin. This idea is supported by embryological evidence from the beetle *Silpha*, the stick insect *Carausius* and the mixed nerve supply of the region's muscles. In the Boreidae (Mecoptera), the first pair of spiracles is anteriorly displaced, in that part of the intersegmental membrane between prothorax and mesothorax has become incorporated into the prothorax (Kaltenbach 1978).

CHAPTER TEN

Evo-devo Perspectives on Homology

> Cells may have different characters now because they were in different situations in the past. They are like identical twins reared apart; they remember their separate experiences.
>
> J.H. Lewis and L. Wolpert 1976: 467

> The initial progenitor field is transformed into a mosaic of regulatory subdomains, and, remarkably, these prefigure the morphological pieces of the body part.
>
> E.H. Davidson 2001: 110

Concepts and Interpretations

From a structuralist perspective, Müller and Newman (1999) have developed the interesting argument that homologous structures, in so far as they are integrated as fundamental parts of a given body plan, become 'attractors of morphological design', a kind of backbone to which further elements of body design may be added.

Hierarchies and Beyond

A hierarchical frame of mind is taken for granted in nearly all approaches to homology. Analysis in terms of hierarchies of structural parts or functional subsystems has been a common strategy in the study of complex systems since the dawn of modern science (Bechtel and Richardson 1993, Zawidzki 1998). However, as forcefully argued by Kauffman (1993), with biological systems this is not the most satisfactory strategy. Ahl and Allen (1996) define a system as hierarchical if it is composed of stable, observable subunits unified by a superordinate relation. It would be fortunate if the features we try to compare were always stable and unified by a

superordinate relation. I suspect, however, that stable and hierarchically unified features would make the world much duller and less interesting than the real biological world.

Advocacy of hierarchy in homology is not universal. Striedter (1999) admits that biological systems consist of a complex network of multiple hierarchies, such that the hierarchical nature of the homology concept remains potentially confusing. Nagy and Grbić (1999) acknowledge that we know very few gene networks in development functioning in a truly hierarchical manner.

Kauffman (1993) predicted that genomic regulatory architectures are more likely to be rich in feedback loops rather than purely hierarchical. This has been repeatedly demonstrated by recent studies. Arnone and Davidson (1997) observe that the linkages between internal genes in genomic networks may connect a given gene to others at several different levels in the same network, something that makes the network other than strictly hierarchical.

Homology: Absolute or Relative?

Until recently, homology was universally regarded as an absolute relationship. Structures either are, or are not, homologous (Striedter and Northcutt 1991, Bolker and Raff 1996). However, by contrasting genes affecting prepattern with genes affecting process, Maynard Smith and Sondhi (1960) have long ago distinguished, in a sense, between positional and special homology. Long before them, Bateson (1894: 24) already had the following observation to offer: "I am aware that Meristic and Substantive Variations often occur together, and that there is a point at which it is not possible to separate [them] satisfactorily. [..] For example, we may see that it is through Meristic Variation that the vertebral column of a Dog may be divided into a number of Vertebrae greater or less than the normal; and though in such cases all the Vertebrae have distinctly canine characters, yet there are nearly always Substantive Variations occurring in correlation with the Meristic Variations, manifesting themselves in a re-arrangement of the points of division between the several groups of Vertebrae, and causing individual Vertebrae to assume characters which are not proper to their ordinal positions".

The first recent author to seriously challenge the all-or-nothing notion of homology was Roth (1984). Her paper opened the way to the more flexible views defended later, for example, by Haszprunar (1992) and myself (Minelli 1996b, 1998).

It has long been acknowledged that homologous parts may be integrated to form nonhomologous complexes. This is one of the arguments leading to the widespread hierarchical notion of homology. On this basis, Striedter and Northcutt (1991) criticised the notion that homologous characters must be based on the same, or homologous, causal elements and mechanisms (a view that had been championed, for instance, by Spemann 1915, Atz 1970, Hodos 1976, Roth 1984 and Wagner 1989). In their opinion, this would be a reductionist approach to the concept of homology, because of the claim that the homology of characters at any one level of organisation can be reduced to the homology of their causal mechanisms. Striedter and Northcutt argued instead that evolution may bring about any level of change at any level of biological organisation, and that these changes may include the causal mechanisms that generate them. This view seems too abstract. To say that an aspect of biological organisation remains the same, irrespective of any evolutionary change it may undergo, will inevitably reduce characters to Platonic ideas. Striedter and Northcutt (1991) point to the complexity of causal relationships that may interrelate the various levels of biological organisation (e.g., because of pleiotropy or multigenic control of morphological traits). Furthermore, during the course of evolution, the causal relationships between characters at different levels of organisation may change. A character at any given level of organisation may thus change by phylogenetic addition, deletion or substitution of elements and causal factors at a lower level. Therefore, Striedter and Northcutt concluded that characters at the same level of organisation may be homologous, even if some of their causal factors or component elements at a lower level are not homologous. I would argue that these circumstances force us to adopt a factorial, or combinatorial, view of homology (Minelli 1998).

Shubin and Wake (1996), Abouheif (1999) and Pigliucci (2001) have made explicit mention of partial homology as the possible relationship between the two traits we are comparing. Wake (1999: 44–45) even goes so far as to affirm that, "Because evolution is a continuous process, [..] homology can only ever be partial, in any real sense." Comparative morphology, especially comparative developmental biology, offer lots of examples to support this view.

Breidbach and Kutsch's (1990) comparative study of the neurones innervating the dorsal longitudinal muscles in several insects is a nice example of factorial analysis of homology. Breidbach and Kutsch compared neurons in juvenile and adult locusts, in larval and adult beetles and in various body segments of these insects. In all species, instars and segments, they

found a common set of 11 neurons: seven motoneurons stemming from the next anterior ganglion and four neurons located in the ganglion of the segment containing the muscles. Also common was the basic structure of the dendritic field. This identical neuronal plan was found between different segments (*serial homology*), between the nymphal or larval stage and the adult of the same species (*ontogeny*), and among different insect species, both hemi- and holometabolous (*phylogeny*).

In vertebrates, the development of spinal and cranial motor neurons offers an intriguing mix of similarities and differences (Jacob, Hacker, and Guthrie 2001). At a molecular level, there is abundant evidence that several transcription factors relevant for body patterning operate in a synergistic and combinatorial way (García-Bellido 1994b, Davidson 2001). Duboule and Morata (1994) suggested the existence of a combinatorial component in the control exercised by homeotic genes on the patterning of the main body axis to the point that analysis of the phenotypic expression may become difficult. An example is the combined function of *Ultrabithorax* and *abdominal-A* in the epidermis of abdominal segments II to VII in *Drosophila*. In this body region, the expression of both genes is required, although not necessarily by the same cells. But in the visceral mesoderm, these genes are not co-expressed and have distinct effects, with *Ultrabithorax* being expressed only in parasegment 7 and *abdominal-A* in parasegments 8–12.

When dissecting homology into its components, one of the most fundamental distinctions is between positional and special homology (Minelli and Schram 1994, Minelli 1998, 2002). According to classic comparative anatomy, body regions are homologous among amniotes despite variation in the number of vertebrae and the position along the body axis, a concept known as 'transposition' (Goodrich 1906, 1913, Burke et al. 1995, Müller and Wagner 1996, P.H.W. Holland 2000). For example, the transition between the cervical and thoracic vertebrae occurs at very different segmental positions in the different species. The equivalence of this transition, irrespective of segmental position, is shown by the circumstance that in mammals as in birds, in fishes as in amphibians, this transition (less clear in fishes than in tetrapods; van der Hoeven et al. 1996) corresponds to the anterior boundary of expression of the *Hoxc6* gene (Gaunt 1994, Burke et al. 1995).

This distinction between positional homology and special homology, obviously more important at the level of organs and body parts, is already relevant at cellular level (Wolpert 1996). For example, homologous cell

types may be located in non-homologous brain regions, while homologous brain regions may contain non-homologous cell types (Striedter 1998). Cell migration, as in the case of the neural crest derivatives, modifies positions, but migrating cells are known to be (or expected to be) specifically labelled before moving, thus offering a further example of dissociation between positional and special homology.

This dissection of homology components is, basically, one of temporal layers. Striedter (1999) acknowledges that homology of adult characters is conceptually independent of the homology of their embryonic precursors. Drawing from his favourite field of comparative neurology of vertebrates, he shows that homology of the embryonic telencephalic zone is a separate issue from the homology of the adult neocortex. Homology of neocortex (as neocortex) is not disproved by the fact that some of its neurons are derived from embryonic regions other than the dorsal telencephalic zone (Anderson et al. 1997). (A question of periodisation nevertheless remains: how are developmental stages defined, e.g., embryo and adult? cf. chapter 4).

Dissection of temporal layers of homology is what Wagner and Gauthier (1999) suggested with respect to the problem of digit homology in the avian hand. It has been long disputed whether bird digits represent digit I, II, and III (conventional view) or digit II, III, and IV (Burke and Feduccia 1997) of the tetrapod pentadactyl limb. Wagner and Gauthier believe that difficulties with this homologisation may be derived from dissociation between the developmental origin of the repeated elements (primordial cartilage condensations) and their subsequent individualisation into fully functional characters (digits). Thus, there would be causal independence (cf. Tabin 1992) between morphogenetic processes creating chondrogenic condensations in the limb and the later developmental individualisation of those elements when they are specified as functional fingers. In the avian case, comparative embryological evidence identifies the primordial condensations as CII, CIII, and CIV, but later in development these condensations differentiate following a frame shift in the developmental identities such that CII becomes digit I, CIII becomes digit II, and CIV becomes digit III. This agrees with Hardy et al.'s (1995) study, which suggests, in the chick limb, the independence of a prepatterning mechanism specifying the digits from a positional information-based process which specifies digit identity.

The question of homology of the amphibian digits is also tricky. Wagner et al. (1999) move from the finding that in the digit-forming region of the

newt *Notophthalmus viridescens*, there is a phase of *Hoxa-11* expression starting with the development of digit III, whereas no corresponding expression is known for either frogs (*Xenopus*) or amniotes. This unique trait of molecular developmental biology would suggest a peculiar origin for the digits of recent urodeles. They would be derived from a lineage of amphibians with two digits only (digits III and IV of the original pentadactyl appendage), which would survive as urodele digits I and II, and the remaining urodele digits would represent an evolutionary innovation.

Another dissection of homology components is invited by the circumstance that in the epidermis (and cuticle) of insects, patterns of differentiation and patterns of antero-posterior or proximo-distal polarity are to some extent under separate control (Nübler-Jung 1987, Nübler-Jung and Grau 1987). This has been shown in *Drosophila* through the analysis of suitable mutants (Gubb and García-Bellido 1982, Held, Duarte and Derakhshanian 1986) and in the cotton seed bug *Dysdercus* by experimental grafting of rotated pieces of cuticle (Nübler-Jung and Grau 1987). The differentiation pattern is determined at the scale of the entire segment (Locke 1960, Nübler-Jung 1977), whereas the cell polarity pattern is dependent on local interactions between cells.

Most ontogenetic features evolve in a mosaic fashion, because developmental integration is historically labile. In the phylogenetic time dimension, characters which appear to be integrated in ontogeny may evolve as separate features. I do not see why this dissociation should specifically characterise the speciation events, as suggested by Fink and Zelditch (1996).

Temporal Serial Homology

Larval and adult homologues are sometimes derived from two temporally distinct phases of activation and differential specification of subsets of a same anlage. The most familiar example is that of the polyphyodont vertebrates (i.e., those that have two or more dentitions). Humans share this trait with most mammals but also with many fishes, amphibians and reptiles. Less obvious is the example of the insect compound eyes and their larval counterparts. In insects such as a cockroach or a grasshopper, there is an obvious morphological and developmental continuity between the compound eyes of the first postembryonic stage and those of later stages, adult included. We just noticed the addition of new ommatidia following each moult. Things are different in the holometabolous insects, whose larvae do not have true compound eyes, but a small cluster of visual

organs called the stemmata. The developmental relationships between the larval stemmata and the ommatidia of the adult compound eyes have been revealed by Paulus (1989) with his study of *Chaoborus* (Diptera). Paulus demonstrated that existence of a common anlage is responsible for production of both larval stemmata and adult ommatidia. The visual organ precursors develop serially in two main waves, in different developmental contexts: embryonic/larval (producing stemmata) and pupal/adult (producing ommatidia).

Most dipteran larvae (e.g., those of *Drosophila*) have a reduced head and no eye at all, nevertheless they have other organs in a relationship of temporal serial homology with corresponding adult structures. The so-called Keilin's organs of the larvae of *Drosophila* have been interpreted as rudimentary legs, topographically corresponding to the legs of the adult fly. Keilin's organs do not grow to become the fly's legs, but disappear at metamorphosis, whereas the leg/wing imaginal discs become activated and give rise to adult appendages. Cohen, Wimmer, and Cohen (1991) demonstrated that the primordia of Keilin's organs and those of the adult leg primordia are part of a single cluster of cells expressing both *Distal-less* and *disconnected*. The two kinds of organs are thus temporal serial homologues.

We can probably apply the concept of temporal serial homology to many structures that undergo complete restructuring during amphibian metamorphosis (Wake and Hanken 1996). In the plethodontid *Eurycea bislineata*, for example, there is compartmentalisation between cells fated to form the larval epibranchial cartilage and cells which will form its adult equivalent (Alberch, Lewbart, and Gale 1985, Alberch, Gale, and Larsen 1986, Alberch and Gale 1986, Alberch 1987, 1989), whereas in the jaw muscles of the anurans, the larval myofibres degenerate and the new adult muscles are formed, at metamorphosis, by myofibers derived from quiescent cells within the larval muscles (Alley 1989).

Underlying temporal serial homology, there is probably a reactivation, later in development, of patterning genes already expressed at earlier stages. A comparable behaviour is known in *Drosophila* for many genes involved in the early embryo in the patterning of the main body axis which are expressed again in the imaginal discs, where they are involved in the patterning of the (paramorphic) body appendages (Bryant 1996).

Genes and Homology

"Can homology ever be definitively demonstrated? I think not" (Wake 1999: 27). In David Wake's opinion, to speak of homology is nothing more, and

nothing less, than to speak of phylogenetic continuity. But continuity of what? Of structures, of genes, of mechanisms?

There is no question that many structures whose homology cannot be meaningfully challenged in terms of comparative morphology are produced by different mechanisms in different animals (e.g., Wilson 1894, Remane 1952, de Beer 1958, 1971, Sander 1983, Roth 1984, Hanken 1986, Henry and Raff 1990, Wray and Raff 1990, Striedter and Northcutt 1991, Striedter 1998). In various amphibians, somites differentiate and develop in the most diverse ways, including 90° rotation as in *Xenopus* (Malacinski et al. 1989). In bony fishes, such as zebrafish, fin muscles arise from migrating precursor cells equivalent to the limb muscle precursors of tetrapod species. But in dogfish embryos, the same muscles are produced through direct epithelial somitic extensions (Neyt et al. 2000). As observed long ago by Baltzer (1952), xenoplastic grafts often give rise to partially equivalent structures, sometimes even in organs that in normal development do not seem to be homologous or are altogether absent in one of the partners in a chimera.

A comprehensive theory of morphological evolution cannot deal with homologues in structural terms only, but it must also explain their origin and fixation (Müller and Newman 1999). No wonder that many attempts have been made at solving this problem by looking at shared developmental genes and pathways.

Nowadays, the concept of homology is no longer restricted to the toolkit of the morphologist, but has colonised the fields of genetics and molecular biology extensively (e.g., Egel 2000, Fitch 2000). I will not discuss the common abuse of the term, when used to denote simple sequence similarity between two macromolecules, a position forcefully rejected by Reeck et al.'s (1987) classic commentary. Rather, I wish briefly to comment on the increasingly common identification of homology with shared expression patterns of some regulatory gene at a given developmental stage. Bolker and Raff (1996) remark that this definition of homology is basically different from the traditional ones, in that it is based on mechanism rather than on structure, and tends to ignore phylogeny. However, Morata and Sánchez-Herrero (1999) find that Snodgrass's (1935) subdivision of the arthropod appendage into coxopodite and telopodite coincides with the two regions defined by the expression of the *extradenticle* gene and the functioning of Hedgehog signalling.

An interesting experimental approach to homology is Smith et al.'s (2000) research on the histology of the stomach and gastrointestinal tract in *Xenopus*, chicken and mice, coupled with the study of the expression

patterns of several genes in the developing guts of the same animals. In all three species, the anterior part of the stomach was histologically similar and showed a common expression pattern, during embryonic development, for two secreted factors, Wnt5a and BMP-4. Also comparable, in both histological and molecular phenotypes, were the posterior nonglandular stomach and pyloric sphincter regions of the chicken and mouse, with the chicken expressing the *six2*, *BMPR1B*, and *Barx1* genes, and the mouse expressing the *Nkx2.5* gene.

Success notwithstanding, it is still doubtful how confidently we can rely on gene expression patterns for the assessment of homology. At no stage during development is there a precise genetic description of the adult organism (Gubb 1998). We have positive proof of how misleading these molecular data may be. In the rhizocephalan parasitic crustacean *Sacculina carcini*, for example, the gene *caudal* is expressed in the thorax, but not in the abdomen – that is, it shows a spatial pattern completely different from that of the other arthropods (Rabet et al. 2001). Abzhanov, Popadić, and Kaufman (1999) performed comparative analysis of the expression patterns of *Ultrabithorax/abdominal-A* in representatives of the different classes of arthropods and found great differences in the anterior boundary of expression of these genes. These authors therefore concluded that *Hox* expression patterns are far from being a safe criterion for establishing the homology of segments and appendages of arthropods belonging to different classes.

A positive consequence of the current interest in comparative gene expression patterns is increased attention to the comparison of processes. This reminds me of García Bellido's (in Wolpert, Ghysen, and García-Bellido 1998) answer to the question, exactly what is conserved in evolution? His answer was: the operation!

I share Gilbert and Bolker's (2001) view that homology of process will be a critically important concept in the future of evolutionary developmental biology. However, I do not wish to restrict the meaning of homology of process, as they do, "to describ[ing] the relationship between patterns that are composed of homologous proteins and that are related by common ancestry" (p. 439). When looking at gene expression patterns as a way to unravel homology, one should be aware that "a gene performs a homologous function in two animals if at least some of its upstream or its downstream linkages (or both) remain the same in the two genomes, and the function it performs is descendant from their common ancestor." The pertinent question thus becomes: "what is a homologous use of a given

gene in a comparison of diverse organisms?" (Both quotes are from Davidson 2001: 201). But this question is still at the level of the gene. Reducing organ homology to gene homology is conceptually and methodologically equivalent to reducing species phylogeny to gene phylogeny, a fault of which systematists are increasingly aware (Maddison 1997, Nichols 2001).

I think that we shall apply the concept of process homology to developmental processes at all levels. This will help us avoid the pitfalls of pleiotropy and co-option. As noted by Galis (1996), when we compare distantly related animals, such as insects and vertebrates, homologous homeobox genes often appear to be involved with the production of non-homologous structures, because of the events happening, in the two systems, downstream of the *Hox* gene expression. Consideration of the whole developmental pathway is required for a sensible assessment of homology. To take the whole developmental pathway into account, it is not enough to consider the expression patterns of more than one gene and the control cascades in which these genes are involved. It is necessary to study, in addition, the epigenetic properties of development (Müller and Newman 1999), that is, the generic properties of molecules, cells and tissues, and the interaction dynamics among them.

Genetic Redundancy, Network Degeneracy, and Homology

Living beings do not care much which gene is encoding a particular function (Akam in the discussion of Wray 1999). Within one developing organism, there often seems to be many equivalent ways of doing the same thing – a circumstance that is usually described as an instance of redundancy. Tautz (1992) did not hesitate to regard redundancy as a necessary requirement for the development and evolution of complex life forms. Redundancy, in particular, has been predicated of genetic networks, but Greenspan (2001) has shown that network behaviours usually attributed to redundancy are due to a different property. For example, knockout mutations are often found to have no apparent phenotypic effect, but this is not an example of redundancy, because the latter implies preservation of the overall network structure and its functional outcome through the substitution of identical elements. Genetic networks are systems of highly interconnected non-identical elements related by non-uniform patterns of connectivity. It is because of this property that the network can produce the same result using different strategies ('degeneracy', as in the case of the genetic code), whereas redundant systems always produce the same result by the same strategy. According to Greenspan (2001), degeneracy of living

systems is not limited to genetic networks. It is amplified, in multicellular organisms, by the plurality of their interacting cell types and organ systems. This is why whole developmental systems can evolve without necessarily showing morphological change, as discovered by Sommer (1997) in vulval fate specification in *Caenorhabditis elegans*.

An interesting difference between redundant systems and degenerate systems is that the former are likely to produce clearly identifiable homologues, whereas the latter are likely to be features with complex and perhaps contradictory homologous components.

Evolutionary Novelties

Müller and Wagner (1991: 243) defined a morphological novelty as "a structure that is neither homologous to any structure in the ancestral species nor homologous to any other structure of the same organism". If we take this definition too literally, of course, there is hardly an evolutionary novelty in the whole history of life. We can easily relax it by adopting a factorial approach to homology. The novel structure will share some homologous components with other structures in the ancestral species, or in the same organism, but these components will be relatively minor with respect to other unique features it will possess.

Meyer (1999) identifies different ways by which evolutionary novelties may originate: by 'reawakening' of existing but silent genetic programmes; through gene duplication and by changing the control of the expression of some genes or by the co-option of genes into a new gene network; and through changes in regulatory elements. His gene-based view seems too restrictive. A touch of epigenetic mechanisms may help to widen the window on the origin of evolutionary novelties. According to Newman and Müller (2000), morphological innovations are literally produced by epigenetic mechanisms rather than by genetic change. Novelties produced by epigenetic factors may later serve as templates around which the overdetermining genetic factors will accumulate.

One of the most extraordinary features of morphological evolution is the explosion of structural disparity among close relatives, in a trait that is otherwise conserved across major taxa. A most conspicuous example is provided by the number and arrangement of microtubules in the axoneme of cilia. The basic structure, with two microtubules in the middle of a crown of nine doublets, is found in such diverse organisms as mammals and ciliate protozoans. Many exceptions do occur, scattered across the

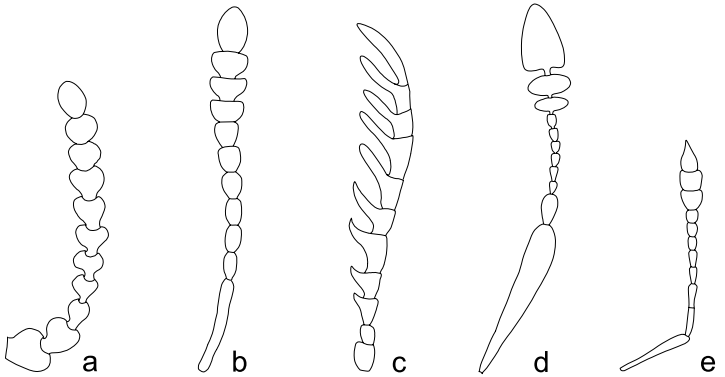

Figure 10.1. Eleven articles are present in the antennae of most beetle species, irrespective of the overall shape of the appendage. The five examples in this figure are: a, *Micromalthus debilis*; b, *Silpha obscura*; c, *Drilus flavescens*; d, *Bythoxenus revelierei*; e, *Apion columbinum*. (Redrawn from various sources after Grandi 1951.)

eukaryotes, but more frequently in some clades, as in insects and especially in Diptera, where the most extraordinary microtubular arrangements have been recorded from the tail of sperm cells.

Less known, but nearly as conspicuous, is the variation in the number of antennal articles in Coleoptera. This number is basically 11, irrespective of the form, be it setiform, moniliform, pectinate, flabellate or geniculate (Figure 10.1). The fact was well known to Bateson (1894), as was the occurrence of rare but conspicuous exceptions. These exceptions are mainly scattered, but become more numerous and conspicuous in a few selected clades, with up to sixty articles in some longhorn beetles.

In centipedes, the poison gland is located in the proximal part of the poison claw itself, to one isolated but extraordinary exception. In the geophilomorph *Henia vesuviana*, the gland is placed in the twentieth body segment approximately, and an extremely long duct brings its secretion to the tip of the poison claw, where the orifice is placed.

Units of Description and Comparison

"How are we to recognize the 'true' characters of organisms rather than imposing upon them arbitrary divisions that obscure the very processes that we seek to understand? [..] No issue is of greater importance in the study of biology" (Lewontin 2001: xvii). Definitely, the single most important

requirement, when addressing a problem of homology, is probably the identification of meaningful units to be compared. Until recently, virtually all assessments of homology involved morphological units, either in adults or earlier developmental stages. New perspectives were explored by the recent turn towards developmentally defined morphological units (e.g., Wagner 1989, Schwenk 2001) or processes (e.g., Butler and Saidel 2000, Gilbert and Bolker 2001).

Modules

Cell–cell interactions, feedback behaviours, short-distance inductions, and long-distance morphogenetic effects notwithstanding, not to mention all forms of neural or hormonal communication, it does not seem to be the case to embrace too literally a holistic view of the organism and its development. If changes in every part of an organism had a non-trivial developmental effect on every other part of the body, evolution could never have occurred. Selection acting of the smallest part would have produced simultaneous selective pressure on all the other body parts. Consequently, the organism would have to be totally rebuilt in response to the slightest selective change (Lewontin 2001).

To describe any of those parts of the phenotype which behave as a relatively independent functionally interacting group of traits, Wagner introduced the concept of module (Wagner 1996, Wagner and Altenberg 1996). A *module* produces an integrated character complex and is thus both a developmental and an evolutionary unit. Characters within such a complex evolve together in a co-ordinate fashion because they are genetically correlated (Cheverud 2001). Modules may arise by differential integration of previously independent characters serving a common functional role, or by parcellation of an originally larger character complex, by selective elimination of pleiotropic effects among characters (Wagner 1996). Complex organs are made by adding new modules, each controlled by a few genes (Fishman and Olson 1997). Based on quantitative trait loci, Mezey, Cheverud and Wagner (2000) found modularity in the correspondence between genotype and the phenotype map of the ascending ramus of the mandible in the mouse.

If modules are units within which developmental interactions are primarily confined, it should be possible to determine the spatial extent of modules by studying patterns of covariation in morphometric data, for example by comparatively analysing the fluctuating asymmetry of presumptive modules. An example of this approach is Klingenberg et al.'s

(2001) study demonstrating that the forewings and hindwings of bumblebees are distinct developmental modules.

Evolutionary Changes in the Discernibility of Developmental Modules: Fusion Versus Non-disjunction

The ontogeny of our braincase provides a good example of the fusion of bones. Many distinct ossification centres give rise to the skull bones whose lines of contact become progressively interdigitated and which, at last, fuse together.

In the literature, the same fusion term is often used to denote what, more correctly, should be called a non-disjunction. This is the case, for example, of a curious feature found in several scattered groups of arthropods – that is, the presence of segments covered by ring-like sclerites, without any trace of the usual distinction between a dorsal (tergal) and a ventral (sternal) component. This behaviour is generally confined to some posterior segments of the body, as in scorpions (segments XIV–XVIII), in thelyphonids (XVI–XVIII), in male snake flies and many caddis-flies (abdominal segment IX), in some psychodid Diptera (abdominal segment IX plus tergum X) and in several Mecoptera (again, ring-like segments in the posterior abdomen). In the miniaturised leptotyphline rove-beetles (Staphylinidae), all sclerites of the prothorax are united together. In phylogenetic terms, these rings (or, at least, a majority of them) may be regarded as the 'fused' equivalent of two or more sclerites that were probably distinct in the ancestors of these arthropods. Strictly speaking, 'fusion' is a process, not a pattern, as implied by these phylogenetic comparisons. In terms of a process (i.e., in terms of developmental events), it is quite probable that most of these rings arise because no distinction between dorsal and ventral sclerites actually develops.

Generally speaking, lack of disjunction (pattern) is much more frequent than real fusion (process). Thus, instances of real fusion are events of greater evo-devo interest.

During the metamorphosis of the European grass frog (*Rana temporaria*), several sets of larval muscles undergo a process of fusion. The levator mandibulae posterior superficialis and levator mandibulae posterior profundus give rise to the levator mandibulae posterior; the levator hyoideus suspensoriohyoideus, levator hyoideus suspensorioangularis, levator hyoideus quadratoangularis and levator hyoideus hyoangularis together become the inner part of the depressor mandibulae; the constrictores branchiales I–IV and levatores arcus branchialis I–III give

rise to the petrohyoidei I–IV. In *Xenopus*, the interarcuales are fused at metamorphosis to form the longissimus dorsi; in adult anurans, the rectus abdominis superficialis and r.a. profundus are fused into a single rectus abdominis (Cannatella 1999).

In the leptostracan crustacean *Nebalia*, the peduncle of the second antenna comprises four articles in the advanced larva, but only three articles in the adult, due to the fusion of the third and fourth larval articles (Olesen and Walossek 2000). In the females of the great majority of the harpacticoid copepods, during the moult from the fifth copepodite to the adult, the genital (= last thoracic) segment and the first abdominal segment fuse to form a large genital double somite. In some genera, this process is delayed and a fused double somite does not form (Huys and Boxshall 1991).

Patterns of gene expression help distinguish between non-disjunction and fusion. Lack of segmental disjunction may be derived from the lack of a specific gene activity, as demonstrated in vertebrates for *lunatic fringe* mutants, which fail to form boundaries between individual somites (Evrard et al. 1998).

Analysis of the expression patterns of presumptive homologues of the *Drosophila* genes *extradenticle* and *dachshund* in the limbs of the woodlouse *Porcellio scaber* and the spider *Steatoda triangulosa* suggests that appendages such as the mouthparts derived from a leg-like ground plan via the elimination or fusion of the intermediate and distal podomeres (Abzhanov, Popadić, and Kaufman 1999). Such modification has been actually seen in the process (Abzhanov and Kaufman 2000a) during differentiation of the maxillipede in *P. scaber*. In early development, this appendage displays expression patterns identical to those in the following pereiopods. Later, the *extradenticle*-expressing cells of the basis expand, and the distal part of the appendage (the telopodite) begins to lose its segmented character.

Germ Layers and Homology

Germ layers are 'temporary developmental modules' which can differentiate, to a more or less large extent, independent of what is going on in the derivatives of the other germ layers. If the development of mesodermal structures is reversibly inhibited in newly hatched nauplius larvae of *Artemia*, ectodermal and endodermal structures go on with differentiation and the missing mesodermal structures are formed, although delayed, when the inhibitory treatment is removed (Hernandorena and Marco 1991). Germ layers are characterised by the exclusive expression of

genes, for example *serpent*, a gene with homeotic properties which specifies endoderm versus ectoderm in the developing gut of *Drosophila* (Reuter 1994).

Traditionally, we recognize two primary germ layers (the ectoderm and the endoderm) and a secondary germ layer (the mesoderm). Lack versus presence of the latter is a criterion for distinguishing two main levels of organisation: diploblastic (e.g., cnidarians) and triploblastic (bilaterians). This simple textbook picture is open to many questions. I am not concerned with recurrent claims, and correspondingly recurrent disclaims, that the mesoderm is present in Ctenophora, which have been traditionally ranked with diploblasts. The question is obviously relevant for our efforts to fit comb-jellies into a phylogenetic scheme, but this is less important, from the evo-devo perspective, than questions such as, are the ectoderm, endoderm and (where present) mesoderm homologous throughout the animal kingdom? Are there animals with more than three germ layers?

As Hall (1998b) clearly describes, germ layers are not immutable building blocks constraining development, but dynamic entities subjected to intensive evolutionary change.

Germ layers are just the most conspicuous of the many levels and ways through which a developing system becomes subdivided into functional units more or less extensively uncoupled from their neighbours.

One of the lowest levels of local autonomy is expressed by mitotic domains. In *Drosophila*, mitotic domain boundaries are cell fate boundaries (Cambridge, Davis, and Minden 1997). Subdivision of the *Drosophila* embryo into two dozen mitotic domains occurs with the fourteenth mitotic cycle prior to segregation of the germ layers (Foe and Odell 1989).

A kind of 'postembryonic mitotic domain' was observed in the beetle *Oryzaephilus surinamensis*, in which ectodermally derived tissues undergo mitotic waves at the end of the first half of each larval stage, whereas in the tissues of mesodermal origin cells divide shortly before ecdysis (Romer 1964). In *Hydra*, the direction of cell division in the ectoderm is somewhat biased in the axial direction, and the endoderm is strongly biased in the circumferential direction (Shimizu, Bode, and Bode 1995). Mitotic domains are not exclusive of insects. In zebrafish, three spatially separate mitotic domains with distinctive cell cycle length and rhythm arise at the so-called midblastula transition. These domains, rather than specifying different cell fates, seem to correspond to distinctive roles in the subsequent morphogenetic movements (Kane et al. 1992). In *Drosophila*, cells in different mitotic domains exhibit specific morphogenetic behaviours,

but many domains with unique morphogenetic activity are functionally identifiable even before their cells start dividing synchronously. Mitotic synchrony is possibly just a superficial but delayed symptom of a specific determination of a cluster of cells (Foe and Odell 1989).

Between the germ layers and the mitotic domains, there is a whole array of more or less independent developmental units, in which we could repeat what Švácha (1992) said of the insect imaginal discs: that these groups of cells do not represent qualitatively different structures, but merely one end of a continuum of more or less autonomous developmental units. Nomenclature notwithstanding, there is no clear-cut difference between the three traditional germ layers, the neural crest of the vertebrates, the imaginal discs of the holometabolous insects, and the set-aside cells from which the adult will be formed in marine invertebrates (such as nemerteans and sea urchins). But there are also processes by which specific morphogenetic factors are allocated to distinct embryonic lineages which are not given any special name. This happens, for example, following the stereotyped cleavage programs adopted by many animal groups, in which signalling centers are reliably positioned together with the cells that respond to their signals (Henry and Martindale 1998).

Brian Hall (1998b, 1999, 2000) has strongly argued in favour of treating the neural crest as a fourth germ layer. The neural crest has been defined as the quintessential feature of vertebrates (Gans and Northcutt 1983, Hall 1999, Butler 2000, Holland and Chen 2001), one of the main keys to their extraordinary adaptive success. In fact, the neural crest parallels the mesoderm as a major source of internal structures, tissues and organs. Its peculiar 'strategy' is the targeted migratory behaviour of its multiple components.

The visceral mesoderm of insects has been also regarded as a separate germ layer (Lawrence 1992, Chauvet et al. 2000). The main homeotic genes have different patterns of expression in somatic and visceral mesoderm respectively (Bienz 1994).

According to Hall (1998b), secondary germ layers, such as the mesoderm or the neural crest, are 'set-aside layers'. I think that we must both accept and reverse this equation. Mesoderm and neural crest represent large clusters of cells which are set aside and thus excluded from the developmental processes that will go on in ectodermal and endodermal derivatives. Therefore, there is no clear-cut criterion for assigning them a different status, with respect to smaller or more localised clusters of cells, such as the adult primordium of the sea urchin or the imaginal discs of the fly.

Groups of cells comparable with the imaginal discs (or the histoblasts) of the holometabolous insects are probably more common than generally believed. For example, the 'nasus', a conspicuous anterior projection found on the head of many termite soldiers, develops from a 'soldier-nasus disc' within just one moult (the last one in the insect's life), as described by Miura and Matsumoto (2000) for *Hospitalitermes medioflavus*.

As for imaginal discs, these are not necessarily limited to only one segment. For example, the so-called genital disc of *Drosophila* consists of three primordia (female genital, male genital and anal) corresponding to as many segments (Chen and Baker 1997).

The typical example of set-aside cells is the adult primordium found in the larvae of type 1 (or maximal indirect development) embryos (Davidson 1991, Cameron, Peterson, and Davidson 1998) belonging to marine invertebrates of the most diverse phyletic lines, including echinoderms, nemerteans and bryozoans. In this kind of development, cell division potentialities and fate specification of the embryonic cell lineages are completely different from those of the set-aside cells. Whereas embryonic cell lineages have a limited mitotic potential and are precociously destined to a fixed fate, the set-aside cells retain extensive proliferative capacities and escape the fate specification affecting the embryonic cells. The embryonically active cells will form the larva, and the set-aside cells will form the adult. According to Peterson, Cameron, and Davidson (1997), separate sets of homologous relationships relate the larval and the adult traits of these animals. These authors argue that the diversity of the adult body plans of these invertebrates reflects diverse pattern formation processes going on in their set-aside cells.

We know (page 47) that the larval body of these invertebrates is not patterned after the zootypic *Hox* gene expression. Problems in tracing homologies between larval and adult features in these animals are therefore serious, to an extent that is not always appreciated. Illuminating in this respect is Lacalli's (1994) revisitation of Garstang's (1894) auricularia hypothesis of the origin of chordates. Garstang distinguished three fields in this larva's body surface: the ventral oral field (possibly homologous to the apical organ of other larvae), the ciliary band and the aboral ectoderm. In chordates, the oral field would have been internalised, giving rise to the neural tube, and the oral field would have been enlarged to form most of the animal's body surface. According to Lacalli, this means that the body cover of a vertebrate is different from that of the auricularia. The *Hox* gene expression formerly associated with the ciliary band

became internalised with it and did not extend to the new ectodermal body cover.

The origin of set-aside cells fits nicely within a Darwinian view of development (cf. Buss 1987), where there is a selective advantage to individual cells and cell clones that are able to reduce the effects of competitions from their neighbours – be it competition for morphogens, nutrients, or other. Evolution of type I development in metazoans (Davidson 1991) was accompanied by the evolution of the 'sequestration' of the definitive germ line within the set-aside cells that will give rise to the adult (Ransick, Cameron, and Davidson 1996, Blackstone and Ellison 2000).

Set-aside cells may be finely interspersed within a sheet of 'ordinary' cells. In *Artemia*, the general epidermis is comprised of proliferating diploid cells. Among them, we find tetra- or octoploid setal cells which do not divide during the first two instars (Freeman and Chronister 1988). This is comparable with the behaviour of the diploid histoblasts – destined to form most of the body wall, musculature and other body parts of the adult *Drosophila* – which are interspersed within polyploid larval cells, destined instead to be destroyed at metamorphosis. Maintenance of diploidy of the imaginal cells is apparently one of the functions of *escargot* gene expression (Hayashi et al. 1993).

The presence of set-aside cells allows a large developmental independence of the 'container' (the larva) from its 'content' (the set-aside cells). As the container develops before its content, we expect that the instar derived from the set-aside cells will be 'more advanced' than the instar in which the set-aside cells were contained, but this is not necessarily true. In the case of the holometabolous insects, as described previously (page 77), the morphology of the larva is generally less conservative than the morphology of the adult. This is not limited to the gross features of external morphology but extends sometimes to the internal anatomy. For example, in many Diptera (Stratiomyidae, Tabanidae, Muscidae), Neuroptera (Myrmeleontidae), and the beetle *Melolontha*, the ventral nerve cord is more primitively segmental in the adult than in the larva (Hanström 1928).

Conventional germ layers and set-aside cells, such as those forming the primordium of the future adult of a sea urchin, represent one extreme of a continuum of morphogenetic individualisation of groups of cells within a developing animal, the other extreme being represented by 'delocalised' unspecialised cells such as sponge archaeocytes or the interstitial cells of the *Hydra*.

Let's re-examine the question of the homology of germ layers and the structures to which they give rise. Homologous structures in different organisms often arise from different germ layers (Hall 1998b), to such an extent that Wagner (1989) considered undermining the germ layer concept itself. Salvini-Plawen and Splechtna (1979) also denied the general possibility of drawing homology between germ layers of different phyla. More cautious is Hall (1998b), who invites distinction between facts and theory, with 'facts' being in his view the existence of homologous germ layers across the animal kingdom and 'theory' being the now disproved belief that homologous structures in different animals are derived from the same germ layer. This distinction between facts and theory is perhaps less clear-cut than Hall (1998b, 2000) would admit. At least a systematic description of all animal embryos in terms of classic germ layers is not immune from a certain danger of typological thinking (Richardson et al. 1999).

Lesser Developmental Units

Different criteria have been used to characterise lesser developmental units for which a complex terminology has been introduced that includes fields, progenitor fields, equivalence groups, territories of gene expression or of action of a morphogen and compartments.

A *field* is a group of cells provided with self-organising and self-regulating properties (Ingham and Martínez-Arias 1992), thus forming a discrete unit of embryonic development (Gilbert, Opitz, and Raff 1996). In a sense, a field is comparable with biological species (for a review of species concepts, see, e.g., Minelli 1993, Mayden 1997). First, it is possible to define a field, or a species, either in terms of what happens inside or in terms of what cuts off the field, or the species, from the surrounding world. In other words, a field is spatially confined within specific boundaries (cf. the isolation species concepts), and cells inside it share gene expression patterns allowed by diffusion, cell adhesion molecules, intercellular electric coupling and so on (cf. the recognition species concept). Consequently, if a species can be defined in terms of a shared gene pool, a field can be defined in terms of shared gene expression. A morphogenetic field is possibly the lowest term in a whole hierarchy of systems in which evolutionary novelties may arise. This happens following quantitative or qualitative changes in one or more gene expression patterns (Gilbert et al. 1996).

Davidson (1993: 666) introduced the concept of *progenitor field*, defined as "a region of an embryo composed of cells whose progeny will constitute a

given morphological structure". Progenitor fields have well-defined spatial boundaries. The term is basically equivalent to the traditional term *anlage*, but Davidson stresses the dynamic biochemical properties of these units, their inception depending on transcription control functions, positive but especially negative, mediated by intercellular signalling. Negative control of transcription prevents specific gene expression in cell lineages other than those constituting the territory. These negative functions are thus required to define the relevant lineage interfaces as field boundaries. For example, in *Drosophila*, the anterior limit of *knirps* expression depends on several interactions with *cis*-regulatory sites to which the negatively acting *hunchback* protein binds, and the posterior limit of expression of the same gene depends on multiple negative interactions at a different *cis*-regulatory locus with the product of the gap gene *tailless* (Pankratz et al. 1992).

Basically identical to a progenitor field is an *equivalence group*, defined (Horvitz and Sternberg 1991) as a set of multipotent cells whose fate is determined by cell interactions between members of the same set. Examples of equivalence groups have been identified in the most diverse animal embryos and are usually singled out from the surrounding cells by interactions involving the Notch/Delta signalling system (Jan and Jan 1995). Lewis and Wolpert (1976) had previously observed that cells that look alike histologically may yet be non-equivalent because of their different position.

From a slightly different perspective, Theisen et al. (1996) proposed the term *territory* to describe the set of cells under the influence of a particular morphogen. Without necessarily identifying a gene product as a morphogen, Raff and Sly (2000) refer to *gene expression territories*.

Rather than in terms of gene expression, *compartments* have been classically defined in terms of cell lineage (García-Bellido, Ripoll, and Morata 1973), as body units uniquely formed by the descendants of a pool of founder cells confined within unbridgeable spatial limits.

Cell populations from adjacent compartments maintain their position because they are unable to mix (Keynes and Stern 1988). This has been shown by grafting experiments in chick embryos, in which cell populations from anterior sclerotome halves only mix with cells from anterior halves of other sclerotomes, and those from posterior halves mix with cells from the posterior halves of other sclerotomes. Anterior and posterior cells no not mix, but form a boundary between them (Stern and Keynes 1987). This property applies to all vertebrates that have been studied. The strictly

similar behaviour of odd and even rhombomeres has been described previously (page 101). Cell sorting based on properties of the cell membrane is at the root of compartmentalisation, but this is a complex process also involving signalling between neighbour compartments, as demonstrated for the boundary between anterior and posterior wing discs in *Drosophila* (Blair and Ralston 1997).

There is no necessary correspondence between compartments and fields, although the very existence of compartments depends on the localised expression of a few genes which gives rise to the boundary between neighbour compartments and maintains it throughout development. However, fields and compartments may overlap whenever gene expression is actually limited by compartment boundaries. In the mouse embryo, for instance, the *MesP* genes and the genes encoding the receptor *EphA4* are only expressed in the anterior half of the somite, whereas *HES1* and *Delta1* are limited to the posterior half (Pourquié 2000).

Cells at compartment boundaries – e.g., those between the anterior and posterior compartments in the wing disc, or those between the alternating anterior and posterior compartments in the ventral epidermis of the *Drosophila* embryo – are the source of morphogens, such as *Wingless* (Lawrence, Sanson, and Vincent 1996).

That compartments are elements of pattern formation but are not involved in differentiation per se, at variance with the behaviour of the developmental units defined as fields, is shown by the fact that in mice compartmentalisation is essential for the maintenance of segment borders in paraxial mesoderm-derived structures, but not for the differentiation of dermomyotome and sclerotome (Hrabë de Angelis, McIntyre, and Gossler 1997). In *Drosophila*, the dorso-ventral patterning of the wing may depend on cell interactions independent of compartment formation (Brook and Cohen 1996). Since the segment rather than the compartment is a unit of morphological pattern, and two compartments are involved in forming an insect segment, cells on either side of the compartment boundaries must communicate with one another to generate a coherent morphological structure (Ingham and Martínez Arias 1992).

Frames of Reference: Muscles and Nerves

Before the advent of developmental genetics and the study of the spatial expression patterns of the so-called developmental genes, comparative anatomists were keen to identify phylogenetically or ontogenetically more

conservative organs or tissues which could provide a reference framework for assessing the homology of other anatomical features. Theoretical support for such a choice was not always obvious. Often, especially in pre-Darwinian literature, it was loaded by metaphysical overtones. For example, the choice of innervation patterns as a frame of reference in establishing positional relationships of vertebrate muscles and bones of arthropod sclerites was sometimes little more than a tribute paid to the most prestigious of the organic systems – a system whose complexity was seen growing along the *scala naturae* to culminate eventually with the human brain.

The choice of the nervous system as the main frame of reference in the assessment of homology was far from universal. Muscles and blood vessels have enjoyed their share of success, in some animal groups at least.

Due to the wealth of functions traditionally assigned to the heart and blood, to the point that the latter was regarded as the essence of life itself, it is not a surprise that some old scientists, such as the eighteenth century Swiss physiologist and polymath Albrecht von Haller, regarded vessels, rather than nerves, as the primary feature of body organisation (Nordenskiöld 1926). This appreciation was not widely shared by later researchers, although vascularisation was mentioned by Remane (1963) in his list of morphological criteria of homology and was used occasionally by vertebrate comparative embryologists. There is some reason for paying attention to these anatomical features, however, because recent studies have demonstrated that blood vessels, in addition to supplying nutrients to the developing organs, may also provide information for morphogenesis. This has been shown in the organogenesis of the pancreas by Lammert, Cleaver, and Melton (2001) and in the development of the liver by Matsumoto et al. (2001).

Muscles and Homology

In the comparative anatomical literature, homologies determined by patterns of muscle insertion are much more frequent than those determined by patterns of vascularisation. Reliance on these patterns has often been backed with the result of investigations on the phylogenetic and ontogenetic continuity of muscular patterns. This was obviously crucial to monographic works such as Matsuda's (1970) volume on the insect thorax, where we find several reassuring examples. Nymphal and adult muscles of the cricket *Gryllus* are nearly the same, with only one muscle being lost during development: this is clearly a good example of muscle continuity in

ontogeny. In other orthopterans, several muscles are lost, either during the last nymphal stage or after the adult stage is reached, but no new muscle is formed during development. The predominant trend in the evolution of the adult thoracic musculature in insects has been a reduction in muscle number. Some muscles present in the primitive wingless insects are still present in the immature winged insects, but are absent in the adult. Of course, some muscles have been added to the thoracic musculature in connection with flight.

Examples of the frequent use of musculature in establishing segmental units in arthropods are Häfner's (1971) study of the dorsal musculature pattern as a series of landmarks to recognise segmental borders in the pig louse *Haematopinus suis* and Shultz's (1993) analysis of the segmentation of the opisthosoma in the uropygid arachnid *Mastigoproctus giganteus*. In this case, muscle morphology provided criteria for distinguishing 'true' sterna from other ventral sclerites. Shultz regarded criteria derived from musculature as unambiguous, even when conflicting with previous interpretations based on external morphology.

Widespread success notwithstanding, analysis of musculature patterns is not immune from problems, technical and conceptual alike. Malicky (1974) observed that it is not that easy to trace homologies of trunk muscles in caddis fly larvae, because their insertions are not strictly conserved. It is not always possible to say whether a given muscle is a single one or a bundle of many different muscles.

Particularly disturbing are the difficulties in tracing homologies between larval and imaginal sclerites and musculature. Problems may be derived from an advanced reduction of some of the presumptive sclerites to which a muscle should be attached, as in the case of the male copulatory organs of insects. As these organs get their musculature from the ninth abdominal segment, they might be interpreted as belonging to it. However, as the tenth sternum is reduced, it is also possible that the phallic musculature is derived from intersegmental muscles originally running between sternum IX and sternum X (Snodgrass 1957). In the honeybee, the imaginal musculature of the thorax develops during the larval stages prior to the production of the skeletal elements to which it will be eventually attached. Most muscles do not grow between final sites of attachment, but many myoblasts are supported by larval muscles already suspended across the body cavity (Daly 1964). In the blackfly *Simulium*, in which some future flight muscles are already developed and attached to the epidermis in the second larval stage, differences in growth rate of the epidermis of different

parts of the mesothorax cause later changes in the position and orientation of these muscles (Hinton 1959, 1961). Morphologists have often used different names for these basically homologous muscles with new points of attachment (Matsuda 1970).

Another problem in tracing homologies of muscles and their attachment points is that a muscle may undergo duplication, as in the case of the jaw muscles in tetraodontiform fishes. The plesiomorphic condition in this order is two main adductor mandibulae muscles; but in some subgroups, these are duplicated, one or more times. The number of adductor mandibulae muscles thus ranges from the original two in triplespines (Triacanthidae) to as many as eight muscles in some filefishes (Monacanthidae). Based on their origins, insertions and relative masses in representative taxa and their congruence with a phylogeny for these taxa, Friel and Wainwright (1997) recognise morphological orthologues and paralogues of the original muscles. Owing to the specific significance given to the word 'orthologous' and 'paralogous' in molecular biology (Fitch 1970), I recommend qualifying these muscles instead as paramorphs, through an easy generalisation of the concept of paramorphism I have originally applied (Minelli 2000b) to the relationships between appendages and main body axis (cf. page 164).

There are even cases in which evidence from muscular insertion patterns seems to point in the wrong direction. This may happen in those groups in which a body part has extensively departed from its morphological setting in the close relatives. In male dipterans, abdominal segments IX and X are often curved ventrally and are associated with the genitalia to form a complex known as the hypandrium. In some groups, a 180° twist gives rise to a hypandrium inversum, with the anus in a position ventral to the genital opening. In the case of the little blood-sucking fly *Phlebotomus garnhami*, it has been claimed that, to allow the postabdominal inversion to occur, the abdominal longitudinal muscles from abdominal segment VI have moved their insertions to segment VII (Just 1973). Bigger problems were faced by Kimsey (1992) in her study of the cuckoo wasps (or gold wasps, Chrysididae). This family is characterised by modification of two or more abdominal segments that are telescoped within the abdomen and may function as an ovipositor. Accompanying the internalisation of these segments are shifts in the position of those muscles which provide for their unusual mobility. Kimsey assumed that a shift in position was more likely to occur than the evolution of an entirely new muscle; nevertheless, one of these muscles does not appear to be homologous to any muscle seen

in the ground plan of the aculeate hymenopterans. One more example of muscles shifting their insertion during development comes from the tendons of the levator mandibulae posterior profundus and the levator mandibulae externus in the frogs, which shift to insert on Meckel's cartilage when the suprarostral cartilage disintegrates during metamorphosis (De Jongh 1968).

Nerves and Homology

In his classic monograph on bird comparative anatomy, Max Fürbringer (1888) established the principle that homologies between muscles in different birds can be correctly established only by reference to their innervation patterns.

One general reason for expecting that the nervous system should be more conserved than other aspects of morphology is its complexity (Nishikawa et al. 1992, Tierney 1996). In vertebrates, further justification for relying on nerves rather than muscles or bones may be that the central nervous system often differentiates far in advance of bones and muscles, as in the head of eutherian mammals (Smith 1996), but this does not apply to animals in other phyla.

In a sense, different body regions are recognised as non-equivalent by the nerves that innervate them. This is the lesson Lewis and Wolpert (1976) derived from Miner's (1956) experiment with a tadpole of the frog *Rana pipiens*, in which a piece of skin was rotated in such a way that skin from the animal's ventral side now covered the back and vice versa. After metamorphosis, the frog reacted to mechanical stimulation of the back or the belly as if the pieces of skin were still in their normal place; that is, the animal wiped its belly when stimulated on the back and vice versa.

In arthropods, innervation is often used as a criterion for identifiying the segmental origin or composition of fused, reduced, or otherwise highly specialised body parts. In the female cockroach *Leucophaea maderae*, for example, Engelmann (1963) recognised six free abdominal ganglia, the latter of them also innervating segments VII through IX, including genital structures. Following the innervation pathways, the author identifies the segmental origin of the different parts of the female reproductive system.

There are problems with this use of innervation as a criterion for delimiting segments. Matsuda (1970), while sometimes using innervation as a key to homology in his comparative analysis of the insect thorax, remarked on the scanty developmental evidence supporting this practice and stressed

the need for adequate studies, extending back to the earliest stages of neurogenesis, but in *Drosophila*, the arrangement of neuroblasts does not help to understand head segmentation (Hartenstein and Campos-Ortega 1984). Matsuda also observed that there may be considerable individual variation in the connections to the muscles a nerve develops. Other difficulties may be derived from the more or less extensive fusion of two neighbouring nerves or by the multiple innervation of one and the same muscle (see also Schmitt 1962, Märkl 1966).

Innervation may shift during development (Striedter 1998). Sewertzoff (1931), for example, reported that, in a lizard, the anlage of the forelimbs are initially innervated by neck nerves. But later these nerves degenerate, and the definitive innervation is provided by more caudal nerves.

There are also problems in arthropods, where motor nerves are usually restricted to the segments in which their respective ganglia are found. But this restriction does not necessarily apply to the integumental sensory nerves. Thus, motor nerves are more reliable indices of segmental limits than sensory nerves, but comparative morphologists should be aware of exceptions, as in the case of the moth *Telea polyphemus*, where the second thoracic ganglion gives off nerves to all three thoracic segments (Snodgrass 1960). Still more important, examples of reorganisation of the patterns of synaptic connectivity during development are known from animals as diverse as insects (Truman 1990, Levine, Morton, and Restifo 1995), frogs (Alley 1990), and *Caenorhabditis elegans* (Hallam and Jin 1998). Lauder (1986) regarded the vertebrate brain as highly plastic and possibly not sufficiently robust to permit phylogenetic inferences.

There are molecular mechanisms supporting the traditional view. In mutant mouse embryos, it has been demonstrated that lack of *Hoxb-1*, one of the *Hox* genes whose expression domain is restricted to one segmental unit (in this case, the fourth rhombomere in the hindbrain) causes altered segmental identity and abnormal migration of motor neurons. In mutants, motor neurons differentiate, but the controlateral vestibuloacoustic efferent and the facial branchiomotor neurons fail to migrate to their proper positions (Studer et al. 1996).

The phylogenetic stability of neuroanatomical characters has been recently tested by Buschbeck (2000). In her elegant study, the phylogenetic relationships of 23 species of Diptera, representing a diversity of lineages such as crane flies, robber flies, hover flies and house flies, were reconstructed based on fine neuroanatomical details of the visual system only.

The phylogenetic relationships suggested by this analysis are similar to those suggested by the traditional macroanatomical traits or by molecular data. The 'phylogenetic signal' contributed by the different parts of the nervous system is not the same. Phylogenetic relationships suggested by the neurons of the second-order visual neuropil, the medulla, are better defined in respect to what is suggested by characters of the deeper visual centre, the lobular plate. These differences, according to Buschbeck (2000), may relate to different functional constraints in the two neuropils.

Summary and Conclusions

If we wish to foster a dialogue between evolutionary biology and developmental biology, we need to ensure that the two disciplines stand on equal metaphysical footing. I think that developmental biology is still heavily biased by finalism, whereas an equation of evolution to progress is by now a matter of the past. A discussion of this problem and some suggestions for a new view of development was the subject of chapter 1.

In this view, development acquires a meaning of its own, rather than simply being the process required to obtain an adult. Consequently, I have suggested that the very origin of many, if not most, of the basic features of an animal's body must be searched for in the adaptive significance these features initially had for development as such, rather than as adult features necessarily prepared through development. This idea was the subject of chapter 2.

If development is not the way by which a programme encoded in the egg is deployed to reach the final, adult condition, we may question whether the metaphor of the programme (more precisely, the metaphor of the genetic programme) is indeed adequate. I think it is not. This is not to deny, of course, the role of genes in development, but to question instead current notions such as the developmental gene and the master control gene. I am sympathetic towards those views according to which the development of the earliest multicellular organisms was mainly caused by generic, rather than genetic, causes. In the course of evolution, the latter acquired an increasingly larger importance and acted as canalisers and stabilisers of developmental processes. These questions were discussed in chapter 3.

Chapter 4 was devoted to developmental time. I suggested that developmental systems first acquired ways to control and pattern the temporal progression of their dynamics and that this offered a blueprint to the subsequent evolution of ways through which control over spatial patterning

was achieved. Further problems concerning developmental time are how to establish homology between developmental stages or events, how to define a larva and what is metamorphosis.

Chapter 5 dealt with the most elementary level of body complexity (i.e., regionalisation or tagmosis). I discussed briefly, in light of comparative morphology and molecular developmental genetics, topics such as number and homology of tagmata, the nature and temporal stability of the boundaries between them and the mostly overlooked relationships between developmental time and the development and patterning of primary and secondary body axes, as well as some problems in morphological and developmental topology.

Cell properties and behaviours, as basic to differentiation and patterning, were briefly analysed in chapter 6, which was devoted to issues of differentiation and body patterning. This was also the place to discuss positional information – a notion I suggested to replace the reciprocal notion of informational position, transpatterning, phylotypic stages, and morphological assimilation in ontogeny and phylogeny, with a coda on regeneration.

Size factors – cell size, cell number, and miniaturisation – were the topics of chapter 7.

Chapters 8 and 9 – respectively devoted to body axes and symmetry and to the nature and origin of segments – included most of the new outlooks, quite probably controversial and hopefully provocative, through which I believe that evo-devo biology should approach the study of animal organisation, in light of joint suggestions from developmental genetics and comparative embryology and morphology. Hot spots of these two chapters were the concept of the dual animal, a relativistic revisitation of the definition of the animal's main body axis, a critique of the conventional description of body structure and morphogenesis in terms of Cartesian axes, and a discussion of two concepts (axis paramorphism and double segmentation) I first introduced in research papers (Minelli 2000a, 2000b, 2001). Unconventional views derived from this way of looking at animal morphology and morphogenesis include the suggestions that the tail of vertebrates is an appendage rather than a part of the main body axis, and the scolex may be the posterior rather than the anterior end of a tapeworm's body.

The last chapter was devoted to homology. Based on evo-devo arguments, I defended the view that we shall follow a factorial approach to homology. That is, that homology is a relative, rather than an absolute

concept. I also discussed, in evo-devo perspective, the merits and weaknesses of homologies drawn on patterns of muscle insertion and innervation. I also suggested that, in the multilayered mosaic of developmental subunits that we can recognise within a developing animal, there is a virtual continuum between the conventional germ layers and the clusters of set-aside cells, such as the imaginal discs of holometabolous insects and the adult primordium of sea urchin larvae.

Following the conceptual background introduced in the first two chapters, I have been articulating a view of development in which there is little space for many old questions which are, in my view, a simple by-product of an unnecessarily complex holistic view of the organism and its development. For example, I have dismissed the 'problem' of the origin of symmetry as simply due to failure to acknowledge that symmetry is the expected 'default product' of largely independent developmental trajectories occurring in parallel within separate modules.

I have also dismissed the fashionable concept that most major developmental 'decisions' are taken early in ontogeny, because this is the way to avoid irreversible mistakes in the long journey towards the adult. I have argued instead that such 'decisions' (i.e., patterning) are only possible within very small systems. But the animal is adequately small only at very early stages. Therefore, patterning is 'decided' at early stages, because at early stages the embryo is small, not the other way around.

With this book, I hope I have succeeded at least in infecting the reader with three ideas.

First, that evolutionary developmental biology urgently needs a bulky injection of facts and concepts from disciplines such as comparative morphology, descriptive embryology and the study of postembryonic development.

Second, that we cannot continue speaking of segments, tagmata or larvae without qualifying these concepts, and many others, adequately – what might not necessarily be the same way in every context.

Third, that evolutionary developmental biology requires much more than the study of a handful of model species, however sophisticated our experimental approaches might eventually be. Animal evolution has explored many more solutions than the chicken, mouse, zebrafish, *Drosophila*, and *Caenorhabditis* would suggest. As Arthur (2002) stated, this is not a reason to despair, but a plea for caution. Wide-ranging comparisons and a combined interdisciplinary approach are required before evolutionary developmental biology may finally be written 'from first principles'.

Honestly, I do not expect that my criticism of the current adultocentric view of development will be accepted easily. I wonder, however, how followers of this view will be able to produce a consistent and sober explanation for the many facts I have tried to interpret in these pages from a less finalistic perspective. Let's see, a few years from now, where the balance between these opposite views will eventually settle.

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