The Evolution of Anisogamy

Darwin identified the existence of separate male and female gametes as one of the central mysteries of evolutionary biology. A century and half later, the question of why male gametes exist remains an intriguing puzzle.

In this, the first book solely devoted to the evolution of anisogamy, top theorists in the field explore why gamete dimorphism characterizes nearly all plants and animals. Did separate male and female gametes evolve as a result of competition, or does anisogamy instead represent selection for cooperation? If disruptive selection drove the evolution of anisogamy, with male gametes focused on search and fusion, and female gametes provisioning the new zygote, why do some algal species continue to produce gametes of a single size? Does sperm limitation, or escape from infection, better explain the need for extremely small, highly mobile sperm?

Written by leaders in the field, this volume offers an authoritative and cutting-edge overview of evolutionary theory.

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A Fundamental Phenomenon Underlying Sexual Selection

Edited by
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PAUL ALAN COX

Introduction: The Evolutionary Mystery of Gamete Dimorphism

We do not even in the least know the final cause of sexuality; why new beings should be produced by the union of the two sexual elements, instead of by a process of parthenogenesis.

Charles Darwin (1862)

The mystery which Darwin struggled with, the existence of sex in the plant and animal kingdoms, continues to fascinate biologists today. While many plant and animal species reproduce sexually, others continue to succeed with asexual reproduction.

Consider, for example, *Prorodon utahensis*, a small animal which flourishes in the hypersaline waters of the Great Salt Lake (Figure 0.1). There are few other forms of life that can tolerate these salinities, which have been measured at up to 27%. The quivering hair-like cilia of *Prorodon* provide its tiny body – scarcely the width of a human hair – with sufficient locomotion to zip about its otherwise lethal environment, consuming organic detritius, cyanobacteria and the salt-tolerant green alga *Dunaliella*. In the shallow waters of the Great Salt Lake, which are too salty for fish, these tiny *Prorodon* are the major hunters, the equivalent of sharks at the microscopic level. Reproduction in *Prorodon* is a simple matter – it simply splits in half. Without resorting to sexual recombination, *Prorodon* is able to lock in its genetic combination for survival and success in this most hostile of environments. Asexual reproduction also grants *Prorodon utahensis* a significant numerical advantage in progeny. A single individual splits, producing two, then four, then eight, then sixteen, then thirty-two genetically identical

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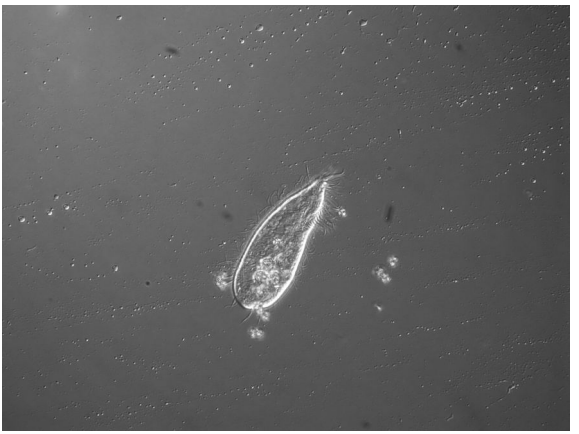

Figure 0.1 Differential interference contrast microphotograph of a drop of water from the Great Salt Lake containing the asexual ciliate *Prorodon utahensis* and the isogamous grean alga *Dunaliella salina*.

offspring. This remorseless process of binary division can rapidly fill a small saline pond or even the Great Salt Lake with hundreds of millions of ciliates from a single immigrant. Were *Prorodon* to reproduce sexually, the number of offspring it produces would be halved, since in a population characterized by separate males and females, only half of the reproductive individuals can produce offspring. Biologically, in terms of population growth, males are of such little consequence that wildlife demographers sometimes simply ignore them. "Why do males exist?" is not a radical feminist slogan, but instead a valid and largely unanswered evolutionary mystery, a mystery which, at the level of female and male gametes, is the essence of this book.

The mystery of sexual dimorphism exists at a variety of modular levels (Cox, 1988), some of which are easily observable without a microscope. The fact that male date palms cannot produce dates, but contribute only pollen was of grave concern to ancient farmers. Stone images carved nearly 3000 years ago in the Assyrian city of Nimrud (about 30 kilometers southeast of present-day Mosul, Iraq) depict winged gods carrying pollen from the male trees of *Phoenix dactylifera* to the female trees. While the necessity of pollination for a successful crop was considered so important that deities were invoked as pollen vectors, the actual function of the pollen was poorly understood. In the third century BC, Theophrastus, a student of Plato, opined in *De Causis Plantarum* that female date palms require pollen as a needed dessicant, otherwise the developing fruit will mold and rot.

A better understanding of the role of pollen in reproduction would require the invention of the microscope two millenia later.

The differential contributions of male and female date palms to one offspring are mirrored in the stunning difference in the number of gametes the two sexes produce. The large female gametes are produced by megaspores embedded in ovules in the carpels of the female flowers. After pollination, the female gametes develop into embryos, the ovules become seeds, and the carpel which surrounds them matures to become the fruit. Because date palms can survive harsh desert conditions, their fruits are regarded with reverence by the nomadic and pastoral people of the Middle East. In modern Oman, a cultivated female date palm annually produces about 4000 dates, with the total harvest from a single tree weighing a total of 40 kilograms. This seems to indicate a large number of female gametes – at least 4000 – to be produced by a single female tree. Yet a single male tree produces millions of pollen grains. Traditional wisdom holds that a "harem" of 50 female date trees – which would produce a total of 200 000 dates – can all be fertilized by the pollen from a single male tree. Since male to female sex ratios at inception in dates is 50:50, this means in traditional Omani orchards, 49 male trees can be culled or destroyed with no measurable loss of date production. We might safely assume that this disparity between male and female reproductive success was mirrored in natural *Phoenix dactylifera* populations prior to their cultivation by humans. The resultant variation in reproductive success is far greater among male trees than among female trees: given adequate pollination, nearly all female trees set fruit, but only a few males have a disproportionately high success in siring offspring. This essential difference in variability of male and female reproductive success drives sexual selection.

Although sexual selection does occur in plants (see Willson, 1994), the consequences of sexual selection in animals are perhaps more familiar. In Jackson Hole, Wyoming, where I write these words, the large elk herd which winters at the National Wildlife Refuge consists of thousands of adult females, but only several hundred reproductive adult males. During mating season, a few extremely large male elk, resplendent with large antlers, each defend a harem of 15 to 20 females with eerie whistle-like calls that pierce the crisp fall air. An alpha bull elk challenges any male aspirant to his harem leadership with snorting, aggressive displays and occasional violence. Head butting and locking of antlers are the major mode of combat. Lacerations, crippling injuries and sometimes death are outcomes of these battles. Usually

the largest male with the largest rack of antlers is ascendent. As a result, although nearly all of the reproductive females in a population produce calves, only a few dominant males sire the next generation of the herd. The cost these male elk pay, for carrying about their large racks each fall, incurring risk of serious injury and even death from ongoing battles is large, yet sexual selection continues to maintain the production of massive displays of antlers.

These differences between male and female, so stunning in larger animals, also have a counterpart in the microscopic realm. Consider, for example, the different sizes of male and female gametes in higher plants and animals. In human beings, a typical sperm is 5 to 7 μm long, while the diameter of a receptive human ovum is 20 times greater. How did this vast difference in size arise?

In this book, the contributors focus on the phenomenon of different-sized male and female gametes, a condition known as anisogamy, which underlies all sexual selection. Why do nearly all animal and plant species have gametes of two different sizes, as opposed to producing gametes of a single size, a condition known as isogamy?

The evolution of anisogamy, one of the major evolutionary riddles to remain unsolved in the nineteenth and twentieth centuries, emerges into the twentieth-first century as potent a mystery as ever. The prevalence of anisogamy in the animal and plant kingdoms – with isogamy characterizing only a few algal species – is an astonishing testament of the evolutionary ascendency of anisogamy as a robust evolutionary solution. Yet, I am reminded of Gertrude Stein's query to Alice B. Toklas as she was wheeled into surgery. "What is the answer?" Stein asked. Toklas responded, "In that case, what is the question?" To paraphrase, if anisogamy is the answer, what was the evolutionary question?

The earliest theories of gametes, emerging from the pre-Renaissance period, were focused on concatenation of future generations within an ovum, much like interlocking Russian matruska dolls. This pre-existence theory was articulated by Nicolas Malebranche in 1673:

> We may with some sort of certainty affirm, that all trees lie in miniature in the cicatride of their seed . . . All the bodies of men and of beasts, which shall be born or produced till the end of the world, were possibly created from the beginning of it. (as quoted in Farley 1982, p. 17).

This matruska-doll theory of the egg was later mirrored in a similar theory for sperm, which were discovered with the newly

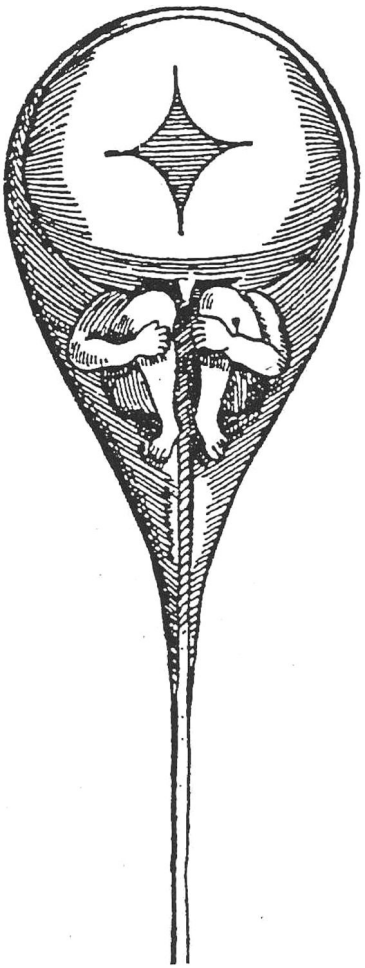

Figure 0.2 1695 drawing of human homonuclus in sperm

invented microscope by Antoni van Leeuwenhoek in 1679. Leeuwenhoek found that ejaculate of a male teams with countless "small animalcules." Although some argued that these animalcules represented parasites within the male testes, it was not long until Nicolas Hartsoeker in 1694 and others argued that these "small animalcules" were in fact containers for entire preformed men (see Farley 1982, Birkhead and Montgomerie, 2009). Retermed "the homonuculus" (Figure 0.2) this new theory suggested that each sperm enclosed a tiny human. These miniature human beings held within them homonuculi for even smaller human beings, which in turn contained even

smaller homonuculi, generation after generation, until the very end of time.

While we may smile at the naiveté of the homonuculus theory of gametes, surprisingly, the discovery of gamete fusion as the fundamental basis for sexual reproduction did not occur until the middle of the nineteenth century. In 1843, embryologist Martin Barry published his note entitled "On the penetration of spermatazoa into the interior of the ovum" based on his discovery of sperm inside the ova of rabbits. Barry's finding triggered Geissen professor Theodor Ludwig Wilhelm von Bischoff in 1847 to propose a theory of sexual reproduction based on gamete fusion. In this theory, he bravely reversed his earlier 1842 claim that entry of sperm to an egg is "absolutely impossible." "I do not hesitate however in declaring absolutely the opposite view, that only the dissolve part of the semen enter the egg" (as quoted in Farley, 1982, p. 56). The gamete contact theory of Barry and Bischoff was highly controversial, resulting in a contentious scientific debate. It required 33 years for von Bischoff's theory to gain traction, and it achieved acceptability only after the careful embryological studies of Oscar Hertwig, who discovered that fertilization of sea urchin eggs requires penetration of a sperm (Austin, 1961). Hertwig also was apparently the first investigator to propose that a division of labor between the sexes gives an advantage to gamete dimorphism:

> In the first place, it is necessary for the nuclear substances of the two cells to become mixed, hence the cells must be able to find one another and unite. Secondly . . . it is equally important that there should be present, quite from the beginning, a sufficient quantity of developmental substance . . . In order to satisfy the first of these conditions, the cells must be motile, and hence active; in order to satisfy the second, they must collect these substances, and hence increase in size, and this of necessity interferes with their motility . . . Nature has solved the difficulty by dividing these properties – which cannot of necessity be united in one body, since they are opposed to one another . . . She has made one cell active and fertilizing, that is to say male, and the other passive and fertilizable, or female. (Hertwig 1901, p. 278.)

Botanists were a bit slower than zoologists to accept the theory of gamete contact as the basis for sexual reproduction. Like early Greek thinkers who believed that semen represented a type of seed, which when planted in a fertile field (e.g. a reproductive female) yielded offspring, mid-nineteenth century botanists such as Hermann Schacht

argued that "the grain of pollen therefore is *not* the fecundating organ . . . *but the egg of the plant*" (Farley, 1982, p. 52). Even so great a botanical observer as Wilhelm Hofmeister claimed that entrance of the pollen tube into the egg is impossible. He instead subscribed to a type of catalytic theory suggesting that the egg is somehow stimulated into developing by the fluid from the pollen tube. Harvard botanist Asa Gray remained agnostic as to gamete contact as late as the sixth edition of his textbook: "Cross fertilization, or Allogamy [is] the action of the pollen of one flower on the pistil of some other flower of the same species" (Gray, 1879, p 216).

Soon, advances in phycology brought gametes, and particularly gamete dimorphism, to the attention of botanists throughout the world. Since algal species, unlike animals, show a variety of gamete types ranging from gametes of identical size (isogametes), to gametes of greatly different sizes (anisogamy), botanists were forced to recognize and explain these differences. The most influential botany textbook of the early twentieth century, that of Strasburger and his colleagues in Germany, suggested that these different forms of gametes represent a type of evolutionary progression:

> Sexual reproduction is met within the vegetable kingdom in very different forms; the extremes are very distinct but are connected by many intermediate forms. . . In the lowest grade we find two completely similar motile cells (gametes); they are evidently to be phylogenetically derived from swarm-spores, but are distinguished from them by undergoing further development after fusion (certain Brown and Green Algae). At a slightly higher stage one of the two gametes is distinguished by its size and by losing the power of movement earlier than the other one; it is the female gamete, and when at rest is sought out and fertilized by the male gamete. At a further stage, this egg-cell has completely lost the power of movement. (Strasburger *et al.* 1921).

With isogamy firmly relegated as an evolutionary relic, the rather vague botanical explanations for the evolution of gamete dimorphism focused on evolutionary progress and efficiency. This sense of isogamous algal species as atavisms which somehow evidence the progressive nature of evolutionary advance is exemplified by E. J. H. Corner in his discussion of seaweed reproduction:

> The remarkable point about these slight differences in gamete behavior, however, is that there should still be plants of relative simplicity satisfied with what seem imperfect, as they are intermediate, steps in

> the evolution of reproduction by eggs, whereas most plants have long ago completed this lap of evolution's race. (Corner, 1964, p. 87).

Yet Corner, unlike other botanists, who were content to merely catalog the differences in gamete types, sought for some type of selective force to explain gamete dimorphism. He proposed what I have termed "the lost child in the woods model," a theory for the evolution of anisogamy based on what could be termed disruptive selection:

> If two persons wish to find each other it is better for one to wait while the other searches. And if on meeting they must journey, it is better if she who waits should be provisioned while he that searches may travel light and fast. The principle of assignation was worked out long ago by gametes . . . Reproduction by eggs has arisen presumably through the selection and inheritance of variations that have helped both the mating of the gametes and the establishment of the zygote as a new plant. The variations have led to the breakdown of female mobility, but out of this has come better provision for the offspring. (Corner, 1964, p. 86).

This suggestion, that in Jack Sprat fashion, a division of labor between male and female gametes allows for efficient search and adequate provisioning of the zygote, is a theme that has been pursued by more recent authors. Lynn Margulis – inventor of the five kingdom system for classification of life – and her son Dorion Sagan argued, "As long as the equality of the parental nuclear contributions is maintained, division of labor leads to efficiency: one parent cell stores food and stays put and the other loses all its excess baggage and moves around." (Margulis and Sagan, 1986, p. 195).

More recent botany textbooks continue to extol this theme of efficiency gained by gamete dimorphism, via both increased fusions through enhanced male motility and increased fitness of the zygote which is largely provisioned by the female gamete.

> With forms inhabiting moving water, isogamous reproduction must be extremely wasteful, and there are evident advantages if one gamete remains relatively stationary, especially if it secretes chemotactic pheromones causing the male gametes to accumulate around it. Moreover, a zygote which begins life with a copious food reserve has a better chance of survival than one with little. Increasing size, however, severely limits mobility, so again advantages can be envisaged in a situation in which one gamete, the male, remains small and motile, and the other, the female, loses motility and specializes in the laying down of food reserves. (Bell, 1992)

This theme of efficiency of a division of labor between male gametes (motility) and female gametes (provisioning) captured the imagination of zoologists and theorists as well. In his elegantly argued book, Michael Ghiselin likened gamete production to a manufacturing enterprise:

> Suppose that a manufacturer of heavy goods such as machine tools wanted to set up an effective program for both selling them and getting them to his customers. He might equip each salesman with a large truck piled high with machinery to be sold on the spot, and have them scout the countryside for customers. This arrangement would have certain advantages, such as immediate delivery, but at the same time a lot of energy would be wasted hauling the stock from place to place. It would be cheaper to use a heavy vehicle only for delivery, and to provide the salesmen with automobiles in which they could cover a far wider area on a given amount of fuel. Extending the analogy, we do not expect a manufacturer to mount his entire plant on wheels and produce goods on the road . . . So it is with sexual roles. Eggs differ from sperm because the female gametes specialize in providing the zygotes with energy and other resources. The male gametes are specialized for uniting the female ones. Any energy used by the females in moving about would necessarily be subtracted from that passed on to the zygotes. Hence the ideal female would be an absolutely passive organism. The male, on the other hand, should concentrate upon obtaining maximal dispersal from a given quantity of energy. (Ghiselin, 1974, p. 101–102)

This general sense of isogamy as a primitive condition from which nearly all plants and animals have escaped driven by selection for specialization of labor between the sexes is appealing in a certain anthropomorphic sense. However, we might remember the anecdote attributed to the physicist Wolfgang Pauli, who when confronted with a laboriously written manuscript by a young physicist, exclaimed, "It is not even wrong" (Peierls, 1960).

Analysis of efficiencies gained due to gamete dimorphism are unlikely to yield a wrong answer, but this surely can't be the whole story, otherwise isogamous species would have perished long ago. Given that small, stripped-down male gametes are indeed far more motile, and that zygotes can better be provisioned by large female gametes lacking flagella, we still must confront the continuing success of species of isogamous algae. Again, referring to Figure 0.1 of the ciliate *Prorodon utahensis* in the Great Salt Lake, which, at the beginning of this essay, I argued benefits from asexuality, please note the glowing ball-like organism at the lower right of the photograph. This is the

green alga *Dunaliella salina*, which can reproduce sexually and is isogamous in its reproductive biology. *Dunaliella salina* is so prolific in the hypersaline northern arm of the Great Salt Lake that, together with the cyanobacterium *Aphanothece halophytica*, it turns the water red, a feature observable from outer space (Roney *et al.*, 2009). If asexuality is the correct answer to a hypersaline environment for *Prorodon*, it is hard to see why 10 to 20 billion or so *Dunaliella* continue to thrive in the Great Salt Lake using the wrong answer.

As a young botanist, I questioned the theories extolling the unparalleled advantages of anisogamy for the entire natural world, particularly since isogamous algae seem to proliferate in hypersaline environments, shallow ponds and near-shore intertidal marine ecosystems. The interesting question, therefore, became in my mind not why anisogamy characterizes most higher plants and animals, but instead why do the relatively few isogamous species continue to thrive? Using simple numerical experiments, beginning with a programmable hand calculator I had with me during fieldwork in Samoa, and then moving to Harvard's mainframe computer (whose computing power today is doubtlessly eclipsed by an ordinary cell phone), I first tested Corner's "lost boy in the woods model" to see if immobility of one of the pair of gametes indeed increases gamete encounters and fusions. My simulations suggested that immobility of one partner does not increase frequency of encounter. Later exploring search theory, as developed for submarine warfare in World War II by Columbia University mathematician Bernard Koopman, I discovered that the probability of an encounter in three dimensions during a random search is not based solely on the velocity of the mobile gamete alone, but instead increases with the *sum of the velocities* of both gametes (Koopman, 1980).

Eliminating female immobility as an advantage for gamete encounter, I wondered if it is possible that a complex adaptive topography, of the type proposed by Sewall Wright (1977), might come into play. Is it possible that anisogamy represents a high-fitness Everest, while extant isogamous species are stranded on smaller fitness foothills, which they cannot reach without first descending through a fitness trough? Together with U.C. Berkeley mathematician James Sethian, I made numerical experiments on a supercomputer of random gamete encounters in three dimensions; Sethian then confirmed the results of these experiments with analytical solutions (Cox and Sethian, 1984; 1985).

Accepting the fact that small male gametes not only can move faster than large female gametes, but also that in the low Reynolds

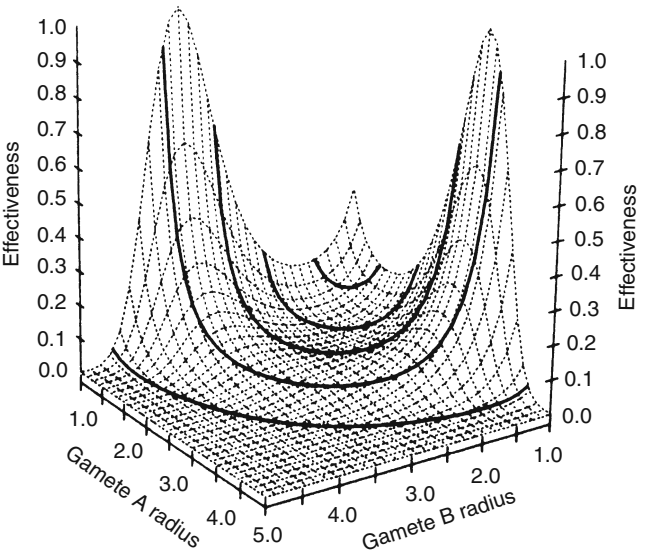

Figure 0.3 Adaptive topography of gamete dimorphism as demonstrated by vulnerability of isogamous populations to invasion by an anisogamous mutant. Note the high adaptive peaks for anisogamy, but the lower region of stability for strict isogamy.

number environment at a microscopic scale, water is a highly viscous medium for gametes, we searched for conditions in which an isogamous population can be successfully invaded by an anisogamous mutant, as well as for the converse situation. Assuming that zygote fitness corresponds with zygote size, we calculated a complex adaptive topography (Figure 0.3), which demonstrates not only global stability for anisogamous populations with a profound size dimorphism, but also smaller regions of local stability for isogamous populations. Thus, isogamy involving small gametes and anisogamy characterized by profound gamete dimorphism were evolutionary stable strategies [ESS] in our analyses (Cox and Sethian, 1984; 1985).

Yet, the answer cannot lie in the evolutionary choice between strict isogamy and strict anisogamy. Distinguished marine biologist Tatsuya Togashi (who is co-editor of this volume) led me to consider not only the existence of isogamous and anisogamous species, but also of slightly anisogamous species – species with nuanced gamete dimorphism – and isogamy with large gametes, both conditions which occur in marine green algae, but neither of which we had

originally predicted to be an ESS (Cox and Sethian 1984; 1985; Togashi and Cox, 2004; Togashi *et al*., 2004; 2006).

Any unified theory of the evolution of anisogamy must therefore not only account for efficiencies of gamete encounter and zygote provisioning generated by anisogamy, but also must account for the persistence and spread of isogamous species, the success of "slightly anisogamous" species, isogamous species with large gametes, as well as the occasional evolutionary reversion from anisogamy to isogamy that appears in the phylogeny of certain marine green algae (Togashi, personal communication).

And so, despite the group selectionist theories of Kalmus (1932) and Scudo (1967), it seems to me that any successful theory of gamete dimorphism must be rooted in the seminal paper of Parker *et al*. (1972), whose theory is affectionately known among evolutionary biologists as "PBS." The PBS theory starts off with some simple, but powerful assumptions. First, PBS assumes for gamete dimorphism to evolve, some disruptive selection is required. Second, PBS assumes that each reproductive individual has only a fixed biomass available for gamete production, and so the greater number of gametes produced, the smaller those gametes must be. Third, PBS assumes that there is some relationship between zygote size and zygote fitness. Thus, gamete dimorphism will evolve only if each decreasing increment of male gamete size disproprionately increases likelihood of gamete fusion, and if each increasing increment of female gamete size increases zygote fitness.

The PBS theory was lucidly described by John Maynard Smith in his essay, *The Evolution of Sex* (1978), and by Graham Bell in his masterful tome, *The Masterpeice of Nature* (1982), both of which should be considered required reading for students interested in the evolution of gamete dimorphism as is also the recent masterful review by Lessells *et al*., 2009. Bell argues that increasing zygote size correlates well with increased complexity of the organism, but readily admits that this trend may not cover all cases. We are reminded of the parable of the tiny mustard seed, whose adult form shelters "the fowls of the heaven" or, at least for tropical botanists, the reality of orchid seeds, among the smallest known to science, which produce plants of remarkable beauty and complexity. So, if PBS is a foundation, but not in any sense the ultimate and final answer, what are the alternatives?

Different thinkers have proposed different ideas such as sperm limitation, paradigms based on cooperation rather than competition, diffusion mechanics, escape from viruses and parasites, and other

ecological constraints for the evolution of anisogamy. Some of the more provocative recent theories on the evolution of anisogamy, like my work with James Sethian, and subsequently Tatsuya Togashi and Former Hughes Research Lab Director John Bartelt, assume that zygote survival depends on size, which in turn is based on the sum of the sizes of the two gametes, and the search efficiency is dramatically increased by reducing the size of the male gametes. These considerations in turn suggest, as noted by George Williams (1975), that "a primeval conflict between the sexes" resulted in selection for small gametes to overcome the discriminatory mechanisms of large gametes, which was the beginning of sexual selection. Thus gamete dimorphism is likely to be a common outcome in evolutionary contests (Maire *et al.*, 2001). Other researchers have studied sperm competition, using empirical evidence from crickets that males which produce numerous small sperm win competitions for fertilization (Gage and Morrow, 2003), or that during sperm competition "males release either too little or too much sperm for females to achieve complete fertilization" (Bode and Marshall, 2007). Continuing the theme of sperm competition, Bjork and Pitnick (2006) argue that sexual selection decreases as sperm size increases, suggesting that isogamy can be approached by increasing sperm size. Yet other researchers, such as Yang (2010) or Roughgarden and Iyer (see this volume, Chapter 3), argue that competition cannot explain the evolution of anisogamy, and that cooperation among gametes is a better paradigm for understanding gamete dimorphism. Another exciting direction is provided by the genetic studies of Nozaki (2008), who finds the *MID* gene of the isogamous green alga *Chlamydomonas reinhardtii* is required to form isogametes of the minus mating type. The *MID* gene also occurs, but only in the male genome, of the colony-forming alga *Pleodorina starrii*, which is oogamous. Thus, there appears to be a direct genetic link between the minus mating type of isogamous green algae and male gametes in oogamous species. Lessells *et al.* (2009) summarize the factors favoring the evolution of small gametes and resultant gamete dimorphism as the gamete-size number trade off, increased gamete motility, and exclusion of cytoplasmic DNA. They suggest that selection for increased zygote survival and zygote size, as well as a larger target for fertilization are the primary factors favoring the evolution of large gametes in anisogamous systems.

To increase dialogue between the different camps, and to bring together the historical pioneers in this field, many of whom are still alive, a few years ago Tatsuya Togashi and I convened in Vienna

a symposium on the evolution of anisogamy. The presentations were so provocative and the exchanges so exciting, we decided to make this conversation accessible to a broader audience, hence this book.

The first chapter, by Geoff Parker, elegantly summarizes PBS and then examines its predictions against empirical evidence. He makes a convincing argument for sperm competition as a major driver of the evolution of aniosgamy. The second chapter, by Hiroyuki Matsuda and Peter Abrams, is an intensive examination of isogamy, based on their seminal paper of 1999. As they note, arguments for the relative rarity of isogamy must also consider arguments for the relative prevalence of anisogamy. In the third chapter, by Joan Roughgarden and Priya Iyer, the assumption that anisogamy evolved from the "primeval conflict between the sexes" is called into question since it ignores "the insight from the seminal group theoretic models – small gametes increase encounter rates." They instead invoke contact rates to propose an individual selection model for anisogamy. In Chapter four, Rolf Hoekstra argues that the evolution of uniparental inheritance of cytoplasmic genomes served to prevent the spread of deleterious mitochondrial genes. He suggests that the evolution of uniparental inheritance and hence anisogamy was driven by the conflict between nuclear and cytoplasmic genes. In chapter five, Kinya Nishimura and Noboru Hoshino, in a fascinating consideration of empirical data from fish, consider the effects of turbulent and non-turbulent flow to derive a model for optimal egg size. Considering predation, starvation and turbulence as major environmental factors impacting zygote fitness, they find that "small floating eggs are adaptive in environments with low biological richness, whereas large demersal eggs are adaptive in environments with high biological richness." David Dusenbery in Chapter six presents a particularly detailed examination of gamete encounter rates in broadcast spawners. He argues that "more effective gamete encounter mechanisms . . . may allow a population to survive at lower population densities," and thus argues for the efficacy of pheromones in gamete attraction. In the final chapter, my colleagues Tatsuya Togashi and John Bartelt compare the predictions arising from fertilization kinetic models to observed gamete size and behavior in a variety of marine algal species. They argue that "gamete behavior plays an important role in increasing search, encounter and fusion rates by concentrating gametes near a light source (phototaxis) or chemical attractant (chemotaxis)," but find that the relationship between "zygote size and survival is still equivocal due to the lack of experimental data."

I don't believe that any of the authors of these chapters would assert that they have the final answer, but I think all of them hope that their contributions will stimulate new thinking and provoke new ideas, particularly among students.

Professor Togashi and I express our gratitude to all of the authors, as well as to Marilyn Asay, and Cambridge University Press for their efforts in bringing this project to fruition. We also thank Yoshiko and Barbara for their patience with us as we have commuted back and forth between Japan and the USA to edit this book.

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GEOFF A. PARKER

1

The origin and maintenance of two sexes (anisogamy), and their gamete sizes by gamete competition

1.1 INTRODUCTION

It is generally assumed (e.g. Maynard Smith 1978, 1982) that ancestrally, gametes were small and isogamous (monomorphic). The evolution of anisogamy (gamete dimorphism) is a crucial transition in evolution (Maynard Smith and Szathmáry, 1995): it represents the evolution of the two sexes, males and females. Following Parker *et al.* (1972), I favor defining a sex in relation to the type of gamete a sexual phenotype carries. A sex is thus an adult phenotype defined in terms of the size of (haploid) gamete it produces: in an anisogamous population, males produce microgametes and females produce macrogametes. A simultaneous hermaphrodite is thus both male and female simultaneously, and a sequential hermaphrodite transforms sequentially from male to female (or vice versa). This definition of a sex differs from one that defines a sex in terms of gamete mating types (e.g. Wiese, 1981; Hoekstra, 1990). Under the Parker *et al.* definition of a sex in terms of gamete size, a mating type is not considered to be a sex, but simply a gametic type (that may or may not be related to gamete size) that shows a preference for fusion with certain other gamete types. In isogamous populations, there is thus one sex (though there may be several mating types). Retaining the definition of a sex for an adult phenotype that produces a given gamete size, and a mating type for a gamete phenotype that has a given characteristic for selective fusion may serve to remove some of the confusions that have arisen in the literature.

The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection, ed. Tatsuya Togashi and Paul Alan Cox. Published by Cambridge University Press.

This initial sexual asymmetry in gamete size, anisogamy, plays the central role in driving all the dramatic pre- and postcopulatory processes of Darwinian sexual selection. This divergence in the two different gamete size-producing morphs, males and females, results in the skewing of parental investment (Trivers, 1972), operational sex ratios (Emlen and Oring, 1977), potential reproductive rates (Clutton-Brock and Vincent, 1991), or whatever index is proposed to account for the intensity of sexual selection.

The present review considers the origin, maintenance, and development of anisogamy and gamete size through the selective pressures of competition between gametes for gametic fusion, and focuses mainly on the work of my colleagues and myself over the past 3–4 decades. The initial selective force of gamete competition, coupled with the evolution of increasing vegetative complexity, usually in the form of complex multicellular organisation (another major transition in evolution; Maynard Smith and Szathmáry, 1995) is argued to offer the most plausible origin of anisogamy from an ancestral isogamous, externally fertilizing state (Parker *et al.*, 1972; Bulmer and Parker, 2002). The subsequent development of internal fertilization greatly reduced the potential for sperm competition, which resulted in a reduction in ejaculate expenditure, allowing sexual selection to become intense and generating high anisogamy ratios (A = ovum cell mass/sperm cell mass, Parker, 1982; for a different definition of anisogamy ratio, see Togashi *et al.*, 2007).

An excellent survey of all theories relating to the evolution of anisogamy (including gamete competition) is given in the recent review of Lessells *et al.* (2009); theories unrelated to gamete competition are also reviewed in the present volume.

1.2 The origin of anisogamy by disruptive selection on gamete size through gamete competition (PBS theory)

Parker, Baker, and Smith (1972) proposed a theory (often referred to as the PBS theory or model, e.g. Bell, 1978, Lessells *et al.*, 2009) for the evolution of anisogamy and two sexes by gamete competition, based on disruptive selection on individuals varying in the size of gamete they produced. The essence of our theory, summarized early in the 1972 paper, relates to two simple assumptions.

> Two very fundamental pressures immediately appear obvious; both would be related to gamete size and would act in opposition. These are

> *numerical productivity* (i.e. the number of gametes produced in unit time by a given parent) and *zygote fitness* (i.e. a measure of the probability that a zygote will survive to reach adulthood and reproduce, and in the shortest time).

We pointed out that, all else equal, in an ancestral population where gametes are shed into the sea, adults would have maximum fitness by producing the maximum number of gametes capable of carrying the haploid chromosomes and surviving to fuse with another gamete, but that there would not be a drive to produce maximum gamete numbers "if parents producing fewer but larger gametes experienced a compensating advantage because of the greater fitness of their offspring" (PBS).

To investigate the implications of these two very simple assumptions about gamete productivity and zygote provisioning, PBS examined a computer simulation with the following more detailed assumptions: (1) there is an ancestral isogamous population with wide variation in the mass, m, of the gametes produced, (2) each individual has the same fixed resources, M, for allocation to gametes, so that there is a direct trade-off between gamete size and number, n, with $n = M/m$, (3) gamete size is controlled by alleles at a single locus, with either diploid control ("small-producing" dominant over "large-producing", or vice versa in different simulations) or haploid control (by the allele carried by the gamete, or by the haploid parent), (4) gametes are released simultaneously by individuals into an external medium (envisaged to be the sea), and fusion between all gametes is random, (5) the size (mass), S, of a zygote is the sum of the masses of the two fusing gametes, i.e. $S_{ij} = m_i + m_j$, and (6) the "fitness", f, of a zygote is an increasing function of zygote mass (f is most simply viewed as survival to reproduction, but includes all aspects related to reproductive success).

The PBS simulations used an explicit function for zygote fitness, namely that $f(S) = aS_{ij}{}^x$, where a is a proportionality constant. Our results, confirmed by further simulations (Parker 1978) to correct an error in the original computations, were as follows. Whatever the genetic system (haploid or diploid control), low values for exponent x caused fixation of the allele for producing the smallest gametes (isogamy with microgametes). When x became sufficiently high, there was a stable polymorphism of alleles for producing the smallest (microgametes) and the largest (macrogametes); all other alleles became lost from the population. At even higher x, the result was fixation of the allele for producing the largest gametes (isogamy with macrogametes). The range of exponent x over which anisogamy is stable depended on the range of gamete sizes

present in the population; as x increased, the range of x allowing anisogamy increased (examples of the simulations are shown in Figures 1.1a, 1.1b). Further, if (i) there is a vast range of gamete sizes in the ancestral population, (ii) $x > 1$, and (iii) $f(2\delta) \to 0$, where δ = the minimum size for a gamete to survive, the surviving adults are approximately equal numbers of two genotypes, one that produces microgametes (proto-sperm producers) and one that produces macrogametes (proto-ovum producers).

The interplay between exponent x (which defines how steeply zygote fitness increases with zygote size) and the ratio of maximum to minimum gamete sizes is thus critical in determining which of the three solutions (i.e. microgamete isogamy, anisogamy, or macrogamete isogamy) is achieved in the ancestral population. The PBS simulations (Parker 1978) suggested that with a very wide range of gamete sizes, the critical exponent x value was 1.0 (i.e. linearity) for the threshold between microgamete isogamy ($x < 1$) and anisogamy ($x > 1$).

Analytical treatments using population genetics approaches (Charlesworth, 1978; Bell, 1978) supported the PBS results. For haploid control of gamete size (the dominant phase of the life cycle for many green algae is the gametophyte which is haploid) they showed that, where θ = the ratio of minimum/maximum gamete size ($\theta = m_{\min}/m_{\max} = n_{\max}/n_{\min}, 0 < \theta < 1$), the range of x for stable anisogamy is obtainable from:

$$2^{-x}\theta^{1-x}(1+\theta)^{x} > 1, \tag{1.1a}$$

$$2^{-x}\theta^{-x}(1+\theta)^{x} > 1. \tag{1.1b}$$

(Figure 1.1c). They also derived the equilibrium allele or genotype frequencies, confirming for both haploid and diploid cases that the ratio of microgamete producers to macrogamete producers approaches equality (a unity sex ratio) as the disparity in size between the two gamete-producing morphs becomes very great.

PBS also discussed their general assumptions about gamete productivity and zygote provisioning in terms of a general and more plausible form for $f(S)$ than the power function $f(S) = aS_{ij}^{x}$, which was used for heuristic purposes in their simulations. They argued that the most plausible form for $f(S)$ would be one in which $f(S) = 0$ up to some minimum S, after which $f(S)$ would rise steeply with decreasing slope. They argued that in complex multicellular organisms, whatever the starting conditions, selection would drive towards conditions favoring anisogamy, and that simple unicellular organisms were, however, more likely to remain isogamous (see Section 1.2.5).

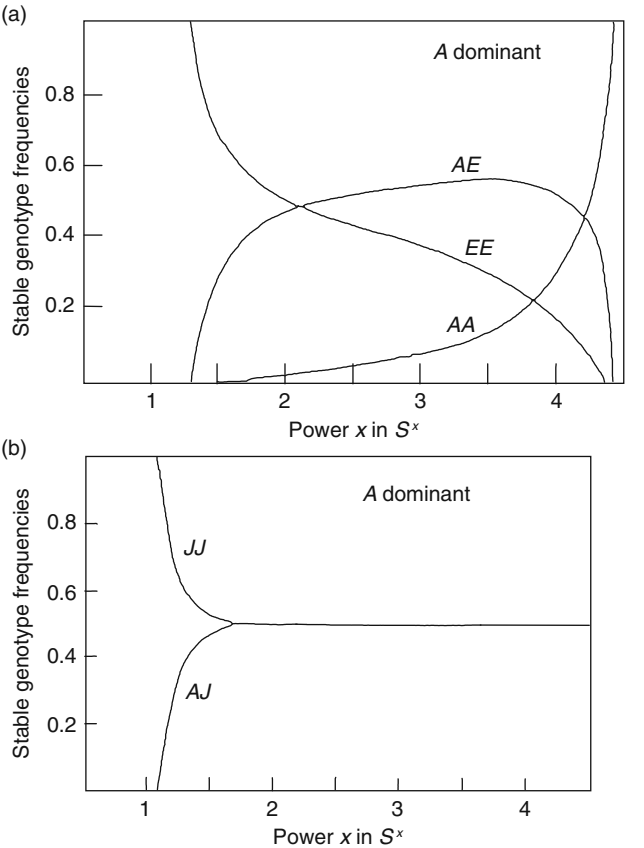

Figure 1.1 Isogamy and anisogamy in the PBS model. (a) and (b) (from Parker 1978) show the stable genotype frequencies attained in the PBS simulations in relation to power x in the zygote fitness function, $f(S) \propto S^x$, starting with three alleles for number of cell divisions, v, that determine gamete productivity, n, where $n = 2^v$. Alleles for low productivity (large gametes) are dominant over those for high productivity (small gametes). In simulation (a) the three alleles are A, C, E, with $v = 1, 3, 5$, giving $n = 2, 8, 32$ (dominance series A: C: E). In (b) the three alleles are A, E, J, with $v = 1, 5, 10$, giving $n = 2, 32, 1024$ (dominance series A: E: J). (c) Charlesworth's (1978) haploid conditions for isogamy and anisogamy in the PBS model. Anisogamy is stable between the upper and lower curves, which show the critical x values from conditions (1a) and (1b); θ = the ratio of minimum/maximum gamete size. Fixation of the smallest gamete size occurs below the lower curve (condition (1a)), and fixation the largest gametes size above the upper curve (condition 1b)). (Redrawn with permission from B. Charlesworth).

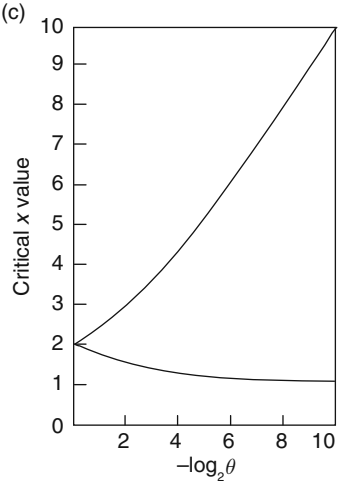

Figure 1.1 (cont.)

Maynard Smith (1978, 1982) used a graphical analysis based on game theory to investigate the basic PBS model, generating conditions for the two types of isogamy or anisogamy. However, caution is required regarding Maynard Smith's isogamous optimum, m^*, which occurs at a zygote size of $S = 2m^*$, where $m^* > \delta$, where δ is the minimum possible gamete size for survival before fusion. This solution, at which $f'(S) = 2f(S)/S$, was shown subsequently to be continuously unstable by Matsuda and Abrams (1999, see also Bulmer and Parker 2002), and also by McNamara *et al.* (2003) in the context of Parker's (1985) analogous ESS solution for biparental care. Thus although an ESS, selection will not converge to it. Maynard Smith's isogamous gamete size ESS, m^*, is nevertheless important in determining how the system behaves in relation to δ (Matsuda and Abrams, 1999; Bulmer and Parker, 2002): we expect isogamy or anisogamy depending on whether $\delta > m^*$ or $\delta < m^*$. If $0 < \delta < m^*$, the system is expected to move towards an anisogamous ESS with $m_1^* = \delta$ and $m_2^* = S^* - \delta$, where $S^* - \delta$ is solved from $f'(S) = f(S)/(S - \delta)$. But when $\delta > m^*$, the system will move to an isogamous ESS with gametes of size δ.

1.2.1 Mating types and the PBS theory

The PBS model was based on random fusion between all gametes. A central problem discussed by PBS and later investigated in detail by Parker (1978), again by computer simulation, was why disassortative fusion for gamete size evolved, in which microgametes fuse only with

macrogametes: essentially, why should proto-sperm and proto-ova become mating types, rather than fuse randomly? This question assumes that disassortative fusion arises under what has been defined as *pseudoanisogamy* (see Scudo 1967, Bell 1982, Lessells 2009), i.e. a state where there is gamete dimorphism but no mating types – or if there are mating types, these fuse independently of size. Several other authors have modelled the evolution of anisogamy starting from ancestral populations without mating types (e.g. Charlesworth, 1978; Bell, 1978; Cox and Sethian, 1985). An alternative possibility is that the prior existence of mating types in the ancestral population played a seminal role in the evolution of anisogamy, a possibility that was first investigated by Charlesworth (1978).

1.2.1.1 *Evolution of disassortative fusion from anisogamy without mating types*

PBS (see also Parker, 1978) used the following argument to account for the evolution of disassortative fusions. Assuming that there is no risk of remaining unfertilised, sperm-producers would be favored if they produced sperm that avoided sperm–sperm fusions and fused selectively with ova. However, ovum-producers would also do better by producing gametes that fuse selectively with other ova. There thus arises a "primordial sexual conflict." PBS argued that sperm producers were likely to "win" this conflict for two reasons: (1) there will be a higher adaptation rate in sperm. The mutation rate is likely to be proportional to the number of gametes produced, and will therefore differ for the two sexes. Sperm producers would have experienced a higher incidence of mutants favoring sperm–ovum fusions than ovum-producers experienced to prevent such fusions, (2) sperm-producers were likely be under stronger selection to avoid sperm–sperm fusions and to favor sperm–ovum fusions, than ovum-producers are to avoid sperm–ovum fusions, especially if sperm–sperm fusions are almost lethal ($f(2\delta) << f(m_2 + \delta)$) and the fitness function $f(S)$ is increasing with decreasing slope (ovum–ovum fusions thus have less than the twice the fitness of sperm–ovum fusions even if $\delta \to 0; f(2m_2) < 2f(m_2 + \delta)$).

PBS argued that even if assortatively fusing ova "won" so that the existing sperm producers became extinct, the system would not be stable: the resulting isogamous population would experience new drives of sperm-producers. In the absence of sperm, the advantage in maintaining antisperm selectivity would be lost and may erode. A mutation for gamete size reduction in one of the existing ovum producers could probably invade with or without this erosion, because it

would possess surface characteristics of the ovum mating type. PBS likened the sperm–ovum relationship to that between parasite and host, with parasitic sperm-producers dependent upon and propagating at the expense of the host ovum-producers.

Parker (1978) investigated the fate of various mutant mating types with size-selective fusion in an anisogamous population with random fusion to examine a further possibility: that mutant assortatively fusing ova (i.e. ova that fuse only with other ova) may fail to encounter other ova, especially when there is: (i) sperm competition and (ii) a time limit for fusion, after which the gamete dies, or cannot fuse for some other reason (for an alternative perspective on size-selective fusion relating to gamete encounters, see Cox and Sethian, 1985). A phenotypic (non-genetic) model considered the fitness of various mutant strategies for selective fusion, each occurring in males and females with equal probability, in a randomly fusing population. Defining the gamete mating type behavior as A = assortative (i.e. fusion with the same gamete size), D = disassortative (i.e. fusion with the other gamete size), and R = random, and using subscripts O for ova and S for sperm, the various strategies become, for example, A_OA_S (the parent produces ova or sperm that fuse assortatively), A_OD_S (the parent produces ova that fuse assortatively and sperm that fuse disassortatively), etc. The population strategy is R_OR_S (random fusion by both gamete types). If the fitness of a zygote arising from an ovum–ovum fusion is standardized as 1, then following the original PBS formulation for $f(S) \propto S^x$, the products of ovum–sperm fusion have fitness $b = (0.5+0.5/n)^x$, and sperm–sperm fusions $c = (1/n)^x$. If all gametes can fuse without difficulty, A_OD_S will obviously be the best strategy ($1 > b > c$). A_OR_S will always invade R_OR_S, and A_OA_S and D_OD_S will sometimes invade, depending on n and x. Strategies D_OR_S, D_OA_S and R_OA_S can be discounted – their fitness can never exceed that of the parental population, R_OR_S.

Whether selection favors selective fusion, and if so, what form of selective fusion, depends on the risk of a gamete's failure to achieve fusion before the gamete dies. In Parker's (1978) model, fertilization of mutant gametes occurred successively during P fertilization time intervals or steps; P can be regarded as a gamete survival time. If the probability of fusion in a given step is T (a measure of the "aptitude for fusion"; Scudo 1967), the probability of not being fused by step P is $(1–T)^P$. The best strategy depended on T and the number of steps (Figure 1.2 shows some results with $T = 0.1$ and $x = 2$). If gametes can survive only one step, random fusion (R_OR_S) was always best, because any mutant selective fusion strategy lost all gametes that "refused" to fuse. But if gametes

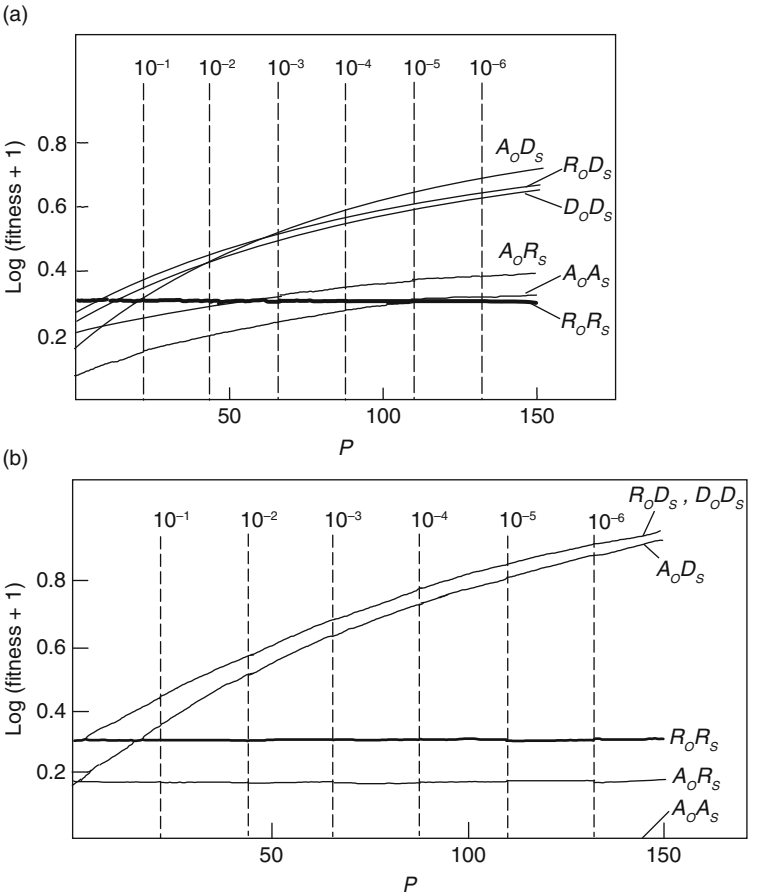

Figure 1.2 Comparison of fitnesses of rare mutant selective fusion strategies after P steps in a fertilization process with the fitness of the population strategy in which both ova and sperm show random fusion (R_OR_S). The broken vertical lines indicate the proportion of R_OR_S gametes that remain unfused at step P; different P values can be regarded as different gamete lifetimes. At low P, random fusion (R_OR_S) always has the highest fitness. A = assortative (i.e. fusion with the same gamete size), D = disassortative (i.e. fusion with the other gamete size), and R = random; subscript O = ova and S = sperm. The proportion of gametes fusing per step is $T = 0.1$, and power $x = 2$. (a) Low anisogamy ratio, with number of sperm per ovum = $n = 10$. (b) High anisogamy ratio, with number of sperm per ovum = $n = 10^5$. With permission from Parker (1978).

could survive several steps, mutant selective fusion strategies were able to invade (Figure 1.2); the most advantageous selective fusion strategy depended on the degree of anisogamy. Thus at low levels of anisogamy (10^1, Figure 1.2a), the first strategy to beat R_OR_S was R_OR_D, followed by D_OD_S, and then A_OD_S (by step P = 19). With T = 0.1, by step P = 22, proportion 10^{-1} of the gamete pool remained unfertilized. As P increased, the fitnesses of the other viable selectively fusing strategies A_OD_S, A_OR_S, and eventually A_OA_S had all overtaken that of R_OR_S.

At high anisogamy ratios (10^5, Figure 1.2b), the fitness of strategies lacking D_S sperm is very small because of the low viability and high probability of sperm–sperm unions. Thus R_OD_S and D_OD_S are favored if they survive only two steps, and A_OD_S beats R_OR_S by P = 17. A_OD_S eventually beats R_OD_S and D_OD_S, but only when almost all of them have fused, requiring a $P > 1020$. Thus if there is simultaneous spawning, to be successful at high anisogamy ratios, A_OD_S mutants must rely on selfing of A_O-ova. They are outcompeted for ova by R_S sperm.

Thus the apparent advantage of A_OD_S over A_RD_S (representing the primordial sexual conflict) can be lost if there is a high anisogamy ratio before the A_O mutant arises from A_R, and if there is a sufficient risk of gamete death before fusion. If randomly fusing gametes are used up quickly in the gamete pool relative to mutant selectively fusing gametes, A_O ova may die before being able to fuse, or be forced to fuse with other mutant A_O ova from the same parent, producing inbred zygotes comparable to the products of apomixis or parthenogenesis. However, disassortatively fusing sperm, D_S, generally have a high advantage when anisogamy would be favored under random fusion. Genetic simulations involving diploid control two loci, one with alleles for fusion behavior and the other with alleles for gamete size, with $T = 0.1$ and $P = 100$, were used to confirm these conclusions. Without sex limitation, fusion genes code for selective or random fusion independently of gamete size produced (i.e. for strategies D_{OS}, A_{OS}, or R_{OS}). This simulation yielded near fixation of disassortative fusion genes (for D_{OS}) in conjunction with stable anisogamy, provided the anisogamy ratio was reasonably high. But because of the polymorphism, random-fusion genes (for R_{OS}) were not entirely eliminated unless n was very high. Simulations with various sex-limited fusion genes showed that genes for the non-limited disassortative fusion strategy, i.e. D_{OS}, won at high anisogamy ratios against all strategies but the one which played the sex-limited strategy, R_OD_S, but at high anisogamy ratios it has only a very small advantage over non-limited disassortative fusion (D_{OS}). I argued that the reasons for the establishment of non-limited disassortative fusion are probably related to avoiding

selfing, and to the cost of maintaining random fusion in ova (in terms of motility, etc.) outweighing the benefits of becoming obligatorily disassortative, e.g. non-motile (Parker, 1978). With asynchrony of spawning, disassortative fusion alleles may do even better than with perfect synchrony, because the probability of remaining unfertilized decreases.

The view of the evolution of anisogamy and disassortative fusion as being the primordial sexual conflict has been attacked by Iyer and Roughgarden (2008), following the line of the "social-selection program," purporting that Darwinian sexual selection is fallacious and that sexual cooperation operates rather than sexual conflict (Roughgarden *et al.*, 2006). They state: ". . . that contradictory to Parker *et al.*'s claim (1972), proto-ova may actually gain from fusing with proto-sperm as compared to selectively fusing with other proto-ova." The purpose of my 1978 paper was exactly to demonstrate that assortatively fusing ova can be selected against if the risk of gamete death before fusion is sufficiently high (Figure 1.2); Iyer and Roughgarden fail to mention that if the risks of gamete death before fusion are *not* sufficiently high, there is sexual conflict because assortatively fusing ova will indeed do best, as my paper showed (Parker, 1978). (In the original PBS model, all gametes fused, so the PBS model was correct to stress that our scenario would favor assortatively fusing ova; Parker (1978) set out to examine what happened when gametes do *not* always manage to fuse.)

Sexual conflict implies a difference between optima for the sexes (see Parker, 1979; Parker, 2006 and other articles in the same volume; Arnqvist and Rowe, 2005); conditions for conflict cannot be deduced by comparison of two separate and different hypothetical populations. For reasons that are therefore entirely unclear, Iyer and Roughgarden (2008) attempt to show that anisogamy does not involve conflict by comparing the fitness of a "female" individual in an isogamous "male" population all producing "egg-size sperm," with that of a similar female in a true male population producing microgametes. The important point for conflict is simply that in a given population, an ovum will do best to fuse randomly if fusion rates are sufficiently low, or to reject fusions with sperm and to accept those with ova if fusion rates are sufficiently high, depending on the risk that an ovum may fail to fuse before it dies (Parker 1978). Iyer and Roughgarden appear to have misunderstood that primordial sexual conflict is *not* in the evolution of (pseudo-) anisogamy per se; it is the conflict arising in an anisogamous population during the evolution of size-disassortative fusion, relating to how much investment proto-sperm and proto-ova receive from the gametes they fuse with.

1.2.1.2 *Evolution of disassortative fusion and anisogamy from ancestral mating types*

However, did disassortative fusion arise as a result of anisogamy (Parker, 1978), or did mating types evolve before the evolution of anisogamy, and act as the initial asymmetry under which anisogamy arises (Charlesworth, 1978)? It seems very likely that mating types arose before gamete dimorphism, since they appear to occur in all forms, isogamous or anisogamous (Wiese *et al.*, 1979; Wiese, 1981; Maynard Smith, 1982; Czárán and Hoekstra, 2004). The evolution of mating types involves reduction in the number of suitable gametes available for fusion (Iwasa and Sasaki, 1987), so this cost must be more than offset by the fitness advantage to a mutant with some form of selective fusion. This cost to the mutant is initially small in isogamous populations (because it can fuse with any other gamete), but the cost increases as the mutation spreads (Iwasa and Sasaki, 1987; Hurst, 1996). In isogamous populations, several mating types may thus spread, and indeed are often found (Hurst, 1996). However, in anisogamous populations, a third gamete size mating type is unable to spread or persist, and a mating type that fuses independently of gamete size is likely to face problems for various reasons (e.g. Parker, 1978; Hoekstra, 1987). A review of the evolution of mating types is given by Lessells *et al.* (2009).

Many approaches (e.g. Charlesworth, 1978; Maynard Smith, 1978; Bulmer, 1994; Bulmer and Parker, 2002) assume that mating types act as the initial asymmetry for the evolution of anisogamy. Using a haploid model (the normal mode of genetic control of gamete size in the Volvocales, Wiese, 1976), Charlesworth (1978) investigated population genetics models with two alleles, A_1, and A_2, at the gamete size locus and two mating-type alleles, M_1, and M_2. He showed that if linkage is sufficiently tight, linkage disequilibrium tends to build up, so that the gamete-size alleles become associated with different mating types, leading to a situation in which there is disassortative fusion with respect to gamete size. The pre-existence of mating types did not affect the conditions for the evolution of anisogamy. Further, selection favors closer linkage between the two loci, even when linkage is initially so loose that the mating-type alleles are equally frequent in microgametes and macrogametes (see also Hoekstra, 1984). Charlesworth plausibly argues that genes coding for size-assortative fusion (as in Parker's model 1978) are a less likely origin of disassortative fusion than an origin via mating-type loci, which are frequently found in isogamous green algae (Lewin, 1976), although there is the initial problem as to how genes on separate

chromosomes could become linked on the same chromosome, if this were not the case originally. Subsequent models of anisogamy have tended to assume close linkage between the mating-type locus and the locus controlling gamete size, or a tight association between gamete size and mating type if the model is phenotypic rather than genetic.

While it seems likely that mating types preceded the evolution of anisogamy and could become closely linked with a gamete-size locus, the problem of explaining conflict concerning selective fusions still remains. Maynard Smith (1982) makes the point that were linkages between the mating-type locus and the gamete-size locus to be selected against, e.g. in the case of sexual conflict outlined by Parker (1978), in which ova may do better to fuse with other ova than with sperm, the fact that mating types may have arisen before anisogamy does not mean that this initial mating-type behavior must be maintained, ensuring disassortative fusion. Any mutant breaking the + and − mating-type rule could spread if favored by selection; an example could be an ovum producer with a mutation that allowed ova to fuse with other ova, despite being of the same mating type. Thus consideration of the evolution of selective fusion in hypothetical conditions of pseudo-anisogamy (random fusions for gamete size with or without mating types) seem important, even if the ancestral population involved mating types. Also, as Charlesworth (1978) points out, in an ancestral species where gamete size is determined non-genetically (see e.g. Wiese, 1981), size disassortative fusion obviously could not evolve by associations between mating-type alleles and gamete-size alleles, but could arise by a mechanism similar to that proposed by Parker (1978).

1.2.2 What determines whether the ESS is isogamy or anisogamy?

An important study by Matsuda and Abrams (1999) discussed the conditions favoring the evolution of the size of isogametes in a population with two mating types, and why isogamy should be relatively rare. Their model followed that of PBS but also included a "mating success" function defining the success of a gamete at fusing with other gametes. They showed that if size is not closely linked to mating type, isogamy can be stable under a wide range of conditions. However, when size is linked to mating type, isogamy is stable only if there are significant direct effects of size on gamete survival and fusion success; even then, isogamy may only be locally stable. They concluded that isogametes larger than the minimum possible gamete size are likely to be

explained by direct effects of size on gamete survival before fusion, rather than by particular forms of the zygote fitness function.

In their computer simulations, PBS used a very simple relation between zygote fitness, f, and zygote size, S, i.e. $f(S) = aS_{ij}^{x}$, though they discussed more general forms.

Randerson and Hurst (2001a) criticised PBS, claiming: (i) that disruptive selection does not lead to anisogamy unless the slope of $f(S)$ is accelerating at the origin (i.e. $x > 1$) and (ii) that this is an unusual assumption for which there is no empirical evidence. In fact, both these claims are incorrect (Bulmer *et al.* 2002). They incorrectly developed the model of Levitan (2000; see also Vance, 1973) used empirically for zygote survival in echinoids, leading to the erroneous conclusion that $f(S)$ under this model is zero below some finite size, above which it is decelerating. They then claimed that anisogamy could not evolve in this case by disruptive selection following PBS. Analyzed correctly, Levitan's (2000) model actually generates a form for $f(S)$ that is accelerating at the origin. However, Randerson and Hurst's claim is incorrect even if $f(S)$ is zero below some finite size, above which it is decelerating, since this form is very similar to a sigmoidal function, and both can give rise to anisogamy (Bulmer *et al.*, 2002).

In response to Bulmer *et al.* (2002), Randerson and Hurst (2002) abandoned their earlier conclusion (Randerson and Hurst, 2001a) that disruptive selection never gives rise to anisogamy under plausible assumptions about the form of $f(S)$, and instead claimed the opposite: that, since a biologically plausible model must assume some finite size below which zygote fitness is zero, all logically consistent conditions appear to lead to anisogamy; PBS could not therefore account for the stable maintenance of isogamy. The flaw in Randerson and Hurst's (2002) argument is that it ignores the fact that if there is a size limit below which zygote fitness is zero, there must also be a size limit below which gamete fitness is zero (Bulmer and Parker, 2002). They nevertheless stimulated an important development in PBS theory, relating to the evolution of isogamy versus anisogamy.

Bulmer and Parker (2002) responded to Randerson and Hurst (2001b) by re-examining the PBS theory, starting from an isogamous population with two mating types (+ with gamete size m_1, and – with size m_2) obeying the PBS size–number trade off (each individual produces a number of gametes, $n = M/m$). They used an evolutionary game-theory approach to determine the existence and continuous stability of isogamous and anisogamous strategies for the two mating types. In addition to the PBS relationship, $f(S)$, between the fitness of a

zygote and its size, $S = m_1 + m_2$, they added the relationship, $g(m)$, between the reproductive fitness of a gamete and its size, m, which is a simpler version of Matsuda and Abram's (1999) "mating success" function. The fitness of + individuals is therefore

$$w_1(m_1, m_2) = \frac{Mg_1(m_1)}{m_1} f(m_1 + m_2),$$

and the reproductive fitness $w_2(m_1, m_2)$ is analogous for – individuals.

Bulmer and Parker (2002) analyzed various forms for $g(m)$ and $f(S)$, including a sigmoidal form based on Vance (1973) and Levitan (2000), and an exponentially diminishing-returns function which is zero below some finite size, above which it is decelerating continuously. The general conclusions for the evolution of isogamy and anisogamy were rather similar for these forms, but the details differ.

For the sigmoidal form (based on Vance 1973), the gamete and zygote survival (or other aspects of success) functions are

$$g(m) = \exp\left(-\frac{\alpha}{m}\right), \tag{1.2a}$$

$$f(S) = \exp\left(-\frac{\beta}{S}\right), \tag{1.2b}$$

where α, β, are positive parameters. This has an isogamous ESS at

$$m^* = \alpha + \beta/4, \tag{1.2c}$$

but to test whether this is continuously stable (i.e. it converges back to the ESS after a small perturbations; see Eshel 1983) requires calculation of the best response (m_1) of an individual of the + mating type to the strategy m_2 played by the – mating type. As m_2 varies, so will the best response, m_1, and we can call the function describing the best response $R(m_2)$. Since the best response to a high m_2 is a low m_1, and vice versa, $R(m_2)$ has a negative slope. If this slope, $R'(m)$, is between –1 and 0, there is continuous stability; but if $R'(m)$ is less than –1, the ESS is continuously unstable. This can be demonstrated in Figure 1.3a–c, which gives results for the Vance functions (1.2a) and (1.2b), by using the "cobweb" technique (Sandefur, 1990). This technique can be explained as follows. Taking a given m value on the broken line in each of Figs. 1.3a–c, the intersect on the curve (above or below it) gives the best reply, $R(m)$. This m value can be extrapolated to its equivalent new m value by finding its intersect, horizontally left or right, on the broken line. For this new m, we can obtain a new $R(m)$, and so on to

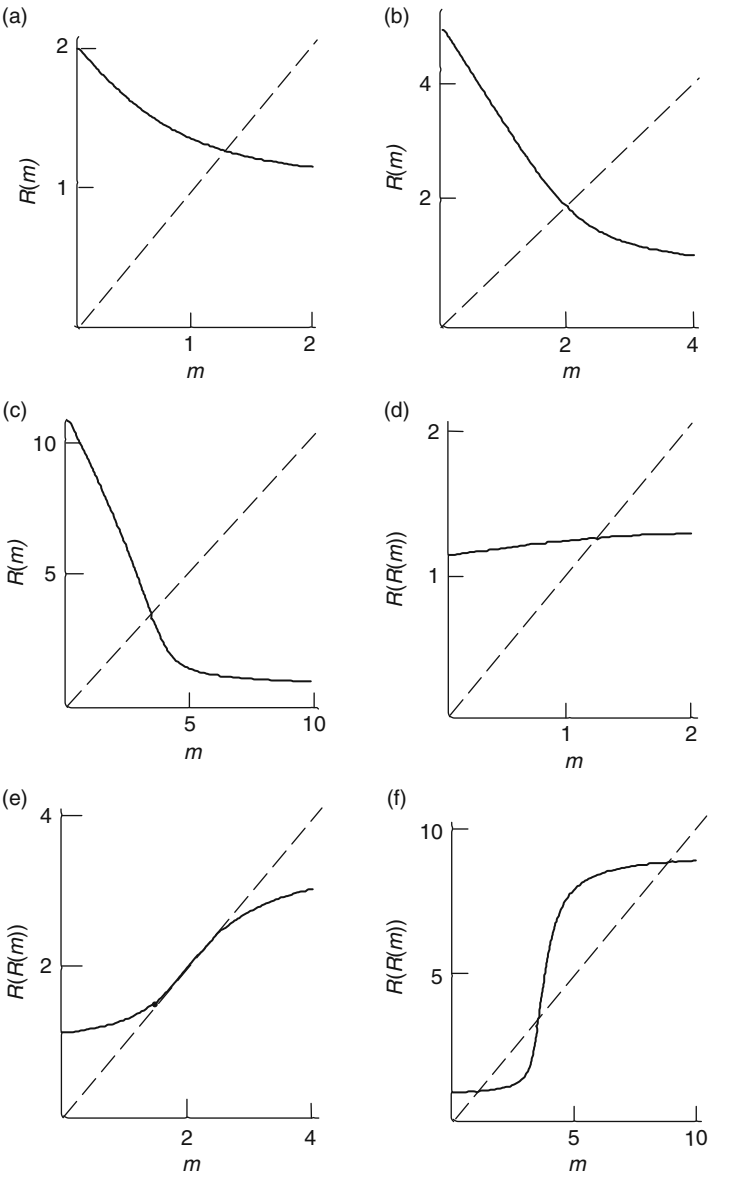

Figure 1.3 Isogamy and anisogamy in the Bulmer and Parker (2002) version of PBS. (a–c) The best response function, $R(m)$, and (d–e) the best response to the best response, $R(R(m))$, using functions (2a) with $\alpha = 1$, and (2b). In (a) and (d), $\beta = 1$. (a) There is a continuously stable isogamous ESS at (1.25, 1.25) because the slope $R'(m) > -1$ at this point; (d) shows that there is no anisogamous ESS. In (b) and (e), $\beta = 4$. (b) There is an

produce a "cobweb." If $R'(m) > -1$ at the ESS (under-compensation; Figure 1.3a), the cobweb converges towards the intersection of the curve and the broken line, which is therefore a stable equilibrium. If $R'(m) > -1$ (over-compensation; Figure 1.3c), the cobweb diverges away from the intersection, which is an unstable equilibrium.

At the isogamous ESS in (1.2c), the slope is

$$R'(m) = -\beta/4\alpha. \tag{1.2d}$$

This is greater than -1 when $\beta < 4\alpha$, as in Figure 1.3a. However, when $\beta > 4\alpha$, the slope is less than -1 (Figure 1.3c), and the isogamous ESS in (1.2c) is not continuously stable. This leads to an anisogamous ESS, which can be found by plotting the "iterated best response function" or "best response to the best response," $R(R(m))$, i.e. the function describing the best responses of an individual of the + mating type to the best responses of the – mating type (Figure 1.3d–f). The anisogamous ESS is a pair of strategies, m_1^* for + and m_2^* for -, which can be found by the points, other than the isogamous ESS, at which $R(R(m))$ intersects the 45° line through the origin (Figure 1.3d–f). There is no anisogamous ESS when the isogamous ESS is continuously stable (Figure 1.3d), but an anisogamous ESS arises when the isogamous ESS is continuously unstable (Figure 1.3f). Figure 1.3b (for $R(m)$) and Figure 1.3e (for $R(R(m))$) relate to the case where β = 4, so that $R'(m) = -1$, i.e. intermediate between isogamy and anisogamy.

In unicellular organisms, Bulmer and Parker (2002) argued that the two survival functions, (1.2a) and (1.2b), are rather similar, so that $\alpha \approx \beta$, resulting in isogamy. As multicellularity began to evolve, α probably remained roughly constant, but β would have increased with the need to provision the embryo. At the stage where β had increased more than fourfold, anisogamy should have replaced the ancestral isogamous state. Thus we would expect a sudden transition from isogamy during the evolution of increasing vegetative complexity (usually multicellularity) as the gamete survival function $g(m)$ and the zygote survival function $f(S)$ separated along the provisioning axis (Figure 1.4).

Caption for Figure 1.3 (cont.)
isogamous ESS at (2, 2) which verges on continuous instability since at this point $R'(m) = -1$; (e) an anisogamous ESS is incipient. In (c) and (f), β = 10. There is a continuously unstable isogamous ESS at (3.5, 3.5) where the slope $R'(m) < -1$; (f) there is an anisogamous ESS at (1.13, 8.87). With permission from Bulmer and Parker (2002).

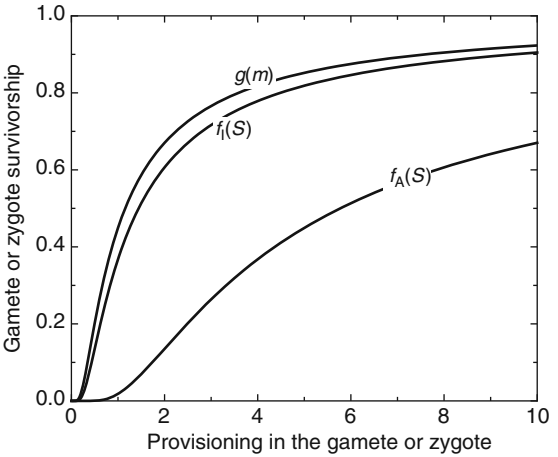

Figure 1.4 Isogamy and anisogamy when there are separate gamete survival and zygote survival functions, $g(m)$ and $f(S)$, in the Bulmer and Parker (2002) model following the Vance function (Equations (1.2a) and (1.2b)). In the ancestral state, the curves for $g(m)$ and $f_I(S)$ are close together and isogamy is stable (shown here with $\alpha = 0.8$ and $\beta = 1.0$). As multicellularity develops, the gamete function $g(m)$ probably remains relatively unchanged in its position on the provisioning axis, but the need to provision the zygote increases, pushing $f(S)$to the right, leading to anisogamy if $\beta > 4\alpha$. In the case shown, $f_A(S)$ has $\beta = 5.0$, which would generate anisogamy.

Bulmer and Parker (2002) also investigated cases (including a modified version of the Vance function) where there is a critical minimum gamete size, δ, below which the gamete dies before fertilization (e.g. Maynard Smith 1978; Bulmer, 1994), and concave functions where $g(m)$ and $f(S)$ are initially zero, e.g. up to δ, and then increase from zero with decreasing slope (δ could be seen as the component due to the chromosomes, and $m - \delta$ the energy reserves that increase survival). These all behaved qualitatively rather similarly with regard to the stability of isogamy and anisogamy, and in each case it was the relationship between the gamete and zygote survival functions that determined whether or not anisogamy evolves.

Thus Bulmer and Parker (2002) have shown that in the ancestral unicellular state, isogamy is likely to be stable because the gamete and zygote survival functions ($g(m)$ and $f(S)$) are likely to have been similar; this leads to isogamy whether these functions are sigmoidal or concave, though in the latter case allowance must be made for a minimal

gamete size. The transition to multicellularity probably left $g(m)$ relatively unchanged while $f(S)$ moved to the right along the provisioning axis, leading to the evolution of anisogamy. The origin of anisogamy is tightly coupled with the evolution of the gamete and zygote survival functions, $g_1(m_1)$ for sperm, $g_2(m_2)$ for ova, and $f(S)$ for zygotes, during their separation during this transition. The theoretical biology of these functions becomes the key to understanding the evolution of the sexes, and would greatly merit further study. The specialisation of micro- and macrogamete sizes after the development of anisogamy are discussed on p. 00–00.

Bonsall (2006) has extended Bulmer and Parker's approach (2002) by developing Equations (1.2a) and (1.2b) to include variability in survival through differential mortality or metabolic damage of gametes or zygotes as a function of their size, and by including a distribution in the number of gametes (or zygotes), $n(T)$, produced by a parent due to mortality over lifespan T. In addition to the classic PBS mechanism, Bonsall concludes that anisogamy can evolve through the effects of variability in mortality or distributed metabolic costs acting on isogamous gametes, by inducing disruptive selection on isogamous gametes favoring individuals that produce smaller gametes to compensate for the costs associated with metabolic damage. Thus in Bonsall's rather complex models, different mechanisms associated with survival disrupt the isogamous state and also affect the evolution of anisogamy; he concludes that the persistence of both the isogamous and anisogamous ESS points is not always assured and calls for a more pleuralistic approach.

1.2.3 The ancestral isogamous state – which came first, smaller or larger gametes?

The early studies of PBS, Charlesworth (1978) and Bell (1978) suggested that anisogamy can evolve only if there is sufficient size variation in the ancestral population to generate it (see Figure 1.1), or if gamete size mutations have sufficiently large effect (but see Maire *et al.*, 2002). PBS argued that whatever gamete size the ancestral isogamous population showed, selection may generally tend to push gamete size towards a zone of the zygote fitness:size relationship, $f(S)$,where anisogamy will occur, depending on the $f(S)$ relation for the ancestral state. PBS envisaged two scenarios: in the first, there is a general reduction in gamete size starting from isogamous macrogametes that then allows invasion by microgametes (PBS, Figure 1.9), and in the second, there is invasion

by macrogametes into an ancestral population of isogamous microgametes, following by a subsequent reduction in the size of the microgametes (PBS, Figure 1.10). The latter scenario is probably the more likely and was proposed by Maynard Smith (1978), who argued that maleness was ancestral, with macrogametes invading an isogamous microgamete (male) population.

Bulmer and Parker's (2002) analysis also suggests that the ancestral stage would have been isogamous unicells (microgametes). As the size of the adult increased (e.g. through the evolution of muticellularity), Bulmer and Parker predicted that $f(S)$ would increase away from its early position near $g(m)$, and during this stage, the isogamous microgamete size would initially have increased. At some threshold in this shift towards larger isogametes, isogamy becomes unstable, and is suddenly replaced by anisogamy through invasion of macrogametes (see Figures 1.3 and 1.4). As increasing adult complexity causes $f(S)$ to move further away from $g(m)$, macrogamete size increases and microgamete size decreases (compare Figures 1.3d, 1.3e, 1.3f) in a manner very similar to that depicted in Figure 10 of Parker *et al.* (1972).

1.2.4 Requirements for PBS theory

The original PBS simulation model made specific assumptions, listed as (1) to (6) on p. 000. However, it is clear that the basic PBS theory, that isogamy and anisogamy can be explained by gamete competition under the two general selective forces of gamete productivity and zygote provisioning, is correct even when most of their detailed assumptions (see p. 000) are relaxed. The initial studies with random gamete fusion (PBS, Charlesworth, 1978) suggested that sufficient genetic variation must exist in the size of gametes produced in the ancestral isogamous population (i.e. requiring mutations of large effect). However, Maire *et al.* (2002) have shown using individual based dynamic simulations that PBS requires neither mutations or large effect nor the pre-existence of mating types; gamete dimorphism can evolve through arbitrarily small mutations in a population without mating types through a mechanism based on evolutionary branching in allele space. It is unlikely that an exact trade-off between gamete size and number must apply, though there must be a sufficiently significant negative relation between size and number. The exact nature of the genetic determination of size and number need not affect the ESS, which can be derived by haploid or diploid models or by game theoretic, phenotypic models. Though the PBS assumption was that

ancestrally, isogametes with a wide range of size variation fused at random to generate anisogamy without mating types (pseudo-anisogamy), the PBS model is not affected if the ancestral state is initially from mating types (e.g. Charlesworth, 1978). Nor is it greatly affected by the fact that encounter rates will be functions of gamete size, though this does increase the range of zygote fitness functions that generate anisogamy (Cox and Sethian, 1984). Simultaneous shedding of gametes within local populations is also not necessary, provided that gametes from different parents compete sufficiently to generate some gamete competition. The theory remains robust even when gamete competition is reduced because of such biological features as internal fertilization, and gamete competition through sperm or pollen competition can help to explain why anisogamy is maintained so ubiquitously in present-day multicells (Parker, 1982). Given that there must logically be some gamete size–number trade-off in an ancestral species in which reproductive expenditure relates mainly to gamete production, the important assumption in PBS relating to the isogamy/anisogamy dichotomy is that zygote fitness increases sigmoidally with zygote mass, or, equivalently increases with diminishing returns from a positive intercept (more detailed models of gamete collisions suggests that an even broader range of functions may allow the switch between isogamy and anisogamy, e.g. Cox and Sethian, 1984). This assumption, the basis of the Smith–Fretwell (1974) model, has considerable empirical support (see e.g. Lessells *et al.*, 2009).

PBS theory therefore appears to be very robust, mainly reliant only on the general assumptions about productivity versus provisioning, which can generate disruptive selection through gamete competition. It has been shown to be challenged only when certain special conditions apply, such as when variability in mortality or distributed metabolic costs acting on gametes are so significant that they can interfere with the basic PBS disruptive selection mechanism involving gamete competition across the productivity–provisioning axis (Bonsall 2006), or when the success of large gametes increases sufficiently as a result of sperm limitation to offset this mechanism (Dusenbery, 2000; 2002; but see Lessells *et al.*, 2009).

1.2.5 Evidence for PBS theory

The claim that a switch from isogamy to anisogamy is likely in the transition from the ancestral unicellular state to the increased complexity of multicellular forms (PBS) as the gamete and zygote survival

functions (Bulmer and Parker, 2002) become sufficiently separated (see Figure 1.4) has some support from comparative studies, particularly of volvocine algae. Knowlton (1974) was the first to note an association between increased complexity and a switch from isogamy to anisogamy in the Volvocales, and this has been supported using modern comparative methods (Randerson and Hurst, 2001a, b). In other chlorophyte algae the same correlation occurs, but is less clear (Bell, 1978). The most extensive analysis (Bell, 1982) involved many algal and protozoan groups, and demonstrated a clear correlation between increasing vegetative organization and increasing gamete dimorphism.

Randerson and Hurst's (2001b) study of the Volvocales controlled for phylogenetic effects, and showed that: (i) the anisogamy ratio (A = macro-/microgamete volume), and (ii) the macrogamete size (see also Bell, 1985), increased with adult size, though these results were sensitive to the mode of analysis and the phylogeny used. They proposed an alternative explanation of these results to PBS, based on the present mode of reproduction in volvocine algae (which may not relate to their ancestral mode of reproduction under which anisogamy evolved). In many colonial forms, male and female colonies clump together and male colonies release sperm in sperm packets; each packet swims as a unit until it reaches a female colony and the individual sperm are released. Randerson and Hurst (2001b) explain the positive correlation between anisogamy ratio and vegetative complexity in terms of the need to produce more (and hence smaller) sperm to fertilize the higher number of eggs in larger female colonies. It remains to be tested whether this is a better explanation of the association between anisogamy ratio and increased multicellularity in Volvocales; although this mode of reproduction does not involve release of separate gametes and random fertilization in the external medium (as assumed by PBS), there may well be sufficient gamete competition under this mode of reproduction to allow the PBS mechanism to operate.

In marine green algae, gametes are typically released individually in one event into the surrounding medium (Togashi *et al.*, 1998; 1999), exactly as the PBS model assumes. However, support for the PBS prediction is controversial, since isogamous species are multicellular, though often with low "vegetative complexity" (*sensu* Bell, 1978; 1982). Generally, isogamous species and slightly anisogamous species produce their gametes through synchronized cell divisions during gametogenesis, so their gametes cannot be larger than their cell size. In contrast, strongly anisogamous species often produce giant female gametes. This is possible because they are typically unicellular and

multinucleate. In certain marine green algal groups, while an increased anisogamy ratio may loosely correlate with increased morphological complexity, it does not correlate with multicellularity, and some forms are large and vegetatively complex, but retain isogamy (Bell, 1978). As with volvocine algae, brown algae generally tend to show the expected PBS correlation between vegetative size and complexity and increased anisogamy and oogamy (Bell, 1978).

Thus although increased morphological complexity is loosely associated with increased gamete dimorphism in algae (Knowlton, 1974; Bell, 1978; 1982; 1985; Randerson and Hurst, 2001b; Iyer and Roughgarden, 2008), it does not accurately predict whether a given species will show gamete monomorphism or dimorphism (Bell, 1978; Madsen and Waller, 1983). There may be number of reasons for this, relating to species ecology and other special conditions. For example, although examined in the context of the evolution of anisogamy in relation to increasing collision probability, Togashi and Cox (2004) have shown that phototaxic responses will be important, and fertilization in shallow water versus deep water can exert a major effect in explaining the variation between isogamy and anisogamy in marine algae (Togashi *et al*., 2007).

While plants and animals appear generally to match PBS predictions, fungi are less accommodating. Fungal modes of reproduction are summarized in various texts (e.g. Alexopoulos, 1962), and though fungi do not contradict the predictions, concurrence with PBS is evident only in certain groups (Bulmer and Parker, 2002). Forms showing the fusion of two naked gametes (planogametes) are closest to the assumptions of the PBS model. The simplest Chytridiomycetes are unicellular, aquatic and the entire thallus is used to produce gametes, and often have isogamous planogametes. Some *Allomyces* species have a simple thallus with specialized reproductive organs, and reproduction involves fusion of motile anisogametes. The most complex Chytridiomycetes such as *Monoblepharis* have developed more complex thalli, and have non-motile female gametes. This trend appears to follow rather closely with the PBS prediction that anisogamy should correlate with vegetative complexity.

In higher fungi, motile free-swimming gametes are absent; instead, there are various forms of transfer of gametic nuclei, some which could possibly be analogous to, or even an extension of, anisogamy under the PBS theory. For example, in spermatization, numerous minute, uninucleate male spermatia are carried by insects, wind, water, etc. to female gametangia or to unspecialized somatic hyphae,

to which they attach and transfer their contents. Other forms are not analogous to PBS, probably because the mode of reproduction has transformed from an ancestral state involving gametes to one in which there is direct transfer of nuclei between hyphae. For example, the haploid spores (basidiospores) resulting from meiosis of Basidiomycetes germinate on moist substrates to form haploid, monokaryotic, hyphae, which usually exist as multiple mating types. When two such hyphae of different mating types meet, they fuse, pass nuclei into each, and typically both mycelia become "dikaryotized" (the donor nuclei divide and migrate from cell to cell forming a dikaryon in the recipient mycelium). The present selective forces are clearly quite different from those envisaged in the PBS theory, but may have arisen from a system similar to that in Chitridiomycetes.

1.3 The Loss of Motility by Female Gametes (Oogamy)

In isogamous populations, the isogametes are typically motile to ensure encounter and fusion. PBS (see also Parker, 1979) argued that once anisogamy has arisen, the evolution of disassortative fusions of ova with sperm may be related to the advantages of loss of motility by ova. Due to the vast numerical predominance of sperm arising through the size–number trade-off at production, and through selection quickly favoring avoidance of sperm–sperm fusions, ova may fuse very quickly with sperm. Thus although loss of motility in ova may ultimately interfere with the ability of ova to fuse with other ova, a mutant that loses ovum motility may suffer little reduction in the probability of fusion of its ova, allowing energy spent on motility to be channelled into increased productivity. Thus as the anisogamy ratio increases and ovum–ovum encounters become rare, selection against disassortative fusion by ova decreases, or actively favors it (Parker, 1978), so that the best strategy for ovum producers may be total commitment to fusions with sperm, with a resulting reallocation of motility expenditure into productivity.

An analysis of gamete motility dimorphism is given by Hoekstra and his co-workers. Hoekstra *et al.* (1984) analyzed the relation between swimming speed of gametes in an isogamous population starting with equal motilities (isomotility) and gametic abilities to locate, and to be located by, other gametes. Gametes produce attractant pheromones and the volume searched by a gamete is proportional to its speed of movement. Motility dimorphism may arise in this isogamous

population via disruptive selection on swimming speed, and no more than two different swimming speeds can coexist in a stable polymorphism. An initial difference in swimming speed of at least twofold leads to a stable motility dimorphism (anisomotility), and loss of motility of one of the gamete types is likely. Hoekstra (1984) extended this model by adding the effect of anisogamy on gamete motility within the PBS framework, but assuming two initial mating types. He concluded that conditions for stable anisogamy were broadened only when the gamete size and mating type loci are closely linked. Anisogamy has no effect on the evolution of anisomotility, and anisomotility no effect on the evolution of anisogamy. Hoekstra (1984) also concluded that anisogamy can also evolve solely as a consequence of its effect on gamete motility (i.e. omitting the basic productivity-provisioning assumptions of PBS), if the size difference between the gamete types is sufficiently large. His assumption that gamete speed is inversely proportional to the gamete cross-sectional area has, however, been disputed (Dusenbery, 2000; 2002; Randerson and Hurst, 2001a).

Cox and Sethian (1985) considered the evolution of motility dimorphism within the framework of the evolution of gamete dimorphism; further models have been constructed by Dusenbery (2000, 2002); see Lessells *et al.* (2009) for a review.

Thus although gamete motility dimorphism may have arisen even before anisogamy evolved, and/or had an important influence on the evolution of anisogamy, it seems likely that macrogametes would anyhow lose their motility as the anisogamy ratio increased, generating oogamy, since the probability of fusion due the vast numbers of tiny sperm rendered the cost of maintaining motility of ova unnecessary (PBS, Parker, 1978). For sperm producers, however, the maintenance or increase of motility would be favored through sperm competition by virtue of increased encounter and ovum penetration possibilities. Many of the adaptations of sperm (e.g. involving vigorous motility, hyaluronidase secretion by the acrosome, and fast migration of the sperm pronucleus towards the ovum pronucleus) can be interpreted in terms of competition between the sperm of several parental variants (PBS).

1.4 Other theories for the origin of anisogamy and their relation to gamete competition

An excellent review of theories for the evolution of anisogamy, and their roles in the differentiation of gamete dimorphism is given by

Lessells *et al*. (2009); for an overview of the inter-relationships between the various theories see their Figure 2.1. Readers are referred to this source, and to other chapters in the present volume for alternative theories for the origin of anisogamy; I discuss them here only very briefly in relation to their impact on the evolution of anisogamy by gamete competition.

1.4.1 Classical views

The earliest analyses of the evolution of anisogamy (Kalmus, 1932; Kalmus and Smith, 1960; Scudo, 1967) were based on maximization of gamete fusions in a population, which can be achieved by division of the total resource for reproduction in a population anisogamously. Though unstated, such a fitness maximization implies group or population selection, which may operate in certain circumstances; it is nevertheless clear that the selective pressure of gamete encounter rate plays a role in the evolution of anisogamy (e.g. Cox and Sethian, 1985; Dusenbery 2000; 2006; Togashi *et al*., 2007; Iyer and Roughgarden, 2008) and is partly implicit in PBS through the fact that productivity (microgamete production) causes microgametes to dominate the fusions of macrogametes (see also Parker, 1978). However, in the original PBS model all gametes were assumed to fuse (or equivalently, gamete survival up to fusion was independent of gamete size), while with the classical theory (and the sperm limitation theory below) there is strong selective pressure to ensure gamete fusion.

1.4.2 Sperm limitation

The "sperm limitation" theory began as a modification of PBS by Cox and Sethian (1984, 1985) and generates an individual selection basis for the classical view that anisogamy relates to increasing gamete encounter rates. In broadcast spawners, infertility resulting from failure to meet another gamete can sometimes be significant (Levitan, 1993; 1996a; 1998) and is likely to select for increased encounter rates, and can be seen both as increasing the "target size" of macrogametes and a driver of anisogamy (e.g. Cox and Sethian, 1985; Levitan, 1996b; 1998; 2000; 2006; Dusenbery, 2000; 2002; 2006; Togashi and Cox, 2004). Assumptions in these models vary, for example, Dusenbery's model (2006) does not include a zygote fitness function, $f(S)$, but instead assumes that a gametes "fertile period" (longevity) increases with its to size, and encounter rates may be increased by attractant

pheromones, phototaxis and other methods. There is empirical evidence that larger-sized eggs can increase fertilization rates (Levitan, 1998; 2006). Togashi *et al*. (2007) introduced the concept of gametic investment per unit volume of the space in which gametes searched for fusion partners, an effect which may explain the prevalence of isogamous species in shallow water and anisogamous species in deep water in marine green algae. Computations show that positive phototaxis is favored, particularly in shallow water, and may increase gamete density to a level where sperm limitation might not be the dominant selective force in the evolution of isogamous or slightly anisogamous marine green algae (Togashi *et al*., 2008). It remains unclear how ubiquitous sperm limitation is as a selective pressure; while sperm limitation may have been a significant selective force ancestrally, fertilization rates in most external fertilizers are currently high (Yund, 2000).

It is important to stress that gamete competition in the form of sperm competition is still operative under sperm limitation, through the size-productivity trade-off (Lessells *et al*., 2009). There are often cheaper ways to increase encounter rates than by increasing ovum size, and Lessells *et al*. (2009) conclude that sperm limitation alone may be insufficient to explain the evolution of anisogamy, though it may well contribute (see also Randerson and Hurst, 2001a).

1.4.3 Conflicts with cytoplasmic elements

This theory originated with Cosmides and Tooby (1981), who proposed that competition between cytotypes with different compositions of cytoplasmic elements would result in selection for increased gamete size, allowing the invasion of nuclear genes coding for microgametes because of the productivity benefits arising through the size–number trade-off. An early population genetic model of this process (Hoekstra, 1987) failed to generate anisogamy, and instead generated a single cytoplasmic allele for gamete size. However, there have been many subsequent modifications of the original intracellular conflict theory for anisogamy; these are reviewed by Lessells *et al*. (2009) who conclude that this theory is unlikely to be the sole explanation for anisogamy, though it may help to maintain gamete dimorphism once this has evolved. Randerson and Hurst's models (1999) suggest that intracellular conflict is less likely to lead to gamete dimorphism in multicellular than in unicellular organisms, contrary to the empirical evidence.

It must also be noted that the two basic PBS assumptions relating to gamete productivity and zygote provisioning cannot be ignored in any theory for the evolution of gamete size; inescapably, there must be a size–number trade-off at gamete production since an adult's reproductive budget must be constrained, and zygote size must affect zygote survival/fitness. Thus although the parasitic organelle theory may be a part of the explanation, it cannot exclude it, unless it can explain anisogamy in situations where PBS would predict isogamy (or vice versa).

1.5 Stability of anisogamy under internal fertilization – why is anisogamy not lost when sperm competition is reduced?

Under PBS conditions, anisogamy arises by gamete competition, through disruptive selection against intermediate-sized gamete-producing genotypes, because of the advantages of provisioning on the one hand, and productivity on the other. Proto-ovum producers have gametes that survive well as zygotes. Proto-sperm producers are able produce so many proto-sperm that they gain most fusions with the proto-ova from proto-ovum producers: gamete competition thus generates anisogamy in ancestral externally fertilizing organisms. Gamete competition – in the sense of competition for fusions – is still present, even if there is severe gamete limitation so that the fusion probability of gametes is low.

We also need to know how anisogamy is maintained despite the many evolutionary changes subsequent to the evolution of early multicellular organisms: for example, how is anisogamy maintained when gamete competition is dramatically reduced due to the evolution of internal fertilization?

1.5.1 Sperm competition and a direct sperm size–number trade off

The PBS model for the evolution of anisogamy relies on external fertilization – fusions occur in a gametic pool, most plausible in the sea. With the onset of internal fertilization (coupled with the much earlier evolution of disassortative fusion) came a dramatic reduction in gamete competition – or more specifically, sperm competition. While PBS argued that gamete competition was responsible for the

evolutionary *origin* of anisogamy, gamete competition in the form of sperm competition (competition between the sperm of different males over the fertilization of a given set of ova; Parker, 1970) has also been claimed to be important for the *maintenance* of anisogamy (Parker, 1982).

Consider a sessile multicellular animal or plant in which groups of individuals spawn simultaneously, and all eggs get fertilized. Suppose that sperm have size δ, defined as the minimum size to carry the chromosomes and to survive until all ova are fertilized (essentially, investment δ includes no investment for zygote provisioning). If a mutant male arises that increases sperm investment to $m > \delta$, it raises the fitness f of any zygote it produces by $b(m)$, from F to $F + b(m)$, where F is the zygote fitness due only to ovum size. Suppose that the direct size–number trade-off applies – a male that increases provisioning in each sperm decreases sperm numbers, so non-mutant males produce (relatively) δ^{-1} sperm, and the mutant produces m^{-1} sperm. The fertilization success of the mutant obeys the "raffle principle" where fertilization success is proportional to proportionate representation in the pool of competing sperm (see Parker, 1998). Thus if there are N competing males, the proportion of eggs gained by the mutant male will be his contribution divided by the total sperm, i.e. $m^{-1}/[m^{-1} + (N-1)\delta^{-1}]$. For sperm to stay small, mutants that increase sperm size must not invade: mutant male fitness must decrease if m deviates above the minimum sperm size δ. Thus for δ to be locally stable, the gradient of male fitness at $m = \delta$ must be negative.

Remembering that $b(\delta) = 0$, Parker (1982) used this technique to show that δ is locally stable, if

$$b'(\delta) < F(N-1)/\delta N, \tag{1.3a}$$

where N = the number of males in the spawning group. If males do not contribute to the zygote, the optimal provisioning for females to invest per egg is given by the following interpretation of the Smith and Fretwell (1974) solution

$$b'(m^*_{\text{ovum}}) = F/m^*_{\text{ovum}}. \tag{1.3b}$$

Assuming that zygote provisioning via sperm has the same benefit as provisioning through ova, (i.e. both affect zygote survival equally), then $b'(m^*_{\text{ovum}}) = b'(\delta)$ and we obtain a simple condition in terms of the anisogamy ratio A (= ovum size/sperm size):

$$A = m^*_{\text{ovum}}/\delta > N/(N-1). \tag{1.3c}$$

If this is satisfied, males should not increase their zygote provisioning, hence anisogamy is stable. Condition (1.3c) is very robust: the anisogamy ratio A must only exceed $N/(N-1)$. With external fertilization, there will generally be high sperm competition (high N) if spawning tends to be synchronous. Thus in large groups, sperm can be almost be as big as ova before a mutant with extra provisioning will spread, and even when only two males compete ($N = 2$), sperm size remains at δ unless A is extremely weak (below 2).

Both selection for increased fertilization probability and sexual selection to outcompete other males by releasing sperm closer to eggs (Parker, 1970) may have driven the evolution of internal fertilization. With internal fertilization, sperm competition is likely to become much reduced, but probably in most groups never entirely absent (see reviews in Smith, 1984; Birkhead and Møller, 1998).

Imagine that sperm competition is rare, and when it occurs, it involves only two males. Such situations are common in internal fertilizers, but also apply to external fertilizers with rare sperm competition, e.g. species in which spawnings occasionally involve both a sneak male as well as the guarding male. Applying the same technique, if a given male faces sperm competition from one other male with low a probability p, the condition to keep sperm minimal becomes

$$b'(\delta) < pF/[\delta(4-2p)] \tag{1.4a}$$

and from the Smith–Fretwell equation (1.3b),

$$A = m^*_{\text{ovum}}/\delta > (4-2p)/p, \tag{1.4b}$$

which, with $p = 1$, is the same as (1.3c) with $N = 2$ (if $p = 1$, two males always compete). The probability p that a male faces sperm competition at a given copulation can be related to the expected probability q that a given female in the population mates twice: $p = 2q/(1+q)$ (Parker *et al.* 1997). Thus condition (1.4b) can be written as

$$q > 2/A. \tag{1.4c}$$

Thus anisogamy is stable provided that the probability that a female mates twice (or releases her eggs with two competing ejaculates) is more frequent than 2/anisogamy ratio.

Again, condition (1.4c) is a remarkably robust one. Anisogamy ratios of vertebrates commonly exceed 10^6; even with $A = 10^3$, anisogamy is stable provided two ejaculates compete in at least 0.2% of clutches.

Increased sperm provisioning is disadvantageous in these two models because, at high anisogamy ratios, a unit increase in investment in each sperm causes significant cost, but trivial benefits. In the "internal fertilization" model a mutant that has double the sperm size has half the sperm number, which reduces its gains under sperm competition from 0.5 to 0.333. Assuming that the original sperm size is due to chromosomes rather than provisioning, it increases its zygote's provisioning only from A to $A + 1$. At high anisogamy ratios, an extra unit of provisioning yields a trivial increase in zygote viability, but a large cost in terms of gamete competition.

This analysis suggests two conclusions. First, provided that a sufficiently high anisogamy ratio had already evolved, the reduction in sperm competition through internal fertilization would be generally insufficient to threaten the stability of anisogamy. Second, apart from the haploid chromosomes, typically no component of sperm mass should be for investment in the zygote (for similar conclusions for isogamy, see Matsuda and Abrams, 1999); all sperm characteristics should relate solely to ensuring fusion with ova. Section 1.6.3 outlines how sperm size may be optimized to maximize fertilization gains under sperm competition.

The external fertilization model (for N competing ejaculates) and the internal fertilization model (where females mate twice with probability q) have in the literature become known the "intensity" and "risk" models in sperm competition games, i.e. games that seek the ESS sperm allocations under a variety of sperm competition scenarios (see Parker, 1998; Parker and Pizzari, submitted) where competition for fertilization follows the raffle principle.

Sperm competition does not always approximate to the raffle principle. In many internal fertilizers such as insects with fixed-volume sperm stores, there is sperm displacement, so that as new sperm are input to the stores, previously stored sperm are displaced. I investigated the case where proportion z of the sperm store is displaced volumetrically by the ejaculate of the last male to mate (Parker, 1978). Again sperm competition is rare and a given male faces sperm competition from another male with probability p. The assumption was that when the mutant male (with sperm size $m > \delta$) mates last, he will have zm^{-1} sperm in competition with $(1 - z)\delta^{-1}$ non-mutant sperm, and when he mates first, he has $(1 - z)m^{-1}$ sperm remaining to compete with the $z\delta^{-1}$ sperm from the first male. This implicitly assumes either that there is little or no seminal fluid (all displacement is by sperm mass), or that the seminal fluid volume is proportional to the sperm

mass. The condition for maintaining sperm at the minimum size, δ, now becomes

$$A = m^*_{\text{ovum}}/\delta > (2-p)/pz(1-z) \tag{1.5a}$$

and substituting $p = 2q/(1+q)$, condition (1.4a) becomes

$$q > 1/Az(1-z). \tag{1.5b}$$

Note that if z = 0.5 (each male's ejaculate is equally represented volumetrically in the sperm stores), condition (1.4b) becomes $q > 4/A$: at intermediate levels of sperm displacement, anisogamy is again robust, but less so than the raffle (cf. condition (1.4c)). However, it is much less robust than the raffle when displacement is either very high ($z \rightarrow 1.0$) or very low ($z \rightarrow 0$).

In the most comprehensive review of insect sperm competition to date, Simmons (2001, Table 2.3) lists 133 species of non-social insects for which there is data on the mean paternity of the last male to mate (P_2), which is related to z. The majority of species have $0.5 < P_2 < 0.95$, i.e. the last male predominates at fertilization. The lowest P_2 value is that of 0.02 for *Anopheles gambiae* (Bryan, 1968), and there are 12 species in the range $0.95 \leq P_2 \leq 1$, with five listed as 1.0 (with standard deviation recorded as 0.00, or not given). Whether this implies that anisogamy could be threatened remains uncertain, since records of 1.00 may represent low sample sizes; there appears to be no sign of large sperm size and small sperm number in these species. P_2 can be very close to 1.0 and yet be sufficient to maintain anisogamy. Parker (1982) cites the example of the ferocious water bug *Abedus herberti* with P_2 = 0.997 (Smith, 1979). Even at this exceptionally high P_2 level, the minimal sperm strategy, δ, appears relatively safe since $A >> z(1-z)$.

So variations in the mechanism of sperm competition from a fair raffle may not greatly affect the stability of anisogamy through sperm competition, under a sperm size–number trade-off.

1.5.2 No sperm competition: sperm size trades-off against mate acquisition or paternal care

In a hypothetical sessile ancestral organism there is every reason that the gamete size–number trade-off should apply; there may be little else on which to expend the resources available for reproduction. The direct size–number trade-off need not apply, however, when other reproductive strategies arise, for example, with the evolution of mobility and

mate searching. I examined the case where increasing sperm size reduces a male's number of matings Parker (1984). Suppose that in a population there is no sperm competition, and k is the optimal proportion of the reproductive budget spent on minimal sperm (size δ) by males in relation to fertility (sperm numbers increase fertility, following the next section). Then $(1-k)$ is spent on gaining matings. The number of matings achieved by a mutant that deviates by producing sperm of size $m > \delta$ is therefore reduced, because only $(1 - km/\delta)$ of the reproductive budget can be spent on gaining matings. With a sex ratio of unity, Parker (1984) showed that the minimal sperm strategy is stable if

$$k > A^{-1}, \tag{1.6}$$

which is a very easy condition to satisfy in vertebrates. A rough measure of k can be taken as the gonadosomatic index (proportion of body mass devoted to testes, see Parker and Ball, 2005). The seahorses (*Sygnathidae*) have probably close to zero sperm competition risk (Stockley *et al.*, 1997; Van Look *et al.*, 2007), and produce remarkably few tiny sperm with high fertilization efficiency (e.g. *Hippocampus kuda*, Van Look *et al.*, 2007). They have a very small gonadosomatic index for fishes (0.15 for *Sygnathus typhle* is the smallest value in Table 1 of Stockley *et al.*, 1997); their anisogamy ratio is such that A^{-1} is many orders of magnitude less than 0.15.

A similar argument may be made for a trade-off of sperm size against paternal care, when this occurs. For example, under near zero sperm competition risk, sea horses have retained minimal sperm, but have responded by producing the tiniest of ejaculates, with a ratio of numbers of sperm ejaculated to eggs fertilized probably less than 2.5, which is comparable to insects and many orders of magnitude smaller than that of most fishes (Van Look *et al.*, 2007). They also show considerable paternal care: increased paternal care appears to have been more favorable than investment in the zygote via increased sperm size.

1.5.3 No sperm competition: sperm numbers increase fertility (sperm limitation)

Sperm limitation can also assist in the maintenance of anisogamy (Parker, 1982). The probability of fertilization, P, is typically an increasing function of sperm numbers surrounding the ovum, or the set of ova. The classical theory for large numbers of tiny sperm is that this is necessary to increase fertility.

First, consider the case where the sperm size–number trade-off applies, so that the maximum sperm number in an ejaculate is proportional to δ^{-1}, and the fitness of a mutant with sperm size $m > \delta$ is $P(m)[F + b(m)]$, where F is the contribution to zygote viability of the ovum, and $b(m)$ the contribution from the mutant male. Remembering that we are now considering a state without sperm competition (one ejaculate fertilizes one set of eggs), applying the techniques of former sections we can see that δ is stable against m if the anisogamy ratio

$$A > P(\delta^{-1})/[P'(\delta^{-1}) \cdot \delta^{-1}] \tag{1.7}$$

(Parker, 1982). Thus, if fertility is high ($P \rightarrow 1.0$), the anisogamy ratio must exceed the reciprocal of the product of sperm number and the gradient of fertilization probability. Applying data on cattle to Equation (1.7), I was unable to conclude whether or not the classical theory (here, that minimal-sized sperm are stable to maximize sperm numbers for fertilization) is plausible Parker (1982). It certainly seems possible that if sperm size trades-off against sperm number, selection to increase fertilization probability may contribute to the stability of minimal-sized sperm.

Can the classical theory also explain why minimal sperm are produced in such large numbers? For this, we need to know the optimal value for k, the proportion of a male's reproductive budget spent sperm, under the selective pressure relating to increasing the probability of fertilization; call this k_P^*. I first concluded that the value of k_P^*, which is set by $P'(k_P^*) = P(k_P^*)/1$ (Equation (17) in Parker, 1984; see also Parker and Pizzari, 2010), could not account readily account for sperm numbers (and hence the proportionate expenditure on sperm, k_P^*) seen in cattle. However, this conclusion was based on interpreting sperm *expenditure* as equivalent to sperm *number* in the published relationship between conception probability and sperm numbers, which is not correct. To derive the result in terms of sperm numbers, let s = the sperm number and D = the energetic cost of each sperm unit. If the costs of gaining a mating are C and the male has an energy budget for reproduction of R, his number of matings is $R/(C + Ds)$, and at each mating he gains a fertility probability of $P(s)$. His fitness is the product $w = P(s)R/(C + Ds)$, which is maximized when $\mathrm{d}w/\mathrm{d}s = 0$. Remembering that $k = Ds/(C + Ds)$, i.e. the proportion of total reproductive effort spent on the ejaculate when C is spent on gaining matings, this gives the result that

$$k_P^* = sP'(s)/P(s). \tag{1.8a}$$

For cattle (see Parker 1982), the number of sperm ejaculated (s) is of the order of 10^{10}, and the probability of conception (P) around 0.75. The gradient of the probability of conception (P') with sperm numbers around the normal ejaculate size could not be evaluated, but from artificial insemination sperm dilution data, it could be deduced to be $<< 10^{-9}$. Until more accurate data can be analyzed, it is therefore not possible to deduced whether (1.8a) suggests a value that is too low to be plausible.

To compare (1.8a) with the ESS proportion of reproductive effort to spend on the ejaculate when there is sperm competition, consider a population where females have a low probability q of mating twice. Fitness can be calculated as $w = v(s)R/(C + Ds)$, where v is the expected value of a mating of a mutant with $s \neq s^*$, where s^* is the ESS sperm number. Thus $v(s) = [(1 - q) + 2qs/(s + s^*)]/(1 + q)$, and we obtain

$$k_{SC}^* = q/2 \tag{1.8b}$$

(Parker and Ball, 2005). Expressed in term of the probability p that a given males faces sperm competition this is $k_{SC}^* = p/(4 - 2p)$ (Parker 1982, 1984). This is a plausible value for relative testes size (Parker 1982). There is also considerable evidence suggesting that relative testes size increases with sperm competition risk in comparative studies across species (e.g. Parker and Pizzari, 2010). Thus sperm competition can readily explain why there are so many sperm are produced in internal fertilizers.

Other theories for why so many tiny sperm are produced by internal fertilizers are reviewed in Pizzari and Parker (2009).

1.5.4 Summary of maintenance of anisogamy

As multicellularity increases, zygote size becomes increasingly advantageous, driving ovum size up. Simultaneously, sperm competition tends to maintain sperm at a minimal size, δ, constrained only by success in fertilization. The anisogamy ratio A (ovum size/sperm size), therefore becomes very large. This high A value generates the fundamental reason why sperm are expected to remain "minimal" i.e. adapted solely to the function of success in fertilization without making any contribution to zygote provisioning. Effectively, any small increase in sperm size to enhance zygote provisioning has trivial benefit, but since

this increase occurs in all sperm of a mutant male, the cost in terms of other selective forces that maintain sperm numbers at a high level is very significant. Thus anisogamy generally remains stable in multicellular plants and animals.

The main agent maintaining "minimal sperm" (or "minimal pollen") is probably sperm (or pollen) competition. Despite reductions in sperm competition with the evolution of internal fertilization in animals (or insect pollination in plants), under a sperm size–number trade-off, with $A >> 1$, sperm competition alone may still be generally sufficient to maintain anisogamy. Further, Parker (1984) has argued that in a species with zero male parental care, but with maternal care, the important index for stability of minimal sperm is not the anisogamy ratio A, but rather the investment ratio, I, which is: total energetic investment in the zygote by the female divided by the energetic cost of a sperm of size δ. For species with female parental care, this makes anisogamy highly robust by ensuring that minimal sperm are stable unless the risk of sperm competition is infinitesimal or zero.

There are also other selective pressures that will militate against increasing sperm size; these include the classical argument that sperm numbers increase fertilization probability, and the fact that sperm size may be traded-off against other reproductive activities such as mate searching and paternal care.

1.6 Optimal sizes of the male and female gametes: anisogamy ratios

What determines the anisogamy ratio (A = ovum mass/sperm mass) in a species? These vary by many orders of magnitude, and though giant sperm do exist where A is low, but greater than unity (e.g. *Drosophila bifurca*, Bjork and Pitnick, 2006), A is usually a very large number.

Following the evolution of internal fertilization, the evolution of the anisogamy ratio, A, becomes complex, with largely independent selective pressures shaping ovum size and sperm size. Ovum size becomes specialized towards provisioning of the zygote, and sperm size is shaped by survival prospect up to fusion and competitive advantages of size under sperm competition.

1.6.1 Gamete size dimorphism from PBS theory

In extending PBS theory to include gamete survival in relation to gamete size (i.e. $g(m)$), as well as zygote survival in relation to zygote

size ($f(S)$), Bulmer and Parker (2002) argued that once gamete dimorphism arises by disruptive selection on an isogamous ancestral population with the same $g(m)$ function for the + and – mating types, the $g(m)$ function differentiates into two forms, $g_1(m_1)$ for microgametes and $g_2(m_2)$ for macrogametes. When a high anisogamy ratio has become established, sperm (size m_1) contributes insignificantly to the size of the zygote, which effectively becomes the ovum size (m_2), i.e. $S = m_1 + m_2 \approx m_2$ (see also Parker 1982). Sperm are selected to maximize $g_1(m_1)/m_1$, so that the optimal sperm size can be found by the Smith and Fretwell (1974) equation

$$[g_1(m_1)]' = g_1(m_1)/m_1. \tag{1.9a}$$

In contrast, ova are selected to maximize $g_2(m_2)f(m_2)/m_2$, where $g_2(m_2)$ is the ovum's survival probability in relation to its size, up to the time of fertilization, and $f(m_2) = f(S)$ is the zygote survival probability.

As multicellularity and complexity increases, function $f(m_2)$ ensures that egg size is typically large in higher organisms. This high provisioning, coupled with the high sperm densities typical when the ova are available for fertilization, make it inevitable that ova have high survival prospects before fusion. Where good data exist (fish, insect, birds, mammals), large changes in sperm numbers have little effect on fertilization probability, which typically approaches 1.0 (Ball and Parker 2000). Thus in most higher organisms, the probability of ovum survival to fusion, $g_2(m_2)$, is likely to be independent of quite large changes in m_2, at least in the general region of typical ovum size, so that ova are selected to maximize $f(m_2)/m_2$, i.e. the optimal ovum size also satisfies the Smith and Fretwell (1974) equation

$$f'(m_2) = f(m_2)/m_2. \tag{1.9b}$$

Effectively, ovum size becomes determined entirely by $f(m_2)$, the principle used to consider conditions for the stability of anisogamy in higher organisms (Parker 1982; see Section 1.5.1).

However, an exception to this principle occurs in species that are often sperm limited, such as certain marine external fertilizers, where fusion probability increases with ovum size (Levitan 1993), so that $g_2(m_2)$ is a significantly increasing function of m_2 in the region of the optimal ovum size. Here the appropriate Smith–Fretwell formulation to derive the optimal egg size is

$$[g_2(m_2)f(m_2)]' = g_2(m_2)f(m_2)/m_2 \tag{1.9c}$$

(Bulmer and Parker 2002), which gives a larger ovum size than equation (1.9b). Levitan (1993, 1996a) gives evidence for such deviations in marine invertebrates.

Once anisogamy develops, other selective forces shape the development of micro- and macrogametes: sperm become or remain very small and retain their motility (Parker 1982, Randerson and Hurst 2001a) and ova become oogamous (non-motile). I next review briefly models for the subsequent specialization of the gamete sizes.

1.6.2 Optimization of ovum size

The fact that sperm contribute little or nothing to the future survival/fitness of the zygote means, as we have seen, that optimal ovum size can be established from the Smith and Fretwell (1974) model. The topic is too large to be reviewed here, but most analyses of optimal ovum size use variants of the Smith–Fretwell approach. This is a non-competitive optimum, which suggests that when resources for reproduction in a species vary, number rather than size of ova, should vary (a prediction often approximated, see Maynard Smith, 1978). Competitive effects between ova are rarely modeled. Under sperm limitation theory, "target gamete" increases in egg size (e.g. Levitan, 1993) arise though egg competition for sperm only if fertilizations remove ova fast enough from the sperm pool that they decrease the fertilization rate of other ova (Levitan, 1996, Lessells *et al*., 2009). Within-clutch sib-competition between developing zygotes can also increase egg size (e.g. Parker and Begon, 1986).

The range of ovum size in vertebrates is vast, with diameters ranging from a tiny 50 μm in the field vole, *Microtus agrestis*, to a massive 15 cm (with volume 1.77 liters) in the shark, *Chlamydoselachus auguineus* (Lombardi, 1998). Examples of two distinct seed sizes in certain plants probably relate to differences in dormancy, dispersal or ecological niches (see Maynard Smith, 1978).

1.6.3 Sperm competition and the evolution of sperm size

Section 1.5.1 outlined reasons why sperm mass should relate only: (i) to carrying the haploid chromosomes, (ii) to optimizing survival before fusion or (iii) to increasing competitiveness at fertilization. On this prediction, sperm should not include any component for provisioning the zygote, unless this arises as material recovered from expenditure

on sperm survival or fertilization competitiveness. However, although sperm are small in total mass compared to ova, like ova, their size variation is vast. For example, a typical bird sperm may be around 150 μm in length (Birkhead and Møller, 1998), but the largest sperm is that of *Drosophila bifurca* which is 58.3 mm long (Pitnick *et al.*, 1995). It is nevertheless well below the mass of the ovum (i.e. $A > 1.0$); Bjork and Pitnick calculate a sperm/egg production rate ratio of 5.8/1. How can we explain these large variations in sperm size?

Parker (1993; see also Snook, 2005; Pizzari and Parker, 2009) argued that increased sperm size may either: (i) unilaterally increase energy reserves, and hence increase sperm survival to fertilization (see also Bulmer and Parker, 2002), or (ii) unilaterally increase sperm tail and motility machinery, which would decrease survival, since energy reserves would be used up faster (Gomendio and Roldan, 1991), or (iii) increase both. Thus if sperm are not fully provisioned by the female, increased sperm size may "buy" either increased survival, or faster motility (i.e. higher competitive loading through increasing swimming speed) or some other competitive advantage under sperm competition, or both simultaneously.

The models in sections 1.6.3.1 to 1.6.3.3 below assume that the sperm characteristics are determined by the parent that produces them; evolutionary conflict between parent and gamete can occur when sperm traits are determined by the haploid gametes of a diploid parent (section 1.6.3.4).

1.6.3.1 Sperm size and number under sperm competition risk: competitive loadings

Most models for the evolution of sperm size generally (like those for ovum size) assume that gamete size is determined by the genotype of the diploid "parent" (Parker, 1993; Parker *et al.*, submitted); obvious exceptions are animals with haplodiploidy, and plants that produce gametes in the haploid phase. Very different conclusions from those given below are generated if sperm size is determined by the genes of the haploid sperm under diploidy (Parker and Begon, 1993).

Much theoretical work has centred on the topic of sperm competition and ejaculate economics (Parker and Pizzari, 2010) and on how sperm competition may influence sperm size and number variation (Pizzari and Parker, 2009). A general approach to size–number variation, using the sperm competition risk model where females mate

twice with probability q and once with probability $(1 - q)$, has been developed by Parker (1993) and Parker *et al.* (submitted).

Calling sperm size or mass m, and sperm number s, a male's total investment in the ejaculate will be proportional to the product, ms. Parker *et al.* (submitted) showed the ESS sperm size and number (m^*, s^*) at a given level of sperm competition risk (q = the probability that a female mates twice) in a population to be

$$m^* = \beta G'(s^*, \mathrm{I}), \tag{1.10a}$$

$$s^* = \beta G'(m^*, \mathrm{I}), \tag{1.10b}$$

where

$$\beta \equiv \left(\frac{R}{D}\right)\left(\frac{2q}{1+q}\right), \tag{1.10c}$$

R is the resource available to a male for reproduction (sperm production and acquiring matings), and D is the cost of one unit of ejaculate (each male spends units of his reproductive budget R per mating on his ejaculate). Hence we obtain the general rule

$$s^* G'(s^*) = m^* G'(m^*) \tag{1.11}$$

(Parker 1993, Parker *et al.* submitted), i.e. the product of sperm number and the marginal gains through increasing sperm numbers, $G'(s^*)$, must equal the product of sperm size times the marginal gains through increasing sperm size, $G'(m^*)$. Though rule (1.11) above indicates how the balance between sperm size and number is attained at the ESS, to deduce how sperm competition shapes sperm size and number, explicit functions for $G(s)$ and $G(m)$ are required.

Parker *et al.* (submitted) investigated three different mechanisms of sperm competition (Figure 1.5). In the simplest mechanism, sperm compete in a fertilization raffle with no constraint on the space available for competing ejaculates, such as may occur in many external fertilizers. This commonly differs from internal fertilizers, where there may be some space constraint on storage of ejaculates due to limitations imposed by the female tract. This constraint is most severe in species with small, fixed-volume sperm stores, as in many insects; in such systems the most recent ejaculate often displaces some of the previously stored ejaculate(s) from a female's sperm stores. Two forms of displacement were studied: (i) displacement mainly by seminal fluid, so that any small changes in sperm mass do not affect the amount of

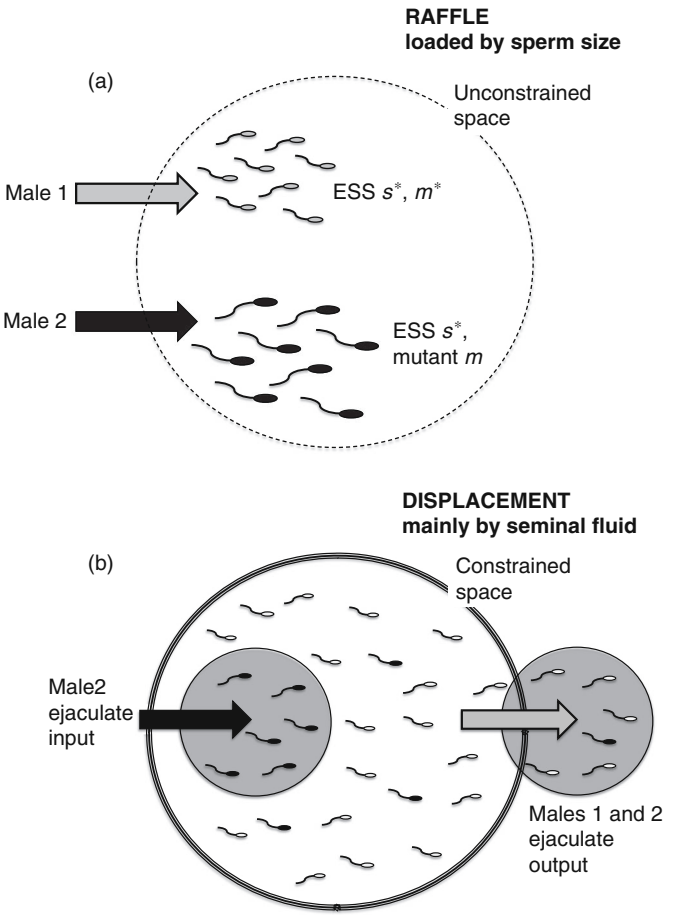

Figure 1.5 Mechanisms of sperm competition investigated in the Parker *et al.* (submitted) models for sperm size and number. (a) Raffle. Males ejaculate into an unconstrained space from which sperm are drawn for fertilization; there is no displacement of previous sperm during ejaculation. The ESS sperm mass is m^* and sperm number is s^*. Mutant male 2 has larger sperm ($m > m^*$). Fertilizations are drawn from the sperm available after both males have ejaculated, after the loaded raffle principle in which the raffle is loaded by sperm size through function $r(m)$ (see Equation (1.12)). (b) Displacement mainly by seminal fluid. During ejaculation of the second male there is continuous displacement of previously stored sperm from the constrained sperm storage space in the female tract following the model of direct displacement with instant mixing (Parker and Simmons 1991). Each sperm is transferred in a fixed volume of seminal fluid that is much larger than the sperm mass, so that deviations in sperm size have a negligible effect on displacement.

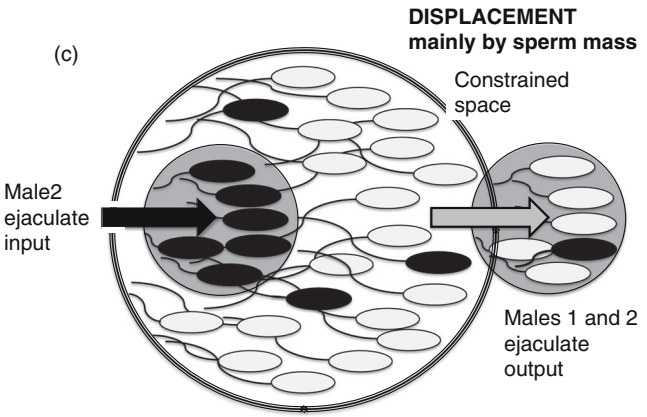

Figure 1.5 (cont.) (c) Displacement mainly by sperm mass. Displacement is as in (b), except that the seminal fluid volume per sperm is negligible, so that for a fixed number of sperm, displacement increases with sperm mass. In both (b) and (c), once ejaculation of the second (last) male is complete, the fertilization follows a raffle loaded by sperm size as in (a). With permission from Parker *et al.* (submitted).

displacement, and (iii) displacement by the sperm mass itself, so that as sperm increase in size, displacement increases. These different mechanisms have different forms for the gain through increases in sperm numbers, $G(s)$, in (1.11) above.

Parker *et al.* (submitted) showed that the product, s^*m^*, was either constant (in the raffle or the sperm mass-displacement models) or highly invariant (the seminal-fluid-displacement model) at a given value for β (which defines the resources available and the sperm competition risk level, q, see (1.10c)), and that s^*m^* increases with the risk of sperm competition, q (Figure 1.6a). Thus, effectively, there is a direct sperm size–number trade-off within the ejaculate allocation s^*m^* at a given level of β.

How sperm size and number are allocated as s^*m^* increases across populations with the risk of sperm competition depends on the marginal gains through increasing sperm numbers, $G'(s^*)$, or sperm size, $G'(m^*)$; see Equation (1.11). If the sperm competition mechanism is a raffle (i.e. no constraint on space for competing ejaculates) then the sperm numbers in the set of sperm that compete for fertilizations (the "fertilization set"; Parker *et al.*, 1990) are directly proportional to the sperm numbers ejaculated. However, the composition of

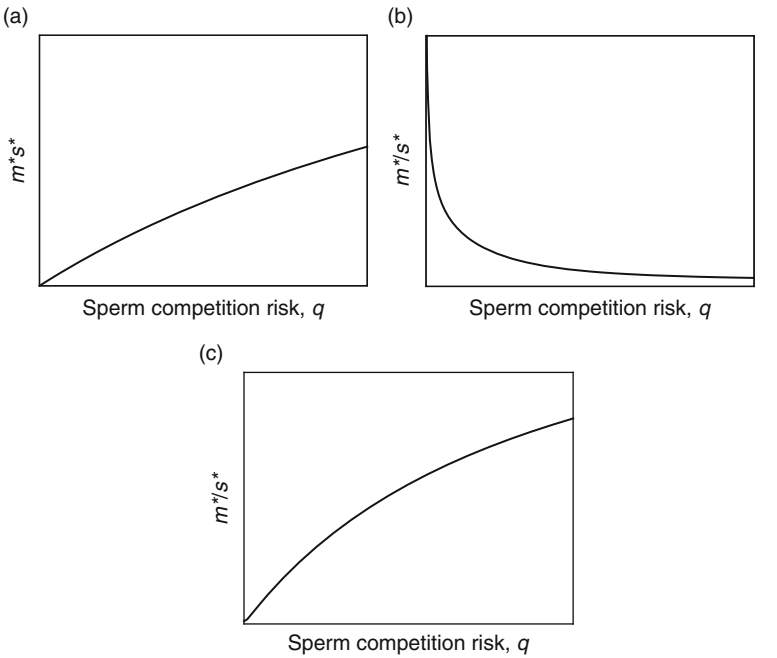

Figure 1.6 Sperm size and number in relation to sperm competition risk, q, in the models of Parker *et al.* (submitted). (a) The total ejaculate expenditure (the product of sperm size and number, m^*s^*, increases with q, and is influenced only by the constants, R, D, and q, in β (see (1.10c)). As m^*s^* increases with q, m^* remains constant if the competitive loading for sperm, r, is a function only of m. Thus the increase in m^*s^* is due entirely to an increase in sperm numbers, s^*, leading to a declining relation between m^*/s^* and q as in (b) (the "default expectation"). This declining relation (b) was also common when the competitive loading due to sperm size, function $r(m)$, was distorted by sperm density, as in Figure 1.7 (c to e). (c) An increasing relation between m^*/s^* and q can occur only if there is greater investment in sperm size than number as risk increases. In Parker *et al.* (submitted), this required sperm density to decrease the slope of $r(m)$, or to increase the intercept of $r(m)$ (see text). With permission from Parker *et al.* (submitted) and Immler *et al.* (submitted).

the fertilization set in the displacement models depends on how much of the first male's sperm has been displaced by the second male to mate. In all three mechanisms studied (Figure 1.5), Parker *et al.* (submitted) assumed that once the sperm numbers from the competing males in the fertilization set has been determined (i.e. after displacement has occurred), sperm then compete in proportion to their

numbers weighted by their size (i.e. a loaded raffle, where the competitive loadings are determined by sperm size). Thus consider a mutant male 1, which deviates from the ESS sperm size, m^*, by having a sperm of size $m \neq m^*$. In the fertilization set, male 1 has s_1 sperm, each with a competitive loading of $r(m)$. They compete against male 2 with s_2 sperm of size m^* each having a competitive loading of $r(m^*)$. Male 1's probability of fertilization is thus

$$\frac{r(m)s_1}{r(m)s_1 + r(m^*)s_2}. \tag{1.12}$$

Parker (1993) and Parker *et al.* (submitted) found that certain forms for increasing $r(m)$ yield a maximum or minimum (Figure 1.7a); for the ESS to be an intermediate sperm size $r(m)$ must be of a form such that a tangent can be drawn from the origin (Figure 1.7b) following the marginal value theorem principle (Charnov, 1976, Parker and Stuart, 1976) and the Smith–Fretwell (1974) model for optimal ovum size. However, if r is solely a function of sperm mass, m, then the ESS sperm size m^* is constant and independent of sperm competition risk, q (Parker, 1993; Parker *et al.* submitted). If m^* is constant with q, and s^*m^* increases with q (Figure 1.6a), then as q increases, the ratio sperm size/number (m^*/s^*) decreases with q (Figure 1.6b). This could be defined as the "default expectation" (Immler *et al.* submitted), i.e. as sperm competition risk increases, relatively more is invested in sperm numbers than in sperm size. But several species show increases in both sperm number and sperm size as risk, q, increases, and some show evidence that this involves relatively more being invested in sperm size than in sperm numbers, since m^*/s^* increases with q (Figure 1.6c). How can this be explained?

One possibility is that increases in sperm density with sperm competition risk arising from the default expectation could distort the form of $r(m)$. Discounting forms for $r(m)$ that yield maxima or minima (Figure 1.7a), consider forms that yield an intermediate ESS sperm size, m^* (Figure 1.7b). If r is a function of sperm density in the fertilization set, as well as a function of sperm size, m^* can vary with sperm competition risk (Parker, 1993; Parker *et al.*, submitted). How such distortions in the form of r through increases in sperm density change the allocation between sperm size and number as sperm competition risk increases is a complex problem. Parker *et al.* (submitted) analyzed how ESS values for sperm size and number (m^*, s^*) should vary with sperm competition risk q, using an exponentially diminishing returns form for $r(m,s)$ that changes with the sperm density in the

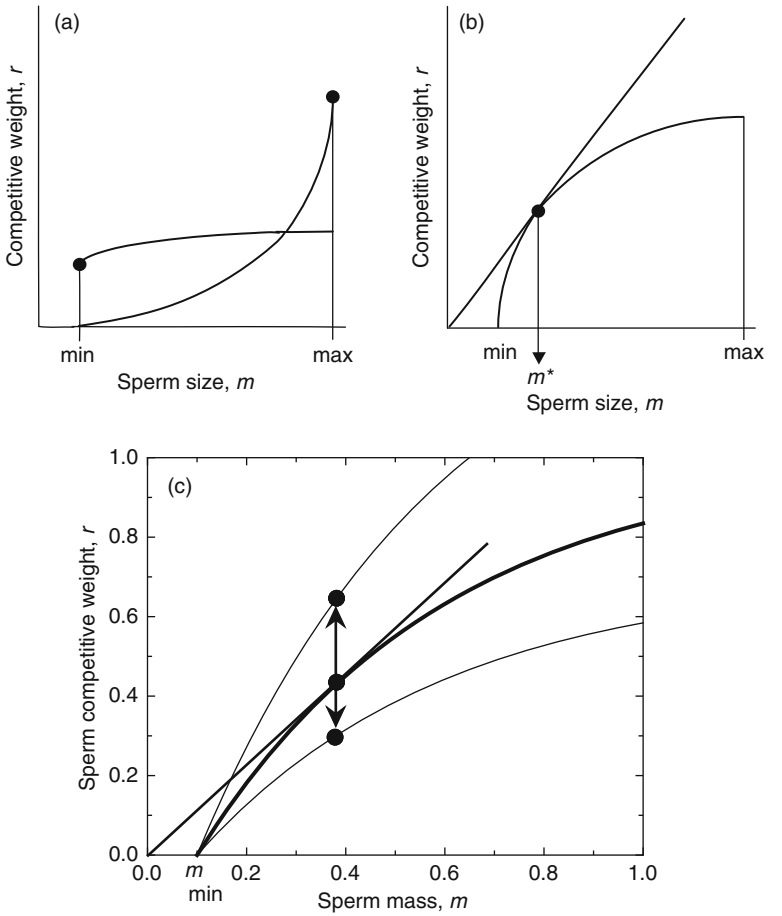

Figure 1.7 A sperm's competitive loading, r, in the fertilization raffle is set by its size, m, and we assume that $r(m)$ is monotonic increasing. (a) shows two forms for $r(m)$ that will not yield an intermediate ESS sperm size, m^*; instead they give a minimum or maximum size a shown by the filled circles. (b) An intermediate ESS sperm size is attained if a tangent can be drawn from the origin to $r(m)$; the filled circle shows the ESS. (c – e) show how the form of $r(m)$ may be distorted, changing the ESS (open circles) as sperm density increases due to increasing risk, q. (c) Increasing sperm density may increase (dotted curve) or decrease (thin continuous curve) the asymptote of $r(m)$. As shown here, this does not affect the ESS m^* in the sperm competition mechanisms in Figures 1.5a and 1.5b, but it does change the ESS m^* in the mechanism in Figure 1.5c.

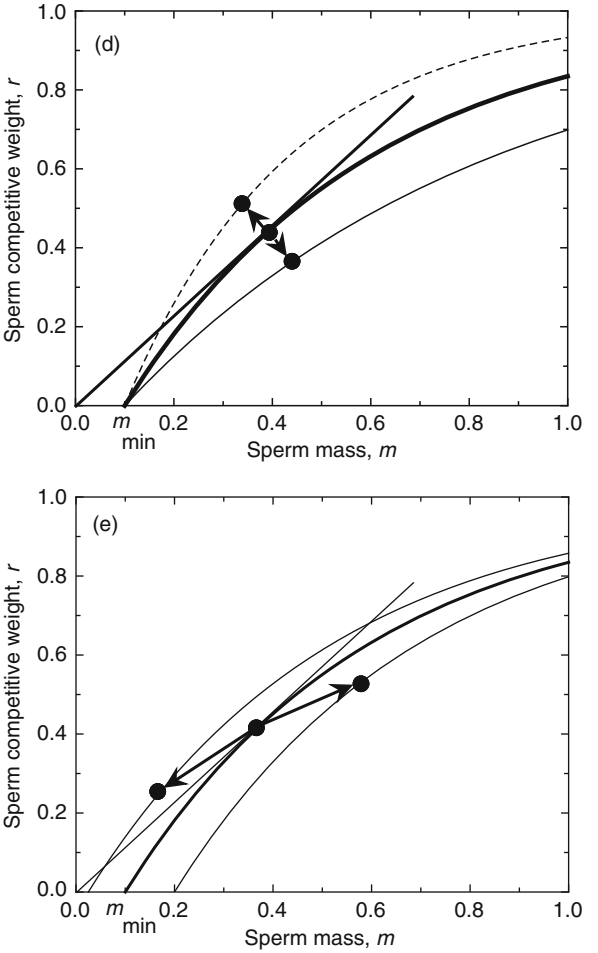

Figure 1.7 (cont.) (d) Increasing sperm density may increase (dotted curve) or decrease (thin continuous curve) the slope of $r(m)$. If the slope increases, m^* decreases, and if the slope decreases, m^* increases. (e). Increasing sperm density may increase (dotted curve) or decrease (thin continuous curve) the intercept of $r(m)$. If the intercept increases, m^* increases, and if the intercept decreases, m^* decreases. (a) and (b) from Parker (1993); (c-e) modified from Parker *et al.* (submitted).

fertilization set in three ways (Figure 1.7c–e). Increases in sperm density were used to change: (i) the asymptote value of $r(m)$ (Figure 1.7c), (ii) the rate at which $r(m)$ rises to its asymptote (the slope of $r(m)$; Figure 1.7d), or (iii) the minimum competitive sperm size (the intercept of $r(m)$; Figure 1.7e).

The results can be summarized as follows. In all models, s^* and s^*m^* increase monotonically with q, but the effect of sperm numbers on sperm size depends critically on how sperm density changes $r(m)$. If sperm density changes the asymptote of $r(m)$, sperm size varies with q only when the displacement mechanism is by sperm mass (Figure 1.5c). Here, if sperm density *increases* the asymptotic value of r, m^* increases with q, and if increasing sperm density *decreases* asymptotic r, m^* decreases with q. In the other two models (Figure 1.5a, b) sperm size is unaffected by a change in the asymptote of $r(m)$ (as shown in Figure 1.7a).

However, in all three models in Figure 1.5, changes in the slope or intercept of $r(m)$ with increasing sperm density cause m^* to change with q (see Figure 1.7b, c): m^* increases with q if increased sperm density increases the intercept or decreases the slope of $r(m)$, and m^* decreases with q if increased sperm density decreases the intercept or increases the slope of $r(m)$.

Most effects of sperm density were found to generate the default relation in which relatively more is spent on sperm numbers than on sperm size as risk increases (i.e. a negative relationship between m^*/s^* and risk, q, as in Figure 1.6b). In the raffle (Figure 1.5a) and displacement by seminal fluid (Figure 1.5b) models, very steeply increasing effects of sperm density on the slope or intercept of $r(m)$ are needed to generate a positive relationship between m^*/s^* and risk, q (Figure 1.6c). However, in the displacement by sperm mass model (Figure 1.5c), this positive relationship could be generated even with linear increasing effects of sperm density.

These effects of sperm density on the competitive value of sperm size have different biological implications. If sperm density decreases the slope of $r(m)$, this suggests that the advantage of sperm size decreases as density increases. If the intercept of $r(m)$ is increased by sperm density, this suggests that sperm size becomes increasingly important in competition as sperm density increases, since the minimum size for a sperm to have any chance of fertilization increases as sperm density increases.

Thus theoretically there are reasons why sperm size may increase, remain constant, or decrease with sperm competition risk across species, and all such patterns have been reported for different groups (Pizzari and Parker, 2009). The ratio of sperm size/number has rarely been studied, but is important since it informs how the trade-off between sperm size and number shifts as the overall ejaculate expenditure, ms, increases with sperm competition risk. The work of Parker

et al. (submitted) suggests that m^*/s^* would most commonly decrease with risk across species or populations (following the default expectation; Figure 1.6b). An increasing relationship between m^*/s^* and risk (Figure 1.6c) suggests that, counter to the default expectation, relatively more is spent on sperm size than on sperm number. This seems most likely to be found only when there are extreme competitive interactions between sperm so that the minimum size for successful sperm increases with sperm density.

Some evidence for these predictions was found in passerine birds and drosophilid flies (Immler *et al*. submitted). As predicted, across both groups, the product *ms* increased with risk (as does testis size in many species, Parker and Pizzari, 2010). In passerines, both sperm size, m^*, and number, s^*, increased with risk, but m^*/s^* decreased (following the default expectation, Figure 1.6b). In drosophilids, sperm size increased with risk, but at an increasing rate – a decreasing rate is predicted by Parker *et al*. (submitted). Further, in drosophilids, sperm number initially increases then decreases with risk (continuous increases in sperm number are predicted by Parker *et al*. submitted). However, the ratio m^*/s^* increased with risk (Figure 1.6c), suggesting that competition becomes more intense with sperm competition density with the minimum sperm size increasing with sperm density.

1.6.3.2 Sperm size and sperm survival under sperm competition risk

Using a similar sperm competition risk approach, Parker (1993) calculated ESS sperm size and number when two males mate sequentially with a female before fertilization occurs: assuming that size increases sperm survival, the ESS sperm size increases (and ESS sperm number decreases) with the time delay between matings and the time from the last mating to fertilization.

1.6.3.3 Sperm size and number under sperm competition intensity

Ball and Parker (1996) developed a model of a continuous fertilization process relating to external fertilizers, such as many fish species, in which eggs and sperm are shed simultaneously. Eggs are fertilized at a rate proportional to sperm density surrounding the egg mass and to the "aptitude for fusion," α; increasing α increases the rate of fertilization (and the proportion of eggs fertilized) at a given sperm density. The

model investigated both the risk range (females mate once with probability (1 – q), and twice with probability q) and intensity range (where N ejaculates compete) of sperm competition, and predicted how ESS sperm size and number, and the ESS level of infertility (the eggs remaining unfertilized after all sperm have died) should vary across species or populations. Increased sperm mass, m, was assumed to increase sperm competitive ability, for example by increasing swimming speed, r, and could affect sperm longevity either positively or negatively through a survival function $\tau(m)$, which defined the length of time a sperm survives if it has mass m. As in other sperm competition games, ejaculate expenditure is traded off against expenditure on obtaining further matings (here spawnings).

This model predicted that, across species, the ESS ejaculate expenditure (s^*m^*) increases (as with the previous risk models) with sperm competition intensity, N. The balance between sperm size and number shifts between two extremes, with one optimum at $N = 1$ (zero competition), and the other at maximum sperm competition ($N \to \infty$). The non-competitive optimum (at $N = 1$) maximizes the total distance travelled by the entire ejaculate in its lifetime, i.e. it conserves sperm longevity to gain the most possible fertilizations from the ejaculate, and has

$$m^* = \left[\frac{r'(m^*)}{r(m^*)} + \frac{\tau'(m^*)}{\tau(m^*)}\right]^{-1}, \tag{1.13a}$$

(Ball and Parker, 1996).

In contrast, the optimum for maximum competition (at $N \to \infty$) maximizes the product of sperm speed and sperm number, i.e. it "snatches" the biggest share of fertilizations by focusing on the first instant of the fertilization process. It has the familiar marginal value form found for risk models by Parker (1993) and Parker *et al.* (submitted):

$$m^* = \frac{r(m^*)}{r'(m^*)}. \tag{1.13b}$$

The ESS sperm size and number are shown in relation to sperm competition intensity, N, in Figure 1.8. Suppose that longevity decreases with sperm size (i.e. $\tau'(m)$ is negative), because the main increase is in tail length, which increases sperm energy expenditure. Then sperm size should increase with N, between the non-competitive and the competitive optima (Figure 1.8a, broken curve). However, if longevity increases

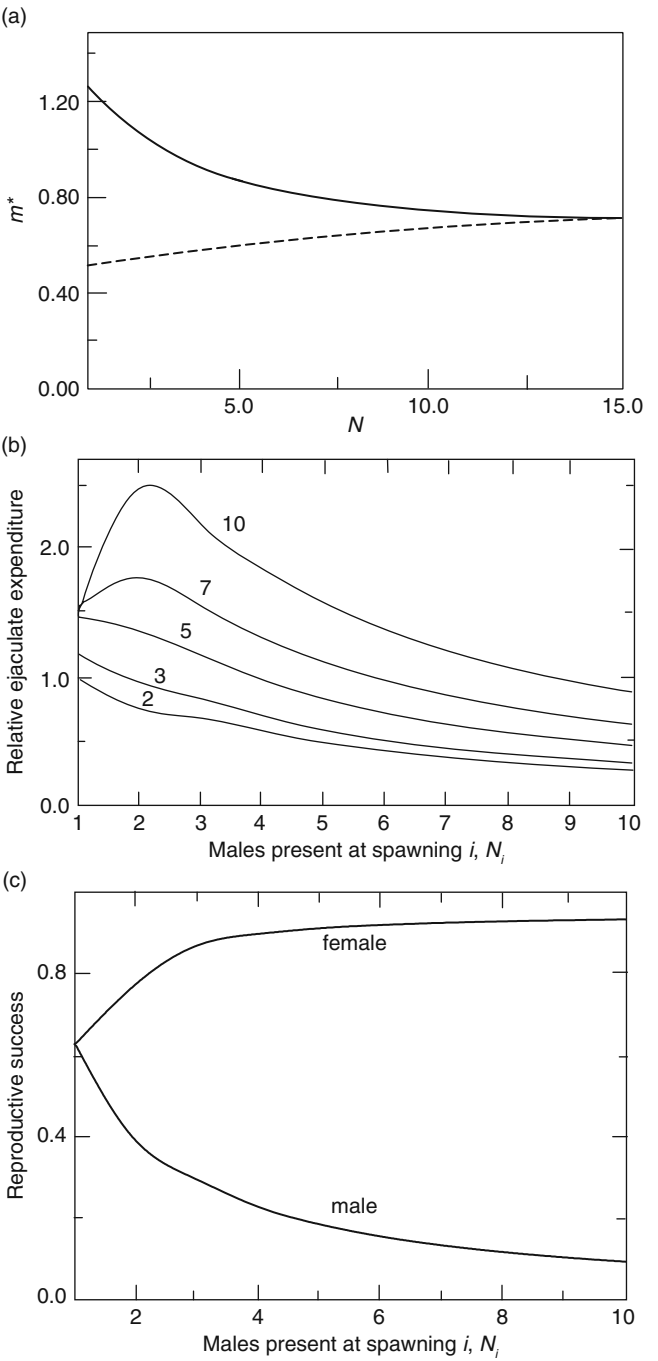

Figure 1.8 (a) Results from the model of Ball and Parker (1996), showing the relation between sperm size, m^*, and sperm competition intensity

with sperm size (i.e. $\tau'(m)$ is positive), then the non-competitive optimal sperm size is greater than that for maximum competition, and sperm size decreases with N (Figure 1.8a, dotted curve). In this continuous fertilization model, sperm numbers, s^*, and the total ejaculate investment, s^*m^*, always increase with N across high sperm competition intensities, whether $\tau'(m)$ is positive or negative. Decreases in s^* are possible only over a range of low sperm competition risk if sperm longevity decreases with sperm size ($\tau'(m)$ is negative), and if infertility is high.

Ball and Parker (1997) extended this continuous fertilization model to investigate how the ESS sperm number, s^*, and the ejaculate expenditure (s^*m^*) should vary within a species or population when males can assess the number of males present at a given spawning. We assumed that the sperm are manufactured and stored before ejaculation, so that the ESS sperm mass m^* is constant and shaped only by the mean number, N, of males present in a species at a spawning, but the ESS sperm number, s^*, can be varied strategically at a given spawning in relation to the number of males present, N_i. In this model, we assumed that increased sperm mass mainly caused increased tail length and hence reduced sperm longevity because of faster use of the sperm's energy reserves. The results for the average expenditure (s^*m^*) in relation to the average intensity, N, are similar to those of the previous model (Ball and Parker, 1996) where males cannot assess the number

Caption for Figure 1.8 (cont.)
across species that vary in the number N of males' ejaculates at spawnings. The lifespan, τ, of a sperm may increase or decrease with sperm mass, m; the upper curve shows that if $\tau(m)$ is increasing, sperm size reduces across species with N; the lower curve for $\tau(m)$ decreasing causes sperm size to increase across species with N. At $N=1$ there is no sperm competition and the non-competitive optimum (Equation (1.13a)) applies (open circles); as sperm competition intensities become very high, $\tau(m)$ has no effect on m^* (Equation (1.13b)) (filled circles). Modified from Ball and Parker (1996). (b) Results from the model of Ball and Parker (1997) showing the relation between ejaculate expenditure, m^*s^*, and sperm competition intensity in species where males can assess N_i, the number of males present at a given spawning, i. Each separate curve represents a species; the average number N of males at spawnings in that species is shown by the curve. (c) Reproductive success achieved by males and females in the model of Ball and Parker (1997) in relation to the number of competing males, N_i, present at spawning i. (b) and (c) With permission from Ball and Parker (1997).

of competitors present at a spawning. However, expenditure in relation to the local sperm competition level varies with N_i, due to strategic adjustments of sperm numbers (Figure 1.8b). Depending on the population average levels of competition, N, two patterns emerge for the relation between and the number of competitors present at a spawning, N_i. If the average N is low, ejaculate expenditure, s^*m^*, declines with N_i; males expend most sperm when on their own ($N_i = 1$) and s^*m^* decreases as N_i increases. At higher average N, s^*m^* increases between $N_i = 1$ and $N_i = 2$, and then decreases as N_i increases (see also Parker *et al.* 1996 for a similar result with instantaneous fertilization). For any given level of average competition, N, and local competition, N_i, reducing α (the aptitude for fusion) increases s^*m^* and reduces the fertility of the spawning.

An interesting effect of the strategic allocation by males, s_i^* sperm when there are N_i males present at a spawning, is that fertility increases with N_i. Thus there is sexual conflict over the number of sperm ejaculated; a given female does best if she spawns when N_i is high, and a given male gains most when N_i is low (Figure 1.8c). Thus females may be selected to prefer to spawn in large groups of males (Shapiro and Giraldeau, 1996) while males should act aggressively to attempt to dispel competitors.

1.6.3.4 Haploid control of sperm size and number under diploidy

A given allele in a diploid parent will be present in only half of the haploid sperm that the parent produces. Though the classical view is that sperm characteristics are determined by the diploid parental genotype, intraejaculate competition can occur if the characteristics of the sperm are determined by the sperm haplotype. Coupled with interejaculate (=sperm) competition, this generates "gamete–parent conflict" because the ESS balance between size and number of sperm in an ejaculate is quite different under haploid control and diploid control (Parker and Begon, 1993). Conflict between haploid and diploid expression is reduced, but is not lost, as interejaculate competition increases, and may have a variety of consequences relating to allocation of sperm size and number, and their variation in natural populations (see Parker and Begon, 1993).

1.7 ANISOGAMY AS A STAGE IN THE EVOLUTIONARY CHAIN OF SEXUALITY

The evolution of sexuality consists of a series of sequential steps, each one catalyzed by its predecessor. Selection for sexual recombination

favored the evolution of fusion and gametes, probably in isogamous populations that released gametes into an external medium (the sea). The evolution of increasing vegetative complexity, usually in the form of complex multicellular organization, generated increasing importance of zygote size for zygote fitness, hailing the origin of anisogamy coupled with the evolution of disassortative fusion from existing linkage of genes for gamete size with those for mating types, if mating types were already present in the ancestral isogamous population (Charlesworth, 1978). If mating types had not already evolved in the ancestral population, early anisogamy without mating types (pseudo-anisogamy) would be followed by selection for disassortative fusions (Parker, 1978). Both pathways would quickly generate the unity sex ratio from Fisher's (1930) principle. This in turn results in sexual selection, both pre- and postcopulatory, and evolution of internal fertilization and reduced sperm competition, with the subsequent specialisation of the sperm cell. The theory of disruptive selection by gamete competition still remains a candidate as the most powerful explanation of the origin of anisogamy (Lessells *et al*., 2009), one of the most important transitions in evolution, which ultimately generated the vast diversity of adaptations that we associate with the two sexes.

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HIROYUKI MATSUDA AND PETER A. ABRAMS

2

The evolutionary instability of isogamy

2.1 INTRODUCTION

Multicellular organisms are usually anisogamous with different mean sizes of gametes of different sexes or mating types. It is generally believed that isogamy (equal gamete sizes of different mating types) is the most primitive mating system (e.g. Maynard Smith, 1978; 1982; Hoekstra, 1987; Maynard Smith and Szathmáry, 1995). Although we observe many isogamous species in algae, fungi and protozoans (for review see Bell, 1982, Hoekstra, 1987), anisogamy has a much wider taxonomic distribution. Attempts to use evolutionary theory to understand this restricted distribution have met with limited success, as have attempts to understand the distribution of isogamy within groups that include both isogamous and anisogamous species, as observed in the freshwater green algal order Volvocales (Randerson and Hurst, 2001). But there are numerous exceptions to fully accept the synthetic theory (Bell, 1978; Hoekstra, 1987). We use a series of mathematical models to help understand what might account for the comparative scarcity of isogamy. The models also produce some new hypotheses that may help explain some of the anomalies in the comparative data.

It is generally believed that the evolution of mating types preceded the evolution of gamete size dimorphism (Hoekstra, 1987; Maynard Smith and Szathmáry, 1995). For example, Hoekstra (1982, 1987) has proposed several possible mechanisms for the evolution of mating types in a randomly mating population. In the simplest mechanism, it is assumed that gamete adhesion was initially brought about

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by two complementary molecules. Each mating type is formed by the loss of one of the two types of molecule. A genotype with both adhesion molecules can eventually be excluded if it has difficulty adhering to an individual with only one type of molecule, or if it must pay a high cost for producing both molecules. This will result in two mating types if selection eventually reduces recombination between the two adhesion loci to zero, as Charlesworth (1978) suggested. Maynard Smith and Szathmáry (1995) interpreted these conditions as being relatively easy to satisfy, although Hoekstra (1987) expressed some reservations. In this chapter, we simply assume that there are two gamete types throughout the study. This is supported by the fact that most isogamous species (as well as all anisogamous ones) have the two mating types (Hoekstra, 1987).

Explaining the comparative scarcity of isogamy is equivalent to explaining the comparative prevalence of anisogamy. Anisogamy is thought to be the origin of all other forms of sexual dimorphism and asymmetry in gender (Darwin, 1871). Anisogamy is also widely believed to be the basis of the two-fold cost of sexual reproduction (Maynard Smith, 1978; Bulmer, 1994). The different theories that have been proposed to explain the evolution of anisogamy can be placed into two broad groups (Bulmer, 1994). Theories in the first group examine disruptive selection acting on gamete size, based on the two conflicting selective forces of search efficiency and postzygotic survival. Parker *et al.* (1972) developed a simple evolutionary game theory model of this scenario, which has been extended by Bell (1978), Charlesworth (1978), Maynard Smith (1978, 1982), Hoekstra (1980, 1987), Parker (1982), Cox and Sethian (1985) and Bulmer (1994). The second group of theories focuses on adaptation to prevent competition between cytoplasmic symbionts (Cosmides and Tooby, 1981; Hurst and Hamilton, 1992) or parasites (Hoekstra, 1990; Hurst, 1990). Uniparental cytoplasmic inheritance prevents destructive competition between unrelated symbionts transmitted in the gametes from the two parents. The small size of one gamete type may be a side-effect of excluding cytoplasmic elements from that gamete type. The relative importance of these two mechanisms for the evolution of anisogamy is still uncertain. However, it is important to note that uniparental inheritance of organelles has been achieved without decreasing male gamete size in some protists and marine green algae (Whatley, 1982; Maynard Smith and Szathmáry, 1995; Kagami *et al.*, 2008). It is therefore unlikely that the second theoretical framework can be the universal explanation for anisogamy.

In this chapter, we focus on the first potential mechanism for the evolution of anisogamy – the coevolution of gamete sizes. Thus, most of our models are modifications of the Parker, Baker and Smith's model (PBS model) (Parker *et al.*, 1972) that was further analyzed by Maynard Smith (Maynard Smith, 1978). Although the original PBS model does not have mating types, we assume that two mating types exist. Charlesworth's (1978) model also has two mating types. But the zygote fitness function that he used precluded isogamy. We are in contexts in which mutations with large effects on gamete size do not occur. This is the difference between the PBS model and its follow-on theory by Maynard Smith (1978), and ours. We also consider the possibility that gamete size has a direct effect on gamete survival or mating success. Finally, we compare the costs of stable isogamy and anisogamy relative to asexual forms and suggest that there is a cost of isogamous sex, and that this may occasionally be greater than the cost of anisogamous sex. We mainly base this chapter on our original paper (Matsuda and Abrams, 1999), adding explanations to help the general reader to understand our ideas.

2.2 Sex-linked model when gamete size is determined independently in each mating type

Here we develop a model in which there is no effect of gamete size on gamete success (see the direct effects of size on gamete fitness section below). First, we develop fitness functions for the case with two mating types, denoted 1 and 2. They are used to develop a model for the dynamics of gamete sizes: the size of type-1 gametes is denoted x_1, and the size of type-2 gametes is denoted x_2. The size of the zygote is assumed to be the sum of gamete sizes fused sexually. The fitness of a zygote increases with its size $(x_1 + x_2)$ according to the function f. The mating success of each gamete (M) is assumed to be independent of its size in this model, though it may depend on the ratio of gamete numbers produced in the population (Togashi *et al.* 2007). Now, the fitnesses of a type-1 individual with gamete size x_1 and a type-2 individual with gamete size x_2 that mate with each other (in a population with average gamete sizes x_1^*, x_2^*) are:

$$F_1(x_1, x_1^*, x_2^*) = M_1(x_1, x_1^*, x_2^*)(R_1/x_1)f(x_1 + x_2), \tag{2.1a}$$

$$F_2(x_2, x_1^*, x_2^*) = M_2(x_2, x_1^*, x_2^*)(R_2/x_2)f(x_1 + x_2), \tag{2.1b}$$

where R_i is the total reproductive investment of an individual of mating type i. The function M_i is the mating success of gamete type i of size x_i. We assume R_i as a constant (Charnov, 1982).

Here we assume that the phenotypic variance in gamete size within each mating type in the population is sufficiently small. So the ratio of type-2 to type-1 gametes can be approximated by $R_2 x_1^* / s R_1 x_2^*$, where s is the sex ratio of individuals: (number of type 1)/(number of type 2). A small variance in size implies also that we may assume that an individual of each mating type mates with an approximately average-sized individual of the other type. It has nothing to do with population size. In this case, Equations (2.1a,b) are rewritten as:

$$F_1(x_1, x_1^*, x_2^*) = M_1(x_1^*, x_2^*)(R_1/x_1) f(x_1 + x_2^*), \tag{2.1c}$$

$$F_2(x_2, x_1^*, x_2^*) = M_2(x_1^*, x_2^*)(R_2/x_2) f(x_1^* + x_2). \tag{2.1d}$$

By associating a size with a mating type, we implicitly assume that the elements determining size in mating type i are either expressed only in type i or are tightly linked to the allele at the sex-determining locus that specifies mating type i. Except for the presence of two mating types and the mating success function, M, these fitness expressions are basically identical to those proposed by Maynard Smith (1978), and follow the basic framework of Parker *et al.* (1972). If there is a significant variance in gamete sizes of either type within the population, the mean sex ratio (number of type 2)/(number of type 1) is generally apart from the inverse ratio of mean sizes, x_1^*/x_2^*. In addition, if there is a large variance in gamete sizes within either mating type, and if reproductive success is a non-linear function of zygote size, the mean reproductive success of a zygote with an x_i gamete may not be well approximated by $f(x_i + x_j)$. Formulae (2.1c) and (2.1d) may then be inaccurate, and the following analysis may be invalid. We relax the assumption of small variance in gamete size below using an individual-based simulation model.

Given that the variance in trait values is sufficiently small to use Equations (2.1c,d), we also assume that the size of gametes of each mating type evolves independently. This is appropriate when size-determining factors for one mating type are genetically uncorrelated with size-determining factors for the other mating type. Given these assumptions, the rates of change of the mean gamete sizes are:

$$\begin{aligned} \mathrm{d}x_1{}^*/\mathrm{d}t &= g_{x1}\partial F_1(x_1, x_1{}^*, x_2{}^*)/\partial x_1|_{x1=x1^*} \\ &= g_{x1}R_1M_1(x_1{}^*, x_2{}^*)\left[-f(x_1{}^* + x_2{}^*)/x_1^{*2} + f'(x_1{}^* + x_2{}^*)/x_1{}^*\right], \end{aligned} \tag{2.2a}$$

$$\begin{aligned} \mathrm{d}x_2{}^*/\mathrm{d}t &= g_{x2}\partial F_2(x_2, x_1{}^*, x_2{}^*)/\partial x_2|_{x2=x2^*} \\ &= g_{x2}R_2M_2(x_1{}^*, x_2{}^*)\left[-f(x_1{}^* + x_2{}^*)/x_2{}^{*2} + f'(x_1{}^* + x_2{}^*)/x_2{}^*\right], \end{aligned} \tag{2.2b}$$

where g_{x1} and g_{x2} are additive genetic variances (Iwasa *et al.*, 1991), partial derivatives are evaluated at $x_1 = x_1{}^*$ and $x_2 = x_2{}^*$, and primes denote derivatives. The genetic variance parameters are often assumed to be a constant in polygenic models, but may vary with time or with mean trait values (Abrams *et al.*, 1993). If the partial derivative of fitness with respect to individual trait value is positive, mutants with slightly larger gamete sizes than the wild type are favored, and gamete size will evolve towards larger. On the other hand, if the partial derivative is negative, gamete size will decrease. It is assumed that there is a biological minimum size, denoted by $x_{\min}$, which is identical for both mating types: below this size a cell cannot fusion effectively as a gamete (Maynard Smith, 1982; Randerson and Hurst, 2002). Such a size threshold is also required as it is necessary for a gamete to at least include a complete genome, but it may be considerably larger than the size of a naked set of chromosomes (Dusenbery, 2000). If this assumption is not made, one may be led to the unrealistic conclusion that a gametophyte should produce an infinite number of gametes of zero mass (Maynard Smith, 1982).

Given above assumptions, the evolutionary equilibrium occurs where Equations (2.2a) and (2.2b) are both equal to zero. The necessary and sufficient conditions for local stability of this equilibrium point differ from the conditions for each gamete size to maximize fitness, conditional on the size of the other gamete type. The latter conditions represent a Nash equilibrium, and are equivalent to each gamete size being univadable by any mutant types with different gamete sizes. We will begin by reconsidering the conditions for a Nash equilibrium, because it helps to understand the full stability conditions for Equations (2.2a,b). The equilibrium point specified by setting equations (2.2a,b) to zero represents a local Nash equilibrium if x_1 maximizes fitness in mating type 1, conditional on x_2, and x_2 maximizes fitness of type 2, conditional on x_1. In both cases, the fitness maximization criterion is simply:

$$\partial^2 F_i / \partial x_i^2 |_{xi=xi^*} < 0 \qquad \text{for } i = 1, 2. \tag{2.3}$$

In the case of isogamy, $x_1{}^* = x_2{}^* = x^*$, the Nash solution is implicitly given by:

$$x^* = f(2x^*)/f'(2x^*). \tag{2.4}$$

This is the same solution that Maynard Smith (1978) obtained using a similar model without mating types. We use x^{**} to denote the gamete size that satisfies Equation (2.4); thus, $2x^{**}$ is the Nash equilibrium zygote size. Expanding Inequality (2.3), using Equation (2.4) shows that the Nash equilibrium isogamous condition is $f''(2x^{**}) < 0$. This means that there is an isogamous equilibrium that is univadable by mutants of small effect on gamete size, provided that Equation (2.4) has a solution for a size where zygote fitness is a decelerating function of size. The Nash solution specified by Equation (2.4) implies that a line passing through the solution x^* must be tangental to the zygote fitness curve, f, at $2x^*$; this graphical technique was first introduced by Maynard Smith (1978). We show three examples using the different fitness functions for zygote in Figure 2.1. The figure also shows the zygote size that would maximize fitness in the absence of sex (denoted z^{**}): a line passing through the origin is tangental to the zygote fitness curve at z^{**}. Isogamous Nash solutions exist for two of the three fitness curves (Figure 2.1A and C).

Condition (2.3) is a local condition for a Nash equilibrium. As pointed out by Maynard Smith (1978, 1982), a mutant with a much smaller size may be able to invade such an equilibrium. Suppose that a mutant with the minimum gamete size ($x_{\min}$) is possible and occurs in mating type 2. If

$$\begin{aligned} &F_2(x_{\min}, x^{**}, x^{**}) = (M_2 R_2 / x_{\min}) f(x_{\min} + x^{**}) > \\ &F_2(x^{**}, x^{**}, x^{**}) = (M_2 R_2 / x^{**}) f(2x^{**}) \end{aligned} \tag{2.5}$$

this mutant can invade an isogamous population where each gamete type has the size specified by conditions setting Equations (2.2a,b) to zero. Inequality (2.5) is satisfied if the ratio $x^{**}/x_{\min}$ is greater than the ratio $f(2x^{**})/f(x_{\min} + x^{**})$. This requirement is satisfied by the fitness functions, f , used in several previous models (Parker *et al.*, 1972, Maynard Smith, 1978, Bulmer 1994). However, there are two reasons why this is not a satisfactory explanation for the predominance of anisogamy. The first is that there is no guarantee that viable mutant gametes that are much smaller than the mean size can occur, be

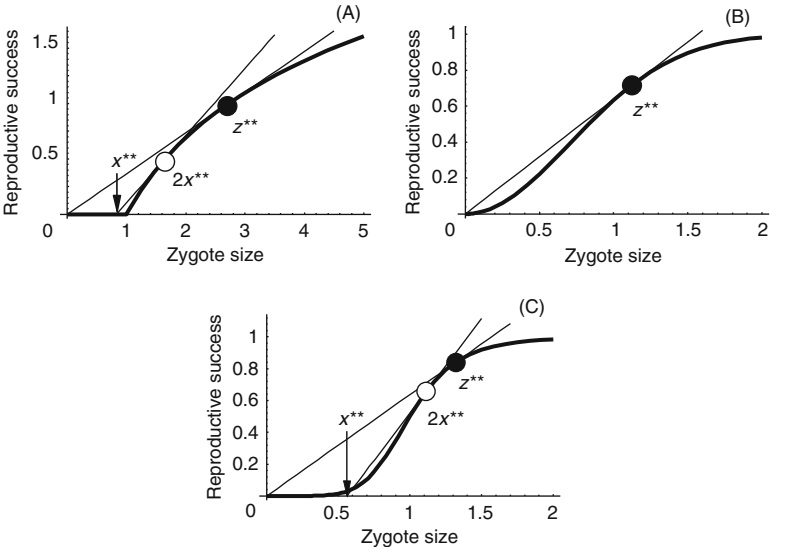

Figure 2.1 (Left) The relationship between reproductive success of a zygote $f(z)$ and its size z for. (A) $f(x) = \max(0, \log z)$, (B) $f(x) = 1 - \exp(-z^2)$ and (C) $f(x) = z^6/(1 + z^6)$. Solid circles (z^{**}) show the optimum zygote size that maximizes the parent fitness in asexual reproduction. This is approximately the zygote size at the anisogamous equilibrium when $x_{\min}$ is close to zero. Open circles ($2x^{**}$) show the symmetric Nash solution of zygote size, in which each gamete size is x^{**} and x^{**} is given by Equation (2.4). Tangents from the origin and from point (x^{**}, 0) give these solutions. See text for details.

viable and breed true. Since Fisher (1930), evolutionary theory has assumed that mutants of large effect are likely to suffer greatly reduced fitness. If only mutants of small effect are viable, then the condition for local stability of isogamy is simply that the zygote fitness function should have a negative second derivative at the equilibrium. The second is that, even if macromutations occur and produced gametes are viable, the zygote fitness function and minimum zygote size may not be always satisfy Condition (2.5). Condition (2.5) may be severe to satisfy if the minimum gamete size is not very small or if zygote fitness declines very rapidly with size, because Condition (2.5) implies that a zygote of half of the optimal size has almost zero probability of survival (Maynard Smith, 1978). Furthermore, it is quite conceivable that zygotes below a certain size have almost no probability of survival. In the absence of comparative data on the shapes of the zygote fitness versus size function, there is little

basis for arguing against zygote fitness functions that produce univadable isogamous Nash equilibria, such as that in Figure 2.1A and C. Charlesworth (1978) investigated a genetic version of a model that is equivalent to ours and found no conditions with an isogamous equilibrium at intermediate sizes. This was because he restricted his attention to a zygote fitness function with a positive second derivative ($f(x) = x^k$, where $k > 1$), which eliminates the possibility of a Nash equilibrium. There is no basis for assuming that fitness can continue to increase indefinitely at an accelerating rate with size, as implied by the function used by Charlesworth. Thus, we suggest that the previous models of the evolution of gamete sizes based on zygote fitness versus gamete number (Parker *et al.*, 1972; Bell, 1978; Maynard Smith, 1978; Hoekstra, 1980) provide little basis for believing that isogamy should be rare or taxonomically restricted.

We now turn to the conditions for the local stability of the dynamic system specified by Equations (2.2a,b). The isogamous equilibrium point of Equations (2.2a,b) is again given implicitly by Equation (2.4). The zygote size at this equilibrium is $2x^{**}$. The necessary and sufficient conditions for the equilibrium to be locally stable are that the trace of the Jacobian matrix of Equations (2.2a,b) is negative and that the determinant of that matrix is positive. When evaluated at the equilibrium point, these two conditions become:

$$f''(2x^{**}) < 0 \tag{2.6a}$$

and

$$(2f - f'x^{**})\left(2f - 3f'x^{**} + 2f''x^{**2}\right) > 0. \tag{2.6b}$$

Condition (2.6a) is identical to the condition for the equilibrium to be univadable (Nash). However, Condition (2.6b) must also be satisfied for local dynamic stability; this condition corresponds to the game-theory concept of convergence stability (Eshel and Akin, 1983; Christiansen, 1991; see Discussion). Replacing $f'x^{**}$ by f [i.e. using Condition (2.4)], Condition (2.6b) reduces to:

$$(f)\left(-f + 2f''x^{**2}\right) > 0. \tag{2.7}$$

Because f is a positive function, it is clear that Condition (2.7) cannot be satisfied if f'' is negative. If f'' is positive, then Condition (2.6a) cannot be satisfied. Therefore, the isogamous equilibrium of the dynamic system is always convergently (or dynamically) unstable. A small deviation in the mean size of one gamete type leads to further selection for

that direction of change in size, and selection for the opposite direction of change in the other gamete type.

Note that this result is independent of the form of the zygote fitness function and, therefore, constitutes a very general explanation for the rarity of isogamy, if gamete size is determined independently in each sex. Gamete sizes appear to be genetically uncorrelated in anisogamous species (Nozaki *et al.*, 2006), and it has been reported that the isogamous alga, *Chlamydomonas reinhardii*, has a mating-type locus consisting of a cluster of very tightly linked genes with diverse functions (Galloway and Goodenough, 1985). Once there is some difference in the mean size of the two gametes, there will be selection for factors that limit the expression of size influencing loci to one mating type. This is expected to lead to evolution away from an isogamous equilibrium.

2.3 Non sex-linked model when gamete size is not associated with mating type

In this section, we compare the results of the preceding section with a model based on the assumption that size-determining loci are not linked to mating-type loci and are expressed in both mating-type loci. Thus, mean sizes of both mating types are the same, and a rare mutant with a different size will occur in both mating types. Consequently, the fitness of a particular gamete size can be expressed without writing separate fitness expressions for each mating type. In this case, therefore, there is no distinction between the sizes of the two gamete types, and the fitness of an allele of size x in a population with size x^* is proportional to $F(x, x^*) = (R/x)[f(x + x^*)]$. Again $\mathrm{d}x^*/\mathrm{d}t$ is proportional to $dF(x, x^*)/dx$, evaluated at $x = x^*$. The fitness function F is identical to that in Maynard Smith's (1978) model. It is easy to verify that, if there is an equilibrium with $f'' < 0$, it is locally stable – that is, stability conditions are the same as for a Nash equilibrium in the sex-linked model. Stability of such an isogamous equilibrium, which is also convergently stable, to invasion by microgametes is described by Inequality (2.5). If microgametes can occur and invade, the result is dimorphism of both mating types: in such cases, microgamete producers would not expunge macrogamete producers, because the advantage of microgamete producers decreases with increasing frequency of microgamete producers. As Charlesworth (1978) has argued, such a situation will favor reduction in recombination, and each size will eventually become associated with a particular mating type. If there are no mating

types, the above fitness function, $F(x, x^*) = (R/x)[f(x + x^*)]$, applies to all individuals and the predicted evolutionary outcome is the same. If there are three or more mating types, the analysis becomes difficult, but such situations appear to be very rare (Hoekstra, 1987).

There are generally two asymmetric (anisogamous) stable equilibria at which one of the gametes has the minimum possible size. We can assume that ${x_1}^* > {x_2}^*$ at that equilibrium without loss of generality. Because of this size relationship, we will refer to x_1 and x_2 as the egg and sperm size, respectively. At the stable equilibrium, sperm size is

$${x_2}^* = x_{\min} \tag{2.8a}$$

and egg size is implicitly given by

$${x_1}^* f'({x_1}^* + x_{\min}) = f({x_1}^* + x_{\min}). \tag{2.8b}$$

The resulting stable zygote size is the value of ${x_1}^* + x_{\min}$ that satisfies Equation (2.8b). Note that if $x_{\min}$ is close to zero, egg size will be very close to the size of an optimally sized asexual offspring. The latter maximizes $(R/x)f(x)$, so it is specified by $xf'(x) = f(x)$.

2.4 Analyses of the Sex-Linked and Non Sex-Linked Models

We present three examples of the evolution of gamete sizes using the three different functional forms of the zygote fitness function, f: (A) $f(z) = \max(0, s\log(z))$, where s is a positive constant; (B) $f(z) = 1 - \exp(-z^2)$ (used by Bulmer 1994) and (C) $f(z) = z^6/(1 + z^6)$. These three functions are roughly similar in form, with zygote fitness being extremely low at small size, accelerating at larger sizes, and then decelerating and leveling off. We assume that $x_{\min} = 0.001$ in Figures 2.1 and 2.2. In case A, the Nash solution is $x^{**} = 0.824$. Because $f(z) = 0$ for $z < 1$, a mutant with any gamete size less than 0.176 will clearly not be able to increase, and we can show that the Nash solution is univadable by any mutant smaller than the Nash value. Thus, this is an example in which microgametes cannot invade the isogamous equilibrium (see Figure 2.1A). However, if size evolves independently in each sex, isogamy is convergently unstable and the gamete sizes approach $x_1 = 2.718$ and $x_2 = x_{\min} = 0.001$, as shown in Figure 2.2A. In case B (Figures 2.1B and 2.2B), there is no positive Nash solution, since $(\partial F/\partial x)$ evaluated at $x = {x_1}^* = {x_2}^*$ is always negative. The

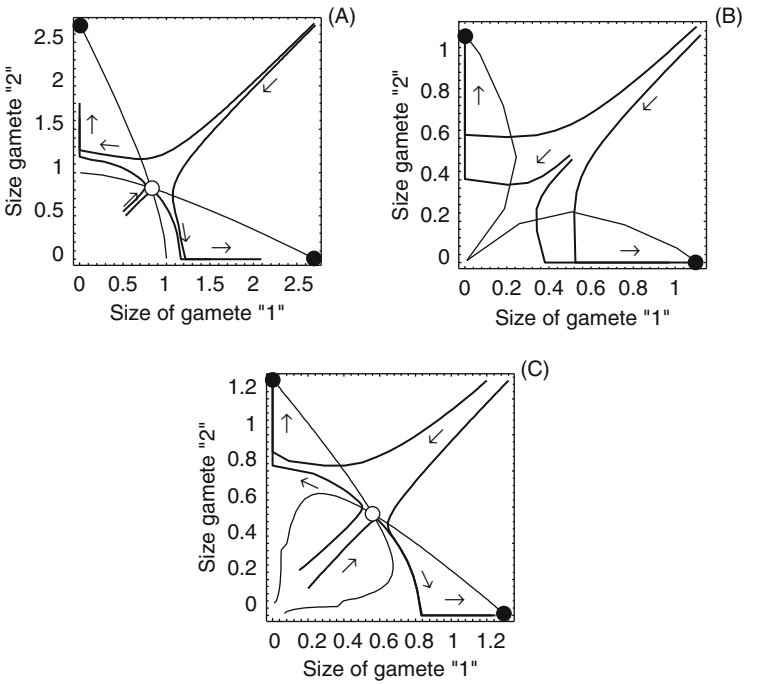

Figure 2.2 (Right) Evolutionary dynamics of egg size (the larger gamete) and sperm size (the smaller gamete) for the three zygote fitness functions used in Figure 2.1. The narrow lines show the isoclines for Equations (2.2a,b) with each fitness function. The bold lines are two pairs of evolutionary trajectories obtained using Equations (2.2a,b); two of these begin near the isogamous equilibrium, and two begin with both gametes having close to the asexual optimal size. The solid circles show the anisogamous equilibria given by Equations (2.8a,b). The open circles show the symmetric (isogamous) Nash solution given by Equation (2.4).

anisogamous equilibrium (1.12, $x_{\min}$) is convergently stable, as shown in Figure 2.2B. In case C, there is a Nash solution (0.561, 0.561). This differs from case A, where a mutant with gamete size $x_{\min}$ can invade the Nash equilibrium in this case. However, even if such small mutants cannot occur, the isogamous Nash solution is convergently unstable when gamete size is determined independently in each sex. In this case, the gamete sizes approach 1.307 and $x_{\min}$, as shown in Figure 2.2C. If size is determined by loci that affect both sexes and are not closely linked to the sex-determining locus, then the isogamous equilibrium in case C is locally stable, but is globally unstable if micromutants are possible.

2.5 COST OF SEX

It is common to associate the twofold cost of sex with anisogamy: parthenogenetic females enjoy a twofold selective advantage because they do not waste energy on producing sons. The current distribution of isogamy and anisogamy may have been influenced by interclade competition between lineages that are similar except for mating type. It is therefore instructive to compare the rate of increase of a population whose gamete sizes are described by the various potential equilibria in each of these examples. In each case, the rate of increase at the anisogamous equilibrium is half the rate of increase of the asexual equilibrium; zygote size is identical in asexual and anisogamous populations, but only half of the individuals in the anisogamous sexual population (i.e. females) contribute significant biomass to the zygotes. The rate of increase at the isogamous equilibrium is reduced by having smaller-than-optimal zygote sizes, even though all individuals contribute significantly to zygote biomass. The ratio of fitness at the isogamous equilibrium to that at the asexual equilibrium is approximately 0.93 in example C and 0.82 in example A. Thus, in each of these cases, the transition from isogamy to anisogamy would result in less than a twofold cost in terms of population growth rate. It is possible for the transition from isogamy to anisogamy to result in an increase in fitness. If we generalize example C, the zygote fitness function is $f(z) = z^n/(1 + z^n)$. There is an isogamous equilibrium for $n > 2$, and this equilibrium is locally stable provided that sex- and size-determining loci are not closely linked. There is also an anisogamous equilibrium for all finite values of n, provided that the minimum gamete size is sufficiently small. The fitness at the isogamous equilibrium, relative to asexual fitness, shows that the fitness of isogamous sexuals is less than half the fitness (rate of increase) of asexual forms for $2 < n < 2.183$. Thus, for a limited range of parameter values in this model, evolving anisogamy increases population growth rate. The population growth rate of the anisogamous form is always approximately half that of the asexual when the smaller gametes are very small. Although a lower growth rate of isogamy relative to anisogamy occurs for a limited range of parameters in this model, the cost of anisogamy is always less than twofold and often substantially less.

Another example is the zygote fitness function, $f(x) = \max [0, b(x - x_0)/(1 + a(x - x_0))]$. This is a Monod function that is zero until $x = x_0$, where x_0 is the minimum size for survival. In this case, the ratio of the fitness of an isogamous species to an asexual species is a function of the product ax_0. In this case, either a large half-saturation

constant, $1/a$, or a small minimum zygote size for survival, x_0, results in a substantial disadvantage of isogamy relative to asexuality, although this is never as large as the twofold cost of anisogamy. Given that we know almost nothing about the form of the zygote fitness versus size function, it is difficult to discuss further the relative rates of increase of isogamous and anisogamous lineages of the same species.

In the next two sections, we extend the basic model considered here in two ways. The first looks at a much more detailed and realistic model to consider whether the assumptions of little variance in size within a gamete type and infinite population sizes, on which we depend in Equations (2a,b), might be have significantly biased the results. The second returns to the simple analytical model, but includes the possibility that a gamete's size can have a direct effect on either its survival or its probability of achieving fertilization (i.e. gamete success).

2.6 Simulation using an individual-based model

To investigate cases in which the variance in gamete sizes within a mating type is large and the population sizes are finite, we use a simple "individual-based model" (DeAngelis and Gross, 1992; Kawata and Toquenaga, 1994). In the individual-based model, the population composition is changed in two steps – selection and mutation. We will consider the selection process first. To avoid complexity in mathematical modeling from genetic structures, we assume that the population consists of N hermaphrodite individuals: each of which invests an amount R for "female" gametes and an amount r for "male" gametes. The following results do not change, irrespective of the ratio R/r. Each individual, i, is haploid and is characterized by two quantitative traits, egg size (x_i) and sperm size (y_i), for individual i ($i = 1, 2, \ldots, N$). (We do not use our earlier notation of x_1 and x_2 to avoid double subscripts.) Individual i produces R/x_i female gametes with size x_i and r/y_i male gametes with size y_i. We assume that the mating success per gamete is 1 for the sex with a smaller total number of gametes, and that the ratio of the abundances of the two gamete types for the sex with the larger number of gametes is:

$$M = \min[1, \Sigma_i(r/y_i)/\Sigma_i(R/x_i)] \text{ and } m = \min[1, \Sigma_i(R/x_i)/\Sigma_i(r/y_i)]. \tag{2.9}$$

If the number of gametes in the population is sufficiently large, the mean reproductive success of gamete i is $\Sigma_j f(x_i + y_j)/N$ for a female

gamete and $\Sigma_j f(x_j + y_i)/N$ for a male gamete. Because of non-linearity of f, the mean fitness of zygotes involving eggs of individual i, $\Sigma_j f(x_i + y_j)/N$, differs from the fitness $[f(x_i + y^*)]$ of an egg that combines with a sperm having the mean sperm size in the population $(y^* = \Sigma_j y_j/N)$. Therefore, the expected fitness of individual i (denoted by H_i) is given by:

$$H_i = F_1 + F_2 = (RM/x_i)\Sigma_j f(x_i + y_j)/N + (rm/y_i)\Sigma_j f(x_j + y_i)/N. \tag{2.10}$$

To generate a phenotypic distribution for the next generation, we pick N numbers independently from 1, 2,..., N with probability $H_i/\Sigma_j H_j$. Thus, individuals with higher expected fitness have a greater chance of being chosen, and may be chosen more than once. After making N selections, we renumber individuals to 1, 2,..., N ($N = 256$ in the simulations here).

In the second step of the simulations, each phenotype may change its trait value because of micromutation. We assume that individual i has a female gamete size $x_i[1 + \exp(a)]$, where a is a uniformly random variable between -0.005 and 0.005. Mutations affecting male gamete size occur in the same way, and are independent of mutations in female gamete size.

The evolution of gamete sizes proceeds by iteration of these two steps. We assume that the initial phenotypic distributions are given by $x_i = x^{**}[1 + \exp(a)]$ and $y_i = x^{**}[1 + \exp(a')]$, where x^{**} is implicitly given by Equation (2.8b) and both a and a' are again independent random variables that are uniformly distributed between -0.005 and 0.005. We used the zygote fitness function from cases A and C of Figures 2.1 and 2.2: $f(z) = \max[0, s\log(z)]$ and $f(z) = z^6/(1 + z^6)$. In both case, isogamy was again convergently unstable under these assumptions. In spite of a significant effect of genetic drift in the population mean trait values and a large variance in the sizes of eggs, mean sperm size decreased and approached the minimum size in all simulations.

2.7 Direct Effects of Size on Gamete Fitness

In this section, we consider some direct effects on gamete success (i.e. either gamete survival or rate of encountering – or success in combining with – members of the opposite gamete type). Because the possible consequences of such effects are best investigated by

returning to our analytical model, we modify the fitness formulae in Equations (2.1a,b) by multiplying each by a survival rate function, denoted $l(x_i - x_0)$, where x_0 is the size that maximizes gamete survival. The function l reaches a maximum value (<1) when its argument is zero. Because we are interested in the stability of an initially isogamous system, we assume that each gamete type has an identical survival function. [After anisogamy has become established, the survival functions for the two mating types might come to differ from each other (see Bulmer and Parker, 2002).] Equations (2.2a,b) then become:

$$\begin{aligned} \mathrm{d}x_1^*/\mathrm{d}t = {} & g_{x1}R_1M_1(x_1^*, x_2^*)[-l(x_1^*)f(x_1^* + x_2^*)/x_1^{*2} \\ & + l(x_1^*)f'(x_1^* + x_2^*)/x_1^* + l'(x_1^*)f(x_1^* + x_2^*)/x_1^*] \end{aligned} \tag{2.11a}$$

$$\begin{aligned} \mathrm{d}x_2^*/\mathrm{d}t = {} & g_{x2}R_2M_2(x_1^*, x_2^*)[-l(x_2^*)f(x_1^* + x_2^*)/x_2^{*2} \\ & + l(x_2^*)f'(x_1^* + x_2^*)/x_2^* + l'(x_2^*)f(x_1^* + x_2^*)/x_2^*]. \end{aligned} \tag{2.11b}$$

The stability conditions for the isogamous equilibrium are more complex than those in the other analyses above. However, the important features can be seen without the full conditions. The first local stability condition, analogous to Equation (2.6a), is that the trace of the Jacobian matrix of System (2.11) is negative; this must again be satisfied at a Nash equilibrium. The second stability condition, that the determinant of the Jacobian is positive, is given by:

$$\begin{aligned} & [R_1R_2\ M_1M_2\,(2fl - xlf' - 2xfl' + x^2f'l' + x^2fl'') \\ & \quad (2fl - 3xlf' - 2xfl' + 3x^2f'l' + 2x^2lf'' + x^2fl'')]/x^6 \\ & \quad = [R_1R_2M_1M_2\,(xlf' + x^2f'l' + x^2fl'')\,(-xlf' + 3x^2f'l' + 2x^2lf'' + x^2fl'')]/x^6 > 0. \end{aligned} \tag{2.12}$$

Because the left-hand side of this inequality is an increasing function of the absolute value of l'', a large magnitude of l'' will always result in convergence stability. In reaching the final expression in Equation (2.12), we used the equilibrium condition $xlf' + xfl' = fl$, which is analogous to Equation (2.4). Because the gamete fitness function, l, has a maximum at $x_i = x_0$, its second derivative is expected to be negative unless the isogamous equilibrium is far from the size that maximizes gamete survival. The magnitude of l'' is a measure of the strength of stabilizing selection due to the direct effects of size on gamete survival and mating success. As one would expect, sufficiently strong direct selection can stabilize an isogamous equilibrium.

This result may be illustrated by considering $l(x) = l_{\max} - (x - 0.5)^2$ and $f(z) = \max(0, s\log(z))$. In this case, the symmetric Nash solution, $x^{**} = 0.725$, is also convergently stable. However, this Nash solution is not globally stable; the basin of attraction of this equilibrium depends on parameter values and is affected by the functional forms of $l(x)$ and $f(z)$. There are two alternative anisogamous equilibria in this system. It is important to note that the system can be shifted to one of the anisogamous equilibria without the immediate invasion of microgamete producers. Cox and Sethian (1985) also found alternative stable equilibria for both isogamy and anisogamy in a model of gamete size evolution where there was direct selection on gamete size due to effects on motility. However, they did not include separate mating types.

2.8 DISCUSSION

The theory presented here adds to the number of potential theories on the stability of isogamy. We first explain how our models account for the rarity of isogamy, and then discuss previous theories.

The models investigated in this chapter suggest that there are two central conditions, either of which could result in the persistence of isogamy. The first is that size-determining factors do not become linked to sex-determining factors in the genome. The second is that there is strong, direct stabilizing selection on gamete size, as the result of size-associated differences in gamete success (i.e. gamete survival or mating success). Of the factors that influence gamete size, it is likely that some will be tightly linked to the sex-determining locus. This provides a mechanism for the beginning of evolution towards anisogamy. Once the mean sizes of gametes of different mating types are unequal, there is further selection to reduce recombination between size and sex loci (Charlesworth, 1978). It is clear that, currently, anisogamous species have achieved tight linkage or sex-limitation of expression of size-determining loci (Nozaki *et al.*, 2006). This may suggest that the path from anisogamy back to isogamy is effectively blocked. Even if there is a change in the environment that leads to a fitness function with a globally univadable isogamous equilibrium (a Nash equilibrium), the linkage of size- and sex-determining factors will prevent the return to that equilibrium. On the other hand, isogamous equilibria are always susceptible to destabilization by a change in the shape of the zygote fitness function that allows an increase of types producing small gametes, or the linkage of size- and sex-determining factors in the

genome. At least many locally stable isogamous equilibria are invadible by gametes that are sufficiently small. All of this suggests that there should be a long-term evolutionary trend away from isogamy.

The explanation favored in previous discussions of this topic is that the isogamous equilibria determined by most zygote fitness functions permit the invasion of microgametes, with the concomitant establishment of anisogamy. This argument depends on: (1) high rates of recombination between sex-determining and size-determining loci, (2) a possibility of viable microgametes arising *de novo* in a species with much larger gametes and (3) zygote fitness functions and minimum gamete sizes that satisfy the invasion criteria given by Inequality (2.5). It is possible that all of these prerequisites are frequently satisfied. We know very little about the genetic architecture determining gamete traits, the shape of zygote size versus fitness relationships, or the possibility of successful macromutants. At most, however, this seems likely to provide one of a number of possible explanations for the comparative lack of isogamy.

The model considered in most detail here is based on approximations that are valid if the phenotypic variance is sufficiently low, if the selection is sufficiently weak, and if fitness of each individual depends only on its own trait value and the population mean trait value (Iwasa *et al.*, 1991; Abrams *et al.*, 1993). These assumptions were relaxed in the analysis using an individual-based simulation model that allowed a large phenotypic variance and strong selection in which difference in fitness between individuals is large. Some assumptions, such as a uniform distribution of mutational effects on gamete sizes, haploidy and a fixed relatively small population size are made in the simulation. However, it is unlikely that these assumptions would affect the basic result; in the presence of linkage between size- and sex-determining factors and in the absence of direct effects of size on gamete success, isogamy is convergently unstable. We do not rule out the occurrence of stable isogamy under some circumstances. But the agreement of the analytical results (based on small variances, infinite population sizes and weak selection) and the simulations suggests that it is unlikely. The analytical results are valid for any form of the zygote fitness function. Thus, it is probable that the instability of isogamous equilibria is a very common feature in natural systems.

As was noted in the Introduction, the comparative patterns of isogamy have proven difficult to explain in some taxa. Knowlton (1974), Maynard Smith (1978) and Bell (1978) argued that larger and/or more complex organisms should have a steeper relationship

between zygote size and zygote fitness. They argued that steeper relationships were more likely to favor anisogamy, and reasoned that this should lead to a correlation between adult size and anisogamy. This trend appears to be true in some algae (Bell, 1978; Madsen and Waller, 1983), but there are many exceptions. The argument is weakened by the lack of concrete knowledge of the form of the zygote fitness functions. The correlation can also be viewed as a consequence of the smaller zygote size that arises from gamete size evolution under isogamy. If smaller zygotes tended to produce smaller adults, this would translate into an association between isogamy and smaller adult size.

The one factor in our models that can produce long-term stability of isogamy is direct stabilizing selection on gamete size. There are many potential reasons for such selection. Gametes in organisms with external fertilization are likely to experience selection on size because size affects mobility, maximum longevity and probability of being consumed by predators. These factors are discussed in Togashi and Bartelt's chapter (Chapter 7). Our results show that isogamy (at a size larger than the minimum possible gamete size) is more likely to be explained by direct effects of size on gamete fitness, rather than by particular forms of the zygote fitness function. From these results it is predicted that isogamy should be more common in species whose gametic phase is a larger fraction of the entire life history. Because smaller forms often have shorter adult phases, this could also account for the association of isogamy with small adult body size (Bell, 1978; Madsen and Waller, 1983).

It is common to associate the twofold cost of sex with the presence of anisogamy (e.g. Maynard Smith, 1978; Bell, 1982; Michod, 1995). However, we suggest that populations with stable isogamy also experience a cost relative to asexual forms, because isogamy results in zygotes that are smaller than the fitness-maximizing size. It is theoretically possible, although perhaps unlikely, that the cost of sexual isogamy exceeds the twofold cost of anisogamy. But at least the disadvantage of anisogamous lineages relative to similar isogamous lineages is likely to be considerably less than twofold.

The isogamous equilibria in our basic models are cases where the size of each gamete type has a higher fitness than all other alternative sizes. Nevertheless, when size-determining loci are sex-linked or sex-limited in their effects, such equally sized gametes cannot persist. Previous work on evolutionary dynamics has provided other examples where univadable equilibria (Nash equilibria or evolutionarily stable strategies (ESSs)) can be dynamically unstable or genetically

unattainable (Eshel and Motro, 1981; Lande, 1981; Eshel and Akin, 1983; Thomas, 1985; Cressman *et al*., 1986; Taylor, 1989; Charlesworth, 1990; Hastings and Hom, 1990; Lessard, 1990; Christiansen, 1991; Abrams *et al*., 1993; Geritz *et al*., 1997; 1998). The isogamous equilibria that arise here are local ESSs but are not "convergence stable" (using the terminology of Christiansen, 1991; Geritz *et al*., 1997; 1998). Eshel and Akin (1983) were the first to point out the possibility of convergence instability of Nash equilibria, in systems of two or more evolving entities. If the evolution of anisogamy in multicellular organisms arose from isogamy, convergence instability is likely to have played a major role in the origin of anisogamy and of other asymmetries of gender.

It has been known that *Drosophila* species have giant sperm (Pitnick and Markow, 1994; Pitnick *et al*., 1995; Karr and Pitnick, 1996). Its lengths are often greater than adult body length. Thus, it is clear that male gametes do not always evolve to the smallest possible size. Only a part of these giant sperm usually enter the egg (Karr and Pitnick, 1996). These phenomena are beyond the scope of the theory in this chapter. The functional significance of giant sperm is still very unclear, but, at least in some cases, it is unlikely that they contribute significantly to zygote nutrition (Pitnick *et al*., 1995).

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3

Contact, not conflict, causes the evolution of anisogamy

3.1 INTRODUCTION

In common with all religions, biology offers a creation story for male and female. Unlike religious myths, biology's creation stories are scientific claims and subject to scientific examination. Today, the mainstream evolutionary theories for the origin of male and female propose that sexual conflict causes the evolution of the distinction between males and females. In this chapter we propose instead that the male/female distinction did not arise from sexual conflict, but as a tactic to maximize the contract rate between gametes. This chapter follows closely the treatment in Iyer and Roughgarden (2008) and Roughgarden (2009).

Anisogamy defines the distinction between the sexes – the male individual or organ is characterized by production of small gametes (sperm) and the female by the production of large gametes (eggs) (Stearns, 1987). Isogamy – the production of equally sized gametes, may be the ancestral condition because its occurrence is restricted to primitive taxa among algae, fungi and protozoa (Bell, 1978). Hence the origin of males and females can be traced back to the evolution of anisogamy from isogamy.

Kalmus (1932) was the first to propose a model for the evolution of anisogamy. He supposed that the size of gametes produced by each sex traded off against the number of gametes produced. Zygotes are formed by collisions between eggs and sperm and hence the number of zygotes produced by a population is proportional to the product of the

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total number of eggs and the total number of sperm produced by its members. Further, assuming an optimal zygote size, Kalmus derived conditions under which an anisogamous population produced more zygotes than an isogamous one. This model was modified by Scudo (1967) to incorporate the depletion of gametes as they underwent fertilization.

The intuition underlying the contact hypothesis for anisogamy is that a parent is dividing the material it can place into eggs and sperm to maximize the number of viable zygotes it produces. If both proto-sperm and proto-egg are the same size, then only so many of them bump into each other. The number of gametic contacts increases as gametes become more numerous, forming a more dense cloud of gametes. If both gametes could be made tiny, then when a dense cloud of tiny sperm mixes with another dense cloud of equally tiny eggs, the highest number of contacts would occur. But egg and sperm cannot both be tiny and still produce zygotes big enough to survive. So, the maximum number of contacts producing viable zygotes then occurs when one of the gametes is nearly the desired zygote size while the other is as small as possible. A sparse cloud of large zygote-sized gametes colliding with a dense cloud of tiny gametes produces more contacts than do collisions between two medium-dense clouds, each with medium-sized gametes. Thus, one possible advantage to gametic dimorphism in size is simply to maximize the number of contacts between gametes that yield zygotes big enough to survive.

The Kalmus contact hypothesis was dismissed during the 1970s by Parker, who argued that selection for anisogamy to maximize contact rate required group selection, and lacked a plausible explanation in terms of individual-level natural selection. As Parker explained in 1978, "Earliest analyses of the evolution of anisogamy (Kalmus, 1932; Kalmus and Smith, 1960; refined by Scudo, 1967) were mainly dependent on group or population selection. Where there is a fixed amount of gametic reserve available in the population, and where gametes require a certain quantity of reserve for survival and development, these authors were able to show that the greatest number of successful fusions occurs when the gametic reserve is divided with a high degree of anisogamy than with isogamy" (Parker, 1978). In 1979, Parker further explained, "Though there are theories for the establishment of anisogamy through group or inter-population selection (Kalmus, 1932; Kalmus and Smith, 1960; Scudo ,1967), the 'anisogamy via disruptive selection' theory [Parker's name for his own theory] relies entirely on immediate forms of selection" (Parker, 1979).

Thus, as of the early 1970s, the contact-rate advantage to anisogamy was believed to require group selection. Indeed, the Kalmus and Scudo papers did demonstrate a population-growth-rate advantage to anisogamy. However, the fact that a trait confers a population advantage does not imply that it cannot also confer an individual advantage. Nonetheless, Parker and colleagues (Parker *et al.*, 1972) introduced a model for the evolution of anisogamy that they claimed explicitly relied on individual selection that continues to remain fundamental to a number of later models.

The Parker *et al.* (1972) model considers a situation where each individual releases a number gametes of one size (where again, number trades-off with size) into the gamete pool. Any two gametes can fuse with each other to form a zygote whose volume is the sum of the volumes of the individual gametes. Further, it is assumed that zygote fitness (survival probability) is an increasing function of its size. The authors find that when the zygote fitness is an accelerating function of zygote size (i.e. increments in zygote size confer more than proportional increments in fitness, henceforth referred to as "disproportionate" fitness dependence on size), disassortative selection favors individuals making extreme-sized gametes over individuals making gametes of intermediate sizes. Parker *et al.* interpret the resulting gamete size dimorphism as conflict between the gamete producers, with anisogamy resulting from the small sperm successfully flooding the gamete pool and the eggs being forced to compensate to preserve zygote viability. To quote Parker, "The primordial sexual conflict concerned the establishment of anisogamy itself. Ovum-producers would fare better without males to 'parasitize' the investment in each ovum. Males are likely to have won this earliest conflict . . ." (Parker, 1979).

This interpretation remains uncontested and influential to this date, and anisogamy is regarded as the first instance of sexual conflict. In his 2006 paper, Parker says "Sexual selection arises ultimately from anisogamy, and a primitive form of sexual conflict may have occurred during the early evolution of anisogamy, such that early ova might profit by fusing with other proto-ova rather than with proto-sperm" (Parker, 2006). And Arnqvist and Rowe claim in their 2005 book on sexual conflict that "there is good reason to believe that "primordial" reproductive conflict in isogamous taxa has led to the evolution of anisogamy, and consequently the sexes" (Arnqvist and Rowe, 2005). Later theoretical work on the evolution of anisogamy has either not contested, or has extended, the interpretation that anisogamy results

from gametic sexual conflict (Charlesworth, 1978; Matsuda and Abrams, 1999; Bulmer and Parker, 2002; Maire *et al.*, 2002).

We have questioned this interpretation of genetic conflict between gametic strategies as the evolutionary cause for anisogamy. Instead we argue that the Parker *et al.* (1972) interpretation comes about by ignoring the insight from the seminal group-theoretic models – small gametes increase encounter rates. To remedy the need to think of contact rates evolution in terms of group selection, we propose an individual selection model for how anisogamy could be selected.

3.2 MODEL

3.2.1 Hermaphroditic populations

We consider a population of hermaphroditic organisms where both egg and sperm sizes are jointly coded by the same gene. Hence these are not two sexes of individuals and a gene that increases to fixation improves the fitness of an individual. We show that the evolutionary dynamics culminates in the fixation of a single allele that in some circumstances, maximizes the gametic size difference. The dynamics in our model always result in an increase in the absolute fitness, and hence do not correspond to sexual conflict. The evolution of increased size difference between the gametes, when it occurs, is a positive adaptation for the individuals in the population.

The selection acting on gamete size is for increased incorporation into zygotes that survive to adulthood. As also assumed by Parker *et al.* (1972) and Charlesworth (1978), we suppose that the probability of gametic encounters depends on the gamete frequencies, and the survival probability of the zygote depends on its size. We assume that the only viable fusions are those between eggs and sperm. (This assumption is relaxed in Iyer and Roughgarden, 2008). We first considered the short-term dynamics that occur when a mutant allele is introduced to a gene pool at equilibrium and that culminates at another equilibrium. We then examined the long-term dynamics of the model by looking, with computer simulations, at a sequence of alleles that invade the population, each such event being modeled by the short-term dynamics.

Suppose the investments by each hermaphrodite in egg production and in sperm production are both equal to Q. We develop a population genetic model for evolution at a locus that codes jointly for the egg size (E) and the sperm size (S), and begin by assuming that the only viable fusions are those between eggs and sperm. The effective

allocation to each sex is fixed at Q because selection makes the allocation to sperm production equal the allocation to egg production (Charnov, 1982)

The fitness of each individual depends on the number of *zygotes* it produces and the probability that each of them survives to adulthood. Zygotes are formed by *collisions* between gametes released into a gamete pool. Hence, under conditions of low gamete density (which is prevalant among free-spawning species, Levitan and Petersen 1995), the total number of zygotes formed in the population is proportional to the product of the number of sperm and the number of eggs. We assume that the fitness dependence of each zygote on its initial size ($z = E + S$) is given by the Vance survival function (Vance, 1973): if the final size to which the zygote grows is z_0, the zygotic growth rate is r and the instantaneous mortality rate is μ, then the development time to adult size is $(z_0 - z)/r$, and the probability of surviving this long is $e^{-\mu\frac{z_0-z}{r}}$. We assume that a zygote of size greater than z_0 has the same survival probability as a zygote of size z_0.

Bulmer and Parker (2002) also claim to consider the evolution of anisogamy assuming the Vance function for zygote survival. However, the function they use is not the Vance survival function, but a modified form proposed by Levitan (2000) that adds to the original Vance equation the additional assumption that the development time is inversely proportional to zygote size. Although this specific allometric assumption may help explain echinoid egg size distribution, there is insufficient reason to expect this Levitan–Vance formula to hold in general.

For subsequent use in the model, we define the function

$$\begin{aligned} w(E,S) &= \frac{e^{k(S+E-z_0)}}{SE}, E+S \le z_0, \\ w(E,S) &= \frac{1}{SE}, \qquad E+S > z_0, \end{aligned} \tag{3.1}$$

where $k = \mu/r$. Hence k measures net survival risk, taking into account both mortality rate and time of exposure.

To study the short-term model dynamics, we first consider the case where there are only two alleles in the gene pool. Suppose allele 1, present in the adult population with frequency x_1, codes for gametes of size (E_1, S_1), and allele 2 present with frequency $x_2 = 1 - x_1$ codes for (E_2, S_2). If the genotype frequencies of genotypes 11, 12 and 22 are a, b and c respectively, then $x_1 = a + b/2$ and $x_2 = c + b/2$. Consider now the heterozygote hermaphrodite with genotype 12. It allocates Q to

sperm, and also Q to eggs. Furthermore, let us suppose it divides its total sperm allocation evenly into producing sperm of the sizes associated with allele 1 and with allele 2, respectively. Hence, it produces $\frac{Q}{2S_1}$ sperm of size S_1 which carry allele 1, and $\frac{Q}{2S_2}$ sperm of size S_2 carrying allele 2. Similarly, the heterozygote divides its total egg allocation evenly into producing eggs of the sizes associated with allele 1 and with allele 2, respectively. Hence, it produces $\frac{Q}{2E_1}$ eggs of size E_1 and $\frac{Q}{2E_2}$ eggs of size E_2. This assumption is consistent with gamete sizes being determined by the genotype of the gamete, and greatly simplifies the model analysis.

3.2.2 Synchronous spawners

Suppose the population consists of N adults who spawn synchronously. Eggs with allele 1 are then produced by the Na adults with genotype 11, each of whom produces $\frac{Q}{E_1}$ eggs, and by the Nb adults with genotype 12, each of whom produces $\frac{Q}{2E_1}$ eggs. Hence the gamete pool contains $Na\frac{Q}{E_1} + Nb\frac{Q}{2E_1} = Nx_1\frac{Q}{E_1}$ eggs of size E_1 carrying allele 1. Similarly, the $Nb + Nc$ adults with genotypes 12 and 22 respectively produce a total of $Nx_2\frac{Q}{E_2}$ eggs of size E2 carrying allele 2. Likewise, the Na 11-genotype hermaphrodites and the Nb 12-genotype hermaphrodites each produce $\frac{Q}{S_1}$ and $\frac{Q}{2S_1}$ sperm of size S_1 respectively. Hence the gamete pool contains $Nx_1\frac{Q}{S_1}$ sperm carrying allele 1 and $Nx_2\frac{Q}{S_2}$ sperm carrying allele 2.

3.2.3 Genotype 11

Zygotes with genotype 11 are formed by collisions between eggs carrying allele 1 and sperm carrying allele 1. Hence the number of such zygotes formed is proportional to the product of the number of eggs in the gamete pool carrying allele 1, $Nx_1\frac{Q}{E_1}$, and the number of sperm carrying allele 1, $Nx_1\frac{Q}{S_1}$. Thus, the number of zygotes formed with genotype 11 is proportional to $\frac{x_1^2}{E_1S_1}$. Each of these zygotes is of the size $E_1 + S_1 \leq z_0$ and survives to adulthood with probability $e^{-\mu\frac{z_0-(E_1+S_1)}{r}}$ if $E_1 + S_1 \leq z_0$ and with probability 1 if $E_1 + S_1 > z_0$. Overall, the number of adults with genotype 11 contributing to the next generation from this spawning event is proportional to $x_1^2 w(E_1, S_1)$, where $w(E, S)$ is defined in Equation (3.1).

3.2.4 Genotype 1.3

Zygotes with genotype 12 can be formed by collisions between the $Nx_1\frac{Q}{E_1}$ eggs with allele 1 and the $Nx_2\frac{Q}{S_2}$ sperm with allele 2. The number of such zygotes formed is proportional to $\frac{x_1x_2}{E_1S_2}$. Each such zygote is E_1+S_2 big, and survives to adulthood with probability $e^{-\mu\frac{z_0-(E_1+S_2)}{r}}$ if $E_1+S_2\leq z_0$ and with probability 1 if $E_1+S_2>z_0$. Hence the number of adults formed by collisions between allele 1 eggs and allele 2 sperm is proportional to $x_1x_2w(E_1,S_2)$. Adults with genotype 12 are also produced by the survival of zygotes formed by collisions between eggs with allele 2 and sperm with allele 1. The number of these adults is proportional to $x_2x_1w(E_2,S_1)$. Hence the total number of adults with genotype 12 contributing to the next generation from this gamete pool is proportional to $x_1x_2w(E_1,S_2)+x_2x_1w(E_2,S_1)$. Likewise, the number of individuals with genotype 22 in the next generation is proportional to $x_2^2w(E_2,S_2)$.

3.2.5 Frequency dependent collisions

The frequency of allele 1 in the new generation is obtained by counting each 11-homozygote offspring twice and each 12-heterozygote once:

$$\begin{aligned}x_1' &= \frac{2x_1^2w(E_1,S_1)+x_1x_2w(E_1,S_2)+x_1x_2w(E_2,S_1)}{2x_1^2w(E_1,S_1)+2x_1x_2w(E_1,S_2)+2x_1x_2w(E_2,S_1)+2x_2^2w(E_2,S_2)}\\ &= x_1\frac{x_1w_{11}+x_2w_{12}}{\bar{w}},\end{aligned} \tag{3.2}$$

where

$$w_{11}=w(E_1,S_1)\qquad w_{22}=w(E_2,S_2)\qquad w_{12}=(w(E_1,S_2)+w(E_2,S_1))/2$$

and $\bar{w}$ is the mean fitness:

$$\begin{aligned}\bar{w} &= x_1^2w(E_1,S_1)+x_1x_2w(E_1,S_2)+x_2x_1w(E_2,S_1)+x_2^2w(E_2,S_2)\\ &= x_1^2w_{11}+2x_1x_2w_{12}+x_2^2w_{22}.\end{aligned} \tag{3.3}$$

Similarly, the frequency of allele 2 in the new generation is

$$x_2' = x_2\frac{x_1w_{12}+x_2w_{22}}{\bar{w}}. \tag{3.4}$$

Equations (3.2)–(3.4) are similar to the standard equations for evolution at one locus with two alleles in a diploid population (Charlesworth, 1978). The dependence of the collision probability on the gamete frequencies is purely due to mass action – the more common a gamete in the gamete pool, more likely it is to get incorporated into a zygote. Describing this mass action by making the number of zygotes proportional to the product of number of sperm and eggs models is what might be thought of as a kind of "frequency dependence" in this evolutionary process. As the fitness coefficients ($w'_{ij}s$) are themselves independent of allele frequencies, this evolutionary process is not formally one of frequency dependence.

In a one-locus, two-allele model, allele 2 increases when rare in a population of allele 1 if $w_{12} > w_{11}$. Allele-2 also displaces allele 1 from a polymorphism if $w_{22} > w_{12}$. With this basic framework, we can analyze the dynamics of the model when alleles can code for any combination of sperm and egg sizes. We assume that gametes need to be of a minimum size m to contain the necessary genetic and cellular material, and hence be viable.

3.3 CONDITION FOR ISOGAMY

We show below that the dynamics of this evolutionary model converge to the maximum of the fitness surface described by $w(E, S)$. This surface is U-shaped over the triangle (m, m), $(m, z_0 - m)$, $(z_0 - m, m)$ in the E–S space, and hence has its maximum at one of the vertices of the triangle. The condition needed for the dynamics to converge to isogamy turns out to be that $\frac{e^{km}}{m} > \frac{e^{k(z_0-m)}}{z_0-m}$. This is because the function

$$f(x) = \frac{e^{kx}}{x}, \qquad m < x < z_0 - m,$$

$$f(x) = \frac{e^{k(z_0-m)}}{x}, \qquad x \geq z_0 - m, \tag{3.5}$$

attains its maximum over $[m, \infty)$ at either $x = m$ or $x = z_0 - m$, and the condition $\frac{e^{km}}{m} > \frac{e^{k(z_0-m)}}{z_0-m}$ implies that $f(m) > f(z_0 - m)$. Now suppose allele i codes for gametes of sizes (m, m) , and allele r codes for eggs and sperm of size (E, S) such that both E and S are larger than m. We show that for all values of E and S, $w_{rr} < w_{ir} < w_{ii}$, implying that a mutant allele i can always increase when rare as well as get fixed in a population of allele r.

Suppose first $E + S < z_0$. Then

$$\begin{aligned} w_{rr}e^{-kz_0} &= \frac{e^{kE}}{E}\frac{e^{kS}}{S} \\ &< \frac{1}{2}\left(\frac{e^{kE}}{E}\frac{e^{km}}{m} + \frac{e^{km}}{m}\frac{e^{kS}}{S}\right) = w_{ir}e^{-kz_0} \\ &< \frac{1}{2}\left(2\frac{e^{2km}}{m^2}\right) = w_{ii}e^{-kz_0}. \end{aligned}$$

Each of these inequalities follows from $f(x)$ being maximized at $x = m$. Hence, we have

$$w_{ii} > w_{ir} > w_{rr}. \tag{3.6}$$

Hence allele i can displace any allele r that codes for gametes of sizes summing to less than z_0.

Now suppose r codes for eggs and sperm of size (E, S) larger than m, such that $E + S > z_0$. Again from $f(x)$ being maximized at $x = m$, it follows that

$$\begin{aligned} w_{rr}e^{-kz_0} &= \frac{e^{kz_0}}{ES} \\ &< \frac{1}{2}\left(\frac{e^{kE}}{E}\frac{e^{k(z_0-E)}}{z_0-E} + \frac{e^{k(z_0-S)}}{z_0-S}\frac{e^{kS}}{S}\right) \\ &< \frac{1}{2}\left(\frac{e^{kE}}{E}\frac{e^{km}}{m} + \frac{e^{km}}{m}\frac{e^{kS}}{S}\right) = w_{ir}e^{-kz_0} \\ &< \frac{1}{2}\left(\frac{e^{2km}}{m^2}\right) = w_{ii}e^{-kz_0}. \end{aligned}$$

Hence, again

$$w_{ii} > w_{ir} > w_{rr}. \tag{3.7}$$

Equations (3.6) and (3.7) imply that the dynamics starting with any set of initial alleles always converge to the fixation of allele i, which is also evolutionarily stable (i.e. no allele can increase when rare in a population with i fixed). Hence under the condition that $\frac{e^{km}}{m} > \frac{e^{k(z_0-m)}}{z_0-m}$, all individuals in the equilibrium are isogamous and make sperm and eggs both of size m.

3.4 CONDITION FOR ANISOGAMY

When $\frac{e^{k(z_0-m)}}{z_0-m} > \frac{e^{km}}{m}$, the dynamics almost always converge to anisogamy with the population monomorphic in either the allele coding for

gamete sizes $(m, z_0 - m)$ or the allele coding for $(z_0 - m, m)$. However, this is not as easy to show as when the dynamics converge to (m, m) because, in general, the value of w_{ij} does not lie intermediate to the values of w_{ii} and w_{jj} , for any two alleles i and j. This means that the initial increase of allele i in a population of allele j (as when $w_{ij} > w_{jj}$) does not imply that the allele i also displaces allele j from the population (as would be the case if also $w_{ii} > w_{ij}$).

We approach this case as follows. First note that the condition $\frac{e^{k(z_0-m)}}{z_0-m} > \frac{e^{km}}{m}$, implies that for any allele i coding for egg and sperm sizes larger than m, $w_{ii} \leq w_{11} = w_{22}$, where allele A_1 codes for gametes of size $(m, z_0 - m)$ and of allele A_2 codes for gametes of size $(z_0 - m, m)$.

Moreover, for any two alleles i and j, the value of w_{ij} is less than the value of w_{kk} for some allele k. This is because if allele i codes for (E_i, S_i) and allele j for (E_j, S_j) , then $w_{ij} = \left[w(E_i, S_j) + w(E_j, S_i)\right]/2 < \text{Max}\left[w(E_i, S_j), w(E_j, S_i)\right]$. And hence $w_{ij} < \text{Max}\{w_{kk}, w_{ll}\}$, where allele-$k$ codes for (E_i, S_j) and allele l for (E_j, S_i). Hence what we have is that for any alleles i, j, $w_{ii} \leq w_{11} = w_{22}$ and $w_{ij} \leq w_{11} = w_{22}$. This implies that the mean fitness function for any set of alleles in the population $\bar{w} \leq w_{11} = w_{22}$. Although this is an infinite-allele model, at any instant of time, there are only a finite number of alleles at the locus under consideration, and hence the dynamics of this one-locus model always increase the mean fitness (Kingman, 1961). Since monomorphisms in alleles A_1 or A_2 maximize the mean fitness, we conclude that a population fixed at either A_1 or A_2 is evolutionarily stable to invasion by any mutants.

Further, let us assume that gametes can be small enough that $\frac{e^{km}}{m} > \frac{e^{kz_0}/2}{z_0/2}$. This condition implies that for each egg and sperm size (E, S) such that $S < z_0/2 < E$, sperm of size m make a greater fitness contribution than the resident sperm, and eggs sized either $z_0 - m$ or $z_0/2$ make a greater fitness contribution than the resident eggs. Hence a population monomorphic in (E, S) can be invaded by an allele that makes gametes of size (E', m) (where E' is either $z_0 - m$ or $z_0/2$), which in turn gets fixed. This population can now be invaded by the allele $(z_0 - m, m)$, whose fixation is an ESS. Hence under the condition that $\frac{e^{km}}{m} > \frac{e^{kz_0}/2}{z_0/2}$, the fixation of no single allele (other than A_1 or A_2) is an ESS.

We have shown so far that under reasonable assumptions, there is a possible route for alleles A_1 or A_2 to replace a single other allele that is fixed in the population. However, evolution proceeds by random mutations, and hence it is possible for the dynamics to lead the

population to a polymorphism consisting of many alleles. It is not obvious analytically that every such path that the dynamics could take will culminate in the fixation of either A_1 or A_2. We use simulations to verify that this is indeed the case.

The simulations are detailed in Iyer and Roughgarden (2008). Every time the population attained equilibrium, a mutant coding for a random egg and sperm size within the possible range was introduced, and the population was allowed to evolve till it reached an equilibrium again. We found that almost all populations are left with either alleles coding for gamete sizes very close to $(m, z_0 - m)$ or, as a mirror image, alleles coding for gamete sizes very close to $(z_0 - m, m)$. For only 4 out of 786 initial values did these simulations not culminate in pure anisogamy – three of these four initial conditions culminated in polymorphisms with either A_1 or A_2 constituting more than 98% of the allele pool. The other exception is when the simulation is initiated with the allele coding for (m, m) and the population is subsequently invaded by $(z_0 - m, z_0 - m)$. A_1 or A_2 can increase when rare in this case, but not take over from this polymorphism.

Hence we show that irrespective of initial conditions, dynamics for a single locus coding for both gamete sizes almost always converges to one of the fitness maxima, corresponding to the fixation of the allele coding for $(m, z_0 - m)$ or $(z_0 - m, m)$. Which of these two alleles gets fixed depends on the priority effect of which mutation occurs first. That anisogamy evolves in a model where the same allele codes for both sperm and egg sizes, and that the anisogamous ESS corresponds to the fixation of a single allele indicates that genetic conflict (between gamete producers) is unnecessary for the origin of anisogamy. Instead, anisogamy evolves when large zygotes are favored, and the difference in gamete sizes maximizes the encounter rate between gametes, and hence the number of zygotes produced.

From the analysis of conditions that lead to isogamy and anisogamy, we conclude that evolution at a single locus coding for both gamete sizes converges to the maximum of the fitness surface corresponding to $w(E, S)$, where E and S take values larger than m. $w(E, S)$ corresponds to the absolute fitness of an individual in a population of homozygotes, all coding for E and S. This surface is U-shaped over the triangle (m, m), $(m, z_0 - m)$, $(z_0 - m, m)$ and decreases outside this triangle. The fitness is maximized by anisogamy with gamete sizes m and $z_0 - m$ if $\frac{e^{k(z_0-m)}}{z_0-m} > \frac{e^{km}}{m}$ and isogamy with gamete size m otherwise. As k increases, the minimum of the U-shaped surface, $(1/k, 1/k)$, shifts

closer to the isogamous vertex, making it more likely that the anisogamous vertices have a higher fitness value than the isogamous vertex. Biologically, this means that as the mortality rate increases relative to the growth rate, selection to minimize the time over which the zygote is exposed to the high mortality rate makes anisogamy fitter than isogamy.

3.5 ECOLOGICAL PREDICTIONS AND TESTS

The ecological and life history predictions from the model are:

(1) Anisogamy is selected when large initial zygotes, which minimize the time spent growing, are selected. Hence, compared to anisogamous species, isogamous species are expected to have lower mortality rates and higher zygotic growth rates to similar final sizes. These traits could be ecologically caused, for example, by greater predation on anisogamous species or higher nutrient availability for isogamous species.

(2) The model predicts isogamous gametes to be of the minimum size possible (m), and anisogamous gametes to be of sizes m and $z_0 - m$ (assuming gametes of size z_0 can be produced. Otherwise anisogamous macrogametes are predicted to be the largest size possible). Hence the gametes and zygotes of isogamous species are predicted to be much smaller than the anisogamous macrogametes and zygotes, and of similar size to anisogamous microgametes.

To test these predictions, data on gamete sizes, initial and final zygote sizes and development time were collected for isogamous and anisogamous *Volvocaceae* (colonial green algae) and *Chlamydomonas* (unicellular green algae) species, as detailed in Iyer and Roughgarden (2008) and online supplementary materials. As predicted, gametes of isogamous *Volvocaceae* species are much smaller than the eggs of anisogamous species and not significantly different in size from the sperm. As also predicted, isogamous *Volvocaceae* species have much smaller zygotes compared to anisogamous species. Data from *Chlamydomonas* species indicate similar but non-significant trends which could be confirmed with data from more species. Pooled data from both taxa indicate that the percent increase in zygote size during development to the mature zygospore stage is significantly higher in isogamous species than in anisogamous species, confirming the model prediction that isogamous zygotes have higher zygotic growth rates.

3.6 DISCUSSION

We have proposed an individual-selection model for the evolution of anisogamy where selection acts at a locus coding for both sperm and egg sizes in an initially isogamous hermaphroditic population. We demonstrate that anisogamy can evolve in this model, corresponding to fixation of a single allele at the locus being considered. We thus show that the evolution of anisogamy need not involve conflict between genetic strategies because the gametic dimorphism that evolves is an individually advantageous trait. Accordingly, we find that a mutant with decreased sperm size, while increasing its own relative fitness as a result of increased presence in the sperm pool, also increases the absolute fitness gained by egg production as a result of increasing the fertilization probability. This implies that contradictory to Parker *et al.*'s claim (1972), proto-ova may actually gain from fusing with proto-sperm as compared to selectively fusing with other proto-ova. Hence we demonstrate how small sperm evolve without being a "cheater" strategy. Thus our model favors the interpretation of anisogamy as being selected for because it results in better zygote survival, as well as higher encounter rates, rather than as an outcome of gametic conflict.

Also, and in contrast to previous models emphasizing zygote fitness dependence on size, we provide a biological basis for the disproportionate (or accelerating) dependence of zygote fitness on size by employing the Vance survival function. Although Bulmer and Parker (2002) also claim to use the Vance function for zygote survival they actually employ a modified form proposed by Levitan (2000) to explain echinoid egg size distribution. With the Levitan–Vance formula, the global fitness maximum is never isogamous, making the evolution of anisogamy seem inevitable. Upon returning to the original Vance function, both isogamy and anisogamy emerge as alternative adaptive outcomes corresponding to alternative ecological conditions.

Specifically, anisogamy is selected to avoid the mortality involved while growing from the smallest possible zygote size to either the final size or the maximum size that can be produced. Hence the parameters of minimum and maximum gamete sizes that can possibly be produced and the optimal final zygote size, along with growth and instantaneous mortality rates fully specify whether isogamy or anisogamy is expected for any particular species. This prediction is partially tested with data from colonial green algae. As expected from the model, we find that isogamous gametes are not significantly different

in size from anisogamous sperm and are significantly smaller than anisogamous eggs, and that isogamous species may have higher growth rates. This is also contradictory to the expectation from the previous models that anisogamous species may have higher growth rates if the higher provisioning in anisogamous eggs is expended in growth.

The observed correlations between adult size and egg size, as well as between adult size and gametic dimorphism in colonial green algae have been taken as evidence for greater disruptive selection on gamete sizes in larger and more complex colonies, and hence indirect evidence for Parker *et al.*'s (1972) model (Randerson and Hurst, 2001). We propose that these correlations could also be explained by larger adults being capable of producing larger gametes, and zygotes of larger colonies having higher mortality rates and smaller growth rates compared to smaller species.

By proposing how males and females need not necessarily be in conflict over parental investment in the zygote, this paper weakens the supposition that conflict between the male and the female strategies is primitive in sexual reproduction, and thus contributes to a "social-selection program" of devising an alternative system of hypotheses to those of sexual selection (Roughgarden, 2009). The distinction between male and female need not have been forged in a primordial gametic battle of the sexes, and sexual conflict is not necessarily evolutionarily primitive or universal.

In conclusion, we have proposed an individual-selection model for the evolution of anisogamy which shows that gamete size dimorphism may be an adaptation that increases gamete contact rates when large zygotes are favored and not a product of genetic conflict. In doing so, we make specific predictions for when isogamy versus anisogamy is expected to be adaptive.

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ROLF F. HOEKSTRA

4

Nucleo-cytoplasmic conflict and the evolution of gamete dimorphism

4.1 CYTOPLASMIC SELECTION MAY CAUSE NUCLEO-CYTOPLASMIC CONFLICT

Several authors have noted that the mixing of cytoplasm following gamete fusion may increase the potential for spread of deleterious cytoplasmic variants through a sexual population (Grun, 1976; Eberhard, 1980; Cosmides and Tooby, 1981). This argument assumes a lack of precise control of intracellular replication and subsequent lack of segregation of the cytoplasmic DNA at gametogenesis. The replication and transmission of nuclear genes is tightly regulated, ensuring no segregation of alleles at mitosis and a fair meiotic segregation in heterozygotes. Thus, in the words of Birky (1983), the nuclear genome is "stringent." In contrast, cytoplasmic genomes are "relaxed": multiple cytoplasmic genomes populate each cell; they can be replicated different numbers of times during a cell cycle (Clayton, 1982) and can be differently transmitted to daughter cells. Throughout this chapter I focus primarily on mitochondria as examples of obligate cytoplasmic organelles, but similar reasoning applies to other cytoplasmic entities like plastids in plants and vertically transmitted endosymbiontic bacteria. The relaxed regulation of mitochondrial replication and segregation increases the scope for within-individual selection among mitochondrial genomes. This may possibly result in the evolution of selfish mitochondrial variants that are able to acquire a within-individual transmission advantage while being harmful to the host organism. A transmission advantage

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of a selfish mitochondrial mutant could result from superior competitiveness in heteroplasmic cells, for example due to a higher replication rate resulting in numerical over-representation in zygotes. A selfish mitochondrial mutation may spread through a population, provided its segregation advantage more than compensates the fitness loss of its individual hosts. Natural selection is thus expected to favor genetic variants that minimize the possibilities for spread of deleterious mitochondrial mutations. One possible mechanism, suggested by Grun (1976), Eberhard (1980) and Cosmides and Tooby (1981), is the evolution of uniparental inheritance of cytoplasmic genomes. Clearly, in a population with uniparental (say maternal) inheritance of mitochondria, the spread of selfish variants is severely hampered, since their presence is limited to the descendants along the female line of the female in which the mutation originally has occurred. Because of the detrimental effects on individual fitness, natural selection is expected to remove this "infected" segment from the population.

4.2 The Evolution of Uniparental Cytoplasmic Inheritance and Anisogamy Driven by Nucleo-Cytoplasmic Conflict

Given that uniparental cytoplasmic inheritance is selectively favored, how is it to be regulated? It is here that the evolution of anisogamy comes into play. A number of different hypotheses have been proposed which link the evolution of uniparental cytoplasmic inheritance and the evolution of anisogamy. These hypotheses assume an initial situation of an isogamous sexual population with biparental cytoplasmic inheritance. The nucleo-cytoplasmic conflict generated by the biparental cytoplasmic transmission either provides direct selection pressure for evolution towards anisogamy, which ultimately results in uniparental cytoplasmic inheritance (Cosmides and Tooby, 1981; Hurst, 1990), or directly selects for the evolution of uniparental inheritance, which may (but need not necessarily) involve the evolution of anisogamy (Hoekstra, 1990b; Hastings, 1992; Law and Hutson, 1992).

Cosmides and Tooby (1981) offer the following scenario for the evolution of anisogamy. Due to the lack of a strict segregation mechanism for cytoplasmic genes, there will be competition in zygotes between the cytoplasmic factors derived from the two parental gametes for preferential representation in the daughter cells. This creates a

selective pressure for increasing the number of copies of cytoplasmic genomes in gametes. Since there is a limit to the number of organelles for a given gamete size, there will be continuous selection on organellar genes to increase gamete size in order to attain a larger input bias. A point will be reached where it becomes advantageous for nuclear genes to make many small gametes instead of a few large ones, since the mass of one large gamete is already sufficient for provisioning the zygote. In this way anisogamy is established. Evolution towards disassortative fusion with respect to gamete size will proceed by selection for cytoplasmic genes to resist fusion with larger gametes, and for nuclear genes to resist fusions between two microgametes. However, I have pointed out that this scenario is not very likely because it requires the (almost) simultaneous occurrence of both a cytoplasmic mutation increasing gamete size and a nuclear mutation increasing gamete productivity (Hoekstra, 1987). When an assumption about increasing zygote fitness with zygote size is added, stable anisogamy may evolve. However, then the model becomes basically identical to Parker, Baker and Smith's model of the evolution of anisogamy (Parker *et al*., 1972).

The models of Hoekstra (1990b), Hastings (1992) and Law and Hutson (1992) all assume nucleo-cytoplasmic conflict resulting from invasion of selfish cytoplasmic variants. This situation favors a nuclear mutation enforcing effective uniparental transmission of cytoplasm. The models differ in their assumptions about fitness effects of the nuclear modifier and about the mechanism involved. They share the same problematic aspect as noted above for the Cosmides and Tooby scenario, namely that the nuclear modifier is only selected during the relatively short time interval in which the population is polymorphic for the selfish and the wild type cytoplasm. As soon as the selfish cytoplasmic mutation is fixed, the nuclear mutation is neutral if it imposes no fitness cost, and will be selected against if it is costly.

Two models were considered by Hoekstra (1990b). In the first model, selfish cytoplasmic mutants invade under biparental inheritance and a nuclear modifier, linked to one of the two mating types, acts to destroy the cytoplasmic DNA from the other mating type gamete shortly after fusion. This situation is reminiscent of chloroplast inheritance in *Chlamydomonas reinhardtii*, where within 30 minutes after zygote formation virtually all the cpDNA genomes in the *minus* chloroplast are destroyed, while virtually all the genomes in the *plus* chloroplast survive (Kuroiwa *et al*., 1982; Nishimura *et al*., 1999). This process appears to be regulated by the mating type locus (Goodenough

et al., 2007). Although this model allows the evolution of uniparental inheritance, it is unsatisfactory because of its rather implausible assumptions. Apart from the problem noted above, that the nuclear modifier mutation must occur almost simultaneously with the selfish cytoplasmic mutation, it also assumes the nuclear mutation to be both closely linked to the mating type locus and able to control the destruction of cytoplasmic DNA in the partner gamete, a combination of effects which in all probability would require at least two or three independent mutations. The second model analyzed in Hoekstra (1990b) aims to be somewhat more realistic in dropping the assumption of close linkage of the nuclear modifier to the mating-type locus, and by assuming that this modifier sacrifices its own cytoplasmic DNA at gamete formation, rather than destroying the cytoplasmic genomes in the other gamete. After all, anisogamy implies "kill off your own cytoplasm" rather than "kill off your partner's cytoplasm" (Randerson and Hurst, 1999). In order to allow the evolution of uniparental inheritance, this model requires direct fitness effects of the nuclear modifier acting in the diploid stage, such that heterozygous zygotes have higher fitness than homozygotes. This guarantees that the nuclear modifier is maintained in the population. The rationale for this latter assumption is that homozygotes for the modifier that sacrifices its own cytoplasm have a low fitness due to shortage of cytoplasmic DNA, while the other homozygotes suffer from the costs of the high cytoplasmic replication activity.

Also the model of Law and Hutson (1992) is highly sensitive to rather special assumptions. A single nuclear mutation which kills its own cytoplasm and is only expressed in gametes of one of the mating types seems unlikely, since such a functionally complicated phenotype probably involves several mutations. Moreover, the modifier does not involve any fitness costs itself. As soon as these assumptions are relaxed, the evolution of uniparental inheritance is prevented.

Hastings (1992) notes that a nuclear mutation sacrificing its own gametic cytoplasmic DNA is less likely to invade than a mutation with the effect that cytoplasmic DNA from the other gamete is prevented from entering the zygote. In his model uniparental inheritance evolves, but the assumption of a single mutation with these effects is implausible, as in the first model of Hoekstra (1990b), discussed above.

Randerson and Hurst (1999) compare a model in which a modifier acts gametically to kill its own organelles (prior to zygote formation) to a model in which a modifier causes destruction of the other

gamete's organelles shortly after zygote formation. They conclude that "kill your own cytoplasm" modifiers are much less effective than "kill your partner's cytoplasm" modifiers. A modifier killing the cytoplasm of its own gamete prior to gamete fusion only increases in frequency if the population is polymorphic for a selfish cytotype. When the selfish cytotype is at fixation there is no advantage to uniparental inheritance, and the modifier will be selected against if it imposes any fitness cost. This is the same problem as discussed above with respect to the other models. On the other hand, in the "kill off your partner's cytoplasm" model the nuclear modifier is stably maintained, even if it imposes a fitness cost, because it can remain associated with the (high fitness) wild type cytoplasm.

4.2.1 Empirical evidence: uniparental inheritance

Although uniparental inheritance of cytoplasmic DNA is widespread, it is by no means exclusive. As summarized in a recent review (Barr *et al.*, 2005), plants, animals and fungi are all characterized by episodes of biparental inheritance. Birky (2001) states in another review: "The most general statement we can make about uniparental inheritance is that in most organisms, some or all progeny inherit organelle genes from only one parent." The fact that some degree of biparental inheritance has been observed in many taxa would indicate that occasional heteroplasmy can be tolerated. (Barr *et al.*, 2005) suggest that low, but non-zero levels of biparental transmission with consequent heteroplasmy and recombination may facilitate mutational clearance in cytoplasmic DNA. With 100% uniparental inheritance, cytoplasmic genomes would be completely asexual and consequently subject to mutation accumulation (Muller's ratchet). On the other hand, in some species uniparental inheritance seems enforced to be practically absolute, particularly in animal species. Despite the fact that an egg contains many orders of magnitude more mitochondria than a sperm, which would already almost guarantee uniparental inheritance as a consequence of numerical dilution of male-derived mitochondria, in mammals an active destruction mechanism of male-derived mitochondria has been demonstrated (Kaneda *et al.*, 1995; Sutovsky *et al.*, 1999). Active degradation of paternal mitochondrial DNA has also been demonstrated in honeybees (Meusel and Moritz, 1993). As mentioned earlier, in the single-celled isogamous green alga *Chlamydomonas reinhardtii*, mitochondria from the *plus* mating-type parent are actively destroyed after gamete fusion, while the chloroplast genome from

the *minus* mating-type parent undergoes the same fate. Other cases are reviewed in Birky (1995).

As emphasized by Birky (1995, 2001), the mechanisms of uniparental inheritance are quite diverse. Cytoplasmic gene inheritance can be blocked by a variety of mechanisms in different species and at different stages of the sexual cycle. Moreover, mitochondria and chloroplasts can be preferentially transmitted from different parents: male and female, in some gymnosperms (Szmidt *et al.*, 1987; Neale and Sederoff, 1989) and from different mating types (*minus* and *plus*) in *Chlamydomonas reinhardtii* (Gillham, 1994). Phylogenetic analysis suggests that cytoplasmic inheritance patterns (uniparental maternal, uniparental paternal, biparental) have changed frequently during evolution (Birky, 1995). This would imply that organelle gene inheritance has been subject to varying selective pressures.

4.2.2 Empirical evidence: selfish mitochondrial DNA

The best-known selfish mitochondrial genes are the petite mutants in yeast (Williamson, 2002). Petite genomes are characterized by large deletions and an inability to respire. Since yeast can switch to fermentation, petites are not lethal, but they do result in a lowered growth rate relative to the wild type. Because they often contain more replication origins than wild type, they can outcompete wild type mitochondria in heteroplasmic cells. Since in yeast mitochondrial transmission is biparental, petite mutations have a transmission advantage. However, because of the fitness cost they impose on the organism, they cannot spread in natural yeast populations.

Other examples of selfish mitochondrial elements in fungi are so-called mitochondrial plasmids, known from some ascomycetous species like *Podospora anserina* and *Neurospora intermedia*. They arise from rearrangements within the mitochondrial genome and have a replicative advantage within cells. Their presence is associated with increased senescence (Bertrand *et al.*, 1985, Griffiths, 1992).

In angiosperms cytoplasmic male sterility (CMS) is caused by mitochondrial mutations. They are selfish in that they suppress male function in hermaphrodites by pollen abortion and (presumably by diversion of resources) cause higher seed production (Manicacci *et al.*, 1998; van Damme and van Delden, 1984). Since mitochondrial inheritance is uniparental, maternal CMS mitochondrial mutations can spread in populations at the expense of wild type mitochondria.

Note, however, that in this case selfish mitochondria do not derive their competitive advantage relative to wild type mitochondria from within-cell competition, but rather from exploitation of the uniparental maternal transmission mechanism. In this case uniparental inheritance does not act as a sieve to prevent such a selfish mitochondrial mutation to spread, but – quite the opposite – allows it to increase in frequency.

Selfish mitochondrial mutations in animals seem to be rare, possibly due to their small mitochondrial genome size. Mutant mitochondrial genomes that replicate faster than wild type genomes have been reported in crickets (Rand and Harrison, 1989).

4.2.3 Evaluation of the theoretical models and comparison of model predictions with empirical evidence

First, the models discussed above are quite sensitive to their assumptions, some of which are implausible. Second, models assuming nuclear modifiers expressed in gametes and causing destruction of cytoplasmic DNA from the partner gamete are more robust and better in explaining the evolution of uniparental inheritance than models assuming the sacrifice of own gametic cytoplasmic DNA.

Active destruction of cytoplasmic genomes has been documented, both in anisogamous taxa (in particular animals) and isogamous taxa (unicellular algae). In anisogamous systems the destruction of mitochondrial DNA in sperm is of the "kill your own mitochondria" type, in the isogamous Chlamydomonas species of the "kill your partner's mitochondria" type. Studies in mammals have demonstrated that the destruction of male mitochondrial genomes involves the ubiquitin-dependent proteolysis of a mitochondrial membrane protein (Sutovsky *et al.*, 2003; Thompson *et al.*, 2003). Ubiquitin is a protein that binds to other proteins and marks them for degradation by the 26S proteasome. The mammalian nuclear encoded mechanism of enforcement of uniparental inheritance by active destruction mechanisms strongly suggests an adaptive value of uniparental cytoplasmic inheritance. This adaptive value may well be the suppression of selfish mitochondrial mutations, but it also has been suggested that uniparental inheritance has evolved to suppress recombination between cytoplasmic genomes, necessary to ensure optimal functional compatibility between the cytoplasmic and nuclear genomes (Sager, 1972; Lane, 2005). The hypothesis that selection for uniparental inheritance

has driven the evolution of anisogamy faces the difficulty that the theoretical models predict the "kill your partner's mitochondria" pattern should occur (Randerson and Hurst, 1999), while active elimination of paternally derived cytoplasmic DNA in anisogamous species actually involves destruction of own mitochondria. Furthermore, the observation that uniparental inheritance is also observed in isogamous species, suggests that anisogamy is not a necessary outcome of selection for uniparental inheritance. Another observation casting doubt on a general link between the evolution of uniparental inheritance and anisogamy is that paternal uniparental inheritance has been observed in a wide range of organisms (Birky, 1995). Therefore, nucleocytoplasmic conflict is unlikely to be a general explanation of the evolution of anisogamy.

4.3 THE EVOLUTION OF ISOGAMOUS BINARY MATING TYPES

Starting with Weismann (1886), hypotheses about the evolution of anisogamy, including the influential paper by Parker *et al.* (1972), have assumed isogamy with undifferentiated gametes as the ancestral situation. Anisogamy is viewed as the primary sexual differentiation which defines males and females, and isogamy as the absence of sexual differentiation, where each gamete can mate with any other. The different characteristics and functional roles of male and females in sexual reproduction both generate and are shaped by the operation of sexual selection (Andersson, 1996). Thus it represents one of the key aspects of life, making an understanding of its evolution a fascinating problem. However, there is a reason to question the classical assumption that anisogamy (unequal size of gametes involved in sexual fusion) represents the basic difference between male and female. Sexual differentiation without morphological differences between the gametes was already described more than a century ago in fungal species belonging to the *Mucorales* (Blakeslee, 1904). In fact, widespread among isogamous species are binary mating systems in which the gametes are differentiated into two mating types, designated for example as *plus* and *minus* as in *Chlamydomonas*, or as *a* and α in *Saccharomyces*. Therefore, an understanding of the evolution of anisogamy from isogamy is not sufficient for understanding the basic asymmetry of sex (Hoekstra, 1982; 1987). We also need to understand why sexual fusions in isogamous species involve gametes differentiated into mating types.

In the remainder of this chapter I review hypotheses which assume that the origin of a binary sexual differentiation predates the evolution of anisogamy.

4.3.1 The role of nucleo-cytoplasmic conflict in mating-type evolution

The idea that nucleo-cytoplasmic conflict may also have provided the driving force for the evolution of binary mating types has been modeled by Hurst and Hamilton (1992). They propose that a system of two mating types has evolved to regulate uniparental cytoplasmic inheritance: one mating type being the transmitter and the other not. Uniparental inheritance is selected in order to minimize the damage from selfish cytoplasmic mutations, just as discussed above for the cytoplasmic conflict models of the evolution of anisogamy. They analyze a three-step scenario (originally outlined by Hoekstra, 1987). Consider a population of Chlamydomonad-like algae which upon induction of the sexual cycle form isogametes which randomly mate in pairs. There is no gametic differentiation of any kind, and any gamete can mate with any other. Cytoplasmic transmission is biparental. The first stage is represented by the occurrence of a cytoplasmic mutation that in the zygote stage destroys the cytoplasmic genome from its mating partner. This mutation is expected to go to fixation under fairly broad conditions. Next a nuclear suppressor mutation enters the scene with the effect of leaving its cytoplasmic genome both unable to destroy its opponent and vulnerable to destruction by that opponent. Then three types of zygotes can be formed: homozygous Suppressor, homozygous Non-suppressor, and heterozygous Suppressor / Non-suppressor. In their model the latter combination has the highest fitness, implying heterozygote advantage and thus stable polymorphism between Suppressors and Non-suppressors. The third step consists in the introduction of a nuclear mutation (Chooser) which prefers its gamete to fuse with one of the opposite suppressor type, thus avoiding non-optimal matings. The Chooser gene will spread to fixation and the population will finally consist of two mating types, of which one transmits cytoplasmic genomes and the other does not.

Hurst and Hamilton (1992) propose that the evolution of binary mating types from a supposed ancestral situation of undifferentiated gametes represents the primary sexual differentiation. They thus introduce a new definition of sexes, based on the asymmetry in cytoplasmic transmission.

Hutson and Law (1993) have analyzed a model which also assumes nuclear-cytoplasmic conflict to be the driving force in the evolution of mating-type differentiation. Their model differs in details of the assumptions of Hurst and Hamilton (1992), but reaches the same conclusions.

4.3.2 Mating-type evolution as a consequence of selection for asymmetry in gamete recognition and adhesion

I proposed that binary mating types have evolved to prevent matings between genetically identical gametes derived from the same mitotic clone Hoekstra (1982). Matings between cells derived from different clones might profit from subsequent genetic recombination. Several related models have been analyzed (Hoekstra, 1982; 1987; Hoekstra *et al.*, 1991; Czaran and Hoekstra, 2004), which all consider a population in which recognition and/or adhesion between gametes is mediated by a diffusible signal and a corresponding receptor. The initial population is assumed to be self-fertile (every gamete both produces the signal and is able to respond; therefore any gamete can mate with any other). A potential problem of these gamete types is self-saturation; e.g. their receptors becoming blocked by their own pheromone. Therefore, mutants with a non-functional signal or a non-functional receptor are supposed to be more efficient in mating (with the original gamete type and with each other) despite a diminished opportunity for mating because they cannot mate with their own type. Under reasonable assumptions the mutants can invade and reach intermediate frequencies. Selection will promote close linkage between the pheromone locus and the receptor locus, and at least under some conditions the original self-fertile gamete type will disappear from the population, leading to the maintenance of just two mating types. One mating type specializes in attraction (coding for pheromone production), the other in becoming attracted (coding for the receptor). The studies by Hoekstra *et al.* (1991) and Czaran and Hoekstra (2004) suggest that for a two mating-type system to evolve the spatial distribution in the population of the various gamete types may play an important role.

4.3.3 Sexual fusion asymmetric from the start?

A rather different model for the evolution of binary mating types has been analyzed by Hoekstra (1990a). The model assumes that the

evolution of asymmetric cell fusion has preceded the evolution of sex, implying that sexual fusions have been asymmetric from the start. It considers the evolution of a dimorphism with respect to cell-fusion characteristics in a population of primitive cells. These cells reproduce exclusively asexually. The evolution towards cell fusion is driven by selection promoting horizontal transfer of an endosymbiontic replicator. This symbiont can induce its host cell to fuse temporarily with another cell, thus infecting the other cell. The next stage is selection for a mutant symbiont which causes its host to avoid fusion with an already infected cell. This mutant will replace the original symbiont, so that finally the population will consist of two cell types, namely uninfected cells and infected cells. Note that all cell fusions in this population are asymmetric, namely between infected and uninfected cells. Once a sexual cycle evolves, this dimorphism in cell-fusion behavior is supposed to be incorporated as a preadaptation into the conglomerate of processes that constitute sex. A similar idea has been suggested by Bell (1993) who proposes that mating-type genes are derived from mobile genetic elements which have been selected to promote sexual exchange in order to spread through a population. Similar to the scenario studied by Hoekstra (1990a), preferential fusions with cells that do not yet contain the transposon will be selectively favored, thus leading to asymmetric sexual fusions.

4.3.4 Empirical evidence

As empirical support for their theory Hurst and Hamilton (1992) point out that binary mating types are absent in the two main groups where sex does not involve mixing of the cytoplasm from both parents, namely the basidiomycetous fungi and most ciliates. This argument makes the reasonable assumption that the danger of nucleo-cytoplasmic conflicts is much reduced in the absence of cytoplasmic mixing, and that therefore uniparental inheritance of cytoplasmic genomes does not have to be regulated. Instead of having a binary mating-type system, these groups are characterized by mating systems with multiple mating types. Matings are allowed between different mating types, and only individuals of the same mating type are sexually incompatible. In some cases the number of mating types can be very large, e.g. more than 10 000 in the basidiomycetous mushroom *Schizophyllum commune* (Raper, 1966). In basidiomycetes hyphal fusions between two monokaryons (haploid mycelia arising after spore formation) is the primary step towards sex. Subsequently,

haploid nuclei are exchanged and reciprocally migrate throughout the existing mycelia to give rise to a dikaryon, but invasion of the cytoplasm from the partner homokaryon is prevented (Casselton, 1985). In most ciliates the process of conjugation involves exchange of the micronuclei through a small bridge connecting the conjugating cells, while the passage of cytoplasm is avoided. Hurst (1995) has reviewed further evidence consistent with the idea that cytoplasmic conflict has driven the evolution of binary mating types. He concludes that there is considerable empirical support.

Nevertheless, there are cases which do not fit the hypothesis. In slime molds sex involves the fusion of isogametes, and multiple mating types may occur (Urushihara, 1992). Also the mating system of yeast is not really consistent with the hypothesis, since there are two mating types, but biparental inheritance of mitochondria. It is true that vegetative segregation rapidly results in homoplasmic lineages, but it is difficult to see how in this system suppression of cytoplasmic conflict requires two mating types.

The model of Hoekstra (1982) predicts binary mating-type differentiation based on separation of production of a diffusible signal and the corresponding receptor. Furthermore it predicts close linkage between the loci governing these phenotypes. The available information on the structure and function of mating-type loci both in the unicellular alga *Chlamydomonas reinhardtii* and in many fungi is consistent with these predictions. In *C. reinhardtii* the mating type loci (MT^- and MT^+) are characterized by several large inversions and translocations, probably contributing to recombinational suppression, the extent of which reaches approximately 1 Mb (Goodenough *et al.*, 2007). Both loci contain large regions not found at the other locus. Thus the mating-type loci contain multiple genes and are to a large extent non-homologous. The presence of genes without homologs is also typical of mating-type loci in other lineages, such as in many fungi (Butler, 2007; Fraser, 2007) and of sex chromosomes of vertebrates and some invertebrates (Charlesworth, 1991; Fraser *et al.*, 2004). In *C. reinhardtii* mating-type genes are involved in the regulation of fertilization competence (including recognition and fusion with gametes of the opposite mating type), initiation of zygote development, and uniparental inheritance of organelle genomes (Goodenough *et al.*, 2007). In fungi genes within the mating-type region typically encode transcriptional regulators on mating-type-specific expression of pheromone and pheromone-receptor genes, and are also involved in the regulation of development following sexual fusion (Stanton and Hull, 2007).

Typically, gametes produce one or more pheromone molecules to which gametes of other the mating type possess the corresponding receptor. For example, in *Saccharomyces cerivisiae*, which has two mating types, designated **a** and α, **a** cells produce **a** pheromone and a receptor for α pheromone, while α cells produce α pheromone and a receptor for **a** pheromone (Herskowitz, 1988; Sprague and Thorner, 1992).

In ciliates, sex involves conjugation between cells of different mating types. Attraction and adhesion between the cells occurs in the initial phase of preconjugation interaction and are mediated by so-called gamones, sexual signal molecules that are either diffusible or membrane bound. According to the gamone–receptor hypothesis, which has received solid empirical support, mating types are produced by differential distribution of gamones and receptors among individuals (Miyake 1981; 1996). Each individual has zero, one or more different types of gamones and zero, one or more different kinds of receptors except the one(s) for its own gamone(s). Therefore, the situation in ciliates is also consistent with the recognition/adhesion model of mating-type evolution.

The model of Hoekstra (1990a) predicts that no symmetric sexual fusions (any gamete can mate with any other) should exist, and that cell fusions in species without a sexual cycle should also be asymmetric. The older literature contains a few reports of symmetric matings in algae (e.g. Pringsheim and Ondracek, 1939), but these studies have been based on light-microscopic observations at a time that mating-type differentiation in isogamous systems was not yet known. I am not aware of any recent study in which symmetric gametic fusions have been demonstrated. Hurst and Hamilton (1992) suspected that perhaps mating types are absent in microsporidians, obligate intracellular parasites of animals, because they lack true mitochondria. However, recently microsporidia have been shown to be fungi with a genomic region homologous to the mating type locus of zygomycetes (Lee *et al.*, 2008), which suggests that they might possess the capacity for sex with mating-type differentiation. The hypothesis of Bell (1993) predicts that mating-type genes are derived from transposons. So far, evidence consistent with this prediction has not been found.

4.3.5 Mating types superimposed on male/female differentiation

Mating-type specificities (bipolar as well as multipolar) may occur next to male/female differentiation. As a consequence, terminological

confusion occurs, since often authors use "sexes" and "mating types" as synonyms. In both the ascomycetous and the basidomycetous fungi a mycelium may act simultaneously as male and female and also have mating-type specificity.

In the ascomycetes, female gametangia are well differentiated within fruiting bodies and the male function may be represented by conidia (mitotically formed spores) or a vegetative hypha. Sex is only possible between different mating types. For example, in *Neurospora crassa* a compatible interaction that initiates the development of fruiting bodies involves two strains of opposite mating type. Mating-type products regulate the initial attraction for cell fusion (fertilization) and coordinated migration of nuclei of opposite mating types into the ascogenous hyphae (Coppin *et al.*, 1997; Debuchy and Turgeon, 2006). Mitochondrial genomes are inherited from the female parent, although a low degree of biparental transmission is common, e.g. in *Neurospora* and *Podospora* (Griffiths, 1992).

In the Basdiomycetes, there are no morphologically differentiated gametes or gametangia, but sex is asymmetric with distinct male and female roles in fertilization. The acceptance of fertilizing nuclei and subsequent migration of these nuclei through the recipient mycelium is a female role. The incoming nucleus, which contributes little or no cytoplasm, can be regarded as the male role. The male nucleus can be derived from a germinating spore, from another monokaryon, or from a dikaryon (the so-called Buller phenomenon).

In ciliates, conjugation can be monozygotic or bizygotic (Miyake, 1996). Monozygotic conjugation usually involves anisomorphous conjugants (cells of different size). The micronucleus from the smaller conjugant enters the larger cell after which fusion occurs with the stationary micronucleus. No cytoplasm from the smaller conjugant is transferred, and the smaller exconjugant usually degenerates. More common is isomorphous bizygotic conjugation. Here one of the two micronuclei from each conjugant migrates into the other conjugant and fuses with the stationary micronucleus. Therefore, both in monozygotic and in bizygotic conjugation a basic male/female distinction is apparent, the migratory micronucleus representing the male role and the recipient cell (which also provides the cytoplasmic environment of the zygote) assuming the female role. Since both conjugants act as male and as female, bizygotic conjugation is equivalent to a mating between hermaphrodites, just as in fungi usually individuals can act both as male and female.

We conclude that there are good reasons to distinguish between sexes and mating types, because in many groups of organisms a basic sexual bipolarity (male/female) is recognizable, while legitimate matings are further restricted by mating-type systems or, as in hermaphroditic higher plants, by self-incompatibility systems (de Nettancourt, 1977). The male/female difference shows up as a typical behavioral or size dimorphism in the cells and/or nuclei involved in sexual fusion. In isogamous species with bipolar mating types the mating-type distinction coincides with a male/female distinction, for example in some isogamous algae with bipolar mating types one gamete type becomes stationary and is approached by the other gamete type, as in *Nephroselmis olivacea* (Suda *et al*., 2004). By analogy to similar behavioral differences between males and females in anisogamous species, the stationary type may be said to adopt the female role and the other the male role. In other systems no morphological or behavioral difference has been detected, but a characteristic dimorphism in cytoplasmic transmission can define a male/female dimorphism, again in analogy to well-known anisogamous systems (Hurst and Hamilton, 1992). However, in some species it has not been possible so far to recognize a male/female bipolarity underlying the mating-type differentiation. This is, for example, the case in *Saccharomyces cerevisiae*. In such cases the male/female terminology seems to be inappropriate.

4.4 THE ORIGIN OF SEXUAL ASYMMETRY

As argued above, the classical assumption that the evolution of anisogamy represents the origin of the male/female dimorphism (Weismann, 1886; Parker *et al*., 1972) is not satisfactory. There is much evidence, some of which is reviewed in this chapter, that in isogamous species a bipolar sexual differentiation exists: the fusing gametes may differ in, for example, recognition and adhesion mechanisms, cytoplasmic transmission and prefusion behavior giving rise to a donor–recipient dimorphism. For this reason Hurst and Hamilton (1992) have proposed to define sexes on the basis of the control of the inheritance of cytoplasmic genomes. It could be argued that this is merely a matter of definition, and that equally well characteristic gametic differences in isogamous systems should be designated as "mating types," restricting the concepts of male and female to anisogamous organisms. However, this "solution" is bound to generate confusion, since in many algae, fungi and ciliates mating types occur which are superimposed on a

more basic binary sexual differentiation, as argued in the preceding paragraph. Therefore, I believe it to be meaningful to extend the concept of sexes to isogamous species also. How should we evaluate the proposal of Hurst and Hamilton (1992) to use the regulation of cytoplasmic inheritance as the most fundamental distinction between the two sexes? First, because of exceptions it cannot be a general explanation (Hurst, 1995; Randerson and Hurst, 2001; Burt and Trivers, 2006). Moreover, other asymmetries exist between binary isogamous mating types. There is not a single well-documented case of a sexual species in which all gametes are equivalent in the sense that any gamete can mate with any other. In other words, sexual asymmetry is the rule. This asymmetry involves a whole suite of characters and may include morphological and behavioral differences, mutual attraction, recognition and adhesion mechanisms and cytoplasmic inheritance. To choose any single character from this list as the defining feature of sexes has the disadvantage that exceptions do occur: not all species with binary sexes have morphologically differentiated gametes, or strict regulation of cytoplasmic inheritance, etc. Ideally, one would like to understand in which temporal order the various asymmetries have evolved, the first being an obvious candidate for the primary distinction between the sexes. Other characteristic differences between the sexes would then have accumulated during evolution, associated with this initial asymmetry and genetically regulated by genes on non-recombining sex chromosomes, or by closely linked genes in complex MAT loci (Bull, 1983; Charlesworth, 1991; Fraser *et al.*, 2004). Further progress in our understanding of the evolution of sexes probably has to come from precise and extensive genetic characterizations of mating-type genes in many species, combined with refined phylogenetic analysis. That such an approach can be illuminating is shown by the work of Nozaki *et al.* (2006). Males of the anisogamous alga *Plerodina starri* have been shown to have an active copy of a gene *PsPlesMID*, which is similar in sequence to the *CrMID* gene of the isogamous *Chlamydomonas reinhardtii*. *CrMID* is only present in the mt^- mating-type locus. Thus, this study may help understand the transition of isogamy to anisogamy in the lineage of the volvocine algae.

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KINYA NISHIMURA AND NOBORU HOSHINO

5

Adaptive significance of egg size variation of aquatic organisms in relation to mesoscale features of aquatic environments

5.1 INTRODUCTION

Following evolution of sexual reproduction, which was most likely characterized by the transition from isogamy (equal-sized gametes) to anisogamy (different-sized gametes) and oogamy (large immotile gametes, eggs, and small motile gametes, sperm), a secondary characteristic related to fertilization resulted in the diversification of the life-history traits of organisms (Parker and Tang-Martinez, 2005; Kokko and Jennions, 2008). An understanding of sexual and reproductive characteristics of organisms is an important aspect of the study of life-history evolution. Various questions arise concerning the evolutionary diversification including sex-specific behaviors and morphologies just before and after mating and the fusion of gametes (Andersson, 1994; Danchin *et al*., 2008).

Size variations – between different gamete types as well as within the same gamete type – evoke questions about the selective pressures on those variations. For example, comparison of + and − type gametes in green algae raises a fundamental question: what selective forces drive gamete size differentiation, and what forces maintain observed size differences between the two types of gametes that unite to create the zygote (Randerson and Hurst, 2001a; 2001b; Togashi *et al*., 2007; Iyer and Roughgarden, 2008)? One of the most influential explanations for patterns of gamete size distribution comes from game theory, using

The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection, ed. Tatsuya Togashi and Paul Alan Cox. Published by Cambridge University Press.

a model that incorporates the mechanism of disruptive selection (Parker *et al.*, 1972).

In oogamous reproductive systems – those characterized by extremely large eggs and small sperm – with external fertilization, variation in male gametes and female gametes is often investigated separately. When the focus is on male gamete (sperm) size, the game-theory explanation stresses the competition among male gametes to fertilize the egg. Such sperm competition selects for numerous, tiny gametes (Ball and Parker, 1996; Gage and Morrow, 2003; Bode and Marshall, 2007).

Because the female gamete (egg) is larger and more conspicuous than the male gamete (sperm), more studies address egg size variation than sperm size variation. The primary explanation for egg size variation is based on simple optimization theory (Vance, 1973b; 1973a; Smith and Fretwell, 1974; Ware, 1975). Modifications to the theory take into account other properties related to egg size that affect the offspring's fitness (Parker and Begon, 1986; Winkler and Wallin, 1987; McGinley, 1989; Nussbaum and Schultz, 1989). These modifications make the primary models more broadly applicable for understanding egg size variation in terrestrial and aquatic organisms of various taxonomic groups (Kaplan, 1980; Sargent *et al.*, 1987; Hutchings, 1991; Iguchi and Yamaguchi, 1994; Hipfner *et al.*, 1997; Hipfner and Gaston, 1999).

In aquatic environments, most organisms are free spawners, releasing their gametes into the water, and fertilization is primarily external. There are other crucial differences between terrestrial and aquatic habitats. Water is 1000-fold more dense a medium than air. Diffusion, turbulent flow and mesoscale currents disperse small particles in the aquatic medium. These factors influence prefertilization processes such as the dispersion of sperm and eggs before fertilization as well as postfertilization processes such as the dispersion, buffeting and transport of the developing embryo in the water column. Such factors might therefore represent selective forces leading to variation in aquatic organisms at early planktonic life stages, including eggs.

One proposed theory suggests that in broadcast-spawning marine invertebrates, e.g. those with external fertilization, the diffusion process affecting the movement of eggs and sperm in water leads to the probability that fertilization is egg size dependent (Levitan, 1993; Podolsky and Strathmann, 1996; Levitan, 2000). In sea urchins, for which detailed fertilization kinetics information is available (Vogel *et al.*, 1982; Levitan, 2000) as well as other biological aspects of early

stages of their life history (Levitan, 1996; Levitan, 2000), this theory explains well the observed patterns of egg size variation in nature (Levitan, 1993; Levitan, 1996; Levitan, 2000).

Another group of animals with principally external fertilization are teleostean. Several patterns of egg size variation, both among and within species, are represented in these fishes (Ware, 1975; 1977; Duarte and Alcaraz, 1989a; Elgar, 1990; Wootton, 1994; Kokita, 2003). Their egg size variation is often conventionally explained by the need to strike an optimal balance between size and number of offspring (e.g. Sargent *et al*., 1987; Hutchings, 1991; Iguchi and Yamaguchi, 1994; Johnston and Leggett, 2002). Even though the impact of the aquatic medium on some early stages of life history of fish species is considerable, to our knowledge, no theory has been proposed that explains egg size variation on the basis of the properties of the medium per se or on the basis of the circumstances under which the eggs enter the medium, except for the theory of the egg size-dependent probability of fertilization as a result of the diffusion of eggs and sperm in the water (Robertson, 1996). We believe it is necessary to consider various features of the aquatic environment in order to understand the patterns of egg size variation in teleostean fish species. In particular, we take into account a major feature of the aquatic environment, namely water movements. We consider two types of water movement, turbulent and non-turbulent flow, to make heuristic inferences about egg size variation in teleostean fishes in a wide range of aquatic habitats. Both turbulent and non-turbulent forces of water movement act on early-stage organisms. We suspect that each of these forces critically influence growth and survival in these fishes.

First, we focus on the influence of the turbulent forces in the medium, water, which surrounds the fertilized eggs and developing embryos. These forces can be considerable. For example, because movement of the dense medium makes the small particles baffle, an appreciable quantity of energy is required to settle eggs on the spawning place in an aquatic medium, compared with that required in a terrestrial environment. In fact, several egg traits function to prevent eggs from being swept away by water turbulence movements, including jelly on the surface of an egg which functions as a glue to attach the egg to a substrate (Hirai, 2003). We thus present an explanation for relationships among egg types, namely, floating eggs versus demersal eggs, egg size and eggs with or without parental care, among fishes in relation to aquatic turbulence.

Second, we consider non-turbluent water movement, particularly water currents. Biologists studying fishes and crustacea have long been aware that mesoscale water currents play an important role in the transport of planktonic eggs or larvae from spawning sites to favorable areas for development (nursery grounds) with a favorable timing (Parrish *et al.*, 1981; Norcross and Shaw, 1984; Sherman *et al.*, 1984; Hinckley *et al.*, 1991; Helfman *et al.*, 1997; Jones *et al.*, 1999; Swearer *et al.*, 1999). Various inferences have been made about life history and population dynamics of aquatic organisms based on such currents (Hjort, 1914; Cushing, 1975). We deduce how egg size should vary in response to the transport of eggs and planktonic larvae by currents from the spawning ground to a downstream nursery ground.

Essentially, we base our inferences on qualitatively validated biological facts. At the same time, we adopt abstractions to describe the environmental factors on which we are focusing and assume *ceteris paribus* (all other things held constant) in order to extend our argument to a wide range of aquatic habitats. Our purpose here is to present the qualitative implications of the selective forces operating on early life-stage traits of aquatic organisms.

5.2 Turbulent Disturbance and Its Influence on Egg Size and Related Traits

5.2.1 An integrative view

The eggs and larvae of all marine animals are usually more strongly affected by the forces of the aquatic medium than by those of air (Denny and Shibata, 1989; Okubo and Levin, 2001). Diffusion, turbulent flow and mesoscale currents disturb and disperse suspended and floating particles in the aquatic medium. For ease of discussion, we here use the term "turbulent disturbance" to describe all types of turbulence in water, whether oceanographic or hydromechanical.

Dispersal of vulnerable eggs and larvae in a large volume of the water with turbulent disturbance subjects them to high mortality. Egg-laying females must deal with the circumstances imposed by the features of the medium and the strength of the forces associated with the medium.

Fish eggs are categorized into several types. Eggs of some fish species are buoyant and float near the water's surface or drift suspended in the water column. Floating and suspended eggs tend to be

dispersed from the spawning site by the turbulent disturbance of the water. Most marine fish species produce buoyant eggs that float or remain suspended in the water column, but some produce demersal eggs. Such eggs have low buoyancy, and settle out of the water column to the bottom of the water body (e.g. eggs of flat fishes) (Minami, 1984). Adherent eggs have jelly or filaments which serve to attach them to substrates such as stones or seaweed (e.g. eggs of sandfish) (Hirai, 2003). Demersal and adherent eggs tend to stay at the site where they were spawned, unlike floating or suspended buoyant eggs, during the egg and larval period.

In species that practice parental care of eggs, eggs must be demersal or adherent, so that they settle near the parent. Therefore, the egg type (roughly, floating versus demersal/adherent), degree of parental care and egg size should be mutually and tightly linked life-history traits targeted by natural selection under the influence of the turbulent disturbance of the medium.

We categorize eggs into just two types, floating or demersal. It is generally known that some species of freshwater fishes (e.g. sticklebacks) lay eggs in a benthic nest. For convenience, we classify demersal/adherent eggs and eggs laid in a nest indiscriminately as "demersal eggs," because all of these egg types remain near the spawning site, which is the important characteristic for our analytical purpose, even though we realize that the label "demersal" is not biologically correct in all cases.

5.2.2 Observed patterns of egg size variation in fish species

Several patterns of variation in egg size distributions have been distinguished in relation to the environment and life-history traits of fish species. First, the mean egg size (about 1 mm) of marine spawning fishes is smaller than that (about 2 mm) of freshwater spawning fishes (Table 5.1). Second, there exists a remarkable egg size difference between fish groups that do and do not practice parental care of eggs (and larvae). Fish groups practicing parental care lay larger eggs than those that do not (Table 5.1). Third, for a wide range of fish taxa, egg size differs among fish species according to spawning habit and egg type (Balon, 1984). Free-floating eggs, which are buoyant and float near the water's surface or remain suspended with in the water column, are smaller than demersal/adherent eggs, which settle to the bottom of the water column or are attached to rocks, gravel or sand (Table 5.1).

Table 5.1 *Egg size according to the categories habitat type, parental care, taxonomic group, and egg type*

Category[a]	Number of species	Egg size (diameter in mm)		Source
		Mean	SD	
Marine fish[b]	306	1.25	1.2840	(Chambers and Leggett 1996)
Pelagic fish	65	1.2	0.12	(Ware 1975)
Marine teleostean	51	1.063[c]	1.8296	(Duarte and Alcaraz 1989b)
Freshwater teleostean[d]	46	2.29	2.2398	(Duarte and Alcaraz 1989b)
Parental care practiced	29	2.458	1.6307	Compiled from (Hirai 2003), (Kawanabe and Mizuno 1989), (Ochiai and Tanaka 1986b) and (Sargent *et al.* 1987)
Salmonid	11	5.786	2.1182	Compiled from (Sargent *et al.* 1987), (Groot *et al.* 1995)
Buoyant	285	1.256[e]	0.4565	(Hirai 2003)
Demersal	146	2.39	1.5285	(Hirai 2003)

[a] Based on terms used in the cited references.
[b] Includes Selachii.
[c] $t = 4.2416$, $df = 95$, $P < 0.000$.
[d] Includes eggs of fish practicing parental care and salmonid eggs.
[e] $t = 12.3233$, $df = 429$, $P < 0.000$.

As these observed relationships do not take into account phylogenetic relationships among taxa, data on individual species are not necessarily evolutionarily independent. Similarly, the abiotic factors considered above are not necessarily independent, but may be complexly interconnected. Nevertheless, this rough picture of egg size distribution implies that egg size is adaptive along each of several environmental and life-history dimensions, namely, a marine versus a freshwater spawning site, parental care of eggs practiced or not practiced and a floating versus a demersal egg type.

5.2.3 The model

We attempted to construct a mathematical model that explains the relationships among egg type (roughly, floating versus demersal/adherent),

degree of parental care and egg size in a group of aquatic organisms, teleost fish species, in which the water's turbulent disturbance is the major explanatory factor. We roughly identified a weak to strong continuum of the turbulent disturbance: along the nearshore–offshore dimension in marine environments, along the lentic–flowing dimension in freshwater environments, and along the rapidly flowing upper–gently flowing lower stream dimension.

First, we need a biological basis by which to validate some of the assumptions utilized in the model. In several fish species, egg size is known to be positively related to larval size at hatching (Duarte and Alcaraz, 1989a; Pepin, 1991). Among fish species, small larvae may be more susceptible to starvation because of the limited energy reserves contained in their yolk sac (Blaxter and Hempel, 1963; Knutsen and Tilseth, 1985; Miller *et al.*, 1988). In addition, small larvae may have short reactive distances and low swimming ability, limiting their ability to search for food (Blaxter, 1986; Miller *et al.*, 1992). The energy intake of actively feeding planktonic larvae is limited by the ability of their developing sensory systems to detect prey, as well as by their gape size, the developmental condition of their feeding and digestive apparatus, and their locomotive ability, all of which clearly increase with larval size (Hunter, 1972; Buskey *et al.*, 1993). Although, predators selectively forage for larger larvae in a proximate choice situation (Litvak and Leggett, 1992; Pepin *et al.*, 1992), larval body size per se reduces the larvae's vulnerability to predators through differentials in encounter rates and escape ability, and through the predator's gape limitation (Blaxter, 1986; Miller *et al.*, 1988; Bailey and Houde, 1989; Pepin and Myers, 1991).

Even though the size of egg produced by a parent is not the sole determinant of the offspring's fitness (Emlet and HoeghGuldberg, 1997; Bertram and Strathmann, 1998; George 1999), the observations presented above suggest that large egg size is advantageous with respect to the size of the individual offspring that emerges from the egg. The size and number of eggs produced are traits of the individual parent and, thus, elements of the fitness of that parent (e.g. Smith and Fretwell, 1974; Parker and Begon, 1986). The simplest, logically complete model that can explain these three life-history characteristics (egg size, parental care of eggs and egg type) and that allows us to discuss optimality must incorporate certain minimum conditions. We postulate that egg type, egg size and egg care are parental traits that are potentially targets of natural

selection such that the surrogate fitness criterion of the spawning parent, W, is maximized:

$$W \propto (\text{number of eggs produced}) \times (\text{survival probability of each offspring}) \quad (5.1)$$

Adopting the conventional egg size optimization model (Smith and Fretwell, 1974), we use the amount of yolk provided by the parent and available to be used for development by the embryo, Y, to represent egg size.

To develop a unified explanation, we present a single surrogate fitness function that includes parameters that are common to a wide range of aquatic environments, and that ignores factors specific to particular aquatic environments. We abstract a weak–strong continuum of the turbulent disturbance of water existing in specific situations, such as along the nearshore–offshore dimension and along the lentic–flowing dimension. Notice again that we use the term "turbulent disturbance" regardless of the type of force, whether oceanographic or hydromechanical. We denote the turbulent disturbance of the medium surrounding the eggs as δ.

5.2.3.1 Number of eggs produced

For demersal eggs, investment in the unhatched egg, such as in jelly or filaments for attaching the egg to a substrate or energy for building the spawning bed or nest against the water's turbulent disturbance, is needed in addition to the investment in yolk. We assume that the investment amount required for settlement of the eggs is an increasing function of the medium's turbulent disturbance δ. For simplicity, the investment is assumed to be $a\delta$, where a is a non-negative constant. We assume that a is 0 for floating eggs and is a positive value for demersal eggs.

Let c be the cost for parental care, such as guarding behavior, which reduces egg mortality. It is noteworthy that, given the turbulent disturbance of the medium, investment for settlement of the eggs is required prior to investment in parental care. For simplicity, the investment for settlement and parental care are evaluated per egg. Even though this simplification is not completely realistic for an empirical analysis, we consider it appropriate for our analytical purpose. The number of eggs, N, produced by a parent with a given proportion of its body mass used for reproduction, R, is a function of egg size, egg type and degree of care effort. The function is given as

$$N(Y, c, a_i, b_{ij}) = \frac{R}{Y + a_i\delta + b_{ij}c}, (i, j = 1, 2), \tag{5.2}$$

where b_{ij} is an auxiliary variable to determine the cost of parental care, $a_1 = 0$, $a_2 > 0$, $b_{1j} = 0$ for any j, $b_{21} = 0$, and $b_{22} > 0$. Specifically, the number of floating eggs, demersal eggs without care investment and demersal eggs with care investment is $N(Y, c, 0, 0) = \frac{R}{Y}$, $N(Y, c, a_2, 0) = \frac{R}{Y + a_2\delta}$ and $N(Y, c, a_2, b_{22}) = \frac{R}{Y + a_2\delta + b_{22}c}$, respectively.

5.2.3.2 *Egg survival*

We assume that the poor–rich food availability continuum and the weak–strong predation risk continuum are positively related, and abstract the environmental continuum as "biological richness." We parameterize biological richness of the environment where the eggs and larvae are placed as u.

We assume an arbitrary S-shaped function for offspring size with respect to egg size

$$G(Y, u) = \frac{f(u)Y^\theta}{\beta + Y^\theta}, \tag{5.3}$$

where θ and β are positive constants. f is a non-decreasing function of biological richness reflecting food availability. We adopt the simple function, φu, to describe the relationship between biological richness of the environment and food availability, where φ is a positive constant. For mathematical convenience, we arbitrarily adopt a non-decreasing function of food availability that reflects biological richness, $f(u) = (\varphi u)^\rho$ where $0 \leq \rho < 1$. Equation (5.3) describes offspring size with respect to egg size and biological richness (Figure 5.1). Choice of the functional

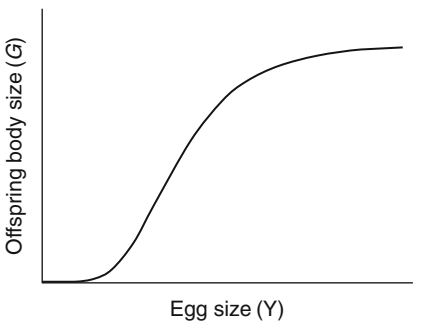

Figure 5.1 Relationship between egg size and larval body size.

form of Equation (5.3) is arbitrary. We adopted a biologically reasonable assumption that the function is convex upward and that a line from the origin is tangental to the convex portion of curve.

We assume that mortality comprises three separable components. The first mortality factor is starvation, which depends on food availability and offspring body size. Assuming that the occurrence of encounters with available food is a Poisson process, the expected waiting time between successive events of food acquisition is reciprocally proportional to the instantaneous encounter rate with suitable prey, which reflects biological richness. We consider the instantaneous death rate under starvation risk to be

$$S_1 = \frac{1}{\phi u G}. \tag{5.4}$$

$1/(\varphi u)$ implies the expected waiting time between successive prey encounters, and $1/G$ implies starvation tolerance. The properties of Equation (5.4) can be qualitatively verified with data from empirical studies of larval physiology (Blaxter and Hempel, 1963; Knutsen and Tilseth, 1985; Blaxter, 1986; Miller *et al.*, 1988; Bailey and Houde, 1989; Pepin and Myers, 1991).

The second mortality factor is predation. Egg-laying females generally choose spawning sites that are relatively safe from predation risk (Parrish *et al.*, 1981; Norcross and Shaw, 1984; Sherman *et al.*, 1984; Hinckley *et al.*, 1991; Epifanio, 1995; Helfman *et al.*, 1997; Jones *et al.*, 1999; Swearer *et al.*, 1999). The instantaneous death rate due to predation is proportional to biological richness u, arbitrarily scaled with a positive coefficient ω_i. Suppose that for a floating egg, ω_i corresponds to ω_1, and for a demersal egg, ω_i corresponds to ω_2; then $\omega_1 > \omega_2$, reflecting that eggs that are more dispersed suffer higher predation mortality.

We assume that the instantaneous death rate due to predation is a decreasing function with respect to parental care effort, c. The same amount of care effort reduces risk less effectively as the turbulent disturbance increases. For mathematical convenience, we assume that the instantaneous death rate under predation risk is

$$S_2 = \frac{\omega_i u}{1 + \varphi c e^{-\delta}}, \tag{5.5}$$

where ϕ is a positive constant. Equation (5.5) reflects all of the features that we consider above.

Third, we assume that the magnitude of the turbulent disturbance, δ, is itself an independent physical mortality factor, and that the

instantaneous death rate is an arbitrary increasing function of δ. In our arguments, this mortality factor is identical regardless of egg type; thus, it cancels out of our argument.

Taking into account the mortality factors s_1 and s_2, the probability of an offspring surviving at a time after passing the critical mortality period is expressed as the exponential function

$$\mu = e^{-(s_1+s_2)}. \tag{5.6}$$

5.2.3.3 *Fitness*

The fitness of the spawner is obtained by multiplying Equation (5.2) by Equation (5.6). Fitness is evaluated as a function of egg type (floating or demersal), egg size and parental care efforts for a given external environmental condition characterized by the turbulent disturbance of the medium and the biological richness. In accordance with our purposes, fitness is expressed as the function

$$W(Y, a_i, b_{ij}, \omega_i, c, u, \delta) = N \times \mu. \tag{5.7}$$

Note again that the adaptive egg types are; (1) floating, if $(a_i, b_{ij}, \omega_i) = (0, 0, \omega_1)$ maximizes the fitness with a positive value of egg size, Y, and no parental care, $c = 0$ (2) demersal with no parental care, if $(a_i, b_{ij}, \omega_i) = (a_2, 0, \omega_2)$ maximizes the fitness function with a positive value of Y and $c = 0$ and (3) demersal with parental care, if $(a_i, b_{ij}, \omega_i) = (a_2, b_{22}, \omega_2)$ maximizes the fitness function with positive values of Y and c.

In our argument, δ is consistently treated as a given environmental parameter. Biological richness u is treated either as a given environmental parameter or as a variable trait that implies the spawner's choice of spawning site along a gradient of biological richness. We consider that egg size, Y, egg type determined by (a_i, b_{ij}, ω_i) and care investment, c, are all targets of natural selection, or that some of them are given conditions. We analyze maximization of fitness in relation to these parameters.

In the numerical evaluation of W, we assign the following basal values to the common parameters: $\rho = 0.5$, $\varphi = 1$, $\beta = 5$, $\theta = 5$, $a_1 = 0$, $a_2 = 0.52$, $b_{11} = b_{12} = b_{21} = 0$, $b_{22} = 1$, $\omega_1 = 1$ and $\omega_2 = 0.80$.

Even though our model is not very complex, non-linear functional relationships in several parts of the model make it difficult to verify our predications. The qualitative predictions of the model can be preserved, however, by changing the mathematical form of each function while keeping its qualitative characteristics, that is, as an

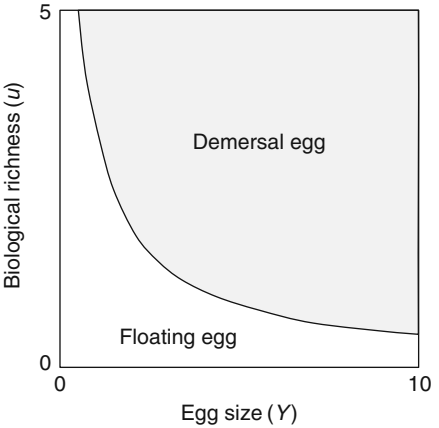

Figure 5.2 Regions in which the optimal egg type is floating or demersal in relation to egg size and biological richness of the spawning site. $c = 0$ and $\delta = 2.5$, where c is the cost of parental care and δ is the turbulent disturbance of the medium.

increasing, decreasing, linear, convex or concave function. Thus, we can qualitatively verify the results of our model by using several functions that possess similar properties.

5.2.4 Floating versus demersal

Our model predicts the well-known egg-type distribution pattern of marine fish species; namely, small floating eggs are adaptive in environments with low biological richness, whereas large demersal eggs are adaptive in environments with high biological richness (Figure 5.2). This pattern reflects the observation not only that free-floating eggs are smaller than demersal eggs, but also that spawning sites of free-floating eggs include oligotrophic pelagic regions (e.g. Paxton and Eschmeyer, 1995).

5.2.5 Optimal egg size and optimal parental care

We analyzed optimal parental care for a given egg size. Our model predicts the observed relationship between egg size and degree of parental care (Gross and Sargent, 1985; Sargent *et al.*, 1987) in which parental care is positively correlated with egg size. We find that there exists a threshold value of egg size below which a parent should invest

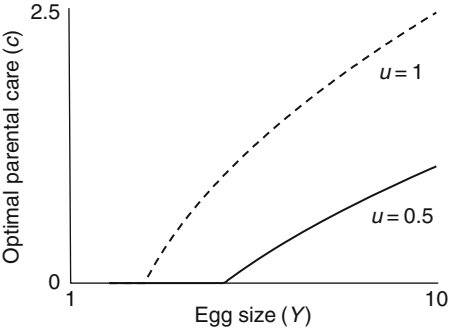

Figure 5.3 Optimal investment in parental egg care in relation to egg size. $u = 1$ indicates a high, and $u = 0.5$ a low, level of biological richness at the spawning site. $\delta = 0.5$.

no care effort. Above the threshold size, the parent should invest care effort, and the optimal amount of parental egg care should increase as egg size increases (Figure 5.3). Because eggs suffer high predation risk in a biologically rich environment (large value of u), the parental care cost is larger when eggs are spawned in such an environment (Figure 5.3).

Next, we consider the case in which egg size is not a given trait, but a target of natural selection, with a parental care cost that is also a target of natural selection, and we investigate optimal trait sets in environmental regimes differing with regard to biological richness and turbulent disturbances in the aquatic environment. High care cost and large egg size are selectively favored in environments with high biological richness and low turbulent disturbance, whereas large egg size and no parental care are favored in environments with low biological richness and a high turbulent disturbance (Figure 5.4).

In broad terms, biological richness is negatively correlated with the turbulent disturbance of water movements, particularly in freshwater environments, when lentic waters are compared with streams. The relationships of parental egg care and egg size in freshwater with the turbulent disturbance of water and biological enrichment are shown by the double-headed arrows in the panels of Figure 5.4. Toward the lower right, the turbulent disturbance is high and biological richness is low, and no parental care with a larger egg tends to be adaptive. Toward the upper left, where turbulent disturbance is low and biological richness is high, parental care, but with a larger egg tends to be adaptive. At intermediate points, a smaller egg with no parental care tends to be adaptive.

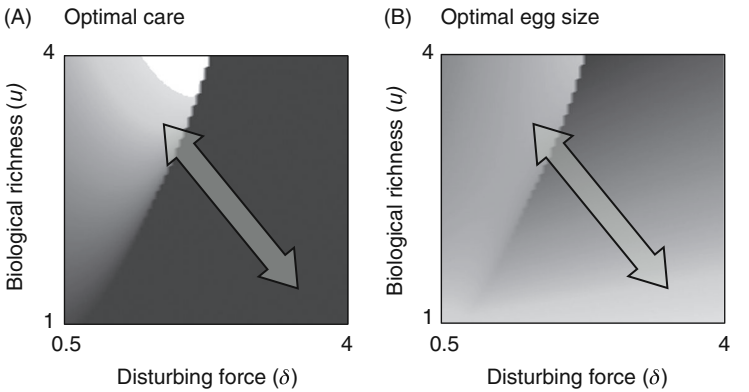

Figure 5.4 Optimal trait set. (A) Parental egg care investment and (B) egg size along continuums of turbulent disturbance and biological richness. Lighter regions indicate high values and darker regions indicate low values. Only the demersal egg type is considered in this analysis.

For example, salmonid species are typical examples of fishes that spawn eggs in the upper reaches of streams, where the current is rapid and, thus, the turbulent disturbance is high. Among freshwater fishes, salmonids have a large egg size (ca. 4.5 mm, see Table 5.1). Parents of salmonids do not, however, pay any egg care cost during the development of the embryos; instead, they construct a spawning bed so that eggs will not be swept away by the current (e.g. Burgner, 1991).

Some Cichlidae, Percidae, Centrarchidae and Antennariidae fish species practice parental care, and their mean egg size is larger than the mean for all freshwater fishes, including both those species that practice care and those that do not (ca. 2.5 mm, see Table 5.1; statistical comparison was not possible because the data categorization was insufficient). Their habitats range from the middle reaches of streams to lentic waters, where the turbulent disturbance of water is relatively low and biological richness is relatively high.

5.2.6 Optimal early life-stage traits

We approach a single-factor explanation for egg size and related traits in teleostean fish species by focusing only on the turbulent disturbance of the medium. Along a gradient of turbulent disturbance, a demersal type egg is favored when the turbulent disturbance is weak (ranges (I) and (II) in Figure 5.5) and a floating type egg is favored when it is strong (range (III) in Figure 5.5).

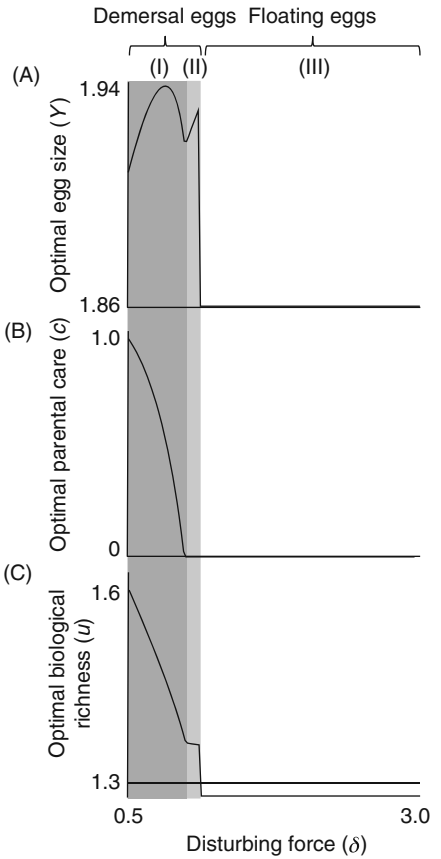

Figure 5.5 Optimal trait sets along the continuum of the turbulent disturbance of water movement. In range (I), eggs are demersal with parental care; in range (II), they are demersal with no parental care; and in range (III), the eggs float in the water column. (A) Egg size, (B) parental egg care investment and (C) biological richness of the environment in which the eggs and larvae are placed.

When the turbulent disturbance is very weak, the egg type is demersal (range (I) in Figure 5.5), egg size is large, and parental care is practiced; moreover, the weaker the turbulent disturbance, the larger the optimal amount of parental care. At the same time, the optimal biological richness of the environment where eggs and larvae should be placed is high in environments where the turbulent disturbance is low, if choice of spawning site is also a selective trait of the parent: the higher the turbulent disturbance, the lower the biological richness of the environment where eggs and larvae should be placed.

When the turbulent disturbance is within bounds (II) (Figure 5.5), the optimal egg type is demersal, but parental care effort should be zero and the biological richness experienced by the eggs and larvae should be lower than that associated with parental care. The higher the turbulent disturbance is in this range, the higher the investment in jelly or other substances should be, to prevent the egg from being swept away by the turbulent disturbance. This extra investment reduces fecundity and makes no contribution to juvenile growth. To compensate for this disadvantage, the egg size should increase with increasing turbulent disturbance to promote higher survival of the larvae. When the turbulent disturbance is high (range (III) in Figure 5.5), a small floating egg spawned in a biologically poor environment along with no parental care is selectively favored.

Optimal biological richness of the environment where eggs and larvae should be placed in accordance with turbulent disturbance of the environment is consistent with the general trend that biological richness is negatively correlated with the turbulent disturbance of water movement (see Figure 5.5C). Oligotrophic environments with high turbulent disturbance are favored by floating-egg spawners, whereas a eutrophic environment with low turbulent disturbance is favored by demersal-egg spawners that practice parental care.

5.2.7 Water turbulent disturbance as an explanatory factor of egg size and related traits

We presented an explanation for the general trends in the distribution patterns of egg size, parental care and egg type in teleost fishes along a single abstract dimension, turbulent disturbance, which reflects a continuum from lentic water to ocean habitat. This attempt is one alternative way to explain egg size of broadcast-spawning fish species by considering a feature of the aquatic environment. The general trend is for egg size in pelagic fish species to be small. This tendency is one part of what we sought to explain. Winemiller and Rose (1993) proposed a simulation model to evaluate how production of plentiful small eggs by pelagic fish is adaptive in relation to a spatially patchy food distribution. Their model affords insight into the adaptive significance of the production of numerous small eggs by pelagic fishes, but Winemiller and Rose (1993) do not address egg size variation in different aquatic environmental regimes.

Even though our scheme of a continuum of turbulent disturbance along the nearshore–offshore and the lentic–flowing waters dimensions and the relationships we derive between the impact of the turbulent disturbance and the survival of eggs and larvae are crude, our model results in a comprehensive yet parsimonious explanation for the observed patterns of egg size and environmental distribution of floating eggs and demersal eggs in marine teleost fish species (Ware, 1975; Minami, 1984; Chambers and Leggett, 1996; Hirai, 2003), and also for the observed relationship between egg size and degree of parental care among those species practicing parental care (Gross and Sargent, 1985; Sargent *et al.*, 1987) (Table 5.1).

5.3 NON-TURBULENT WATER MOVEMENT AND EGG SIZE

In the previous section, we considered the influence of the turbulent disturbance of the medium on early life-stage characteristics of teleost fish species. It is known that non-turbulent forces, particularly directional water currents, transport planktonic eggs and larvae. Biologists studying fishes and crustacea have realized that this transport plays an important role in relocating the developing embryos to a favorable place with favorable timing (Parrish *et al.*, 1981; Norcross and Shaw, 1984; Sherman *et al.*, 1984; Hinckley *et al.*, 1991; Helfman *et al.*, 1997; Jones *et al.*, 1999; Swearer *et al.*, 1999). In this section, we make the deductive inference that egg size is adaptive and is designed to optimize the developmental schedule of the embryo and larva on the transporting current from the spawning site to the nursery ground. We begin by reviewing the common features of buoyant suspended or floating eggs and developing larvae, from which we can infer the adaptive features of egg size in non-turbulent water currents.

5.3.1 Planktonic eggs and larvae in water currents

5.3.1.1 Periods of endogenous and exogenous nutrition

Eggs are packed with nutrition for the development of the embryo to the larval stage, when the organism gains the ability to feed exogenously. Whether or not the embryos develop at the spawning site, newly hatched fish larvae are planktonic and drift with the water currents. This characteristic is shared by most fish species, even though a diversity of other biological and environmental features affect the species.

Initially, larvae may be lecithotrophic; that is, they may have some amount of unabsorbed yolk in their body. Generally, lecithotrophic larvae with extensive yolk reserves have an extended developmental period during which they rely on endogenous nutrition before they exhaust their yolk supply and must rely on exogenous nutrition (Hunter, 1972; Buskey *et al*., 1993). The energy intake of actively feeding planktonic larvae is limited by the ability of their developing sensory systems to detect prey, as well as by their gape size, the developmental condition of their feeding and digestive apparatus, and their locomotive ability (Hunter, 1972; Buskey *et al*., 1993).

Generally, eggs have no defense against predation without parental care. Moreover, just after hatching, the larvae are small and have little locomotive ability; thus, they are also vulnerable to predation by predators such as planktivorous fishes (Fuiman and Batty, 1994), cnidarians (Duffy *et al*., 1997), and ctenophores (Cowan and Houde, 1993). Therefore, owing to their immaturity and small body size, newly hatched larvae are at risk of predation as well as of starvation (Hjort, 1914; Blaxter and Hempel, 1963; Knutsen and Tilseth, 1985; Blaxter, 1986; Miller *et al*., 1988; Miller *et al*., 1992). We argue, however, that the spawners generally choose spawning sites that are relatively safe from predation risk (Parrish *et al*., 1981; Norcross and Shaw, 1984; Sherman *et al*., 1984; Hinckley *et al*., 1991; Epifanio, 1995; Helfman *et al*., 1997; Jones *et al*., 1999; Swearer *et al*., 1999).

5.3.1.2 Currents transport eggs and larvae

Susceptible planktonic larvae are passively moved by any mesoscale water movements that occur. Ocean currents are known to transport eggs and larvae of several pelagic fishes such as walleye pollock (*Theragra chalcogramma*), cod (*Gadus morhua*) and herring (*Clupea pallasii*) (Marteinsdottir *et al*., 2000; Hinckley *et al*., 2001). Offshore or onshore tidal currents also transport larvae of such fishes as sailfin sandfish (*Arctoscopus japonicus*) and grass puffer (*Fugu niphobles*). Buoyant eggs and the larvae that hatch from them are exposed to the forces of water movement from the time of spawning, but larvae that hatch from demersal eggs are exposed only after they enter their planktonic life stage.

In fact, some fish species that have at least an early planktonic stage are spawned upcurrent, whereas their nursery ground is downcurrent (Norcross and Shaw, 1984). For example, many coral-reef fish species spawn at reef edges, and their larvae are carried off by the currents that flow past the reef (Helfman *et al*., 1997). Coastal fish

species of the Pacific Northwest of North America spawn offshore, and the larvae are transported inshore to the nursery ground by surface wind drift (Parrish *et al.*, 1981). The demersal and buoyant eggs of several fish species are spawned on the seafloor or near the bottom of the water column and then transported to the nursery grounds by upwelling or buoyancy regulation (Bailey *et al.*, 2003; Hare *et al.*, 2005). Newly hatched larvae of chum salmon are transported by freshets fed by melting snow from their upstream riverine spawning grounds to river-mouth or estuarine nursery grounds (Ochiai and Tanaka, 1986a; Groot and Margolis, 1991), and larvae of some freshwater fish species are transported by stream flow from the spawning grounds in the upper reaches of rivers to nursery grounds in downstream lakes (Hall *et al.*, 1991; Cooperman and Markle, 2003).

5.3.1.3 *Transporting currents determine the environment of planktonic eggs and larvae*

In the water column, currents determine where developing planktonic embryos experience external biotic conditions. Typically, but not always, the primary productivity and bioabundance needed for the larva's subsequent trophic levels are higher downcurrent than upcurrent because the water movement transports suspended solids downcurrent (Norcross and Shaw, 1984). Therefore, one possible scenario is that the organisms constituting the prey of the planktonic larvae, as well as those that prey on the larvae, are more abundant downcurrent (Sherman *et al.*, 1984). The transporting currents, therefore, have the important role of placing the embryo in an appropriate location, with appropriate timing, for adequate survival and growth during its developmental period. Fisheries scientists have long argued about spawning location and timing, and about nursery ground location and the timing of the arrival of embryos or larvae at that location, in relation to the transport mechanism, because these factors affect the recruitment of fish stocks and fishery population dynamics (Hjort, 1914; Cushing, 1975; Norcross and Shaw, 1984; Miller *et al.*, 1988).

5.3.1.4 *Life-history design in an environment with water currents*

A primary factor affecting embryos that rely on endogenous nutrition and require no exogenous foods is safety. Larvae with exogenous nutritional requirements, on the other hand, need prey organisms of appropriate size for them to handle at the place where they start feeding, in

conjunction with, hopefully, an absence or low abundance of predatory organisms that prey on the vulnerable larvae. From an evolutionary biology perspective, natural selection can act on the schedule according to which embryos or larvae remain at an upcurrent spawning site, move from the spawning ground to a downstream nursery ground, and arrive and stay at the nursery ground, and by so acting, determine the life-history trajectory of these organisms. We argue that the relationships between egg size and the amount of time that the developing embryos remain at the spawning site, on the one hand, and the time needed for transport from the spawning ground to the nursery ground and their size and status upon arrival at the nursery ground on the other, can be used to deduce adaptive life-histories of fish species that pass the early stages of their life in water currents.

Prior to our rigorous analysis, we suggest that the following probable relationships exist between environmental conditions and the development and survival of an embryo: (1) egg development and hatch timing depend on egg size and water temperature, (2) growth and survival of the embryos or larvae depend on not only available food and predation risk during transport, respectively, but also their size and status (locomotive or not) and (3) current transport affects the locations associated with the developmental stages and determines the levels of food availability and predation risk to embryos or larvae drifting along the current.

5.3.2 The model

An egg size model for aquatic organisms with a planktonic early life stage that allows us to discuss optimality must incorporate certain minimum conditions. We postulate that a current flowing from the spawning site to the nursery grounds transports eggs or larvae, and that there exists an arbitrary monotonic distribution of biological richness determining food availability and predation risk along the current.

The gradient of biological richness along a current flow is defined on an axis, x, in one-dimensional space as an arbitrary function $\lambda(x)$, where the spatial domain is standardized as $x \in (0, 1)$. First, let us assume that the gradient of biological richness along the current flow is a decreasing function of x. This means that biological richness is highest at $x = 0$, around which the spawning site is located, and decreases toward the nursery ground. In this situation, we deduce that the eggs at the spawning ground suffer from the highest absolute predation risk, and available prey with high density around the

spawning ground is not utilized by endogenous-nutrition-dependent embryos that leave the spawning site on the current flow. Moreover, by the time the larvae hatch and start exogeneous feeding, they will have been carried to a relatively oligotrophic area.

In this case, utilization or acceptance of the current flow for passive transport during the early stage of life history should be excluded. Given this environmental gradient, settlement of the embryo at the spawning site should be favored, from the perspective that the site will be advantageous later for the exogenously feeding larvae, with the condition that the eggs and vulnerable larvae might be guarded by their parent. We do not consider this situation further here because under these circumstances the organism's life history should not depend on the water currents.

Instead, let us consider the situation where the gradient of biological richness along the current flow increases with x. In this case, biological richness is low at the spawning site and highest at the nursery ground. That this qualitatively postulated situation can occur is supported by several empirical studies of spawning sites and larval life history (Helfman *et al.*, 1997; Marteinsdottir *et al.*, 2000; Bailey *et al.*, 2003). For mathematical convenience, to evaluate the mesoscale aggregative pattern of biological richness around the nursery ground, we adopt a half-truncated normal distribution, $\lambda(x) = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{(x-1)^2}{2\sigma^2}}$, where σ is a constant, to express relative biological richness along the current flow.

In this situation, eggs or larvae enjoy relative safety in the upcurrent area because of the low predation risk, at the expense of a low density of available food, and during transport, exogenously feeding larvae enjoy a high density of available food, but at the expense of a high predation risk in the downcurrent area. Thus, under these circumstances, the early life-history trajectory of aquatic organisms can be optimized to take advantage of the biological gradient of the water current.

The instantaneous food-encounter rate and instantaneous predator-encounter rate at location x are proportional to $\lambda(x)$, arbitrarily scaled with the proportionality coefficients φ and ω, respectively, where $0 < \varphi$ and $0 < \omega$. The current transports the eggs or larvae from $x = 0$ to $x = 1$ with velocity v. Suppose that the spawning ground is located at $x = x_0$ $(0 < x_0 < 1)$ and the nursery ground is located at $x = 1$. It takes an amount of time $T = \frac{1 - x_0}{v}$ for drifting particles to travel from the spawning ground, $x = x_0$, to the nursery ground, $x = 1$.

For simplicity, we treat eggs and larvae with yolk sacs indiscriminately as embryos that rely on endogenous nutrition and are

dependent solely on the absorption of yolk for their growth and development. Let Y be the amount of yolk in an egg, representing egg size, and let α be the yolk absorption rate, which depends mainly on the external temperature. The endogenous nutrition period, from newly spawned egg until all the yolk is absorbed, is assumed to be $t_e = Y/\alpha$. This approximation can be verified with the data of several empirical studies (Laurence and Rogers, 1976; Miller *et al.*, 1988; Pepin, 1991; Chambers and Leggett, 1996).

Let p be the proportion of the endogenous-nutrition-dependent developmental time spent at the spawning site. Thus, the embryo spends $t_1 = pt_e$ at the spawning site and $t_2 = (1 - p)t_e$ drifting on the current while relying on endogenous nutrition. After yolk absorption, the larva requires exogenous nutrition acquired by active feeding.

A certain non-zero value of p is assigned to larvae that develop from adherent or demersal eggs, and $p \approx 0$ in the case of larvae developing from planktonic eggs. We assume that the value of p reflects an extra investment f, in addition to the amount of yolk per egg, on the part of the parent, such as adherent materials surrounding the eggs for attachment to stones or seaweed. Thus, the total amount of energy invested in one egg is $Y_T = Y + f(p)$, where f is an increasing function of p; that is, $f(p) = \eta p^{\zeta}$, arbitrarily chosen for mathematical convenience, and η and ζ are positive constants. Because our interest is the size variation of the eggs themselves, we show not Y_T, but Y as egg size in the following.

During the fraction of the endogenous nutrition period that the embryo remains at the spawning site, t_1, the survival probability of an individual is $s_1 = e^{-\omega\lambda(x_0)t_1}$, and during time t_2, when the embryo is drifting in the current, the survival probability is $s_2 = e^{-\omega\int_0^{t_2}\lambda(x(z))\mathrm{d}z}$, where $x(z)$ is the location at time z (z is an auxiliary variable for integration).

After the initiation of active feeding, growth depends on the external food supply and on the larva's own body size as it affects its food acquisition ability. Survival depends on the potential predation hazard in the environment and also on the larva's own body size as it affects both its ability to avoid predation and the likelihood that it will not die of starvation. We model the growth and survival processes of the larva after the initiation of reliance on exogenous nutrition as follows:

$$\frac{\mathrm{d}B}{\mathrm{d}t} = g\left(\frac{c_p\phi\lambda(x(t))e}{1 + c_p\phi\lambda(x(t))h}\right)^{\theta}, \tag{5.8}$$

$$\frac{\mathrm{d}s_3}{\mathrm{d}t} = -m\left(\frac{1}{c_p\phi\lambda(x(t))}\cdot\frac{1}{B^{\xi}} + c_e\omega\lambda(x(t))\right)s_3(t), \tag{5.9}$$

where B is the body size of the larva after it begins exogenous feeding, s_3 is the probability of survival after initiation of exogenous feeding and m is the potential death rate.

The fraction in parentheses in Equation (5.8) is the food acquisition rate per unit time; e and h are the average energy and handling time, respectively, of an available prey item; $c_p\varphi\lambda(x(t))$ is the instantaneous encounter rate with an available food item and c_p is the ability of the larva to catch encountered prey, which is an increasing function of the body size of the larva, B (Miller *et al.*, 1988). For mathematical convenience, we adopt $c_p = 1 - e^{-\mu B}$, where μ is a positive constant. g and θ are positive constants, and the right-hand side of Equation (5.8) represents the instantaneous conversion of acquired energy to body mass.

The first term in the parentheses of Equation (5.9) indicates the risk of starvation, which depends on the interval between prey encounters and the larva's susceptibility to starvation. If we assume that the occurrence of encounters is a Poisson process, then the expected waiting time between successive events of food acquisition is reciprocally proportional to the instantaneous encounter rate with suitable prey. We also assume that the susceptibility to starvation is a function of body size. The first fraction of the first term in the parentheses of Equation (5.9) is the expected waiting time between successive prey encounters. The second fraction of the first term in the parentheses indicates the larva's susceptibility to starvation, in relation to body size, where ξ is a positive constant. Thus, the first term in the parentheses implies death caused by starvation, and the properties of the mathematical expression can be qualitatively verified with data from empirical studies of larval physiology (e.g. Cushing, 1975).

The second term in the parentheses of Equation (5.9) indicates the hazard of predation. $\omega\lambda(x(t))$ is the instantaneous encounter rate with a predator at x, and c_e is the probability of being eaten by the predator encountered. This probability is a decreasing function of B, that is, $c_e = e^{-\rho B}$, where ρ is a positive constant. The properties of this mathematical expression can also be qualitatively verified with data from empirical studies of larval physiology (e.g. Cushing, 1975; Miller *et al.*, 1988). We assume that the size of the larva at the beginning of exogenous feeding is proportional to its original egg size. This can also be qualitatively verified with data from empirical studies (Miller *et al.*, 1988; Chambers and Leggett, 1996).

The exogenously feeding larva is transported by the current during time $t_3 = T - t_2$ to arrive at the center of the nursery ground.

Equations (5.8) and (5.9) are numerically solved for $t = 0$ to $t = t_3$ under the initial conditions $s_3(0) = 1$ and $B(0) = Y$.

The probability of larval survival, $S = s_1 s_2 s_3(t_3)$, times larval body size, $B_l = B(t_3)$, just until arrival at the nursery ground, is a surrogate criterion of offspring fitness. The number of eggs produced by a parent with a given proportion of its body mass used for reproduction, R, is R/Y_T. Thus, the parental fitness can be defined as $W \propto \frac{R}{Y_T} \cdot S \cdot B_l$. In the numerical evaluation of W, the common parameters and the basal values used are $v = 0.1$, $a_0 = \alpha = 0.5$, $b_0 = \beta = 1$, $\sigma = 0.1$, $g = 1$, $e = 1$, $h = 1$, $\theta = 1$, $\mu = 0.5$, $\eta = 4$, $\zeta = 3$ and $\rho = 0.2$.

We also examined the sensitivity of the results to changes in some of the common parameters. The model is constructed in accordance with a policy of simplification. We adopt simple functional relationships, such as increasing, decreasing, linear, convex and concave, among several variables for each process incorporated into the model. We then qualitatively verify the results of our model by using several functions that possess similar properties.

5.3.3 Optimal egg size

The positioning of the larva at each developmental stage at the most appropriate location along the current depends on the spawning site location, the current velocity and the water temperature, which controls the developmental rate of the embryo. In addition to these factors, the amount of yolk (egg size) affects the rate of embryo development, the time at which the larva begins exogenous feeding for a given yolk absorption rate and the size of the larva at the time it begins to feed, which affects its initial ability to avoid predators. A certain egg size can be expected to maximize parental fitness, that is, the number of surviving offspring times the body size of the larvae arriving at the nursery grounds, in such a current regime.

We evaluate optimal egg size in relation to the relative distance from the spawning site to the nursery ground. If this distance varies among populations with different spawning sites, then we can expect adaptive egg size variation among the spawning sites, given appropriate adjustment for environmental variety other than the factor of interest. Furthermore, if related species share the nursery ground, but have different spawning areas, then we can also expect adaptive egg size variation among these related species given appropriate adjustment for the phyletic constraint.

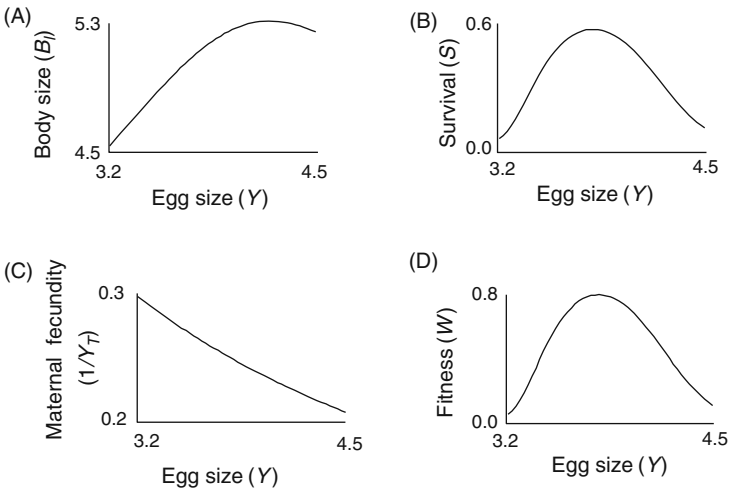

Figure 5.6 (A) Larval survival, (B) larval body size upon arrival at the nursery ground, (C) number of eggs produced by a parent given a reproductive investment of unity and (D) parental fitness with respect to egg size, Y. $p = 0$, $\varphi = 1$, and $\omega = 1.5$, where p is the relative retention time, that is, the proportion of the endogenous-nutrition-dependent developmental time spent at the spawning site, and φ and ω represent relative food richness and predation risk along the current flow, respectively.

Even though large exogenous larvae emerged from large eggs can resist starvation, the long period of vulnerability to predation risk of planktonic eggs or larvae drifting on the current cancels the size advantage conferred by tolerance to starvation. Therefore, larger egg size does not necessarily accomplish the highest survival probability on arrival at the nursery ground (Figure 5.6A). Even though larvae from large eggs have a large body size at the initiation of exogenous feeding, a long drifting period without feeding would cancel the size advantage. Therefore, larger egg size also does not necessarily result in the largest larval body size on arrival at the nursery ground (Figure 5.6B). Furthermore, survival is not necessarily maximized at the same egg size as that which maximizes larval body size (compare Figure 5.6A and B). If maternal fecundity is taken into account (Figure 5.6C), an intermediate egg size results in the highest level of parental fitness (Figure 5.6D).

The distance from the spawning site to the nursery ground affects optimal egg size via the mechanisms underlying survival and

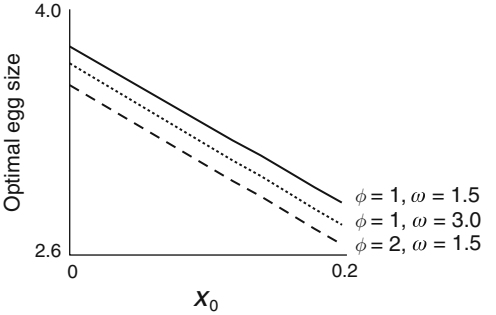

Figure 5.7 Optimal egg size in relation to the location of the spawning site given a nursery ground located at $x = 1$ and $p = 0$. Parameters: solid line ($\omega = 1.5$, and $\varphi = 1$), dotted line ($\omega = 3.0$, and $\varphi = 1$) and dashed line ($\omega = 1.5$, and $\varphi = 2$). The meanings of p, ω and φ are given in the legend of Figure 5.6.

growth at each place along the current for a given egg size. Suppose that a certain egg size is optimal for females that consistently spawn a certain distance from the nursery grounds. If eggs spawned close to the nursery ground were of the same size, then the developing embryo would spend a large proportion of the time with non-feeding ability and a vulnerable state in relatively food-rich and also highly risky regions close to the nursery ground, and, thus, it would have a lower probability of survival and a smaller larval body size on arrival at the nursery ground. It follows that optimal egg size is smaller for females that spawn close to the nursery ground than for those that spawn far from the nursery ground (Figure 5.7). We can deduce the effects of food and predator conditions along the current as well as the effect of distance from the spawning site to the nursery ground. In our model, φ and ω represent relative food richness and predation risk, respectively, along the current flow. Both parameters reflect prey and predator abundances along the current flow from the spawning site to the nursery ground, and they can be expected to vary both latitudinally and seasonally. Thus, for the same species, we can expect adaptive egg size to also vary both latitudinally and according to the spawning season, along with the variation in these factors.

If prey abundance is relatively high along the current, then early initiation of exogenous feeding associated with a small egg size means an extended food acquisition period [Figure 5.7, solid line (basal condition) versus dashed line (high prey abundance)].

If the predation risk is relatively high along the current, the developing embryo should not be transported downcurrent while

still completely unable to avoid predators, that is, as an egg or yolk-sac larva. A short yolk-absorption period (i.e. a small egg size) and, consequently, a short inactive period accompanied by the early initiation of active feeding and the ability to avoid predators are required. If this causal mechanism, which has the additional side effect of increasing the fecundity of the parent producing the eggs, is dominant over the commonly postulated preferred condition for exogenously feeding larvae, (i.e. large and vigorous larvae, which are favored for food acquisition and predator avoidance), then egg size should be small [Figure 5.7, solid line (basal condition) versus dotted line (high predation risk)].

In summary, high bioabundances (in both predation threat and food abundance) along the currents that transport embryos and larvae from spawning sites to nursery grounds should cause the optimal egg size to be small.

5.3.4 Optimal egg size and retention at the spawning site

Retention at the spawning site is characteristic of adherent eggs and some types of demersal eggs. For example, sandfishes spawn adherent eggs on cobblestones in shallow coastal waters, and the embryos develop on the cobbles (Ochiai and Tanaka, 1986a). Smelt and herring also lay adherent eggs on cobblestones or seaweed, and these embryos remain at the spawning site during a portion of their developmental period (Ochiai and Tanaka, 1986a). Salmonid species spawn demersal eggs in pebble spawning beds, and a portion of the development of the embryos takes place at the spawning beds (e.g. Burgner, 1991). The duration of the retention period at the spawning site should be a key adaptive characteristic in the life history of aquatic organisms. In our model, parameter p represents the proportion of the egg/larval developmental period that takes place at the spawning site.

We evaluate optimal retention time at the spawning site and optimal egg size in relation to the distance from the spawning site to the nursery ground. If this distance varies among populations with different spawning sites, then we can expect adaptive retention time and egg size variations among spawning sites, given appropriate adjustment for environmental variety other than the factor of interest. If related species share a nursery ground, but use different spawning areas, then we can also expect adaptive variations among these related species, given appropriate adjustment for phyletic constraints.

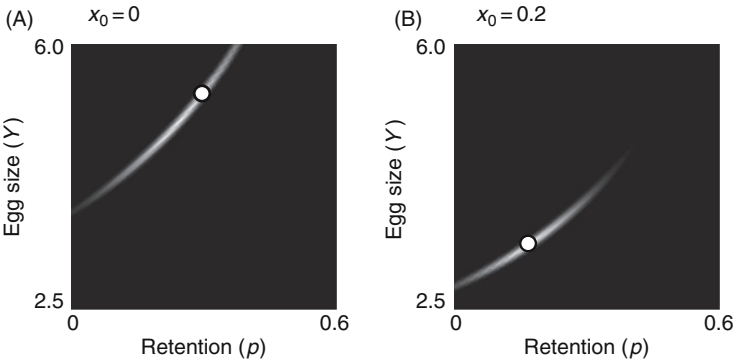

Figure 5.8 Fitness contours for given values of the retention parameter p and egg size Y for a spawning site (A) far from or (B) close to the nursery ground. Fitness is high in the light area and low in the dark area. Circles indicate optimal trait sets. The shared parameters are $\omega = 1.5$ and $\varphi = 1$. The meanings of ω and φ are given in the legend of Figure 5.6.

If both "egg size" (Y) and "retention time at the spawning site" (p) are parameters that maximize fitness, optimal egg size is larger when coupled with retention at the spawning site for a certain time period (Figure 5.8). Both optimal egg size and optimal retention at the spawning site decrease for a female that spawns close to the nursery ground relative to those for a female that spawns far from the nursery ground (see Figure 5.8A and B).

Food abundance and predator threat along the current flow affect optimal egg size differently if retention at the spawning site is also a trait that maximizes fitness. We separately analyze the effect of food richness and predation threat. The higher the food richness is along the current (i.e. the larger the value of φ), the smaller the optimal egg size and the shorter the optimal retention time (compare Figure 5.9B and A). A higher predation threat (i.e. a larger value of ω) leads to a larger optimal egg size and a longer optimal retention time at the spawning site (compare Figure 5.9B and C). The latter prediction of egg size is not identical to the prediction when retention time is not considered a factor. This implies that selection pressures acting on egg size of adherent and demersal eggs are, in part, different from those acting on floating or suspended buoyant eggs.

If we consider variation in food abundance and predation risk simultaneously, the effect of the two factors on optimal egg size and retention time is composite. Suppose that food abundance and predation risk become high at the same time, as expected. If the effect of food

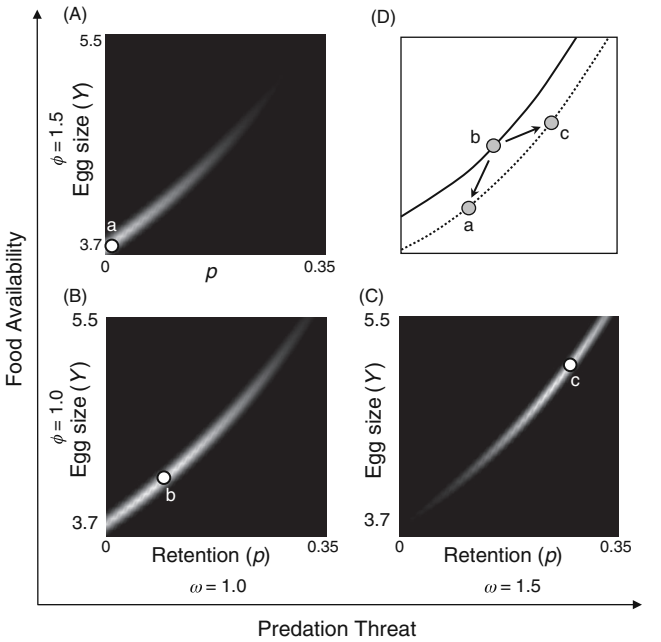

Figure 5.9 (A)–(C) Optimal egg size and optimal retention at the spawning site (circles) on the parameter plane of egg size (Y) and the retention parameter (p) for given parameter sets of predation risk (ω) and food richness (φ). (D) A schematic diagram showing how the optimal trait set (b) responds to increases in the predation hazard (c) and food richness (a) parameters.

availability is dominant, then the optimal egg size would tend to be smaller and the optimal retention time shorter (from b to a in Figure 5.9D). In contrast, if the effect of predator threat is dominant, then a similar change would cause the optimal egg size to become larger and the optimal retention time to become longer (from b to c in Figure 5.9D).

5.3.5 Ecological implications

We deduced that the duration of the retention period at the spawning ground and egg size variations of fish species with a planktonic stage, at least during the early part of their life history, could be evaluated as adaptive life-history traits in terms of the mesoscale water movements that transport the larvae. Distance from the spawning grounds to the

nursery grounds along with predation threat and food availability during the trip from the spawning grounds to the nursery grounds would both exert selection pressure on egg size and retention time at the spawning grounds. Long distance from the spawning grounds to the nursery grounds, and high predation risk and/or low food availability during the trip from the spawning grounds to the nursery grounds, would selectively favor larger egg size. If retention time at the spawning ground is also a trait acted on by natural selection, then the selected retention times and egg sizes would be positively correlated.

Biologists studying the life histories of fishes and crustacea have long been aware that favorable timing is critical for transport of planktonic eggs and larvae from spawning sites to nursery grounds, particularly in early developmental stage (Parrish *et al*., 1981; Hinckley *et al*., 1991; Helfman *et al*., 1997; Jones *et al*., 1999; Swearer *et al*., 1999). The general features of the environmental gradient and the fundamental response of the developing eggs and larvae of many fish species to a gradient during transport have been acknowledged by various studies of fish biology (Hjort, 1914; Hunter, 1972; Cushing, 1975; Miller *et al*., 1988; Buskey *et al*., 1993; Helfman *et al*., 1997; Marteinsdottir *et al*., 2000; Bailey *et al*., 2003).

To our knowledge, the evolutionary biology literature contains, unfortunately, no explicit data that can be used to evaluate our predictions empirically. Some studies of fish biology, however, include a few anecdotal descriptions that imply the validity of our deductions. Cod (*Gadus morhua*), for example, spawn nearshore, and then the eggs and larvae are transported by offshore currents to an offshore nursery ground, and eggs spawned closer to shore are larger than those spawned relatively far from the shore (Marteinsdottir *et al*., 2000).

Another piece of evidence supports our deductive inference. Flat fishes are known to spawn at various depths in the water column both inshore and offshore, and then eggs and larvae are transported by onshore currents to nursery grounds in shallow nearshore waters (Minami, 1984). Generally, spawning grounds that are deeper are farther from the shallow-water nursery ground. Several bastard halibut species (Paralichthyidae) spawn in Wakasa Bay, Japan, each in a specific place and at a specific depth, while sharing the same nursery ground. Eggs of the female *Paralichthys olivaceus*, which spawns in relatively deep offshore waters, are larger than those of a female *Tarphops oligolepis*, which spawns in relatively shallow inshore waters (Minami, 1984). These observations support the prediction of our model

regarding the relationship between egg size and distance from the spawning ground to the nursery ground.

5.4 FEMALE GAMETE SIZE VARIATION IN AQUATIC ENVIRONMENTS

For most primitive organisms and some groups of higher-order organisms, at least during the early planktonic life stages, an aquatic medium is an essential environmental condition. Some life-history traits of the organisms related to processes such as the fusion of gametes and the early development of the zygote should therefore reflect the features of the aquatic medium.

In free-spawning organisms, the process of collision of heterotypic gametes to form zygotes is a crucial process in the view of the microscopic movement of small particles in the aquatic medium. Several life-history studies of algae have argued that this process importantly affects the evolution of isogamous or anisogamous reproduction (Cox and Sethian, 1985; Togashi and Cox, 2004; Dusenbery, 2006; Iyer and Roughgarden, 2008). In marine benthic invertebrates with a planktonic stage, which readily evolved given an oogamous reproductive system, female gamete size variation is also explained by the selection force expressed as the probability that gametes will encounter one another in the water to complete the fertilization process (Strathmann and Vedder, 1977; Vogel *et al.*, 1982; Levitan, 1993; Levitan, 1996; Podolsky and Strathmann, 1996; Levitan, 2000; Petersen *et al.*, 2001; Podolsky, 2001; 2004).

Robertson (1996) attempted to explain egg size variation in tropical reef fishes on the basis of Levitan's (1993) fertilization probability theory. His hypothesis, however, was not supported by his data. Fish species inhabit larger spaces during their early life stages than do benthic invertebrates such as sea urchins. Therefore, mesoscale turbulent and non-turbulent water movements would act on the developing embryos of fish (Parrish *et al.*, 1981; Norcross and Shaw, 1984; Sherman *et al.*, 1984; Hinckley *et al.*, 1991; Helfman *et al.*, 1997; Jones *et al.*, 1999; Swearer *et al.*, 1999).

Our explanation of the effects of turbulent disturbance on egg size evolution is highly generalized, but applicable for understanding the big picture of egg size variation across a wide range of environments. Our explanation of the effects of a non-turbulent disturbance, on the other hand, is capable of fine-scale predictions about egg size variation. Moreover, these two explanations are not mutually exclusive.

We should also consider other factors and mechanisms for explain the egg size variation in the organisms of aquatic environment. An explanation for the smallness of pelagic eggs has been proposed. Winemiller and Rose (1993) proposed a simulation model showing that production of plentiful small eggs by pelagic fish is adaptive in relation to a spatially patchy food distribution, but they do not address variation in egg size among different aquatic environmental regimes.

Several environmental factors are known to influence egg size variation in aquatic environments. For example, water temperature affects egg size. In general, egg size is smaller under higher temperature conditions (Johnston and Leggett, 2002; Kokita, 2003; Laptikhovsky, 2006; Llanos-Rivera and Castro, 2006). Latitudinal variation in egg size may partly reflect water temperature gradients (Thresher, 1988; Kokita, 2003), and it has also been argued that a lower level of dissolved oxygen in the water can be a selection factor against large egg size (Einum *et al.*, 2002; Rombough, 2007). Consideration of factors related to the features of the medium other than the water movements, such as temperature, and dissolved oxygen, enables us to understand other aspects of natural selection for egg size in the primary environment of life.

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DAVID B. DUSENBERY

6

Gamete encounters

6.1 INTRODUCTION

A gamete must encounter a partner gamete before it can contribute to the formation of a new individual with a new array of genetic information. Thus, it is natural to consider whether the advantage of anisogamy might be that it aids gamete encounters. Since it is plausible that both a larger number of smaller gametes can be formed and a larger gamete presents a larger target for another gamete, it is plausible to suppose that the combination of a few large and many small gametes might lead to more encounters than an equal investment in gametes all of the same size. Thus, anisogamy might have evolved from isogamy under selection for higher rates of encounter between gametes of two mating types.

In order for the encounter process to be a strong evolutionary force, encounters must be relatively rare. In situations where each gamete is likely to encounter many partners, there may be selective pressures for gametes to play hard-to-get and select for fertilization by only the most competent of partners (correlating with better genes). On the other hand, if the probability of encountering any suitable gamete is low, there will be selective pressure to produce gametes that have the greatest chance of encountering any suitable partner. In oogametic species, these two situations are frequently called sperm competition and sperm limitation. Thus, a critical question becomes whether a given gamete is likely to encounter many or no fitting partners.

The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection, ed. Tatsuya Togashi and Paul Alan Cox. Published by Cambridge University Press.

In this chapter, we consider the primitive situation of broadcast spawning, where sperm limitation seems most likely. The chapter is organized as: (1) evidence of the occurrence of sperm limitation, (2) a model of the encounter process, (3) features of gametes that influence encounter rates between gametes, (4) the resulting selective pressure for anisogamy and (5) questions for the future.

6.2 Occurrence of sperm limitation

6.2.1 Unfertilized eggs

Theories of reproduction have often assumed that all eggs are fertilized and sperm limitation is rare; see references in Levitan *et al.*, 1991. However there is abundant evidence that eggs frequently go unfertilized in nature, and sperm limitation is common. In this section, we take a brief look at the evidence, which is of several kinds: features that appear to have evolved to diminish sperm limitation, laboratory experiments and field observations. The general question of the occurrence of sperm limitation has been reviewed by Yund (2000).

6.2.2 Evolved features to avoid sperm limitation

The most obvious way to avoid sperm limitation is to increase the local concentration of gametes. With a given investment in gamete tissue, concentration can be increased by producing more, smaller gametes. Beyond this, a population can compress either the spatial or temporal extent over which gametes are distributed.

6.2.2.1 Concentration of gametes

In general, compressing the spatial extent of gamete distribution can be accomplished by aggregation of the parents or giving gametes appropriate properties. A simple method of concentrating gametes in shallow waters is to make them buoyant so that they float to the surface. In recent decades, it has indeed been discovered that many corals release buoyant gametes, which become concentrated in a thin layer at the surface (Harrison *et al.*, 1984; Benayahu and Loya, 1986; Wilson and Harrison, 2003). This simple strategy has the potential to increase the effective concentration by large factors.

Some gametes are known to exhibit phototaxis. In shallow water, this behavior might also lead to increased concentration at the surface, if they swim toward light.

6.2.2.2 Synchronized spawning

Synchronized spawning provides a means of increasing effective concentration by compressing the temporal extent of gamete release.

The most dramatic examples of this behavior occur in corals. The majority of coral species are broadcast spawners. In most species, gonads develop over a period of months, but release of gametes is confined to a much shorter period – often less than an hour at a certain time of day on a certain day of a specific lunar month (Harrison *et al.*, 1984; Shlesinger and Loya, 1985; Benayahu and Loya, 1986; Richmond and Hunter, 1990; Wilson and Harrison, 2003). Such synchronous spawning requires some kind of coordination between different individuals. Since no simple mechanisms to accomplish this are apparent, their occurrence suggests some complicated adaptations, such as a means of detecting the phase of the moon, in addition to the time of day.

In addition to corals, it has more recently been found that many species of calcified, siphonous tropical green algae (of the order Bryopsidales), spawn for only a few minutes a day on a few days of the year (Clifton, 1997; Hay, 1997).

These examples of synchronous spawning suggest that contemporary species have evolved under selective pressure to increase the effective concentration of gametes, and that sperm limitation must have been important. It may not occur all the time, but it must have be frequent enough to have been a potent force in evolution.

Unfortunately, the reliability of this argument is undermined by at least one alternative hypothesis: that synchronous spawning is a mechanism to reduce predation on gametes by saturating predators. So, we now take a look at more direct evidence.

6.2.3 Laboratory observations

Yund (2000) reviewed eight reports of laboratory studies of fertilization in marine free-spawners. He summarized his findings: "most experiments simultaneously demonstrate the potential for both severe sperm limitation and highly successful fertilization." The results depend dramatically on the precise conditions of the experiments, and it is difficult to know which conditions are most appropriate to natural settings.

6.2.4 Field surveys

Some data (Levitan, 1993, Table 1) indicate that under natural conditions fertilization success varies widely and is sometimes less than 1%.

More recent data (Yund, 2000, Table 1) suggests that it is common for more than 50% of eggs to be fertilized.

These direct observations and experiments indicate that sperm limitation can occur in nature but is rare in common conditions. However, the observations are on highly evolved species that may have developed mechanisms to overcome limits on sperm in their evolutionary history. It should be kept in mind that small advantages (say 1%) can have important influences in the course of evolution.

The best we can probably conclude is that sperm limitation probably occurred sufficiently often to influence evolution. Now, we consider what features of gametes might be selected, if it did happen.

6.3 MECHANISMS OF ENCOUNTER

We consider a basic model of the encounter process in order to identify gamete features that are likely to influence encounter rates.

6.3.1 Gamete encounter rates

6.3.1.1 Assumption of uniform conditions

For the greatest generality, it is assumed that organism density is uniform and there are no preferred spatial directions. In addition, there are no surfaces to be encountered or influencing fluid flow or chemical diffusion other than those of the other gametes. Although none of these assumptions is strictly accurate in any real situation, they often represent reasonable approximations over the range of distances relevant to gamete swimming.

In highly turbulent environments, turbulent flows can play a major role in bringing gametes together (Denny and Shibata, 1989; Denny *et al.*, 2002; Crimaldi and Browning, 2004). However, here it is assumed that any current is uniform, carrying all gametes in parallel. Consequently, the encounter rate is dependent on swimming of the gametes and the current does not influence the encounter rates. In principle, very small gametes might also rely on Brownian motion (Dusenbery, 2009), but actual gametes are larger and its influence is not considered here.

6.3.1.2 Fertilization formula

Researchers engaged in experimental studies of fertilization have often (Levitan *et al.*, 1991; Levitan, 1993; Levitan, 1996; Styan *et al.*, 2005;

Hodgson *et al.*, 2007) utilized the general rate model of Vogel *et al.* (1982). This mathematical model is the same as one used for chemical reactions, equating the rate of reaction (or encounter or fertilization) to the mathematical products of the concentrations of each class of reactant (A and B molecules or male and female gametes) and a rate constant, β, which has units of volume/time and is a measure of the rate at which reactants sweep the environment for a partner. The basic concepts of this theory are illustrated in Dusenbery (1992), Chapter 16.

This model attempts to describe the course of reaction at any time under any combination of concentrations. Even though the rate constant lumps several effects together (speed of movement, target size and reaction probability), the resulting equations can be quite complicated, involving an exponential of an exponential. For example, the most often used is Equation 13 of Vogel *et al.* (1982), for the fraction of eggs fertilized after the reaction has run to completion, assuming only a fraction of the egg surface is capable of being fertilized

$$\varphi_\infty = 1 - \exp\left[-\frac{\beta S_0}{\beta_0 E_0}\left(1 - e^{-\beta_0 E_0 \tau}\right)\right], \tag{6.1}$$

where S_0 and E_0 are the initial concentrations of sperm and eggs, β is the rate constant for fertilization, β_0 is the rate constant for encounters between sperm and egg, and τ is the average period sperm are active.

Not only is this equation symbolically complicated, it is conceptually problematic. It explains the fraction of eggs ultimately fertilized (φ_∞) by invoking the rate at which eggs are fertilized (β). This doesn't provide much of a mechanistic explanation. The equation is often fit to data. But, with five parameters, many equations of comparable complexity could be fit to the limited data available in these experiments, and any fit might well be coincidental.

The mathematics can be simplified, if we make some additional assumptions. A well-known mathematical result is that the exponential equals an infinite series: $e^x = 1 + x/1! + x^2/2! + x^3/3! + \ldots$ With $x < 1$, each term is smaller than the preceding terms, and, for sufficiently small x, $1 - e^x$ can be approximated by x, with the error going to zero as x gets smaller and smaller. This approximation is extremely useful and frequently used in physics. So, for an assumption of extreme sperm limitation, where only a small fraction of eggs are fertilized,

$$\varphi_\infty \approx \frac{\beta S_0}{\beta_0 E_0}\left(1 - e^{-\beta_0 E_0 \tau}\right) \ll 1. \tag{6.2}$$

If egg concentrations, E_0, are sufficiently low that most sperm search for their full active period ($\beta_0 E_0 \tau \ll 1$), further simplification is possible:

$$\varphi_\infty \approx \frac{\beta S_0}{\beta_0 E_0} \beta_0 E_0 \tau \approx \beta S_0 \tau. \tag{6.3}$$

This enormous simplification is the reason that most researchers focused on gamete evolution generally start with an assumption of sperm limitation. The simplification allows them to deal with more specific and meaningful mechanistic models, but the results are entirely consistent with this low-concentration and sperm-limitation approximation.

6.3.1.3 Encounter formula

Like most other researchers focused on the evolution of gametes, I use the classic colliding spheres model. It assumes there are two kinds (A and B) of objects distributed randomly and moving in random directions. Each kind has uniform size (radii r_A or r_B) and speed (v_A or v_B). Encounter occurs when individuals of the two different kinds approach each other to within a certain distance; we measure this encounter distance from center to center and symbolize it r_e. With these assumptions, the following formula has been derived for chemical reactions by (Boltzmann, 1964 (1896), footnote 2, p. 78, Equation 65) and (Kauzmann, 1966, Equation 5–15), for predator–prey encounters by (Gerritsen and Strickler, 1977), and for gamete encounters by (Cox and Sethian, 1985):

$$Z_{AB} = \pi {r_e}^2 \frac{3{v_A}^2 + {v_B}^2}{3v_A} n_A n_B, \quad v_A \geq v_B, \tag{6.4}$$

where the ns are densities (number of bodies per unit volume of the environment). They are the same parameters as the concentrations of sperm and eggs, above.

6.3.2 Factors influencing encounter rates

Equation 6.4 is fairly complicated, so consider the independent parameters that contribute to gamete encounter rates.

6.3.2.1 Speed

Swimming speed parameters appear only in the fraction, which itself has the dimensions of speed. Thus, it can be viewed as an effective speed for encounter:

$$v_e \equiv \frac{3{v_f}^2 + {v_s}^2}{3v_f}, \tag{6.5}$$

where subscripts "f" and "s" refer to the faster and slower speeds.

If both types have the same speed ($v_A = v_B = v$), the effective speed is $v_e = 4v/3$. If one type is stationary, the effective speed is simply the speed of the motile type. So the movement of the slower type doesn't have a large effect; at most, it adds one-third to the encounter rate.

This non-linear and unequal contribution of speed to the encounter rate is potentially quite important. It may well be the fundamental reason for the evolution of distinctive types of gametes, and explain why one type is commonly immobile. For example, suppose that there is a trade-off between motility and investment in each gamete such that twice as many immobile gametes can be produced as motile gametes. Then a change from isogamy with all gametes motile to anisogamy with one type being immobile would reduce the encounter speed to 0.75 of the previous value, but increase the density of one type of gamete by a factor of two for a combined increase to 1.5 of the previous encounter rate. This result contrasts with the balanced trade-off that would occur if all the parameters contributed proportionally, e.g. if $v_e = v_A = v_B$.

6.3.2.2 Encounter distance

The factor πr_e^2 is the cross-sectional area for encounter (σ_e) – a good measure of target size. If we consider that an encounter occurs when physical contact is made, the encounter distance is the sum of the radii of the two gametes ($r_e = r_A + r_B$). In this respect, larger gametes would be advantageous. Note that the increase is proportional to an area (r_e^2), so (with increasing size) it increases faster than the diameter, but slower than the volume. Parallel to the case of speed, the smaller gamete contributes less to the encounter rate than the larger one.

The definition of the reaction rate parameter, β, mentioned above, is such that the rate for encounters (β_0, using the established symbol) is $\beta_0 = \sigma_e v_e$.

Using the cross-section and effective speed of encounter, Equation 6.4 can be simplified in various ways:

$$Z_{AB} = \pi {r_e}^2 \, v_e \, n_A \, n_B = \sigma_e \, v_e \, n_A \, n_B = \beta_0 \;\; n_A \, n_B. \tag{6.6}$$

This provides a clearer view of the important factors, and these parameters all contribute in a proportional manner, independently of one another.

Another way in which encounter distance can be increased is by using attractant chemicals. It is well-established that many anisogametic species employ attractant chemicals. For example, a reviewer of published observations on algae and fungi (Kochert, 1978) concluded: "In nearly every case where one gamete is relatively immobile (customarily termed the female gamete) that gamete will be found to produce a pheromone which attracts the male gamete."

The basic idea is that one gamete type releases a pheromone chemical and the other type responds to it and directs its own locomotion up the gradient of pheromone to the gamete that is the source of the pheromone. If the motile gamete responds efficiently, the source gamete has effectively increased its target size, possibly by orders of magnitude.

6.3.2.3 Numbers

From Equations 6.4 and 6.6, the encounter rate is proportional to the densities of each of the types of gamete. This confirms the obvious that more gametes will increase encounter rates, but demonstrates that the density of each type contributes independently of the density of the other type, in the sense that the rate is proportional to the product of the two densities

The effective density of gametes available at any time depends not only on how many are released, but also on how long they survive and remain active. At any time, the available number is proportional to the average duration of this active period (t_G). This is important because extending the active period may involve tradeoffs with size or swimming speed.

For example, careful analysis of gametes in one species of intertidal alga (*Monostroma angicava*) found that the larger (female) gametes swam for longer periods of time than the smaller (male) gametes (Togashi *et al.*, 1997). Analysis of the data suggests that the active period might be proportional to gamete volume. According to Figure 18 of that work, the median duration for female gametes was approximately twice that of the males. Estimating gamete volume as width squared times length (more accurate than the author's length cubed), the ratio of volumes is 1.99, suggesting that the period a gamete remained active and fertile might be proportional to its volume. A simple hypothesis for

a mechanism that would produce this relation is that the gametes swim until they run out of energy or some other consumed resource that is initially present in proportion to volume.

6.4 PREDICTED EVOLUTION OF ANISOGAMY

With these concepts of basic factors that determine encounter rates between gametes, we now develop specific models of trade-offs in gamete characteristics and size. We then analyze the selective pressures acting on the size of each of the two types of gametes.

6.4.1 Assumptions

6.4.1.1 Size dependence

A basic assumption of our analysis is that all the important features of gametes are consequences of their size. Thus, we can simplify the analysis by focusing on those parameters that influence encounter rate (swimming speed, encounter distance and gamete numbers) and are hypothesized to have size dependence. This focus is facilitated by converting the equation for the encounter rate ($Z_{AB} = r_e^2 v_e n_A n_B$) to the corresponding proportionality with the relevant parameters ($Z_{AB} \propto r_e^2 v_e n_A n_B$), where $\propto$ is a symbol for "proportional to." Considering only the size-dependent proportionalities, the expressions for each of the parameters (r_e, v_e, n_A, n_B) can also be reduced to appropriate proportionalities, simplifying the expression for the encounter rate to the components relevant to this analysis.

6.4.1.2 Cargo of chromatin

An important feature of gametes is that it is essential that they carry a cargo including the haploid genome. They often carry other important materials, but the genome is essential and a significant proportion of the gamete volume, at least in sperm.

For example, analysis of observations on the gametes of 15 species of algae, fungi and sperm of invertebrate aquatic animals revealed that all chromatin volumes had similar radii (r_C) within 50% of 1 μm (0.9 ±0.3 SD) (Dusenbery 2006). So we take this as a given, universal value ($r_C = 1$ μm).

The analysis is simplified if we make the dimensions of the bodies relative to this chromatin size, which is the same for both

sexes. Thus the size of a gamete is represented by the dimensionless parameter $\gamma_G \equiv r_G/r_C$. Since $r_G \geq r_C$ we must have $\gamma_G \geq 1$. Little relevant data was found for isogametes, but among 10 invertebrate species, the chromosomes appeared to occupy 0.25–0.8, median 0.42, of the sperm volume (Dusenbery, 2000). This suggests that an appropriate value for sperm is typically $\gamma_S = 1.34$, with a range of 1.1–1.6.

6.4.1.3 Gamete investment

There are good reasons to expect that individuals of each mating type make similar investments in producing gametes (Smith, 1978, p. 157), and that the investment in each gamete is proportional to its volume (References in Levitan, 1991). This suggests that each sex produces a similar total volume of gametes, as has been observed (Togashi *et al.*, 1997). We assume that there are two sexes that allocate equal volumes (V_T) of cytoplasm to their gametes. This total gamete volume is taken as fixed, for the purposes of the analysis.

Our question is how should this volume be divided between gametes for the greatest number of encounters between gametes – either sex can produce a few large gametes or many small ones. Quantitatively, the number of gametes produced of type G is $n_G = V_T/V_G = (3/4\pi)V_T r_G{}^{-3}$, where V_G is the volume of an individual gamete of type G and r_G is its radius. With our focus on size proportionality, $n_G \propto r_G{}^{-3}$ or, using the dimensionless size,

$$n_G \propto \gamma_G{}^{-3}. \tag{6.7}$$

This assumption provides a strong reason for gametes to be small – the smaller they are, the more can be produced and the higher their concentration, with everything else the same. So the difficult question is why one type of gamete is larger.

6.4.1.4 Fertility model

One possible reason for a larger gamete is that larger gametes may have a longer active lifetime. Previous observations (Togashi *et al.*, 1997) and theory (Dusenbery, 2002) suggest that the time interval over which gametes are active may be an important feature distinguishing between gamete types. In order to more easily incorporate this feature into the theoretical model, we shift from assuming a single spawning event to a steady-state model.

Consider a steady state where R_V is the rate at which gamete volume is produced in each unit volume of the environment (units of s^{-1}). The effective total volume of active gametes at any time is $V_{T'} = R_V t_G$, where t_G is the average time a gamete of type G is active (units of s). Different types of gametes may have different fertile periods, but the volume rate of initial production is assumed identical for both types. The number of active gametes in the environment is then $n_G = V_{T'}/V_G = R_V t_G / V_G = (3/4\pi) R_V t_G {r_G}^{-3}$. With our focus on size dependency, this can be simplified to the proportion $n_G \propto t_G {r_G}^{-3}$ or

$$n_G \propto t_G \, {\gamma_G}^{-3}. \tag{6.8}$$

Note that, although we are interpreting t_G as the time a gamete is active, it could represent other factors affecting the fraction or probability that a gamete is fertile. For example, the actual time period could be the same t for all gametes, but gametes might have a variable probability p of being fertile or p might represent the fraction of a gamete surface area available for functional contact with a partner, as has been considered (Vogel *et al.*, 1982). In either case, the equations with $t_G = pt$ would be an appropriate description, and the analysis would still be relevant. Considering this generality, I refer to t_G as the fertility of the gamete as well as its fertile period.

Assuming that the fertile period is proportional to the cytoplasm volume of the gamete (as suggested by observations described above), let t_V represent the constant of proportionality, and we have $t_G = pt, t_V, t_G = t_V (4/3)\left(r_G^3 - r_C^3\right)$. Note that t_V has units of $s{\cdot}m^{-3}$, while R_V has units of s^{-1}. Thus, their product has dimensions of reciprocal volume as does the number density. This helps explain the derived relationship:

$$n_G = \frac{V_T{}'}{V_G} = \frac{R_V t_G}{V_G} = R_V t_V \frac{\left({r_G}^3 - {r_C}^3\right)}{{r_G}^3} = R_V t_V {\gamma_G}^{-3}\left({\gamma_G}^3 - 1\right). \tag{6.9}$$

Note that this assumes that the cost of producing a gamete is proportional to its total volume (including the chromatin, r_G^3), but the fertile period depends on only the cytoplasmic volume (excluding the chromatin, $r_G^3 - r_C^3$) because chromatin volume cannot contribute additional resources (such as fuel) to fertility.

We are interested in relative changes in encounter rates with changing size (γ_G). All the parameters that do not depend on size are expected to have identical values for all the gametes of a species, and we can simplify the relations to consider only the proportional size

dependence. Thus, the parameters R_V and t_V, are unnecessary to this analysis, and Equation 6.9 can be simplified to the proportion $n_G \propto \gamma_G^{-3}(\gamma_G^3 - 1)$.

Note that the parentheses in the right-hand side of Equation 6.9 enclose an expression $(\gamma_G^3 - 1)$ representing the effect of size on fertility. Fertility is proportional to cytoplasmic volume (excluding the chromatin volume). To explore the consequences of changing the strength of the size effect on fertility, introduce the exponent ϕ and apply it to the parentheses, obtaining

$$n_G \propto {\gamma_G}^{-3}({\gamma_G}^3 - 1)^{\phi}. \tag{6.10}$$

With this relation, $\phi = 1$ is equivalent to fertile period proportional to cytoplasm volume, as above, while $\phi = 0$ is equivalent to fertile period independent of size. Intermediate values of ϕ provide a way of varying the strength of this relationship.

6.4.1.5 Pheromone model

Another possible reason for a larger gamete is that larger gametes may be able to release sufficient chemical attractant that they effectively increase their target size.

At the size of gametes, chemicals are ordinarily transported by diffusion faster than by the flow of the water that suspends them. Since diffusion is much more predictable than flows in the turbulent world of our size scale, we can make much more reliable predictions than are possible for, say, insect sex attractants.

We assume that one gamete type, called the egg, is immobile, but releases an attractant chemical at a rate J_V per unit volume. Thus, we are assuming that the release rate is proportional to the cytoplasmic volume of the egg. The other gamete type, called sperm, swim and direct their swimming up a gradient of the attractant, if the attractant concentration at the sperm's location is above some threshold concentration, C_{Th}.

The chemical flux (J) from an egg of radius r_E is a flux per unit volume (J_V) times the cytoplasmic volume, excluding the chromatin, so

$$J = \frac{4\pi}{3} J_V({r_E}^3 - {r_C}^3) = \frac{4\pi}{3} J_V {r_C}^3({\gamma_E}^3 - 1). \tag{6.11}$$

Substituting this into the equation for concentration around a steady point source (Dusenbery, 1992, p. 66; Dusenbery, 2009, Equation 9.5), the concentration at distance r from the egg is

$$C(r) = \frac{\frac{4\pi}{3} J_V {r_C}^3 ({\gamma_E}^3 - 1)}{4\pi D r} = \frac{J_V {r_C}^3}{3Dr} ({\gamma_E}^3 - 1). \tag{6.12}$$

If we assume that the sperm has a detection threshold C_{Th}, the distance from the egg at which it encounters the pheromone is

$$r_P = \frac{J_V {r_C}^3}{3DC_{Th}} ({\gamma_E}^3 - 1). \tag{6.13}$$

If we define a dimensionless parameter for this distance $\gamma_P \equiv r_P / r_C$ and a dimensionless parameter collecting all the physical constants relevant to pheromone effectiveness, $P \equiv J_V {r_C}^2 / 3DC_{Th}$, we obtain the simple relation

$$\gamma_P = P({\gamma_E}^3 - 1) \tag{6.14}$$

Note that the value of P is treated as a given for any particular species, and this equation can be used as the proportion $\gamma_P \propto ({\gamma_E}^3 - 1)$.

The appropriate value of P is not at all clear, and a wide range of values is plausible, depending primarily on the energy efficiency of pheromone production by the egg and the threshold for pheromone detection by the sperm. In a previous work, I assumed values equivalent to $P = 0.01$ (Dusenbery, 2000). With reasonable values for the other parameters, this value occurs with a threshold concentration of about 10^{-8} molar, which seems plausible.

6.4.1.6 Speed of swimming

In some previous publications, I have assumed that cells and organisms have a universal power density, which leads to the prediction that small organisms, swimming at low Reynolds numbers where Stokes' law applies, will swim at speeds proportional to their linear size. This model seems to work well in comparing the swimming speeds of a wide range of species (McMahon and Bonner, 1983, p. 150; Okubo, 1987; Mann and Lazier, 1991, Figure 2.03, p. 15; Dusenbery, 1996, p. 45).

However, there is evidence that, in comparing gametes within a species, an assumption of constant force (F) may be more appropriate. For example, a species may have evolved a propulsion mechanism that has developed to an optimum that generates the same force independently of the size of the gamete. Using Stokes' law, this assumption leads to the relation $v_G = F / 6\pi\eta\, r_C \gamma_G \propto {\gamma_G}^{-1}$. And speed is inversely

proportional to linear size, instead of directly proportional ($v_G \propto \gamma_G{}^1$) as found under the constant-power-density model. Intermediate between these would be a constant-speed model ($v_G \propto \gamma_G{}^0 = 1$).

A more recent analysis of the limited data available, indeed, suggests that across genera the constant power density model fits best, while within genera the constant force model fits best (Dusenbery, 2009). In light of these correlations, the constant force assumption is probably the best assumption for considering the evolution of anisogamy, where the important differences are within a species.

In order to explore the consequences of other possible strengths of the relationship between size and speed, for our gamete model, we express the size dependence of speed as

$$v_G \propto \gamma_G{}^{\theta}. \tag{6.15}$$

Then, $\theta = -1$ provides the correct relationship for a constant-force assumption, while $\theta = 0$ provides a speed independent of size, and intermediate values may approximate actual relationships.

6.4.2 Selective pressures

With these concepts, we now consider whether and how constraints on encounters between gametes can provide the disruptive selection to form and maintain gametes of distinctly different size within a species.

6.4.2.1 At the limits of extreme dimorphisms

Often, a good way of dealing with a complex relationship, such as Equation 6.4, is to see what happens with extreme parameter values where the mathematical relationship can be approximated by a simpler one. In this case, assume that the gametes have specialized into a large, immobile egg (E) and small, motile sperm (S). Then the encounter speed is the speed of sperm swimming, $v_e = v_S$ and the encounter distance can be approximated by the egg radius, r_E, since the smaller sperm radius makes a relatively small contribution to encounter distance. With these, and the other more specialized assumptions, the effects on fitness can be represented by the gamete radius raised to the appropriate power.

The analysis is simplified by the fact that the overall fitness is proportional to the product of all the factors considered. This includes

the factors in Equation 6.6 with additional factors representing the active life span of gametes (as in Equation 6.8). Past theories have emphasized the size dependence of zygote survival; in order to include them in the analysis, a factor representing zygote fitness is also included. With each factor represented as a power of the radius, the overall fitness can be expressed as the product of some power of the egg radius and some power of the sperm radius ($r_E{}^x r_S{}^y$).

If the exponent of the egg radius is positive ($x > 0$) and the exponent of the sperm radius is negative ($y < 0$), then the system experiences disruptive selection, with eggs under pressure to get larger and sperm to get smaller. This is the kind of selective pressure necessary to develop and maintain anisogamy.

Table 6.1, after Dusenbery, 2002, summarizes this type of analysis for eight hypotheses that have been proposed for explaining anisogamy by effects of size on the effectiveness of gamete encounters and/or survival of the resulting zygote. The analysis confirms that each hypothesis can potentially provide the disruptive selection necessary to maintain large egg size and small sperm. More importantly, it demonstrates how different factors can work together to provide the necessary disruptive selection.

But, this analysis is for a situation in which there are already large differences in the sizes of gametes. It explains how anisogamy and oogamy can be maintained. However, more detailed analyses indicate that, in at least some of them, the advantage of two sizes does not occur when both gametes are small, and there is a barrier in the fitness landscape to the evolution of gametes from small isogametes to anisogametes (Dusenbery, 2000). So we now consider a more detailed analysis of evolution under some of these assumptions.

6.4.2.2 Size dependence of fertility

Current knowledge and calculations suggest that most isogametes may be too small for pheromone attractants to be effective (Dusenbery, 2000; Dusenbery, 2009); see Figure 6.2, below. So, assume that encounter occurs on physical contact. The encounter distance is $r_e = r_{Contact} \equiv r_A + r_B = r_C(\gamma_A + \gamma_B)$, defining a dimensionless encounter/contact distance,

$$\gamma_{Contact} \equiv \frac{r_{Contact}}{r_C} = \gamma_A + \gamma_B \tag{6.16}$$

Table 6.1 *Comparison of anisogamy/oogamy models*

Parameter	Zygote[a]	Zygote[b]	Diffusion[c]	Hoekstra[d]	Search[e]	Pheromone[f]	Target[g]	Longevity[g]
n_E or V_E^{-1}	r_E^{-3}	r_E^{-3}	r_E^{-3}	r_E^{-3}	r_E^{-3}	r_E^{-3}	r_E^{-3}	r_E^{-3}
n_S or V_S^{-1}	r_S^{-3}	r_S^{-3}	r_S^{-3}	r_S^{-3}	r_S^{-3}	r_S^{-3}	r_S^{-3}	r_S^{-3}
σ_E	–	–	}	r_E^{0}	r_E^{2}	r_E^{6}	r_E^{2}	r_E^{2}
v_S	–	–	$r_E\, r_S^{-1}$	r_S^{-2}	r_S^{-1}	r_S	–	–
t	–	–	}	–	–	–	–	$r_E^{2}\, r_S^{2}$
f	r_E^{6}	$r_E^{>3}$	$r_E^{>2}$	$r_E^{>3}$	r_E^{3}	–	$r_E^{>1}$	–
Product:	$r_E^{3}\, r_S^{-3}$	$r_E^{>0}\, r_S^{-3}$	$r_E^{>0}\, r_S^{-4}$	$r_E^{>0}\, r_S^{-5}$	$r_E^{2}\, r_S^{-4}$	$r_E^{3}\, r_S^{-2}$	$r_E^{>0}\, r_S^{-3}$	$r_E\, r_S^{-1}$

Subscript E refers to eggs, S to sperm. $r^{>x}$ is gamete radius to any exponent greater than x. The symbol V is gamete volume, t is the time period over which gametes are active, and f is the fraction of encounters that result in a zygote that survives to become a reproductive adult. The product of the six parameters is the measure of fitness, in this situation. The relations are taken from the models in the limits $r_E >> r_S$, $v_E = 0$.

[a] Parker *et al.* (1972); [b] Charlesworth (1978); [c] Schuster and Sigmund (1982) did not work out how much smaller the zygote exponent could be, but this analysis indicates that it can get down close to 2; [d] Hoekstra (1984); [e] Cox and Sethian (1985); [f] Dusenbery (2000); [g] Dusenbery (2002).

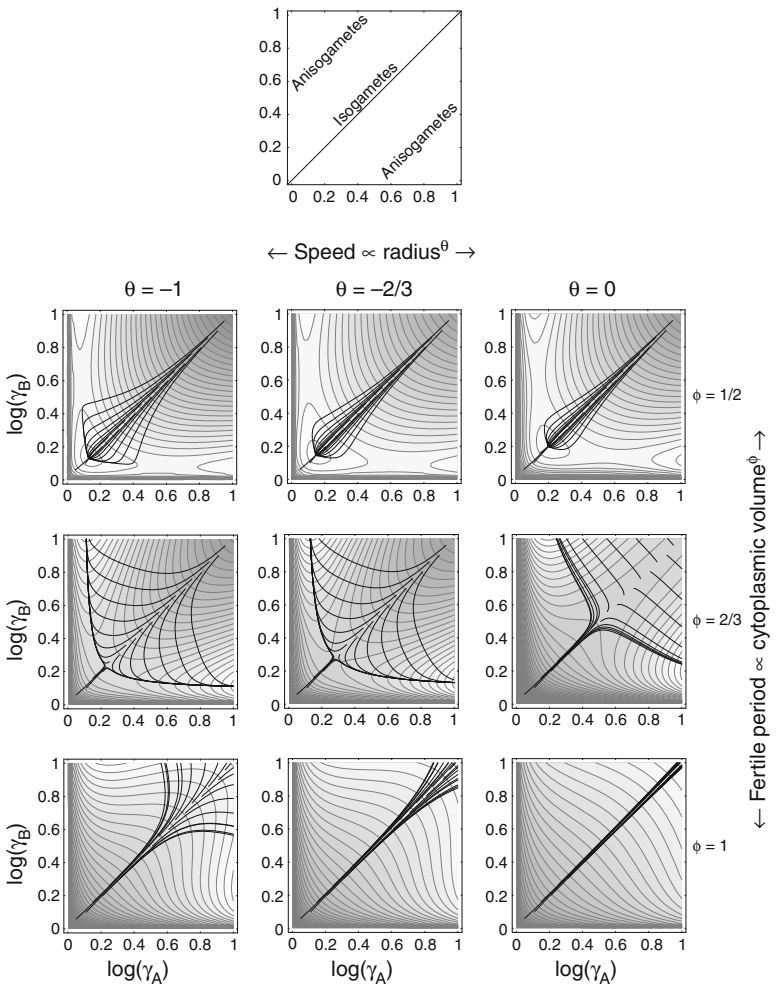

Figure 6.1 Contour plots of fitness landscapes and paths to higher contact encounter rates. The axes represent the sizes of the two types of gametes, in terms of the log of the radius relative to that of the chromatin volume. Thus, 0 corresponds to a gamete that is all chromatin with no cytoplasm to support motility, and 1 corresponds to a gamete with a diameter 10 times larger. Encounter rates for each combination of gamete size are indicated by gray contours, with lighter fills for higher encounter rates. The black lines record the paths expected for the evolution of a mating system selected for higher encounter rates. These paths start at points spaced along the isogamous diagonal, but displaced from it by a small factor (≈ 1.02). Each column of plots includes rates for a particular speed–size relationship. The left column ($\theta = -1$) is for the constant-force

and the target size is $\pi rc^2(\gamma_A + \gamma_B)^2$. Substituting these relations into Equation 6.4, the encounter rate is proportional as[1]

$$Z_{AB} \propto (\gamma_A + \gamma_B)^2 ({\gamma_A}^3 - 1)^{\phi} ({\gamma_B}^3 - 1)^{\phi} \frac{3{\gamma_A}^{2\theta} + {\gamma_B}^{2\theta}}{3{\gamma_A}^{3+\theta}\,{\gamma_B}^3}, \quad {\gamma_A}^{\theta} \geq {\gamma_B}^{\theta}. \quad (6.17)$$

This equation is too complex for most of us to understand how the encounter rate varies with the sizes of the two gametes, so computer modeling is used. Some results are shown in Figure 6.1 and elsewhere (Dusenbery, 2006; Dusenbery, 2009).

The contours are a direct representation of the notion of the "fitness landscape" in which elevation is proportional to fitness, and selection pressures populations to evolve upslope to higher levels of fitness. The figure also includes a sample of paths by which nearly isogamous populations are predicted to evolve as they move up the local slope to higher encounter rates, as they accumulate small genetic changes leading to higher fitness. In doing this, it is assumed that initially there is some inherited difference between gamete types that causes a small difference in size. This initial size difference could be a consequence of any difference between mating types, including chance differences due to genetic linkages between different genetic loci.

The starting isogamete sizes and the plot size range were chosen to cover the sizes of most relevance to the evolution of anisogametes. With γ in the range 1 to 10 and a typical chromatin radius of $r_C = 1$ μm, the gamete radii are in the range 1 to 10 μm. Plots are presented using

Caption for Figure 6.1. (cont.)
assumption, where speed is inversely proportional to radius, the middle column ($\theta = -2/3$) is the best estimate of the little data available and the right column corresponds to speed independent of size ($\theta = 0$). Each row of plots includes rates for a particular fertile- period–size relationship, ranging from the top row of period proportional to the square root of volume ($\phi = 1/2$) to proportionality to volume ($\phi = 1$). More details in (Dusenbery, 2006; Dusenbery, 2009).

[1] This relation differs from Equation 2 in Dusenbery 2000, because the latter assumed that speed is based on power proportional to the cytoplasmic volume of the gamete, while the calculation here assumes that speed is some power of the whole volume. The two formulas are equal at the most important values $\phi = 0$ and 1.

various assumptions for the values of parameters θ and ϕ, which control the strengths of assumed relationships.

The results displayed in Figure 6.1 indicate that the size dependence of the swimming speed (θ) is relatively unimportant, within the range of plausible values.

In contrast, the strength of the relationship between size and fertility makes for quantitatively different evolutionary pathways. With fertility proportional to the square root of cytoplasmic volume ($\varphi = 1/2$), all the considered sizes of isogametes evolve toward isogametes of an optimal small size, although larger ones pass through anisogamitic states on their way to the optimum.

With fertility proportional to some gamete area, the 2/3 power of cytoplasmic volume ($\varphi = 2/3$), all the considered sizes of isogametes evolve toward extreme anisogamy, with the smaller gamete approaching an optimal size, but the larger increasing in size without limit (among factors considered here).

With fertility proportional to cytoplasmic volume ($\varphi = 1$), isogametes evolve toward larger sizes, initially without much divergence from isogamy, but after reaching some size (that depends on the value of θ), they start to become increasingly dimorphic. An argument suggests that the minimum value supporting anisogamy is $\varphi > 1/3$ (Dusenbery, 2009).

These results clearly demonstrate that anisogamy can evolve under conditions of encounter by physical contact, if the fertility depends on gamete size in an appropriate way. The required relationship is a plausible representation of reality, but the range of effective values is relatively narrow.

6.4.2.3 Size dependence of pheromone attractants

The results summarized in Table 6.1 indicate that pheromone attractants can potentially provide the strongest selective force for a larger gamete. With target size increasing with the sixth power of radius or the square of volume, pheromone attraction has a stronger size dependence than any other proposed mechanism.

However, detailed analysis (Dusenbery and Snell, 1995; Dusenbery, 2000) indicates that, for small sources, chemicals diffuse away faster than they can be produced. Consequently, there is a minimum gamete size for effective production of attractant pheromones.

Considering Equations 6.14 and 6.16, whatever the value of P, for sufficiently large eggs, the encounter distance for pheromone detection grows much faster with larger eggs (as the third power of radius) than for physical contact (as the first power). Since target size varies as the square of encounter distance, the difference is amplified (powers of 6 and 2).

It is appropriate to recognize that our assumption of pheromone transport by diffusion will break down at some distance from the egg, where flow of the surrounding water begins to dominate the transport process. In the absence of better estimates of this limit, I assume that this effect becomes important at a distance of 1 cm and limit the value of γ_P to 10^4. Considering Equation 6.14, our assumption of pheromone effectiveness $P = 0.01$ implies that eggs larger than $\gamma_E = 100$ ($r_E = 100\ \mu m$) do not increase their target size any more from pheromone attraction, because of this limitation.

Using relations 10 and 15 for sperm and eggs, as appropriate, in Equation 6.6, we obtain the pheromone encounter rate proportionality

$$Z_{SE} \propto {\gamma_e}^2 {\gamma_S}^{\theta} \frac{({\gamma_S}^3 - 1)^{\phi}}{{\gamma_S}^3} \frac{({\gamma_E}^3 - 1)^{\phi}}{{\gamma_E}^3}, \tag{6.18}$$

where γ_e is the larger of γ_P or γ_C, but not more than 10^4 (equivalent to 1 cm).

Unfortunately, little is known about the amounts of pheromone released or the sensitivity of gametes to such pheromones, and it is difficult to estimate what the minimum size might be. The effect of this uncertainty is illustrated in Figure 6.2.

The figure demonstrates what a strong effect pheromone attraction can have, once the source is sufficiently large. But the necessary size depends strongly on the pheromone effectiveness, leaving much uncertainty as to the critical size where pheromone attraction becomes practical. My best guess is that a reasonable general value for the effectiveness is $P = 0.01$. This indicates a minimum radius on the order of 10 μm, which is a factor of 2–4 times the size of typical sperm and isogametes (Dusenbery, 2009, Table 20.1). So, most isogametes are likely too small to employ pheromone attraction to increase encounters.

Overall, the analysis suggests that a probable scenario is that isogametes are sometimes in a situation where fertility increases with gamete size in a way that is approximated by $\phi = 2/3$. This generates selective pressures, as revealed in Figure 6.1, for the larger gamete type to become still larger. And, at some point, it may become

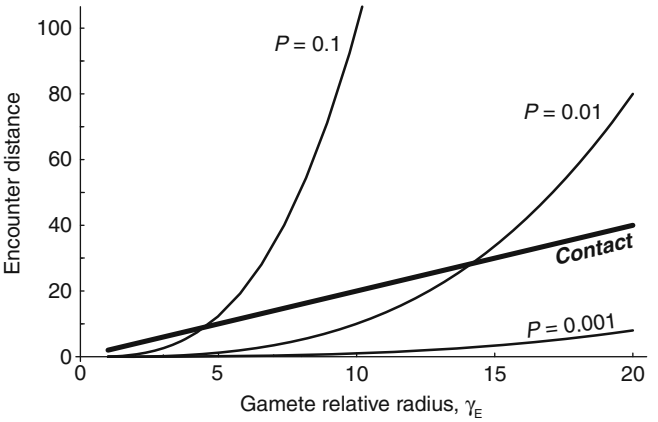

Figure 6.2 Gamete size influence on encounter distance. Encounter distance is relative to chromatin radius (r_C), estimated as typically equal to about 1 μm (Dusenbery, 2000). *Contact* is encounter by physical contact, Equation 6.16. The other curves are based on Equation 6.14, where P is a measure of pheromone effectiveness proportional to the ratio of the rate of pheromone release per volume of gamete to the threshold sensitivity of the mobile gamete (Dusenbery, 2000, Equation 5). Note that, with $P = 0.01$, gametes must be larger than $\gamma_E = 14$ for pheromone attraction to be effective, while a 10-fold more effective pheromone system ($P = 0.1$) reduces the minimum size to $\gamma_E = 5$.

large enough to reach the threshold size for pheromones to become effective. Then, there will be selective pressure for that type to specialize in releasing a chemical attractant and give up motility, while the other improves on a chemotactic mechanism for the attractant. This provides even stronger selective pressure for the larger gamete to become larger still.

This hypothesis was explored quantitatively using computer modeling (Dusenbery, 2006; Dusenbery, 2009). In the example shown in Figure 6.3, the strength of sperm swimming-speed dependence on size was intermediate between constant force and constant speed ($\theta = -2/3$). The strength of fertility dependence on size was also intermediate ($\phi = 2/3$). Pheromone effectiveness was the best estimate, $P = 0.01$. Pheromone attraction was assumed limited to a distance of 1 cm, equivalent to $\gamma_E = 100$.

In this scenario, even equal-sized gametes are distinctive because one type is immobile and gives off a pheromone while the other swims toward higher concentrations of the pheromone. The analysis

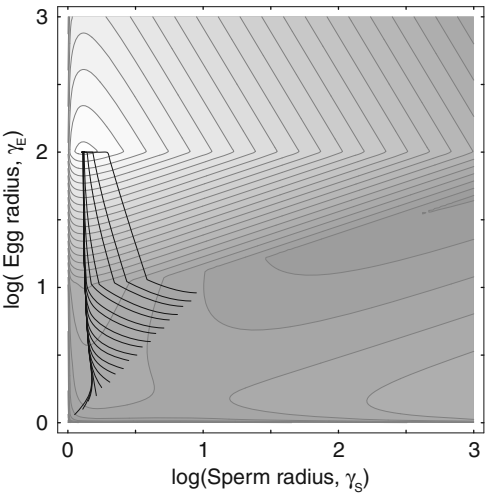

Figure 6.3 Evolutionary paths of gametes under the most plausible set of parameter values. The gray contours represent the encounter rate between gametes. The black lines are the paths of evolution of gametes from populations starting near the isogamous diagonal in the range $\gamma_G = 1$ to 10. The strength of sperm swimming speed dependence on size is intermediate between constant force and constant speed ($\theta = -2/3$). The strength of fertility dependence on size is also intermediate ($\phi = 2/3$). Pheromone effectiveness is the best estimate, of $P = 0.01$. Pheromone attraction is assumed limited to a distance of 1 cm, equivalent to $\gamma_E = 100$. Along the paths, encounter rates ranged from 0.9 to 431 000, and they all end with the same large eggs and small sperm. More details in (Dusenbery, 2006; Dusenbery 2009).

demonstrates that during the initial stage of evolution, the egg type gets larger, under the influence of the greater fertility of larger gametes, while the sperm type evolves toward an optimal size (with a radius 1.2 to 1.4 times that of the chromatin volume, depending on egg size). Then, the egg reaches a critical size ($\gamma_E = 10$, with assumed $P = 0.01$) for pheromone attraction to become effective, and there is even stronger pressure for eggs to increase in size. This increase continues until the range of action of the pheromone has become large enough for water currents to distort the gradients (a limit of 1 cm assumed, equivalent to $\gamma_E = 100$).

This result suggests general mechanisms for the evolutionary sequence: isogamy – > anisogamy – > oogamy. In fact, there exists evidence that this sequence has occurred independently in several different lineages (Baccetti, 1985; Margulis and Sagan, 1986).

6.4 CONSEQUENCES OF MORE EFFECTIVE ENCOUNTER MECHANISMS

An important consequence of more effective gamete encounter mechanisms is that they may allow a population to survive at lower population densities. For example, Levitan invoked sperm limitation as a potential explanation for the slow recovery of Caribbean sea-urchin populations after the 1983 population crash (Levitan, 1991).

At lower population densities, the population may well have failed to recover and gone extinct. Thus, looking at evolutionary time spans, more effective gamete encounter mechanisms may allow lineages to persist over longer time spans.

Lineage longevity is important because it is critical to the evolution of more complex organisms. For example, molecular evidence suggests that single-celled organisms existed two billion years before multi-cellular organisms with more than 10 cell types evolved (Hedges *et al.*, 2004). It took another billion years of evolution before complexity reached 100 cell types (Valentine, 1994) and terrestrial plants invaded the land, providing food for land animals. Since all the large plants and animals most familiar to us have evolved in only the last half a billion years, it is reasonable to suppose that anisogamy was well established long before these forms evolved.

Complexity is important because it is necessary to overcome the physical constraints on large body size, such as mechanical strength and material transport (especially water in trees and oxygen in animals).

Thus, we arrive at an explanation for the big question: why do all large organisms reproduce by a form of sexual reproduction in which there are two types of gametes – a small one produced in large numbers and specialized for transport and a larger one that is relatively immobile?

The largest and most complex organisms (trees and animals) employ very different mechanisms for bringing gametes together, including wind-borne pollen, animal-borne pollen and internal fertilization. In the case of wind-borne pollen, it is easy to see selective pressure for small pollen because small particles sink more slowly and would be carried further by winds. However, for animal pollination and internal fertilization it is more difficult to see similar selective pressure for the same gamete types.

It is probable that this pattern in many contemporary organisms is a remnant of their evolutionary history when their ancestors were aquatic organisms that engaged in broadcast spawning and the mechanisms analyzed in this paper were relevant. If this is the

case, we have a physical explanation for why male and female organisms are so familiar.

6.6 QUESTIONS FOR THE FUTURE

A primary value of theoretical analysis of this kind is to help focus research on questions that are likely to be of general importance to the origins of anisogamy. In particular, it seems to me that the following questions are important:

- What is the relationship between gamete size and active period or other aspects of fertility? This question can be easily studied by common techniques. The main barriers are researcher interest and availability of appropriate gametes.
- What are suitable values for release rates of pheromone attractants, and how do these depend on gamete size? The development of increasingly sensitive analytical chemistry equipment makes studies of this kind more practical and we can expect to see gamete attractant pheromones identified and release rates quantified for much larger numbers of species.
- What are appropriate values of threshold concentrations for response by motile gametes to attractant pheromones? This is subject to the same developments in chemical technology as the previous question. In addition, the widespread availability of video cameras and image analysis software provide improved ways of studying gamete behavior.
- What is the actual sequence of changes that have occurred in evolutionary history that lead to anisogamy? In the past, fossils provided little hope of answering such questions. But now the revolution in molecular methods raises the expectation that it will be possible to provide some specific answers.

Overall, I am optimistic that much more progress can be made in understanding the origins of anisogamy in the coming decades than in the previous few.

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7

Evolution of anisogamy and related phenomena in marine green algae

7.1 INTRODUCTION

External fertilization is a reproductive strategy, common to invertebrates and algae in aquatic environments. In this chapter, we focus mainly on marine green algae, and as well as other algae if they are useful for understanding the evolution of anisogamy. In contrast to the consistency of oogamous animals, various types of mating systems from isogamy to pronounced anisogamy are observed in marine green algae; this facilitates comparisons of different reproductive strategies. This biological group, therefore, represents a superb opportunity to examine patterns of sexual allocation and gamete size versus a suite of ecologically relevant factors (e.g. dioecious versus monoecious life history, size of gametophytes, density of gametophytes in nature and type of habitats). This study makes remarkable progress in the study of the evolution of anisogamy beyond theoretical speculation. If we know how the evolution of anisogamy happened, we might begin to see why it happened.

In green algae, the freshwater green algal order Volvocales has played an important role as the traditional testing ground for Parker, Baker and Smith's model (hereafter the PBS model) (Parker *et al.*, 1972, see also Parker's chapter, chapter 1) (Knowlton, 1974; Madsen and Waller, 1983; Bell, 1985; Randerson and Hurst, 2001a). Although it is useful for study since this group has various types of mating systems from isogamy to strong anisogamy, it violates an important assumption of the PBS model. The PBS model assumes broadcast fertilization

The Evolution of Anisogamy: A Fundamental Phenomenon Underlying Sexual Selection, ed. Tatsuya Togashi and Paul Alan Cox. Published by Cambridge University Press.

without any parental care. But this is not the case for most anisogamous Volvocalean species, which release sperm in packets (Bell, 1985). Each sperm packet freely drifts. When it encounters a female colony it breaks up and the individual sperm fertilize all of the eggs in the colony. Additionally, zygotes are not released into the medium in some species of Volvocales (Nozaki, 1988). This may be regarded as maternal care (postfertilization protection) if it is actually helpful for zygote survival (Randerson and Hurst, 2001a). In contrast, marine green algae release their individual gametes of both sexes separately into the surrounding medium. Also, in marine green algae, we don't need to take the functional relationship between the fitness of a gamete and its size into account since gametes are specialized solely for fusion, rather than for longer-term survival, as would be the case for the adult (Bulmer and Parker, 2002). Thus, marine green algae are safely regarded as using broadcast fertilization without parental care. These situations are exactly what the PBS model assumes (see Parker's chapter for details).

7.2 Uniparental inheritance of cytoplasmic genes and gamete size

One interesting set of models explains the evolution of anisogamy as an adaptation for preventing nuclear–cytoplasmic conflict (hereafter the conflict hypothesis) (Cosmides and Tooby, 1981, see also Hoekstra's chapter, chapter 4, for more details). The conflict hypothesis proposes that, if sperm are small, they carry a minimal amount of cytoplasm. This may ensure uniparental inheritance of cytoplasmic genes (e.g. mitochondoria, pastids, bacteria and intracellular symbionts) (e.g. Hurst and Hamilton, 1992). However, theoretical difficulties associated with anisogamy as a mechanism for achieving uniparental inheritance have been suggested (Randerson and Hurst, 2001b). Also, there are numerous exceptions to the rule that the larger gamete should donate the organelles to the zygote (Reboud and Zeyl, 1994). Particularly, in marine green algae, the association of isogamy and binary mating types with uniparental inheritance of cytoplasmic genes has been reported (Kagami *et al.*, 2008). It is also worth noting that uniparental inheritance of organella can be mediated either by making sperm very small or by the unisexual destruction of cytoplasmic genes just after or before entry into the zygotes (Hurst and Hamilton, 1992). For example, the nuclear mating-type genes of Chlamydomonads determine the inheritance of organella such that

mitochondoria are inherited from the minus type but chloroplasts are inherited from the plus type, despite the fact that the zygote inherits both types (Bennoun *et al.*, 1991).

7.3 GAMETE SIZE AND BEHAVIOR

Information on patterns of gamete size and behavior exists in some previous papers. In marine green algae, the gametes are generally pear-shaped and biflagellated. We observe various types of mating systems from isogamy to strong anisogamy. Particularly, isogamous or slightly anisogamous species are taxonomically widespread (e.g. Chihara, 1969; Melkonian, 1980; Togashi *et al.*, 1997), a distinction from other biological groups. These gametes not only have specific mating types, but may also have a phototactic system with an eyespot in both sexes (see Figure 7.1A and B). Such gametes initially show positive phototaxis prior to mating, swimming upward in the water column towards the light at the sea surface, with the planozygotes (i.e. motile zygotes) exhibiting negative phototaxis (e.g. Chihara, 1969; Melkonian, 1980; Togashi *et al.*, 1999). It has been suggested that the eyespot evolved in the most primitive green flagellate taxa (Melkonian, 1982). In marine green algae, phototactic behavior corresponds well with the presence or absence of an eyespot which is an important organelle for algal phototaxis, although the eyespot itself is not the photoreceptor (Melkonian and Robenek, 1984).

In contrast to these isogamous algae, Bryopsidales marine green algae are markedly anisogamous (Okuda *et al.*, 1979; Togashi *et al.*, 1998; Clifton and Clifton, 1999). In seven common genera (*Bryopsis*,

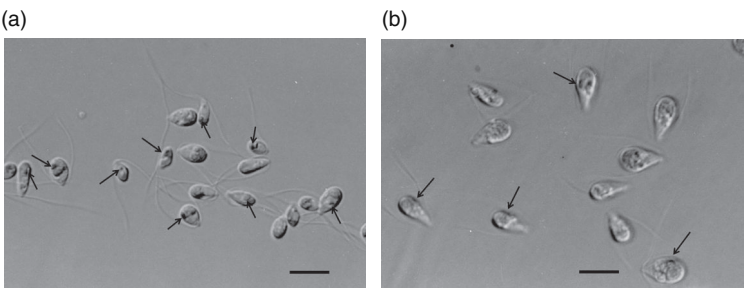

Figure 7.1 Gametes of *Monostroma angicava*. a: male (5.90±0.09 × 2.96±0.03, length × width, μm); b: female (7.53±0.05 × 3.70±0.03, μm) (Togashi *et al.*, 1997). Arrowheads indicate eyespots. Scale bar = 5 μm.

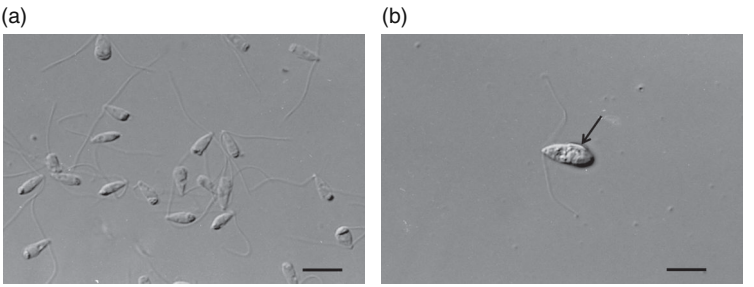

Figure 7.2 Gametes of *Bryopsis plumosa*. A: male (5.04±0.10 × 2.36±0.05, length × width, μm); B: female (8.96±0.20 × 4.83±0.11, μm) (Togashi *et al.*, 1998). Arrowhead indicates an eyespot. Scale bar = 5 μm.

Caulerpa, *Derbesia*, *Halimeda*, *Penicillus*, *Rhipocephalus*, *Trichosolen* and *Udotea*), male gametes of all species are biflagellated and similar in size, and seem to be too small to maintain a phototactic system (Figure 7.2A). However, species-specific differences are considerable in the size of female gametes. In *Bryopsis*, *Caulerpa*, *Halimeda*, *Trichosolen* and *Udotea flabellum*, female gametes are biflagellated and have a phototactic system with an eyespot (Figure 7.2B). They show positive phototaxis prior to mating, as do many isogametes and slight anisogametes. [In *Bryopsis corymbosa*, sexually unfused female gametes change phototaxis to negative a long time after liberation (Togashi *et al.*, 1998). In some other intertidal green algae, such as *Enteromorpha* (Jones and Babb, 1968) and *Spongomorpha* (Miyaji, 1985), sexually active gametes show positive phototaxis, while sexually inactive or parthenogenetic gametes are negatively phototactic or show no phototaxis. Therefore, phototactic behaviors of gametes and sexuality might be related to each other.] Some exceptions have been observed: gametes of both sexes do not have a phototactic system in markedly anisogamous species of *Derbesia* (Togashi, 1998). Female gametes of some species the genus *Bryopsis* attract male gametes using a sexual pheromone (Togashi *et al.*, 1998). Such pheromonal attraction systems would considerably increase the fertilization collision cross-section (Okubo and Levin, 2001; Togashi *et al.*, 2006, see also the pheromonal male gamete attraction systems in external fertilizers section below). Immediately after sexual fusion, their planozygotes show negative phototaxis, as do those of isogamous and slightly anisogamous species. In *Caulerpa*, female gametes cover relatively large (0.5–2.0 m) distances within a short time (5–10 min) (Clifton and Clifton, 1999). In these markedly anisogamous species (i.e. *Bryopsis*, *Caulerpa*, *Derbesia*, *Halimeda*, *Trichosolen* and

Udotea flabellum), the ratio of the volume of male gametes to that of female gametes ranges approximately from 1:2 to 1:45. In contrast, much higher levels of anisogamy are observed in *Penicillus*, *Rhipocephalus phoenix* and other *Udotea* spp (e.g. $1{:}10^4$). These strongly anisogamous species produce very large spheroid female gametes (>100 μm in length) with stephanokont flagella arrayed along a membranous sheet-like tail. Such female gametes do not have a phototactic system. In *Udotea*, patterns of gametogenesis, gamete morphology and gamete behavior of *U. flabellum* are similar to those of *Halimeda* and *Caulerpa*. In contrast, such patterns of other species of *Udotea* (*U. abbottiorum*, *U. caribaea* and *U. cyathiformis*) are similar to those of *Penicillus* and *Rhipocephalus*.

Some previous theories suggest that both mating types should produce gametes of the minimum viable size in the most ancestral isogamous mating system (Maynard Smith, 1978). However, in marine green algae, it appears that the size of isogametes is often between those of male and female anisogametes (Togashi *et al.*, 2006). But there are exceptions. For example, isogametes of *Blidingia minima*, which have binary types, are smaller than such intermediate-sized isogametes and have no phototactic system (Tatewaki and Iima, 1984).

7.4 PHOTOTAXIS AND FERTILIZATION SUCCESS

Theorists argue that positively phototactic gametes may gain significant advantages, especially in shallow water, by being able to search for potential mates in the top two-dimensional surface rather than in the three-dimensional water volume (Cox, 1983; Cox and Sethian, 1985). Thus, in aquatic systems, positive phototaxis may increase mating efficiency, as well as maintain the position of gametes in photosynthetically advantageous areas (Geider and Osborne, 1992). Negative phototaxis of planozygotes facilitates settlement on the intertidal substratum in these advantageous areas, preventing the zygotes from drifting out to deep waters as they might if phototaxis remained positive.

The behavior of phototactic gametes has been experimentally tested in *Monostroma angicava* (Togashi *et al.*, 1999). This alga is a slightly anisogamous species, but both male and female gametes have a phototactic device with an eyespot. Thus, gametic behavior is not different between the sexes. Both male and female gametes show positive phototaxis in response to a white light source as described above (Figure 7.3A and B) though they do not respond to a red light source. A gamete's probability of finding another gamete in a given time interval may

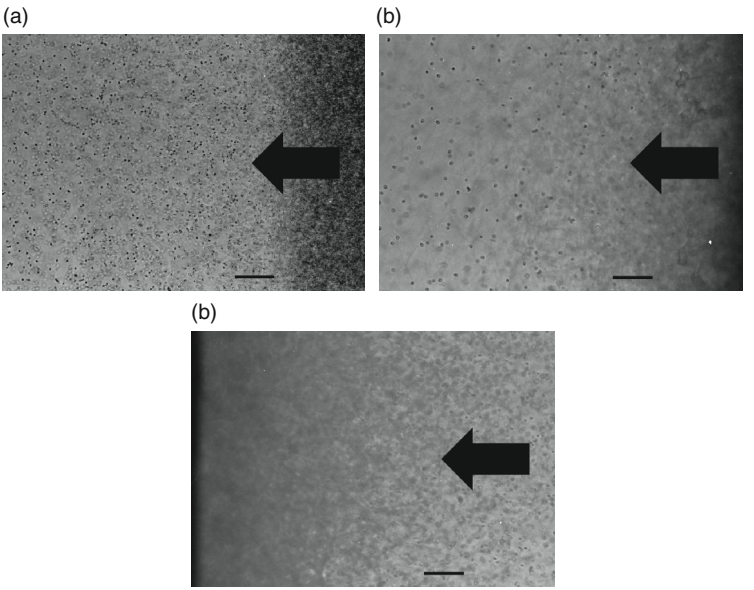

Figure 7.3. Phototactic behaviors of gametes and planozygotes of *Monostroma angicava*. A: male gametes; B: female gametes; C: planozygotes. Arrows indicate the direction of the light. Phototactic responses of test cells were investigated by analyzing individual swimming paths to exclude effects of other light-dependent responses, such as photokinesis and photophobic responses (Togashi *et al*., 1999). In cases where gametes of both sexes are released together and are illuminated from one side, male and female gametes swim side by side toward the light source. Therefore, sexual gametic fusions may not occur frequently until gametes reach the water surface both in laboratories and nature. Scale bar = 100 μm.

increase as it swims more rapidly (Hoekstra *et al*., 1984; Cox and Sethian, 1985). However, swimming velocity does not differ significantly between these two illuminating light sources. It is therefore suggested that the search ability of the gamete itself might not vary between phototactic and non-phototactic conditions. The number of zygotes formed during the mating process may be expressed as the product of the number of encounters between male and female gametes and the fraction of encounters that result in sexual fusion. In their study, with high densities of male and female gametes mixed in test tubes, almost all minor (fewer in number) gametes fused sexually within 10 min under all of their experimental conditions. Considering the swimming velocity of male and female gametes and

the depth of seawater in the experiments, almost all gametes of both sexes may be able to reach the water surface within the experimental time. After dilution of the gamete suspensions by half, however, mating efficiency in test tubes illuminated by white light from above was much higher than that in dark controls. This suggests that male and female gametes gather at the water surface by their positive phototaxis, thus increasing the rate of encounters. Mating efficiency decreases if the test tubes are illuminated from above by white light and also shaken. The possibility that illumination itself rather than the change from three dimensions to two in search patterns was responsible for increased mating efficiency may be eliminated as well. These densities used in their experiments very closely simulate the natural conditions, as estimated in the reproductive investment, gamete size and number section below. Considering that planozygotes immediately change to negative phototaxis as described above (Figure 7.3C), positive phototaxis of male and female gametes might actually be an adaptive trait for increasing the rate of gametic search, encounter and sexual fusion, rather than for the dispersal of zygotes, as previously reported for zoospores of some marine algae (see Reed *et al.*, 1988). This may be applicable to many other marine green algae, because they show the same gametic behavior as this alga. Slight anisogamy that is frequently observed in marine green algae might be maintained by the adaptive need for an eyespot and other phototactic devices in both sexes for two-dimensional (surface) encounters, thus limiting selection for decreased size in male gametes. In deep waters, it would be difficult for gametes showing positive phototaxis to reach the sea surface. Some species of the genus *Derbesia* are often observed in relatively deep waters [e.g. 9–12 m deep (Chapman *et al.*, 1964)]. Not surprisingly, their male and female gametes do not show any phototaxis (Togashi, 1998). The advantage of positive phototaxis of gametes has also been supported by studies using numerical models based on experimental data (Togashi and Cox, 2004; Togashi *et al.*, 2004).

7.5 Pheromonal male gamete attraction systems in external fertilizers

In population genetic models that do not include the assumption that zygote survival is positively related to its volume, another assumption of fitness based on gamete swimming speed and pheromonal attraction area is made with no empirical support (Hoekstra, 1984; Hoekstra *et al.*, 1984). In these models, gamete swimming speed is considered as

independent of gamete size (or is taken to be inversely proportional to the area of a section through the center of spherical gamete). The evolution of gamete motility dimorphism in cases where there is already anisogamy and the evolution of anisogamy in cases where there is already gamete motility dimorohism is analyzed. A gamete's probability of finding another gamete in a given time interval is proportional to its swimming speed. A gamete's probability of being found by another gamete in a given time interval is proportional to the area of its attraction surface. Also, the existence of two pseudo-mating types based on pheromonal gamete attraction is assumed in isogamy. [There are two varieties of gametes: one produces pheromone, and the other responds to it. But, differing from the cases where the real mating types exist, any two gametes can fuse.] Consequently, it has been concluded that anisogamy has no effect on the evolution of anisomotility, and that anisomotility has no effects on the evolution of anisogamy. One of these models suggests that, after a pheromonal gamete attraction system evolves in an isogamous population, selection will soon favor the evolution of stable anisogamy (Hoekstra, 1984).

There are many reports on gamete attraction systems in sexual reproduction of anisogamous organisms. The most intensively studied biological group might be algae (see Maier and Müller, 1986 for review). In marine green algae, pheromonal attraction systems have been found only in anisogamous species (Togashi *et al.*, 1998). As we introduced above, it appears that such pheromonal attraction systems evolve to compensate for the difference in phototactic behavior between non-phototactic males and positively phototactic females: if the chemotactic attraction of the female gametes leads the male gametes to the right place for sexual fusion, perhaps to the two-dimensional space of the sea surface, the male gametes would have no need to retain phototactic ability, and they might gain a high power of search ability by minimizing their cell size. Thus, anisogamy in which a female gamete with a phototactic system is fertilized by a male gamete without a phototactic system might have coevolved with the development of the chemotactic attraction system. Although male and female gametes cluster and agglutinate to each other with the tips of their flagella, to the authors' knowledge, no pheromonal attraction system has been reported in isogamous or slightly anisogamous species with the same phototactic behavior between male and female (Løvlie and Bryhni, 1976; Togashi *et al.*, 1997).

The difference in phototactic behavior might not be necessary to evolve pheromonal attraction systems in algae. A Scytosiphonales brown alga, *Scytosiphon lomentaria* produces slightly anisogamous male

and female gametes that have a phototactic system, including an eyespot in both sexes (Tatewaki, 1966). Their gametes show negative phototaxis that is common in brown algae. Female gametes first settle on the substratum and begin to secrete a sexual pheromone to attract male gametes. Female gametes stop secreting a sexual pheromone immediately after they have fused with male gametes. So unfused male gametes swim away to find another partner.

An exception to the combination of anisogamy and a pheromonal attraction system is observed in a freshwater green alga, *Chlamydomonas moewusii* var. *rotunda* (Tsubo, 1961). In the genus *Chlamydomonas*, there are some isogamous heterothallic species (e.g. *C. moewusii* var. *rotunda*, *C. moewusii*, *C. eugametos*, *C. reinhardi*, *C. moewusii* var. *tenuichloris*). A pheromonal attraction system has been found only in *C. moewusii* var. *rotunda*: gametes of mating-type plus are attracted by gametes of mating-type minus. Such a behavior is not observed in vegetative cells. In anisogamous species of the genus *Chlamydomonas*, another pheromonal attraction system has been reported in *C. alloensworthii*. The structural formula of this pheromone is suggested to be a 2,3-dimethil-5-(triprenylcarboxymethyl)-1,4- benzohydroquinone-1-(β-xyloside) based on the spectral, chemical and physical properties (Starr *et al.*, 1995).

Oogamous organisms including echinoid species (e.g. Vogel *et al.*, 1982; Levitan, 1998) as well as many other anisogamous broadcast spawning taxa have no pheromonal attraction system. The most obvious examples might exist in red algae (Hommersand and Fredericq, 1990). They are generally anisogamous: most species which reproduce sexually are oogamous. Eggs stay in the gametangia until they are fertilized. Such a gamete behavior might be adaptive because gametes of both sexes are always immotile without flagella. So any pheromonal attraction system would be impossible. Thus, the generality of models based on pheromonal attraction to discuss the evolutionary mechanisms of anisogamy is still uncertain. Additionally, no pheromonal pseudo-mating types have been observed in real organisms. The effects of pheromonal attraction systems on fertilization dynamics have been addressed using a numerical simulation method based on experimental data of marine green algae (Togashi *et al.* 2006).

7.6 SYNCHRONOUS GAMETE PRODUCTION AND RELEASE

Successful search, encounter and fusion of sexually different type of gametes can be increased by concentrating gametes in time as well as

space. So synchrony of production and release of male and female gametes may be important ecological conditions for successful external fertilization (e.g. Levitan, 1991; Serrão *et al.*, 1996). Also, the possible adaptiveness of phototactic concentration of gametes could be most easily tested in a species whose phenology includes an overlap between the season of sexual reproduction and a succession of noctural great low tides (i.e. late winter). In such a species, it would be of great interest to know if gametes are released at night when phototactic swarmers (i.e. gametes and planozygotes) do not have light of sufficient intensity or spectral quality to orient their trajectories towards the surface, or if gametes are released under stormy conditions where water turbulence would reduce the positive impact of phototactic swarming. Using a slightly anisogamous marine green alga, *Monostroma angicava*, a study that combines field observations on the Pacific coast of Japan and laboratory experiments affords important insights as follows (Togashi and Cox, 2001).

Many species in algae have been known to release gametes on a lunar or semilunar cycle (e.g. Fletcher, 1980; Norton, 1981; Neumann, 1989; Brawley, 1992). In some species, the cue appears to be truly lunar, but more often algae respond to the tidal height accompanying the lunar phase, often in association with a cycle of gametogenesis. Field observations confirmed that sexual reproduction of *M. angicava* occurs almost on a semilunar cycle near full and new moons from February to June, but gamete release appears to be related to tidal height rather than to the lunar phase per se. If plants are submerged by heavy seas, gamete release is postponed. Also, gamete release generally occurs during daytime low tide. Such conditions are the most favorable for positive (in gametes) or negative (in planozygotes) phototaxis. Laboratory experiments suggest that gamete release may be induced by a water-borne substance secreted by mature gametophytes under illuminated conditions, and that it may be the same or very similar for both sexes. Such a system to control gamete release of *M. angicava* is different from those previously reported for any other marine green algae, especially some species of the genus *Ulva* that form dioecious gametophytic thalli morphologically similar to those of *M. angicava*, although they are two cell-layered. In *Ulva mutabilis*, gamete release is controlled by an extracellular swarming inhibitor (Stratmann *et al.*, 1996). When the density of matured gametophytes is kept high, their gametes remain non-motile in the gametangia. If the medium is changed at this stage, gametes are immediately released. These results are completely opposite to those in *M. angicava*. In *U. mutabilis*, gametogenesis is controlled by two groups of chemical sporulation inhibitors

(Stratmann *et al.*, 1996), one which is excreted into the environment and the other which exists between the two layers of thallus cells. Gametogenesis can be induced by removing both inhibitors from single-cell layered fragments of gametophytic thalli. Similar phenomena have been observed in other *Ulva* species (Hiraoka and Enomoto, 1998). However, this system is unlikely in *M. angicava*, because gametogenesis is not induced immediately after cutting the thallus into small disks, and because, unlike *Ulva*, the presence of sporulation inhibitors between the cell layers cannot occur in the single-cell layers of *Monostroma* species. There likely is variability between taxonomically related and morphologically similar algae in the mechanisms that control gamete release and gametogenesis.

Systems that depend on tidal height to control gamete release would be impossible in deep waters. For example, in *Bryopsis plumosa* that inhabits the middle and lower intertidal zones, liberation of gametes in both sexes of *Bryopsis* is triggered by light (Burr and West, 1970). Blue and UV-A are the most effective wavelengths for light induction of gamete liberation (Mine *et al.*, 1996). The time required for liberation of gametes is not different between male and female (Togashi *et al.*, 1998). Male and female gametes appear to be synchronously liberated in the early morning in nature. Through the continuous monitoring in the field, Clifton has actually shown several species of Bryopsidales marine green algae often release gametes on the same morning (Clifton, 1997). Numerous algae species release gametes into the water column during a single brief pulse of sexual reproduction (5–20 min). However, closely related species release gametes at different times. The consistently precise timing of gamete release in the early morning, regardless of tide level or lunar phase, suggests that gamete release may depend on environmental factors such as light level and action spectrum to stimulate gamete release rather than larger-scale patterns of water motion (Clifton and Clifton, 1999).

7.7 Reproductive Investment, Gamete Size and Number

In many previous theoretical studies on the evolution of anisogamy, completed after the study of Parker *et al.* (1972), it is always assumed that each type of gamete is produced from the same fixed amount of reproductive biomass investment. Also, as the simplest example, they assume that size variance of gametes arises through the different number of cell divisions of the same biomass of the gametic material. Thus,

these assumptions suggest the trade-off between gamete size and number. However, there are few experimental studies that support these assumptions based on experimental data of real organisms. Every cell might wish to become two cells. However, it is usually held very carefully in check. This is also an example that such a process is well regulated.

Here, we show an example of the trade-off between gamete size and number in a marine green alga. As the PBS model predicted, if more small gametes can be produced than large ones, there may be a numerical advantage, although the correlation between gamete life span and size, which might be positive, should not be ignored. However, it is often difficult to give experimental evidence to support this prediction in many marine algal species because of the highly complicated structures of gametophytes and extremely large numbers of gametes produced. Togashi *et al.* suggest that a marine green alga, *Monostroma angicava* seems to be a useful material for such studies (Togashi *et al.*, 1997). *M. angicava* has a heteromorphic biphasic life history (Tatewaki, 1972). After several months of summer dormancy, the microscopic diploid sporophytes produce quadriflagellate asexual haploid zoospores through meiosis. Each zoospore develops into a macroscopic dioecious gametophyte that is iteroparous (i.e. individuals reproducing several times). In both sexes, vegetative cells that are located on the outside of simple one-cell layered gametophytes are changed into gametangia (Figure 7.4A and B). They produce slightly

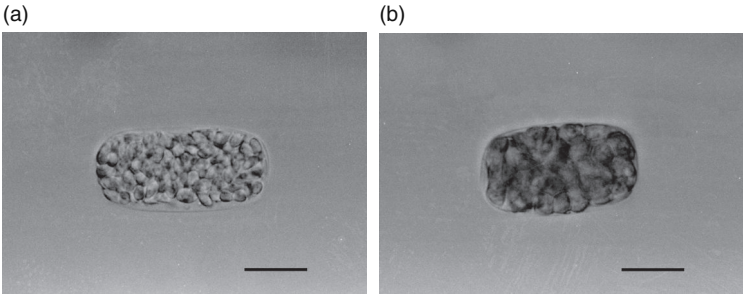

Figure 7.4 Gametangia of *Monostroma angicava*. A: male (140.3±2.3 × 24.9 ±0.3, Area of cross-section, μm^2 × length, μm); B: female (142.0±2.3 μm^2 × 25.1±0.5 μm) (Togashi *et al.*, 1997). The volume of a gametangium, which is cylindrical in shape, is estimated from the area of its optical cross-section and its length. The density of gametangia (individuals per 100 μm^2) and the area of the whole and of the sexually matured part of the gametophyte (cm^2) in both sexes are 68.2±1.8 and 66.8±1.4, 73.9±7.6 and 73.7±7.6, and 32.8±3.1 and 29.9±3.3, respectively (Togashi *et al.*, 1997).

anisogamous gametes (Figure 7.1A and B). Fertilization of these gametes results in sporophytes again.

Body length and width of female gametes are a little, but significantly, larger than those of male gametes, though the ratios of body length to body width are not significantly different between male and female gametes (see Figure 7.1). Other characterstics, except for gamete size, are not significantly different between male and female (see Figure 7.4). The number of gametes per gametangium can be counted by staining gametic nuclei with the DNA-localizing fluorochrome DAPI (4′-6-diamidino-2-phenylindole). Male gametangia have 64 or 128 nuclei per gametangium. Female gametangia have 16, 32 or 64 nuclei per gametangium. The majorities of male and female gametangia have 64 (2^6) and 32 (2^5) gametic nuclei, respectively.

In some isogamous unicellular freshwater green algal species of the genus *Chlamydomonas*, Schmeisser *et al.* (1973) and Wiese *et al.* (1979) have reported the existence of two different pathways of gametogeneis (i.e. with or without mitosis) that may produce large and small gametes, respectively. Wiese *et al.* (1979) and Wiese (1981) suggest that the potential to produce anisogametes may be based on the existence of such two pathways of gemetogenesis. In the example of a marine green alga *M. angicava*, since the volume of gametangium does not differ between male and female, anisogamy of this alga may be caused by the difference of the number of cell divisions during gametogenesis. Assuming that gametes of both sexes are similar in shape, the volume ratio of gametes can be calculated as follows: (body length of male gametes)^3:(body length of female gametes)^3 = $(5.90)^3$:$7.53)^3$ = 1:2.1. This is very consistent with the prediction based on the mechanism to produce anisogametes suggested above. Even in hermaphroditic species, individuals seem to expend almost equal efforts on male and female reproductive functions (e.g. Rietema, 1971 for an anisogamous marine green alga, *Bryopsis hypnoides*).

These results not only indicate that total reproductive biomass investments for gamete production do not differ between male and female, but also support the assumption that a gamete dimorphism may occur from a difference in the number of cell divisions during gametogenesis. This is apparently a primitive condition commonly assumed in many previous theoretical studies on the evolution of anisogamy (e.g. Parker *et al.*, 1972; Bell, 1978; Charlesworth, 1978; Maynard Smith, 1978; Parker, 1978; Hoekstra, 1980; Cox and Sethian, 1985). To create the variation of gamete size, it seems that macromutations would not be required, but, small genetic changes would be

enough, since just the change in the number of cell divisions during gametogensis can produce gametes of different sizes.

7.8 GAMETE MOTILITY

Cox and Sethian (1985), criticizing the explicit exclusion of effects of gamete size on the rates of encounter in the model of Parker *et al.* (1972) and their followers, measured collision rates between gametes as a function of size, assuming an inverse relationship between gamete size and velocity within a population of a single species. They used these results, together with considerations of zygote fitness, to show the existence of a driving force for the evolution of anisogamy. Hoekstra *et al.* (1984) predict that cases of gamete motility dimorphism with a small difference would not be expected between two types of gametes, although Hoekstra (1984) permits a stable dimorphism with a smaller difference between two motility variants under certain conditions. However, there are few quantitative empirical studies on gamete motility. Marine green algae might be useful materials to study the relationship between gamete size and motility because they produce various sizes of gametes due to the wide range of the degree of anisogamy. Particularly, a slightly anisogamous species *Monostroma angicava* and a markedly anisogamous species *Bryopsis plumosa* give us the insight to predict the relationship between cell size and motility (Togashi *et al.*, 1997 for *M. angicava;* Togashi *et al.*, 1998 for *B. plumosa*). The sizes of male and female gametes of *M. angicava* are between those of *B. plumosa*, as shown in the gamete size and behavior section.

In *M. angicava*, swimming velocities of gametes of both sexes and planozygotes do not change greatly, even when water temperatures and irradiances are varied. Water temperature during the reproductive season of this alga in the field appears to be around 5 °C. Swimming velocities of male and female gametes gradually slow down over time: the mean maximum swimming velocities of gametes at 5 °C are 182.7 $\mu m\ s^{-1}$ for males and 158.4 $\mu m\ s^{-1}$ for females. The proportions of swimming gametes decrease over time in both sexes: almost all gametes stopped swimming by 72 h (male) and 84 h (female) after liberation. This is one example of a case where gamete motility is slightly different between the two sexes.

In *B. plumosa*, although male gametes change their swimming velocity according to water temperature, swimming velocities of gametes of both sexes and planozygotes are not sensitive to irradiance. The mean maximum swimming velocities of male and female gametes at

an estimated water temperature during their reproductive season (18 °C) are 220.4 μm s^{-1} and 122.7 μm s^{-1}, respectively.

In general, swimming organisms with small body size such as the single cell of an algal gamete have small (1) Reynolds numbers (i.e. the ratios of the inertial forces to the viscous forces). In other words, movement is governed by viscous forces, and inertial forces can be safely ignored (see Purcell, 1977; Bray, 1992). Viscous force depends on the size of the organism, related to its linear dimension (L), velocity (v) and the viscosity of the liquid (η), according to the equation: viscous force = $vL\eta$ (see Bray, 1992). The mean length of gametes of *M. angicava* is 5.90 μm in males and 7.53 μm in females, and the mean maximum swimming velocity of gametes is 182.7 μm s^{-1} in males and 158.4 μm s^{-1} in females. Similarly, the mean length of gametes of *B. plumosa* is 5.04 μm in males and 8.96 μm in females, and the mean maximum swimming velocity of gametes is 220.4 μm s^{-1} in males and 122.7 μm s^{-1} in females. Experimental data also suggest that the variance of gamete swimming velocity is often very small. The ratio of the viscous force of male gametes to that of female gametes can be calculated as follows: $182.7 \times 5.90 : 158.4 \times 7.53 = 1:1.1$ for *M. angicava* and $220.4 \times 5.04 : 122.7 \times 8.96 = 1:0.99$ for *B. plumosa* because the viscosity of seawater does not differ for gametes of either sex. [As pointed out by Cox and Sethian (1985), drag forces at low Reynolds numbers (i.e. the ratio of inertial forces to viscous forces) are determined by the cross-sectional radius orthogonal to the direction of travel, according to Stoke's law. In cases of marine green algal gametes, since the ratio of gamete length to width is usually similar, it might be safe to calculate the thrust with length, which is easier to correctly measure.] These results of gametes of two species of marine green algae with different sizes of gametes well support Cox and Sethian's assumption that viscous forces, as the driving forces of gametes, are the same for all gametes (Cox and Sethian, 1985). This means that the thrust generated by the flagella of a gamete is independent of gamete size. Also, it appears to be true that small-sized male gametes have a large swimming velocity and the large-sized female gametes have a small swimming velocity. The number of flagella doesn't always increase gamete motility. For instance, stephanokont (multiflagellate) female gametes, which are produced in some Bryopsidales marine green algae (e.g. some species of the genera, *Penicillus*, *Rhipocephalus* and *Udotea*) are usually very large, and are almost non-motile despite flagellar motion (Clifton and Clifton, 1999). To the authors' knowledge, the presence of anisomotile gametes in isogamous species has not been reported in

marine green algae, though it has been known in some other biological groups (see Maier, 1993 for review). The two components (i.e. high motility and low survival) seem to actually trade off against each other. Swimming duration time of gametes is positively related to their size (volume as the source of their energy.) Life for most sperm is very short.

In some previous papers on the evolution of anisogamy, different assumptions of the relationship between gamete size and speed were made. For example, in one study, it was assumed that thrust of a gamete is proportional to its volume (Dusenbery, 2000). This implies that the velocity of a gamete is proportional to its radius. However, we find no empirical data that directly support such an assertion.

7.9 SEX RATIO

Sex ratio of gametophytes at maturity is closely related to the evolution of anisogamy, as shown below. In many organisms, including animals and land plants with separate sexes, the ratios of males to females is approximately even in nature, regardless of the sex determination mechanisms (e.g. Maynard Smith, 1978; Charnov, 1982; Seger and Stubblefield, 2002). A consensus about the issue of why balanced sex ratios evolve so often is that, in situations of a skewed sex ratio, individuals that produce offspring that are predominately of the minor sex will enjoy greater reproductive success, so sex ratios will tend to equalize (Fisher, 1930; Charnov, 1982; Maynard Smith, 1982; Bulmer, 1994). As a result, the most favored strategy for an individual usually is to produce offspring with a 1:1 sex ratio. However, highly skewed sex ratios have been observed among some animals in nature, including invertebrates where there is a parent–offspring conflict, local mate competition among males, or local resource competition among females (Karlin and Lessard, 1986 for more information and references). In plants, sex allocation is more complicated and involves geographical and environmental factors (Cox, 1981; Charnov, 1982; Lloyd, 1982). Genetic, behavioral, physiological and environmental factors may affect an individual's relative resource allocation to each sex (Karlin and Lessard, 1986). Several kinds of questions arise in considerations of sex-ratio evolution, including how are 1:1 sex ratios maintained, and how sex ratios are affected by fluctuating environments. The information on sex ratios at inception (i.e. sex ratio of zoospores) and at maturity (i.e. sex ratio of gametophytes matured) of the marine green alga *Bryopsis plumosa* through laboratory culture

experiments controlling the whole life history, as well as field observations (Togashi and Cox, 2008) give answers to such questions. Sex ratios could be different at inception and at maturity due to differential mortality during development (Hamilton, 1967).

Gametophytes of *B. plumosa* are dioecious, and unicellular but multinucleate. Their sporophytes are also unicellular, but, have a single giant nucleus (see van den Hoek *et al*., 1996). They have a haplodiplontic life cycle: separate haploid and diploid stages during the species life cycle. Gametes develop in gametangia within the haploid gametophyte. Fertilization gives rise to the microscopic diploid sporophyte. The appearance of macroscopic features consistently related to sexual function allows visual recognition of sexual reproduction in the field. In *Bryopsis*, these involve rapid gametangial development (typically overnight) that follows changes in color, different between male (yellowish orange) and female (dark green). These are macroscopic and easily detected in the field as an indication of fertility. The sex ratio at inception is studied by experimentally inducing zoosporogenesis, since it is difficult to observe in nature. Zoospores (Figure 7.5) germinate into new gametophytes with one sex (i.e. male or female). The sex of different gametophytes produced from a single sporophyte is checked in the laboratory. The sex ratio was analyzed at maturity based on data collected in the field. In this study, a statistically significant biased sex ratio was not detected both at inception and maturity. In this alga, meiosis appears to occur in the sporophyte during zoosporogenesis (Kapraun, 1994). The two mating types might consist of

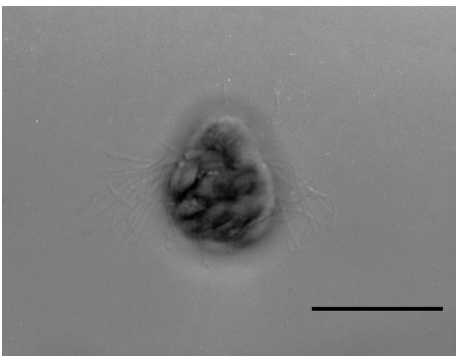

Figure 7.5 Stephanokont zoospore of *Bryopsis plumose* (25.3±5.6 × 20.5±2.7, length × width, μm) (Togashi and Cox 2008). Released zoospores swim slowly for a while. No phototaxis was observed. Scale bar = 30 μm.

non-homologous complexes of closely linked genes occupying homologous positions at the same chromosomal locus (i.e. idiomorphs). Thus, they may segregate as single alleles during meiosis. Also, Fisherian selection of sex ratio holds for this alga.

7.10 DIOECIOUS VERSUS MONOECIOUS LIFE HISTORY

Both dioecious and monoecious life histories are observed in marine green algae. For example, within Bryopsidales, we can analyze the relationship between life history and mating system. In Udoteaceae, most of the species are dioecious.(e.g. *Halimeda discodea*, *H. goreaui*, *H. incrassate*, *H. monile*, *H. opintia*, *H. simulans*, *H. tuna*). In Caulerpaceae, all of the species are monoecious (e.g. *Caulerpa cupressoides*, *C. mexicana*, *C. racemosa*, *C. serrulata*, *C. sertularioides*, *C. verticillata*), which release gametes from different parts of the same thallus (Clifton and Clifton, 1999). In contrast, both dioecism and monoecism mix in Bryopsidaceae (e.g. dioecious: *Bryopsis plumosa*, *B. maxima*; monoecious: *B. hypnoides*, *B. ryukyuensis*) (e.g. Tatewaki, 1977; Togashi *et al.*, 1998). Molecular phylogenetic studies suggest that the monoeciuos species have derived from the dioecious ancestors (Hanyuda *et al.*, 2000; Lam and Zechman, 2006). In marine green algae, therefore, most of the monoecious species are unlikely to be primitive. Actually, they are usually markedly anisogamous. Because we observe no significant difference between monoecious and dioecious species, whether gametophytes are dioecious or monoecious is not a significant consideration for our analytical models below.

7.11 FERILIZATION KINETICS MODEL

One set of models views anisogamy as an adaptation to maximize gamete encounter rate (Schuster and Sigmund, 1982; Hoekstra, 1984; Hoekstra *et al.*, 1984; Dusenbery, 2000). There are many difficulties in conducting laboratory or field experiments using real gametes, mainly because of their extremely small sizes and large numbers. Here we show a fertilization kinetics model with an analytical approach based on experimental observations of marine green algae, in which fused gametes are properly removed from the mating population (see Togashi *et al.*, 2007 for more details). This turns out to be a good alternative to wet experiments. In previous population genetic models of the evolution of anisogamy, the numbers of zygotes formed are often estimated based just on the relative frequencies of gametes;

fused gametes (i.e. those that have formed zygotes) are not excluded from the mating population count (Charlesworth, 1978). This might suffice in models of oogamous echinoderms where the number of sperm released would be generally much larger than that of eggs, but in most cases it is important to remove fused gametes from the mating population in order to correctly estimate the number of zygotes formed, especially for isogamous or slightly anisogamous species.

Though real gametes of marine green algal species are pear-shaped (Figures 7.1–7.4), gametes may be idealized as spheres for our study purposes (Cox and Sethian, 1985). According to Stoke's, law drag forces are determined by the cross-sectional radius orthogonal to the direction of travel at the low Reynolds numbers relevant here. Thus, the use of spherical gametes is plausible. Our approach to understanding the fertilization dynamics of marine green algae begins with a mathematical model for collisions similar to that used for the kinetics of hard sphere gases (Goldberger and Watson, 2004), but includes some important factors to be considered in this biological application. It is assumed that gametes of two mating types (+) and (-) are distributed randomly throughout a well-mixed volume. The radii of the gametes are r_+ and r_- for the two mating types, respectively. The initial number density of gametes is determined from the gametic investment, G, expressed as a fraction of the total volume, together with the size of the gametes as follows:

$$N_+ = \frac{G}{r_+^3} \text{ and } N_- = \frac{G}{r_-^3},$$

i.e. each gametophyte is assumed to devote the same amount of energy or mass to the production of gametes. We also like to parameterize with the zygote size

$$Z = r_+^3 + r_-^3$$

and the volumetric anisogamous ratio

$$\alpha = \left(\frac{r_-}{r_+}\right)^3,$$

which is related to the usual anisogamy ratio $A = \dfrac{r_+ - r_-}{r_+}$ by $\alpha = (1 - A)^3$. Note that anisogamy increases as α decreases, and we have isogamy when $\alpha = 1$.

We begin with an analysis of the anisogamous case ($r_+ \neq r_-$). The zygote number density, $\zeta(t)$, that is produced as a result of collisions

between gametes of (+) and (–) types can be determined from the collision rate equation.

$$\frac{\mathrm{d}\zeta(t)}{\mathrm{d}t} = \sigma \cdot \langle v_{\mathrm{rel}} \rangle \cdot (N_+ - \zeta(t)) \cdot (N_- - \zeta(t)), \tag{7.1}$$

where t is the time since release of the gametes, σ is the collision cross-section

$$\sigma = \pi \cdot (r_+ + r_-)^2$$

and $\langle v_{\mathrm{rel}} \rangle$ is the root mean square relative velocity given by

$$\langle v_{\mathrm{rel}} \rangle = \sqrt{\bar{v}_+^2 + \bar{v}_-^2}.$$

As discussed in the section above, the force generated on the gamete is independent of its size. Thus, the average velocity, according to Stoke's law, is inversely proportional to the radii, i.e.

$$\bar{v}_+ = \frac{S}{r_+} \text{ and } \bar{v}_- = \frac{S}{r_-}.$$

With initial conditions of $\zeta(0) = 0$, and constraint that $r_+ \neq r_-$, we can solve (7.1) to give

$$\zeta(t) = \frac{N_+ \cdot N_- \cdot \left(1 - e^{\beta \cdot t}\right)}{N_+ - N_- \cdot e^{\beta \cdot t}} = \left(\frac{G}{Z}\right) \cdot \frac{(1+\alpha) \cdot \left(1 - e^{\beta \cdot t}\right)}{(\alpha - e^{\beta \cdot t})}, \tag{7.2}$$

where

$$\beta = (N_- - N_+) \cdot \sigma \cdot \langle v_{\mathrm{rel}} \rangle = \frac{G \cdot (1 - \alpha^2)}{Z \cdot \alpha} \cdot \gamma$$

and

$$\gamma = \sigma \cdot \langle v_{\mathrm{rel}} \rangle = \left(\pi \cdot S \cdot Z^{1/3}\right) \cdot \left(\frac{\left(1 + \alpha^{1/3}\right)^2 \cdot \left(1 + \alpha^{2/3}\right)^{1/2}}{\alpha^{1/3} \cdot (1+\alpha)^{2/3}}\right).$$

For the isogamous case, $r = r_+ = r_-$, the number of zygotes, $\zeta_{\mathrm{iso}}(t)$, is determined by the collision rate equation

$$\frac{\mathrm{d}\zeta_{iso}(t)}{\mathrm{d}t} = \sigma \cdot \langle v_{\mathrm{rel}} \rangle \cdot (M - \zeta_{\mathrm{iso}}(t))^2, \tag{7.3}$$

where

$$M = \frac{G}{r^3} = \frac{2 \cdot G}{Z},$$
$$\sigma = 4 \cdot \pi \cdot r^2,$$
$$\langle v_{\text{rel}} \rangle = \sqrt{2} \cdot \bar{v}, \text{and}$$
$$\bar{v} = \frac{S}{r}.$$

Solving (7.3) for $\zeta_{\text{iso}}(0) = 0$ gives

$$\zeta_{\text{iso}}(t) = 2 \cdot (G/Z) \cdot \frac{2 \cdot (G/Z) \cdot \gamma_1 \cdot t}{(1 + 2 \cdot (G/Z) \cdot \gamma_1 \cdot t)}, \tag{7.4}$$

where

$$\gamma_1 = \gamma(\alpha = 1) = \left(\frac{4 \cdot \pi \cdot S \cdot Z^{1/3}}{\sqrt[6]{2}} \right).$$

To visualize the behavior of $\zeta(t)$, we plot Equations (7.2) and (7.4) with representative values of parameters we use in typical simulations in Figure 7.6. Here, we can verify that for each series with the same G, the initial formation rate decreases with α, but as the curves reach saturation (for instance near $t = 2200$ s for $G = 4 \times 10^{-6}$), they cross over, so that the largest number of zygotes is formed for the largest α beyond that point. Also, as the density of gametes decreases (smaller G) the crossover takes longer to reach. Note that nearly the same behavior is produced when Z is increased, i.e. when zygotes are larger, they are slower to form and fewer in number. The crossing curves do not all

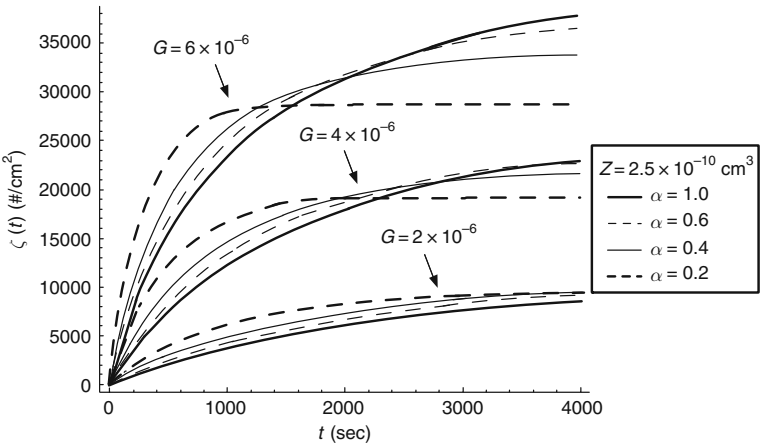

Figure 7.6 Zygote formation as a function of time for fixed zygote size.

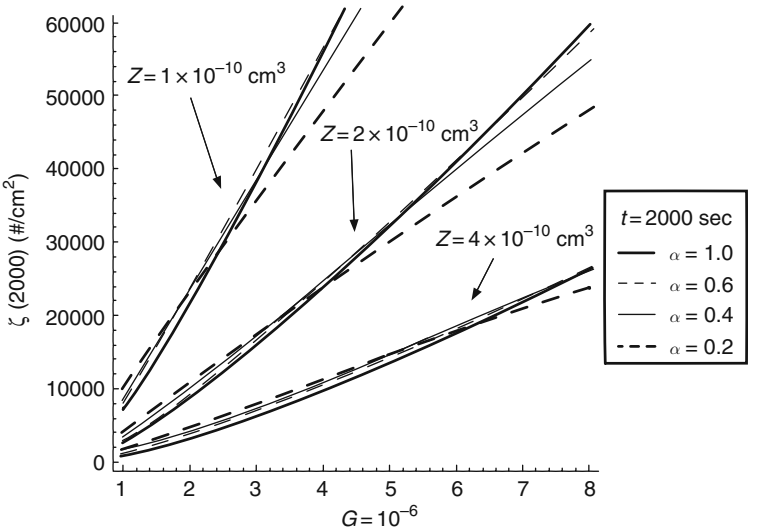

Figure 7.7 Zygote formation as a function of gamete investment at a fixed time.

intersect at the same point, but in a small region that we call the "cross-over time zone (CTZ)."

The location of the CTZ is significant because if we decide to count zygotes formed up to a specific cut-off time, say $t_x = 2000$ s, and compare zygote counts at lower gamete densities with those at higher gamete densities, then at lower densities the counts from curves of higher anisogamy (smaller α) may exceed those of lower anisogamy (higher α). Or, at higher densities, if the cross-over point has been passed, the counts from curves of lower anisogamy will be highest. This is best shown in Figure 7.7, another example plot of Equations (7.2) and (7.4) at fixed time ($t_x = 2000$) for several zygote sizes, while varying the dimensionless parameter G, the total gametic investment of each gametophyte. G can be thought of a dilution or concentration factor that measures how the gametes are initially spread throughout the medium. We call the crossing region here the "critical cross-over region (CCR)".

It is easy to see that, for each series with the same Z, at gamete densities below the CCR, zygote counts for highly anisogamous conditions are largest, while at G values above the CCR, lower anisogamy prevails, and that the CCR increases as Z increases. This is shown more clearly in Figure 7.8 for a fixed zygote size and two different cut-off times. These results suggest the hypothesis that isogamous species

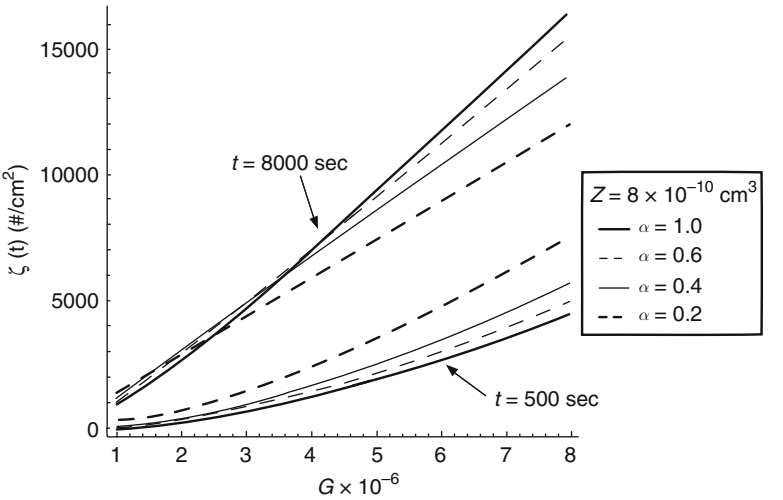

Figure 7.8 Zygote formation at two different fixed times showing that isogamy dominates for times greater than the CCR, while anisogamy predominates for shorter times.

should dominate in shallow water, and conversely, anisogamous species should dominate in deep water. Since shallow water would often provide environmental conditions suitable for growth and reproduction, the total gametic investment of each gametophyte (G) would easily and often exceed the CCR in shallow water, and vice versa. Positive phototaxis of isogamous gametes of both sexes and resultant high 2D collision rates would also increase gamete densities in shallow water. Some previous models on the evolution of anisogamy assume that gamete size is independent of its swimming speed (Hoekstra, 1984; Hoekstra *et al.*, 1984). In contrast, this study suggests that gamete size determines the fertilization kinetics. Numerical computer simulation is another alternative to difficult laboratory or field experiments with tedious examination and counting (e.g. Togashi and Cox, 2004; Togashi *et al.*, 2004). In particular, such an approach is useful to study the effects of special gamete behaviors as phototaxis and chemotaxis (sexual pheromone) on fertilization dynamics.

7.12 COST OF SEX

In standard theory, it has been argued that, if deleterious intracellular parasites are disregarded, isogamous sexual reproduction has no direct

costs, and the familiar "two-fold cost of sex" only arose later with the evolution of anisogamy: parthenogenetic females enjoy a two-fold selective advantage because they do not waste energy on producing sons (e.g. Maynard Smith, 1978). However, are such isogamous systems always free of cost? Hastings has quantified the costs of intracellular parasites or deleterious selfish genomes (Hastings, 1999). Such costs are likely to be high particularly in isogamous sexual reproduction. Matsuda and Abrams have shown that, isogamy can only be evolutionary stable when there are significant direct effects of gamete size on gamete survival and mating success, and that, when isogamy is stable, it results in a lower population growth rate than asexual forms (Matsuda and Abrams, 1999). Thus there could be a cost even in isogamous mating systems. They, however, have estimated the numbers of zygotes formed with a very simple assumption: it just depends on the ratio of gamete numbers in the mating population. With the detailed fertilization kinetics model as shown above, however, we can compare zygote formation in the case of isogamy with mating types to that without mating types.

In the case of no mating types, the number of zygotes, $\zeta_{\mathrm{non}}(t)$, is determined by the collision rate equation

$$\frac{\mathrm{d}\zeta_{\mathrm{non}}(t)}{\mathrm{d}t} = \frac{1}{2} \cdot \sigma \cdot \langle v_{\mathrm{rel}} \rangle \cdot (N - 2 \cdot \zeta_{\mathrm{non}}(t))^2 \tag{7.5}$$

where σ and $\langle v_{\mathrm{rel}} \rangle$ are the same as in the case of isogamy, but the initial number of gametes is

$$N = \frac{2 \cdot G}{r^3} = \frac{4 \cdot G}{Z}.$$

Solving (7.5) for $\zeta_{\mathrm{non}}(0) = 0$ yields

$$\zeta_{\mathrm{non}}(t) = \frac{1}{2} \cdot \frac{N^2 \cdot \gamma_1 \cdot t}{1 + N \cdot \gamma_1 \cdot t} = 2 \cdot (G/Z) \cdot \left(\frac{4 \cdot (G/Z) \cdot \gamma_1 \cdot t}{1 + 4 \cdot (G/Z) \cdot \gamma_1 \cdot t} \right), \tag{7.6}$$

where γ_i is the same as in the isogamous case with mating types. As before, we can compare and visualize the behavior of $\zeta_i(t)$ and $\zeta_{\mathrm{non}}(t)$ by plotting Equations (7.4) and (7.6) with representative values of the parameters used above (Figure 7.9). Clearly the non-mating-type case has a higher zygote formation rate for all time, even as the asymptote value of $2 \cdot (G/Z)$ is approached. A substantial disadvantage of isogamy relative to asexuality remains, no matter what the value of γ_1. These results suggest that another cost of sex exists.

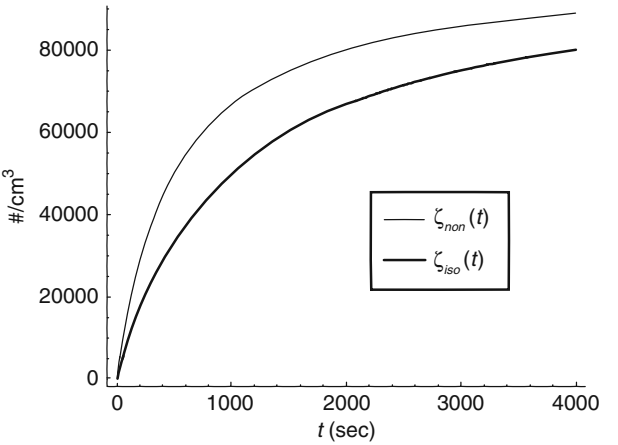

Figure 7.9 Comparison of mating versus non-mating isogamous zygote formation. as a function of mating time.

7.13 EVOLUTION OF GAMETE SIZE WITHOUT MATING TYPES

Here we show an ESS (evolutionarily stable strategy) model to better understand the evolutionary dynamics of a primitive non-mating-type gamete size, developed with reference to the PBS theory, which is based on total numbers of zygotes formed and the zygote survival rates (Togashi *et al.*, in press). Mating types are not included since it has been suggested that primitive mating systems did not have mating types. The mating types seem to have evolved in such a primitive isogamous ancestor prior to the evolution of anisogamy since most of the existing isogamous species have mating types in marine green algae. The mating-type locus is supposed to be linked to the gamete-size locus with selection to suppress crossing over between these loci (Charlesworth, 1978). The fertilization kinetics model above is applied to calculate the numbers of zygotes formed.

Consider a seawater tank containing a number of gametophytes of two varieties, "a (mutant)" and "b (wild type)." The two varieties are identical except that they produce non-mating type gametes of different sizes with radii r_a and r_b respectively. The gametophytes release their gametes into a well-mixed volume. We follow the gametophyte demography: gamete production and release, zygote formation and zygote survival to produce gametophytes in the next generation. At the beginning of sexual reproduction we observe N_a and N_b numbers of

gametophytes per unit volume and at the end of sexual reproduction we find N'_a and N'_b gametophyte densities. We define the invasion success ratio for "a" as

$$R_a = \frac{N'_a N_b}{N_a N'_b}, \tag{7.7}$$

so that $R_a > 1$ indicates that variety "a" can successfully invade variety "b." In the analysis that follows, $g_a(0) = \frac{G \cdot N_a}{m_a}$ and $g_b(0) = \frac{G \cdot N_b}{m_b}$ are the initial number densities of gametes of the varieties "a" and "b" respectively, where G is the constant total volumetric gametic investment of each individual gametophyte and $m_x = r_x^3$ is proportional to the gamete's volume.

Note that, differing from the fertilization kinetics model above, gametes of originals and invading mutants fuse in this model. Since there are no mating types in this model, all possible combinations of gamete fusion are possible: a–a, a–b and b–b. We are interested in the number density of such zygotes (number of zygotes per unit volume) $z_{aa}(t), z_{ab}(t)$, and $z_{bb}(t)$ respectively as a function of time, t, during the mating period.

The relative velocity between any two gametes can be expressed in terms of their vector velocities. The magnitude of the relative velocity is the square root of the scalar product of the velocity with itself:

$$\overline{v_{xy}^{\text{rel}}} = \sqrt{\left(\vec{v}_x - \vec{v}_y\right) \cdot \left(\vec{v}_x - \vec{v}_y\right)} = \sqrt{\vec{v}_x \cdot \vec{v}_x - 2\vec{v}_x \cdot \vec{v}_y + \vec{v}_y \cdot \vec{v}_y}.$$

Taking the average of the terms leads to

$$\left\langle v_{xy}^{\text{rel}} \right\rangle = \sqrt{\langle \vec{v}_x \cdot \vec{v}_x \rangle - 2\langle \vec{v}_x \cdot \vec{v}_y \rangle + \langle \vec{v}_y \cdot \vec{v}_y \rangle} = \sqrt{\langle \vec{v}_x \cdot \vec{v}_x \rangle + \langle \vec{v}_y \cdot \vec{v}_y \rangle},$$

since we assume that $\vec{v}_x$ and $\vec{v}_y$ are random and uncorrelated, and thus $\langle \vec{v}_x \cdot \vec{v}_y \rangle = 0$. The average swimming velocity is inversely proportional to gamete radius, so that $\langle v \rangle = C/r$, where C is a constant. We then have

$$\left\langle v_{xy}^{\text{rel}} \right\rangle = \sqrt{\langle v_x \rangle^2 + \langle v_y \rangle^2} = \left(\frac{C}{r_x \cdot r_y}\right) \cdot \sqrt{r_x^2 + r_y^2}.$$

The effective collision area or cross-section of two colliding gametes is

$$\sigma_{xy} = \pi \cdot \left(r_x + r_y\right)^2.$$

As gametes collide we assume that they fuse and form a zygote. The gamete density thus decreases over time as shown by

$$g_a(t) = g_a(0) - 2 \cdot z_{aa}(t) - z_{ab}(t)$$
$$g_b(t) = g_b(0) - 2 \cdot z_{bb}(t) - z_{ab}(t).$$

Note that two "x" gametes are removed for each z_{xx} zygote formed, while only one "x" gamete is removed by the formation of a z_{xy} zygote.

The kinetic theory of collisions then gives us

$$\begin{aligned}
\frac{dz_{aa}(t)}{dt} &= \frac{1}{2} \cdot \sigma_{aa} \cdot \left\langle v_{aa}^{rel} \right\rangle \cdot g_a(t)^2 \\
\frac{dz_{ab}(t)}{dt} &= \sigma_{ab} \cdot \left\langle v_{ab}^{rel} \right\rangle \cdot g_a(t) \cdot g_b(t) \\
\frac{dz_{bb}(t)}{dt} &= \frac{1}{2} \cdot \sigma_{bb} \cdot \left\langle v_{bb}^{rel} \right\rangle \cdot g_b(t)^2,
\end{aligned} \tag{7.8}$$

where the factor of ½ keeps us from double counting collisions between gametes of the same type.

If we adjust the time scale with

$$T = \frac{1}{2} \cdot \sigma_{bb} \cdot \left\langle v_{bb}^{rel} \right\rangle \cdot g_b(0) \cdot t \tag{7.9}$$

and normalize the zygote densities to give a non-dimensional representation, then Equations (7.8) become

$$\begin{aligned}
\frac{d}{dT}\left(\frac{z_{aa}(T)}{g_a(0)}\right) &= n \cdot r^{-2} \cdot \left(\frac{g_a(T)}{g_a(0)}\right)^2 \\
\frac{d}{dT}\left(\frac{z_{ab}(T)}{g_a(0)}\right) &= \frac{(1+r)^2 \cdot \sqrt{1+r^2}}{2\sqrt{2} \cdot r} \cdot \left(\frac{g_a(T)}{g_a(0)}\right) \cdot \left(\frac{g_b(T)}{g_b(0)}\right) \\
\frac{d}{dT}\left(\frac{z_{bb}(T)}{g_b(0)}\right) &= \left(\frac{g_b(T)}{g_b(0)}\right)^2,
\end{aligned} \tag{7.10}$$

where

$$n = \left(\frac{N_a}{N_b}\right) \qquad r = \left(\frac{r_a}{r_b}\right)$$

Thus we see that the entire collision dynamics is determined by this set of coupled, non-linear differential equations which depend only on two dimensionless constants n and r. This is a new finding. The equations are difficult to solve analytically, but with some typical values of the two parameters it is easy to integrate them numerically.

We define $z'_{xy} = S(m_x + m_y) \cdot \tilde{z}_{xy}$ as the number density of zygotes that survive to produce gametophytes for the next generation, where $\tilde{z}_{xy}$ is the steady state value of $z_{xy}(t)$, and where $S(m_x + m_y)$ is the zygote survival rate. As suggested in the PBS model, it might be reasonable to

assume that the zygote survival rate is a function of zygote size (volume) which is the sum of the volumes of the two constituent gametes $(m_x + m_y)$. In this study, we introduce the survival rate function

$$S(m_x + m_y) = \frac{1}{2} + \frac{1}{2}\tanh\left[s \cdot \left(\frac{m_x + m_y}{2m_h} - 1\right)\right], \tag{7.11}$$

which produces the sigmoidal relationship (Figure 7.10). We show how this type of survival rate function determines ultimate ESS gamete sizes. "s" is the slope of this function. $2m_{\mathrm{h}}$ is the value of the zygote volume for which the survival rate is 50%.

We assume each zygote produces two gametophytes, each of which have one gamete size allele, as below. Such an assumption seems to be reasonable, as shown in the fertilization kinetics and the evolution of anisogamy section below. In marine green algae, zygotes generally grow into diploid sporophytes that produce haploid zoospores through meiosis, each of which germinates into a new gametophyte. This means that the number density of gametophytes in the next generation is given by

$$N'_{\mathrm{a}} = 2 \cdot z'_{\mathrm{aa}} + z'_{\mathrm{ab}} \quad \text{and} \quad N'_{\mathrm{b}} = 2 \cdot z'_{\mathrm{bb}} + z'_{\mathrm{ab}}.$$

Using Equation (7.11), the invasion success ratio, R_{a}, may be written as

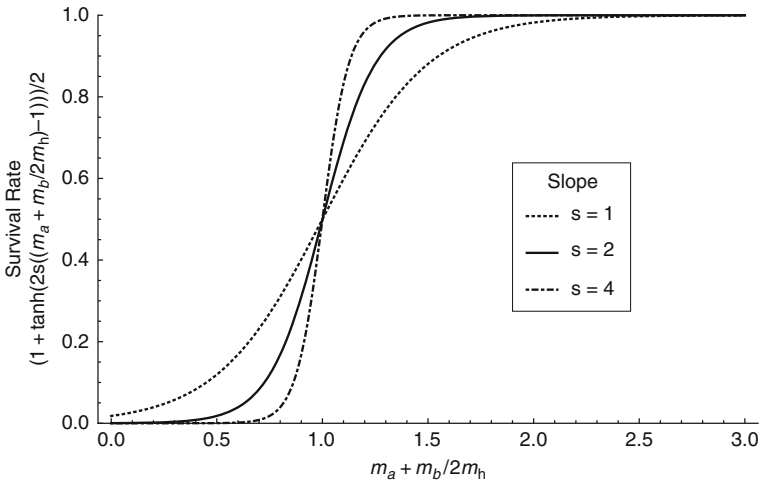

Figure 7.10 Zygote survival rate as a function of zygote volume.

$$R_a = \frac{N'_a N_b}{N_a N'_b} = \left(\frac{N_b}{N_a}\right) \cdot \left(\frac{2 \cdot S(m_a + m_a) \cdot \tilde{z}_{aa} + S(m_a + m_b) \cdot \tilde{z}_{ab}}{2 \cdot S(m_b + m_b) \cdot \tilde{z}_{bb} + S(m_a + m_b) \cdot \tilde{z}_{ab}}\right).$$

These are determined by numerical integration of the differential Equations (7.10) for specific n and r.

A contour plot of R_a as a function of m_a/m_h and m_b/m_h generally shows a characteristic saddle shape (Figure 7.11a). Here we see in the region of the saddle point at approximately (1.2, 1.2) that $R_a \approx 1$ with $\frac{\partial^2 R_a}{\partial m_a^2} < 0$ and $\frac{\partial^2 R_a}{\partial m_b^2} > 0$, or in other words, R_a has a maximum with respect to m_a and a minimum with respect to m_b (Bulmer and Parker, 2002;

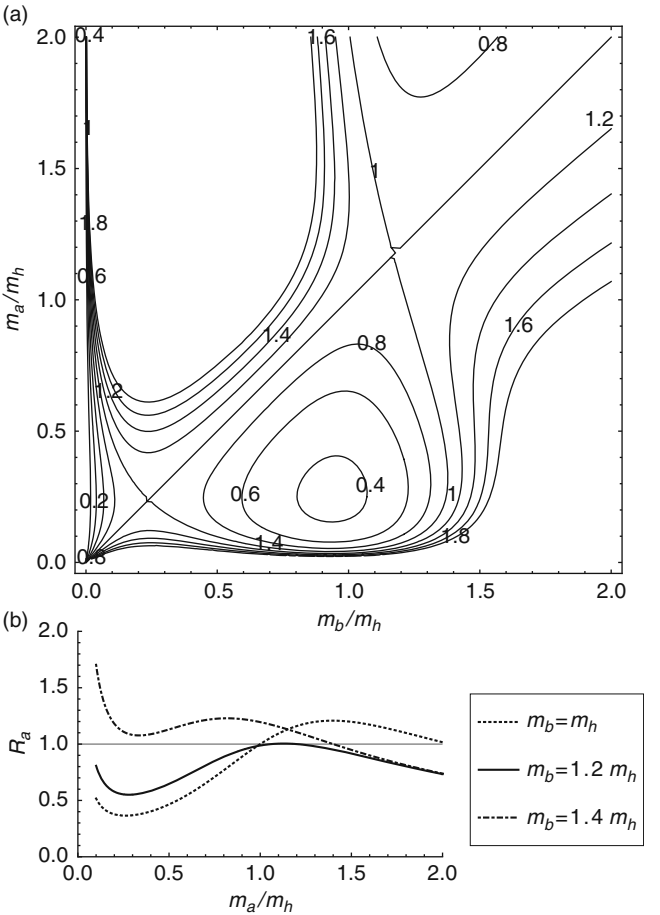

Figure 7.11 Invasion success ratio (R_a). A: contour plot of R_a as a function of m_a/m_h and m_b/m_h for $n = 0.001$ and $s = 4$; B: Cross-sections of a.

Bonsall, 2006). Cross-sections through the surface plot near the saddle point for $m_b/m_h = 1.0, 1.2, 1.4$ are shown in Figure 7.11b. Here we see that $m_b/m_h = 1.2$ is stable against invasion. If $m_b > 1.2 \cdot m_h$ then smaller "a" gametes will invade over time until $m_b/m_h = 1.2$. Likewise if $m_b < 1.2 \cdot m_h$, then larger "a" gametes invade. Thus, when the zygote survival function is sigmoidal, these cross-section figures suggest that if $m_b \neq 1.2 \cdot m_h$, then "b" is invaded by any "a" mutant with a single gamete volume (m_a) closer to the value ($1.2 \cdot m_h$) than a single gamete volume of "b" (m_b). Ultimately over an evolutionary series of mutations, the gametophytes should produce gametes whose sizes remain in the close vicinity of $1.2 \cdot m_h$ and then be stable against further invasion.

7.14 Fertilization kinetics and the evolution of anisogamy

Because isogamy is assumed to be ancestral, there has been a long history of interest in the emergence of anisogamy beginning with Darwin (Darwin, 1871). Some remarkable theoretical developments in the evolutionary mechanisms of anisogamy have occurred recently; nevertheless, it has been suggested that no model is capable of offering a universal explanation (Randerson and Hurst, 2001b). One important unsolved question involves the role of underwater fertilization kinetics in the evolution of anisogamy. Are the kinetics of zygote formation sufficient to explain the evolution of anisogamy with small sperm and large eggs as the sperm-limitation hypothesis suggests (Levitan, 1996)? Here we address this question by coupling observations on marine green algae and an analytical model based on kinetic collision theory and evolutionary game theory. They afford significant insights.

The sperm-limitation hypothesis suggests that anisogamy evolves because a larger egg has an increased chance of being fertilized since it offers a large target for sperm to escape from sperm limitation rather than sperm competition (Levitan, 1998). In habitats where turbulent water conditions may seriously dilute gametes of both sexes as in the intertidal zones, sea urchins seem to be often under sperm-limited conditions (Levitan, 1996). However, is it the rule for external fertilizers? Many marine green algae exhibit adaptations to avoid sperm limitation [e.g. synchronous spawning (Togashi and Cox, 2001) and aggregation using phototaxis (Togashi *et al.*, 1999) and sexual pheromones (Togashi *et al.*, 1998)]. Another question as to the sperm-limitation hypothesis is whether higher encounter rates between male and female gametes might alone provide an adequate fitness

advantage. Using a model of sea urchin fertilization kinetics (Vogel *et al.*, 1982), this point has been repeatedly addressed and it is commonly suggested that higher encounter rates of gametes are unlikely to compensate for egg numbers that would be disproportionately reduced when the size of eggs increases (Podolsky and Strathmann, 1996, see also Randerson and Hurst, 2001b). Large eggs would be fitter because of their higher fertilization rates, but not disproportionately so. Podolsky and Strathmann have shown that the number of zygotes formed per unit of egg material decreases monotonically with increasing egg size, in spite of the fertilization advantage of larger eggs (Podolsky and Strathmann, 1996). In contrast, as shown in the above section, the fertilization kinetics of marine green algae indicate that, as a function of the gametic investment for various anisogamy ratios, there is generally a cross-over region for each series where, for gametic investments larger than the cross-over region, isogamy prevails, with the highest number of zygotes formed, while for gametic investments smaller than the cross-over region, anisogamy dominates (Togashi *et al.*, 2007). This model has realistic advantages over the sea-urchin model. These conclusions are consistent with the predictions of the sperm-limitation hypothesis and well explain distributions of marine green algae in the fields. But both models of sea urchins and marine green algae are not evolutionary models. Therefore, our approach to address this long-pending question of the mechanisms of the evolution of anisogamy should be based on an evolutionary model coupled with the study of three-dimensional gamete swimming and fertilization processes.

For the evolution of anisogamy, the PBS model proposes two conflicting selection forces acting on gamete size: the number of gametes and the fitness of the zygotes formed. The number of small gametes could be increased, but they would have little contribution of resources to the zygotes. For disruptive selection on gamete size, the PBS model requires that zygote fitness must be positively related to its volume at least over part of its size range (see also Bulmer and Parker, 2002) because it assumes the advantage of large zygotes provisioned mainly by large eggs should compensate a loss of the number of zygotes formed. It appears that gamete number and size actually trade off against each other, as shown above. Thus, we are also interested in whether any disproportional relationship between zygote size and survival is really needed for the evolution of anisogamy.

Suppose we observe a seawater tank containing a number of gametophytes of three varieties, “a”, “b” and “c” that spawn mating-type

gametes, each with radius r_a, r_b, and r_c respectively, into a well-mixed volume without spatial variations. We will treat the mating-type case here. In our analysis we assume that variety "a" and "b" are both of one mating type, while "c" is of the opposite type. This means that only zygotes z_{ac} and z_{bc} are formed from the collisions of "a" and "b" with "c", respectively, and that no "a–b" collisions produce zygotes.

If we denote by $z_{xy}(t)$ the zygote density (number of zygotes per unit volume) at time t, and $g_a(t)$ and $g_b(t)$ are the densities of "a" and "b" gametes respectively, then we have

$$\begin{aligned} z_{ac}(t) &= g_a(0) - g_a(t) \\ z_{bc}(t) &= g_b(0) - g_b(t). \end{aligned} \tag{7.12}$$

We also know that

$$g_c(T) = g_c(0) - [g_a(0) - g_a(T)] - [g_b(0) - g_b(T)], \tag{7.13}$$

since one "c" gamete is removed each time an "a" gamete or "b" gamete is removed by collision with a "c" gamete and subsequent fusion to form a zygote.

We define a successful invasion by "a" to be when the proportion of "a" gametophytes between "a" and "b" in a succeeding generation is larger than that in the current generation. In the current generation this proportion is

$$\frac{N_a}{N_a + N_b} \tag{7.14}$$

where N_x is defined as the initial gametophyte density of type "x". It's also important to remember that $N_c = N_a + N_b$.

In the next generation we can estimate the proportion of "a" by

$$\frac{z'_{ac}(\hat{t})}{z'_{ac}(\hat{t}) + z'_{bc}(\hat{t})} \tag{7.15}$$

where $\hat{t}$ is considered the end of the current generation, either due to finite gamete lifetime or depletion, interrupting external events or passage of a sufficiently long time for all zygotes to form, and $z'_{xy}(\hat{t})$ is the number of "x–y" zygotes that survive into the succeeding generation. This estimate is appropriate because it has been experimentally determined that each diploid sporophyte with two mating-type genes produces haploid zoospores with a 1:1 ratio of one mating type to another (e.g. Togashi and Cox, 2008).

Now we can define the invasion ratio, $R_a(\hat{t})$, to measure the invasion success of "a" by

$$R_a(\hat{t}) = \left(\frac{N_a + N_b}{N_a}\right)\left(\frac{z'_{ac}(\hat{t})}{z'_{ac}(\hat{t}) + z'_{bc}(\hat{t})}\right) \tag{7.16}$$

Here, when $R_a \leq 1$ we can say that "b-c" is stable against invasion by "a". Conversely, when $R_a > 1$ we say that "a" invades successfully. From Equation (7.16) it is clear that $R_a > 1$ when

$$\frac{N_b \cdot z'_{ac}(\hat{t})}{N_a \cdot z'_{bc}(\hat{t})} > 1. \tag{7.17}$$

The remainder of our analysis deals primarily with the consequences of Equation (7.17), but further understanding requires knowledge of the time dependence of the zygote-formation process. As in the above sections, our approach is based on the kinetic theory of collisions.

We assume that gametes are uniformly distributed and moving under water in random, uncorrelated directions. The number of collisions depends on the product of the densities of the two gametes, their collision cross-sectional area, and their average relative velocity as the following equations:

$$\begin{aligned} \frac{dz_{ac}(t)}{dt} &= -\frac{dg_a(t)}{dt} = \sigma_{ac} \cdot \left\langle v_{ac}^{rel} \right\rangle \cdot g_a(t) \cdot g_c(t) \\ \frac{dz_{bc}(t)}{dt} &= -\frac{dg_b(t)}{dt} = \sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle \cdot g_b(t) \cdot g_c(t). \end{aligned} \tag{7.18}$$

Here σ_{xy} is the collision cross-section of "x" and "y", and $\left\langle v_{xy}^{rel} \right\rangle$ is their average relative velocity. Our model encompasses the entire motion description of the gametes as determined experimentally. As in the non-mating type case, to simplify our analysis, we will adjust the time scale with the substitution

$$T = g_c(0) \cdot \sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle \cdot t \tag{7.19}$$

and introduce the relative collision rate function

$$K = \frac{\sigma_{ac} \cdot \left\langle v_{ac}^{rel} \right\rangle}{\sigma_{bc} \cdot \left\langle v_{bc}^{rel} \right\rangle}. \tag{7.20}$$

The relationship between gamete size and swimming velocity is only used to determine the relative collision rate ratio K. Even if we used other relationships (if any) the rest of our analysis would remain unchanged.

Equations (7.18) become

$$\frac{\mathrm{d}}{\mathrm{d}T}\left(\frac{g_{\mathrm{a}}(T)}{g_{\mathrm{a}}(0)}\right) = -K \cdot \left(\frac{g_{\mathrm{a}}(T)}{g_{\mathrm{a}}(0)}\right) \cdot \left(\frac{g_{\mathrm{c}}(T)}{g_{\mathrm{c}}(0)}\right), \tag{7.21}$$

$$\frac{\mathrm{d}}{\mathrm{d}T}\left(\frac{g_{\mathrm{b}}(T)}{g_{\mathrm{b}}(0)}\right) = -\left(\frac{g_{\mathrm{b}}(T)}{g_{\mathrm{b}}(0)}\right) \cdot \left(\frac{g_{\mathrm{c}}(T)}{g_{\mathrm{c}}(0)}\right) \tag{7.22}$$

and we see that the entire time dynamics of zygote formation and gamete depletion are characterized by the single entity "K," and the initial densities of each gamete. By eliminating the common term $\left(\frac{g_{\mathrm{c}}(T)}{g_{\mathrm{c}}(0)}\right)$ between Equations (7.21) and (7.22), followed by integration, we find that

$$\left(\frac{g_{\mathrm{a}}(T)}{g_{\mathrm{a}}(0)}\right) = \left(\frac{g_{\mathrm{b}}(T)}{g_{\mathrm{b}}(0)}\right)^{K}. \tag{7.23}$$

Substitution of Equations (7.13) and (7.23) into Equation (7.22) yields

$$\frac{\mathrm{d}G(T)}{\mathrm{d}T} = -\frac{G(T)}{1+N} \cdot \left[E\left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}, \frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right) + N \cdot \left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}\right) \cdot G(T)^{K\left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}, \frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right)} + \left(\frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right) \cdot G(T)\right]. \tag{7.24}$$

Here we have made a number of substitutions of dimensionless parameters to aid understanding and analysis:

$$G(T) = \left(\frac{g_{\mathrm{b}}(T)}{g_{\mathrm{b}}(0)}\right), \tag{7.25}$$

$$N = \frac{N_{\mathrm{a}}}{N_{\mathrm{b}}}, \qquad n_x = \frac{g_x(0)}{N_x}, \tag{7.26}$$

$$E\left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}, \frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right) = 1 - \left(\frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right) + N \cdot \left[1 - \left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}\right)\right], \tag{7.27}$$

where n_x is the number of gametes produced by a single gametophyte of "x" in the current generation. With Equation (7.24), we now have a single equation which determines the complete time-dependent behavior of all zygote formation and gamete depletion, controlled by only three dimensionless quantities: N, $\left(\frac{n_{\mathrm{a}}}{n_{\mathrm{c}}}\right)$, and $\left(\frac{n_{\mathrm{b}}}{n_{\mathrm{c}}}\right)$.

Because each gametophyte invests the same gametic volume in producing gametes, the volume of each gamete, "x," is proportional to $\frac{1}{n_x}$. This means that

$$\left(r_x/r_y\right) = \left(n_y/n_x\right)^{\frac{1}{3}}.$$

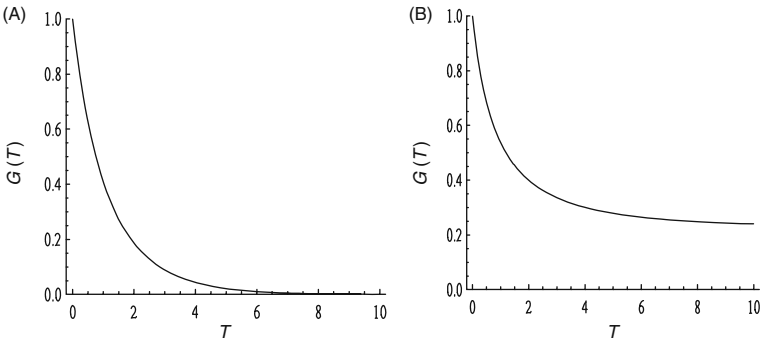

Figure 7.12 The results of numerical integration of Equation (7.24). A: N = 0.001, $\frac{n_a}{n_c} = 1.2, \frac{n_b}{n_c} = 0.3, E = 0.7(>0); C: N = 0.001, \frac{n_a}{n_c} = 1.2, \frac{n_b}{n_c} = 1.3, E = -0.3(<0)$.

We can now rewrite Equation (7.20) as

$$K = \frac{(n_b/n_c)^{\frac{2}{3}}}{(n_a/n_c)^{\frac{2}{3}}} \left(\frac{1 + (n_a/n_c)^{\frac{1}{3}}}{1 + (n_b/n_c)^{\frac{1}{3}}} \right)^2 \sqrt{\frac{1 + (n_a/n_c)^{\frac{2}{3}}}{1 + (n_b/n_c)^{\frac{2}{3}}}}$$

and we see that K is a function of only (n_a/n_c) and (n_b/n_c).

Figure 7.10 shows the results of numerical integration of Equation (7.24) for two typical scenarios: $E>0$ (Figure 7.12a) and $E<0$ (Figure 7.12b). When $E>0$, "c" gametes are in excess so that "a" and "b" gametes are depleted before "c." The opposite is true for $E<0$. It is worth noting that for shorter times, up to about the knee of the exponential decay, the two curves are similar, but we may expect to see different evolutionary behaviors when comparing a system whose generation time $\hat{t}$ is short with one having longer $\hat{t}$.

The form of the zygote survival function must have changed considerably during evolution (Bulmer and Parker, 2002). Dusenbery (2000), in his comparative analysis of a number of ecological models of anisogamy, determined the critical importance of the relationship of zygote size and fitness or survival rate into the next generation. Here, it might be inappropriate to apply the Vance inverse exponential function for zygote survival that is based on the experimental observations that the time for a planktonic larva to reach a size of which it can independently feed not depending on zygotic provisioning relates inversely to egg size, which can be approximated as zygote size (Vance, 1973; see also Levitan, 2000). Larvae that hatched from larger eggs might actually reduce predation risk, which might be much

higher at their planktonic stage, due to their reduced developmental time. However, marine green algae generally do not have such a planktonic stage due to negative phototaxis of planozygotes, and we choose a different approach.

We define the survival rate of a zygote as S_{xy}, so that $z'_{xy}(\ddot{t}) = S_{xy} \cdot z_{xy}(\ddot{t})$, and treat it's dependence on zygote volume as

$$S_{xy} = \left(\frac{1}{n_x} + \frac{1}{n_y}\right)^m, \tag{7.28}$$

since the gametic investment for each gametophyte is the same.

The PBS model requires that zygote survival proportional to zygote volume to the power m, with $1.8 < m < 2.5$, for disruptive selection on gamete size and the evolution of anisogamy (see also Bulmer and Parker, 2002) because it assumes the advantage of large zygotes provisioned mainly by large eggs should compensate a loss of the number of zygotes formed. We consider below whether such an assumption is really needed to explain the evolutionary mechanisms of anisogamy as well.

Returning now to the conditions for successful invasion, from the analysis above, Equation (7.17) can be written as

$$\left(\frac{n_a/n_c}{n_b/n_c}\right)\left[\frac{1 - G\left(\hat{t}\right)^{K\left(\frac{n_a}{n_c}, \frac{n_b}{n_c}\right)}}{1 - G\left(\hat{t}\right)}\right] \cdot \left(\frac{(n_b/n_c) \cdot (1 + n_a/n_c)}{(n_a/n_c) \cdot (1 + n_b/n_c)}\right)^m > 1. \tag{7.29}$$

Proceeding further analytically is difficult; we can, however, compute the values in Equation (7.29) numerically and test its behavior over a wide range of m, $\hat{t}$, N, $(n_a/n_c)$and(n_b/n_c). Also, note that Equation (7.29) suggests that the evolutionary pathways of gamete size have to do with the ratios of gametes of "a" and "b" types to that of "c" type which are produced by an individual gametophyte, but, don't depend on initial gamete densities (and the numbers of gametophytes) of each type.

We show in Figure 7.13 a table of invasion success diagrams for several values of m and $\hat{t}$ with $N = 0.001$. This is a reasonable choice for N, given that we are investigating an invasion scenario. Interpretation of these diagrams is aided by consulting Figure 7.14 where we indicate six zones, each of which shows the nature of the invading mutant where there is colored shading in the diagram in the respective zone. Figure 7.13 shows in the first row with $m = 0$ (survival is independent of zygote volume) that only invasions of smaller males or smaller females are successful. Although there is opportunity for anisogamy to evolve,

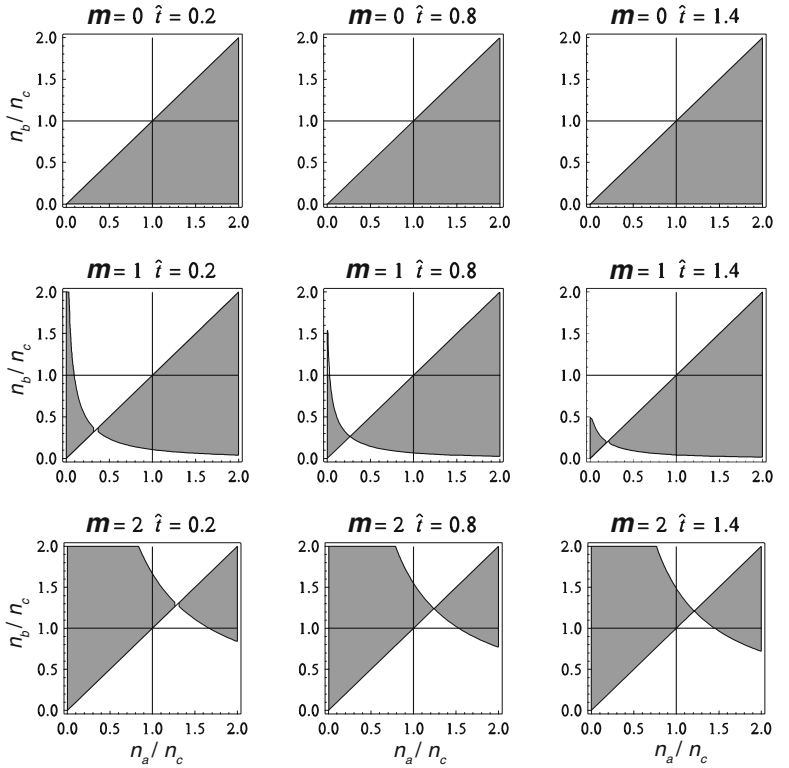

Figure 7.13 Successful invasion regions (see also Figure 7.12).

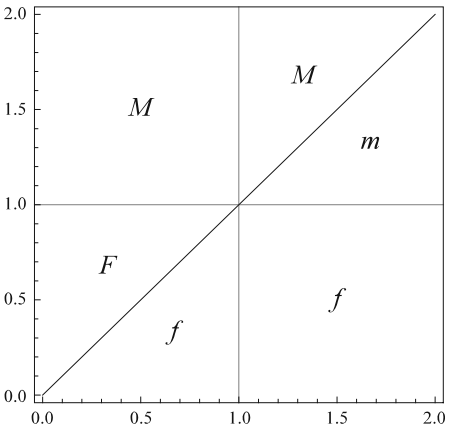

Figure 7.14 Invasion success diagram consulting various invading mutants: *m*: smaller male; *M*: lager male; *f*: smaller female; *F*: larger female.

there are no evolutionary pathways available for larger females to invade and with every successful invasion, the zygote size becomes smaller. In the third row of Figure 7.13 we have the case $m = 2$ representing the PBS theory. Here we see that not only are smaller males and females able to invade as with $m = 0$, but there are substantial regions that allow invasion by both larger males and larger females. Finally, the middle row of Figure 7.13 shows successful invasions when $m = 1$, i.e. there is linear proportionality between zygote survival and volume. Here we see again that smaller males and females can invade successfully, but there is a small region where larger females are successful. This region represents already largely anisogamous starting zygotes that can become even more anisogamous with the introduction of substantially larger females.

An interesting example when $m = 1$ is in the analysis of evolutionary pathways where size changes are due to mutations that result in changing of the number of cell divisions during gametogenesis, as actually observed in marine green algae (see the reproductive investment, gamete size and number section above). This means that (n_a/n_c) and (n_b/n_c) are integral positive or negative powers of 2. For example, Figure 7.15 shows the allowed invasionary pathways for

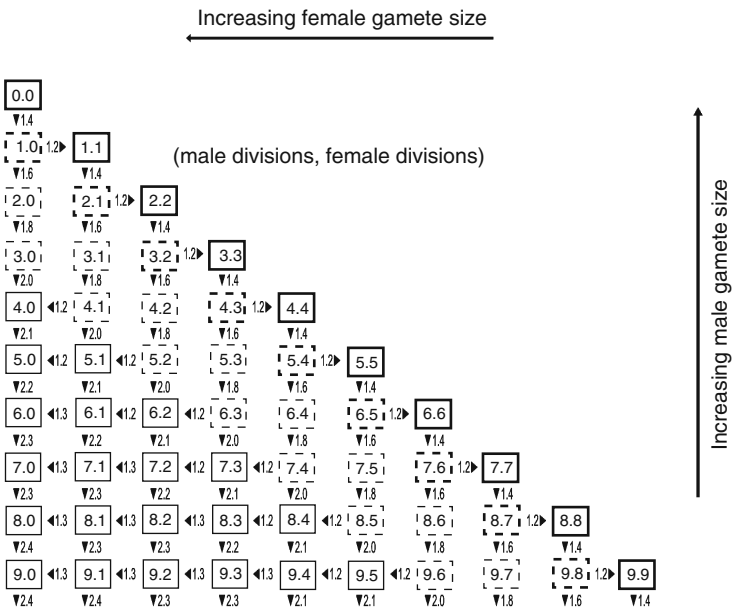

Figure 7.15 Allowed invasionary pathways for $\hat{t} = 0.5$.

$\hat{t} = 0.5$ with $(n_a/n_b) = 2^{\pm 1}$ (see also Figures 7.13 and 7.14). Note that the arrows show the possible directions for successful invasion. The isogamous and slightly anisogamous varieties are always invaded by smaller males and smaller females. The more anisogamous varieties are always invaded by larger females and smaller males. Finally, the bridge varieties are always invaded by smaller males, but females either larger or smaller can successfully invade. Thus, the pathways for blue are down and right unless they go down to a bridge variety. The pathways for green are down and left – they cannot enter the bridge region. Thus, we have a simple demonstration of evolutionary pathways that lead to highly anisogamous varieties without the need for assumptions of survival depending more than linearly on volume.

Our analysis here is for a well-mixed volume. As soon as we add any of the possible gradients, such as gravity, temperature, concentration, light or any boundaries or surfaces, the problem becomes much more complex and we will need more experimental data to guide the analysis. But field experiments using sea urchins suggest that the influence of gamete traits on fertilization is not overwhelmed by sea conditions (Levitan, 1993). Natural selection for enhanced fertilization may act on females as well as males. As shown above, isogamous or slightly anisogamous species of marine green algae generally produce gametes of both sexes with a phototactic device, which actually facilitates fertilization on the two-dimensional surface of seawater with a high efficiency and might be a constraint to reduce gamete size. But, zygote size of such species is generally smaller than that of strongly anisogamous species (Togashi *et al.*, 2006). Thus, we suggest that the assumption that the advantage of large zygotes should compensate a loss of the number of zygotes formed is not really needed for the disruptive selection of gamete size. Also, it is important to note that our results do not depend on initial gamete densities (and the numbers of gametophytes) of each type.

7.15 PARTHENOGENESIS

From laboratory and culture experiments, parthenogenesis (i.e. development without sexual fusion of gametes) has been known in algae. For example, gametes of both sexes of a slight anisogamous alga, *Monostroma angicava*, develop parthenogenetically into cysts that are morphologically indistinguishable from those developed from zygotes formed by sexually fused gametes (Tatewaki, 1969). In contrast, in *Bryopsis*, which are markedly anisogamous, only female gametes often develop parthenogenetically (Togashi, 1998). A large amount of

resources seems to be needed for parthenogenesis. In the field, however, such parthenogenetic development is suggested to be very improbable because gametes will not settle on the substratum to develop into cysts for more than 1 or 2 days unless they fuse to form zygotes: unsettled gametes are likely to drift out to photosynthetically disadvantageous deep waters as the yellow-green scums on the surface of seawater that are often observed after the spring tides (Togashi *et al.*, 1997). The frequency of parthenogenesis in natural populations also seems to be low in other marine algae (De Wreede and Klinger, 1988). Therefore, parthenogenesis does not play an important role in reproduction of marine green algae.

7.16 CURRENTS AND TURBULENCE

At macroscopic levels, under turbulent hydrodynamic conditions experienced by marine organisms living in intertidal and subtidal habitats, such as surf, orbital wave motion or heavy currents, concentration of gametes either spatially or temporally may be disturbed (Denny and Shibata, 1989; Denny *et al.*, 1992). However, gametes of both sexes are often released under calm conditions with the mechanisms shown in the synchronous gamete production and release section above. At microscopic levels, for gametes swimming in the water, Reynolds numbers are very low (1), which implies that the viscous forces of the surrounding water dominate the inertial forces of the swimming gametes (Purcell, 1977). Therefore, gamete swimming motion is scarcely affected by any turbulence in water. Thus, the effects of currents and turbulence in water on sexual reproduction of marine green algae are not usually serious.

7.17 TESTS OF THE PBS MODEL

The PBS model suggests that gamete size would be disruptively selected if zygote fitness is positively related to its volume at least over part of its size range. Although this assumption might be plausible, it has few empirical supports, except for some indirect ones. Multicellular organisms with higher complexity and large size would be more likely to require more provisioning for safety development because larger zygotes would require less time to reach a given adult size for reproduction, and would suffer less mortality at the juvenile stage. So, first, the PBS model predicts that, as the adult size increases, so does the selection for anisogamy (Parker *et al.*, 1972; Maynard Smith, 1978).

Further, as organisms increase their size, they should increase their zygote size. In such cases, a greater proportion of zygote volume should come from the egg. Thus, second, egg (female gamete) size is expected to increase with adult size. The first prediction about adult size and the degree of anisogamy has been suggested in the freshwater green algal order Volvocales (Knowlton, 1974; Bell, 1985). Actually, isogamy is commonly observed in simple unicellular species requiring little reserve material. On the other hand, complicated multicellular species, which may need a certain amount of reserve material in early stages of development, often have marked anisogamy. However, exceptions have been founded (Madsen and Waller, 1983). Such an assumption that the conditions generating anisogamy become more probable during the transition from uni- to multicellularity is questionable particularly in marine green algae because isogamous species are usually multicellular. It has been also reported that zygote size increases with adult size in the Volvocales (e.g. Bell, 1985). In these reports, however, the problem of phylogenetic non-independence is not considered. The mating systems will largely reflect phylogenetic specializations because closely related species tend to share many characters through common descent rather than through independent evolution (Harvey and Purvis, 1991). Thus, gamete size will be strongly constrained by phylogeney. Although their results are sensitive to the methods used to analyze the phylogeny, Randerson and Hurst's first phylogenetically controlled comparative tests of the PBS model (Randerson and Hurst, 2001a) supported these two predictions using the phylogeny of the Volvocales (Coleman, 1999). However, they have proposed a simple alternative explanation for the first prediction based on a constraint due to the mode of sexual reproduction as follows. As the size of female gametophytes increases, they would invest more materials for sexual reproduction (i.e. production of gametes). Sperm in a single packet can fertilize all of the eggs in a female spheroid. So males should increase the number of sperm to fertilize all of such eggs. The number of sperm in a single packet depends on the number of cell divisions during gametogenesis. But this might not the case for egg number (this is not true at least for some marine green algae, as shown in the reproductive investment, gamete size and number section above). Additionally, the size range of the sperm packet might be narrow. These factors would also lead to an increase in the degree of anisogamy. Actually, egg number is positively related to sperm number. Thus, they conclude that it is still unclear that the Volvocales satisfies these predictions (Randerson and Hurst, 2001a). However, we suppose that,

if their alternative explanation is true, it should be confirmed that the size of a sperm packet remains small as the degree of anisogamy increases [they assume the investment in a sperm packet and in an egg is approximately equal, in another paper (Randerson and Hurst, 2001b)]. Also, they have actually shown the degree of anisogamy increases as the size of female gametes increases. Therefore, some questions still remain for their alternative explanation.

With regard to zygote survival, it might be interesting to note that embryogenesis is very slow in *Caulerpa*, with little or no cellular differentiation observed in the laboratory up to 4 months after gamete fusion (Clifton and Clifton, 1999). Such lengthy terms of zygote development have also been reported in some other Bryopsidales marine green algae (e.g. Roth and Friedman, 1977; Meinestz, 1980). As shown above, these algae are strongly anisogamous with large zygotes. Thus, this delay might be actually useful for these algae to survive through unfavorable seasons. For marine organisms including algae, seasonality is known to be ecologically significant on Caribbean coral reefs (Clifton, 1997).

7.18 GAMETE SURVIVAL

Bulmer and Parker take the functional relationship between the fitness of a gamete and its size as well as the relationship between the fitness of a zygote and its size into account (Bulmer and Parker, 2002). They use the evolutionary game theory to determine the stability of isogamous and anisogamous strategies. Assuming these two fitness functions are similar at the most primitive stage where they assume organisms are unicellular, they show this leads to isogamy, irrespective of whether these functions are sigmoidal or concave in shape. (If the functions are concave, a minimal gamete size is needed for this result.) Further, they suppose, as multicellularity develops, the zygote fitness function favors larger zygotes, while the gamete fitness function remains unchanged. This leads to anisogamy. Bonsall also explores the role of mortality acting on gametes, as well as zygotes as a factor that influences the evolution of gamete size (Bonsall, 2006). He shows that variable mortality through differential survival or metabolic damage affects the evolution of anisogamy: variable gamete and zygote survival can induce disruptive selection on isogametes. However, in marine green algae, gametes are specialized solely for fusion, rather than for longer-term survival, as would be the adult. Thus, it might be unnecessary to take the functional relationship between the fitness of a gamete and its size into account.

7.19 ANISOGAMY AND HABITATS

As shown above, the study on the fertilization dynamics of marine green algae indicates that there is a cross-over region of mating success for each series with the same zygote size and various anisogamy ratios as a function of gamete density: at gamete densities below the cross-over region, zygote counts for highly anisogamous conditions are largest, while at gamete densities above the cross-over region, lower anisogamy prevails. This suggests that the mating system might be linked to environmental conditions that affect gamete density.

Comparative data on the field distribution of marine green algae clearly support this hypothesis (Togashi *et al.*, 2007). As the preferred zygote size gets larger, anisogamy tends to dominate. Also, as the depth of habitat increases, anisogamy increases. In deep waters, positive phototaxis would not be effective. In marine green algae, mating systems may be roughly classified into four types: (1) isogamy with phototactic gametes of both sexes, (2) slight anisogamy with phototactic gametes of both sexes, (3) marked anisogamy with non-phototactic male gametes and phototactic female gametes and (4) strong anisogamy with non-phototactic gametes of either sex. The anisogamy ratios, $\alpha = (r_-/r_+)^3$, separating them are approximately 0.88, 0.37 and 0.13, respectively. In green algae, although strongly anisogamy species are observed in marine environments, oogamous species are found only in freshwater environments (e.g. Volvocales, see Nozaki *et al.*, 2000). We suppose that oogamous species might have adapted to unstable, sometimes severe, environmental conditions where large amounts of resources for zygotes are required. This is likely to be related to the fact that, compared with marine environments, freshwater is relatively unstable; for instance, water levels often fluctuate seasonally.

7.20 MULTICELLULARITY

Organisms with small size and unicellularity might be actually ancestral in evolution and have advantages for effective mineral absorption in early planktonic forms by increasing the surface area to the whole volume (Bulmer and Parker, 2002). Such advantages might play an important role in generating selection against large gametes and zygotes. Bulmer and Parker suggest that this is a factor militating against the evolution of anisogamy and multicellularity in planktonic forms (Bulmer and Parker, 2002). This idea is plausible. Many unicellular organisms have, however, anisogamous mating systems in

nature. In marine green algae, isogamous species are generally multicellular. Further, anisogamous species often have unicellular multinucleate cell systems, particularly in the order Bryopsidales. These facts indicate that multicellularity is not likely to be a major driving force for the evolution of anisogamy.

7.21 SUMMARY

In marine green algae, fertilization kinetics has profound consequences for the evolution of anisogamy. Reproductive strategies, including gamete size and behavior, and mechanisms to synchronously release gametes are tightly linked each other and closely related to environmental conditions of their habitats, particularly the depth of water: the anisogamy ratio is positively related to water depth. This is explained well by the fertilization dynamics model. The field distribution of isogamy and anisogamy may have been influenced by competition between species that are similar except for mating systems. Recent studies seem to indicate that the evolution of anisogamy and the uniparental inheritance of cytoplasmic genes are likely to occur independently at least at an early stage of the evolution of anisogamy in this biological group. However, this does not mean that uniparental inheritance of cytoplasmic genes plays an unimportant role in later stages. The evolutionary model based on experimentally supported fertilization kinetics suggests that higher encounter rates between male and female gametes *alone* is unlikely to provide an adequate explanation of the evolution of anisogamy. An appropriate zygote survival function is also required. However, this relationship need not be superlinear for the evolution of anisogamy. Also, gamete behavior plays an important role in increasing search, encounter and fusion rates by concentrating gametes near a light source (phototaxis) or chemical attractant (chemotaxis). Although a larger zygote might actually bring about a disproportionate gain in fitness, the relationship between zygote size and survival is still equivocal due to the lack of experimental data. This is one of the more important unsolved questions in the study of anisogamy.

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