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Conflict and the Evolution of Genetic Systems

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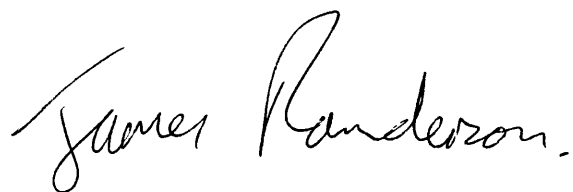
Conflict and the Evolution of Genetic Systems

submitted by James Peter Randerson
for the degree of PhD
of the University of Bath
2001

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"You do not really understand something unless you can explain it to your grandmother"

Albert Einstein

Summary

Within-group conflict (whether that group is an ant colony, an organism or a genome) has been identified as an important evolutionary force. Indeed, it is somewhat paradoxical that groups are able to operate in the face of such conflict. The problem is that selfishness is frequently the best option for the lower level unit. For example, genes that gain an 'unfair' transmission advantage, or worker bees that lay male eggs (so neglecting their colony duties), are expected to have the edge over their co-operative colleagues. The fact that actual conflict emerges far less frequently than theory predicts, suggests that there are mechanisms at the group level to keep lower level units in check. Such mechanisms, it is claimed, may have evolved in response to the appearance of 'selfish' variants. In this thesis, I examine some of these claims in more detail. For example, did meiosis and anisogamy evolve in response to the invasion of particular 'selfish' genetic elements? I show that in both cases this seems unlikely. In the case of anisogamy there are numerous other suggestions for the selective advantages of gamete dimorphism. I describe the first phylogenetically controlled, comparative test of the dominant explanation and critically review the others. One consequence of anisogamy is that it opens up a further arena for conflict between nuclear and cytoplasmic genes. Male-killing parasites are one manifestation of such conflict. I investigate some of the consequences of these parasites for host evolution. In particular I explore, theoretically, the evolution of host resistance and of host mating systems in response to male-killer invasion. While I find that the presence of a male-killer can, in theory, impose selection for male mate-choice, field data suggest that this explanation does not apply in the case of an East African butterfly.

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Chapter 1. The Tragedy of the Commons

Picture a pasture open to all. It is to be expected that each herdsman will try to keep as many cattle as possible on the commons.As a rational being, each herdsman seeks to maximise his gain. Explicitly or implicitly, more or less consciously, he asks, "What is the utility to me of adding one more animal to my herd?" This utility has one negative and one positive component.

- 1. The positive component is a function of the increment of one animal. Since the herdsman receives all the proceeds from the sale of the additional animal, the positive utility is nearly +1.*
- 2. The negative component is a function of the additional overgrazing created by one more animal. Since, however, the effects of overgrazing are shared by all the herdsmen, the negative utility for any particular decision-making herdsman is only a fraction of -1.*

Adding together the component partial utilities, the rational herdsman concludes that the only sensible course for him to pursue is to add another animal to his herd. And another; and another.... But this is the conclusion reached by each and every rational herdsman sharing a commons. Therein is the tragedy. Each man is locked into a system that compels him to increase his herd without limit - in a world that is limited. Ruin is the destination toward which all men rush, each pursuing his own best interests in a society that believes in the freedom of the commons. Freedom in the commons brings ruin to all.

(Hardin 1968)

Hardin's eloquent warning about the impending population crisis is, if anything, more relevant today than ever. We need only examine the near collapse of North Sea fish stocks, grid-lock on the roads and the impending greenhouse climate to see that in an overpopulated and wealthy world, complete personal freedom can only lead to disaster. When the gain to an individual of his actions exceeds his share of the costs then, in a *laissez faire* society, the tragedy of the commons cannot be avoided.

Yet despite the threat of impending doom, society survives (at least so far). Current crises notwithstanding, by and large we do manage to gain the benefits of group living without the whole system crumbling into self-interested calamity: how? Society survives because of laws and regulations governing the actions of individuals. In theory, rules drafted by society protect the common good, stopping individuals from playing the system to their own advantage. The bobby on the commons beat counts the cows every night to make sure no one has sneaked any extras into the herd. If they have, they are punished.

The 'tragedy of the commons' and its resolution have strong resonance in evolutionary biology. At many stages on the scale of evolutionary complexity, single competing units have come together to form a super-unit (single genes → hypercycles, hypercycles → prokaryotic cells, prokaryotes → eukaryotes, single cells → multicells, single organisms → groups (Maynard Smith & Szathmari 1995)). Like the nomadic herdsmen opting to settle and graze their cattle together, this offers numerous advantages, notably in terms of division of labour. But just like the herdsmen, there is the potential for disaster.

Much recent effort in evolutionary theory has been directed at understanding how these transitions from lower to higher levels of organisation can come about, without succumbing to Hardin's

calamity (Maynard Smith & Szathmary 1995; Szathmary & Maynard Smith 1995; Keller 1999). How are the interests of the individual tamed and channelled for the common good? A number of answers have emerged, for example relatedness (Hamilton 1964), mutual policing (Frank 1995) and reciprocation (Axelrod & Hamilton 1981), but it is becoming clear that conflict is a pervasive force. The potential for tragedy lurking beneath the surface of an apparently harmonious group (be it an ant colony or a genome) has had a profound influence on the way such groups operate (Hurst *et al.* 1996; Partridge & Hurst 1998).

Without contraries is no progression - William Blake

“Evolution is the control of development by ecology.” In the light of recent evolutionary advances it has become clear that Van Valen’s (1973) statement is overly simplistic. Numerous examples of evolution uncoupled from ecological perturbation have emerged, suggesting that there is an inherent force driving evolutionary change. This perpetual selective engine is conflict between individuals and between units within groups (Hurst *et al.* 1996; Partridge & Hurst 1998; Pomiankowski 1999).

Partridge and Hurst (p. 2003 Partridge & Hurst 1998) define conflict as occurring, “... when the spread of an allele at one locus in a population lowers the fitness of either the individuals in which it resides or of other members of the same population. The spread of this ‘harmful’ allele therefore results in natural selection for suppressers at other gene loci, which reduce the phenotypic effects of the original allele.” It has been proposed that numerous aspects of biology have been moulded by conflict: meiosis and recombination (Haig & Grafen 1991; Haig 1993), eusociality (Hurst 1997), plant embryology (Haig 1986), genomic imprinting (Haig & Graham 1991; Moore & Haig 1991), gametogenesis (Hurst & Pomiankowski 1991), mating systems (Jiggins *et al.* 2000), sexual selection (Lande & Wilkinson 2000), to name but a few (for more examples see (Hurst *et al.* 1996)).

Its leverage does not stop, however, even after the evolution of group level mechanisms that keep lower units in check. One example is the inheritance of cytoplasmic genomes. Organisms with biparental cytoplasmic inheritance are vulnerable to fast-replicating, ‘selfish’ variants of cytoplasmic genomes (be they organelles or symbionts). Such a fast replicator is capable of outcompeting the ‘unselfish’ type when the two are paired in a zygote. As a result of this within-cell advantage, the ‘selfish’ type can spread through a population even if it imposes a cost on host cells. An example of such a fast-replicator would be ‘petite’ mutants in yeast (reviewed by Jinks 1964). These have a deletion in the mitochondrial genome that impairs respiratory function, but allows faster replication.

Just like the herdsmen, the benefits of the selfish act (fast-replication and hence greater than 50% transmission) outweigh the cost to the group (the cell) so the strategy can be successful even if it lowers the cell’s fitness by up to 50%. Such ‘selfish’ variants are prevented from spreading if the nuclear genes enforce uniparental inheritance of cytoplasmic genomes. Under these circumstances, fast-replication is of no benefit, because transmission is either 100% or 0% (decided by the nuclear genes). Hence, with a level selective playing field, the wild-type outcompetes the ‘selfish’ variant due to its cost.

Uniparental inheritance simply sets up another arena for conflict: that over the sex ratio. Nuclear genes favour a 1:1 sex ratio because they achieve transmission equally through males and females. Assuming that males and females are equally costly to produce, if there is any deviation from 1:1 in the population, an individual producing more of the rarer sex will be at an advantage. Such an individual will

be over-represented in the following generation (*i.e.* its grandchildren) because of the reproductive advantage of those rare-sex offspring. Such selection for producing the rarer sex will always tend to bring the population back to a 1:1 sex ratio (Fisher 1930).

This argument does not apply to cytoplasmic genes. They only benefit by the production of the cytoplasm-transmitting sex (typically the female) and hence favour a female biased sex ratio. Hence, even though they may be locked into uniparental inheritance, they have other means at their disposal to 'selfishly' promote their own transmission. One example is to divert host resources from male function to female function. In plants this is manifested as Cytoplasmic Male Sterility (CMS) (Frank 1989). By sabotaging pollen development, the cytoplasmic genes skew the male-female investment ratio in favour of females. Thus increasing their own reproductive success at the expense of the nuclear genes. So, while the particular manifestation of conflict changes form, the relentless internal engine driving the evolution of genetic systems hardly skips a beat.

How best to model evolution?

For an age in which every base in the human genome has (more or less) been documented, it is perhaps paradoxical that, to all practical purposes, we are still ignorant as to the relationship between genotype and phenotype. Since natural selection is concerned with the relative fitness of alternative alleles in a population, one might imagine that this presents insurmountable problems for modelling evolutionary processes. Much progress has been made, however, using modelling techniques that ignore genetics; namely optimisation theory (Parker & Maynard Smith 1990) and game theory (Maynard Smith & Price 1973; Maynard Smith 1982). The former deals with situations in which an individual's fitness is frequency-independent (*i.e.* it does not depend on the phenotypes of other individuals in the population). The latter can be applied to cases in which an individual's fitness is frequency-dependent (*i.e.* it is determined by the collection of other phenotypes present in the population (Maynard Smith 1982)). Such approaches are particularly valuable when one is considering traits whose genetic basis is unknown or complex (*e.g.* sophisticated behavioural strategies). In such situations, a preoccupation with the underlying genetics would seem inappropriate.

However, a population geneticist might argue that, without considering a trait's genetic basis, one is ignoring a vital component of its evolution (Karlín 1975). A popular example is sickle cell anaemia (Maynard Smith 1982; Hammerstein 1996). Consider a naïve biologist encountering the problem for the first time. She would find individuals with three different blood types, each with radically different relative fitnesses. The biologist would conclude that this is a system in evolutionary flux, in which the inferior blood groups are on their way to extinction. With a knowledge of the traits' genetics however, we come to a very different conclusion. Despite the fitness variation between phenotypes, the system can be in equilibrium. This is possible, because, in each generation, matings between the most fit heterozygote genotypes produce less fit homozygous offspring.

A streetcar named selection

If the genetics of a system regularly affects the course of its evolution, where does this leave the phenotypic approach? A number of authors have suggested distinguishing long and short-term evolution

when modelling a system (Eshel 1996; Hammerstein 1996; Marrow *et al.* 1996; Weissing 1996). They propose that while genetic details will be important in the short term, the final destination of an evolutionary trajectory approximates well to the predictions of phenotypic models. A vivid formulation of this idea is Hammerstein's 'Streetcar theory' of evolution (Hammerstein 1996; Marrow *et al.* 1996). He views a population's evolution as the route taken by a streetcar (the tracks constraining its route are excluded for the purposes of the analogy). The streetcar moves from temporary stop to stop, representing equilibrium points on its evolutionary journey (the sickle cell anaemia case would be one such temporary stop). In each case it is prompted to move on by the embarkation of a new passenger (a mutation). When this novel mutation alights, it perturbs the population's equilibrium and takes it to the next stop. The particular route depends on the detailed genetics of mutations that arise at each stop, but the final destination bears a close resemblance to that predicted by phenotypic models. At the final destination there are no new mutations capable of perturbing the streetcar.

This view of evolution offers hope to both sides in the debate, depending on what stage of the streetcar's journey one is interested in. Phenotypic models are able to inform us about the population's final destination, but more complex models incorporating genetics are needed to describe the route to that end point. Importantly though, Hammerstein specifically excludes situations involving non-Mendelian genetics (Hammerstein 1996). By implication therefore, where 'selfish' genetic elements are involved, one should be wary of the predictions of phenotypic models.

I tackle this point in Part I, where I show that the evolution of meiosis as a defence mechanism against particular 'selfish' genetic elements (as suggested by 'end-point' models) is unlikely (See Part I introduction). Part II begins by exploring whether anisogamy could have evolved as a generalised defence against fast-replicating cytoplasmic parasites. My analysis suggests that the presence of such parasites is unlikely to account for the transition from isogamy to anisogamy.

This is by no means the only suggested mechanism for the evolution of anisogamy, however. In Chapters 4 and 5, I examine more broadly the alternatives to the conflict argument. Chapter 4 is the first phylogenetically-controlled comparative test of the dominant model for the evolution of gamete dimorphism (Parker *et al.* 1972). While the results broadly support the model (but are subject to the particular phylogeny used) I note that the test group (the green algal order Volvocales) are not the ideal testing ground because they violate some of the model's assumptions. Furthermore, there is a simple alternative explanation for the observed trends. In light of these results I review the numerous models that have been proposed to explain small sperm and large eggs (Chapter 5). While many have merits, I conclude that it is unlikely that any one model can provide a universal explanation applicable to all taxa.

Part III looks at some of the consequences of anisogamy. Specifically, under uniparental inheritance of cytoplasm, another arena for conflict is opened up; that between nuclear and cytoplasmic genes over the sex ratio. One manifestation of this conflict is male-killing bacteria: vertically-transmitted, cytoplasmic parasites that kill their host when they find themselves in a male (Hurst & Majerus 1993; Appendix I Randerson 2000). While this is an act of suicide for the particular bacteria, it can be selectively favoured if the bacteria's clonal relatives in the male host's sisters benefit (*e.g.* by sibling cannibalism amongst the hosts).

I show in Chapter 6, that the invasion of such parasites can select for host resistance to either the male-killing action or transmission of these parasites. Furthermore, the evolution of host resistance may account for the maintenance of more than one strain of male-killer in a host population. In the absence of resistance, the models predict that the 'best' male-killer will outcompete any others.

Male-killer infection at high frequency in the host population has also been correlated with changes in host mating system (Jiggins *et al.* 2000). In the East African butterfly *Acraea encedon*, populations with male-killing bacteria (*Wolbachia*) at high prevalence exhibit unusual mating behaviour. In such populations, one finds large congregations of females which gather, apparently, in search of mates. It has been argued that these congregations might represent role-reversed leks, at which females congregate and males choose amongst them. In Chapter 7, I model the proposal that the highly female-biased nature of such populations might select for male mate-choice in favour of uninfected mates. I find that such a scenario is theoretically plausible, but that the male-killing parasite will be selected out of the population unless male choice is error-prone. Chapter 8 is a test of this model in the field.

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Part I

“The Honesty of the Mendelian Shuffle” (p. 305 Crow 1991)

In chapter 2 (Hurst & Randerson 2000), I model the evolution of meiosis (Hamilton’s “gavotte of chromosomes” (p. 175 1975)) in response to particular varieties of selfish genetic element. As discussed in the introduction, the invulnerability of genetic systems to particular assailants has been proposed as an important factor influencing their evolution. However, I find disagreements between the conclusions of what I call defence/Evolutionary Stable Strategy (ESS) models and population genetic modelling by modifier analysis.

The former can be characterised as follows. Suppose that genetic system X is less vulnerable to selfish element S than genetic system Y. A defence argument might assume that X evolved from Y in response to the presence of S. I show that while it may well be true that X offers a defence against S, it does not necessarily follow that the Y→X transition occurred because of S. As an aside, I note that I am using the term ‘ESS’ somewhat loosely compared with its usual definition (Maynard Smith 1982). I am considering cases in which the success of S depends on the collection of genetic systems (strategies) that exist in the host population. Crucially, ‘defence’ arguments examine cases in which all members of the population adopt the same strategy, then ask whether this strategy is resistant to change (*i.e.* invasion of S). In this regard there are similarities with the ESS concept. However, unlike the traditional definition, I do not consider competing parasitic strategies whose success depends on the other parasitic strategies in the population.

My conclusions strongly support the argument of Szathmary and Maynard Smith (1995) that, “transitions must be explained in terms of immediate selective advantage to individual replicators.” They cite the example of multiple DNA replication origins in eukaryotes. These were necessary in order to allow the rapid replication of a large genome. However, the increase in genome size did not select for multiple replication origins. In Chapter 2 (Hurst & Randerson 2000), I demonstrate inconsistencies between ‘defence’ and population genetics arguments with respect to the evolution of meiosis.

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Chapter 2. Transitions in the evolution of meiosis

Laurence D. Hurst and James P. Randerson (2000)

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Transitions in the evolution of meiosis

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Keywords:

eukaryotes;
meiosis;
recombination.

Abstract

Meiosis may have evolved gradually within the eukaryotes with the earliest forms having a one-step meiosis. It has been speculated that the putative transition from a one-step meiosis without recombination to one with recombination may have been stimulated by the invasion of *Killer* alleles. These imaginary selfish elements are considered to act prior to recombination. They prime for destruction (which occurs after cell division) the half of the cell on the opposite side of the meiotic spindle. Likewise the transition from one-step to two-step meiosis might have been stimulated by a subtly different sort of imaginary distorter allele, a *SisterKiller*. These are proposed to act after recombination. It has yet to be established that the presence of such distorter alleles could induce the transitions in question. To investigate these issues we have analysed the dynamics of a modifier (1) of recombination and (2) of the number of steps of meiosis, as they enter a population with one-step meiosis. For the modifier of recombination, we find that invasion conditions are very broad and that persistence of *Killer* and modifier is likely through most parameter space, even when the recombination rate is low. However, if we allow a *Killer* element to mutate into one that is self-tolerant, the modifier and the nonself-tolerant alleles are typically both lost from the population. The modifier of the number of steps can invade if the *SisterKiller* acts at meiosis II. However, a *SisterKiller* acting at meiosis I, far from promoting the modifier's spread, actually impedes it. In the former case the invasion is easiest if there is no recombination. The *SisterKiller* hypothesis therefore fails to provide a reasonable account of the evolution of two-step meiosis with recombination. As before, the evolution of self-tolerance on the part of the selfish element destroys the process. We conclude that the conditions under which *SisterKillers* promote the evolution of two-step meiosis are very much more limited than originally considered. We also conclude that there is no universal agreement between ESS and modifier analyses of the same transitions.

Introduction

The early evolution of eukaryote meiosis is poorly understood both in terms of what happened and why. Following Cleveland (1947), several authors have proposed a gradual evolution of meiosis in which the earliest organisms had a one-step meiosis (reviewed in Maynard Smith & Szathmary, 1995; Kondrashov, 1997). In such a

meiosis a diploid cell would allow homologues to pair and then undergo one reduction division resulting in two haploid cells (i.e. $2N \rightarrow 2 \times N$). This might have evolved into one-step meiosis with recombination. This in turn could have evolved into the now almost universal two-step meiosis in which the reductional phase is preceded, perhaps paradoxically, by a doubling of the number of chromatids (i.e. $2N \rightarrow 4N \rightarrow 2 \times 2N \rightarrow 4 \times N$). The first reduction division ($4N \rightarrow 2 \times 2N$) is meiosis I and the second ($2 \times 2N \rightarrow 4 \times N$) is meiosis II. The point at which sex was invented is unclear (Kondrashov, 1994). Whether this was the history of the evolution of two-step

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meiosis is unclear and colleagues argue that four-strand (i.e. two-step) meiosis might be the ancestral condition, it being the most easily achieved modification of mitosis (Cavalier-Smith, 1995).

Direct evidence for any of the hypothetical more primitive forms of meiosis is sketchy, although Cleveland's extensive observations (see, e.g. Cleveland, 1947) suggest that one-step meioses might exist in some organisms (for a thorough review of such primitive meioses see Raikov, 1978, 1995). One of the more convincing instances is in *Urinympha*, a parabasalid (Hypermastigote), in which the two products are seen to fuse immediately after division (Cleveland, 1951). Even in this example the facts are ambiguous. There also exist reports in the dinoflagellate *Cryptocodinium* (Beam *et al.*, 1977) and the apicomplexan *Eimeria* (Canning & Morgan, 1975). Here the claims are supported by DNA measurements.

Why the putative transitions might occur has also attracted some speculation (see, e.g. Kondrashov, 1994). Haig and colleagues (Haig & Grafen, 1991; Haig, 1993) have suggested that transitions in the forms of meiosis might have been the result of selection to counter certain forms of selfish elements. Although these hypotheses have received some advocacy (Hurst, 1993a; Maynard Smith & Szathmary, 1995), they have neither been tested nor subjected to theoretical scrutiny. Here we provide theoretical analysis of (1) the transition from one-step meiosis to one-step with recombination and (2) the evolution of two-step meiosis with recombination.

A theoretical issue

Many hypotheses for the evolution of genetic systems suggest one system to be superior to another because it is less vulnerable to a certain type of selfish element (for review see Hurst *et al.*, 1996). Grun (1976), for example, hypothesizes that uniparental inheritance of cytoplasmic genes evolved from biparental inheritance, as a population with uniparental inheritance is less likely to be invaded by cytoplasmic selfish genes. More generally, these hypotheses consider that system X evolved from system Y as a 'defence' against selfish gene S. More precisely, if we suppose that all individuals have system X, we can show that the population is less likely to be invaded by selfish gene S than if all the population had system Y. Models of this variety are comparable to ESS forms of analysis as they suppose populations to be monomorphic either for condition X or for condition Y and analyse the stability of each population rather than explicitly modelling the transition. We shall refer to such arguments/models therefore as defence/ESS analyses.

How should we evaluate these ideas? At the very least such differences can be seen as group selective effects of the possession of one genetic system rather than another. To explain the transition ($Y \rightarrow X$), however, is it theo-

retically adequate to hypothesize about a class of selfish genes that is less likely to invade a population with system X than one with system Y? The alternative is to examine the dynamics of the transition by asking about a population starting with system Y being affected by selfish gene S and ask about a modifier allowing some individuals to adopt system X.

Modifier models of this variety have, for example, been analysed in the case of the evolution of uniparental inheritance (Hoekstra, 1990; Hastings, 1992; Randerson & Hurst, 1999) and have, for the most part, confirmed Grun's defence argument. Is it generally true that defence/ESS and modifier models will agree? The modifier analysis is the more complex of the two modes of analysis. Therefore, if the defence/ESS analysis is a mathematical shortcut to the same solutions, then there is no reason not to adopt this method, if only long-term outcomes are of interest (for a discussion of the same problem under Mendelian inheritance see Marrow *et al.* (1996) and references therein). This issue is, by example, in part the concern of the present paper. We use the transitions in the evolution of meiosis as a case study and perform both types of analysis so as to compare their results.

One-step meiosis and the evolution of recombination

A population with recombination is resistant to some *Killer* alleles

Haig and Grafen postulate the existence of a sort of selfish element that acts against a 'nonheritable' target in one-step meiosis. This we understand to be an allele which, when heterozygous, acts early in meiosis as if to label something in the half of the cell that the wild-type allele will segregate into (in the absence of recombination). After the two products of meiosis divide, the labelled half dies. If this provides some net benefit for the surviving cell, then this allele will spread. Such distorter alleles are not the same as, but are similar to, classical meiotic drive genes, such as *t-complex* of mice and *Segregation Distorter* of *Drosophila* (Lyttle, 1991). Recombination in this system protects from such a '*Killer*' allele. This is because recombination between the *Killer* locus and the centromere forces the *Killer* allele to go to the half of the cell that the allele has primed to die after cell separation, thereby preventing its spread.

This can be demonstrated more precisely. Consider a one-step meiosis in which the probability of recombination between the *Killer* locus and the centromere is r (note that recombination between *Killer* and centromere ensures that the *Killer* ends up in the cell that the *Killer* has targeted). Consider also that the cells that survive following the death of their meiotic sister have a fitness $(1 + g)$, where g is some gain to fitness. If p is

the frequency of the *Killer* allele (K) and q the frequency of the wild-type (k), where $p + q = 1$, then in the next generation (p' , q') the alleles frequencies will be

$$\begin{aligned}\bar{W}p' &= (1-r)pq(1+g) \\ \bar{W}q' &= q^2 + rpq(1+g)\end{aligned}$$

where \bar{W} is the sum of the right-hand sides. The selfish allele can invade if $dp'/dp > 1$, when the allele is rare ($p \approx 0$). Solving for this condition reveals that the *Killer* allele can invade if

$$g > r/(1-r).$$

If there is no recombination in the system or if the *Killer* locus is near the centromere, then $r = 0$ and the condition for invasion is simply that there must be some benefit to the surviving cell in having its sister die (reduced competition is likely, cannibalism is another possibility). Fixation is then the only equilibrium. However, if recombination between centromere and the *Killer* locus is common ($r = 0.5$), then invasion of the *Killer* allele is most unlikely ($g > 1$), i.e. the death of one cell must allow a doubling of fitness of the survivor. Were this found, death of one of the two cells by any means would be globally advantageous. Recombination is thus a good defence against *Killer* alleles.

In the above recursions we have assumed that when the *Killer* allele is homozygous the two products of meiosis are mutually destroyed (hence there is no term in p^2). Furthermore, when a *Killer* allele ends up in the cell it has primed for destruction, we have supposed that the cell dies as the *Killer* allele is not tolerant to its own activity. If we consider an allele that is self-tolerant, then recombination is not a block to invasion. Assuming self-tolerance to be cost free, the recursions for such an allele (again p) will be

$$\begin{aligned}\bar{W}p' &= \beta p^2 + pq[(1-r)(1+g) + r] \\ \bar{W}q' &= q^2 + prq\end{aligned}$$

where \bar{W} is the sum of the right-hand sides and β an arbitrary fitness parameter. The self tolerant *Killer* can then invade if

$$g(1-r) > 0.$$

If $0 \leq r < 1$, then invasion is guaranteed if $g > 0$, i.e. as before some benefit to death is received by the surviving cell. Recombination therefore is no preventative measure against self-tolerant *Killers*. Similarly, a *Killer* that labels after recombination occurs is not prevented from spreading (Haig & Grafen, 1991).

Could recombination evolve in response to *Killer* alleles?

The above model demonstrates that *Killer* alleles that are not self-tolerant will be prevented from invading if

recombination between the *Killer* locus and the centromere is adequately common. That such a population cannot be invaded by a particular class of selfish element is not the same as a demonstration that the transition could have occurred because of such elements. Indeed, at first sight it is far from clear that the presence of a *Killer* allele would create the conditions favouring the spread of a modifier of recombination. The only direct effect of the modifier must occur in *Kk* heterozygotes. Recombination would act to force the *Killer* allele into the cell due for death, but would not prevent the death of this cell. Recombination therefore has no direct effects on the net amount of cell death, at least within the first generation (this can be contrasted with a suppressor that simply prevents *Killer* from acting). The issue can, however, only be resolved through analysis of a modifier of recombination entering a population with one-step meiosis and the *Killer* alleles.

Consider then a new polymorphic locus with two alleles: M is the active and dominant modifier locus that allows recombination between *Killer* and centromere at a rate r . Individuals homozygous for m have no recombination, just the one-step meiosis. There is, we shall assume, a cost (s) to the action of this modifier that is the same for all cells undergoing recombination (i.e. Mm and MM cells both have fitness $1-s$). The modifier we assume to be unlinked to the *Killer* locus.

To make the system a little more realistic, and to be consistent with our next model, we also consider that the attempted action of the *Killer* locus has some cost associated with it, t , that also afflicts all gametic products that survive from *Kk* matings. There is, as before, some benefit to the survivors, g . When death of one of the two meiotic products occurs, fitness of the single cell that survives in these recursions happens always to be the same, i.e. $(1-t)(1+g)$. We shall therefore consider that they have net fitness $(1+\gamma) = (1-t)(1+g)$. There are four haploid genotypes, MK , Mk , mK and mk . These exist at frequencies, x_1 , x_2 , x_3 and x_4 , respectively. From the above assumptions the following recursions may be derived:

$$\begin{aligned}\bar{W}x'_1 &= (1-s)(1+\gamma)(1-r)(x_1x_4 + x_1x_4/2 + x_2x_3/2) \\ \bar{W}x'_2 &= (1-s)r(1+\gamma)(x_1x_4/2 + x_2x_3/2 + x_1x_2 + x_2^2 + x_2x_4) \\ \bar{W}x'_3 &= (1-s)(1+\gamma)(1-r)(x_1x_4/2 + x_2x_3/2) + x_3x_4(1+\gamma) \\ \bar{W}x'_4 &= (1-s)(1+g)r(x_1x_4/2 + x_2x_3/2) + x_2x_4(1-s) + x_4^2\end{aligned}$$

where \bar{W} is the sum of the right-hand sides. After linearization, we can calculate the leading Eigenvalue (λ) for the matrix describing the transformation of x_1 and x_2 at given values of x_3 and x_4 . The invasion of the modifier is possible if $\lambda > 1$. Solving for this reveals a condition on the maximum cost of the modifier (s_{max}) consistent with its invasion. The precise solution is lengthy, but for realistic values of γ the condition can be well approximated to

$$s_{\max} \approx \frac{x_3 - 1 - r + \sqrt{r^2(1 - 2x_3)^2 + (1 - x_3)(2r(1 + 2x_3) + 1 - x_3)}}{2(1 - r)(1 - x_3)}$$

If $r = 0.5$ then this resolves to

$$s_{\max} \approx \frac{2x_3 - 3 + \sqrt{9 - 8x_3}}{2(1 - x_3)}$$

Under this circumstance, if the modifier is cost free, it can then invade so long as the selfish allele exists. For a modifier of arbitrary cost, a certain critical frequency of the selfish element must be reached before invasion is possible: the higher the cost, the higher the minimum frequency the *Killer* must achieve. The above approximation and the precise solutions for varying γ are shown in Fig. 1.

Note that in the absence of the modifier the *Killer* allele will spread to fixation if it can invade, so it is safe to assume that all frequencies are attainable (this finding is robust to allowance for incomplete killing action). When the *Killer* allele is extremely common almost any cost can be tolerated. The reason for this is, at least in part, that the modifier forces linkage disequilibrium between itself and the wild-type allele. As the *Killer* spreads, the wild-type allele has the advantage of being in disproportionately many of the crosses that result in viable progeny (*KK* homozygotes are inviable, whereas *Kk* and *kk* diploids produce viable progeny). This effect of linkage disequilibrium was confirmed through simulation.

At the other extreme, when the modifier has no effect ($r = 0$), then, not unexpectedly, the condition resolves to $s < 0$, i.e. the modifier must be directly advantageous. More generally, for a given frequency of the selfish element, the lower the value of r , the lower the maximum cost of the modifier consistent with invasion.

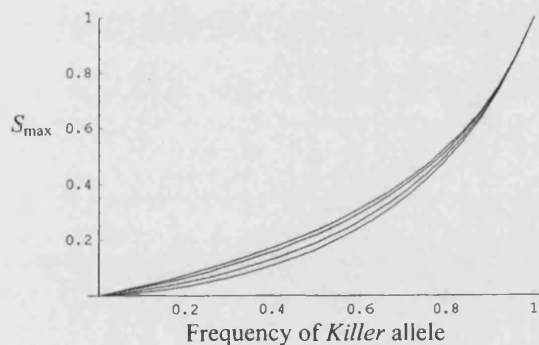


Fig. 1 Maximum value of the cost of the modifier of recombination (with $r = 0.5$) consistent with its invasion, as a function of the frequency of the *Killer* allele in the population. The uppermost line is the approximation provided in the text, the following lines are (in descending order) for $\gamma = 0.1$, $\gamma = 0.3$ and $\gamma = 0.5$. For invasion the cost of the modifier must sit below the curve. For invasion of *Killer*, $\gamma > 0$ must hold.

However, even at very low r , as the distorter allele tends to fixation, so the maximum cost tends to unity. Selection on a population with such a nonself-tolerant *Killer* allele must be extreme as at fixation the population is also extinct! The load imposed by the *Killer* is hence extraordinarily large and a little recombination allows the rare production of *k* haploid cells from *Kk* diploids. The precise analytical solution, at an arbitrary value of γ , is plotted in Fig. 2.

To investigate the fate of the modifier it is necessary to simulate the system. This reveals that persistence of both the modifier and the *Killer* allele is common, be it either as a stable equilibrium or as a limit cycle. Indeed, as suggested by the analytical results, even if r is very low (e.g. $r = 0.001$), so long as the cost of the modifier is also low, persistence is possible. Plots of the parameter space consistent with the persistence of the modifier allele are given in Fig. 3. Note that with a costly modifier, persistence of the modifier is only possible if the *Killer* allele also persists.

There exists another *Killer* against which recombination might act as a protective agent, i.e. that results in the death of one of the two cells in *Kk* diploids, regardless of the rate of recombination. This type of *Killer* is viable when homozygous but cannot rescue its own killing effect when heterozygous. We can, however, see no especially good reason to expect such a pattern of mortality. After extensive simulation, we find that the spread of such an allele does not create the conditions for the persistence of the modifier of recombination.

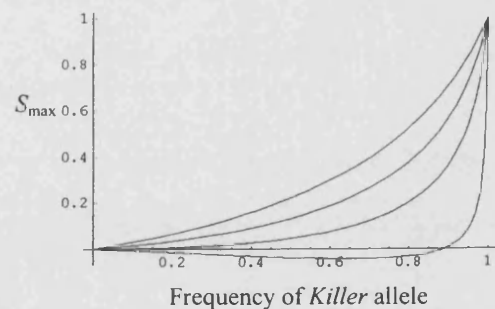


Fig. 2 The maximum cost of the modifier, consistent with invasion as a function of the frequency of the selfish element (x_3) at four different values of the recombination rate. Here γ , the net gain to surviving cells, is arbitrarily set to 0.1. Four lines are shown. The uppermost line is for $r = 0.5$, this is followed by $r = 0.25$, $r = 0.1$ and $r = 0.01$. The form of the lines is not greatly affected by the value of γ within realistic limits ($0 < \gamma < 1$).

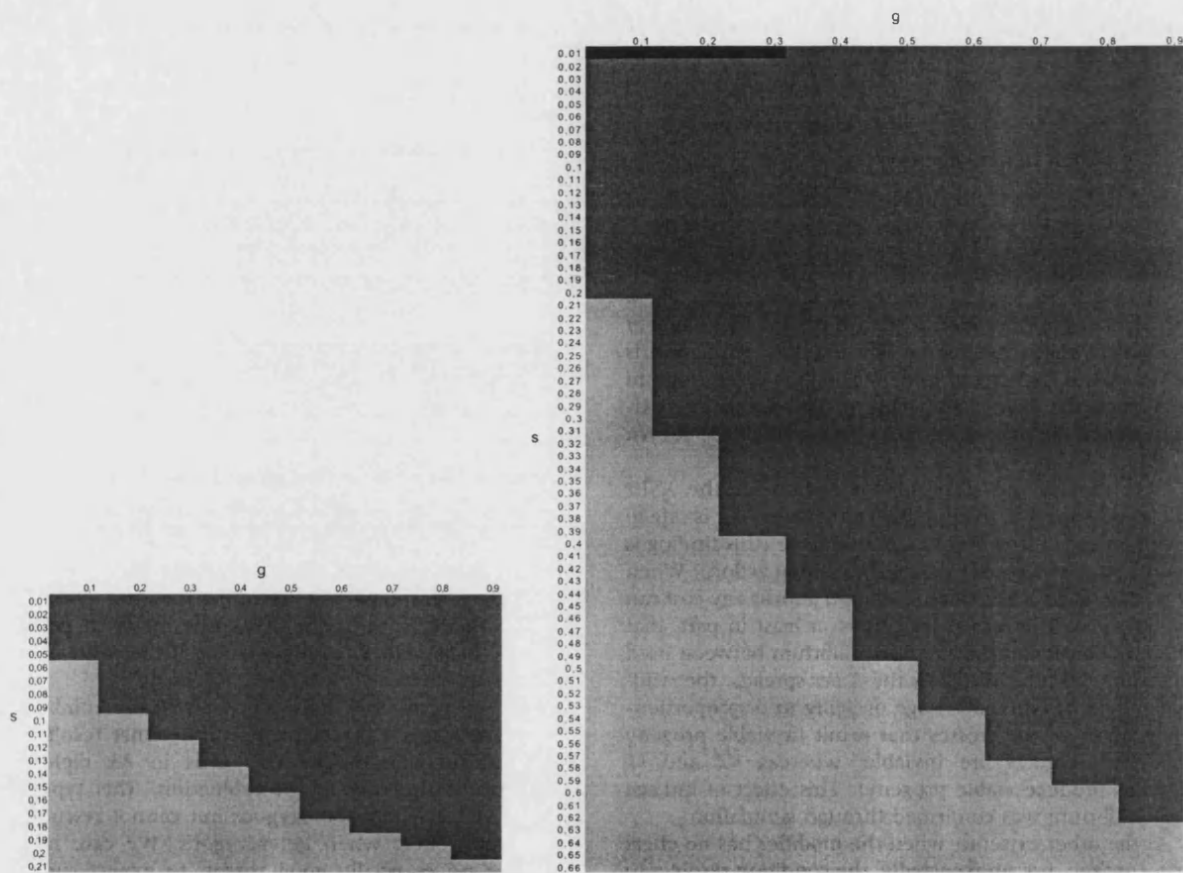


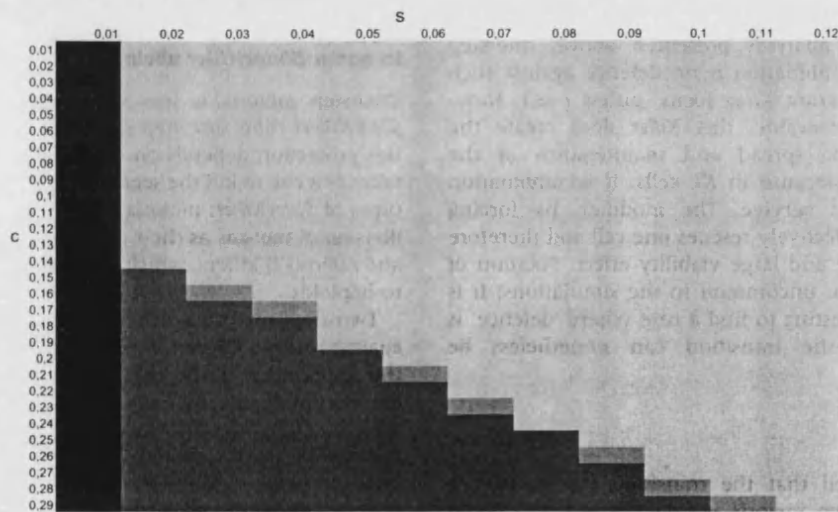
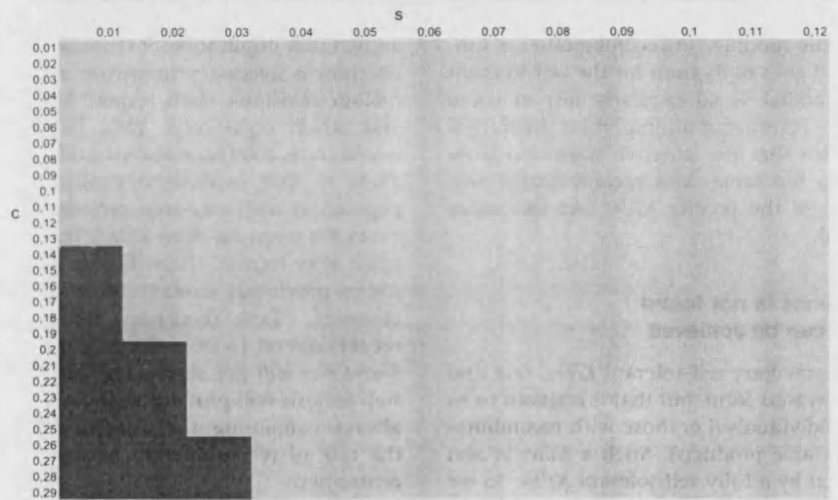
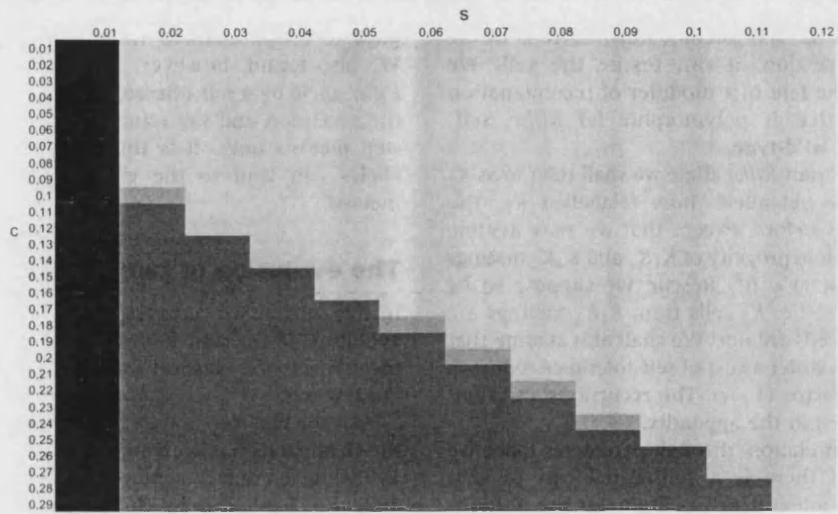
Fig. 3 Map of the parameter space in which the modifier of recombination, in a population with one-step meiosis, can persist alongside the *Killer* allele. Two plots are provided: in (a) $r = 0.001$ and in (b) $r = 0.5$. The black boxes represent space in which the modifier invades, removes the *Killer* and is then lost due to its own cost, s . In dark grey space the modifier invades and remains in equilibrium with the *Killer* allele. In light grey space the modifier is unable to invade.

Self-tolerant *Killers* and the subsequent evolution of the system

The sort of nonself-tolerant selfish elements that are envisaged have never been observed. That nonself-tolerance might not have been observed is to some

extent expected (Hurst, 1993a). As a *Killer* allele spreads the production of homozygotes becomes commonplace. If the allele is not self-tolerant the homozygotes must produce no viable progeny. We might then expect very strong selection favouring any mutant *Killer* allele that is self-tolerant, i.e. capable of neutralizing its own action.

Fig. 4 Map of the parameter space in which the self-tolerant *Killer* (K_2) allele invades a population with the modifier of recombination and the nonself-tolerant *Killer* (K_1). Four plots are provided to illustrate the effect of a range of parameters on the fate of the second *Killer* and the modifier. Note that the self-tolerant *Killer*, even at quite high costs (c), eliminates both the modifier and the nonself-tolerant *Killer*. The parameter values for each of the three plots are as follows: (a) $r = 0.5$, $g = 0.3$, $t = 0.1$; (b) $r = 0.1$, $g = 0.2$, $t = 0.1$; (c) $r = 0.5$, $g = 0.2$, $t = 0.1$. We have also investigated $r = 0.1$, $g = 0.3$, $t = 0.1$. This is not shown as the space is uniformly light grey for costs of tolerance up to the limit that we considered. Black space is that in which the modifier invades and removes K_1 . The costly modifier is then, in turn, lost. Light grey space is that in which K_2 invades and removes both K_1 and the modifier from the population or that in which K_2 invades and ousts K_1 but is then itself removed by the modifier. The end result in both of these scenarios is nonpersistence of the modifier. Mid-grey space (a very rare feature) is that in which all three alleles are maintained in the population. Dark grey space is that in which K_2 could not invade owing to its high cost. It is evident that the frequency of recombination brought about by the modifier has a significant bearing on whether K_2 can invade. If r is high then the modifier of recombination has a strong effect of K_1 and hence at equilibrium, both the *Killer* and the modifier are at low frequency. K_2 's advantage when it is itself rare stems from the fact that it will not end up killing itself if recombination occurs. Hence, in the case of high r (and therefore low frequency of the modifier), K_2 's advantage is relatively slight and must be weighed against the cost (c) of resistance.



Were this so, however, recombination is likely to be less effective as, when the self-tolerant *Killer* allele is in the cell due for destruction, it can rescue the cell. We therefore model the fate of a modifier of recombination in a population that is polymorphic for *Killer*, self-tolerant *Killer* and wild-type.

The new self-tolerant *Killer* allele we shall refer to as K_2 as opposed to the old allele, now relabelled K_1 . The assumptions are as before, except that we now assume that the viable haploid progeny of K_1K_2 and K_2K_2 matings have a fitness cost $(1 - t)^2$. Rescue we suppose to be haploid cell specific, i.e. K_2 cells from K_1K_2 matings are viable, but the K_1 cells are not. We shall also assume that viable K_2 type cells suffer a cost of self-tolerance reducing their fitness by a factor $(1 - c)$. The recursions and their derivation are given in the appendix.

Searching, by simulation, through parameter space we find that, even if there is a substantial cost to self-tolerance, the self-tolerant *Killer* eliminates the nonself-tolerant version (Fig. 4). Once this has occurred, if the modifier is costly, the modifier of recombination is lost. The population then goes to fixation for the self-tolerant *Killer*. That the modifier is so regularly lost in these simulations must be considered an important theoretical objection to the idea that the putative transition from one-step meiosis to the same with recombination was due to the activity of the precise *Killer* loci that were originally envisaged.

A case where defence is not found but the transition can be achieved

There exists an intermediary self-tolerant *Killer*, one that is inviable in homozygous form, but that is resistant to its own action in Kk individuals (i.e. those with recombination produce two viable products). Such a *Killer* is also likely to be displaced by a fully self-tolerant *Killer*, so we have not analysed its population genetics in detail. However, we do wish to note one finding. According to the defence/ESS analyses presented above, one-step meiosis with recombination is no defence against such a partially self-tolerant *Killer* locus, unless $r = 1$. However, when polymorphic, this *Killer* does create the conditions for the spread and maintenance of the modifier. This is because in Kk cells, if recombination occurs, both cells survive. The modifier, by forcing recombination, effectively rescues one cell and therefore has an immediate and large viability effect. Fixation of the modifier is not uncommon in the simulations. It is heuristically interesting to find a case where 'defence' is not found but the transition can nonetheless be achieved.

Summary

Above we showed that the transition from one-step meiosis to one-step meiosis with recombination could

have come about if a nonself-tolerant *Killer* allele, acting prior to recombination, were afflicting the population. We also found, however, that the replacement of the *Killer* allele by a self-tolerant *Killer* caused the reversal of the evolution and the return to a population with one-step meiosis only. It is thus far from clear that *Killer* alleles can lead to the evolution of novel forms of meiosis.

The evolution of two-step meiosis

In this section we consider the next step in the gradual evolution of meiosis, namely the evolution of two-step meiosis (i.e. the classical form of meiosis found in most eukaryotes).

That the first step in two-step meiosis is a duplication of the chromatids has been argued to be paradoxical (Hurst, 1993a), given that the 'intention' of meiosis is to reduce a diploid to haploid cells. This has, however, been argued against (Cavalier-Smith, 1995). Haig and colleagues have argued that duplication of chromatids allied with recombination is necessary to ensure a randomization of the meiotic divisions, with respect to which is reductional and which equational. This, in turn, it is argued is necessary as a defence against a different form of distorter allele to that evoked to explain recombination in a population with one-step meiosis. The sort of distorter evoked is termed a '*SisterKiller*'. The killing allele we shall again refer to as K , the wild-type being k . Like the *Killer* alleles previously evoked, *SisterKillers* have never been observed. These imaginary selfish elements act after recombination to label the pole of the cell to which the *SisterKiller* will not segregate. Thus, a Kk cell doing one-step meiosis will produce a single surviving product, this always containing the *SisterKiller* allele, regardless of the rate of recombination between the *SisterKiller* and centromere.

A population with two-step meiosis is resistant to some *SisterKiller* alleles

Two-step meiosis is less vulnerable to the action of *SisterKillers* than one-step meiosis. The precise logic for this protection depends on which division the *SisterKiller* takes as a cue to kill the segregating cell. We consider two types of *SisterKiller*: meiosis I killers, which take the first division of meiosis as their cue to act (i.e. $4n \rightarrow 2 \times 2N$), and meiosis II killers, which act when the diploids reduce to haploids.

Two-step meiosis with recombination is a defence against meiosis I *SisterKillers*. Imagine a Kk diploid. After the duplication of the chromosomes there will be four chromatids. If recombination occurs between the centromere and the *SisterKiller* locus then after the first division the two progeny will both be Kk . If not, the product of meiosis I will be KK and kk . Thus, if the *SisterKiller* acts at meiosis I and if there is recombination, the *SisterKiller* acts

to destroy itself, i.e. both *Kk* cells die. Two-step meiosis can also protect against a *SisterKiller* that acts after the second division. If there is no recombination, half the cells will die, these half being the two containing *K*.

To see this more precisely consider a sexual species with two-step meiosis and recombination. Let p be the frequency of the *SisterKiller* and q the frequency of the wild-type (null) allele ($p + q = 1$). Let us suppose that the death of two of the products of a two-step meiosis increases the fitness of the two survivors by g . Recombination between the centromere and the *SisterKiller* goes on at rate r , prior to the first division. For a *SisterKiller* that acts during meiosis I, the recursions will be

$$\begin{aligned}\bar{W}p' &= (1-r)pq(1+g) \\ \bar{W}q' &= q^2\end{aligned}$$

where \bar{W} is the sum of the right-hand sides. Invasion of the *SisterKiller* is possible if $dp'/dp > 1$ when $p \approx 0$. For invasion, then $g > r/(1-r)$ must hold. If there is free recombination between *SisterKiller* and centromere ($r = 0.5$), then, as for the *Killer* allele in one-step meiosis with recombination, the surviving cells must have a doubling of fitness. If, however, the allele is centromeric ($r = 0$), then some gain is necessary for the distorter allele to spread. Recombination with two-step meiosis thus protects against noncentromeric meiosis I *SisterKillers*.

If, by contrast, we consider a *SisterKiller* that acts in meiosis II then the recursions become

$$\begin{aligned}\bar{W}p' &= rpq(1+g) \\ \bar{W}q' &= q^2 + (1-r)pq(1+g).\end{aligned}$$

For invasion of such a *SisterKiller*, $r(1+g) > 1$ must hold. If recombination is free ($r = 0.5$), then invasion requires that $g > 1$, i.e. a doubling of fitness is required. If there is no recombination ($r = 0$) then invasion is impossible ($g > \infty$). Meiosis II *SisterKillers* can only invade if the recombination event between centromere and *SisterKiller* locus is near obligatory ($r \approx 1$).

Overall, then, two-step meiosis with recombination is an effective block to the invasion of some *SisterKillers*. However, in coming to this conclusion, we have again presumed that *SisterKillers* are not immune to their own activity. How will self-tolerance affect a meiosis I *SisterKiller*? Unlike the previous model, if there is recombination, then the two *Kk* cells produced after meiosis I will be rescued. For a cost-free self-tolerant *SisterKillers* acting at meiosis I the recursions are identical to those for a self-tolerant *Killer* as described above (Haig, 1993). The invasion conditions are therefore also that $g(1-r) > 0$, i.e. unless recombination always takes place between *SisterKiller* and centromere ($r = 1$) invasion is every bit as easy as in a population with one-step meiosis and requires only that there is some gain to the death of half of the products of meiosis ($g > 0$).

Comparably, two-step meiosis is no defence against a self-tolerant *SisterKiller* acting in meiosis II. For such a killer the recursions are:

$$\begin{aligned}\bar{W}p' &= pq(rg + 1) + \beta p^2 \\ \bar{W}q' &= q^2 + (1-r)pq.\end{aligned}$$

Invasion is possible if $rg > 0$, i.e. there is some recombination ($r > 0$) and some gain to the death of half of the products of meiosis. To consider whether the transition from one-step meiosis to two-step meiosis, we therefore consider only the nonself-tolerant *SisterKiller*.

Does the presence of a *SisterKiller* create the conditions for the evolution of two-step meiosis?

In this section we consider the population genetics of a modifier (M) of the form of meiosis entering a population with one-step meiosis and *SisterKiller*. The action of this modifier is as before associated with some cost, s . We consider two situations. First, the population with one-step meiosis is affected by a *SisterKiller* which, on finding itself in a two-step meiosis, acts in meiosis I. The second case is when the *SisterKiller* acts in meiosis II. We here therefore presumed that the action of the *SisterKiller* is dependent on some cue, possibly termination of recombination, in which case it would act in meiosis I. An alternative cue would be the imminent reduction of the $2N$ state to the N state, in which case meiosis II might be the time of action.

A modifier of the form of meiosis with a SisterKiller acting in meiosis I

To analyse the dynamics of a modifier converting a one-step into a two-step meiosis, we need to make a few more assumptions. A two-step meiosis will produce four progeny but a one-step one produces only two. The transition may trivially be achieved if the four are the same fitness as the two, as two-step meiosis is then a doubling of fitness. This is very unrealistic. It is then better to suppose that each of the four has a fitness $1/2$, rather than 1, assuming all else to be equal. Under this circumstance, in the absence of any direct costs on the modifier, one-step and two-step meiosis are of the same fitness. Likewise, it is necessary to suppose that the fitness of each product of a two-step meiosis in which half the progeny die is $(1+g)/2$. Again we can consider $(1+\gamma)/2 = [(1-t)(1+g)]/2$, where t is some cost to the action of the distorter. The modifier (M) we again presume to be dominant and to freely recombine with the *SisterKiller* locus. There are four haploid genotypes, MK , Mk , mK and mk . These exist at frequencies, x_1 , x_2 , x_3 and x_4 , respectively. From the above assumptions the following recursions may be derived:

$$\begin{aligned}\bar{W}x'_1 &= (1-s)(1-r)(1+\gamma)(2x_1x_2 + x_1x_4 + x_2x_3) \\ \bar{W}x'_2 &= (1-s)(2x_2^2 + 2x_2x_4) \\ \bar{W}x'_3 &= (1-s)(1-r)(1+\gamma)(x_1x_4 + x_2x_3) + 2x_3x_4(1+\gamma) \\ \bar{W}x'_4 &= 2x_2x_4(1-s)2x_4^2.\end{aligned}$$

After linearization, we can calculate the leading Eigenvalue (λ) for the matrix describing the transformation of x_1 and x_2 at given values of x_3 and x_4 . The invasion of the modifier is possible if $\lambda > 1$. Solving for this reveals a

$$s_{\max} = \frac{x_3 + 2 + r_2 + \sqrt{(1 - r_2)^2(1 - 2x_3)^2 + (1 - x_3)(3 - 2r_2(1 + 2x_3) + 3x_3)}}{2r_2(1 - x_3)}$$

condition on the maximum cost of the modifier (s_{\max}) consistent with its invasion. This presents two solutions:

$$s_{\max} < -gx_3$$

and

$$s_{\max} < \frac{\gamma(1 - r - 2x_3) - r - 1}{(1 + \gamma)(1 - r)}$$

If invasion of the selfish *SisterKiller* is possible ($g > 0$, $x_3 > 0$), both of these solutions are negative. Thus the modifier cannot be either neutral or costly but must be directly advantageous (and invasion is not always guaranteed then). This was confirmed by simulation. We conclude that a *SisterKiller* acting at meiosis I, far from creating the conditions for a transition in the evolution of meiosis, provides conditions that impede the transition!

This result is to be expected. The modifier only has an effect on segregation ratios in *Kk* heterozygotes. If there is no recombination between *K* locus and the centromere then half the products of meiosis die, these being the ones carrying the wild-type allele. This is as would happen in the absence of the modifier. If there is recombination then after meiosis I the two cells are both *Kk*. Given that we must suppose that the *SisterKiller* is not self-tolerant, these two cells kill each other. At least one of these must contain the modifier. Thus, the modifier either has no net effect and only imparts costs, or acts to kill itself.

A modifier of the form of meiosis with a SisterKiller acting in meiosis II

The modifier of meiosis in a population with a *SisterKiller* that acts in meiosis II has almost exactly the same recursions as the modifier of meiosis acting in one-step meiosis to allow the evolution of recombination, in a population with *Killer*. If r_1 is the rate of recombination specified in the recursions for the one-step meiosis and r_2 the rate of recombination for the recursions of the meiosis II acting *SisterKiller*, then the second series of recursions can be derived from the first by replacing r_1 with $1 - r_2$. This makes verbal sense. The *SisterKiller* acting in meiosis II gains if there is recombination, whereas the *Killer* in one-step meiosis loses if there is recombination (it ends up in the dead cell). Either way, half the meiotic products die. Conversely, if there is no recombination in the two-step meiosis again half of the products die, these both bearing the *SisterKiller* (they kill

each other). If there is no recombination in the one-step meiosis *Killer* ends up in the one surviving cell. So, generally, the maximum value of the cost of the modifier is approximately:

If $r_2 = 0.5$ then the condition on the invasion of the modifier is as it was for $r = 0.5$ in the one-step meiosis, i.e.

$$s_{\max} \approx \frac{2x_3 - 3 + \sqrt{9 - 8x_3}}{2(1 - x_3)}$$

From inspection of the above equations we find, importantly, that although the modifier can invade when $r_2 = 0.5$, the selection on the modifier is strongest if there is no recombination. This is because in the absence of recombination the two products of meiosis I are *KK* and *kk*. The *KK* cell then divides, producing two *K* cells that kill each other, so providing an advantage to the two surviving *k* cells. This greatly strengthens the linkage disequilibrium between *k* and *M*. Thus, the modifier is most likely to spread, given arbitrary costs, if it prevents recombination and allows two-step meiosis.

One might also consider a case in which the *SisterKiller* that, on finding itself in a two-step meiosis, does not 'know' whether to act in meiosis I or meiosis II. If it were to act in meiosis I one half the time and in meiosis II half the time, then selection on the modifier of the form of meiosis will, half the time, be that of the modifier in the meiosis I case (i.e. invasion is impossible) and half the time the selection will be that associated with the meiosis II *SisterKiller* (i.e. invasion is possible). The case of the modifier associated with the meiosis II acting *SisterKiller* hence provides the most permissive conditions for the evolution of the modifier of the form of meiosis.

Summary

From the above two sets of results we may conclude (a) that the presence of meiotic I *SisterKillers* impedes the transition in the evolution of meiosis, (b) that the evolution from one-step to two-step meiosis could be driven by *SistersKillers* acting at meiosis II but (c) this process fails convincingly to account for the evolution of recombination associated with two-step meiosis. Conclusion b is also subject to the same criticisms as before, i.e. the effect goes away if one allows for the evolution of self-tolerance (Figs 4a,c and 5). The recursions for this are as given in the appendix, only again with the substitution of r with $1 - r_2$.

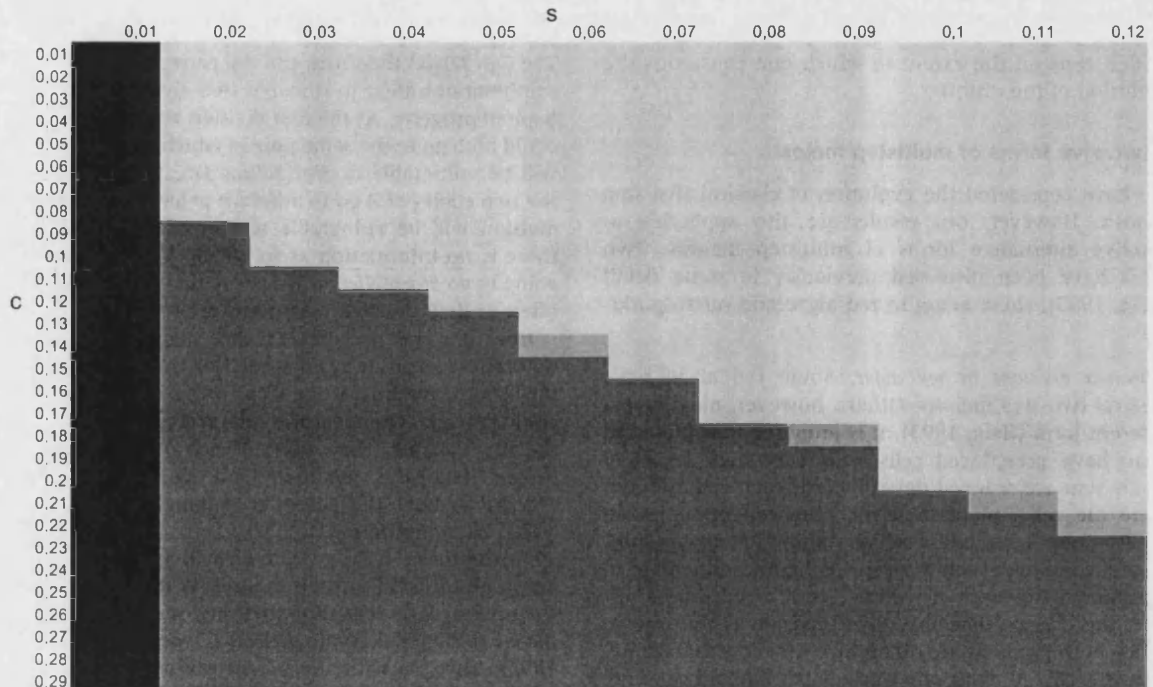


Fig. 5 Map of the parameter space in which the self-tolerant *SisterKiller* allele invades a population with the modifier of recombination and the nonself-tolerant *SisterKiller*. In this case, $g = 0.2$, $t = 0.1$ and $r = 0$. The degree of shading represents the same results as in Fig. 4, but in this case the modifier is of the form of meiosis. Note that invasion of the self-tolerant *SisterKiller*, even at quite high costs (c), leads to loss of both the modifier of the form of meiosis and the nonself-tolerant *SisterKiller* from the population.

Discussion

The evolution of meiosis

We have analysed two putative transitions in the evolution of meiosis and broadly found against the hypothesis (Haig & Grafen, 1991) that distorter alleles could have induced the transitions. In the case of one-step meiosis, we found that, although the presence of the selfish element does allow the spread of the modifier of recombination, this evolution can be corrupted by the evolution of the selfish element: a self-tolerant *Killer* that is viable in homozygous form destroys the process. By contrast one that is inviable in homozygous form greatly promotes the process, despite the fact that the resulting form of meiosis is not resistant to invasion of such a *Killer*. The answers therefore depend upon how we suppose the system to be constrained.

In the case of the transition from one-step to two-step meiosis, we confirmed that, if every individual is performing a two-step meiosis, then a nonself-tolerant *SisterKiller* cannot invade under many circumstances. However, we cannot support the conclusion that *SisterKillers* allow the evolution of two-step meiosis with recombination. It can support a model for one- to

two-step meiosis with minimal recombination, but this is also sensitive to the evolution of self-tolerance in *SisterKillers*. Importantly, it is also sensitive to the presence of *SisterKillers* that act in meiosis I, as these impede the evolution of two-step meiosis. Again therefore whether the process works depends upon precisely what we assume about when and how *SisterKillers* act.

Although it would be premature to reject the *SisterKiller* model, we can be sure that the conditions under which it works are very much more limited than previously considered. Further, we have not, for example, considered the case of the heritable target locus, i.e. one that is polymorphic. Such a model will require more extensive analysis as one must allow for three loci (*Killer/Non-Killer* (K/k), *Target/Non-Target* (T/t) and modifier (M/m)). Such a set up might be more permissive of the evolution of recombination as a cell that was $KkTt$ cannot have both cells survive if recombination between K and t were to occur, i.e. the sort of self-tolerance that we envisage is not possible in this cross. It is, however, possible in $KkTT$ cells, but here no distortion can ever occur. Equally, however, the evolution of recombination could drive the evolution of a *Killer-Target* tightly linked gene cluster that could not be broken by recombination.

Recombination would then not be able to spread. We would guess therefore that, again, the plausibility of the models rests on the extent to which one constrains the evolution of the distorter.

Alternative forms of multistep meiosis

We have considered the evolution of classical two-step meiosis. However, our results are also applicable to putative alternative forms of multistep meiosis. Two types have been discussed previously in some detail (Haig, 1993), these being in red algae and microsporidians.

Reduction divisions in red algae. Some red algae have classical two-step meiosis. Others, however, may have a different form (Haig, 1993). It is proposed that these red algae have pre-reduced cells with very high levels of ploidy that are reduced down by half each cell division. Were the cell diploid then the only cell division will result in one *A* and one *a* cell (assuming heterozygosity). As argued above, such a system is highly vulnerable to *SisterKillers*. However, if we start with a cell that is $4N$, then the chance that any given division is the one in which both products are different is now less than unity. That is a $4N$ cell, with one nucleus per haploid genome, will be *AAaa* and the first division could give *AA* and *aa* in which case *SisterKilling* would be advantageous. But it could also give two *Aa* cells in which case *SisterKilling* would be disadvantageous, if not lethal for the *Sister-Killer*. With higher ploidy and proportionally more divisions the invasion of *SisterKillers* is made harder still (Haig, 1993).

Could this extra 'confusion' afforded by the extra division steps have led to the evolution of the extra steps? Consider the $2N \rightarrow 4N$ transition. The analysis here is the same as the analysis of the evolution of two-step meiosis with free recombination (independent segregation of nuclei is more or less equivalent to free recombination). As established above, the modifier analysis does not support greatly the hypothesis that *SisterKillers* could have induced the transition when recombination is found: if the *SisterKillers* are not self-tolerant then extra divisions impede the process as the death of the meiotic products in the first step eliminates the modifier as well; if the *SisterKillers* are self-tolerant then variation tends to be abolished and the modifier is redundant but costly.

Microsporidian meiosis. It remains unclear whether microsporidians have an unusual form of meiosis. Nonetheless, an 'interpretation' (Haig, 1993) in terms of *SisterKillers* has been provided to explain the complex form of the putative alternative (Canning, 1988). It is conjectured that two haploid cells fuse to produce a diploid. This diploid then goes through a mitosis but with no cell division. If the diploid was a heterozygote, there now

exist two heterozygous nuclei. The chromosomes now pair up with their homologue, prior to nuclear fusion. The two nuclei then fuse and the paired up chromosome combinations align to undergo two divisions to produce haploid progeny. At the first division the two copies of *A* could both go to the same pole in which case this division will be vulnerable to *SisterKillers*. On the other hand, if the two copies of *A* go to different poles, then the second division will be vulnerable to *SisterKillers*. However, as there is no information as to which way the division is going to go *SisterKillers* will destroy their identical copy as often as they destroy their nonclone mate (Haig, 1993).

How are we to consider this suggestion? First, in theoretical terms, it again is unclear that the modifier of meiosis (converting a one-step to this strange two-step) could spread. Whenever the cells segregate *Aa* and *Aa* in the first division, they will kill each other and, as seen before, take the modifier with them. Thus, again, *SisterKillers* have the potential to impede the transition, rather than promote it.

Furthermore, it is far from clear that Microsporidians are at all unusual in their meiosis. A reinterpretation of the unclear facts regarding their meiosis argues that they have a normal eukaryote meiosis (Flegel & Pasharawipas, 1995). This fits with the recent revision of their taxonomic status. It presently looks as though they are fungi (or closely related) (Edlind *et al.*, 1996). Evidence of mitochondrial genes found in their nuclear genome (Germot *et al.*, 1997) indicates that their lack of mitochondria is most likely a derived characteristic, probably associated with parasitic status.

Theoretical concerns

If a system *X* is invulnerable to selfish gene *S*, while closely related system *Y* is not, does it follow that *X* can evolve from *Y* when *S* is present? The results provided above indicate that it is inappropriate to assume that 'defence'/ESS analyses and modifier analyses need agree. Leaving aside the issue of self-tolerance, we find that defence and modifier models do agree with respect to one-step meiosis evolving to one-step meiosis with recombination under pressure from *Killer* alleles. Likewise, the pressure of *SisterKillers* that act in meiosis II can induce the transition with the new form of meiosis being immune to the selfish element. However, two-step meiosis is immune to *SisterKillers* that act in meiosis I, but their presence hinders rather than favours the transition in question. Inversely, we found a class of selfish element that one step-meiosis with recombination is no more protected against than one-step meiosis without recombination, but that can nonetheless induce the transition. In summary then, there exists no universal correspondence between the defence/ESS analysis and the modifier analysis. To ask therefore whether a transition can be favoured by nongroup

selective forces, it is necessary to perform a modifier analysis.

One might also ask whether it is surprising that a class of selfish element can be imagined that can invade in one system but not the other? It is our contention that if one were to compare any two genetic systems in sexual species, it is always possible to define a type of selfish element that can afflict at least one of the two that cannot (or is less likely to) affect the other. Defining the putative selfish elements is a matter of ingenuity alone. Species with biparental inheritance of organelles, for example, are vulnerable to fast replicating deleterious organelles, while those with uniparental inheritance are not (Hoekstra, 1990; Hastings, 1992). By contrast, species with uniparental inheritance of organelles are vulnerable to the spread of sex-converting cytoplasmic factors (e.g. feminizers), while species with biparental inheritance are not (Hurst, 1993b). The validity of such a hypothesis should then not be measured by the fact that such a selfish gene is imaginable, but rather, at least in some small part, by whether such selfish genes are likely and have ever been observed. The alleles that we consider here, as postulated by Haig and Grafen, have never been observed. We do not know how to evaluate how likely they are.

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Appendix

Recursions for a population with self-tolerant and nonself-tolerant *Killer* alleles and a modifier of recombination

Consider a sexual population of single-celled organisms with a one-step meiosis. The meiosis is affected by *Killer* alleles. At the *Killer* locus there exist three alleles. K_1 and K_2 are *Killer* alleles and k is the wild-type. K_2 is a self-tolerant *Killer*, while K_1 not self-tolerant. In K_1k and in K_2k matings in which there is no recombination between K and the centromere, the *Killer* allele labels the opposite pole for destruction and hence the only product of this one-step meiosis is a single haploid cell bearing the *Killer* allele.

K_1 and K_2 differ in their behaviour in KK meioses and in Kk meioses with recombination. K_1 is not self-tolerant and hence in K_1K_1 diploids both products of meiosis are

$$\begin{aligned}\bar{W}x'_1 &= ((1-r)(1-s)(1+g)(1-t))(x_1x_5 + x_1x_6/2 + x_2x_5/2) \\ \bar{W}x'_2 &= ((1-r)(1-s)(1+g)(1-t))(x_1x_6/2 + x_2x_5/2) + x_2x_6(1+g)(1-t) \\ \bar{W}x'_3 &= (1-c)(1-s)(1-t) \left((1+g)(1-t)(x_1x_4/2 + x_1x_3 + x_2x_3/2) + (1-t)(x_3^2 + x_3x_4) \right) \\ &\quad + ((x_3x_5 + x_3x_6/2 + x_4x_5/2)((1-r)(1+g) + r)) \\ \bar{W}x'_4 &= (1-c)(1-t) \left(0.5(1+g)(1-t)(1-s)(x_1x_4 + x_2x_3) + (1+g)(1-t)x_2x_4 + x_3x_4(1-t)(1-s) \right) \\ &\quad + 0.5(1-s)(x_3x_6 + x_4x_5)((1-r)(1+g) + r) + x_4^2(1-t) + x_4x_6(1+g) \\ \bar{W}x'_5 &= (1-s)((x_1x_5 + x_2x_5/2 + x_1x_6/2)(r(1+g)(1-t)) + x_3x_5 + x_3x_6/2 + x_4x_5/2)r(1-t) + x_5^2 + x_5x_6 \\ \bar{W}x'_6 &= (1-s)((1+g)(x_1x_6 + x_2x_5) + x_3x_6 + x_4x_5)(r(1-t)/2) + x_5x_6 + x_6^2\end{aligned}$$

destroyed. Likewise, in K_1k meioses, if recombination has occurred, the K_1 allele is in the cell due to die and cannot rescue it. In contrast, K_2 can rescue the dying cell and K_2K_2 matings produce two viable cells. K_1K_2 diploids produce one viable cell, this bearing K_2 , it being immune to the action of both *Killers*. All haploid cells bearing K_2 suffer a cost c of self-tolerance. There is also a cost to

attempted distortion, t . This acts in a multiplicative fashion. All viable progeny of meiosis in which distortion was attempted suffer the costs. If one of the two cells of a meiosis dies, the other receives a gain of fitness, g .

The modifier locus has two alleles, the dominant modifier M and the wild-type null allele, m . The modifier acts to allow recombination between the *Killer* locus and the centromere at a rate r . The modifier freely recombines with the *Killer* locus, being unlinked to it. Modification ensures a cost s that acts on all products of meiosis in which the modifier was active. The costs of modification in MM and Mm individuals are assumed to be the same.

There are six haploid genotypes: K_1M , K_1m , K_2M , K_2m , kM and km . These exist at frequencies x_1 , x_2 , x_3 , x_4 , x_5 and x_6 , respectively. Hence, there are 21 different possible matings. The frequency of each mating and the fitness of their haploid progeny are shown in Table 1. From this we may derive the following recursions:

where \bar{W} is the sum of the right-hand sides of the equations.

The recursions for the self-tolerant *SisterKiller* allele acting at meiosis II in a population polymorphic for nonself-tolerant *SisterKiller* and the modifier of the form of meiosis (one step \rightarrow two step) are identical, excepting that one must substitute $r \rightarrow 1-r$.

Table 1 The 21 matings, their frequencies and the haploid products with their associated fitnesses.

Mating	Freq	K_1M	K_1m	K_2M	K_2m	kM	km
$K_1M \times K_1M$	x_1^2	-	-	-	-	-	-
$K_1M \times K_1m$	$2x_1x_2$	-	-	-	-	-	-
$K_1M \times K_2M$	$2x_1x_3$	-	-	$(1-c)(1+g)(1-t)^2(1-s)/2$	-	-	-
$K_1M \times K_2m$	$2x_1x_4$	-	-	$(1-c)(1+g)(1-t)^2(1-s)/4$	$(1-c)(1+g)(1-t)^2(1-s)/4$	-	-
$K_1M \times kM$	$2x_1x_5$	$(1-r)(1-s)(1+g)(1-t)/2$	-	-	-	$r(1-s)(1+g)(1-t)/2$	-
$K_1M \times km$	$2x_1x_6$	$(1-r)(1-s)(1+g)(1-t)/4$	$(1-r)(1-s)(1+g)(1-t)/4$	-	-	$r(1-s)(1+g)(1-t)/4$	$r(1-s)(1+g)(1-t)/4$
$K_1m \times K_1M$	x_2^2	-	-	-	-	-	-
$K_1m \times K_1m$	x_2^2	-	-	-	-	-	-
$K_1m \times K_2M$	$2x_2x_3$	-	-	$(1-c)(1+g)(1-t)^2(1-s)/4$	$(1-c)(1+g)(1-t)^2(1-s)/4$	-	-
$K_1m \times K_2m$	$2x_2x_4$	-	-	-	$(1-c)(1+g)(1-t)^2/2$	-	-
$K_1m \times kM$	$2x_2x_5$	$(1-r)(1-s)(1+g)(1-t)/4$	$(1-r)(1-s)(1+g)(1-t)/4$	-	-	$r(1-s)(1+g)(1-t)/4$	$r(1-s)(1+g)(1-t)/4$
$K_1m \times km$	$2x_2x_6$	-	$(1-c)(1-t)/2$	-	-	-	-
$K_2M \times K_2M$	x_3^2	-	-	$(1-c)(1-t)^2(1-s)$	-	-	-
$K_2M \times K_2m$	$2x_3x_4$	-	-	$(1-c)(1-t)^2(1-s)/2$	$(1-c)(1-t)^2(1-s)/2$	-	-
$K_2M \times kM$	$2x_3x_5$	-	-	$(1-c)(1-t)(1-s)r + (1-r)(1+g)/2$	-	$r(1-t)(1-s)/2$	-
$K_2M \times km$	$2x_3x_6$	-	-	$(1-c)(1-t)(1-s)r + (1-r)(1+g)/4$	$(1-c)(1-t)(1-s)r + (1-r)(1+g)/4$	$r(1-t)(1-s)/4$	$r(1-t)(1-s)/4$
$K_2m \times K_2M$	x_4^2	-	-	-	$(1-c)(1-t)^2$	-	-
$K_2m \times K_2m$	x_4^2	-	-	-	-	-	-
$K_2m \times kM$	$2x_4x_5$	-	-	$(1-c)(1-t)(1-s)r + (1-r)(1+g)/4$	$(1-c)(1-t)(1-s)r + (1-r)(1+g)/4$	$r(1-t)(1-s)/4$	$r(1-t)(1-s)/4$
$K_2m \times km$	$2x_4x_6$	-	-	-	$(1-c)(1+g)(1-t)/2$	-	-
$kM \times kM$	x_5^2	-	-	-	-	$(1-s)$	-
$kM \times km$	$2x_5x_6$	-	-	-	-	$(1-s)/2$	$(1-s)/2$
$km \times km$	x_6^2	-	-	-	-	-	1

Part II

The Evolution of Anisogamy

"I am fundamentally mixed, male with female, parent with offspring, warring segments of chromosomes that interlocked in strife millions of years before Europe as a whole existed or saw any of the human violence that became later embedded in my ancestry." (p. 135 Hamilton 1996)

In many organisms, gametes come in two varieties. In some cases they are differentiated into sperm and eggs (*i.e.* anisogamous). In others, the gametes are the same size (*i.e.* isogamous) but act differently ('behavioural anisogamy'). Typically, one isogamete attaches to the substratum and produces a pheromone that attracts the other isogamete (*e.g.* Chlorophyta: *Chloromonas paupera*, *Cosmarium botrytis*, *Nephroselmis olivacea* (Maier 1993), Phaeophyta: *Ectopcarpus siliculosus* (Kochert 1978)). In still other cases, gametes are identical in both size and behaviour, but are differentiated into two mating types (plus and minus or α and α) which only fuse asymmetrically.

Assuming isogamy to be the ancestral condition (Jennings 1920; Margulis & Sagan 1986; but see Alexander & Borgia 1979) one can ask, why did the gametes change in relative size? This question defines one of the oldest debates in evolutionary genetics (authorised translation Weismann 1886; Butschli 1889; Weismann 1902; Hertwig 1909; Ghiselin 1974; Hoekstra 1987) and has provoked intense theoretical interest in recent decades (*e.g.* Parker *et al.* 1972; Charlesworth 1978; Maynard Smith 1978; Cosmides & Tooby 1981; Dusenbery 2000).

The idea that anisogamy represents a division of labour between the sexes was originally postulated by a group of German authors (authorised translation Weismann 1886; Butschli 1889; Weismann 1902; Hertwig 1909). They reasoned that the act of fertilisation imposes conflicting demands on gamete size. Gametes must be able to find one another, but once this happens they must have sufficient material to produce a viable zygote. It was proposed that to meet these demands simultaneously, the gametes of the two sexes have specialised on different functions: sperm on mate finding and eggs on zygote provisioning. However, no mechanism was suggested by which the transition from isogamy to anisogamy could have come about.

These ideas were developed by a number of authors (Kalmus 1932; Kalmus & Smith 1960; Scudo 1967; for a review see Hoekstra 1987). Kalmus (1932) asked whether in an anisogamous population, a greater number of viable zygotes are formed in a given short period of time than in an isogamous population. His model adopted the same tools used in classical statistical mechanics models of the collision of particles. He assumed that each gamete's speed is independent of its size and that gametes move randomly. He did not assume the pre-existence of mating types.

The model predicts that the mean number of successful zygotes (and hence the growth rate of the population) will be greater for an anisogamous population, provided the size difference between the gametes is greater than 6-fold. At first sight, this result seems counter-intuitive. In such a population, one would imagine that the number of viable zygotes that can be produced each generation is limited by the number of macro-gametes. If total gamete material is divided equally between macro and micro-gametes

(as the model predicts) then the number of macro-gametes will necessarily be less than half the number of gametes in an isogamous population. Hence, the total number of viable zygotes should be limited. However, because Kalmus assumes that fusions occur in a short period of time, the number of zygotes produced is always much smaller than the number of macrogametes. Hence, macro-gametes are not limiting.

Scudo (1967) examined whether this result could be an artefact of the assumption that there is only a limited time window for gamete fusion. This assumption is necessary for the statistical mechanics formulation because it deals with elastic collisions between gametes (*i.e.* gametes are not removed from the gamete pool when they fuse, so the number of gametes of each type available at the end of the process is the same as at the start).

To establish whether Kalmus' result is robust to inelastic fusion, Scudo constructed models which assume that gamete density was a function of three factors: the rate of gamete production, the rate of gamete death and the rate of gamete removal due to fusion (Scudo 1967). Different scenarios determining the rate of gamete death were considered; namely predation and time dependent natural mortality. The models upheld the original qualitative result that anisogamy permits more rapid population growth rate than isogamy provided the size difference between gametes is large. As pointed out by Ghiselin (1974) and Hoekstra (1987) however, these models rely on group selection. In both cases they consider an advantage to anisogamy in terms of an increased population growth rate. This stems from an increased number of fertilisation events when gametes are dimorphic.

The first model couched in terms of an advantage to the individual of producing a different sized gamete was proposed by Kalmus and Smith (1960) (this was originally stated as a verbal argument, but was later formally modelled by Hoekstra (1987)). They considered a diploid population in which there is a gene a for small gamete production. Next, suppose a dominant mutation A arises such that Aa and AA individuals produce larger gametes. The mutation is expected to increase in frequency because it is found in larger fitter zygotes. However, at some point its spread will be checked because unions between the larger, relatively immobile gametes occur infrequently. They suggest that a stable equilibrium will eventually arise between large and small gamete producers. As we shall see, this reasoning broadly underpins the dominant model for the evolution of anisogamy (Parker *et al.* 1972).

In marked contrast to such models, since the early '90s there has been growing interest in a series of models supposing that small sperm might have evolved to limit the paternal inheritance of cytoplasmic genes (Hurst 1990; Law & Hutson 1992; Hutson & Law 1993). One major class of these theories attempts to explain the evolution of anisogamy in terms of conflict between nucleus and cytoplasm. Biparental cytoplasmic inheritance is vulnerable to subversion by 'selfish' cytoplasmic genomes that replicate faster than the wild-type. These parasites can be any cytoplasmically inherited genome whose replication is free from the constraints of the cell cycle (*e.g.* organelles, bacteria, microsporidia), not just cytoplasmic entities (such as bacteria) that we would traditionally regard as parasitic. Such mutants can be selectively favoured because of the within-cell advantage they enjoy when paired with the wild-type cytotype in a zygote (Chapter 3; Randerson & Hurst 1999). They are capable of spreading in a population despite harming the fitness of cells they inhabit.

I examine whether such conflict could have imposed selection for anisogamy as a generalised defence against such mutants. Subsequently, I explore the question of gamete dimorphism more broadly. In Chapter 4 (Randerson & Hurst 2001a), I provide the first phylogenetically controlled comparative test of the Parker, Baker and Smith model (Parker *et al.* 1972). I then go on to evaluate recent theoretical and empirical advances in the field and ask what prospects there are for a general solution to the anisogamy problem (*i.e.* one that applies to all taxa)(Randerson & Hurst 2001b).

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Chapter 3. Small sperm, uniparental inheritance and selfish cytoplasmic elements: a comparison of two models

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Small sperm, uniparental inheritance and selfish cytoplasmic elements: a comparison of two models

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Abstract

It has previously been suggested that small sperm size may be an adaptation to achieve uniparental inheritance of organelles, and hence to prevent the spread of selfish cytoplasmic elements. Such an explanation for anisogamy implies a mechanism whereby the male gamete eliminates its own cytoplasm prior to fusion with the egg. A model has been presented demonstrating the invasion and persistence of a modifier that acts gametically to kill its own organelles. Here we show, however, that this model is far from robust; indeed, if any cost is associated with the modifier it cannot persist. We also show that despite an empirically demonstrated association between anisogamy and multicellularity, this result also applies if the analysis is applied in the multicellular case. This class of model contrasts with the majority of analyses in which the modifier kills off the incoming gamete's organelles. We show that these models are highly robust, even if uniparental inheritance is imperfect.

Introduction

Why are organelles uniparentally inherited, and what is the link between uniparental inheritance and the small size of sperm relative to eggs? Uniparental inheritance of cytoplasmic elements is widespread amongst the eukaryotes (Sears, 1980; Whatley, 1982; Birky, 1983), and in anisogamous organisms, the transmitting gamete is often the larger egg (Cosmides & Tooby, 1981). This is by no means a general rule, however, and several examples of paternal inheritance have been uncovered (Neale *et al.*, 1990; Faure *et al.*, 1994; Mason *et al.*, 1994). There are also examples of biparental inheritance, though these appear to be very much the exception (e.g. *Pelargonium zonare* (Birky, 1983)). Work on isogamous organisms suggests that the degree of gamete size differentiation may not be a good predictor of the mode of cytoplasmic inheritance. In *Chlamydomonas*, acellular slime moulds and numerous other isogamous organisms, organelle inheritance is almost entirely uniparental (see references

in Hurst, 1994), whereas in yeast, the control over inheritance appears to be much less stringent with both uni- and biparental inheritance occurring. An attempt to explain this variety in terms of the typical level of inbreeding in a species has been made previously (Hurst, 1994). Although modern molecular techniques are revealing that uniparental inheritance of cytoplasmic elements is not as ubiquitous as once thought, there is still the theoretical challenge presented by its predominance. A further challenge is to explain the tantalizing link with anisogamy, and to ask whether selection in favour of uniparental inheritance also led to the size difference between sperm and eggs.

Several models have been put forward to explain the evolution of uniparental inheritance of cytoplasmic elements (for review see Birky, 1995). Many of these are unlikely to be general; for example Sears & VanWinkle-Swift (1994) have proposed that in *Chlamydomonas reinhardtii*, uniparental inheritance of chloroplasts is an adaptation to combat nitrogen starvation. Their Salvage-Turnover-Repair (STOR) model proposes that cpDNA from the mt-gamete is degraded to act as sustenance for the developing zygote. However, this explanation cannot be applied to anisogamy, which implies a mechanism whereby the male gamete

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eliminates its own cytoplasm. Nor can it explain cases in which the sperm eliminates its own cytoplasm prior to fusion with the egg (Mogensen & Rusche, 1985). Hence, while this explanation may hold in the specific case, it seems unlikely that it can provide a general solution to the problem of why uniparental inheritance evolved.

An alternative class of potentially general model is that based on the conflict of interests between the nuclear and cytoplasmic genomes (Cosmides & Tooby, 1981; Partridge & Hurst, 1998). Under the 'Conflict Hypothesis', uniparental inheritance is interpreted as an adaptation to prevent the mixing of different cytoplasmic genomes in the zygote. The 'Conflict Hypothesis' might more strictly be considered as an array of subtly different hypotheses. For example, one sort of model assumes that there is a general cost associated with the possession of two sets of cytoplasmic genomes in the same zygote (Hurst, 1990; Hurst & Hamilton, 1992; Frank, 1996). This assumption is based on negative fitness consequences for the host due to antagonistic interactions between cytoplasmic elements. Clearly, if cytoplasmic mixing *per se* is bad for the nuclear genes then a nuclear modifier that prevents such mixing will be favoured. Although these models predict uniparental inheritance of cytoplasmic elements, evidence for a synergistic cost to possession of different cytoplasmic genomes is sparse (but see discussion). Although it would be premature to reject these models on these grounds, it might be instructive to ask if other models with more robust assumptions might also account for uniparental inheritance.

One such set of models supposes the cytoplasmic element to be directly deleterious and gain greater than Mendelian inheritance rates when inheritance is biparental. These models might consider a deletion in an organelle genome, permitting fast replication while being deleterious or a deleterious vertically transmitted bacterium, virus or related pathogen. Petite mutants in yeast are an example of the first.

Within this class of models, most attention has been paid to the dynamics of a modifier that kills off the cytoplasmic factors of the partner's gamete (Hoekstra, 1990; Hastings, 1992; Hurst, 1996). This appears to be the case in the isogamous, unicellular alga *C. reinhardtii* in which chloroplast DNA from the - type parent is broken down in the zygote by gene products from the + type. The success of the 'kill off the partner's cytoplasm' modifier in such models stems from the fact that the modifier is able to remain in linkage disequilibrium with the fitter cytotype. If the modifier starts off associated with the nonselfish cytotype and uniparental inheritance is perfect, then the modifier never suffers the fitness costs associated with the selfish cytotype. What happens though if uniparental inheritance is not perfect and the tight linkage disequilibrium between the modifier and the nonselfish cytotype is broken down? Can the modifier still invade? Following Hastings (1992), we investigate the robustness of the 'kill off the partner's

cytoplasm' model in the absence of perfect uniparental inheritance by allowing some degree of 'cytoplasmic leakage' from the 'paternal' gamete. We also extend this analysis to the multicellular case.

The success of this model however, leaves a problem: How can we explain the evolution of anisogamy, which implies 'kill off your own cytoplasm' rather than 'kill off the partner's cytoplasm', in terms of this subcategory of the 'Conflict Hypothesis'? An attempt to solve this problem was made by Law & Hutson (1992) who consider a subtly different modifier. They model the spread of a nuclear modifier that enforces uniparental inheritance by bringing about the destruction of the cytoplasm of the gamete in which it is found, a mechanism that could lead to anisogamy.

The Law & Hutson (1992) model considers specifically the invasion of vertically transmitted deleterious bacterial symbionts as the driving force behind selection for uniparental inheritance. It should be noted, however, that the mathematics could equally well apply to fast-replicating organelle genomes. Indeed, in mammals vertically transmitted bacteria and viruses have never been reported, so a theory to explain anisogamy in this lineage is not especially convincing if it requires their existence. The previous modelling attempt simplified the analysis of this model by supposing the modifier was neutral and that the vertical transmission rate was one (i.e. a cytoplasmic factor inherited from only one of two parents was transmitted to all). Given that vertical transmission may well be less than one both for symbionts (Hurst, 1993) and for organelle genomes (Birky, 1995), it is worth enquiring as to whether the model is especially sensitive to alternations in this parameter. Given too that a cost of modification is likely for many different reasons, it is worth asking what happens if the modifier is costly. We show that in contrast to the 'kill the partner's cytoplasm' modifier, the 'kill your own cytoplasm' modifier envisaged by Law and Hutson is dependent upon the assumption of a cost-free modifier. An infinitely small cost ensures that the modifier never persists.

A number of authors (Parker *et al.*, 1972; Knowlton, 1974) have suggested that there may be a link between anisogamy and multicellularity. As we shall discuss, multicellularity will change the dynamics of the selfish element. It is then worth asking whether the 'kill off your own cytoplasm' modifier can persist in this circumstance? We find quite the opposite.

Can a modifier that enforces uniparental inheritance by killing off the partner's cytoplasm invade?

We investigate the dynamics of two types of nuclear modifier that enforce uniparental inheritance of cytoplasmic elements in a unicellular and a multicellular population. First we consider the invasion conditions for

a modifier that eliminates the cytoplasm of the other gamete at fusion (a 'kill off the partner's cytoplasm' modifier). Although this analysis is not new (Hoekstra, 1990; Hastings, 1992), we include it to contrast the invasion conditions of such a modifier with a modifier that eliminates its own cytoplasm prior to fusion (a 'kill off your own cytoplasm' modifier). In all cases, we consider the invasion of a modifier that is unlinked to the mating type as this would offer the hardest invasion conditions (costly meetings of the modifier where both gametes end up with no cytoplasm are still possible). Linkage to the mating type locus could of course occur subsequently (Hurst, 1996).

The unicellular case

Invasion of the selfish element

Consider a population of haploid, sexual, unicellular organisms. When two individuals mate they undergo fusion and their nuclear DNA recombines. Following recombination, the cell undergoes meiosis. Once this process has taken place there is the potential for both parental cytotypes to be present in the progeny. However, we assume that this heteroplasmic stage is short-lived and is resolved by successive rounds of asexual mitotic division such that the parental cytotypes segregate between progeny. This happens for example in yeast (*Saccharomyces cerevisiae*), where virtually all daughter cells from heteroplasmic zygotes are homoplasmic after about 10 subsequent mitotic divisions (Birky, 1995). So, for the purposes of the model, we assume that the heteroplasmic stage makes no contribution to fitness.

Now consider the invasion of a selfish cytoplasmic element (*A*) that has a replicative advantage over the wild-type (*a*) due to a deletion in its chromosome. We assume that the cells with the selfish cytotype have a reduced fitness $1 - t$ compared to the wild-type fitness of 1. Also, we assume a replicative advantage to the selfish cytotype such that in matings with the wild-type a proportion k of the progeny receive the selfish cytotype and $1 - k$ of the progeny receive the wild-type (where $k > 0.5$). If p and q represent the frequencies of the selfish cytotype and the wild-type, respectively, then the recursion equations for the values of p and q in the next generation are

$$p' = \frac{p^2(1-t) + 2pqk(1-t)}{\varpi}$$

$$q' = \frac{q^2 + 2pq(1-k)}{\varpi}$$

where ϖ is the sum of the numerators.

The conditions for invasion of the selfish cytotype are found by solving $dp'/dp > 1$ when $p = 0$. This reveals that for invasion,

$$k > 1/[2(1-t)].$$

So, as previously shown by Hurst (1994): for invasion, the selfish cytotype must have a transmission advantage and must not be too deleterious to the host cell.

Model 1 – invasion conditions for the 'kill off the partner's cytoplasm' modifier

Consider a novel nuclear modifier (*M*) that appears in the population while the selfish cytotype is still rare and is unlinked to the mating type locus. The modifier, in comparison to the wild-type allele *m*, enforces uniparental inheritance of cytoplasmic elements by destroying the cytoplasm of the partner cell just prior to fusion with a mate. Such a modifier is likely to have three associated costs: Firstly there will be a metabolic cost associated with production of the cellular machinery for modification itself. Secondly, the zygotes from *Mm* matings are likely to suffer a fitness cost due to them receiving half the normal amount of cytoplasm. This cost is directly analogous to the two-fold cost of sex (Maynard Smith, 1978), which as Hastings (1992) has pointed out is effectively a two-fold cost of anisogamy. Thirdly, in matings between two cells containing the modifier, the zygote is likely to suffer a substantial cost due to its greatly depleted cytoplasm. These matings can arise because the modifier is initially unlinked to the mating type locus and expression is not sex-specific. We assume that all gametes with the *M* allele suffer a cost of possession (ϕ). This also represents the cost of receiving a depleted cytoplasm. In addition, we assume the fitness of the progeny *MM* matings to be $1 - s$, compared to the wild-type fitness 1, so the s cost is an extra cost of *MM* matings. (Note that this assumption does not affect the invasion conditions for the modifier because we assume that when rare the modifier does not meet itself.) For clarification, the cost of the selfish element (t) applies just after fusion and meiosis, to meiotic products that have the *A* cytotype.

The frequencies of the genotypes *MA*, *Ma*, *mA* and *ma* are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation and the derivation of the invasion conditions are given in Appendix 2.

Since the modifier destroys the incoming cytoplasm, it always remains in linkage disequilibrium with the cytotype with which it was initially associated. Clearly, invasion is not possible when the modifier is initially associated with the selfish cytotype. This is because in the absence of 'paternal' leakage, the modifier always remains in linkage disequilibrium with the costly cytotype.

Alternatively, the modifier may initially be associated with the wild-type cytotype. In this case, invasion conditions are

$$\phi < tx_3[2k(1-x_3) + x_3].$$

This is plotted in Fig. 1. As the cost of the selfish element increases, and as its frequency in the population

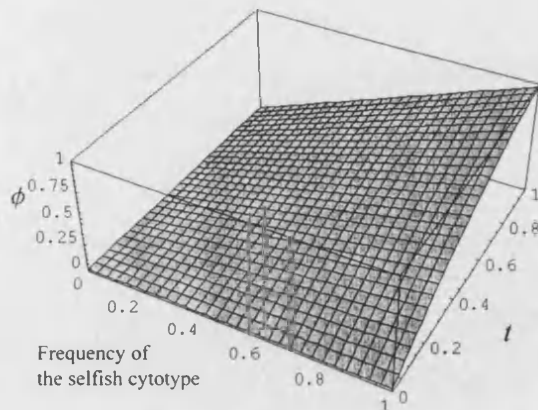


Fig. 1 Invasion conditions for the 'kill off the partner's cytoplasm' modifier in the unicellular case, when it comes in initially associated with the wild-type cytotype. For invasion, the cost of the modifier (ϕ) must lie beneath the sheet. The selfish cytotype's replicative advantage, k , is arbitrarily set to 0.8, but adjusting this parameter does not have a large effect on the invasion conditions. At high k -values, the sheet is more convex, indicating that invasion is easier, whereas at low k -values the sheet is more concave, indicating that invasion is harder. As the cost imposed by the selfish element increases, the modifier can have a higher associated cost and still invade. For all frequencies of the selfish cytotype in the population greater than one, the fitness of the modifier is higher than the average fitness in the population. This is because of the tight linkage disequilibrium between it and the cost-free wild-type cytotype. As the proportion of the population with the selfish cytotype increases, the average fitness in the population decreases. Hence, the relative fitness of the modifier increases allowing it to invade despite having a higher associated cost (ϕ).

increases, the modifier can impose a greater cost and yet still be able to invade. Invasion is also tolerated at a higher cost if k is high, especially when the selfish element is at low frequency. Taken together, these conditions suggest that the more 'virulent' the selfish element, the more costly a modifier can be and still invade. The modifier can never become associated with the selfish cytotype due to the tight linkage disequilibrium between it and the wild-type. Hence, it will always be fitter than the population average. As the average population fitness goes down (i.e. as t , k and x_3 increase), the modifier's advantage over the wild-type (m) increases, so invasion can still occur despite a greater cost of modification (ϕ).

Model 2 – invasion conditions for the 'kill off your own cytoplasm' modifier

Again, we consider a novel nuclear modifier (M) that appears in the population while the selfish cytotype is still rare and is unlinked to the mating type locus. In contrast to the previous model, this modifier enforces uniparental inheritance by destroying the cytoplasm of

its own cell just prior to fusion. We assume the same associated costs as in the previous case.

In addition to causing uniparental inheritance, a further potential effect of such a modifier is that by producing smaller gametes it can produce more of them. We do not consider this potential numerical advantage, and by so doing we (1) provide a general model applicable to both isogamous and anisogamous organisms, and (2) isolate the selection operating on anisogamy that results from reduction of organelle mixing alone. The aim is that by teasing apart the effect of organelle size and the effect of cytoplasmic mixing on fitness, we may be able to obtain a prediction about which mechanism of cytoplasmic inheritance we should observe based on conflict between nuclear and cytoplasmic genes alone.

The frequencies of the genotypes MA , Ma , mA and ma are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation are given in Appendix 2. For the condition that M is initially rare, these recursion equations, after linearization (i.e. elimination of terms in x_1^2 , x_2^2 and x_1x_2) resolve to

$$\begin{aligned}x'_1 &= \frac{(1-t)(1-\phi)(x_1x_3 + x_2x_3)}{\varpi} \\x'_2 &= \frac{(1-\phi)(x_1x_4 + x_2x_4)}{\varpi} \\x'_3 &= \frac{(1-t)x_3[(1-\phi)(x_1 + x_2) + x_3 + 2kx_4]}{\varpi} \\x'_4 &= \frac{x_4[(1-\phi)(x_1 + x_2) + 2(1-k)x_3 + x_4]}{\varpi}\end{aligned}$$

where ϖ is the sum of the numerators.

For invasion, the leading eigenvalue of these equations must be greater than one. After simplification, we obtained the following invasion conditions for the modifier:

$$\phi < \frac{tx_3(1-x_3)(1-2k)}{tx_3 - 1}$$

This is plotted in Fig. 2. Invasion conditions are more lenient when the cost (t) of the selfish element is high. This is because, at high t , the fitness of those individuals with the selfish element is low and so the benefits of modification are high. Invasion conditions are also more lenient when the selfish element is at intermediate frequency. When the selfish element is rare, the modifier is unlikely to be associated with it and hence often pays the cost of modification without receiving any benefit. Conversely, when the selfish element is common, the chances of mating with a gamete with wild-type cytoplasm and hence gaining the benefits of modification are also low.

A further point not illustrated in Fig. 1 is that the modifier's advantage is greatest when the replicative advantage of the selfish element is high. This can be illustrated by considering the gain to the nuclear genome of modification in various matings when $k = 1$. Firstly, if

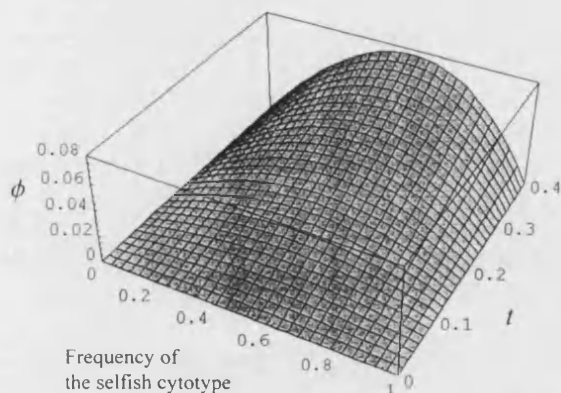


Fig. 2 Invasion conditions for the 'kill your own cytoplasm' modifier in the unicellular case. The replicative advantage of the selfish cytotype (k) has been arbitrarily set to 0.8 (note that the region above $t = 0.375$ is not relevant because the selfish cytotype cannot invade with a cost of this size). For invasion, the cost of the modifier (ϕ) must lie beneath the sheet. Hence, as the cost of the selfish cytotype increases, the modifier can have a larger associated cost and still invade. Also, when the frequency of the selfish cytotype is either low or high, the cost tolerated for invasion goes down. This is because when the selfish cytotype is uncommon, the chances of the modifier being associated with it are low, so in many cases the cost of modification is suffered without any benefit. Similarly, when the selfish cytotype is common, a modifier associated with the selfish cytotype is unlikely to mate with a gamete that has wild-type cytoplasm. Hence, the modifier gains its greatest advantage when selfish element is at intermediate frequency. Note that the invasion conditions are much more stringent for the 'kill off your own cytoplasm' modifier than for the 'kill off the partner's cytoplasm' modifier.

the selfish element can perfectly outcompete the wild-type, a cell that has the selfish cytotype can only gain by modification, no matter who it mates with. If it fuses with a wild-type cell, it gains greatly by ensuring that its progeny all have wild-type cytoplasm. If it fuses with a cell containing the selfish element then it loses nothing by modification because its progeny would all have been saddled with the selfish cytotype anyway. On the other hand, if the modifier is initially associated with the wild-type cytotype, then modification has no advantages or disadvantages. Whether it mates with a cell with the wild-type or the selfish cytotype, then either way its progeny will all end up with the same cytoplasm as its partner. Invasion of the modifier does become less likely as k decreases, but a perfect transmission rate (i.e. $k = 1$) is not a necessary condition for the model to work. Hence, Law & Hutson's (1992) model, although sensitive to changes in k , is not undermined by them.

Simulation results

We investigated the subsequent invasion of both the 'kill off your own cytoplasm' and 'kill off the partner's

cytoplasm' modifiers by simulation. Unsurprisingly, this revealed that the modifier could only increase when the population is polymorphic for the selfish cytotype. Clearly, once the selfish cytotype reaches fixation there is no advantage to uniparental inheritance because it does not prevent the progeny from suffering the costs of the selfish element. The 'kill off your own cytoplasm' modifier has very little impact on the spread of the selfish cytotype and, owing to this rapid spread, the modifier does not invade very far. If even a small cost is associated with the modifier then it is lost from the population once the selfish cytotype reaches fixation. By contrast, the spread of the 'kill off the partner's cytoplasm' modifier is far more robust to the addition of an associated cost and over a wide range of parameter space will reach a stable equilibrium with the selfish cytotype. Therefore, in a unicellular organism, the 'kill off the partner's cytoplasm' model appears to be far more robust than the 'kill off your own cytoplasm' model.

The multicellular case

It has previously been suggested (Parker *et al.*, 1972; Knowlton, 1974) that there may be a link between multicellularity and anisogamy. If then the 'kill off your own cytoplasm' model of uniparental inheritance is more robust in the multicellular case, this might be taken as supportive evidence for such a model. We investigate this possibility by examining how multicellularity affects the invasion and subsequent spread of both types of modifier.

One difference that multicellularity makes is that it changes the recessiveness of the selfish element. In the single-celled case segregation of the selfish element into different cell lines allows selection to see the deleterious effects of the cytotype on cell fitness. However, comparable selection in a multicellular organism will lead to a variety of cell types within one organism. Selection against these organisms is unlikely to be as strong as it would be against a homoplasmic unicell. This leads to an effective recessiveness of the selfish cytotype, which is likely to affect the dynamics of any modifier.

Invasion of the selfish cytotype

Consider now a population of haploid, sexual, multicellular organisms. The life cycle of these organisms consists of a haploid multicellular phase, a haploid gametic phase and a diploid zygotic phase. Fusion and segregation occur as in the unicellular case, but the products of zygotic meiosis then go on to become multicellular organisms. As in the unicellular case, the individual cells have the potential to start out being heteroplasmic. One possibility is that any heteroplasmy is resolved within a few cell divisions so that each cell in the organism contains cytoplasmic elements of only one type. Different cells can nonetheless have different cytotypes. Such a situation occurs, for example, in the geranium (*Pelargonium zonale*) where a heteroplasmic

zygote will mature into an adult consisting of clonal sectors of homoplasmic mutant and wild-type cells (Birky, 1995). Another possibility is that heteroplasmy is maintained throughout the multicellular stage. The essential point, however, is that the cost of the selfish element is to some extent recessive in the multicellular stage, but the element itself is still over-represented in the gametes.

If a selfish cytotypic similar to that considered above arises in the population, its replication advantage over the wild-type will thus be seen in matings between gametes of different cytotypic as an over-representation of the selfish element in the resulting organism. Hence, the relative proportions of the selfish (A) and the wild-type (a) cytotypes will be k and $1 - k$, respectively. We assume the gametes will also have the same proportions, but that gametes are homoplasmic. The fitness of individuals with heteroplasmic cells and/or a mixture of cells, homoplasmic for different cytotypes, is assumed to be $1 - ht$ where $h = f(k)$. If $h = 1$ then these individuals have the same fitness as the selfish cytotypic, whereas if $h = 0$ these individuals have the same fitness as the wild-type.

If the frequency of gametes with the selfish cytotypic is p and the frequency of those with the wild-type is q then the frequency of gametes with the two cytotypes in the next generation's gamete pool is

$$p' = \frac{p^2(1-t) + 2pqk(1-ht)}{\omega}$$

$$q' = \frac{q^2 + 2pq(1-k)(1-ht)}{\omega}$$

where ω is the sum of the numerators.

Invasion conditions for the selfish cytotypic are obtained by solving $dp'/dp > 1$ when $p = 0$. This reveals that for invasion

$$k > 1/[2(1-ht)].$$

So, a progressively higher replication advantage is necessary for invasion as $h, t \rightarrow 1$. Note that in comparison with the unicellular case, the invasion conditions are always broader provided that $h < 1$. This is because heteroplasmic individuals do not suffer the full effect of the cost associated with the selfish cytotypic (t).

The h parameter describes the fitness of heteroplasmic individuals. It seems sensible that this fitness should depend on the relative proportions of cells with the different cytotypes, and hence on k , the selfish element's replicative advantage. Evidence from work on a number of mitochondrial diseases suggests that a large proportion of mitochondria (typically 80–90%) must be dysfunctional for symptoms to become apparent (Bodnar *et al.*, 1993; Moraes *et al.*, 1993; Damian *et al.*, 1995; Oldfors *et al.*, 1995; Frank & Hurst, 1996). This suggests that k must be fairly high for the progeny of a cross between parents with a wild-type and a selfish cytotypic to have a fitness significantly lower than unity. Hence, it

seems likely that an appropriate function describing h in terms of k would be $h = k^n$ where n is a constant greater than one. This function has the property that the organism only suffers a significant fitness cost when a large proportion of cells are infected with the selfish element (i.e. when k is high). In the absence of better empirical evidence, this function is only intended to be illustrative. We shall assume for graphical representation that $n = 6$.

Model 1 – invasion conditions for a 'kill off the partner's cytoplasm' modifier

Consider the invasion of a modifier (M) that enforces uniparental inheritance by destroying the cytoplasm of the incoming gamete. It has similar costs associated with it to those discussed for the unicellular case. The frequencies of the genotypes MA , Ma , mA and ma are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation are given in Appendix 2.

Again, since there is perfect linkage disequilibrium between the modifier and the cytotypic there are effectively two mutually exclusive invasion conditions. However, it is easy to show that if the modifier is initially associated with the selfish cytotypic then it cannot invade.

For the modifier that is initially associated with the wild-type cytotypic, invasion conditions can be found by solving dx_2/dx_2 with $x_1 = x_2 = 0$ and $\omega = x_3^2(1-t) + 2x_3x_4(1-ht) + x_4^2$.

This reveals that for invasion

$$\phi < tx_3[2h(1-x_3) + x_3].$$

This is plotted in Fig. 3. As in the unicellular case, the invasion conditions for the modifier, in terms of its associated cost, become more lenient as the cost of the selfish element increases and its frequency in the population increases. Also, as h increases, invasion when the selfish element is at low frequency becomes possible at a lower cost (ϕ).

For both the multicellular and the unicellular cases, simulations were carried out showing that if the modifier can invade, it can only go to fixation and hence eliminate the selfish element if it is costless. Otherwise, both the modifier and the selfish element are retained in the population in stable equilibrium.

Model 2 – invasion conditions for a 'kill off your own cytoplasm' modifier

Again, we now consider the invasion of a modifier (M) that enforces uniparental inheritance by destroying the cytoplasm of the gamete it is in. The costs associated with the modifier are the same as those discussed for the unicellular case. The frequencies of the genotypes MA , Ma , mA and ma are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation are given in Appendix 2. For the

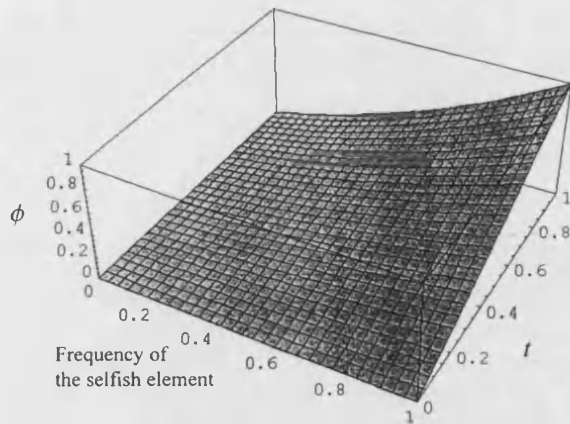


Fig. 3 Invasion conditions for the 'kill off the partner's cytoplasm' modifier in the multicellular case, when it comes in initially associated with the wild-type cytotype. The selfish cytotype's replicative advantage, k , is arbitrarily set to 0.8 and $h = k^{\circ}$ (see discussion in the text). For invasion, the cost of the modifier (ϕ) must lie beneath the sheet. Hence, as the cost of the selfish cytotype increases, the modifier can have a larger associated cost and still invade. As in the unicellular case, invasion of the modifier is possible with a higher associated cost as the frequency of the selfish element increases.

condition that M is initially rare, these recursion equations, after linearization resolve to

$$x'_1 = \frac{(1-t)(1-\phi)(x_1x_3 + x_3x_3)}{\varpi}$$

$$x'_2 = \frac{(1-\phi)(x_1x_4 + x_2x_4)}{\varpi}$$

$$x'_3 = \frac{x_3[(1-\phi)(1-t)(x_1 + x_2) + (1-t)x_3 + 2k(1-h)x_4]}{\varpi}$$

$$x'_4 = \frac{x_4[(1-\phi)x_1 + (1-\phi)x_2 + 2(1-k)(1-h)x_3 + x_4]}{\varpi}$$

where ϖ is the sum of the numerators.

For invasion, the leading eigenvalue of these equations must be greater than one. Hence, for invasion of the modifier,

$$\phi < \frac{tx_3[(2h-1)(x_3-1)]}{tx_3-1}$$

This is plotted in Fig. 4. Even if the population is polymorphic for a selfish cytotype with a massive replicative advantage and substantial cost, the invasion conditions of the modifier can only tolerate a minimal cost. This is because the full cost of the selfish element is masked in heteroplasmic individuals.

Note that for invasion $h > 0.5$, so the fitness of heteroplasmic individuals must be less than half that of individuals which have only the selfish cytotype. To take $h = k^{\circ}$ as an illustrative example, solving $k^{\circ} = 0.5$ reveals that the replicative advantage of the selfish cytotype necessary for invasion of the modifier must be very large

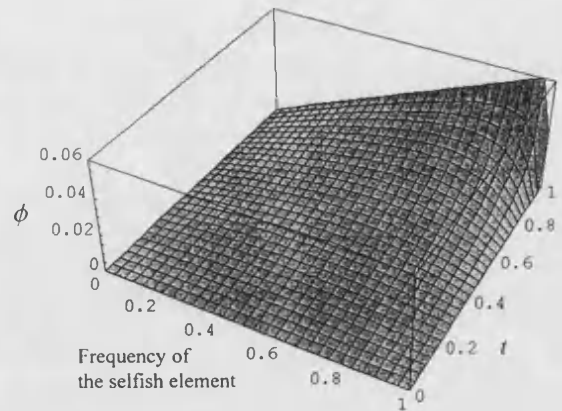


Fig. 4 Invasion conditions for the 'kill off your own cytoplasm' modifier in the multicellular case. The selfish cytotype's replicative advantage (k) has been set to 0.9, which is the minimum permissible for invasion of the modifier. For invasion, the cost of the modifier (ϕ) must lie below the sheet. As with the unicellular case, invasion is possible at a higher cost as the cost of the selfish cytotype (t) increases. Also, invasion conditions are more lenient at intermediate frequencies of the selfish cytotype because the modifier is both likely to be associated with the selfish element and likely to be able to mate with a wild-type individual. Note that the invasion conditions are much more stringent for the 'kill off your own cytoplasm' modifier than for the 'kill off the partner's cytoplasm' modifier.

($k \geq 0.9$). Invasion is unlikely at both low and high frequencies of the selfish cytotype. If the selfish element is uncommon then it exists mainly in heteroplasmic individuals, so with its cost (t) masked, the advantages of modification are not great. Conversely, if the selfish element is common then the chances of gaining the benefits of modification by mating with an individual with the wild-type cytotype are slim.

Simulation results

Even if invasion is possible, simulation revealed that the persistence of the modifier is unlikely. Under conditions for invasion, the replicative advantage of the selfish cytotype is so great that it rapidly goes to fixation. The modifier does initially increase while the population is polymorphic but only very slightly, and as before is rapidly lost when the selfish cytotype reaches fixation if it has any associated costs.

To summarize the results of the analyses carried out so far, it seems that the 'kill off the partner's cytoplasm' model for enforcing uniparental inheritance is by far the more robust in both the unicellular and the multicellular cases. It will tolerate a cost associated with the modifier and does not require successive invasions of selfish cytoplasmic elements to be maintained in the population. Also, invasion of the 'kill off your own cytoplasm' modifier does not seem any more likely in the multicellular case. Hence, this analysis is not able to explain the association between multicellularity and anisogamy.

The effect of leakage on the 'kill off the partner's cytoplasm' model

The 'kill off the partner's cytoplasm' modifier spreads in the population by effectively hitch-hiking with the 'nonselfish' cytotype. This is possible because provided the modifier originates with the better cytotype, it remains in linkage disequilibrium with that cytotype. However, in many cases some degree of biparental inheritance of cytoplasmic elements has been shown to occur (see references in Birky (1995) and Mason *et al.* (1994)). Our preliminary conclusion that the 'kill off the partner's cytoplasm' model is the more robust can therefore only be justified if the modifier can still spread while the assumption of perfect linkage is relaxed.

The 'kill off the partner's cytoplasm' model was altered to incorporate some degree of cytoplasmic leakage from the 'paternal' gamete (i.e. the one whose

The unicellular case

The model is identical to the 'kill off the partner's cytoplasm' model in a unicellular population discussed earlier, except that there is some degree of cytoplasmic leakage into the zygote from the *m* gamete in *Mm* matings. The frequencies of the genotypes *MA*, *Ma*, *mA* and *ma* are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation are given in Appendix 2. After linearization these resolve to

$$\begin{aligned}x'_1 &= \frac{(1-t)(1-\phi)[x_1x_3 + x_1x_4g + x_2x_3f]}{\varpi} \\x'_2 &= \frac{(1-\phi)[x_2x_3(1-f) + x_1x_4(1-g) + x_2x_4]}{\varpi} \\x'_3 &= \frac{(1-t)[(1-\phi)(x_1x_3 + x_1x_4g + x_2x_3f) + x_3x_3 + 2kx_3x_4]}{\varpi}\end{aligned}$$

$$x'_4 = \frac{(1-\phi)(x_2x_3(1-f) + x_1x_4(1-g) + x_2x_4) + 2x_3x_4(1-k) + x_4x_4}{\varpi}$$

cytoplasm is destroyed). The parameter l describes the proportion of cytoplasm in the zygote derived from the 'paternal' gamete (hence, $l=0.5$ corresponds to

where ϖ is the sum of the numerators.

For invasion, the leading eigenvalue of these equations must be > 1 . Hence, for invasion of the modifier,

$$\frac{(1-\phi)g((t-1)(\phi + \varpi - 1)(x_3 - 1) + (1-\phi)(f-1)(t-1)x_3 + \varpi(1 + x_3 - fx_3 - tx_3))}{\varpi^2} > 1.$$

biparental inheritance). Again, this will result in the production of heteroplasmic zygotes. As before, we assume that the replicative advantage of the selfish cytotype results in a higher proportion of gametes containing the selfish element than was present in the zygote initially. When cytoplasmic inheritance is biparental, and the input frequency of the selfish element is 0.5, the output frequency is k . Similarly, when the input frequency is l , the over-representation of the *A* cytotype in the products of *mA/Ma* matings and *MA/ma* matings is described by the parameters f and g , respectively, where

$$\begin{aligned}f &= \frac{lk}{lk + (1-l)(1-k)} \\g &= \frac{k(1-l)}{k(1-l) + l(1-k)}.\end{aligned}$$

It was convenient to use two parameters to describe the over-representation of the leaked selfish cytotype so that we could discuss a single parameter (l) to represent leakage, whether it is the *A* or the *a* cytotype that is being leaked. If leakage is expressed in terms of the input frequency of the selfish cytotype then $g=f$.

Solving for ϕ produces an expression that is too complicated to reproduce. However, the invasion conditions are represented graphically in Fig. 5. These show that invasion is possible despite some degree of leakage; however, as leakage increases, the invasion conditions become more stringent with respect to the cost of the modifier (ϕ). Also, as the cost of the selfish element increases, invasion is permitted at higher values of ϕ .

The multicellular case

The model is identical to the 'kill off the partner's cytoplasm' model in a multicellular population discussed earlier, except that there is some degree of cytoplasmic leakage into the zygote from the *m* gamete in *Mm* matings. Since this leakage alters the relative proportions of the *a* and *A* cytotypes in the resulting progeny, it will also affect their fitness. Hence, for example, if an individual has a proportion g cells with the *A* cytotype, its fitness is $1 - g^r t$. This is analogous to $1 - k^r t$ in the multicellular case without leakage. The frequencies of the genotypes *MA*, *Ma*, *mA* and *ma* are x_1 , x_2 , x_3 and x_4 , respectively. The recursion equations for the frequency of each genotype in the next generation

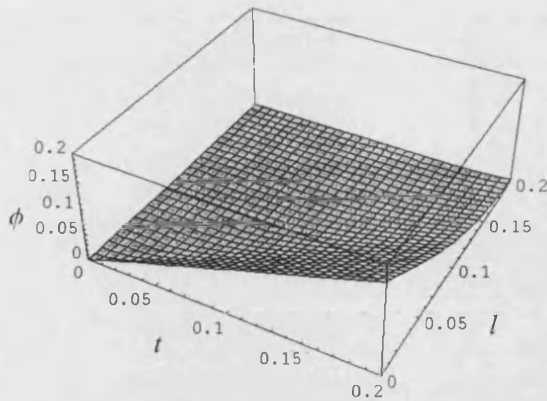


Fig. 5 Invasion conditions for the 'kill off the partner's cytoplasm' modifier in the unicellular case with leakage. The replicative advantage of the selfish element (k) has been arbitrarily set to 0.65. In this case, the cost of selfish cytotype (t) cannot be greater than 0.2 or the selfish element cannot invade. Also, the frequency of the mA genotype in the population (x^3) has been arbitrarily set to 0.8. For invasion, the cost of the modifier (ϕ) must lie beneath the sheet. It is therefore evident that as the degree of leakage decreases and the cost of the selfish cytotype increases, invasion conditions become less stringent with respect to cost.

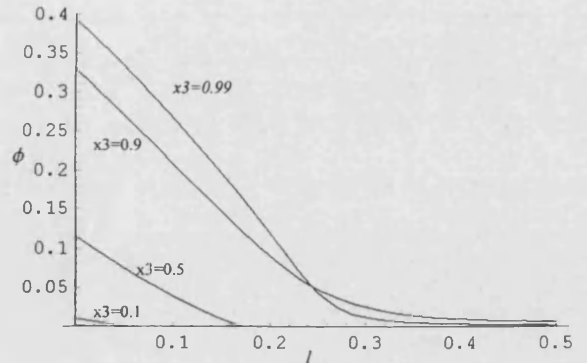


Fig. 6 Invasion conditions for the 'kill off the partner's cytoplasm' modifier in the multicellular case with leakage. The replicative advantage of the selfish element (k) has been arbitrarily set to 0.65, and its cost (t) has been set to 0.4. Each line represents invasion conditions for different frequencies of the selfish cytotype in the population (i.e. different x_3 values). For invasion, the cost of the modifier (ϕ) must lie below the line. Hence, it is evident that as leakage increases, the range of ϕ -values tolerated decreases. Also, as the frequency of the selfish cytotype increases, the cost tolerated increases. This is because as the selfish cytotype becomes more common, the average fitness of the population decreases. Hence, the relative fitness of the Ma genotype increases. So despite the breakdown of linkage disequilibrium by leakage, the modifier's advantage increases as the frequency of the selfish cytotype increases.

are given in Appendix 2. After linearization these resolve to

$$\begin{aligned}
 x'_1 &= \frac{(1-\phi)[x_1x_3(1-t) + x_1x_4g(1-g''t) + x_2x_3f(1-f''t)]}{\varpi} \\
 x'_2 &= \frac{(1-\phi)[x_2x_3(1-f)(1-f''t) + x_1x_4(1-g)(1-g''t) + x_2x_4]}{\varpi} \\
 x'_3 &= \frac{(1-t)(1-\phi)x_1x_3 + x_1x_4g(1-g''t)(1-\phi) + x_2x_3f(1-f''t)(1-\phi) + (1-t)x_3x_3 + 2k(1-ht)x_3x_4}{\varpi} \\
 x'_4 &= \frac{x_2x_3(1-\phi)(1-f)(1-f''t) + x_1x_4(1-g)(1-g''t)(1-\phi) + x_2x_4(1-\phi) + 2x_3x_4(1-k)(1-ht) + x_4x_4}{\varpi}
 \end{aligned}$$

where ϖ is the sum of the numerators.

For invasion, the leading eigenvalue of these equations must be >1 . Hence, for invasion of the modifier,

$$1 < \frac{(\phi-1)^2fx_3(g-1)(f''t-1)(g''t-1) - \varpi(\phi-1)(g+g''t(x_3-1) + x_3 - gx_3 - tx_3)}{\varpi^2} - \frac{\varpi(\phi-1)(1-fx_3 - f''tx_3 + f''+1tx_3) - (\phi-1)^2(g+g''t(x_3-1) + x_3 - gx_3 - tx_3)(1-f''tx_3 + f''+1tx_3)}{\varpi^2}$$

Solving for ϕ produces an expression that is too complicated to reproduce. However, the invasion conditions are represented graphically in Fig. 6. As in the unicellular case, a higher cost of the modifier (ϕ) is permitted as leakage decreases and the cost of the selfish element (t) increases. The absolute value of leakage that still permits invasion is higher than in the unicellular

case, especially if the selfish element is at high frequency. This is because the fitness consequences for a modifier that becomes associated with the selfish element by

leakage are more severe in the unicellular case. These modifiers will suffer the full cost of the selfish element (t), whereas in a heteroplasmic multicellular organism this cost is masked and the selectively favoured Ma genotype can be recovered when gametes are formed. Hence, it seems that the 'kill off the partner's cytoplasm' model is more tolerant of leakage in the multicellular case.

Comparison of the unicellular and multicellular cases by simulation

The spread of the 'kill off the partner's cytoplasm' modifier was investigated by simulation. In the results presented below, the cost suffered by zygotes *MM* matings (s) is not considered so the only cost to the modifier is the cost of modification (ϕ). The inclusion of the s parameter did not change the results qualitatively.

In the multicellular case, the modifier was able to remain in the population over a larger range of parameter values than in the unicellular case (see Fig. 7). This is in part due to the more lenient invasion conditions for the selfish cytotype in the multicellular case. However, the selfish cytotype and the modifier can reach an internal equilibrium at lower values of the modifier's associated cost (ϕ) when the population is multicellular. As discussed, this is due to the modifier experiencing less deleterious selection in heteroplasmic individuals while still being able to recreate the favoured *Ma* genotype.

The 'kill off the partner's cytoplasm' model also appears to be more robust to leakage in the multicellular case (Fig. 8), although conditions for spread in a unicellular population will tolerate some degree of leakage. Hence, this analysis reinforces the conclusion that of the two models for achieving uniparental inheritance, the 'kill off the partner's cytoplasm' model is by far the more robust.

Discussion

There are two broad classes of model that invoke a conflict of interests between the nuclear and cytoplasmic genomes as the driving force behind the evolution of uniparental inheritance. One class is based on cytoplasmic mixing being detrimental to host fitness *per se* due to negative synergistic interactions between cytoplasmic elements (Hurst, 1990; Hurst & Hamilton, 1992; Frank, 1996). Under these conditions, any nuclear mechanism to avoid cytoplasmic mixing will be favoured. Although evidence for negative synergistic fitness effects is sparse, we are aware of one suggestive case. Ziegler & Davidson (1981) carried out interspecific somatic cell hybridizations between Chinese hamster and mouse cell lines. In crosses where the mitochondria were left intact, the hybrids grew poorly or degenerated after a short time. However, in crosses where the hamster parent cells were pretreated with the mitochondria-specific dye rhodamine-6G (R6G), nearly all hybrids grew vigorously. The effect of R6G pretreatment is to block transmission of cytoplasmic determinants to hybrid cells, so the enhanced growth effect observed may have been due to a prevention of the synergism between mitochondrial determinants.

The second class of conflict models explains uniparental inheritance as a nuclear defence against fast-replicating cytoplasmic elements, be they symbionts or

organelles. Following Law & Hutson (1992), we model the invasion of a modifier that enforces uniparental inheritance by killing off the cytoplasm of its own gamete just prior to gametic fusion. In a population of either unicellular or multicellular organisms, the modifier can only invade if the population is polymorphic for a selfish cytotype. This is because when the selfish cytotype (i.e. one with a deletion in its genome) is at fixation there is no advantage to uniparental inheritance. In a polymorphic population, whenever the modifier is associated with the selfish cytotype it improves its fitness in the next generation by breaking this association. However, this advantage is temporary because the modifier does not stay in linkage disequilibrium with the wild-type cytotype. As a consequence, it does not impose much of a check on the spread of the selfish element, which rapidly goes to fixation. Once this happens, the modifier stops increasing because there is now no advantage to uniparental inheritance. At this point, if there is any cost to the modifier (i.e. $s > 0$ or $\phi > 0$), it is lost from the population.

As with the previous model, recurrent invasions of selfish cytoplasmic elements are necessary to raise the modifier to an appreciable frequency in the population. We show that if any cost is attached to the modifier, then these invasions must occur at regular intervals simply for the modifier to remain in the population. This necessity for frequent re-invasion would seem to weaken the model. By contrast, the 'kill off the partner's cytoplasm' model predicts maintenance of a costly modifier in stable polymorphism. This model would therefore seem to be the more robust.

Previous authors have noted an association between anisogamy and multicellularity, implying that perhaps we should expect the 'kill off your own cytoplasm' model to be more robust in the multicellular case. Conditions for invasion in a multicellular population, however, appear to be even more stringent. This is because heteroplasmic individuals only suffer a significant fitness cost when the proportion of the selfish cytotype relative to the wild-type is very large (i.e. when the replicative advantage of the selfish element is large). For the selfish element to impose an appreciable cost on individuals from *Aa* matings (and hence for the modifier to invade), the replicative advantage of the selfish element and its associated cost must be very high. However, under these conditions the selfish element rapidly goes to fixation and the modifier only reaches a low frequency.

Our analysis suggests that of the two mechanisms of achieving uniparental inheritance by the conflict model (excluding negative synergistic effects of cytoplasmic elements), the only robust model is that which involves a 'kill off the partner's cytoplasm' type modifier. This modifier gains its advantage by remaining in linkage disequilibrium with the wild-type cytotype. It is therefore able to spread by hitch-hiking with the nonselfish cytotype. Although this model would seem to have

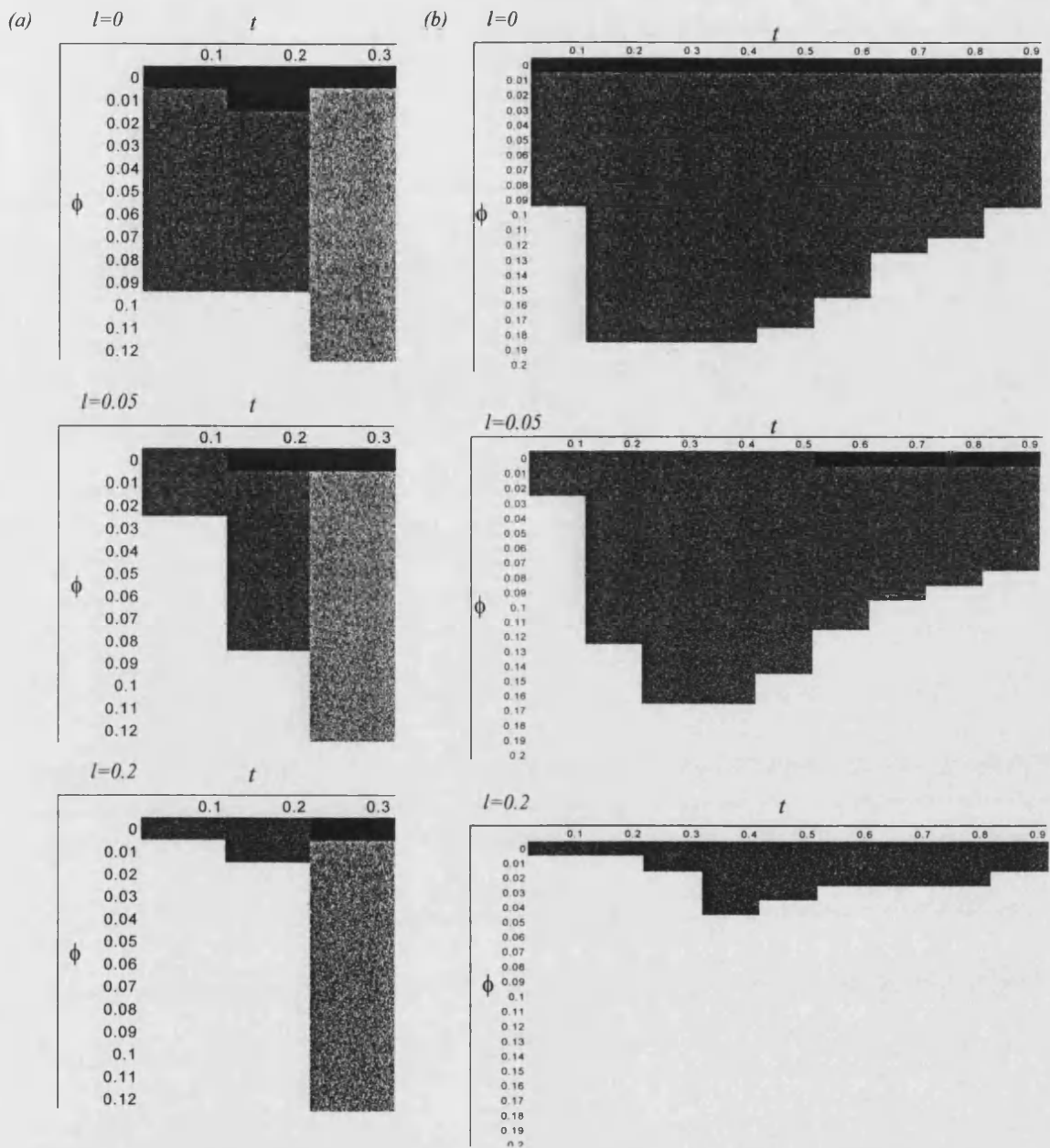


Fig. 7 Representation of simulation for the 'kill off the partner's cytoplasm' model with leakage in the (a) unicellular and (b) multicellular cases. The modifier was started off in linkage disequilibrium with the wild-type cytoplasm. Combinations of different values of ϕ and t were simulated at three leakage values ($l=0, 0.05, 0.2$) and other parameters were set to $k=0.65$ and $h=k^6$. Four possible outcomes are represented: noninvasion of the selfish cytoplasm, and hence the modifier (shown in light grey); invasion of the selfish cytoplasm, but not the modifier (shown in white); invasion of both, followed by loss of the selfish cytoplasm and loss or retention of the modifier depending on whether it has an associated cost (shown in black). Persistence of the modifier and the selfish cytoplasm in the population after 200 000 generations (shown in dark grey).

difficulty explaining the evolution of anisogamy, its predictions are met in the case of uniparental inheritance in *C. reinhardtii* (Sears & VanWinkle-Swift, 1994). As

predicted, the gene products responsible for destruction of the chloroplast DNA from the - type parent are encoded by genes from the + type.

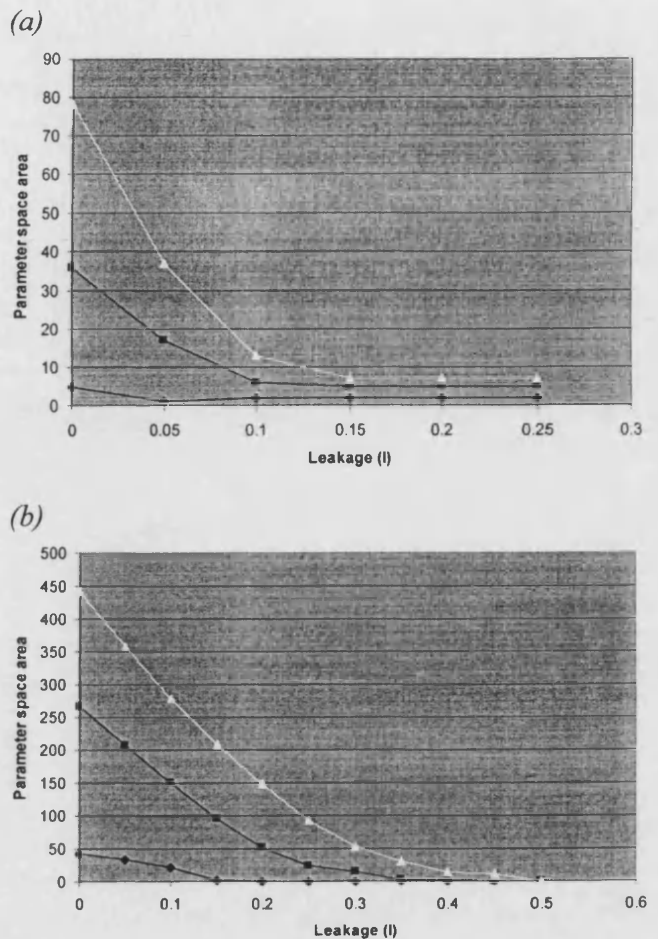


Fig. 8 Representation of robustness to leakage in the (a) unicellular and (b) multicellular cases. Parameter space area for a range of l and ϕ -values in which both the modifier and the selfish cytotypic are retained in the population after 200 000 generations are quantified for different leakage values. The three plots on each graph represent different values of k ($k = 0.55$ – diamonds, $k = 0.65$ – squares, $k = 0.75$ – triangles). In the multicellular case, the higher absolute number of these outcomes is due to the greater range of parameter space over which the selfish cytotypic can invade. However, the more rapid collapse of the model in the unicellular case with increasing leakage suggests that the ‘kill off the partner’s cytoplasm’ model is less robust to leakage in the unicellular case.

We examined the robustness of the ‘kill off the partner’s cytoplasm’ model further by allowing this linkage disequilibrium to be broken down by cytoplasmic leakage from the ‘paternal’ gamete (i.e. the one whose cytoplasm is normally destroyed). As previously shown (Hastings, 1992) the model will tolerate some leakage without breaking down, but it seems that the invasion conditions are more robust in the multicellular case than in the unicellular case. This is because the fitness of a multicellular organism that is heteroplasmic is intermediate between the fitness of individuals with only the wild-type cytotypic and those with only the selfish cytotypic. Hence, becoming associated with the selfish cytotypic by leakage has less severe fitness consequences for the modifier in the multicellular case than in the unicellular case, and the selectively favoured *Ma* genotype can be recovered at the gametic stage.

Given the variety of mechanisms for achieving uniparental inheritance in the natural world, must this branch of the ‘Conflict Hypothesis’ therefore be rejected as a general explanation for uniparental inheritance? The

conflict model may be capable of explaining this variety, in terms of subsequent evolution in response to the selective pressures set up by the evolution of a ‘kill off the partner’s cytoplasm’ model of uniparental inheritance. Consider a sexual population with two mating types and some degree of inbreeding. A ‘kill off the partner’s cytoplasm’ modifier is linked to one of the mating type alleles. Such a population is vulnerable to invasion by a cytoplasmic mutant that destroys its own gamete whenever it is in the nontransmitting (‘male’) gamete (in effect causing cytoplasmic male sterility (Cosmides & Tooby, 1981; Hurst *et al.*, 1996)). The mutant has nothing to lose by this because it is not going to be transmitted anyway, but can potentially gain by ensuring that its clonal relatives are never found in less-fit inbred individuals. It can also gain if there is gamete competition by ensuring that its host gamete does not take resources away from transmitting gametes in which it is present. This will lead to selection on the nuclear genes to pre-empt their own destruction by killing off the selfish cytoplasmic element first. One outcome of such an arms race between the

cytoplasmic and nuclear genes is a situation in which the male gamete eliminates as much of its own cytoplasm as possible, as soon as it is no longer needed (by definition a 'kill off your own cytoplasm' modifier). In the case of symbionts that are never needed, one would predict that they are never allowed into sperm cells. Indeed, there is some evidence that this may be the case (Buchner, 1965). In general, though, the argument predicts that organisms with a 'kill off your own cytoplasm' mechanism of uniparental inheritance should be associated either with inbreeding or with gamete competition.

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Appendix 1

List of parameters

- p frequency of the A cytotype.
 q frequency of the a cytotype.
 x_1 frequency of the MA genotype.
 x_2 frequency of the Ma genotype.
 x_3 frequency of the mA genotype.
 x_4 frequency of the ma genotype.
 t fitness cost of possession of the selfish cytotype.
 ϕ fitness cost of modification suffered by Mm gametes.
 s fitness cost associated with extreme cytoplasm depletion suffered by MM gametes.
 h proportion of the fitness cost t suffered by individuals with some wild-type and some selfish cytotype cells.
 n describes the relationship between k and h in the expression $h = k^n$.
 ω the weighted mean fitness of the population.
 l the proportion of 'leaked' cytoplasm in the zygote from the paternal gamete.

$$f = \frac{lk}{lk + (1-l)(1-k)}$$

$$g = \frac{k(1-l)}{k(1-l) + l(1-k)}$$

Appendix 2

Nonlinearized recursion equations for the modifier M in a haploid, sexual, unicellular population (Model 1 - 'kill off the partner's cytoplasm').

$$x'_1 = \frac{(1-t)(1-\phi)[x_1x_3 + x_1x_4 + x_1x_1(1-s) + 2kx_1x_2(1-s)]}{\omega}$$

$$x'_2 = \frac{(1-\phi)[x_2x_3 + x_2x_4 + x_2x_2(1-s) + 2x_1x_2(1-s)(1-k)]}{\omega}$$

$$x'_3 = \frac{(1-t)[(1-\phi)(x_1x_3 + x_1x_4) + x_3x_3 + 2kx_3x_4]}{\omega}$$

$$x'_4 = \frac{(1-\phi)(x_2x_3 + x_2x_4) + 2x_3x_4(1-k) + x_4x_4}{\omega}$$

Linearized recursion equations (i.e. eliminating terms in x_1x_1 , x_2x_2 and x_1x_2 because the modifier is assumed to be infinitely rare):

$$x'_1 = \frac{(1-t)(1-\phi)[x_1x_3 + x_1x_4]}{\omega}$$

$$x'_2 = \frac{(1-\phi)[x_2x_3 + x_2x_4]}{\omega}$$

$$x'_3 = \frac{(1-t)[(1-\phi)(x_1x_3 + x_1x_4) + x_3x_3 + 2kx_3x_4]}{\omega}$$

$$x'_4 = \frac{(1-\phi)(x_2x_3 + x_2x_4) + 2x_3x_4(1-k) + x_4x_4}{\omega}$$

where ω is the sum of the numerators.

Hence, $\omega = 1 + 2ktx_3(x_3 - 1) - tx_3^2$.

In matrix form, the equations for x'_1 and x'_2 can be represented as follows:

$$\begin{pmatrix} x'_1 \\ x'_2 \end{pmatrix} = \begin{pmatrix} a & b \\ c & d \end{pmatrix} \begin{pmatrix} x_1 \\ x_2 \end{pmatrix}$$

where

$$a = (1-t)(1-\phi)x_3$$

$$b = (1-t)(1-\phi)x_4$$

$$c = (1-\phi)x_3$$

$$d = (1-\phi)x_4$$

Equations x'_1 and x'_2 can also be represented as follows:

$$\begin{pmatrix} x'_1 \\ x'_2 \end{pmatrix} = \lambda \begin{pmatrix} x_1 \\ x_2 \end{pmatrix}$$

where λ is the leading eigenvalue of equations x'_1 and x'_2 :

$$\lambda = a + b - ad + bc$$

In this case,

$$\lambda = \frac{(\phi-1)(\phi(t-1)-1+2ktx_3(t-2)(x_3-1)+2tx_3^2-t^2x_3^2)}{(1+2kt(x_3-1)x_3-tx_3^2)^2}$$

For invasion of the modifier, $\lambda > 1$. So the invasion conditions are

$$\phi < tx_3[2k(1-x_3) + x_3].$$

Nonlinearized recursion equations for the modifier M in a haploid, sexual, multicellular population (Model 1 - 'kill off the partner's cytoplasm').

$$x'_1 = \frac{(1-\phi)[(1-t)(x_1x_3 + x_1x_4 + x_1x_1(1-s)) + 2kx_1x_2(1-s)(1-h)]}{\omega}$$

$$x'_2 = \frac{(1-\phi)[x_2x_3 + x_2x_4 + x_2x_2(1-s) + 2x_1x_2(1-s)(1-k)(1-h)]}{\omega}$$

$$x'_3 = \frac{(1-t)(1-\phi)x_1(x_3 + x_4) + (1-t)x_3x_3 + 2k(1-h)x_3x_4}{\omega}$$

$$x'_4 = \frac{(1-\phi)(x_2x_3 + x_2x_4) + 2x_3x_4(1-k)(1-h) + x_4x_4}{\omega}$$

Nonlinearized recursion equations for the modifier M in a haploid, sexual, unicellular population (Model 2 - 'kill off your own cytoplasm').

$$x'_1 = \frac{(1-t)(1-\phi)[(1-s)(x_1x_1 + 2kx_1x_2) + x_1x_3 + x_2x_3]}{\omega}$$

$$x'_2 = \frac{(1-\phi)[2x_1x_2(1-s)(1-k) + x_2x_2(1-s) + x_1x_4 + x_2x_4]}{\omega}$$

$$x'_3 = \frac{(1-t)x_3[(1-\phi)(x_1 + x_2) + x_3 + 2kx_4]}{\omega}$$

$$x'_4 = \frac{x_4[(1-\phi)(x_1 + x_2) + 2(1-k)x_3 + x_4]}{\omega}$$

Nonlinearized recursion equations for the modifier M in a haploid, sexual, multicellular population (Model 2 - 'kill off your own cytoplasm').

$$\begin{aligned}
 x'_1 &= \frac{(1-\phi)[x_1x_1(1-s)(1-t) + 2kx_1x_2(1-h)t] + (1-t)(x_1x_3 + x_3x_3)}{\bar{w}} \\
 x'_2 &= \frac{(1-\phi)[2x_1x_2(1-s)(1-k)(1-h)t + x_2x_2(1-s) + x_1x_4 + x_2x_4]}{\bar{w}} \\
 x'_3 &= \frac{x_3[(1-\phi)(1-t)x_1 + (1-\phi)(1-t)x_2 + (1-t)x_3 + 2k(1-h)t x_4]}{\bar{w}} \\
 x'_4 &= \frac{x_4[(1-\phi)x_1 + (1-\phi)x_2 + 2(1-k)(1-h)t x_3 + x_4]}{\bar{w}}.
 \end{aligned}$$

Nonlinearized recursion equations for the modifier M in a haploid, sexual, unicellular population (Model 1 - 'kill off the partner's cytoplasm', with leakage).

$$\begin{aligned}
 x'_1 &= \frac{(1-t)(1-\phi)[x_1x_3 + x_1x_4g + x_2x_3f + x_1x_1(1-s) + 2kx_1x_2(1-s)]}{\bar{w}} \\
 x'_2 &= \frac{(1-\phi)[x_2x_3(1-f) + x_1x_4(1-g) + x_2x_4 + x_2x_2(1-s) + 2x_1x_2(1-s)(1-k)]}{\bar{w}} \\
 x'_3 &= \frac{(1-t)[(1-\phi)(x_1x_3 + x_1x_4g + x_2x_3f) + x_3x_3 + 2kx_3x_4]}{\bar{w}} \\
 x'_4 &= \frac{(1-\phi)(x_2x_3(1-f) + x_1x_4(1-g) + x_2x_4) + 2x_3x_4(1-k) + x_4x_4}{\bar{w}}
 \end{aligned}$$

where

$$\begin{aligned}
 f &= \frac{lk}{lk + (1-l)(1-k)} \\
 g &= \frac{k(1-l)}{k(1-l) + l(1-k)}.
 \end{aligned}$$

Nonlinearized recursion equations for the modifier M in a haploid, sexual, multicellular population (Model 1 - 'kill off the partner's cytoplasm').

$$\begin{aligned}
 x'_1 &= \frac{(1-\phi)[x_1x_3(1-t) + x_1x_4g(1-g^n)t + x_2x_3f(1-f^n)t + x_1x_1(1-s)(1-t) + 2kx_1x_2(1-s)(1-h)t]}{\bar{w}} \\
 x'_2 &= \frac{(1-\phi)[x_2x_3(1-f)(1-f^n)t + x_1x_4(1-g)(1-g^n)t + x_2x_4 + x_2x_2(1-s) + 2x_1x_2(1-s)(1-k)(1-h)t]}{\bar{w}} \\
 x'_3 &= \frac{(1-t)(1-\phi)[x_1x_3 + x_1x_4g(1-g^n)t + x_2x_3f(1-f^n)t] + (1-t)x_3x_3 + 2k(1-h)t x_3x_4}{\bar{w}} \\
 x'_4 &= \frac{x_2x_3(1-\phi)(1-f)(1-f^n)t + x_1x_4(1-g)(1-g^n)t + x_2x_4(1-\phi) + 2x_3x_4(1-k)(1-h)t + x_4x_4}{\bar{w}}
 \end{aligned}$$

where

$$\begin{aligned}
 f &= \frac{lk}{lk + (1-l)(1-k)} \\
 g &= \frac{k(1-l)}{k(1-l) + l(1-k)}.
 \end{aligned}$$

Chapter 4. A comparative test of a theory for the evolution of anisogamy

James P. Randerson and Laurence D. Hurst (2001)

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A comparative test of a theory for the evolution of anisogamy

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Why are sperm small and eggs large? The dominant explanation for the evolution of gamete size dimorphism envisages two opposing selection pressures acting on gamete size: small gametes are favoured because many can be produced, whereas large gametes contribute to a large zygote with consequently increased survival chances. This model predicts disruptive selection on gamete size (i.e. selection for anisogamy) if increases in zygote size confer disproportional increases in fitness (at least over part of its size range). It therefore predicts that increases in adult size should be accompanied by stronger selection for anisogamy. Using data from the green algal order Volvocales, we provide the first phylogenetically controlled test of the model's predictions using a published phylogeny and a new phylogeny derived by a different method. The predictions that larger organisms should (i) have a greater degree of gamete dimorphism and (ii) have larger eggs are broadly upheld. However, the results are highly sensitive to the phylogeny and the mode of analysis used.

Keywords: anisogamy; uniparental inheritance; conflict hypothesis; gamete size; Volvocales; Volvocaceae

1. INTRODUCTION

Gametes come in two varieties in many organisms. In some cases they are a different size (i.e. sperm and eggs), but in others the gametes are the same size (i.e. isogametes) but are differentiated into two mating types (plus and minus or α and β). Assuming isogamy is the ancestral condition we can ask why did the gametes change in relative size? A number of theories have been presented (Parker *et al.* 1972; Cosmides & Tooby 1981; Hoekstra 1982; Cox & Sethian 1985; Dusenbery 2000) (for a review see Hoekstra 1987). Here we test the dominant explanation for the evolution of gamete dimorphism (Parker *et al.* 1972; Maynard Smith 1978; Bulmer 1994).

Parker, Baker and Smith's (Parker *et al.* 1972) model (henceforth the PBS model) for the evolution of gamete dimorphism proposes two opposing selection pressures acting on gamete production: the number of gametes produced and the fitness of the resulting zygote (which is assumed to be some function of its size). Gamete number and gamete contribution to zygote fitness necessarily trade off against each other.

A male produces multiple sperm and can therefore fertilize numerous eggs whilst contributing little to the zygote. In this regard, males parasitize the investment in eggs provided by females. However, for disruptive selection on gamete size to occur the PBS model requires that, at least over part of its size range, zygote fitness must increase disproportionately with volume (i.e. an increase in zygote size must confer a more than proportional increase in its fitness). It is suggested that this is more likely in multicellular organisms than in unicellular organisms (Parker *et al.* 1972). In the former, the transition from zygote to complex multicellular organism requires more provisioning, so an increase in zygote size is likely to confer a significant benefit. Conversely, increased provisioning on the part of a unicellular organism need

not lead to a significant increase in zygote fitness (Parker *et al.* 1972; Bell 1985). The PBS model therefore predicts that, as adult size increases, so does selection for anisogamy (Parker *et al.* 1972; Maynard Smith 1978). One should therefore observe a greater degree of anisogamy in larger species.

A further prediction concerns the relationship between egg size and adult size. If a larger zygote brings about a disproportionate gain in fitness in larger species then egg size should also increase with adult size (Bell 1985). Clearly, increased egg size might lead to an increase in the degree of anisogamy; the two could form part of the same prediction. However, this is not necessarily the case. Increased anisogamy could come about by a decrease in sperm size with egg size staying the same.

On the other hand, the PBS model predicts that the degree of gamete dimorphism will be greatest in organisms with the largest zygotes. Therefore, as organisms become larger, not only should their zygotes increase in size, but also a greater proportion of zygote volume should come from the egg. Thus, an increase in egg size with adult size is a strong prediction of the model.

The green algae have been the traditional testing ground for the PBS model (Knowlton 1974; Madsen & Waller 1983; Bell 1985). Members of the group exhibit variation in both size and gamete dimorphism (e.g. *Chlamydomonas reinhardtii* is isogamous and unicellular, whilst the oogamous *Volvox rousetii* has up to 50 000 cells per colony) (see the references in electronic Appendix B available on The Royal Society Web site). Tests of the PBS model's prediction that the degree of anisogamy should increase with adult size have yielded equivocal results. While Knowlton (1974) and Bell (1985) suggested that the pattern may be found in the Volvocales, data accumulated by Madsen & Waller (1983) on pond-living algae indicated numerous exceptions (see also Bell 1978). Furthermore, in agreement with the PBS model, Bell (1985) reported that zygote size increases with adult size in the Volvocales. However, interpretation of all these

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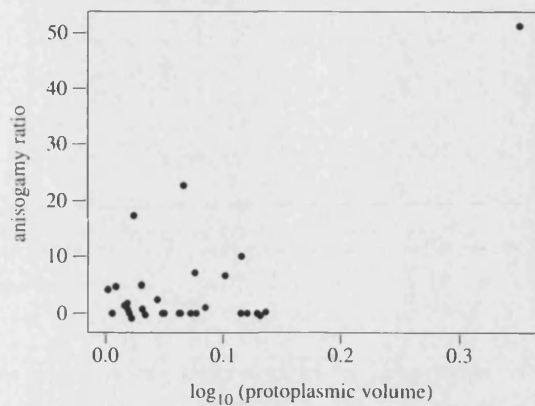


Figure 1. Contrasts in the anisogamy ratio versus contrasts in \log_{10} (protoplasmic volume) using the Coleman (1999) phylogeny (Spearman's $s = -0.002$, $n = 31$ and $p > 0.05$).

results is impossible as in no case was phylogenetic non-independence taken into account. The new phylogeny (Coleman 1999) of the Volvocales now makes the appropriate test possible using the method of independent contrasts (Purvis & Rambaut 1995). Furthermore, we derived our own phylogeny in order to investigate how sensitive the results of this test are to the particular tree used.

2. METHODS

(a) The existing phylogeny

The phylogeny (see electronic Appendix A available on The Royal Society's Web site) is an amalgamation of the two cladograms published by Coleman (1999) using variations in the two internal transcribed spacers (ITS-1 and ITS-2) flanking the 5.8S ribosomal gene. This phylogeny will subsequently be referred to as the Coleman (1999) phylogeny. One of Coleman's

(1999) cladograms was derived using a parsimony method. Given the potential unreliability of this method of tree reconstruction, we derived an alternative phylogeny in order to test the robustness of our comparative tests to the particular tree used.

(b) Our phylogeny

The taxa and GenBank accession numbers used were a subset of those in Coleman's (1999) study. Where that phylogeny supported a monophyletic clade consisting entirely of different isolates of one species, we used a representative member of that species. However, if a species was predicted to be paraphyletic, more than one isolate was used in the analysis. The original phylogeny (Coleman 1999) reported GenBank entry U67020 as being *Pandorina morum* (UTEX871). It is in fact a *Volvox dissipatrix* isolate that appears elsewhere in the phylogeny. We only included this once in our analysis.

The phylogeny contains 44 taxa representing 38 different species. The species represented by more than one different isolate are as follows: *Pleodorina indica* (two isolates), *Eudorina illinoisensis* (two isolates), *P. morum* (four isolates) and *V. dissipatrix* (two isolates). The DNA sequence for alignment was a region encompassing the two internal transcribed spacers (ITS-1 and ITS-2) flanking the 5.8s ribosomal gene. The largest of these sequences was 1273 nucleotides long. The sequences were aligned using CLUSTALX (available at <http://www.hgmp.mrc.ac.uk/Registered/Menu/alphabet.html>). The reason for the length disparity between our sequences and Coleman's (1999) sequences is that we included the RNA gene whereas she did not.

A distance matrix was calculated using TREE-PUZZLE (formerly known as Puzzle). This programme uses quartet puzzling, a maximum-likelihood technique that reconstructs all possible groups of four taxa (quartets) that can be formed from n sequences. These quartet trees then serve as starting points for reconstructing a set of optimal n -taxon trees (Strimmer & Von Haeseler 1996). The distance matrix computed by TREE-PUZZLE was fed into Weighbor (Bruno *et al.* 2000). This is a

Table 1. Summary of results using both phylogenies and the raw data alone

test	raw data (no phylogeny)	Coleman (1999) phylogeny	Weighbor phylogeny
anisogamy ratio versus \log_{10} (protoplasmic volume)	$F = 41.2$, d.f. = 30, $p < 0.001$ and $r^2 = 58.7$ Spearman's $s = 0.77$ and $p < 0.005$	$F = 23.4$, d.f. = 31, $p < 0.001$ and $r^2 = 41.9$ Spearman's $s = -0.002$ and n.s.	$F = 65.9$, d.f. = 36, $p < 0.001$ and $r^2 = 64.3$ Spearman's $s = 0.53$ and $p = 0.005$
\log_{10} (anisogamy ratio) versus \log_{10} (protoplasmic volume)	$F = 35.5$, d.f. = 30, $p < 0.001$ and $r^2 = 55.0$ Spearman's $s = 0.74$ and $p < 0.005$	$F = 0.23$, d.f. = 31, $p = 0.64$ and $r^2 = 0$ Spearman's $s = -0.038$ and n.s.	$F = 13.7$, d.f. = 36, $p = 0.001$ and $r^2 = 26.1$ Spearman's $s = 0.47$ and $p = 0.005$
\log_{10} (egg or isogamete volume) versus \log_{10} (protoplasmic volume)	$F = 29.9$, d.f. = 25, $p < 0.001$ and $r^2 = 55.5$ Spearman's $s = 0.75$ and $p < 0.005$	$F = 13.9$, d.f. = 24, $p = 0.001$ and $r^2 = 34.9$ Spearman's $s = 0.29$ and n.s.	$F = 9.2$, d.f. = 27, $p = 0.005$ and $r^2 = 23.3$ Spearman's $s = 0.44$ and $p < 0.02$
sperm number versus egg number	$F = 49.3$, d.f. = 17, $p < 0.001$ and $r^2 = 75.5$ Spearman's $s = 0.87$ and $p < 0.005$	$F = 19.7$, d.f. = 16, $p < 0.001$ and $r^2 = 53.9$ Spearman's $s = 0.65$ and $p < 0.005$	$F = 17.9$, d.f. = 19, $p = 0.001$ and $r^2 = 47.1$ Spearman's $s = 0.68$ and $p < 0.005$
egg number versus \log_{10} (protoplasmic volume)	$F = 47.4$, d.f. = 25, $p < 0.001$ and $r^2 = 66.4$ Spearman's $s = 0.82$ and $p < 0.005$	$F = 52.1$, d.f. = 26, $p < 0.001$ and $r^2 = 66.3$ Spearman's $s = 0.43$ and $p < 0.02$	$F = 33.0$, d.f. = 30, $p < 0.001$ and $r^2 = 51.6$ Spearman's $s = 0.49$ and $p < 0.005$

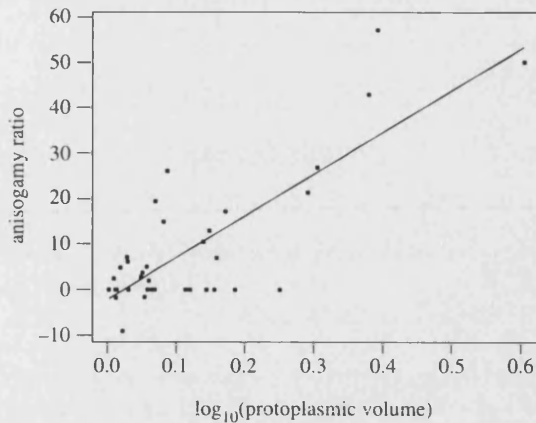


Figure 2. Contrasts in the anisogamy ratio versus contrasts in \log_{10} (protozooplasmic volume) using the Weighbor phylogeny (Spearman's $\rho = 0.47$, $n = 37$ and $p < 0.005$). The regression analysis was highly significant ($F = 65.9$, d.f. = 36, $p < 0.001$ and $r^2 = 64.3$). Furthermore, the intercept was not significantly different from zero ($p = 0.28$).

weighted neighbour-joining method of tree construction that produces quantitatively and qualitatively similar results to maximum-likelihood methods but is much faster. Furthermore, it does not suffer from the problem of long-branch attraction inherent in standard neighbour-joining methods. We used the HKY model of substitution with gamma-distributed rates.

Our phylogeny (henceforth referred to as the Weighbor phylogeny) (see electronic Appendix A) is similar to that reported by Coleman (1999). In many cases groups of species are preserved in a similar topology to the original phylogeny, but the relationships between these blocks are different. Many of the conclusions that Coleman (1999) drew from her phylogeny are also supported here. For example, we also see a split between the four-celled *Gonium* species and those with more than four cells. Furthermore, we also find an association between *Volvulina* species and *P. morum*. Most strikingly, our phylogeny also reinforces the notion that *Volvox* is a paraphyletic genus.

(c) The tests and the data

The PBS hypothesis predicts a positive correlation between contrasts in the degree of anisogamy and contrasts in the amount of provisioning that goes into adult growth (Parker *et al.* 1972). *A priori*, one would expect protozooplasmic volume (i.e. volume of an individual protoplast \times number of cells) to reflect this degree of provisioning most accurately. Using 'colony cell number' as such a measure is vulnerable to variations in cell size and 'colony volume' would include the space enclosed by hollow spherical colonies and so does not truly represent provisioning. Subsequently, we therefore used protozooplasmic volume as our measure of provisioning in the adult. Most cells that were not spherical were prolate spheroids (i.e. a spindle-shaped ellipsoid). If a , b and c are the radii of the three axes, then their volume equals $4/3\pi abc$.

The upper limit of the range was used in the analyses when different literature sources disagreed on the value of a parameter for a particular species. This is the most appropriate measure as it ensures that we are considering mature colonies and not juveniles.

The anisogamy ratio is defined as macrogamete volume divided by microgamete volume. For cases in which this

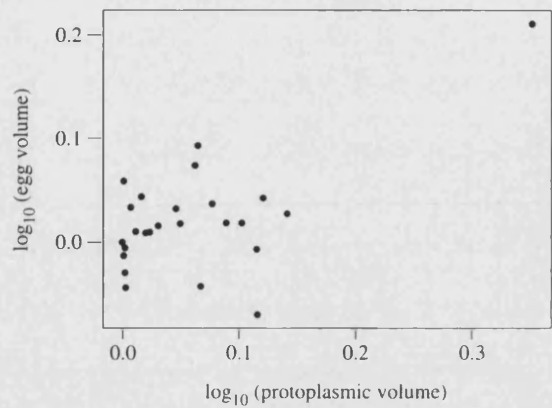


Figure 3. Contrasts in \log_{10} (egg volume or isogamete volume) versus contrasts in \log_{10} (protozooplasmic volume) using the Coleman (1999) phylogeny (Spearman's $\rho = 0.29$, $n = 24$ and $p > 0.05$).

information was not available (particularly in species with sperm packets), the number of sperm in a packet was used as the anisogamy ratio. This assumes that the investment in a sperm packet and in an egg is approximately equal. Hence, if there are 32 sperm in a packet then each one is 1/32 the volume of an egg. While this is not ideal, given that sperm packets appear to be approximately the same size as eggs, we do not believe that this approximation adds a large degree of error.

Both of the model's predictions were tested using the raw data alone and the method of independent contrasts (Purvis & Rambaut 1995) using both phylogenies. We noted that one large contrast in the Coleman (1999) phylogeny had a disproportionate affect on the results (e.g. figure 1). We therefore based our conclusions on non-parametric statistics applied to the data, although the equivalent parametric statistics are reported in table 1.

3. RESULTS

(a) Does anisogamy increase with adult size?

The raw data (not taking phylogenetic history into account) support the PBS model's prediction that \log_{10} (female gamete volume/male gamete volume) should increase with \log_{10} (protozooplasmic volume). The two are very significantly correlated (Spearman's $\rho = 0.77$ and $p < 0.005$) (table 1).

However, when using the Coleman (1999) phylogeny, we found no significant correlation between contrasts in \log_{10} (female gamete volume/male gamete volume) and contrasts in \log_{10} (protozooplasmic volume) (Spearman's $\rho = -0.002$, $n = 31$ and $p > 0.05$) (table 1 and figure 1). Furthermore, this result was not affected by the decision to log the anisogamy ratio before calculating contrasts. There was no significant correlation between the unlogged ratio and \log_{10} (protozooplasmic volume) (Spearman's $\rho = -0.038$, $n = 31$ and $p > 0.05$) (table 1).

However, this result was highly sensitive to the particular phylogeny used in the analysis. When we used the Weighbor phylogeny there was a very significant correlation between contrasts in the anisogamy ratio and contrasts in \log_{10} (protozooplasmic volume) (Spearman's $\rho = 0.47$, $n = 37$ and $p < 0.005$) (table 1 and figure 2). Furthermore, this

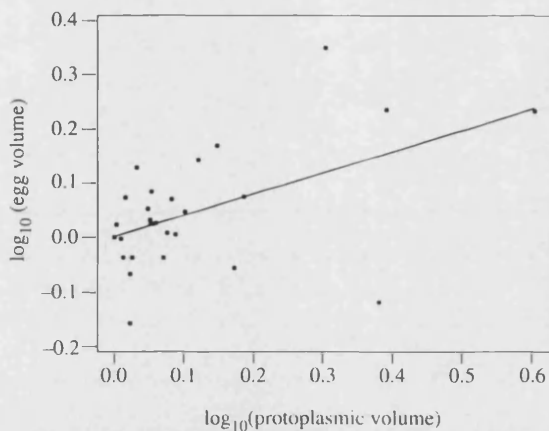


Figure 4. Contrasts in \log_{10} (egg volume or isogamete volume) versus contrasts in \log_{10} (proto-plasmic volume) using the Weighbor phylogeny (Spearman's $\rho = 0.44$, $n = 28$ and $p < 0.02$). The regression analysis was highly significant ($F = 9.2$, d.f. = 27, $p < 0.005$ and $r^2 = 23.3$). Furthermore, the intercept was not significantly different from zero ($p = 0.93$).

was not sensitive to the decision to log the anisogamy ratio (Spearman's $\rho = 0.53$, $n = 37$ and $p < 0.005$) (table 1).

(b) Does egg size increase with adult size?

A further prediction of the PBS model is that zygote size (and, hence, egg size) should increase with adult size. As previously reported by Bell (1985), the raw data support this prediction: \log_{10} (proto-plasmic volume) and \log_{10} (egg or isogamete volume) are significantly correlated (Spearman's $\rho = 0.75$ and $p < 0.005$) (table 1).

However, this result changed when the group's phylogenetic history was taken into account. There was no significant correlation between \log_{10} (egg or isogamete volume) and \log_{10} (proto-plasmic volume) when using the Coleman (1999) phylogeny (Spearman's $\rho = 0.29$, $n = 24$ and $p > 0.05$) (table 1 and figure 3). This result contradicts the prediction of the PBS model. On the other hand, there was a significant correlation between contrasts in egg size and contrasts in \log_{10} (proto-plasmic volume) when we used the Weighbor phylogeny (Spearman's $\rho = 0.44$, $n = 28$ and $p < 0.02$) (table 1 and figure 4).

One possible reason for the discrepancy between the two results is that the Coleman (1999) phylogeny may have reconstructed ancestral nodes incorrectly. Certainly, one would expect both phylogenetic methods to reconstruct the relationships between terminal taxa most accurately.

A different test uses contrasts between these terminal taxa only and, thus, does not rely on correct ancestral node reconstruction. We used this method in order to ascertain what proportion of terminal closest relative species pairs supported the PBS model's hypothesis prediction with respect to egg size. Support was taken to be cases in which both adult size and egg size increased or both decreased.

Where the phylogeny suggested that two species were unambiguously each other's closest relative (e.g. *Astrephomene gubernaculifera* and *Astrephomene perforata*), they were used in the analysis. In other cases, where one species could have multiple others as its closest relative

(e.g. *Volvox obversus* with *Volvox carteri f. nagariensis* and *Volvox carteri f. kawasakiensis*), the second taxon making up the contrast was chosen at random. This analysis yielded 13 species pairs using the Coleman (1999) phylogeny for which there were egg size data. Of these, ten contrasts supported the hypothesis and three did not. Binomial analysis showed that this represents a significant deviation from a random distribution of supporting and non-supporting contrasts ($p = 0.035$).

This result was reinforced by the Weighbor phylogeny. We identified 11 contrasts using the same criteria for node selection as outlined above. Of these, nine supported the hypothesis. This is also a significant deviation from a random distribution ($p = 0.027$).

In summary, our results were highly sensitive to the mode of analysis and the particular phylogeny, although the general trend of the data was in favour of the PBS model. We noted that an incorrect phylogeny would be more likely to destroy rather than create the strongly significant trends that we observed in our data.

4. DISCUSSION

(a) Implications for the PBS model

We report, to the authors' knowledge, on the first phylogenetically controlled tests of the Parker *et al.* (1972) (PBS) model for the evolution of anisogamy. These tests were conducted using a published phylogeny of the Volvocales (Coleman 1999) and a phylogeny constructed using the same sequences but by a different method. Two trees were used in order to give some indication of the robustness of our results to phylogenetic inaccuracy. The results are somewhat equivocal (table 1) depending on the mode of analysis, the statistical tests used and the particular phylogeny, but they suggest acceptance of the PBS model.

Both the raw data and independent contrasts using the Weighbor phylogeny supported both PBS predictions unambiguously (table 1). However, the results from independent contrasts using the Coleman (1999) phylogeny depended on the mode of analysis and statistical tests used. We noted the strikingly different results obtained with the two different trees but suggest that it would be very unlikely for the effects we observed in the Weighbor analysis to have been created by a bad phylogeny. If anything, an incorrect tree would have removed any trend that existed in the data.

(b) Alternative models

While the data were broadly supportive of the PBS model, we believe that it would be unfair to suggest that this model is uniquely capable of explaining the data. Furthermore, it is not clear whether the PBS model would predict the observed pattern in the Volvocales.

Despite their role as the traditional testing ground for the PBS model (Knowlton 1974; Madsen & Waller 1983; Bell 1985), the Volvocales violate a number of its assumptions. First, the PBS model assumes 'broadcast fertilization' (*sensu* Yund 2000), i.e. gametes are released in one event, but individually into the surrounding medium. This is not what happens in the vast majority of Volvocalean species with dimorphic gametes. All but one of the species in our data set with different sized gametes release sperm in packets (see the references in electronic

Appendix B). The typical sequence of events is as follows (Iyengar & Desikachary 1981). First, colonies of both sexes clump together. This is then followed by the release of sperm packets (as opposed to individual sperm) by males, which contain up to 512 sperm.

Evidence from echinoids suggests a possible adaptive reason for releasing sperm in packets. Sperm tend to be much more short lived than eggs, presumably because of their small size (Pennington 1985; Levitan *et al.* 1991). This effect is reduced to some extent in the sea urchin *Strongylocentrotus franciscanus* if sperm are at high concentration (Levitan *et al.* 1991). The effect is termed the 'respiratory dilution effect' (Chia & Bickell 1983). Dilute sperm consume more oxygen, but the amount of oxygen consumed over the sperm's lifetime is fixed; hence, dilute sperm live longer. Swimming as a group may effectively act to increase the local sperm concentration and, hence, longevity. Adopting a collective size comparable to an egg might also make the sperm less vulnerable to predators.

Each sperm packet (effectively a dwarf male) (Bell 1985, p. 252) swims as a unit until it reaches a female colony. Here, the sperm packet enters the colony, breaking up in the process. The individual sperm then typically fertilize all the eggs therein. Rather than broadcast fertilization, it is perhaps better to regard the Volvocales as 'brooding organisms' (*sensu* Yund 2000). These are organisms in which females retain their eggs so that fertilization occurs internally with sperm that have been released into the medium.

Reproduction by a sperm packet alone may explain the trends in gamete dimorphism and egg size we observe in the Volvocales. If, as females get bigger, they place increased reproductive effort into making more eggs, then gamete dimorphism could result. Since all the sperm in a packet fertilize all the eggs in a female spheroid, males would be expected to increase the number of divisions in the packet in order to ensure that all eggs are fertilized. The data did indeed support an increase in egg number with adult size. There was a significant correlation between contrasts in \log_{10} (protoplasmic volume) and egg number using both phylogenies (Weighor phylogeny, Spearman's $\rho = 0.49$, $n = 31$ and $p < 0.005$ and Coleman phylogeny, Spearman's $\rho = 0.43$, $n = 27$ and $p < 0.02$) (table 1).

The number of sperm in a packet is always 2^N , where N is the number of divisions, whereas this is not the case for egg number (see the references in electronic Appendix B). Hence, as the number of eggs and sperm increases, the inaccuracy in matching the number of eggs and number of sperm increases also, with sperm number tending to overshoot egg number by larger and larger amounts. This effect, combined with the fact that sperm packet size must probably remain within a fairly narrow size range for efficient swimming, will result in increased anisogamy. More sperm making up a similarly sized packet necessarily means smaller sperm.

Clearly, this is unlikely to represent a universal explanation for the evolution of anisogamy that would be applicable to a wide range of groups. We propose it merely as an alternative explanation that could explain the results we have presented. Indeed, as this female-driven hypothesis would predict, contrasts in the number of sperm per packet correlate very strongly with contrasts in the number of eggs to be fertilized (Spearman's $\rho = 0.65$,

$n = 17$ and $p < 0.005$). Bell (1985) reported this finding previously, but he did not take the phylogenetic relationships between species into account.

A second PBS model assumption that is violated by the Volvocales is that of no zygote provisioning or protection post-fertilization. In some species there is the potential for maternal care of the zygote because it is not released into the medium immediately. For example, in *V. c. f. kawasakiensis* the zygotes are retained in the female colony for four days before the colony disintegrates and they are released (Nozaki 1988).

What impact do our findings have on other models of the evolution of anisogamy (for a review see Hoekstra 1987)? One competing set of models attempts to explain the evolution of anisogamy as an adaptation for preventing nuclear-cytoplasmic conflict (Grun 1976; Eberhard 1980; Cosmides & Tooby 1981). The 'conflict hypothesis', as these models have collectively been called, proposes that sperm are small so that they carry a minimal amount of cytoplasm. This ensures uniparental inheritance of cytoplasmic genes (e.g. mitochondria, plastids, bacteria and intracellular symbionts) (Hurst 1990; Hastings 1992; Hurst & Hamilton 1992; Law & Hutson 1992; Frank 1996; Randerson & Hurst 1999).

It is not clear exactly what the conflict hypothesis predicts regarding the relationship between adult size and anisogamy. One argument is that multicellularity might increase the effective recessivity of a deleterious cytoplasmic variant and, hence, lead to selection for nuclear enforcement of uniparental inheritance. However, detailed modelling has shown that this is not the case (Randerson & Hurst 1999).

Another suggestion is that organisms undergoing many rounds of asexual division between sex might be less vulnerable to mitotic 'selfish' cytoplasmic variants (Hastings 1999). This might predict that multicellular organisms should be more vulnerable to 'selfish' variants that would impose selection for uniparental inheritance, although this is not clear. Our results are therefore not particularly informative in assessing the validity of the conflict hypothesis. However, we also note the theoretical difficulties associated with anisogamy as a mechanism for achieving uniparental inheritance (Randerson & Hurst 1999) and the numerous exceptions to the 'rule' that the larger gamete should donate the organelles to the zygote (Reboud & Zeyl 1994).

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An electronic appendix to this paper can be found at <http://www.pubs.royalsoc.ac.uk>.

Appendix 1

Figure 1

Phylogeny of the green algal order Volvocales (Volvocaceae, Chlorophyta). This is an amalgamation of two cladograms derived by Coleman (1999). In that paper, one cladogram contains mainly species in the genus *Volvox* and was constructed using maximum likelihood. The second was constructed using PAUP and contains mainly the other Volvocalean species with some *Volvox*. Amalgamation of the two cladograms was possible due to the appearance of some species in both cladograms. If there were any points of disagreement, the phylogenetic relationship predicted by the maximum likelihood tree was used. Branch-lengths calculated in the original paper were used.

Figure 2

Phylogeny derived using TREE-PUZZLE and Weighbor.

Figure 1

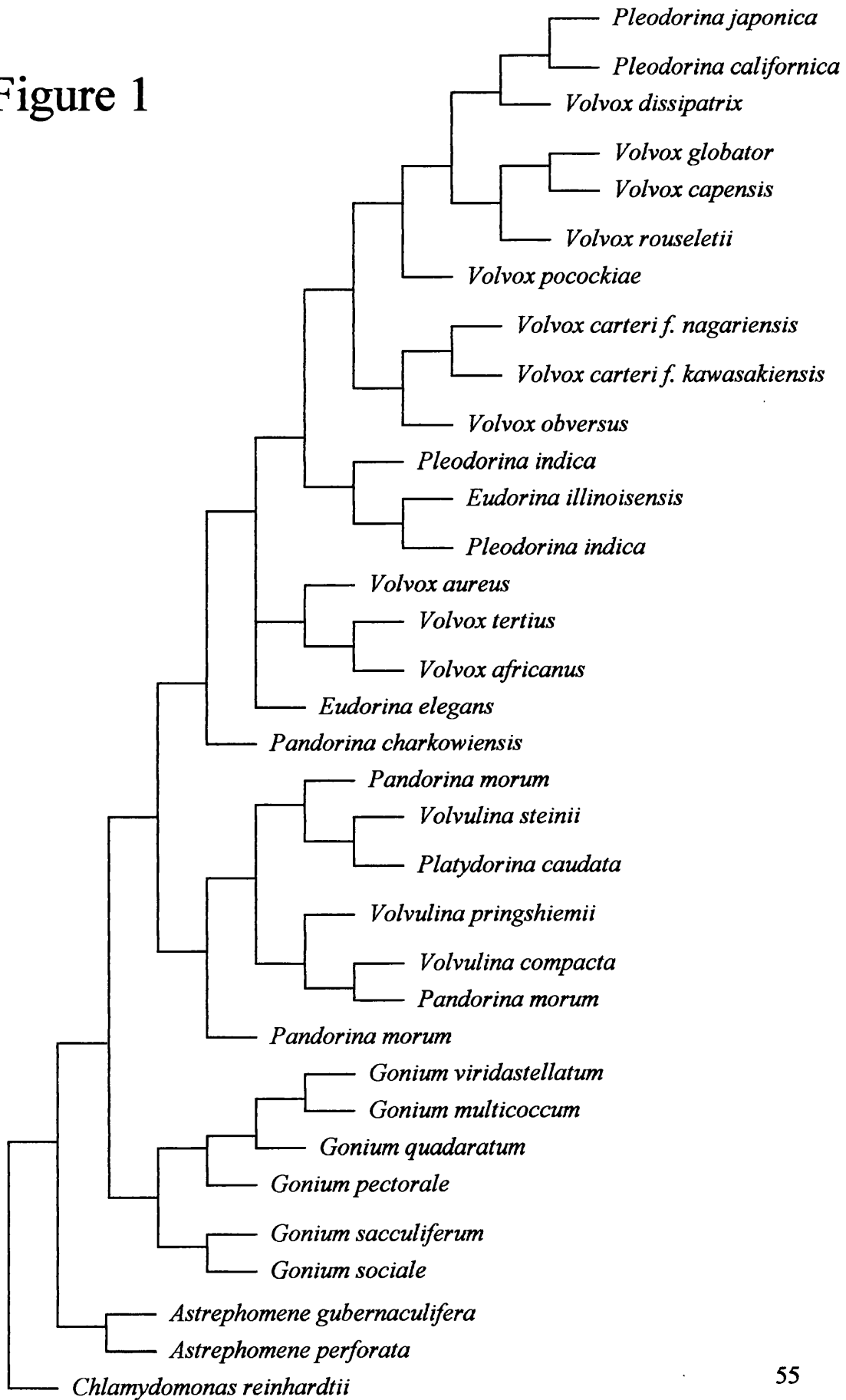
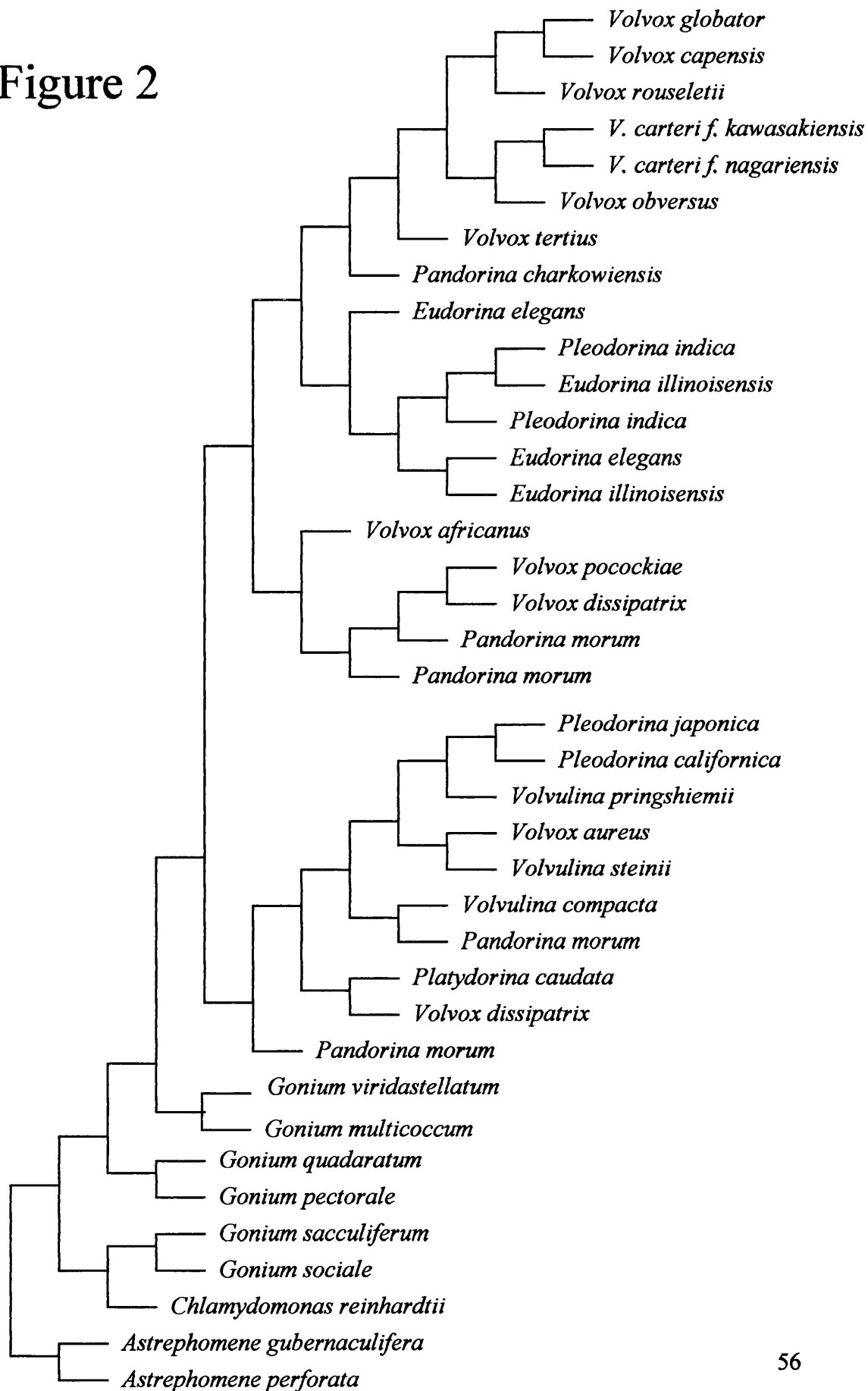


Figure 2



Appendix 2

The data used to calculate contrasts are presented in Table 1. Data on cell number, somatic cell diameter and relative size of sperm and egg for 31 species were gathered from published sources. We detail below how we obtain the measure of colony size (protoplasmic volume).

- Where possible, the upper limit of the range in the literature was used for all measurements (including diameter, cell number and number of sperm in a sperm packet). This is the most appropriate measure as it ensures that we are considering mature colonies and not juveniles.
- Calculating cell volume - Most cells that were not spherical were prolate spheroids (i.e. a spindle-shaped ellipsoid). If a , b and c are the radii of the three axes, then the volume of such a shape is $4/3\pi abc$.
- Colony size - This is defined as the protoplasmic volume of a 'somatic' cell multiplied by the number of cells. Where colonies had one hemisphere with one sized cell and another hemisphere with a different sized cell, it was assumed that half of the cells were of each size.
- Anisogamy ratio - This is defined as macrogamete volume divided by microgamete volume. For cases in which this information was not available (particularly in species with sperm packets), the number of sperm in a packet was used as the anisogamy ratio. This assumes that the investment in a sperm packet and in an egg is roughly equal. Hence if there are 32 sperm in a packet then each one is $1/32$ the volume of an egg.

Table 1

Data used to calculate contrasts in protoplasmic volume and anisogamy ratio. NB the citation in the 'Protoplasmic volume' column refers to the source of the estimate of 'somatic' cell diameter we used.

Table 1.

Species	No. cells	Protoplasmic volume (μm^3)	Classification *	Egg or isogamete volume (μm^3)	Anisogamy ratio
<i>Astrephomene gubernaculifera</i>	128 (Stein 1958b)	6.10×10^6 (Stein 1958b)	I	3.05×10^3	1 (Stein 1958b)
<i>Astrephomene perforata</i>	128 (Nozaki 1983)	4.60×10^5 (Nozaki 1983)	I		1 (Nozaki 1983)
<i>Chlamydomonas reinhardtii</i>	1 (Benson-Evans & Antoine 1996)	8.18×10^3 (Benson-Evans & Antoine 1996)	I	9.05×10^2	1 (Iyengar & Desikachary 1981)
<i>Eudorina elegans</i>	32 (Benson-Evans & Antoine 1996)	2.32×10^5 (Pentecost 1984)	SP	4.19×10^3	64 (West & Fritsch 1927)
<i>Eudorina illinoisensis</i>	32 (Iyengar & Desikachary 1981)	7.19×10^4 (Iyengar & Desikachary 1981)	SP		64 (Fritsch 1935)
<i>Gonium multicocum</i>	32 (Nozaki & Kuroiwa 1991)	9.77×10^4 (Nozaki & Kuroiwa 1991)	I	3.05×10^3	1 (Nozaki & Kuroiwa 1991)
<i>Gonium pectorale</i>	16 (Stein 1958a)	7.56×10^4 (Bold & Wynne 1978)	I	1.50×10^3	1 (Stein 1958a)
<i>Gonium quadratum</i>	16 (Nozaki 1993)	1.12×10^4 (Nozaki 1993)	I	6.97×10^2	1 (Nozaki 1993)
<i>Gonium sacculiferum</i>	4 (Bold & Wynne 1978)	6.02×10^3 (Bold & Wynne 1978)	I		1 (Bold & Wynne 1978)
<i>Gonium sociale</i>	4 (Benson-Evans & Antoine 1996)	1.18×10^4 (Benson-Evans & Antoine 1996)	I	5.24×10^2	1 (Benson-Evans & Antoine 1996)
<i>Gonium viridastellatum</i>	16 (Nozaki 1989)	2.83×10^4 (Nozaki 1989)	I	6.97×10^2	1 (Nozaki 1989)
<i>Pandorina charkowiensis</i>	32 (Iyengar & Desikachary 1981)	2.64×10^5 (Iyengar & Desikachary 1981)	A	3.76×10^3	4.19 (Thompson 1954)
<i>Pandorina morum</i>	32 (Benson-Evans & Antoine 1996)	2.94×10^5 (Bold & Wynne 1978)	I or A	5.24×10^2	1 (Nozaki 1979)
<i>Platydorina caudata</i>	32 (Harris & Starr 1969)	2.62×10^5 (Harris & Starr 1969)	SP	3.05×10^3	32 (Harris & Starr 1969)
<i>Pleodorina californica</i>	128 (Smith 1950)	2.13×10^6 (Fritsch 1935)	SP		128 (West & Fritsch 1927)
<i>Pleodorina indica</i>	64 (Doraiswami 1940)	3.57×10^5 (Doraiswami 1940)	SP	1.15×10^3	64 (Doraiswami 1940)
<i>Pleodorina japonica</i>	128 (Nozaki <i>et al.</i> 1989)	1.04×10^6 (Nozaki <i>et al.</i> 1989)	SP	1.41×10^4	128 (Nozaki <i>et al.</i> 1989)
<i>Volvox africanus</i>	6000 (Iyengar & Desikachary 1981)	9.63×10^5 (Iyengar 1933)	SP	4.77×10^4	128 (Iyengar & Desikachary 1981)
<i>Volvox aureus</i>	3200 (Pentecost 1984)	3.62×10^5 (Pentecost 1984)	SP	7.23×10^3	32 (West 1916)
<i>Volvox capensis</i>	20000 (Pocock 1933c)	4.21×10^7 (Pocock 1933b)	SP	1.25×10^5	512 (Pocock 1933a)
<i>Volvox carteri f. kawaskiensis</i>	3000 (Nozaki 1988)	1.57×10^6 (Nozaki 1988)	SP	1.03×10^4	128 (Nozaki 1988)
<i>Volvox carteri f. nagariensis</i>	8000 (Iyengar & Desikachary 1981)	1.85×10^6 (Iyengar & Desikachary 1981)	SP		128 (Bold & Wynne 1978)
<i>Volvox dissipatrix</i>	31800 (Iyengar & Desikachary 1981)	1.33×10^8 (Iyengar & Desikachary 1981)	SP	3.35×10^4	256 (Iyengar & Desikachary 1981)
<i>Volvox globator</i>	22000 (Iyengar & Desikachary 1981)	7.37×10^5 (Pentecost 1984)	SP	1.10×10^4	256 (Iyengar & Desikachary 1981)
<i>Volvox obversus</i>	4000 (Kam <i>et al.</i> 1974)	2.09×10^6 (Kam <i>et al.</i> 1974)	SP	3.88×10^4	128 (Kam <i>et al.</i> 1974)
<i>Volvox pocockiae</i>	1500 (Starr 1970)	2.65×10^6 (Starr 1970)	SP	1.13×10^5	64 (Starr 1970)
<i>Volvox rouseletii</i>	50000 (West 1916)	1.05×10^8 (Iyengar & Desikachary 1981)	SP	1.41×10^4	512 (Iyengar & Desikachary 1981)
<i>Volvox tertius</i>	2000 (Iyengar & Desikachary 1981)	7.63×10^5 (Iyengar & Desikachary 1981)	SP	6.55×10^5	32 (Iyengar & Desikachary 1981)
<i>Volvolina compacta</i>	16 (Nozaki & Kuroiwa 1990)	6.70×10^4 (Nozaki & Kuroiwa 1990)	I		1 (Nozaki & Kuroiwa 1990)
<i>Volvolina pringsheimii</i>	16 (Starr 1962)	2.83×10^4 (Starr 1962)	I	1.77×10^3	1 (Starr 1962)
<i>Volvolina steinii</i>	32 (Stein 1958b)	7.06×10^4 (Stein 1958b)	I	1.59×10^3	1 (Stein 1958b)

* I = isogamy, A = anisogamy (gamete dimorphism but no sperm packets), SP = reproduction by sperm packet.

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Chapter 5. The uncertain evolution of the sexes

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Abstract

What forces gave rise to the evolution of the size difference between sperm and eggs? For many years, it has been all but accepted wisdom that the answer was laid out by Parker *et al.* However, their model requires an unusual and unverified assumption regarding the relationship between zygote size and fitness. Although the first phylogenetically controlled test of the comparative predictions of the model is consistent, the results have a simple alternative interpretation. Furthermore, recent work has formalised different theoretical frameworks that require less unusual assumptions. These postulate, for example, that, under sperm limitation, a larger egg will have an increased chance of being fertilised, either because its own mass offers a larger target for sperm or because larger eggs can produce a greater quantity of attraction pheromone. Other frameworks either point to small sperm preventing transmission of cytoplasmic symbionts and/or organelles or having a motility advantage. At present, however, no model is capable of offering a universal explanation.

There are many differences between males and females (*e.g.* ornaments, size and fighting ability), but only one is universal, namely the size difference between sperm and eggs. Assuming isogamy to be ancestral, we can ask: why did the gametes change in relative size? This question defines one of the oldest debates in evolutionary biology (see references in Refs. 1 and 2), but has, for many³⁻⁵, been satisfactorily answered by the seminal work of Parker *et al.*⁶ and its subsequent developments⁷⁻¹³. But are the model's assumptions reasonable? More importantly, do the assumptions and the predictions of the theory command empirical support?

We argue that the empirical base for the established model is weak. It makes an unusual and untested assumption, and the limited support for its comparative predictions could have a simple alternative explanation¹⁴. Therefore, it is helpful to ask what other advantages there might be to small sperm and large eggs.

The Parker, Baker and Smith model

Consider an isogamous, externally fertilising species similar to the single-celled green alga, *Chlamydomonas*. Although the gametes are the same size, they occur in two varieties, (plus and minus-types). What forces will drive the evolution of anisogamy in such a population? Parker, Baker and Smith⁶ (the PBS model) propose two selection pressures acting on gamete production: the number of gametes and the fitness of the resulting zygote (assumed to be some function of its size) (Box 1). Imagine a mutant plus-type producing multiple small gametes. Such a mutant can therefore fertilise numerous proto-eggs, whilst contributing little to the zygote. These proto-sperm parasitise the investment provided by proto-eggs.

Although it is easy to see why such a 'cheating' strategy should evolve, it is less obvious how the original large gamete strategy could be maintained in the face of such exploitation. Why should minus-types not also produce large numbers of small gametes? Under many circumstances, this is just what theory predicts. However, PBS identifies the conditions under which gamete dimorphism can be stable. The crux of the PBS model is the relationship between zygote size and fitness. For disruptive selection on gamete size to occur, PBS requires that zygote fitness must increase disproportionately with volume (*i.e.* increments in zygote size must confer more than proportional increments in fitness), at least over part of its size range (Box 1). This assumption is by no means standard^{12,15,16} and we are aware of no direct supportive evidence.

Testing the assumptions and predictions

The evidence that does exist appears to contradict the PBS model's assumption. While the model is vague about the mechanistic basis of disproportionate increases in fitness with zygote size (Box 1), a reduced developmental time could conceivably be a component. However, while larger eggs do develop more quickly (and are hence exposed to reduced predation risk) this does not outweigh the associated loss of fecundity (Box 2).

However, there is indirect evidence in favour of the model. PBS argues that the necessary conditions are more likely to be fulfilled in multicellular organisms than in unicellular organisms⁶. In the former, the transition from zygote to complex multicellular organism requires more provisioning. Hence, zygote size is likely to be crucially important to survival. Conversely, zygote number will be the prime fitness determinant in unicellular organisms^{6,12,17}. The PBS model therefore predicts that, as adult size increases, so does selection for anisogamy^{6,7}. The combination of these two forces (*i.e.* increasing anisogamy and increasing zygote size with adult size) results in a second prediction; namely that egg size is expected to increase with adult size^{14,17}.

The most recent comparative test¹⁴ of the PBS predictions (in the green algal order Volvocales (Box 3)) found in favour of the hypothesis on both counts, although this result is sensitive to the phylogeny used. However, it is unclear whether this group satisfies the PBS assumptions (Box 3). Furthermore, the authors propose a simple, alternative explanation for the observed correlation between the degree of gamete dimorphism and adult size¹⁴ (Box 3). Regarding the second of the PBS predictions, evidence from a variety of broadcast spawners (*i.e.* species in which both sperm and eggs are released into the environment) suggests that egg size does indeed increase with adult size (see references in Refs. 14 and 18). However, there are exceptions¹⁹.

Where does this leave PBS? Given the above qualifications, we clearly cannot assert that PBS provides an adequately supported explanation for the evolution of anisogamy. However, it has other importance. Notably, it indicates a requirement to demonstrate that the advantage to being large is great enough to counter the advantages of making numerous smaller gametes. Simply identifying advantages to small sperm and larger eggs is not, in itself, adequate.

Large egg, large target?

PBS assumes that isogamy evolved under conditions of sperm competition²⁰. By contrast, Levitan argues that ‘anisogamy and copulation evolved because of sperm limitation rather than sperm competition’²¹. If sperm limitation were the ancestral condition, would adaptations that maximise encounter rates between gametes not be expected?

One such adaptation could be increased egg size²² (Box 4), so that sperm have a larger target to hit. Data from three sea urchin species (*Strongylocentrotus* spp.) provide support for this argument. These report a tendency for egg size to correlate negatively with mean sperm concentration^{23,24}. It is suggested that, in these species, divergent demographic conditions have imposed different selective pressures on egg size. In dense populations, where sperm competition is the norm, eggs may be small so that large numbers can be produced (even small eggs have little trouble being found by sperm). Conversely, where populations are less dense (and hence sperm limitation is the norm) eggs may be larger in order to increase fertilisation. Large eggs provide an easier target for sperm to hit²⁵.

Data from the Volvocales however, suggest that an explanation based on fertilisation advantage is unlikely to be general. In most anisogamous members of this group, the adult female spheroid acts as a target for sperm packets rather than the eggs themselves¹⁴. Hence, the above argument does not apply.

We can nonetheless ask whether in broadcast spawners sperm limitation is likely to be the exception or the rule and whether the advantage of higher encounter rates might alone provide an adequate fitness advantage. As regards the first issue, the question is whether an adequate proportion of eggs go unfertilised because of a dearth of sperm. This seems commonly to be the case^{26,27}, particularly in habitats in which turbulent water conditions rapidly dilute gamete plumes²⁸. Indeed, many species exhibit adaptations to reduce sperm limitation (*e.g.* synchronous spawning and aggregation).

As regards the second question, a model of sea urchin fertilisation kinetics²⁹ suggests that higher encounter rates are unlikely to compensate for reduced egg numbers (Box 4). Larger eggs are fitter, because of their fertilisation advantage, but not disproportionately so. Hence, doubling the size of eggs increases their fitness individually, but does not outweigh the fact that there are half as many. This point was made previously by Podolsky and Strathmann³⁰. They showed by a rearrangement of Vogel *et al.*'s model²⁹ that the number of zygotes produced per unit egg material decreases monotonically with increasing egg size, in spite of the fertilisation advantage of larger eggs.

Extra volume for free?

The rigid trade off between egg size and volume depends crucially, however, on the assumption that eggs cannot somehow get volume for free (or at least 'on the cheap'). If this were so, gamete producers could double the size of their eggs (perhaps by hydration), while reducing the number by less than half. But can eggs get volume for free? Podolsky and Strathmann³⁰ note that, among the echinoderms, increased egg size does not generally result in decreased organic concentration. Where it does, the decrease is not sufficient to make up for the loss in fertility caused by reduced egg number. These authors suggest that there may be 'physical constraints' that make it difficult to change the organic concentration of an egg. These include the structural integrity of the egg, enzyme concentration and its effect on reaction kinetics, the mechanics of cell division and the egg storage capacity of the female³⁰.

Data from a range of echinoderm species and covering egg sizes spanning *ca.* five orders of magnitude showed that energy content and dry, ash-free egg weight scale directly with egg volume³¹ (but see Ref. 32). It seems therefore that, at least in one group (Echinodermata), the assumption that volume is a good proxy for investment is met. Consequentially, although Levitan²² identifies an important component of egg fitness, the model is unlikely to provide a complete explanation.

Another adaptation providing 'volume on the cheap' are the jelly coats that surround some externally fertilised eggs³³. Jelly is cheaper to produce than egg mass³⁴ and so may represent an energetically inexpensive means of increasing the target size. If such adaptations are easily produced they may confound any egg size/fertilisation effect³⁰. Farley and Levitan³³ have shown that while jelly coats do

indeed increase fertilisation probability, volume per volume, it is not as effective as egg mass. Hence, prezygotic selection for increased egg size is not eliminated by the presence of a jelly coat.

Pheromone as 'volume on the cheap'

The production of a sperm attraction pheromone is another adaptation that effectively allows 'volume on the cheap'. In this case though, the extra volume is not solid matter, but a zone of attraction around the egg, which increases its probability of fertilisation. Jantzen *et al.*³⁵ show that the advantages of attraction are considerable, giving eggs a greater than 50-fold increase in fertilisation success.

A recent model¹⁶ suggests that pheromone production may solve the anisogamy problem. Dusenbery^{16,36} assumes that the radius of the pheromone sphere is a function of the volume available for pheromone production because rate of production is proportional to egg volume. Hence, the volume of the pheromone sphere is proportional to egg volume cubed. Substituting this as the new egg volume into the modified form of Vogel's *et al.*'s model²⁹ (Eqn III in Box 4) reveals that, over part of its range, there is a more than proportional increase in the fraction of eggs fertilised with egg volume (Fig. 1). Hence, if we accept Dusenbery's relationship between egg volume and pheromone sphere radius, then the increased fertilisation success of larger eggs is not counterbalanced by the associated decrease in fecundity. Larger egg producers are fitter.

How sensitive is this finding to the assumption¹⁶ that pheromone sphere radius is proportional to egg volume? By substituting different relationships between egg volume and pheromone sphere into Eqn III Box 4 we can determine the minimum exponent that will produce a more than proportional increase in pheromone sphere size with egg size. When this exponent is roughly greater than 2 (Fig. 1) then there begins to be a noticeable disproportional fertilisation advantage to larger eggs. If, however, the pheromone cloud volume is a function of egg volume (rather than egg volume squared or cubed) then the model fails. Importantly, Dusenbery's model does not consider the possibility that pheromone production rate can be uncoupled from egg size (perhaps by increased investment in the relevant metabolic pathway). Indeed Jantzen *et al.*³⁵ assert that the strength of the pheromone effect is such that egg size is of lesser importance if pheromone production rates are adjustable.

In the absence of data on the egg volume/pheromone production relationship and its adaptability, the validity of Dusenbery's model is uncertain. Furthermore, as many anisogamous, broadcast spawning taxa do not exhibit pheromonal attraction^{21,37} the generality of any such model is questionable.

Why are sperm small?

There can be little doubt that PBS's trade off between sperm size and number must represent at least one important aspect of a solution to the anisogamy problem. One component of this is a simple numerical advantage when the mutant proto-sperm producer is initially rare (although the correlation between gamete longevity and size should not be ignored²¹). Sperm might be small for other reasons however.

Size and speed

One set of models views anisogamy as an adaptation to maximise gamete encounter rate^{16,38-41}. Most of these models postulate that divergent gamete sizes are selected because of their effect on gamete motility. The importance of sperm swimming speed in fertilisation has been demonstrated in the sea urchin *Lytechinus variegatus*, where faster sperm have a fertilisation advantage under sperm competition⁴². However, speed was shown to trade off against longevity. This suggests that under sperm limited conditions, slow, long-lived sperm may be at an advantage. The presence in many isogamous species of anisomotile gametes^{43,44} also indicates the importance of motility differences. But by equal measure, their existence demonstrates that gamete dimorphism is not necessary for differences in motility.

Hoekstra^{39,40} explored the relationship between anisomotility and anisogamy. His model assumes a starting point of two pheromonal pseudo mating-types (*i.e.* two gamete types exist, those that produce, and those that respond to pheromone, although any two gametes can fuse successfully) in an isogamous population⁴⁰ and incorporates a motility advantage to anisogamy (*i.e.* smaller gametes travel faster).

This broadens the conditions for the evolution of anisogamy beyond those predicted by PBS, provided that there is close linkage between loci that determine mating type, pheromone production/recognition and gamete motility. In particular, there need not be a trade off between gamete size and number (gamete producers make the same number of gametes regardless of gamete size in his model). Furthermore, there is no need for PBS's unusual zygote fitness assumption.

These models have been strongly criticised on several grounds¹⁶. The major criticism is that Hoekstra⁴⁰ assumes that the swimming speed of a gamete is inversely proportional to its cross-sectional area. This assumption is based on all gametes generating equal thrust, but experiencing drag in proportion to their cross-sectional area. Dusenbery¹⁶ disagrees with the first of these assertions, advocating instead that thrust should be proportional to gamete volume (Box 5). This implies that a gamete's velocity is proportional to its radius.

Although plausible, to our knowledge, there is no direct empirical data to support the assertion that a gamete's thrust is proportional to its volume. Indeed, the data on gamete swimming speed are equivocal, although there might be a trend for smaller gametes to travel faster (Fig. 2).

However, under the supposition that small gametes travel slower, Dusenbery¹⁶ formulated a different model which focuses on gamete encounter rate as the prime determinant of fitness. It investigates size/speed adaptations that allow maintenance of population density when individuals are dispersed. In this regard the model is group selectionist and consequently should be regarded with caution.

The model assumes that the energy a gamete can devote to locomotion is proportional to its volume. Initially, random gamete motion (approximated by the hard-sphere model of gases) with no pheromone attraction is considered. Dusenbery¹⁶ predicts that anisogamy offers the advantage of increased gamete encounter rate, with initial evolution towards anisogamy occurring via one gamete

becoming smaller and non-motile (because it has no energy to devote to locomotion). We believe that the absence (to our knowledge) of organisms in which the smaller of the two gametes is non-motile is a serious shortcoming of this model. Indeed, it is unclear how the transition between such a situation and that of a pheromone-producing large gamete (described above) could occur.

More generally, we note that these theories probably cannot represent a universal explanation. The red algae (Rhodophyceae), for example, have sessile macrogametes and dispersing microgametes (although gamete immotility may be a derived trait). There is, however, no gamete propulsion or pheromonal attraction³⁷, so microgametes move at the whim of local water currents (but see Ref. 45 for evidence of potential sexual selection). Hence, in this case, anisogamy appears not to depend on motility. Furthermore, anisogamy and oogamy in the green algal order Volvocales is almost exclusively associated with reproduction by sperm packets. This implies that any motility advantages associated with small sperm are unlikely to apply, because most sperm movement occurs while they are packaged together. More generally, the effects of gamete motility on gamete encounter in broadcast spawners are often overwhelmed by turbulent water conditions²⁸.

Control of cytoplasmic inheritance

In most isogamous species, a gamete's mating type determines not only which other gametes it can mate with, but also which gamete transmits its cytoplasmic genes to the zygote⁴⁶. It has been postulated that gamete size dimorphism could be a generalised mechanism for achieving the same end; namely enforcing uniparental inheritance (UPI) of both organelles and cytoplasmic symbionts⁴⁷⁻⁴⁹. Importantly, these ideas can explain why, in several taxa (*e.g.* tunicates and ferns), sperm cytoplasm is sloughed off just before sperm entry into the oocyte⁴⁹. These observations are contrary to the prediction of PBS, which emphasises the advantages of large net zygote size. Comparably, the final stage of sperm maturation in mammals involves the removal of a quantity of cytoplasm. Whether this is an adaptation for preventing transmission of cytoplasm⁴⁹ or simply a means to make a lithe sperm is unclear.

What is becoming clear, however, is that the validity of the argument is very sensitive to the precise assumptions of the model, most particularly with respect to the costs of biparental inheritance. In one set of models, a direct cost to mixing is evoked⁴⁹⁻⁵¹. Others⁵²⁻⁵⁶ consider a deleterious cytoplasmic factor that is capable of 'fast-replicating' at the expense of cell fitness. At present, however, the evidence for negative synergistic effects of cytoplasmic mixing is sparse⁵⁶ (but see Ref. 57). By contrast, the second sort of mutant is well described, yeast (*Saccharomyces cerevisiae*) 'petite' mutants being a good model for such a 'fast-replicator'. Importantly however, recent models⁵⁶ suggest that the spread of petite-like mutants (or bacterial equivalents) can promote the evolution of uniparental inheritance, but are unlikely to provide conditions that are conducive to the evolution of small sperm.

Consider an isogamous population with biparental inheritance of a deleterious cytoplasmic factor. If the cytoplasmic factor 'fast-replicates', the cost to individual cells can be outweighed by the mutant's replication advantage and it can spread. Now let us consider nuclear modifier alleles that impose

uniparental inheritance. This can occur in one of two ways: (1) a modifier acting in the gamete that destroys its own cytoplasm (possibly prior to fusion); and (2) one that acts following fusion to destroy cytoplasm from the partner. By definition, anisogamy implies the first of these mechanisms. However, although the ‘destroy the partner’s cytoplasm’ modifier can spread and be maintained under a wide range of conditions^{52,53,55}, one that destroys its own gamete’s cytoplasm is much more restricted⁵⁶.

The crucial difference between the two mechanisms concerns the extent of linkage disequilibrium between the nuclear UPI modifier and the mutant-free cytotype. Consider the case of the modifier that destroys the cytoplasm of its partner. As long as the nuclear mutation arises in a cell that is mutant-free, it will always stay in linkage with that cytotype (notwithstanding cytoplasmic leakage⁵⁶ from the ‘paternal’ gamete). By contrast, a modifier that destroys its own cytotype (as in anisogamy) breaks up this linkage between the nuclear modifier and the ‘unselfish’ cytotype. Hence, the nuclear modifier does not benefit from repeated association with the relatively fit cytotype. Detailed modelling shows that, maintenance of the modifier in the population (even if it is neutral) requires frequent, and repeated⁵⁸ invasions of ‘selfish’ cytoplasmic replicators (*e.g.* organelles or symbionts), but if it is deleterious, it is likely to be lost⁵⁶.

In summary, the model for which there is evidence for the assumed costs, although capable of explaining uniparental inheritance, is probably incapable of explaining the evolution of small sperm. By contrast, the model for which the assumptions are not well supported is capable of this. Further, there exist numerous exceptions^{59,60} to the ‘rule’ that cytoplasmic genes should be inherited via the egg.

Prospects for a general solution

After over a century of theoretical speculation on the evolution of males and females, what progress has been made? Although many of the diverse theoretical strands have merits, all have problems that prevent their acceptance as a general solution.

PBS presents a simple model with few assumptions. In this regard, it is attractive. However, the assumptions are unusual and lack empirical support. The comparative predictions of the model are upheld within the Volvocales¹⁴, but this group appears not to satisfy the assumptions. Furthermore, simple alternative explanations are possible (Box 3). Levitan²², by contrast, argues that eggs become large to increase sperm encounter rates. The best calculations, however, suggest that the advantages of “being seen” are unlikely to outweigh the costs associated with producing fewer eggs. Under certain assumptions, the same limitation may not apply to eggs that produce pheromones¹⁶. However, to our knowledge, there is no empirical evidence to say whether such assumptions are valid.

Models that invoke a link between gamete size and motility^{16,39,40} fail to explain anisogamy in groups with gamete dimorphism, but immotile gametes. They also fail to explain reproduction by sperm packet. The status of these models is also hampered by confusion over the relationship between gamete size and swimming speed. The cytoplasmic hypothesis has achieved considerable support as a mechanism for the evolution of mating types⁴⁶. However, at least in terms of protection against selfish cytoplasmic replicators, this scenario is unlikely to explain why sperm are small⁵⁶.

Some progress has been made, in that we can now define the strengths and weaknesses of the various theories with reasonable confidence. Furthermore, we can identify the critical issues that will allow a better understanding. Are there, for example, costs to cytoplasmic mixing^{49,56}? What is the relationship between gamete size and swimming speed? This question could be resolved with good empirical or comparative data, as could the problematic relationship between egg size and pheromone production. Is it ever the case that increments in zygote size give disproportionate increases in zygote fitness?

Regarding development in theory; as with explanations for sex⁶¹, the next generation of models may need to synthesise the likely forces into more unified models. We might also have to resign ourselves to the notion that there is no single solution applicable to all taxa. Each group may have to be treated individually.

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Fig. 1. Pheromones and fertilisation

Modified versions of Volgel's²⁹ equation relating egg size to fertilisation rate (Equation III Box 4). Here we illustrate how different relationships between egg volume and pheromone attraction sphere radius affect an egg's chance of fertilisation if it increases in size. Substituting egg volumeⁿ into the original equation, the three curves are (from left to right), $n = 2$, $n = 2.5$ and $n = 3$. The last of these corresponds to Desenberg's^{16,36} assumption that the radius of the attraction sphere is proportional to egg volume. Note that for $n = 2$ there is no disproportional increase in fertilisation proportion with egg volume.

Fig. 2. Do smaller gametes swim faster?

Data (from the literature)¹⁶ of gamete swimming speed ($\mu\text{m s}^{-1}$) versus gamete volume (μm^3). When we use all the data, the regression is not significant ($P = 0.26$, $r^2 = 12.5$, $N = 12$), but suggests a negative trend ($y = 176 - 25.6 x$; upper regression line). However, Grubb's test identifies the extreme point (red circle) as a statistical outlier ($\alpha = 2.38$, $P < 0.05$). With this point removed, the negative trend of swimming speed against volume becomes significant ($P = 0.038$, $r^2 = 39.6$, $N = 11$). The regression equation is $y = 168 - 30.3 x$ (lower regression line), suggesting that smaller gametes travel faster.

Figure 1

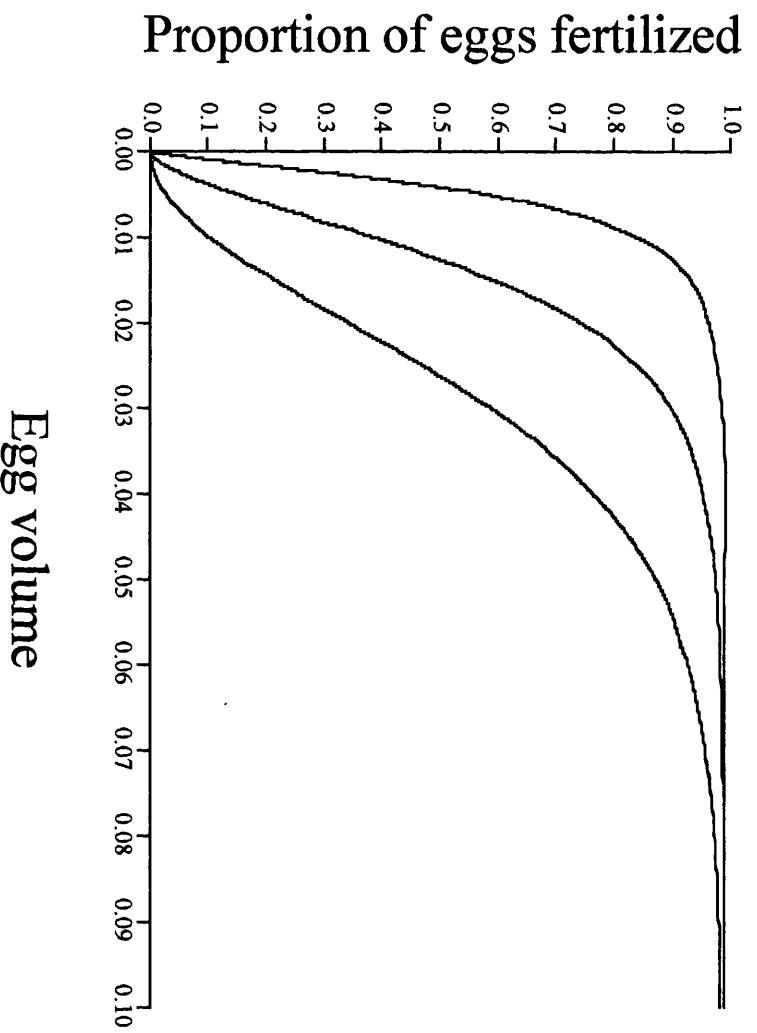
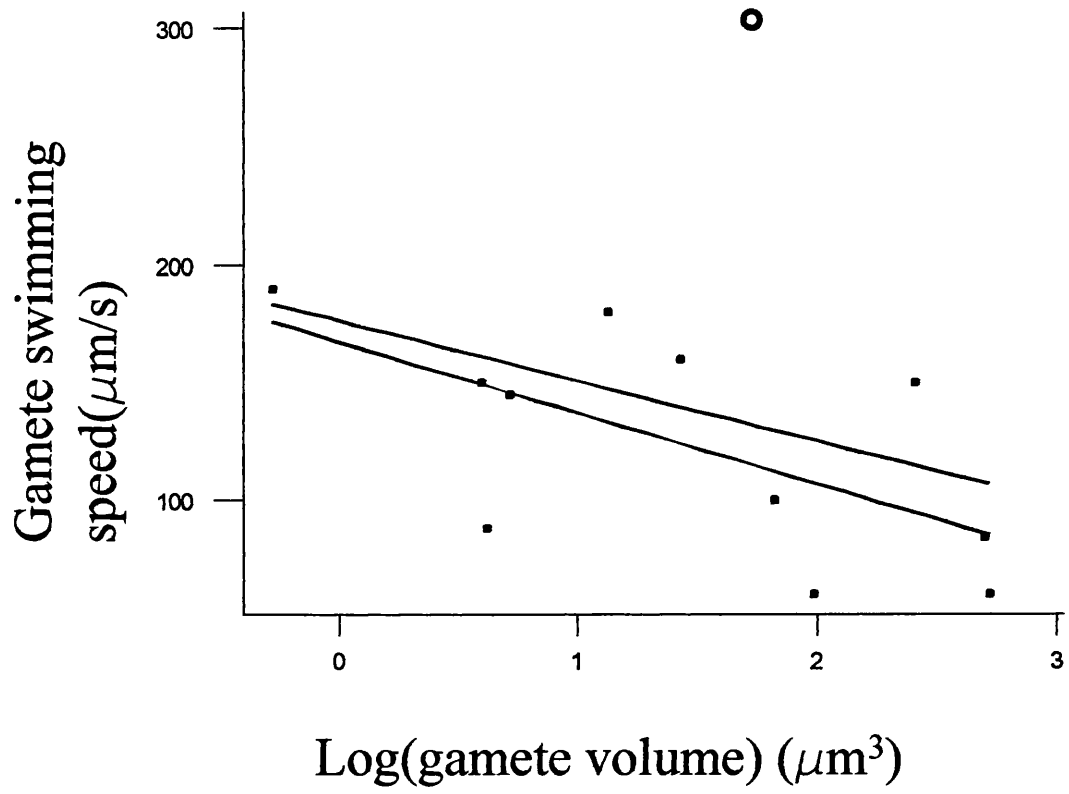


Figure 2



Box 1. The Parker, Baker, Smith theory in Bulmer's formulation

The following is based heavily on Bulmer's^a description of the Parker, Baker and Smith^b (PBS) Model. Let us assume that an individual produces n gametes of size m , but that total gametic investment is limited to M (so $M = nm$). plus-type individuals produce n_1 gametes of size m_1 , and minus-type individuals produce n_2 gametes of size m_2 . There is consequently a trade off between gamete number and size, with $n_i = M/m_i$.

Let us further assume that the probability of zygote survival (s) is some increasing function of the size of its two constituent gametes, $s(m_1 + m_2)$. For the sake of argument, we consider two survival functions,

$$s_1(x) = 1 - e^{-x} \quad \text{[I]}$$

$$s_2(x) = 1 - e^{-x^2} \quad \text{[II]}$$

Equation I gives diminishing fitness returns with increasing investment over its whole range (Fig. I). Equation II gives increasing returns for increased investment at least for $x < 0.71$ (Fig. I). Bulmer^a treats the problem as an asymmetric game.

The fitnesses (w) of + and - type gamete producers are shown by Eqns III and IV:

$$w_1(m_1) = \frac{M}{m_1} s(m_1 + m_2) \quad \text{and} \quad \text{[III]}$$

$$w_2(m_2) = \frac{M}{m_2} s(m_1 + m_2). \quad \text{[IV]}$$

Selection on plus-type gametes will maximise $w_1(m_1)$ for given m_2 , whereas selection on minus-type gametes will maximise $w_2(m_2)$ for given m_1 . The evolutionary outcome will be the result of these two processes.

Note that if $m_1 \ll m_2$, then Eqn (III) becomes Eqn V:

$$w_1(m_1) \cong \frac{M}{m_1} s(m_2). \quad \text{[V]}$$

It is clear from Eqn V that further decreases in m_1 (the size of plus-type gametes) lead to ever increasing fitness gains, provided that $s(m_2) > 0$. Therefore, plus and minus-type gametes are selected to become infinitesimally small regardless of the relationship between zygote size and fitness.

To avoid this clearly artefactual result, one can reasonably assume that there is some minimum gamete size for viability^{c,d} (0.01 for the sake of illustration). We can now investigate the predicted outcomes when the different functions relating the size of a zygote to its fitness are considered.

For the first fitness function s_1 (Eqn I), the fitness of a plus-type individual $w_1(m_1)$ decreases with increasing gamete size (m_1) regardless of the size of the minus-type gamete (m_2). The same applies for minus-type gamete producers, and both mating types end up producing gametes of the minimum possible size (in this case, 0.01). This result is intuitively sensible because, with diminishing fitness returns with zygote size, one large zygote is less fit than the combined fitness of two zygotes half the size. Under these conditions, PBS predicts isogamous gametes of the minimum size for viability.

Disruptive selection on gamete size leading to anisogamy occurs if we use fitness function s_2 (Eqn 2). From Fig. II, one can see that $w_1(m_1)$ has a maximum value when $m_1 = 1.1$, given $m_2 = 0.01$. Conversely, $w_2(m_2)$ has a maximum value when $m_2 = 1.1$, if $m_1 = 0.01$. There is no stable solution with both gamete types at some intermediate size. Hence, disruptive selection and anisogamy are the result.

Fig. I Two hypothetical functions (Eqn I and Eqn II) relating a size of a zygote to its fitness. PBS predicts selection for gamete dimorphism only when there are disproportionate fitness increments associated with increases in zygote size (*i.e.* with s_2 and not s_1). The axes are in arbitrary units.

Fig. II Eqn V using zygote fitness function s_2 with the smallest size of a viable gamete set to 0.01. It describes the relationship between the size of a gamete and its fitness when there is a disproportional fitness gain for increases in zygote size. The arrow shows the optimum size for the larger of the two gametes. The axes are in arbitrary units.

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Figure I - Box 1

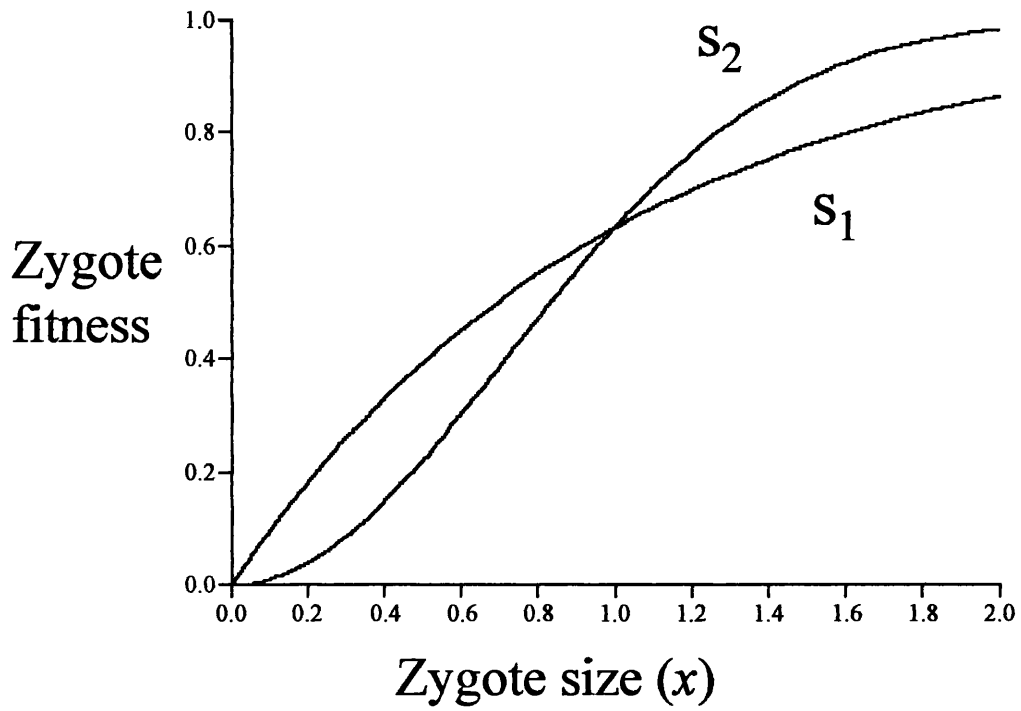
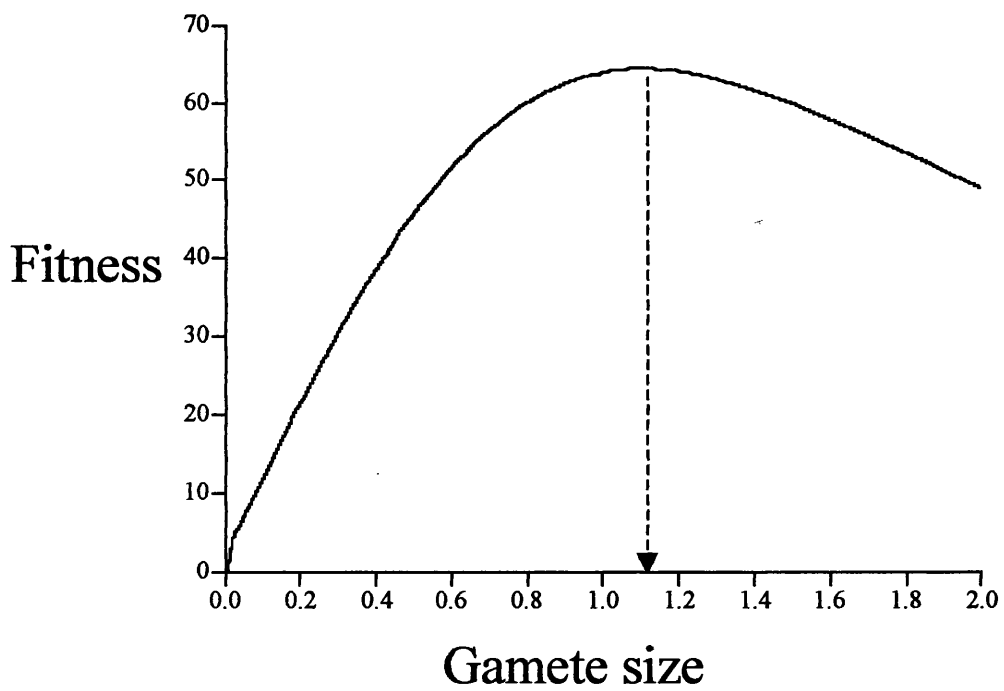


Figure II - Box 1



Box 2. Size, development time and fitness

The Parker, Baker and Smith^a (PBS) model requires that there be a disproportionate relationship between zygote size and fitness for anisogamy to be selected. While the precise mechanistic nature of this relationship is not specified, one component could be the developmental time of the zygote. If larger zygotes develop faster, they will be exposed to reduced predation risk. The question remains, however, will they be disproportionately fitter?

Analysis of this problem is possible within echinoids (sea urchins). If a planktonic larva spends a long time in the water column before metamorphosing into an adult, it will be exposed to increased predation risk. Therefore, large zygote size might be favoured to curtail this larval period. Recent data from echinoids support the following relationship between the development time (T) of an egg and its size (Fig. I and Eqn I)^b:

$$(T) = (S_{fp}/S_a - 1) + T_{fp}. \quad [I]$$

where S_a is absolute egg volume, S_{fp} is the egg volume necessary to provide enough energy for a facultative planktotroph larva to develop successfully without feeding until it metamorphoses. T_{fp} is the development time of such a larva.

If we now assume that there is a constant risk of predation per unit time, it is possible to visualise the relationship between egg size and the chance of surviving to adulthood. Hence the chance of survival (f) becomes (Eqn II):

$$f = 1 - [(S_{fp}/S_a - 1) + T_{fp}]p, \quad [II]$$

where p ranges from 0 to 1 and is the proportion of larvae predated per unit time. From Fig. II, it is clear that there are no disproportionate increases in our substitute for fitness (chance of surviving predation) and egg size in this curve. Hence, it seems unlikely that the relationship between egg size and developmental time will rescue the PBS model's untested assumption.

Fig. I

An empirically supported relationship between egg volume (S_a) and developmental time (T) in echinoids (Eqn 1). Following the original paper^b, values used are $S_{fp} = 0.0103 \text{ mm}^3$ and $T_{fp} = 14$ days (at 20°C).

Fig. II

The relationship between egg volume (S_a) and chance of survival (f) (Eqn II). This is based on Eqn I, and assumes that there is a constant per time predation risk (p). For the purposes of graphical representation $p = 0.01$.

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Figure I - Box 2

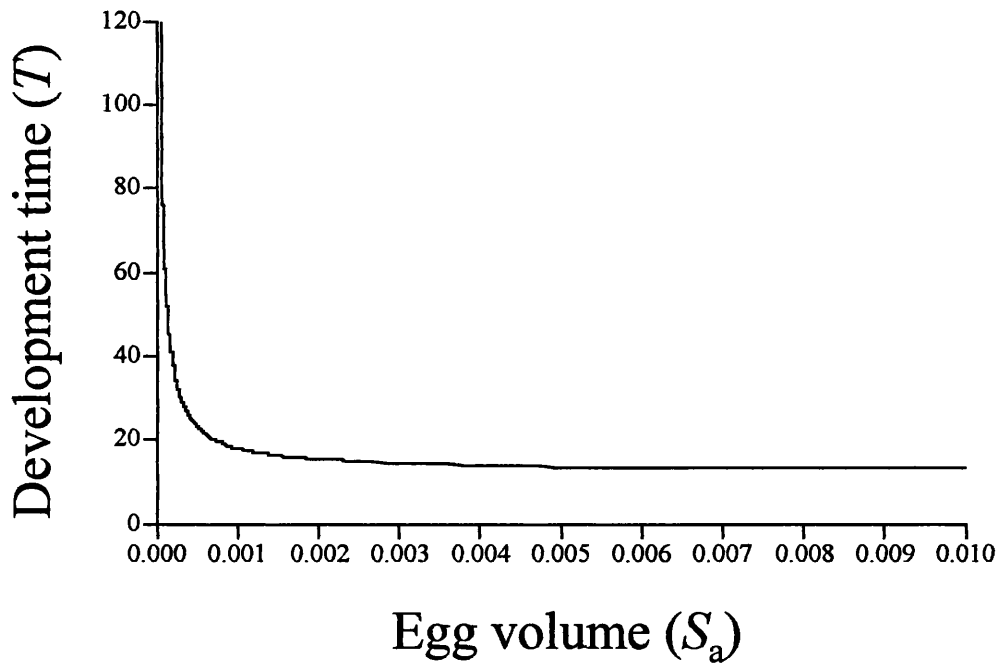
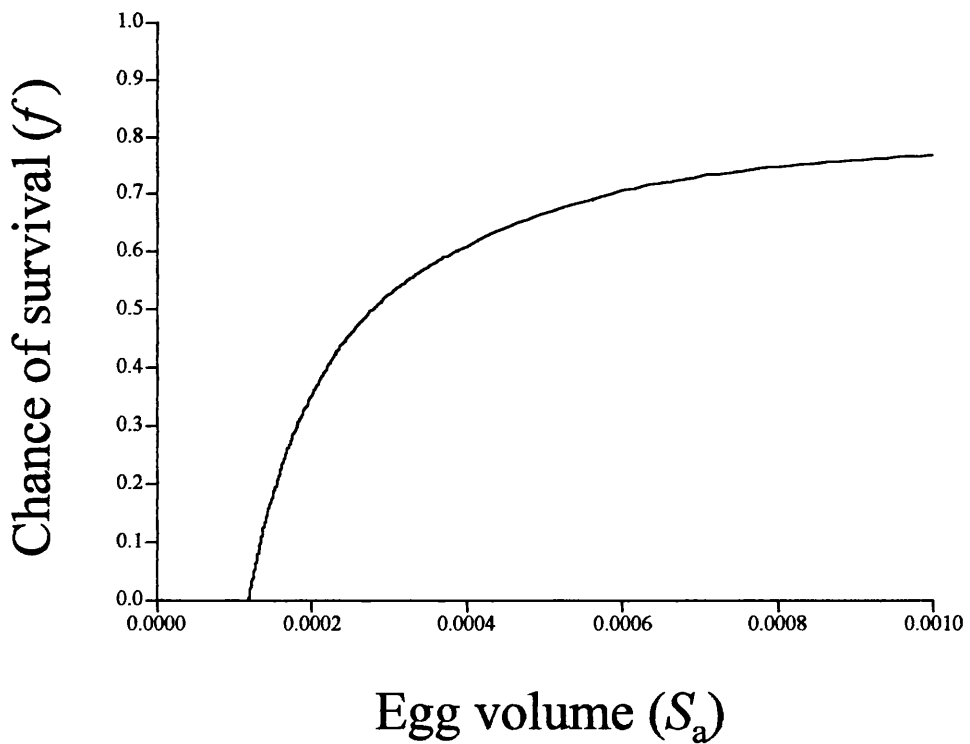


Figure II - Box 2



Box 3. Testing PBS in the green algal order Volvocales

The traditional testing ground for the PBS model and its variants has been the green algal order Volvocales^{a-d}. Members of the group exhibit variation in both size and gamete dimorphism (e.g. *Chlamydomonas reinhardtii* is isogamous and unicellular, whereas the oogamous *Volvox rousetii* has up to 50000 cells per colony).

It has been noted, however, that the Volvocales might not, in fact, be the ideal group for testing PBS because they violate some of the model's assumptions^d. Firstly, in some species there is the potential for a form of maternal care because developing zygotes are maintained within the maternal spheroid before release. Secondly, in most species, sperm are not released separately or *en masse* as a 'broadcast fertilisation' event. Rather, they are released as discrete packets that penetrate a female spheroid as a unit, fertilising all the eggs therein. Hence, neither sperm nor eggs are 'free-swimming'.

The first phylogenetically controlled comparative test^d of PBS using the Volvocales appears to support the model's^e prediction that the degree of anisogamy should increase with egg size. However, Randerson and Hurst (Chapter 4)^d have proposed a simple, alternative explanation for this trend. As females get bigger, they place increased reproductive effort into making more eggs. Since all the sperm in a packet fertilise all the eggs in a female spheroid, males would be expected to increase the number of divisions of the packet to ensure that all eggs are fertilised. The number of sperm in a packet is always 2^N , where N is the number of divisions, but this is not the case for egg number. Hence, as the number of eggs and sperm increase, the inaccuracy in matching the number of eggs and number of sperm also increases, with sperm number tending to over-shoot egg number by increasing amounts. This effect, combined with the fact that sperm packet size must probably remain within a fairly narrow size range for efficient swimming would result in increased anisogamy. More sperm making up a similarly sized packet necessarily means smaller sperm. Consistent with this model, the data support a strong correlation between egg number and sperm number^d.

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Box 4. Fertilisation advantage to large eggs

How does a fertilisation advantage for large eggs affect the Parker, Baker and Smith (PBS) model? An empirically supported^a model^b of sea urchin fertilisation kinetics forms the basis of our discussion.

The model assumes that sperm attach to the first egg they come into contact with, regardless of whether fertilisation occurs. It incorporates egg concentration (E_o), virgin sperm concentration (S_o), sperm swimming velocity (v) and half-life (τ), and egg cross-sectional area (σ_o). The rate of sperm and egg collisions (β_o) is given by Eqn I:

$$\beta_o = v \sigma_o \quad \text{[I]}$$

A second rate constant, β is the rate of fertilisation, such that β/β_o is the average proportion of sperm contacts necessary for fertilisation to occur. The model was derived by calculating the average number of potential fertilisers per egg and then using the Poisson distribution to estimate the probability of an egg not being fertilised. Hence, the proportion of eggs fertilised (φ_x) is (Eqn II)

$$\varphi_x = 1 - e^{-\frac{\beta S_o}{\beta_o E_o} (1 - e^{-\beta_o E_o \tau})} \quad \text{[II]}$$

The model does not incorporate sperm chemotaxis, which has not been documented for any echinoid species^{b,d}. If Eqn II is expressed in terms of volume, rather than in terms of cross-sectional area, we get Eqn III:

$$\varphi_x = 1 - e^{-\frac{c S_o}{E_o} (1 - e^{-\pi v E_o \tau (\frac{3\phi}{4\pi})^{2/3}})} \quad \text{[III]}$$

where ϕ is egg volume and $c = \beta/\beta_o$.

Inspection of Fig. I shows that Levitan's argument^e is unlikely to solve the PBS dilemma. Larger eggs are fitter, but not disproportionately so. We note that Levitan also considers the influence of egg size on both fertilisation success and development time^f.

Fig. I The relationship between the volume of an egg and its chance of fertilisation. Eqn III is based on Vogel's model of fertilisation kinetics in sea urchins^b.

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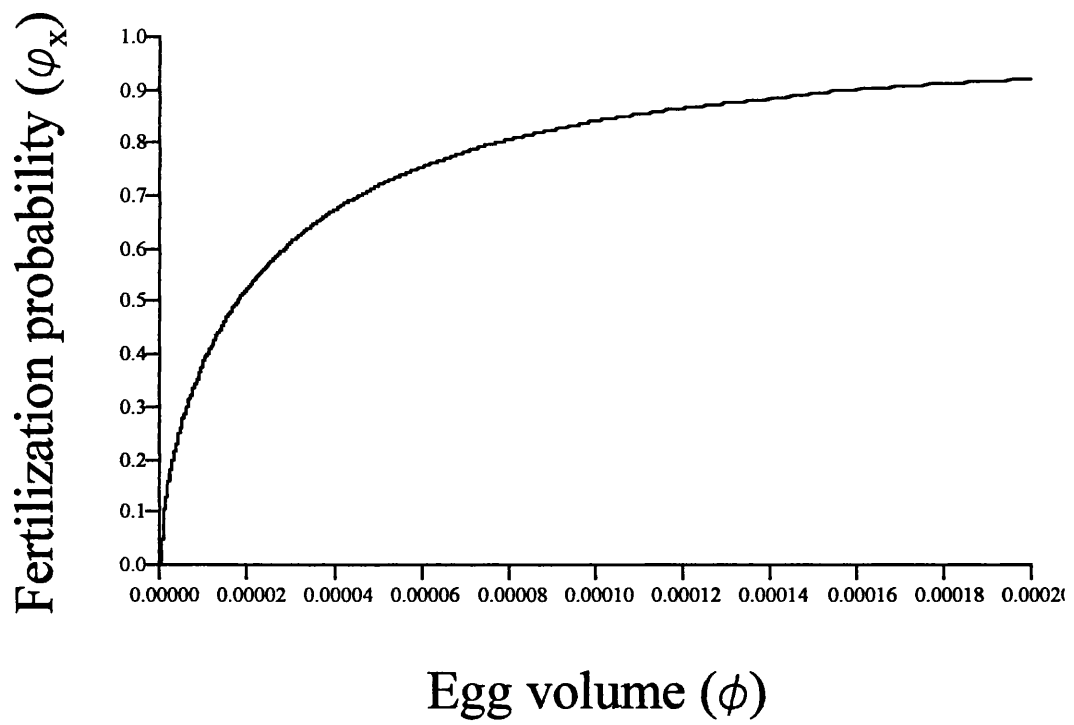
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Figure I - Box 4



Box 5. Do small gametes swim slower or faster than large gametes?

Gametes generally operate at Reynold's numbers (Re) less than unity. If c is the velocity of the gamete (assuming it is spherical), r is its radius, ρ is the density of the medium and ϵ is the viscosity of the liquid, then (Eqn I):

$$Re = 2 r c \rho / \epsilon. \quad [I]$$

Gamete motion under these conditions is described by Stoke's equation for a sphere moving in a liquid. This states that the resistance (F_w) due to movement is (Eqn II):

$$F_w = 6 \pi \epsilon c r. \quad [II]$$

The scenario described by Hoekstra^a is that the force (F) generated by the gamete is independent of its size (so F is constant). By placing $F = F_w$ and solving for c , we obtain,

$$c \propto 1/r. \quad [III]$$

The alternative, advocated by Dusenbery^b, is to suppose that the force generated by a gamete is proportional to its volume (*i.e.* $F \propto r^3$). In that case, solving for c , we obtain (Eqn IV)

$$c \propto r^2. \quad [IV]$$

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Part III

Consequences of Anisogamy

“Two souls dwell, alas! in my breast” Goethe, Faust Pt.1

Whether or not anisogamy evolved as a defence against selfish cytoplasmic fast-replicators, its appearance opened up a whole new arena for potential conflict over the sex ratio. Such potential exists because cytoplasmic genes (*e.g.* organelles, symbionts) are only transmitted via eggs (and hence by females) while nuclear genes are transmitted equally by both sexes. Thus, cytoplasmic genes are under selective pressure to bias the sex ratio in favour of females (Cosmides & Tooby 1981) while nuclear genes favour a 1:1 sex ratio (Fisher 1930). The following three chapters explore the consequences of such nuclear/cytoplasmic conflict, with particular reference to male-killing parasites (Hurst *et al.* 1997).

Selfish cytoplasmic parasites: sideshows or ringmasters?

Selfish cytoplasmic elements represent superb evolutionary case studies in the conflict between different levels of selection (in this case between cytoplasmic and nuclear genes)(Hurst *et al.* 1996; O'Neill *et al.* 1997; Bandi *et al.* 2001). But are they more than merely evolutionary curiosities? To what extent do they have wider consequences for their hosts' evolution? Furthermore, have hosts evolved mechanisms to keep errant cytoplasmic genes in check?

Previous studies have investigated the link between such parasites and various features of host biology (speciation (Hurst & Schilthuizen 1997; Werren 1998), eusociality (Hurst 1997), mate choice (Moreau *et al.* 2001), sex chromosome evolution (Hurst 1995)). In Part III, I explore the wider consequences of male-killer infection. In Chapter 7, I model host resistance to a male-killer infection (Randerson *et al.* 2000b), considering two different mechanisms of host resistance. One possibility is a female-based resistance where infected females block transmission of the bacteria to their offspring. Alternatively, infected males might block bacterial action and hence prevent themselves from being killed. In the absence of such resistance, theory does not expect the stable maintenance of two or more parasite strains. The 'best' male-killer is expected to oust all others from the host population. However, I show that in the context of such resistance, stable maintenance of more than one male-killer variant is possible.

Chapters 8 and 9 (Randerson *et al.* 2000a) investigate the possibility that a male-killer infection can lead to sex role-reversal in the host population. Theory suggests (Kvarnemo & Ahnesjo 1996) that factors influencing the Operational Sex Ratio (*i.e.* the ratio of males to females who are ready to mate in a population at a given time (Emlen & Oring 1977)) will affect the intensity of both competition and mate choice exhibited by each sex. Selfish cytoplasmic elements, because of their profound influence on host sex ratio are expected to influence these traits. In Chapter 8 (Randerson *et al.* 2000a), I investigate the theoretical possibility that a male-killer infection can select for a further type of host resistance gene: one that allows males to choose mates on the basis of their infection status. This model is focused on a well

documented male-killer infection in the Ugandan butterfly *Acraea encedon* (Jiggins *et al.* 1998; Jiggins *et al.* 2000). Chapter 9 is a test of the model's predictions in the field.

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Chapter 6. The evolutionary dynamics of male-killers and their hosts

James P. Randerson, Nick G. C. Smith and Laurence D. Hurst (2000)

Heredity, 84 152-160

The evolutionary dynamics of male-killers and their hosts

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Male-killing bacteria are cytoplasmic sex-ratio distorters that are transmitted vertically through females of their insect hosts. The killing of male hosts by their bacteria is thought to be an adaptive bacterial trait because it augments the fitness of female hosts carrying clonal relatives of those bacteria. Here we attempt to explain observations of multiple male-killers in natural host populations. First we show that such male-killer polymorphism cannot be explained by a classical model of male-killing. We then show that more complicated models incorporating the evolution of resistance in hosts can explain male-killer polymorphism. However, this is only likely if resistance genes are very costly. We also consider the long-term evolutionary dynamics of male-killers, and show that evolution towards progressively more 'efficient' male-killers can be thwarted by the appearance of host resistance. The presence of a resistance gene can allow a less efficient male-killer to outcompete its rival and hence reverse the trend towards more efficient transmission and reduced metabolic load on the host.

Keywords: male-killer, polymorphism, resistance genes, sex-ratio.

Introduction

Male-killing bacteria belong to the class of cytoplasmic elements which spread by manipulating the sex-ratio of their hosts (Hurst, 1993; Hurst *et al.*, 1997). Male-killers have been found in a number of bacterial genera, most notably *Spiroplasma*, *Rickettsia* and *Wolbachia* (Williamson & Poulson, 1979; Werren *et al.*, 1994; Hurst *et al.*, 1999a), and male-killing has been reported in a wide variety of insect taxa (Hurst, 1993; Hurst *et al.*, 1997). In the case of 'early male-killing' (*sensu* Hurst, 1991) sex-specific pathogenesis is thought to have evolved in male-killing bacteria as a consequence of their almost exclusively vertical (i.e. egg to brood) means of transmission between hosts (Hurst, 1991). Bacteria in male hosts are at an evolutionary dead-end, so male-killing has a fitness cost of zero (from the bacterial point of view). But the death of males can augment the fitness of all remaining brood members, including female hosts carrying clonal relatives of the bacteria which killed males.

This positive effect of male-killing is termed fitness compensation, and can occur for a number of reasons: reduced intrabrood competition, reduced inbreeding or direct benefits from egg cannibalism (Hurst *et al.*, 1997;

and references therein). It is fitness compensation which allows male-killers to spread in a host population, despite infected male hosts being killed and infected female hosts bearing a fitness cost (Hurst, 1991).

Classical models of male-killers (Hurst, 1991; Hurst *et al.*, 1997) have considered a number of parameters such as the vertical transmission efficiency, cost borne by infected females, and the level of fitness compensation. We consider a host population that possesses two strains of male-killers. We find that male-killer polymorphism (*sensu* Ford, 1971) is not a stable solution. As long as both male-killers are able to invade deterministically a host population free of male-killers, then only one male-killer will be maintained in the host population. The male-killer with the higher Basic Rate of Increase or *BRI* (defined later) will always out-compete the other. *BRI* is a function of the three parameters mentioned above.

This theoretical result of no male-killer polymorphism appears to be contradicted by the empirical data of a number of field studies (Hurst *et al.*, 1999). In an extreme case, four different male-killer strains were isolated from individuals of the host *Adalia bipunctata* found on a single street in Moscow (M. E. N. Majerus & J. G. H. V. D. Schulenburg, pers. comm.). How can we reconcile the theoretical and empirical data?

It is, by definition, a property of all selfish elements that their spread creates the context for the spread of

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host resistance genes. We ask whether the evolution of host resistance could provide conditions for male-killer polymorphism. The evolution of resistance to cytoplasmic sex-ratio distorters has received some theoretical consideration (Uyenoyama & Feldman, 1978), although not in the context of polymorphism.

Here we ask what would happen if a 'weaker' male-killer were introduced into a host population which had evolved resistance to a 'stronger' male-killer?

One male-killer

We specify a model of male-killing similar to those of previous studies (Hurst, 1991; Freeland & McCabe, 1997; Hurst *et al.*, 1997). We assume that the male-killer, MK, is transmitted to a proportion α of a female's brood. Transmission is exclusively vertical (no horizontal transmission). All infected males die, whereas infected females suffer a viability fitness cost U . Empirical evidence for such a cost has been found for *Rickettsia* infection of *Adalia bipunctata* (Hurst *et al.*, 1994). Fitness compensation benefits all surviving members of the brood. We assume that it is a function of the number of males killed (i.e. the amount of fitness to be redistributed), and the number of survivors (i.e. the number of individuals amongst whom redistribution takes place). Fitness compensation is maximized when brood fitness is unaffected by male death, when the fitness of dead males is perfectly redistributed amongst the surviving brood. We assume fitness compensation to be a proportion ϕ of this theoretical maximum, so the compensation received by survivors as a consequence of male death is given by

$$\phi = 1 + \phi \left(\frac{1}{1 - \alpha/2} - 1 \right) = 1 + \frac{\phi\alpha}{2 - \alpha}. \quad (1)$$

Note that this is an increasing function because as α and hence male death increases, brood fitness is shared amongst an increasingly small number of individuals.

We assume an infinite panmictic outbred population with discrete generations. Recursion equations can be expressed in terms of infected and uninfected females because all breeding males are uninfected. The proportion of adult females infected by MK is p , the proportion of uninfected adult females is q , and W is the sum of the right-hand sides.

$$Wp' = p\alpha(1 - U)\phi \quad (2)$$

and

$$Wq' = p(1 - \alpha)\phi + q. \quad (3)$$

The invasion conditions for MK are found when:

$$\left. \frac{dp'}{dp} \right|_{p=0} > 1,$$

is satisfied, i.e. when the 'Basic Rate of Increase' (BRI) is positive, where

$$BRI = \alpha(1 - U)\phi - 1. \quad (4)$$

Note that W does not feature in this equation because as $p \rightarrow 0$, $W \rightarrow 1$.

This formula gives a measure of the 'strength' of a male-killer, and shows that if a male-killer is to spread, then the fitness compensation ($\phi > 1$) must be high enough to account for viability effects ($U > 0$) and imperfect transmission ($1 > \alpha > 0$).

The equilibrium value of MK (p^*) is found by solving for $p' = p$, which gives

$$p^* = (1 - \phi\alpha + \phi U\alpha)/(1 - \phi + \phi U\alpha). \quad (5)$$

As reported previously (Hurst, 1991), perfect transmission of a male-killer theoretically leads to fixation of the male-killer ($p^* = 1$ when $\alpha = 1$), otherwise p and q are maintained in polymorphism ($p^* < 1$ when $\alpha < 1$). Fixation of a male-killer would lead to population extinction because of the severe shortage of males.

Two male-killers

We now define a second male-killer, MK_2 . All male-killer parameters are defined separately for MK_1 and MK_2 , except for the fitness compensation parameter ϕ (subscripts denote the male-killer to which each parameter applies). One could imagine a situation in which ϕ differed between male-killers, for example if killing occurred at a different time during development. However, for the sake of simplicity we assume that ϕ applies equally to both male-killers. The two male-killers are never found in the same host because there is no horizontal transmission, so the recursions are a simple extension of those for a single male-killer.

$$Wp'_1 = p_1(BRI_1 + 1) \quad (6)$$

$$Wp'_2 = p_2(BRI_2 + 1) \quad (7)$$

$$Wq' = p_1(1 - \alpha_1)\phi_1 + p_2(1 - \alpha_2)\phi_2 + q. \quad (8)$$

Equation (6) is equivalent to eqn (2), and eqn (8) is equivalent to eqn (3) when $p_2 = 0$.

The condition for MK₂ to invade a population already containing MK₁ at equilibrium is given by the solution to

$$\left. \frac{\partial p'_2}{\partial p_2} \right|_{p_1=p'_1} > 1,$$

which is given by

$$BRI_2 > BRI_1. \quad (9)$$

So MK₂ can invade in the presence of MK₁ if its *BRI* is higher. Note that this result stands even if we were to assume that ϕ applies differently to the two male-killers, as ϕ is a component of each male-killer's *BRI*.

To determine whether a stable polymorphism of male-killers is possible, we need to find the intersection of $p'_1 = p_1$ and $p'_2 = p_2$. We find that at $p'_1 = p_1$,

$$p_1 = p_1^* - p_2 p_1^* (BRI_1 / BRI_2) \quad (10)$$

and at $p'_2 = p_2$,

$$p_1 = p_1^* (BRI_2 / BRI_1) - p_2 p_1^* (BRI_1 / BRI_2). \quad (11)$$

It is clear by inspection that these lines are parallel, with gradient $p_1^* BRI_1 / BRI_2$. Therefore, a neutral equilibrium is only possible if the lines have the same intercept, i.e. if $BRI_1 = BRI_2$. Hence for stable polymorphism both male-killers must have the same *BRI*. These equations are illustrated graphically in Fig. 1. Note that

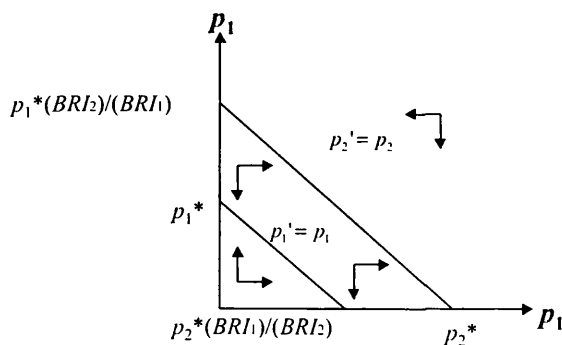


Fig. 1 Illustration that stable male-killer polymorphism is impossible in a panmictic, nonresistant population. The graph shows plots of p_1 (the frequency of MK₁) against p_2 (the frequency of MK₂) for $p'_1 = p_1$ and $p'_2 = p_2$. The only stable points for the system are at $p_1 = 0$ and $p_2 = p'_1$ or $p_1 = p'_1$ and $p_2 = 0$, unless $BRI_1 = BRI_2$. So, a neutral equilibrium is only possible if the *BRI* of the two MKs is the same. However, this does not necessarily mean that α and U must be the same.

the direction of the vectors makes it impossible that a stable limit cycle will result.

This means that a contest between two male-killers is decided solely on which of the two has the higher *BRI*. This makes intuitive sense because in a three-allele haploid system with no frequency dependence, one would expect the most fit allele simply to outcompete the others. The reason that in this case the 'better' male-killer does not remove the uninfected 'allele' is that uninfected individuals are created each generation. The system mimics a mutation–selection equilibrium in which the uninfected 'allele' is maintained by a high 'mutation' rate, i.e. by imperfect transmission of the male-killer.

Note that it is not necessarily the case that the male-killer with the higher *BRI* will also have the higher equilibrium value, although the general trend is for *BRI* to increase with equilibrium frequency.

Resistance genes

We consider two alternative resistance genes: a maternal-effect gene and a filial-effect gene. Both resistance genes act in the diploid host to reduce male-killing, and are inherited in an autosomal fashion. The resistance genes differ in their sex-dependent effects, with the maternal-effect gene acting to reduce vertical transmission and the filial-effect gene acting to cure males of the male-killer. Qualitatively similar results were obtained for the two models of resistance, so we present only the filial-effect resistance gene here. Details of the maternal-effect resistance can be found at <http://www.bath.ac.uk/Departments/BiolBioch/hurst.htm> (hereafter referred to as URL1).

The filial-effect resistance gene (*R*) acts in a dominant fashion in the male host, so that the proportion of males killed is gx rather than x . We assume that *R* imposes a cost c on males, and that this cost acts multiplicatively so that the fitness of males homozygous for *R* is $(1 - c)^2$. This combination of a dominant allele with multiplicative costs assumes that the *R* gene codes for an antibiotic whose effectiveness does not increase above the haploid dosage, but whose production cost varies with the copy number of the *R* allele. The recursion equations of the model are shown in Appendix 2.

The spread of a resistance gene

The conditions for *R* to invade a population containing a male-killer at equilibrium were obtained by modifier analysis (see URL1) and confirmed by simulation. *R* can always invade if it is not costly ($c = 0$) so long as $p^* > 0$. Furthermore, *R* can invade even if the cost of resistance is considerable, especially if the transmission advantage of the male-killer is particularly high and the resistance

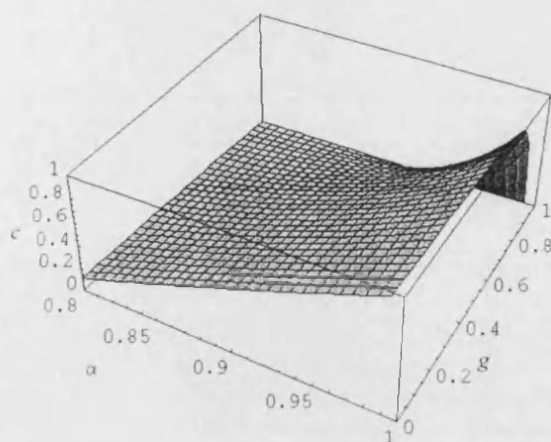


Fig. 2 Invasion conditions for the resistance gene in the presence of a male-killer at equilibrium ($\phi = 0.5$ and $U_1 = 0.01$). For invasion, the cost of the resistance gene must lie beneath the sheet. Note that as the transmission efficiency of MK_1 increases, the invasion conditions become less stringent. This is because transmission of the gene through saved males becomes increasingly significant as the population becomes more female-biased. The transmission reduction parameter g has little effect on the cost tolerated by the invasion conditions except when g approaches unity. Invasion cannot occur at $g = 1$ because this represents zero transmission reduction.

gene is highly effective (g low). The invasion conditions for a costly resistance gene are plotted in Fig. 2.

Simulation shows that a costly resistance gene, if it can invade, will always reach a stable equilibrium within the population and reduce the equilibrium level of the male-killer. However, this result is dependent on the population being infinitely large. If the resistance gene is highly effective (g low) then the male-killer can be reduced to such low frequencies that it would almost certainly be lost from a finite population. We therefore incorporated into our simulations a cut-off threshold, with frequencies falling below this threshold put to zero. The results in this paper were obtained using a cut-off of 10^{-7} , but qualitatively identical results were obtained with a cut-off of 10^{-13} .

Male-killer polymorphism with resistance genes

The proposed scheme for the evolution of male-killer polymorphism with resistance genes involves two steps. First, the spread of a costly resistance gene should 'weaken' the resident male-killer. The results above confirm that this can happen, although sometimes both the male-killer and the resistance gene will be lost from the population. The second step is for a second 'weaker' male-killer, unaffected by the resistance gene, to spread

at the expense of the 'stronger' male-killer. The 'stronger' male-killer declines in frequency, thereby causing a decrease in the frequency of the resistance gene, and hence reducing the degree to which the 'stronger' male-killer is affected by host resistance. Such frequency-dependent selection, if sufficiently damped, should allow the stable maintenance of a male-killer polymorphism. Owing to the complexity of the recursions and because of a need to incorporate cut-offs, we have used simulations to investigate whether this intuitive argument is correct.

The ability of the second male-killer to invade is not determined simply by its *BRI*. Instead, we define a General Rate of Increase (*GRI*) such that

$$GRI = \alpha(1 - U)\phi - W_f, \quad (12)$$

where W_f is the mean female fitness, and where *GRI* has to be positive for a male-killer to invade. With no male-killers or resistance genes, $W_f = 1$ and so $GRI = BRI$. However, with a male-killer and resistance gene in polymorphism, W_f will be lower because of reduced male-killing (the resistance genes spread because of their effects on mean male fitness). When a male-killer (say MK_1) is at equilibrium within a host population without resistance:

$$W_f = \alpha_1(1 - U_1)\phi_1. \quad (13)$$

This means that for MK_2 to invade when MK_1 is at equilibrium, $GRI_2 > 0$ simplifies to $BRI_2 > BRI_1$, as obtained above (see 'Two male-killers').

These considerations of *GRI* mean that MK_2 will always invade and eliminate MK_1 if $BRI_2 > BRI_1$, and that MK_2 is unlikely to invade unless $BRI_2 > 0$. This leaves an intermediate region of $BRI_1 > BRI_2 > 0$ in which male-killer polymorphism might occur. Simulations indicate that MK_2 can invade and eliminate MK_1 even if $BRI_1 > BRI_2$, and that male-killer polymorphism does occur within the bounds described. This polymorphic zone is shown in Fig. 3. These figures represent the outcomes of a series of simulations in which α_1 and α_2 were varied while U_1 and U_2 were constant and equal.

To characterize the parameter space more rigorously, we carried out parameter scans similar to those represented in Fig. 3 for varying resistance gene and male-killer cost parameter values. An example of such a parameter scan is shown in Fig. 4.

The two models of host resistance yield similar likelihoods of male-killer polymorphism. As suggested by the intuitive argument presented above, both the resistance gene's cost and its effectiveness affect the likelihood of male-killer polymorphism. As the cost of

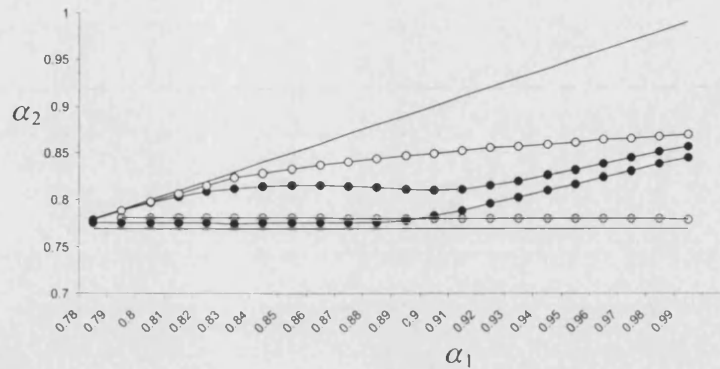


Fig. 3 Plot illustrating the parameter space in which we observe male-killer polymorphism. It is a representation of a series of simulation runs for both resistance genes (filial-effect and maternal-effect) at different combinations of α_1 and α_2 . For all simulations $\phi = 0.5$ and $U_1 = U_2 = 0.01$. Also, the characteristics of the two resistance genes are held constant ($g = d = 0.5$, $c = 0.01$). For the parameter values specified, a male-killer cannot invade if $\alpha < 0.769$ because its *BRI* will be less than zero. Polymorphism is therefore impossible if MK_2 has a transmission efficiency of less than 0.769. Polymorphism is also impossible above the line $\alpha_1 = \alpha_2$ because if MK_2 has the higher *BRI* then it will always oust MK_1 from the population regardless of host resistance. The presence of the resistance gene allows MK_2 to completely outcompete MK_1 in some circumstances, even if it has a lower *BRI*. However, near the lower boundary of the region in which MK_2 can invade, it is unable to oust MK_1 from the population. This results in stable maintenance of the two male-killers. The region enclosed by the open dotted lines represents parameter space in which polymorphism results for the filial-effect model (that described in the text). The equivalent region for the maternal-effect model is enclosed by the closed dotted lines (see URL1).

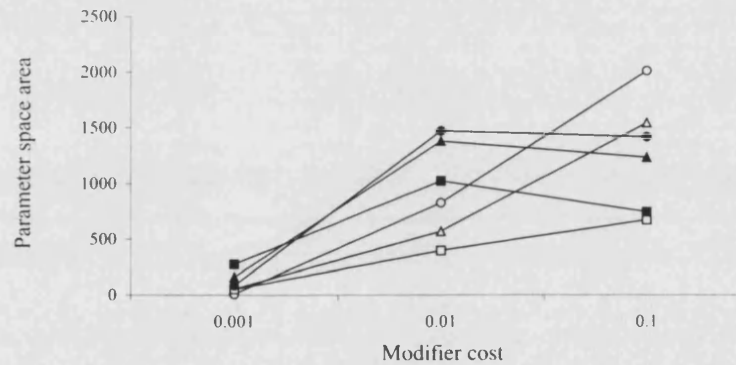


Fig. 4 Outcome of a more extensive scan of parameter space. Each point on the graph represents the area of the polymorphic zone in individual scans such as Fig. 3. These individual scans were undertaken for quantitatively different resistance genes, so g , d and c were varied. For each scan there are 2784 different combinations of parameter values that could theoretically allow polymorphism. These lie between the lowest α value that will allow invasion and the line $\alpha_1 = \alpha_2$. The parameter space area on the y -axis is therefore the number of these parameter combinations that resulted in polymorphism. The closed symbols represent results for the maternal-effect gene and the open symbols represent results for the filial-effect gene. The different shaped symbols represent different values of g and d : circle = 0.2, triangle = 0.5 and square = 0.8. The major trends visible in the scan are that polymorphism is less likely for a low-cost resistance gene, and polymorphism is more likely for a more effective resistance gene (i.e. one that reduces transmission or action by a lot). It is evident that neither class of resistance gene is substantially more likely to result in polymorphism.

resistance c is reduced, the zone of polymorphism diminishes. A high cost is required for sufficient frequency-dependent damping. When c is high, then as resistance gene effectiveness is increased (g lowered), the

likelihood of male-killer polymorphism is also increased. This is because a less effective resistance gene has a smaller effect on MK_1 , thus creating a smaller potential zone of polymorphism.

Discussion

Male-killer polymorphism

We have shown that stable coexistence of two male-killer strains within a single population is impossible if no host resistance is permitted. The male-killer with the higher *BRI* will always outcompete the other and spread to its equilibrium value. In contrast, we have shown that stable male-killer polymorphism is possible if the host can evolve resistance to a male-killer. Parameter scans indicate that male-killer polymorphism is only likely if the resistance gene is costly (roughly speaking, above 1%). Such costs may seem unrealistically high, but such is the deleterious effect of the male-killer on its hosts that resistance genes many times more costly can spread.

There are alternative explanations for the observations of male-killer polymorphism. (i) Such observations may include individuals from different populations: if different male-killers are present in different populations then male-killer polymorphism may be incorrectly inferred. Proximity of individuals need not imply interbreeding. (ii) Population substructure might enable male-killer polymorphism, with immigration of 'weaker' (i.e. lower *BRI*) male-killers balancing selection in favour of the 'stronger' male-killers (i.e. higher *BRI*). (iii) The observation of male-killer polymorphism does not necessarily imply a stable polymorphism: if new strains of male-killers are continuously arising then a mutation–selection balance will maintain transient polymorphisms. In the same way that we cannot assess the likelihood of the resistance gene explanation until we know about the critical parameters (such as resistance cost), these alternative models cannot be evaluated without explicit models and the necessary parameter information (such as migration and *de novo* appearance of male-killers).

Long-term dynamics of male-killers and their hosts

What effect would one expect host resistance to have on the long-term dynamics of a host/male-killer system? Will it evolve towards a particular evolutionarily stable state? In the absence of host resistance, new male-killers can oust the incumbent male-killer if they have a higher *BRI*. This means that *BRI* has to increase over time, with an associated tendency (but not strict requirement) for male-killer equilibrium frequency to increase also. Like a ratchet mechanism, evolution can proceed stepwise in one direction, but is prevented from going in reverse. One would therefore expect male-killers in extant populations to have high transmission efficiency and impose a modest cost on their hosts.

The evolution of host resistance breaks the ratchet. If the resistance gene is costless, then the male-killer can be deterministically driven from the population. Even if the resistance gene is costly, then as long as it is highly effective it can 'cure' the population of a male-killer by reducing the male-killer down to an absorbing lower boundary.

Even if the spread of the resistance gene does not automatically eliminate the male-killer, host resistance can still cause the ratchet to click backwards. If the second male-killer is unaffected by the resistance gene, then it can eliminate the first male-killer even if it has a lower *BRI*. In many cases this second male-killer will oust the first from the population completely, which in turn will lead to the extinction of the resistance gene if costly. Host resistance can therefore cause the ratchet to click back in the opposite direction.

Empirical studies have indicated that the vertical transmission frequency of male-killers is typically in the region of 80–90% (Hurst *et al.*, 1992, 1997), although values in excess of 99% have been reported (Majerus *et al.*, 1998). A number of ideas have been put forward to explain why transmission efficiency rarely exceeds 90% (Hurst *et al.*, 1997).

One suggestion is that the observed transmission efficiency is the maximum the male-killer is capable of, given the constraints imposed by the host (Hurst *et al.*, 1996). The observed transmission value may therefore be the outcome of an arms race between the bacterium and its host. A second possibility is that higher transmission efficiency is possible, but that male-killers reaching such high values of α will send their host population extinct because of the severe shortage of males. Clade selection may impose a higher-order filter on evolution to very high vertical transmission efficiencies. A third suggestion is that transmission efficiency may trade off against the cost imposed on the host. This could plausibly arise if both cost and transmission are dependent on bacterial density in the host cells. From our analysis, we have shown that *BRI* depends on both U and α . Hence, if there is a trade-off between α and U then one could imagine a point at which further increases in transmission efficiency actually lead to a reduction in *BRI*, and would be selectively disfavoured (Hurst *et al.*, 1997). There is some recent evidence for a link between bacterial load and CI level for *Wolbachia* in *Nasonia vitripennis* (e.g. Perrot-Minnot & Werren, 1999).

Our results suggest three further possibilities. First, in the absence of resistance, we show that one male-killer can eliminate another male-killer by having a higher *BRI*, despite having a lower transmission efficiency. Secondly, the spread of host resistance can lead to complete elimination of a male-killer. Thirdly, we show

that if host resistance has evolved to a resident male-killer, a second male-killer can eliminate the resident male-killer even with a lower *BRI* and lower transmission efficiency.

The evolution of host resistance

We have shown that the evolution of host resistance affects both the likelihood of the male-killer polymorphism and the long-term dynamics of male-killers and their hosts. To what extent are our results dependent on our specific models of host resistance, and is there any evidence of resistance genes in nature?

Although conclusive evidence of host resistance to male-killers is lacking, a number of studies have suggested that the effectiveness of a male-killer varies with host genotype (Cavalcanti *et al.*, 1957; Malagolowkin & Poulson, 1957).

A huge variety of alternative resistance genes is imaginable. We have chosen two models of resistance, which we consider to represent the middle ground, in that it is possible to imagine both resistance genes that will spread more easily, and resistance genes that will spread less easily.

Why might the evolution of host resistance be harder than suggested by our models? Hurst *et al.*, (1997) consider the consequences of a resistance gene causing increased longevity in males. If the resistance gene increases male life span by only a small time, it may actually be selectively disfavoured. This is because a male that dies as a larva is still incapable of passing on his genes, and furthermore contributes less fitness compensation to the surviving members of the brood. In order to spread, a resistance gene must allow some males to reach maturity. We have neglected this problem, by assuming that the fitness compensation parameter is unaffected by the resistance genes. In effect, we consider that our resistance genes enable a complete transition from infected to uninfected, without any intermediate classes.

On the other hand, the evolution of host resistance could be easier than suggested by our models. If a resistance gene were to reduce *U* as well as the transmission of male-killers, then its spread would be more strongly favoured.

There is a pressing need for empirical data to back up the theoretical approach adopted in this paper. We need to know if resistance genes exist, and if so, how they act. It seems reasonable to suppose that the qualitative results reached in this paper (male-killer polymorphism with resistance genes is possible) are probably independent of the exact form of a resistance gene. Any quantitative results however (resistance gene cost must be of the order

of 10^{-2} or above), are probably highly dependent on the exact nature of host resistance to male-killers.

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RESISTANCE TO MALE-KILLERS

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Appendix 1 — Parameters and abbreviations

- MK₁, male-killer one.
- MK₂, male-killer two.
- p₁, frequency of MK₁.
- p₂, frequency of MK₂.
- q, frequency of uninfected individuals.
- α, transmission efficiency of the MK.
- U, cost imposed on infected females.
- φ, proportion of the theoretical maximum fitness compensation received by surviving brood members.
- φ, fitness compensation to surviving brood because of male death.
- p*, equilibrium frequency of a particular bacterial strain in a nonresistant host population.
- BRI, Basic Rate of Increase of a particular male-killer.
- R, filial-effect resistance gene.
- c, cost imposed by a resistance gene on the sex in which it acts.

g, reduction in bacterial action in infected males with the R gene.
 d, reduction in bacterial transmission from mother to eggs because of maternal resistance (see URL1).

Appendix 2 — Details of the filial-resistance model

The genotype frequencies are represented as follows (males: y, females: x)

R gene status	No MK	MK ₁ (females only)	MK ₂ (females only)
No R gene	y ₁ , x ₁	x ₄	x ₇
Het	y ₂ , x ₂	x ₅	x ₈
Hom	y ₃ , x ₃	x ₆	x ₉

Het, heterozygous; Hom, homozygous.

Recursion equations for the dynamics of a two male-killer system with the filial-effect resistance gene are as follows

	y ₁	y ₂	y ₃
x ₁	y ₁ + x ₁	$\frac{1}{2}(y_1 + y_2 + x_1 + x_2)$	y ₂ + x ₂
x ₂	$\frac{1}{2}(y_1 + y_2 + x_1 + x_2)$	$\frac{1}{4}(1 + y_2 + x_1 + 2x_2 + x_3)$	$\frac{1}{2}(y_2 + y_3 + x_2 + x_3)$
x ₃	y ₂ + x ₂	$\frac{1}{2}(y_2 + y_3 + x_2 + x_3)$	y ₃ + x ₃
x ₄	y ₁ (1 - α ₁) + x ₄ α ₁ + x ₁ (1 - α ₁)	$\frac{1}{2}((1 - \alpha_1)(y_1 + x_1 + x_2) + \alpha_1(x_4 + x_5) + y_2(1 - g\alpha_1))$	y ₂ (1 - gα ₁) + x ₅ α ₁ + x ₂ (1 - α ₁)
x ₅	$\frac{1}{2}((1 - \alpha_1)(y_1 + x_1 + x_2) + \alpha_1(x_4 + x_5) + y_2(1 - g\alpha_1))$	$\frac{1}{4}((1 - \alpha_1)(y_1 + x_1 + 2x_2 + x_3) + (1 - g\alpha_1)(2y_2 + y_3) + \alpha_1(x_4 + 2x_5 + x_6))$	$\frac{1}{2}((1 - \alpha_1)(x_2 + x_3) + \alpha_1(x_5 + x_6) + (1 - g\alpha_1)(y_2 + y_3))$
x ₆	y ₂ (1 - gα ₁) + x ₅ α ₁ + x ₂ (1 - α ₁)	$\frac{1}{2}((1 - g\alpha_1)(y_2 + y_3) + (1 - \alpha_1)(x_2 + x_3) + \alpha_1(x_5 + x_6))$	y ₃ (1 - gα ₁) + x ₆ α ₁ + x ₃ (1 - α ₁)
x ₇	y ₁ (1 - α ₂) + x ₇ α ₂ + x ₁ (1 - α ₂)	$\frac{1}{2}((1 - \alpha_2)(y_1 + y_2) + \alpha_2(x_7 + x_8) + (1 - \alpha_2)(x_1 + x_2))$	y ₂ (1 - α ₂) + x ₈ α ₂ + x ₂ (1 - α ₂)
x ₈	$\frac{1}{2}((1 - \alpha_2)(y_1 + y_2 + x_1 + x_2) + \alpha_2(x_7 + x_8))$	$\frac{1}{4}((1 - \alpha_2)(1 + y_2 + x_1 + 2x_2 + x_3) + \alpha_2(x_7 + 2x_8 + x_9))$	$\frac{1}{2}((1 - \alpha_2)(y_2 + y_3 + x_2 + x_3) + \alpha_2(x_8 + x_9))$
x ₉	y ₂ (1 - α ₂) + x ₈ α ₂ + x ₂ (1 - α ₂)	$\frac{1}{2}((1 - \alpha_2)(y_2 + y_3 + x_2 + x_3) + \alpha_2(x_8 + x_9))$	y ₃ (1 - α ₂) + x ₉ α ₂ + x ₃ (1 - α ₂)

Male death occurs in broods from mothers x_4 - x_9 , and the number of males that die (and hence the amount of fitness compensation) is dependent both on the mother's and the father's genotype

Parental genotypes	Appropriate fitness compensation term
$J_1 \times x_4$	$1 + \frac{\phi x_1}{2 - x_1}$
$J_1 \times x_6, J_2 \times x_6, J_3 \times x_4, J_3 \times x_5, J_3 \times x_6$	$1 + \frac{\phi g x_1}{2 - g x_1}$
$J_2 \times x_5$	$1 + \frac{(0.75g x_1 + 0.25x_1)\phi}{2 - (0.75g x_1 + 0.25x_1)}$
$J_1 \times x_5, J_2 \times x_4$	$1 + \frac{(0.5g x_1 + 0.5x_1)\phi}{2 - (0.5g x_1 + 0.5x_1)}$
All matings involving x_7, x_8 and x_9	$1 + \frac{\phi x_2}{2 - x_2}$

Females in the next generation then suffer viability costs dependent on their genotype. In females it is the cost imposed by the male-killer, whereas males suffer the viability cost of R

Genotype	Viability costs
$x_1 \times x_2 \times x_3 \times J_1$	—
J_2	$(1 - c)$
J_3	$(1 - c)^2$
$x_4 \times x_5 \times x_6$	$(1 - U_1)$
$x_7 \times x_8 \times x_9$	$(1 - U_2)$

The invasion conditions for the R gene may be obtained by modifier analysis. The resulting expression is too long to reproduce here, but can be viewed at URL1. However, see Fig. 2 for a graphical representation.

Appendix 2 (extension) – Details of Resistance Gene Models

This is an extended version of the “Appendix 2” that appears in the published paper.

We consider (in separate models) two alternative resistance genes; a maternal-effect gene (R_m) and a filial-effect gene (R_f). The latter is discussed in more detail in the paper and referred to there as R . Both resistance genes act in the diploid host to reduce male-killing, and are inherited in an autosomal fashion. The resistance genes differ in their sex-dependent effects (Figure 1). The recursion equations for the two models (with two male-killer strains) are given below.

The maternal-effect resistance gene acts in a dominant fashion in the female host to reduce the transmission of male-killers to her eggs, with the proportion of eggs infected reduced from α to $d\alpha$ (where $0 < d < 1$). We assume that R_m imposes a cost c on females, and that this cost acts multiplicatively so that the fitness of females homozygous for R_m is $(1 - c)^2$.

The filial-effect resistance gene (R_f) acts in a dominant fashion in the male host to completely eliminate male-killers from a proportion $1 - g$ (where $0 < g < 1$) of infected male embryos, so that the proportion of males killed is $g\alpha$ rather than α . We assume that R_f imposes a cost c on males, and that this cost acts multiplicatively so that the fitness of males homozygous for R_f is $(1 - c)^2$.

Mathematical Details of the Models

The genotype frequencies are represented as follows:

males: y , females: x

	No MK	MK ₁ (females only)	MK ₂ (females only)
No R gene	y_1, x_1	x_4	x_7
Heterozygous for R gene	y_2, x_2	x_5	x_8
Homozygous for R gene	y_3, x_3	x_6	x_9

Invasion of the Maternal-Effect Resistance Gene

Recursion equations for the dynamics of a two male-killer system with the maternal-effect resistance gene were obtained by following the scheme in Figure 1. It is necessary to carry out recursions on individuals because fitness compensation affects broods as a whole.

	y_1	y_2	y_3
x_1	$x_1 + y_1$	$\frac{1}{2}(x_1 + y_1 + y_2 + x_2)$	$y_2 + x_2$
x_2	$\frac{1}{2}(x_1 + y_1 + y_2 + x_2)$	$\frac{1}{4}(x_1 + y_1 + 2y_2 + 2x_2 + y_3 + x_3)$	$\frac{1}{2}(y_2 + x_2 + y_3 + x_3)$
x_3	$y_2 + x_2$	$\frac{1}{2}(y_2 + x_2 + y_3 + x_3)$	$y_3 + x_3$
x_4	$x_1(1-\alpha_1) + y_1(1-\alpha_1) + x_4\alpha_1$	$\frac{1}{2}((1-\alpha_1)(x_1 + y_1 + y_2 + x_2) + \alpha_1(x_4 + x_5))$	$y_2(1-\alpha_1) + x_2(1-\alpha_1) + x_5\alpha_1$
x_5	$\frac{1}{2}((1-d\alpha_1)(x_1 + y_1 + y_2 + x_2) + d\alpha_1(x_4 + x_5))$	$\frac{1}{4}((1-d\alpha_1)(x_1 + y_1 + 2y_2 + y_3 + 2x_2 + x_3) + d\alpha_1(x_4 + 2x_5 + x_6))$	$\frac{1}{2}((1-d\alpha_1)(x_2 + y_2 + y_3 + x_3) + d\alpha_1(x_5 + x_6))$
x_6	$y_2(1-d\alpha_1) + x_2(1-d\alpha_1) + x_5d\alpha_1$	$\frac{1}{2}((1-d\alpha_1)(x_2 + y_2 + y_3 + x_3) + d\alpha_1(x_5 + x_6))$	$y_3(1-d\alpha_1) + x_3(1-d\alpha_1) + x_6d\alpha_1$
x_7	$x_1(1-\alpha_2) + y_1(1-\alpha_2) + x_7\alpha_2$	$\frac{1}{2}((1-\alpha_2)(x_1 + y_1 + y_2 + x_2) + \alpha_2(x_7 + x_8))$	$y_2(1-\alpha_2) + x_2(1-\alpha_2) + x_8\alpha_2$
x_8	$\frac{1}{2}((1-\alpha_2)(x_1 + y_1 + y_2 + x_2) + \alpha_2(x_7 + x_8))$	$\frac{1}{4}((1-\alpha_2)(x_1 + y_1 + 2y_2 + 2x_2 + y_3 + x_3) + \alpha_2(x_7 + 2x_8 + x_9))$	$\frac{1}{2}((1-\alpha_2)(x_2 + y_2 + y_3 + x_3) + \alpha_2(x_8 + x_9))$
x_9	$y_2(1-\alpha_2) + x_2(1-\alpha_2) + x_8\alpha_2$	$\frac{1}{2}((1-\alpha_2)(x_2 + y_2 + y_3 + x_3) + \alpha_2(x_8 + x_9))$	$y_3(1-\alpha_2) + x_3(1-\alpha_2) + x_9\alpha_2$

Male death occurs in broods from mothers $x_4 - x_9$. In each case, the surviving brood members receive fitness compensation depending on the mother's genotype. Hence, the number of progeny in each row of the table above must be multiplied by the appropriate fitness compensation term:

Maternal Genotype	Appropriate fitness compensation term	Abbreviation in invasion term
x_4	$1 + \frac{\phi\alpha_1}{2 - \alpha_1}$	f^4
x_5, x_6	$1 + \frac{\phi d\alpha_1}{2 - d\alpha_1}$	f^5
x_7, x_8, x_9	$1 + \frac{\phi\alpha_2}{2 - \alpha_2}$	NA

Females in the next generation then suffer the viability costs of carrying the resistance gene and the male-killer, dependent on their own genotype.

Female Genotype	Viability costs
x_1	-
x_2	$(1-c)$
x_3	$(1-c)^2$
x_4	$(1-U_1)$
x_5	$(1-U_1)(1-c)$
x_6	$(1-U_1)(1-c)^2$
x_7	$(1-U_2)$
x_8	$(1-U_2)(1-c)$
x_9	$(1-U_2)(1-c)^2$

The invasion conditions for R_m in the presence of MK_1 only were found by modifier analysis. Linearised recursions were obtained for the three genotypes in which R_f is heterozygous and MK_2 is absent (y_2, x_2, x_5). x_1, y_1 and x_4 were taken to be at their equilibrium frequencies in the absence of R_f . In matrix form, the linearised recursions become:

$$\begin{bmatrix} y_2' \\ x_2' \\ x_5' \end{bmatrix} = \begin{bmatrix} a & b & c \\ d & e & f \\ g & h & i \end{bmatrix} \begin{bmatrix} y_2 \\ x_2 \\ x_5 \end{bmatrix} = \lambda \begin{bmatrix} y_2 \\ x_2 \\ x_5 \end{bmatrix}$$

Hence for invasion, the leading eigenvalue, λ of the resultant 3x3 matrix must be greater than one. It therefore follows that:

$$1 < a + bd + e - ae + cg - ceg + bfg + cdh + fh - afh + i - ai - bdi - ei + aei$$

This revealed that for invasion:

$$1 < (-4 + \alpha_1 c^2 d f_5 (U_1 - 1) (p^* - 1 - p^* f_4 + \alpha_1 p^* f_4) + 2 p^{*3} (1 - f_4 + \alpha_1 f_4) (1 - f_4 + \alpha_1 f_4 U_1)^2 + p^{*2} (1 - f_4 + \alpha_1 f_4 U_1) (8 f_4 - 8 - 6 \alpha_1 f_4 - \alpha_1 d f_5 + \alpha_1 f_4 f_5 + \alpha_1 d f_4 f_5 - 2 \alpha_1^2 d f_4 f_5 - 2 \alpha_1 f_4 U_1 + \alpha_1 d f_5 U_1 - \alpha_1 f_4 f_5 U_1 - \alpha_1 d f_4 f_5 U_1 + 2 \alpha_1^2 d f_4 f_5 U_1) + p^* (10 - 10 f_4 + 4 \alpha_1 f_4 + \alpha_1 d f_5 - \alpha_1 f_4 f_5 - \alpha_1 d f_4 f_5 + \alpha_1^2 d f_4 f_5 + 6 \alpha_1 f_4 U_1 - \alpha_1 d f_5 U_1 + \alpha_1 f_4 f_5 U_1 + \alpha_1 d f_4 f_5 U_1 - \alpha_1^2 d f_4 f_5 U_1^2) + c (2 - \alpha_1 d f_5 + \alpha_1 d f_5 U_1 - p^{*2} (1 - f_4 + \alpha_1 f_4 U_1) (2 f_4 - 2 - 2 \alpha_1 f_4 - \alpha_1 d f_5 + \alpha_1 f_4 f_5 + \alpha_1 d f_4 f_5 - 2 \alpha_1^2 d f_4 f_5 + \alpha_1 d f_5 U_1 - \alpha_1 f_4 f_5 U_1 - \alpha_1 d f_4 f_5 U_1 + 2 \alpha_1^2 d f_4 f_5 U_1) + p^* (4 f_4 - 4 - 2 \alpha_1 f_4 + \alpha_1 f_4 f_5 - 2 \alpha_1 f_4 U_1 - \alpha_1 f_4 f_5 U_1 - \alpha_1^2 d f_4 f_5 U_1 + \alpha_1^2 d f_4 f_5 U_1^2))) / (4(p^* - 1 - p^* f_4 + \alpha_1 p^* f_4) (p^* - 1 - p^* f_4 + \alpha_1 p^* f_4 U_1)^2)$$

The conditions for invasion of R_m when $\phi = 0.5$ and $U_1 = 0.01$ are plotted in Figure 2.

Invasion of The Filial-Effect Resistance Gene

Recursion equations for the dynamics of a two male-killer system with the filial-effect resistance gene:

	y_1	y_2	y_3
x_1	$y_1 + x_1$	$\frac{1}{2} (y_1 + y_2 + x_1 + x_2)$	$y_2 + x_2$
x_2	$\frac{1}{2} (y_1 + y_2 + x_1 + x_2)$	$\frac{1}{4} (1 + y_2 + x_1 + 2x_2 + x_3)$	$\frac{1}{2} (y_2 + y_3 + x_2 + x_3)$
x_3	$y_2 + x_2$	$\frac{1}{2} (y_2 + y_3 + x_2 + x_3)$	$y_3 + x_3$
x_4	$y_1(1-\alpha_1) + x_4\alpha_1 + x_1(1-\alpha_1)$	$\frac{1}{2} ((1-\alpha_1) (y_1 + x_1 + x_2) + \alpha_1(x_4 + x_5) + y_2 (1-g\alpha_1))$	$y_2 (1-g\alpha_1) + x_5\alpha_1 + x_2(1-\alpha_1)$
x_5	$\frac{1}{2} ((1-\alpha_1) (y_1 + x_1 + x_2) + \alpha_1(x_4 + x_5) + y_2 (1-g\alpha_1))$	$\frac{1}{4} ((1-\alpha_1)(y_1 + x_1 + 2x_2 + x_3) + (1-g\alpha_1)(2y_2 + y_3) + \alpha_1(x_4 + 2x_5 + x_6))$	$\frac{1}{2} ((1-\alpha_1) (x_2 + x_3) + \alpha_1(x_5 + x_6) + (1-g\alpha_1)(y_2 + y_3))$
x_6	$y_2 (1-g\alpha_1) + x_5\alpha_1 + x_2(1-\alpha_1)$	$\frac{1}{2} ((1-g\alpha_1)(y_2 + y_3) + (1-\alpha_1)(x_2 + x_3) + \alpha_1(x_5 + x_6))$	$y_3 (1-g\alpha_1) + x_6\alpha_1 + x_3(1-\alpha_1)$
x_7	$y_1(1-\alpha_2) + x_7\alpha_2 + x_1(1-\alpha_2)$	$\frac{1}{2} ((1-\alpha_2)(y_1 + y_2) + \alpha_2(x_7 + x_8) + (1-\alpha_2)(x_1 + x_2))$	$y_2 (1-\alpha_2) + x_8\alpha_2 + x_2(1-\alpha_2)$
x_8	$\frac{1}{2} ((1-\alpha_2)(y_1 + y_2 + x_1 + x_2) + \alpha_2(x_7 + x_8))$	$\frac{1}{4} ((1-\alpha_2)(1 + y_2 + x_1 + 2x_2 + x_3) + \alpha_2(x_7 + 2x_8 + x_9))$	$\frac{1}{2} ((1-\alpha_2)(y_2 + y_3 + x_2 + x_3) + \alpha_2(x_8 + x_9))$
x_9	$y_2 (1-\alpha_2) + x_8\alpha_2 + x_2(1-\alpha_2)$	$\frac{1}{2} ((1-\alpha_2)(y_2 + y_3 + x_2 + x_3) + \alpha_2(x_8 + x_9))$	$y_3 (1-\alpha_2) + x_9\alpha_2 + x_3(1-\alpha_2)$

Again, male death occurs in broods from mothers $x_4 - x_9$, but in this case, the number of males that die is not solely dependent on the mother's genotype. Infected males have a chance of surviving infection if they carry R_6 , so the proportion of males dying is also affected by the father's genotype. The fitness compensation received by the brood therefore depends on the combination of the father and mother's genotype.

Parental Genotypes	Appropriate fitness compensation term	Abbreviation in invasion term
$y_1 x_4$	$1 + \frac{\phi\alpha_1}{2 - \alpha_1}$	$f1$
$y_1 x_6, y_2 x_6, y_3 x_4, y_3 x_5, y_3 x_6$	$1 + \frac{\phi g\alpha_1}{2 - g\alpha_1}$	NA
$y_2 x_5$	$1 + \frac{(0.75g\alpha_1 + 0.25\alpha_1)\phi}{2 - (0.75g\alpha_1 + 0.25\alpha_1)}$	NA
$y_1 x_5, y_2 x_4$	$1 + \frac{(0.5g\alpha_1 + 0.5\alpha_1)\phi}{2 - (0.5g\alpha_1 + 0.5\alpha_1)}$	$f5$
All matings involving x_7, x_8 and x_9	$1 + \frac{\phi\alpha_2}{2 - \alpha_2}$	NA

Females in the next generation then suffer viability costs dependent on their genotype. In females it is the cost imposed by the male-killer, while males suffer the viability cost of R_f .

Genotype	Viability costs
$x_1 x_2 x_3 y_1$	-
y_2	$(1-c)$
y_3	$(1-c)^2$
$x_4 x_5 x_6$	$(1-U_1)$
$x_7 x_8 x_9$	$(1-U_2)$

Invasion conditions for R_f were obtained by modifier analysis in the same way as for R_m .

This revealed that for invasion:

$$1 < (-4 + 2p^*^3(1 - f5 + \alpha_1 g f5)(1 - f1 + \alpha_1 f1 U_1)^2 + p^*^2(1 - f1 + \alpha_1 f1 U_1)(4f1 - 8 - 2\alpha_1 f1 + 4f5 - 2\alpha_1 f5 + 2\alpha_1 f5 f1 - 2\alpha_1^2 f5 f1 - 3\alpha_1 f5 g - 2\alpha_1 f1 U_1 + \alpha_1 f5 U_1 - 2\alpha_1 f1 f5 U_1 + 2\alpha_1^2 f1 f5 U_1) + p^*(10 - 8f1 + 2\alpha_1 f1 - 2f5 + 2\alpha_1 f5 - 2\alpha_1 f1 f5 + \alpha_1^2 f1 f5 + \alpha_1 f5 g + 6\alpha_1 f1 U_1 - \alpha_1 f5 U_1 + 2\alpha_1 f1 f5 U_1 - \alpha_1^2 f1 f5 U_1^2) - c(p^* - 1 - p^* f1 + \alpha_1 p^* f1 U_1)(2 - \alpha_1 f5 + \alpha_1 f5 U_1 + 2p^*^2(1 - f5 + \alpha_1 f5 g)(1 - f1 + \alpha_1 f1 U_1) - p^*(4 - 2f1 - 2f5 + \alpha_1 f5 g + 2\alpha_1 f1 U_1 + \alpha_1 f5 U_1))) / (4(p^* - 1 - p^* f1 + \alpha_1 p^* f1)(p^* - 1 - p^* f1 + \alpha_1 p^* f1 U_1)^2)$$

The conditions for invasion of R_f when $\phi = 0.5$ and $U_1 = 0.01$ are plotted in Figure 3.

Figure 1. A scheme describing the mode of action of both resistance genes. The two resistance genes are combined to enable comparison, but it is envisaged that they act separately, in different populations. Note that the cost and action of R_f and R_m occur at different points: respectively in brood males and in the mother.

Figure 2. Invasion conditions for the maternal-effect resistance gene in the presence of a male-killer at equilibrium ($\phi = 0.5$ and $U_f = 0.01$). For invasion, the cost of the resistance gene must lie beneath the sheet. Note that as the transmission efficiency of MK_1 increases, the invasion conditions become less stringent. This is because transmission of the gene through saved males becomes increasingly significant as the population becomes more female biased. The transmission reduction parameter d has little effect on the cost tolerated by the invasion conditions except when d approaches unity. Invasion cannot occur at $d = 1$ because this represents zero transmission reduction.

Figure 3. Invasion conditions for the maternal-effect resistance gene in the presence of a male-killer at equilibrium ($\phi = 0.5$ and $U_f = 0.01$). Comparison with Figure 2 indicates that the invasion conditions for the two genes are qualitatively and quantitatively very similar.

Figure 1.

Action of Resistance Genes

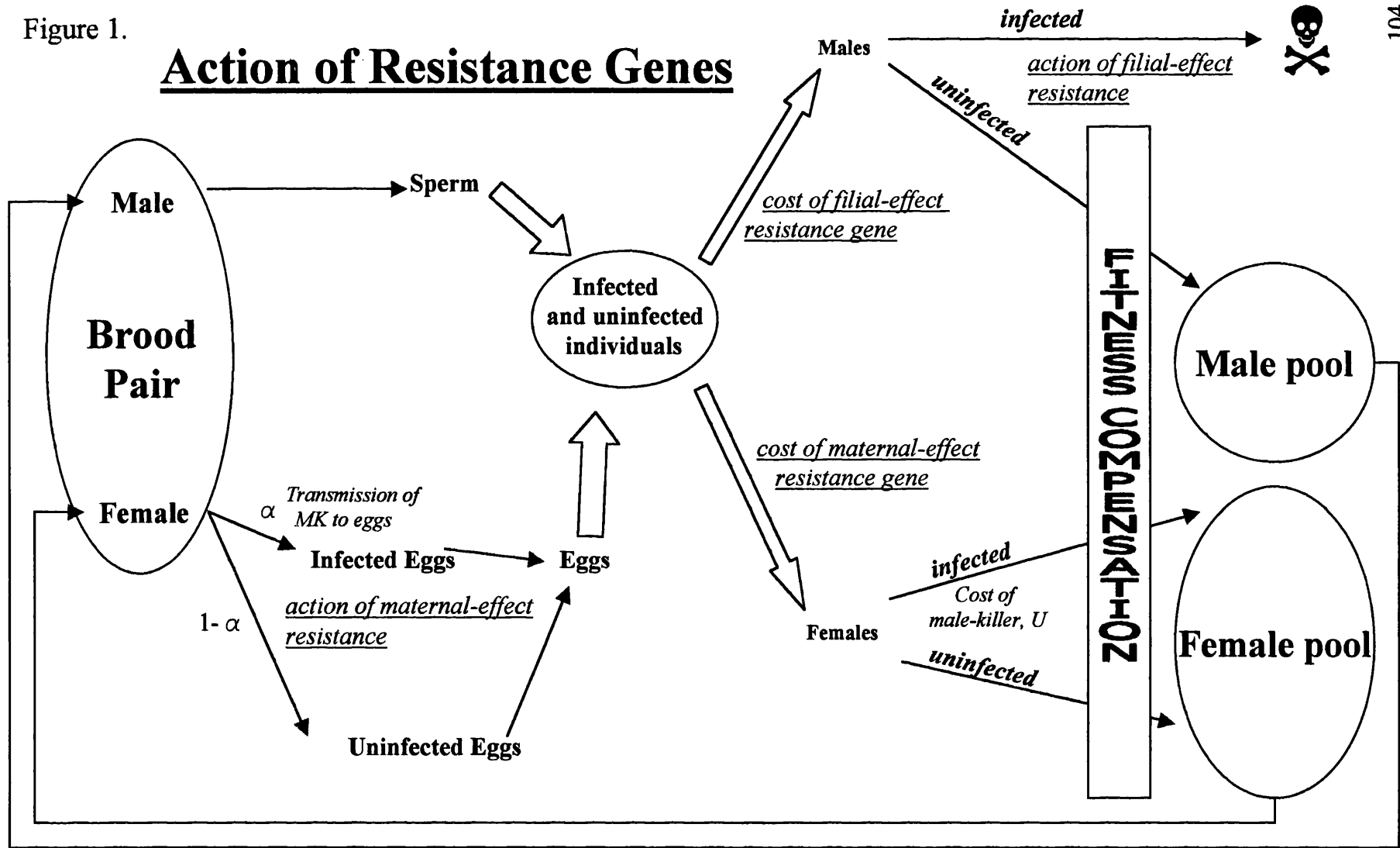


Figure 2.

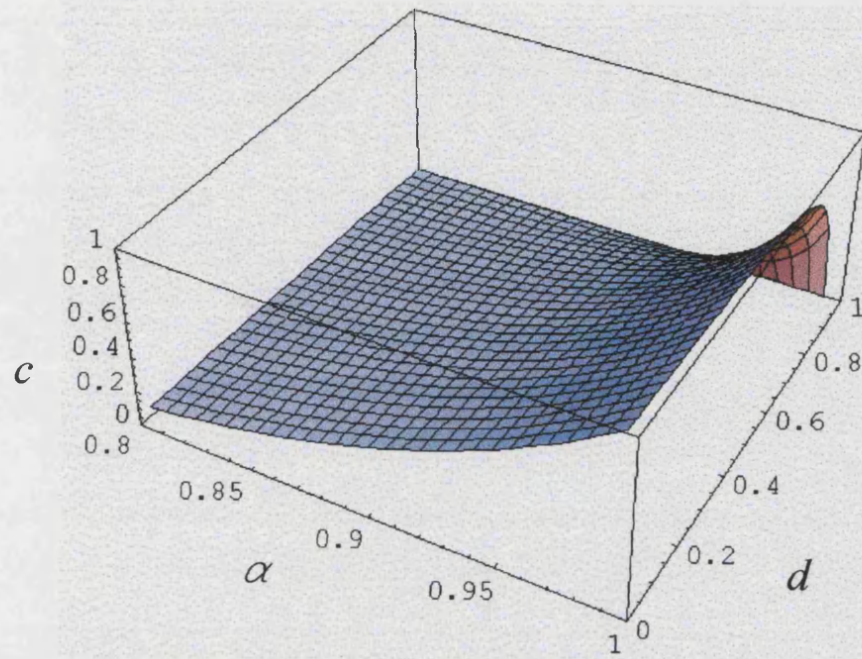
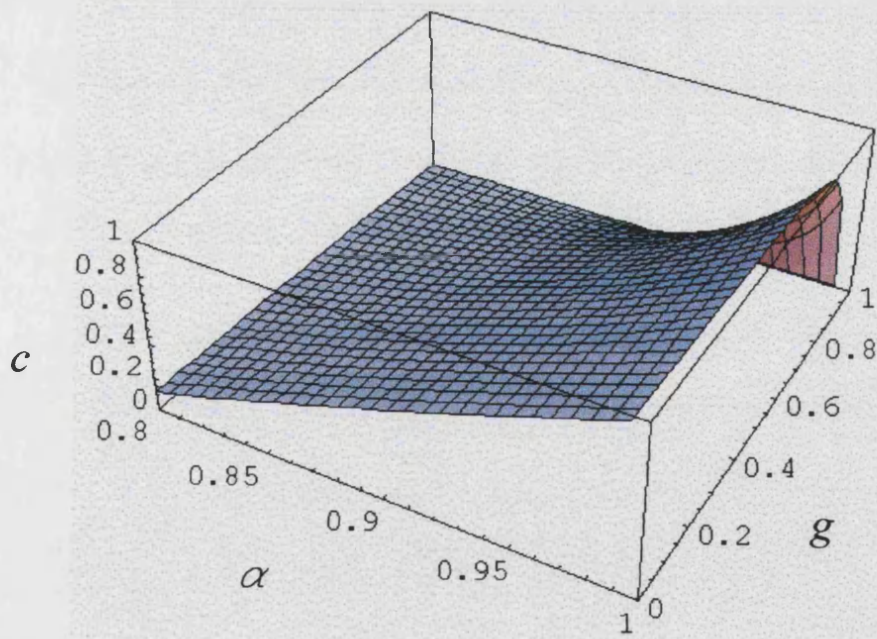


Figure 3.



Chapter 7. Male killing can select for male mate choice: a novel solution to the paradox of the lek

James P. Randerson, Francis M. Jiggins and Laurence D. Hurst (2000)

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Male killing can select for male mate choice: a novel solution to the paradox of the lek

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In lekking species, intense directional selection is applied to aspects of the male genotype by female choice. Under conventional quantitative genetics theory, the expectation is that this will lead to a rapid loss in additive genetic variance for the trait in question. However, despite female choice, male variation is maintained and hence it pays females to continue choosing. This has been termed the 'paradox of the lek'. Here we present a theoretical analysis of a putative sex-role-reversed lek in the butterfly *Acraea encedon*. Sex-role reversal appears to have come about because of infection with a male-killing *Wolbachia*. The bacterium is highly prevalent in some populations, such that there is a dearth of males. Receptive females form dense aggregations, and it has been suggested that males preferentially select females uninfected with the bacterium. As with more conventional systems, this presents a theoretical problem exactly analogous to the lek paradox, namely, what maintains female variation and hence why do males continue to choose? We model the evolution of a male choice gene that allows discrimination between infected and uninfected females, and show that the stable maintenance of both female variation and male choice is likely, so long as males make mistakes when discriminating between females. Furthermore, our model allows the maintenance, in a panmictic population, of a male killer that is perfectly transmitted. This is the first model to allow this result, and may explain the long-term persistence of a male killer in *Hypolimnas bolina*.

Keywords: male killer; sex ratio; lek; mate choice; *Acraea encedon*; *Hypolimnas bolina*

1. INTRODUCTION

Standard population genetics theory would predict that intense directional selection imposed by female choice will lead to a rapid loss of additive genetic variance for the male trait in question. Should this occur, the benefits of female choice disappear. This presents us with the 'paradox of the lek', namely, why do females continue to choose between males when the genetic benefits of choice are so small?

In the most convincing resolution of the paradox (Pomiankowski & Møller 1995), the authors turn the problem around. Rather than asking what maintains female choice in the absence of male variation, they ask what maintains variation despite strong directional selection? They argue that intense directional selection will favour modifiers that increase the number of genes and the average contribution of a locus to phenotypic variance in males. Continued variability of the male trait is hence expected in their model and consequently so is the maintenance of female choice—the paradox disappears. To back up their case, they present data showing that additive genetic variance is in fact higher in sexually selected traits and not lower as the traditional argument would predict. Here we present an alternative solution to the lek paradox inspired by the biology of *Acraea encedon* and its male-killing bacterium.

Certain Ugandan populations of the butterfly *Acraea encedon* are infected with the male-killing bacterium *Wolbachia*. As is typically the case with this bacterial group, infection of new individuals does not occur horizontally (i.e. to conspecifics that the host encounters), but

vertically, via the mother's eggs. One consequence of this is that bacteria finding themselves in a male have effectively zero fitness (there is no transmission via sperm) (O'Neill *et al.* 1997; Werren 1997). In *A. encedon*, and numerous other hosts (Hurst *et al.* 1996; Hurst 1993; Majerus *et al.* 1998), bacterial pathogenesis is sex limited such that the *Wolbachia* will only kill their host if they find themselves in a male (Jiggins *et al.* 1998, 2000a). Killing occurs at the egg stage, so infected females typically produce a clutch in which only half the eggs hatch, these are almost all females. While this is an act of suicide for the parasite, bacterial fitness in males is zero anyway, so they have nothing to lose. However, their clonal relatives in the dead males' sisters probably benefit through female host cannibalism of their dead brothers (Hurst & Majerus 1993).

One remarkable feature of the *Wolbachia* infection of *A. encedon* is the high prevalence of the bacteria in females (78–100%) (Jiggins *et al.* 2000b). As a consequence, many populations have a marked shortage of males. What is unique about this system though, is that bacterial prevalence is correlated with a change in the butterfly's mating system. Under normal circumstances, males seek out and compete for matings with females that are dispersed around larval food plants. In populations where the male killer is at high frequency, however, females form dense aggregations in grassy areas near prominent landmarks (e.g. trees). At these sites, up to 351 female butterflies have been found congregated in a small arena measuring roughly 10 m × 20 m (Jiggins *et al.* 2000b).

Several pieces of evidence suggest that females aggregate at these sites in order to attract mates. First, in mark-release-recapture experiments, virgin females were more likely to remain at these sites than mated females,

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suggesting that females congregate in order to mate. Second, aggregating females exhibit a range of mate-attracting behaviours (Jiggins *et al.* 2000b).

These observations are reminiscent of the common male phenomenon of lek formation. Classical leks are situations in which '... males aggregate when advertising for females, females are unimpeded in their choice of mates, and males are thought to make no contribution to the females' effort besides their sperm.' (Ryan 1997, p. 179). In such circumstances, it is females that are the 'in-demand' sex, as they have the slower reproductive rate. Hence, with many males laid out before them, females can afford to be choosy about whom they mate with.

In a highly female-biased population it is likely that the balance of which is the 'in-demand' sex will swing towards males. In such situations, it is expected that males rather than females will exercise choice when selecting a partner (Emlen & Oring 1977). One piece of evidence suggesting that males may use the sites to discriminate between infected and uninfected females is that uninfected females are more likely to be mated than infected females (Jiggins *et al.* 2000b). Assuming that males are choosing, this system poses a theoretical problem that is exactly analogous to the paradox of the lek. Namely, can the male-killing bacterium be maintained in a population in which males are discriminating against it?

At first sight this seems rather unlikely. Assuming that a choice gene can invade, its presence in the population confers a mating disadvantage on infected females relative to uninfecteds. The prevalence of the bacterium will therefore decrease, and consequently males with the choice gene will find it increasingly easy to realize the benefits of choice (because uninfected females will be easier to find and hence mate searching will be less costly). Such a positive feedback process could well lead to loss of the male killer (MK) altogether. If this is the case, then male mate choice is likely to be a rather transient phenomenon.

Our models are able to explain both the invasion and maintenance of a male choice gene coupled with retention of the male-killing bacterium. Crucially, male discrimination must have some degree of inaccuracy if choice is to be maintained.

2. THE SIMPLE MODEL

Fisher's explanation for the near ubiquity of the 1:1 sex ratio rests on the expectation that if the population deviates from equal numbers of males and females, any individual that produces an excess of the rarer sex will gain an advantage (provided investment in males and females is equal) (Fisher 1930). If this change is heritable then the gene responsible will spread, returning the population to equal proportions of males and females. A host population infected with a male-killing bacterium will by necessity be female biased, and so for similar reasons, most autosomal modifiers that promote male production will be favoured by selection (primary sex-ratio compensation is an exception). Attempts to model host genes that resist cytoplasmic sex-ratio distorters have been made previously (Randerson *et al.* 2000; Caubet *et al.* 2000; Werren 1997, and references therein; Uyenoyama & Feldman 1978).

(a) *Male killer only*

We specify a model of male killing similar to those that have been proposed previously (Randerson *et al.* 2000; Freeland & McCabe 1997; Hurst 1991). MK transmission is entirely vertical (mother to egg), with a proportion α of a mother's eggs being infected. (All parameters are listed in Appendix A.) There is no horizontal transmission (i.e. infectious transmission to conspecifics encountered by the host) and the bacteria do not enter sperm. All males infected with the bacterium die as eggs and their fitness is redistributed amongst the rest of the surviving brood. The MK benefits from this because it is mainly females (i.e. transmitters of the bacterium) that receive the fitness handout. We assume this 'fitness compensation' to be a function of the amount of male death that occurs and hence of α . If the death of males is perfectly compensated amongst the rest of the brood (i.e. no fitness is wasted), then the fitness augmentation received by survivors is equal to the term in brackets in equation (1). We assume fitness compensation to be a proportion ϕ of this theoretical maximum, so the compensation received by survivors as a consequence of male death is

$$\varphi = 1 + \phi \left(\frac{1}{1 - \alpha/2} - 1 \right) = 1 + \frac{\phi\alpha}{2 - \alpha}. \quad (1)$$

Note that this is an increasing function because as α and hence male death increases, the amount of fitness to be distributed increases, while brood fitness is shared amongst an increasingly small number of individuals.

In addition, we assume that the MK has a direct effect on adult female fitness U . There is evidence that the male-killing bacteria *Rickettsia* imposes a viability cost on females of its host *Adalia bipunctata* (Hurst *et al.* 1994). However, in *A. encydon* there is some suggestion that the bacteria may in fact benefit female hosts (Jiggins *et al.* 2000a). Either scenario can be incorporated depending on which operator precedes the U -parameter: positive values of U refer to a fitness cost, while negative values refer to a fitness benefit.

We assume for the purposes of deriving the MK invasion and equilibrium conditions that the population is infinite and panmictic with discrete generations. Recursion equations are expressed in terms of infected and uninfected females since all breeding males are uninfected. The proportion of infected adult females is p , while the proportion of uninfected adult females is q . W is the sum of the right-hand sides.

$$Wp' = p\alpha(1 - U)\varphi, \quad (2)$$

and

$$Wq' = p(1 - \alpha)\varphi + q. \quad (3)$$

The invasion conditions for MK are found when

$$\left. \frac{dp'}{dp} \right|_{p=0} > 1,$$

is satisfied, i.e. when

$$U < \frac{\alpha\varphi - 1}{\alpha\varphi}. \quad (4)$$

The equilibrium value of MK (p^*) is found by solving for $p' = p$, which gives

$$p^* = \frac{1 - \varphi\alpha + \varphi\alpha U}{1 - \varphi + \varphi\alpha U}. \quad (5)$$

This is a stable equilibrium. As reported previously (Hurst 1991), perfect transmission of a MK theoretically leads to fixation of the MK ($p^* = 1$ when $\alpha = 1$), otherwise p and q are maintained in polymorphism ($p^* < 1$ when $\alpha < 1$). Fixation of a MK would lead to population extinction because of the severe shortage of males.

3. INVASION OF THE CHOICE GENE

We propose a modifier of male choice that allows discrimination between infected and uninfected females. Although the mechanism for such choice is hypothetical, one could imagine a situation in which males use chemical cues to single out uninfected females. The potential advantages for such a gene are large, especially if the MK is at a high frequency. Males with the choice gene will be able to ensure that half of their offspring are male—the 'in-demand' sex.

We assume an autosomal, dominant choice gene that acts in males, but has no effect on females. There are two rounds of mate choice. In the first round, a male with the gene will encounter female genotypes (N.B. we use this term to refer to the presence or absence of both the choice gene and the bacterium) at their frequency in the population. If he encounters an uninfected female he accepts her and mates. If on the other hand he encounters an infected female he rejects her and moves on to the second round of mating. In the second round he accepts any female he encounters. We assume that the act of rejection and passage to the second round imposes a cost of extra mate searching, c ; there is a cost associated with a second bite at the cherry. Males without the choice gene always accept the first female they meet in the first round, hence they never suffer the cost of mate searching. One could imagine numerous other ways in which to model male choice and with no data on how males may be choosing this is perhaps as good as any. It seems unlikely that alterations, such as allowing multiple rounds of mating, would significantly change the qualitative predictions of the model, but this remains to be proven.

Generations are discrete and both sexes mate only once. Although the latter assumption is unlikely to be met in the field, it is necessary to prevent the model from becoming overly complicated. For convenience at this stage we also assume an infinite panmictic population. This provides the most permissible conditions for choice gene invasion because male mate selection in the first round of mating has no effect on the distribution of available female genotypes in the second round; the second bite at the cherry is as good as the first. We also assume at this stage that males and females do not compete with each other (males and females have a separate mean fitness W'_m and W'_f , respectively).

A further aspect of the model is that males may not be able to distinguish perfectly between infected and uninfected females. The parameter m describes the proportion

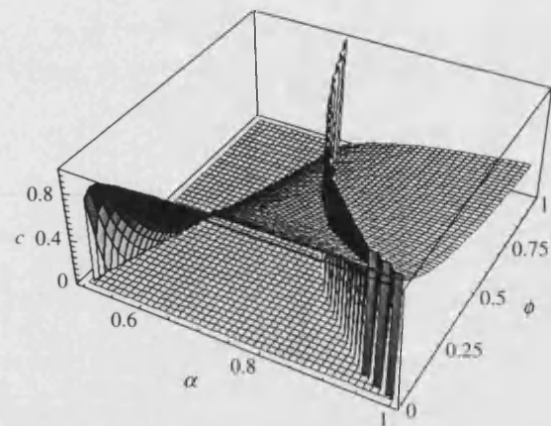


Figure 1. Invasion conditions for the simple choice gene (infinite population size) when $U = 0$ and $m = 0$. The vertical sheet represents the invasion conditions for the MK with parameter combinations to the right of the sheet allowing invasion. The other sheet represents invasion conditions for the choice gene. Invasion is permitted below the sheet. Note that for all parameter space in which the MK can invade, the choice gene can invade also. The choice gene is capable of invading in much of the parameter space despite a high cost of mate searching.

of occasions on which a male with the choice gene makes a mistake in the first round (i.e. either rejecting an uninfected female or accepting an infected female). Hence, $m = 0$ corresponds to perfect discrimination between infecteds and uninfecteds, $m = 0.5$ corresponds to an absence of choice, and $0.5 < m < 1$ would refer to choice for infected females. The recursion equations for the system and invasion conditions for the choice gene are reported in the electronic Appendix B, which can be found at The Royal Society Web site.

(a) 'Perfect' choice

We first consider the specific case of perfect discrimination ($m = 0$). Under these conditions, it is clear from figure 1 that as long as the MK can invade, the choice gene can invade also. What is more, very often it can do so despite a massive cost (c) of mate searching. Simulations were performed in order to determine the ultimate outcome of the system. In these the MK was allowed to reach its equilibrium frequency, then the choice gene was introduced at low frequency in males. If any genotype reached very low frequency (i.e. 10^{-6}), it was set to zero and the system was allowed to run to its equilibrium point. These simulations reveal that once the choice gene has invaded the host population, it almost always removes the MK completely. Results in which the choice gene and the MK are maintained in polymorphism only begin to appear when the cost of mate searching is of the order of 0.2 or greater, and even then they are rare. So, it appears that this simplest of models is unlikely to explain the results obtained from the *A. encedon* system. Although the presence of a MK can promote the evolution of male choice, if discrimination between infecteds and uninfecteds is perfect then the MK is exceedingly unlikely to be maintained in the population (let alone at the sort of frequencies we see in *A. encedon* populations).

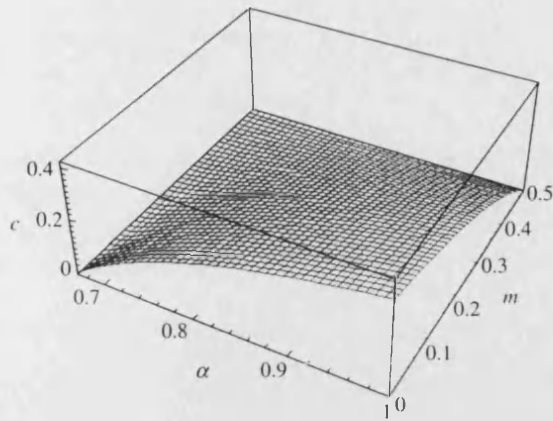


Figure 2. Invasion conditions for the simple choice gene (infinite population size) when $U=0$ and $\phi=0.5$. Only values of α for which the MK can itself invade are shown. Note that for all values of m below 0.5 the choice gene can invade if costly (this corresponds to some degree of discrimination between infected and uninfected females). Also note that as the frequency of mistakes in male choice increases (i.e. increasing m) invasion conditions become more stringent.

(b) Error-prone choice

Figure 2 shows the invasion conditions for the choice gene when $0 < m < 0.5$. This corresponds to some degree of error in discrimination between infected and uninfected females. It is clear that the choice gene is still capable of invading the population, although as the probability of errors increases, the invasion conditions become more stringent. An extensive scan of parameter space revealed that when $0 < m < 0.5$, results in which both the MK and the choice gene are maintained in the population are much more frequent. What is more, as choice becomes more inaccurate (i.e. increasing m) the proportion of results in which the MK is at high frequency increases. The model therefore predicts that for maintenance of the MK at high frequency, the frequency of male mistakes must be high.

(c) The effect of male sampling in the first round of mating

We have so far assumed that male choice in the first round of mating has no effect on female genotype frequencies in the second round. This would only be the case in an infinite population. We now consider the more realistic case of finite population size and hence the effect of male sampling. Let N_t be the total population size. We assume that this is invariable, but that the number of males and females (respectively N_y and N_x) can change (N.B. $N_y + N_x = N_t$). This amounts to a model in which an excess of juveniles are produced by each of the different male-female combinations, but that these individuals then compete for N_t spaces as adults in the environment. This assumption seems reasonable as long as the population is not so female biased that there are not enough males to maintain the population size.

To allow the calculation of N_x and N_y after changes in genotype frequencies in each generation, we now assume that males and females do not have separate mean fitnesses, but that there is a single population mean

fitness. Hence, the recursions for the more complicated model are the same as those for the simple model, except that W_x and W_y are replaced by a population mean fitness W (the sum of the relative fitnesses of all male and female genotypes). A further change from the simple model is that female genotype frequencies in the second round of mating (reported in electronic Appendix B) are now affected by male sampling in the first round. The model was investigated by simulation.

It is evident that invasion conditions for the choice gene are more stringent in a finite population than for the simple model. Intuitively this is sensible because the benefits of male choice are reduced. In the second round, some proportion of the uninfected females will have already been removed by mating in the first round, so the chance of meeting a 'desirable' female in the second round is reduced compared with the previous model. So the second bite at the cherry is not as good as the first. Despite this reduced likelihood of invasion however, the choice gene is still capable of invading and persisting in polymorphism with the MK. Figure 3a-d summarizes the parameter space in which we obtain results, where the choice gene and the MK both persist in polymorphism. If discrimination is perfect (i.e. $m=0$), then the MK is always ousted from the population by the choice gene. However, if there is even a slight amount of error (i.e. $m>0$), then both the MK and choice gene are retained at equilibrium, although for very low values of m the MK will be at very low frequency. The model predicts that invasion of the choice gene followed by persistence of the MK is most likely when male discrimination is imperfect, but with few mistakes, and when the cost of mate searching is low. For maintenance of both the MK and the choice gene at high frequency, the model predicts that male mistakes must be fairly common, the MK should impose a low cost (or preferably a benefit) to females and the cost of mate searching should be low.

(d) 100% transmission

Up to this point we have only considered MKs that are not transmitted to all of a female's eggs. What happens if the MK has perfect vertical transmission ($\alpha=1$)? Can the choice gene invade and reach an equilibrium at which both the MK and the choice gene are maintained? This arises as an interesting question when we consider the case of the butterfly *Hypolimnas bolina*, a MK host (see §4) (Hurst 1993).

When $\alpha=1$, so long as the MK can invade it will go to fixation in the population under conditions of panmixis. Hence, if a choice gene is going to have any effect on the MK's dynamics then it only has a short time-window in which to invade before the population goes extinct. An alternative sequence of events would be that a MK with a very efficient (but not perfect) vertical transmission prompted the evolution of a male choice gene and then subsequently improved its transmission to $\alpha=1$. This is more likely because it does not require the evolution of male choice in a very short time-period.

For convenience we investigated the first scenario because it was possible to address it using our existing model. Simulations were set up similar to those above, except that the choice gene was introduced after an

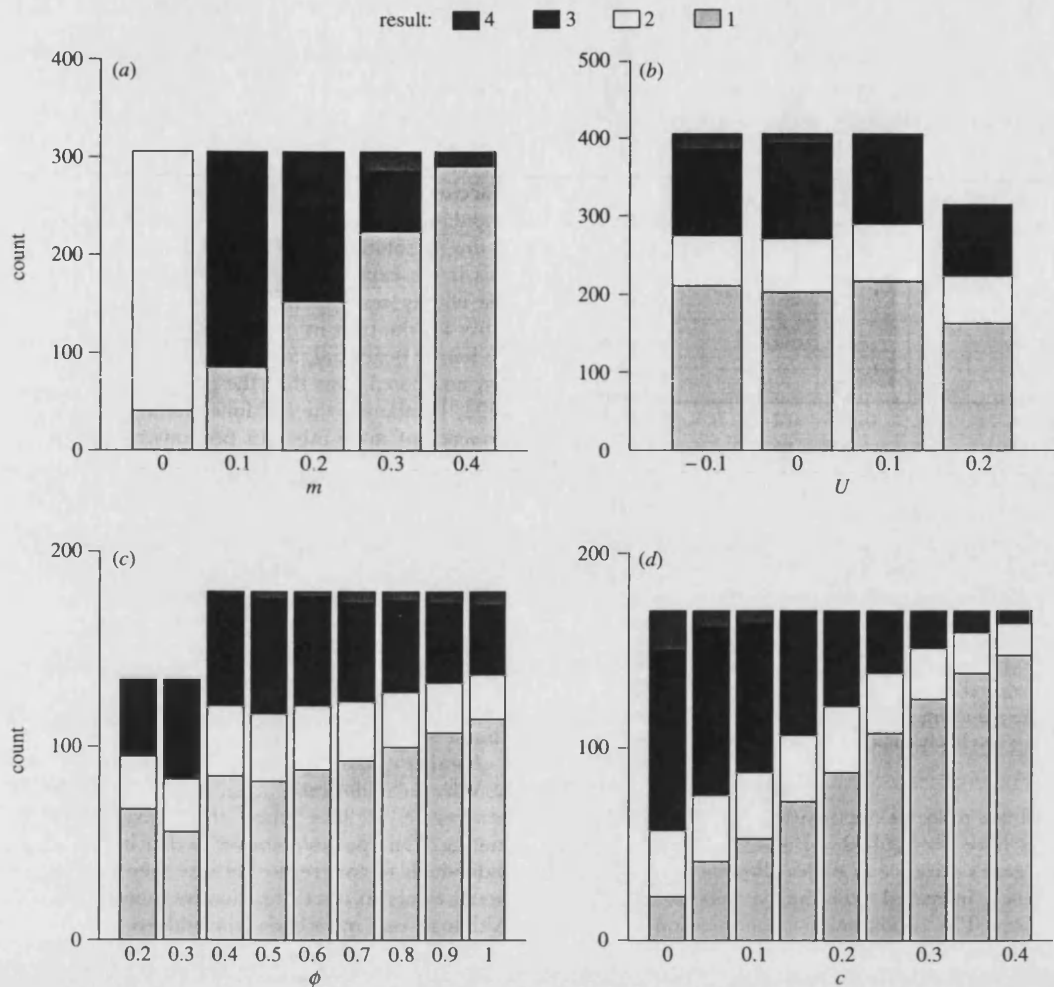


Figure 3. (a–d) Results of a parameter scan for the more complicated choice model (incorporating the effects of male sampling of female genotypes in the first round of mating). Simulations were conducted so that the MK reached its equilibrium frequency before introduction of the choice gene at low frequency. The system was again allowed to reach equilibrium and the result was classified as one of the following: 1, failure of the choice gene to invade; 2, invasion of the choice gene followed by loss of the MK; 3, maintenance of both choice gene and MK in the population at polymorphic frequencies; 4, polymorphism with both the MK and choice gene at high frequency (i.e. more than half of males exhibiting choice, and the MK frequency is in excess of 60%). The following parameter values were used in all possible combinations (the empirical estimate of $\alpha = 0.95$ from the closely related MK in *Acraea encedana* was used throughout; Jiggins *et al.* 2000a): m , range 0–0.4, step 0.1; U , range –0.1–0.2, step 0.1; ϕ , range 0.2–1, step 0.1; c , range 0–0.4, step 0.05 (all ranges inclusive). Ignoring those combinations for which the MK cannot invade, this gives 1534 combinations. In (a–d) the ‘count’ label on the y -axis refers to the number of each class of result for each value of the focal parameter.

arbitrary value of 20 generations and not when the MK had reached its equilibrium frequency. The results are presented in figure 4. In a number of cases, the system had not reached equilibrium after 500 000 generations. These were considered as results in which the MK and the choice gene are maintained. Although there are generally fewer results than for $\alpha = 0.95$, in which both the MK and choice gene are at high frequency, it is clear that persistence of both is a common result. So, the evolution of male choice for uninfected females could lead to the long-term survival of a host population infected with a MK that would otherwise have sent it extinct. To the best of our knowledge this is the only model that allows

population maintenance when vertical transmission is perfect (and is unaffected by host genotype) in the absence of population structure (but, see Heuch 1978; Heuch & Chanter 1982).

4. DISCUSSION

We have demonstrated that, in theory, the presence of a MK at equilibrium in its host population can select for the evolution of male choice for uninfected females. Such a choice gene gains its advantage because by frequently allying itself with the uninfected cytotypic, it ensures that in the next generation it will be represented in a brood

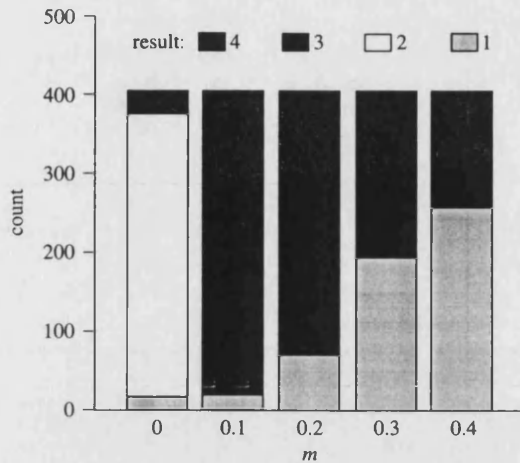


Figure 4. Results of a parameter scan for the more complicated choice model when $\alpha = 1$ (i.e. perfect vertical transmission). The data are similar to those in figure 3, but the range of parameter combinations scanned is slightly larger. In this case the values of the U -parameter considered were extended to 0.4. This gives an extra 491 parameter combinations for which the MK can invade. This was done because a MK with perfect transmission can invade even if it imposes a high cost. Note that retention of the MK and the choice gene is a common result, although maintenance at high frequency is less likely than for $\alpha = 0.95$.

with a 1:1 sex ratio, as opposed to one in which most of the males have been killed. In a female-biased population, the gene's presence in males allows it to take advantage of their increased reproductive success as the 'in-demand' sex. The conditions for the invasion of such a choice gene are benign, despite the incorporation of male mistakes and a cost of mate searching. Furthermore, as long as there are mistakes in male choice the MK is maintained in the population, which preserves the benefits of male discrimination. We thus provide a novel and simple solution to the lek paradox.

(a) *Relevance to the *Acraea encedon* system*

What are the model's predictions regarding the particular case of *A. encedon*? Observations suggest that in populations exhibiting female-aggregating behaviour, the MK tends to be at high frequency (Jiggins *et al.* 2000b). As is evident from figure 3a–d, although results in which both the MK and the choice gene are retained are common, those in which both are at high frequency are less so. This is because, in many cases, when the choice gene invades it brings down the frequency of the MK appreciably. However, there are cases in which the choice gene goes almost to fixation without making much of an impression on the MK. The model predicts that for both the choice gene and the MK to be at high frequency at equilibrium, male mistakes should be common, the cost of mate searching should be low and the MK should impose a low cost, or be beneficial to females. The first of these predictions has yet to be tested, the second is probably true given that females aggregate and the third has been suggested by previous work (Jiggins *et al.* 2000a).

(b) *The paradox of the female lek*

The strongest prediction from the models is that both the MK and the choice gene will be retained in the population as long as there are mistakes in male choice. This is the case even if there is no cost to the extra mate searching involved in rejecting an infected female in favour of one that is uninfected. Hence, if male choice is inaccurate then the MK cannot be 'chosen out' of the population. This is an intriguing result because it provides a simple solution to the lek paradox. In general terms, this is the expectation that by the act of mate selection, the choosy sex rapidly eliminates all population variability for the trait in question. Once this occurs, choice is no longer beneficial, and hence if the act of choosing has any associated costs then the gene will be lost (Andersson 1994; Pomiankowski & Møller 1995). In our model, the presence of male mistakes prevents the MK from being eliminated, so the paradox of choice without variation does not arise. Mistakes effectively result in a frequency-dependent cost to choice; as the choice gene invades and in the process pushes down the frequency of the MK, choosy males are more likely to encounter uninfected females in the first round. Hence, there comes a point at which the MK is so uncommon that it is detrimental to be choosing. This is because choosers will sometimes reject 'desirable' females and suffer the cost of mate searching. At this point, the fitness of choosers and non-choosers is equivalent.

Another question posed by conventional lekking species is: Why do 'undesirable' members of the chosen sex opt to congregate at leks when they have little chance of mating? One possible answer is that it pays 'undesirable' individuals to congregate because they benefit from occasional errors in discrimination by choosers (Kokko 1997). Although our models do not address this issue directly, we note that error in male discrimination is an essential component of the maintenance of variation in our model. Choice mistakes could well be an important general force maintaining leks.

(c) *The maintenance of perfect male killers*

Classical models of male killing predict that if the MK is transmitted perfectly to the entire brood (i.e. $\alpha = 1$), then it will rapidly go to fixation (Hurst 1991). In the process this will eliminate its host population due to the extreme dearth of males. This result applies even if one considers the evolution of host resistance. One class of resistance gene that has been modelled is that which acts in males to prevent bacterial killing. Invasion of such a gene always results in either fixation or loss of the MK when $\alpha = 1$ (Randerson *et al.* 2000). A further class of resistance genes that acts in females to reduce bacterial transmission to the eggs is able to maintain a perfectly transmitted MK at polymorphic frequencies. However, in this case, α no longer equals unity (Randerson *et al.* 2000). How then can we make sense of empirical evidence for a long-term infection of the butterfly *Hypolimnys bolina* with a perfectly transmitted male-killing cytoplasmic element?

It has been known for some time that the butterfly *Hypolimnys bolina* has a maternally inherited sex-ratio (SR) condition in which SR females give rise to all-female broods (Clarke *et al.* 1983; Hurst 1993). On a

number of the West Fijian islands, Simmonds found biased sex ratios due to the presence of a polymorphism for SR and normal females (Simmonds 1923, 1926, 1928). He also found that the SR condition was associated with high egg mortality and early larval mortality. Later work has established that the individuals killed are all males (Clarke *et al.* 1975). By revisiting sites at which the SR polymorphism was previously documented, Clarke *et al.* (1983) showed that in some instances, the polymorphism has existed for at least 150 generations. Assuming that the male-killing trait does indeed achieve 100% vertical transmission, this poses a theoretical problem. As described above, previous models of male killing in a panmictic population, predict that a MK with perfect vertical transmission will rapidly go to fixation, sending its host population extinct in the process (Hurst 1991). Our results suggest a novel explanation for the stable persistence of the SR condition over time. It is clear that provided male choice appears before the MK goes to fixation, it can prevent population extinction and allow the stable maintenance of the MK at polymorphic frequencies. This would manifest itself as a polymorphism for SR females and normal females. Clarke *et al.* (1983, p.230) suggest however that male mate choice is unlikely, stating that 'The butterflies flew and mated freely in our heated greenhouses and there was no indication that males mated preferentially with bisexual or unisexual females, or with those of any particular pattern.' This appears to be just an assertion however, not backed up by any controlled mate-choice experiments, so it is possible that they simply did not notice any mate choice that was in fact occurring (particularly if it was rather inaccurate).

At present it is not clear how general the phenomenon of male mate choice is in male-killing systems. However, there is some evidence from *Armadillidium vulgare* that females not infected with a feminizing *Wolbachia* are more likely to be mated than those that are infected (J. Moreau and T. Rigaud, personal communication). Moreover, empirical and theoretical work on meiotic drive in stalk-eyed flies has suggested that mate choice may be an important factor in the population dynamics of other types of sex-ratio distorter (Lande & Wilkinson 2000; Pomiankowski & Hurst 1999; Reinhold *et al.* 1999; Wilkinson *et al.* 1998).

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APPENDIX A. LIST OF PARAMETERS

α , proportion of eggs from an infected female that contain the male-killing bacterium.
 c , cost of mate searching, i.e. the cost suffered by males that reject a female in the first round of mating and move to the second round.
 ϕ , proportion of dead male fitness that is redistributed amongst the rest of the brood.
 φ , fitness compensation received by survivors in MK broods.
 U , cost imposed by the MK on infected females as adults.

m , proportion of occasions on which a male makes an error (in either direction) when discriminating between infecteds and uninfecteds in the first round of mating.

p , frequency of the MK in females.

q , frequency of the uninfected cytotype in females.

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As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.

An electronic appendix to this paper can be found at (http://www.pubs.royalsoc.ac.uk/publish/pro_bs/rpb1446.htm).

Appendix B - Details of the Simple Male Choice Model.

The genotype frequencies are represented as follows (males: y , females: x)

	No MK	MK (females only)
No choice gene	y_1, x_1	x_4
Heterozygous for the choice gene	y_2, x_2	x_5
Homozygous for the choice gene	y_3, x_3	x_6

The frequencies of matings in which there is no choice (i.e. those involving y_1) are simply the product of the respective male and female genotype frequencies. In our notation, $x_1y_1 = m_{11}$, $x_2y_1 = m_{21}$ etc. The frequencies of matings in which there is male choice (i.e. those involving y_2 and y_3) are as follows:

Genotypes involved	Notation	Frequency
x_1y_2	m_{12}	$y_2 (x_1(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{12})$
x_2y_2	m_{22}	$y_2 (x_2(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{22})$
x_3y_2	m_{32}	$y_2 (x_3(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{32})$
x_4y_2	m_{42}	$y_2 (x_4 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{42})$
x_5y_2	m_{52}	$y_2 (x_5 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{52})$
x_6y_2	m_{62}	$y_2 (x_6 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{62})$
x_1y_3	m_{13}	$y_3 (x_1(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{12})$
x_2y_3	m_{23}	$y_3 (x_2(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{22})$
x_3y_3	m_{33}	$y_3 (x_3(1-m) + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{32})$
x_4y_3	m_{43}	$y_3 (x_4 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{42})$
x_5y_3	m_{53}	$y_3 (x_5 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{52})$
x_6y_3	m_{63}	$y_3 (x_6 m + ((1-m)(x_4 + x_5 + x_6) + m(x_1 + x_2 + x_3))(1-c)x_{62})$

m is the probability of a mistake by a male and c is the cost of mate searching (suffered if a male enters the second round of mate searching). x_{12} , x_{22} , x_{32} etc. refer to the female genotype frequencies in the second round of mating. In the simple model in which population size is assumed to be infinite (so male choice in the first round has no effect on females genotype frequencies in the second round) these are the same as the original female genotype frequency. Recursion equations were obtained following the assumptions in the section "Invasion of the choice gene" (so $x_1 = x_{12}$, $x_2 = x_{22}$ etc.). It was necessary to carry out recursions on adult individuals because fitness compensation affects broods as a whole.

$$W_x x_1' = W_y y_1' = m_{11} + m_{12}/2 + m_{21}/2 + m_{22}/4 + m_{41}(1-\alpha)\phi + m_{42}(1-\alpha)\phi/2 + m_{51}(1-\alpha)\phi/2 + m_{52}(1-\alpha)\phi/4$$

$$W_x x_2' = W_y y_2' = m_{12}/2 + m_{13} + m_{21}/2 + m_{22}/2 + m_{23}/2 + m_{31} + m_{32}/2 + m_{42}(1-\alpha)\phi/2 + m_{43}(1-\alpha)\phi + m_{51}(1-\alpha)\phi/2 + m_{52}(1-\alpha)\phi/2 + m_{53}(1-\alpha)\phi/2 + m_{61}(1-\alpha)\phi + m_{62}(1-\alpha)\phi/2$$

$$W_x x_3' = W_y y_3' = m_{22}/4 + m_{23}/2 + m_{32}/2 + m_{33} + m_{52}(1-\alpha)\phi/4 + m_{53}(1-\alpha)\phi/2 + m_{62}(1-\alpha)\phi/2 + m_{63}(1-\alpha)\phi$$

$$W_x x_4' = (1-U)[m_{41} \alpha\phi + m_{42} \alpha\phi/2 + m_{51} \alpha\phi/2 + m_{52} \alpha\phi/4]$$

$$W_x x_5' = (1-U)[m_{42} \alpha\phi/2 + m_{43} \alpha\phi + m_{51} \alpha\phi/2 + m_{52} \alpha\phi/2 + m_{53} \alpha\phi/2 + m_{61} \alpha\phi + m_{62} \alpha\phi/2]$$

$$W_x x_6' = (1-U)[m_{52} \alpha\phi/4 + m_{53} \alpha\phi/2 + m_{62} \alpha\phi/2 + m_{63} \alpha\phi]$$

The invasion conditions for the choice gene in the presence of the MK were found by modifier analysis. Linearised recursion equations were obtained for the three genotypes in which the choice gene is heterozygous (y_2, x_2, x_5). The system was taken to be in equilibrium for the male killer. Hence $x_4 = p^*$ (equation 5), $x_1 = 1-p^*$, $y_1=1$ and $x_3 = y_3 = x_6 = 0$. In matrix form, the linearised recursions become:

$$\begin{bmatrix} y_2' \\ x_2' \\ x_5' \end{bmatrix} = \begin{bmatrix} a & b & c \\ d & e & f \\ g & h & i \end{bmatrix} \begin{bmatrix} y_2 \\ x_2 \\ x_5 \end{bmatrix} = \lambda \begin{bmatrix} y_2 \\ x_2 \\ x_5 \end{bmatrix}$$

Hence for invasion, the leading eigenvalue, λ of the resultant 3x3 matrix must be greater than one. It therefore follows that:

$$1 < a + bd + e - ae + cg - ceg + bfg + cdh + fh - afh + i - ai - bdi - ei + aei$$

This revealed that for invasion:

$$c < \frac{(1-2m)(2\alpha\phi - 1 - 2(\phi-1)(1+\alpha(U-1)\phi))}{(1+2\alpha(U-1)\phi)(\alpha\phi(1+U(m-1)-2m) + (1+\phi)m-1)}$$

Finite Population Size

If the population size is finite, the female genotype frequencies in the second round of mating (i.e. x_{12}, x_{22} etc.) will not be the same as those in the first round due to unequal sampling by males. If N_y and N_x are the numbers of males and females in the population respectively, then the frequencies of female genotypes in the second round of mating are:

$$x_{12} = (x_1(N_x - N_y) + (y_2 + y_3)x_1 m N_y) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

$$x_{22} = (x_2(N_x - N_y) + (y_2 + y_3)x_2 m N_y) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

$$x_{32} = (x_3(N_x - N_y) + (y_2 + y_3)x_3 m N_y) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

$$x_{42} = (x_4(N_x - N_y) + x_4 N_y(y_2 + y_3)) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

$$x_{52} = (x_5(N_x - N_y) + x_5 N_y(y_2 + y_3)) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

$$x_{62} = (x_6(N_x - N_y) + x_6 N_y(y_2 + y_3)) / ((N_x - N_y) + (y_2 + y_3)N_y((x_4 + x_5 + x_6)(1-m) + m(x_1 + x_2 + x_3)))$$

Chapter 8. Testing a solution to the paradox of the lek: do males choose *Wolbachia*-free females in a butterfly (submitted)

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Abstract

In some species, females select mates on the basis of a particular conspicuous male characteristic. However, the strong directional selection applied by such mate choice is expected to eliminate population genetic variance for the trait being chosen. This presents a logical problem for understanding the evolution of mate choice in lekking species; namely, how is such choice maintained in the face of limited variation. This is the ‘paradox of the lek’. One possible solution to the paradox is provided by selfish genetic elements, whose ‘drive’ might be capable of maintaining deleterious alleles despite mate choice against them. We have tested this hypothesis in the sex role reversed mating system of *Acraea encedon*. Here a male killing *Wolbachia* bacterium can reach prevalences in excess of 95%, and this is associated with virgin females forming large congregations that males visit in order to mate. Given the extremely female-biased sex ratio, it has been argued that male preference for uninfected females would be selectively favoured. Indeed, theory suggests that both male choice genes and the female trait (the infection) can be stably maintained. This idea is supported by a previous finding that uninfected females at congregations are more likely to have mated. Here we test the hypothesis that males are choosing. Captive-bred males were released at two congregation sites and their mating partners were collected. The infection frequency in these ‘chosen’ females was then compared with that in the population. Contrary to the predictions of the role-reversed lek hypothesis there was a higher proportion of infected individuals amongst the ‘chosen’ females at both sites. Furthermore, we were unable to reproduce the result that uninfected females are more likely to have mated. These results argue strongly against the hypothesis that males are choosing uninfected mates.

Introduction

Typically, it is females that choose mates based on some aspect of male phenotype (*e.g.* antlers, plumage). Such mate choice is capable of producing intense directional selection on the chosen trait (Ryan 1997). This, however, poses a logical problem for theories of sexual selection. Strong selection might be expected to eliminate genetic variance for the chosen trait. With variation extinguished, there will be no advantages to the female choice, so why do females continue to choose. This is the 'paradox of the lek' (Pomiankowski & Moller 1995).

A possible solution to the paradox is provided by selfish genetic elements. These are genes which spread despite their deleterious effects on the 'host' organism because they break Mendel's rules and gain a transmission advantage to the next generation (Hurst *et al.* 1996). Even if males choose to mate with females that do not carry these elements, they can potentially be maintained in a population because of this 'drive'. Some support for this hypothesis is provided by stalk-eyed flies, where females are thought to choose males which either do not carry the meiotic drive allele or do carry suppressors of meiotic drive (Wilkinson *et al.* 1998; Reinhold *et al.* 1999; Lande & Wilkinson 2000).

We have tested whether an analogous process has led to the evolution of male mate choice in a sex-role reversed mating system. The East African butterfly *Acraea encedon* is infected by a male-killing *Wolbachia* bacterium (Jiggins *et al.* 1998; Hurst *et al.* 1999). Male-killing is a tactic employed by maternally inherited parasites whereby the sons of infected mothers are killed. This enhances the fitness of the parasite's clonal relatives in the dead sons' sisters (Hurst & Majerus 1993; Hurst *et al.* 1997; Randerson 2000) and allows it to spread in the host population. The *Wolbachia* infection in *A. encedon* is unusual because the bacterium is at remarkably high frequency (78-100% of individuals infected (Jiggins *et al.* 2000)) resulting in extremely female biased sex ratios. Furthermore, a link has been demonstrated between the high prevalence of the bacterium and the butterfly's mating system (Jiggins *et al.* 2000). In those populations where the parasite is at high frequency, females form dense aggregations at traditional sites (usually grassy areas near landmarks such as trees and hilltops). These aggregations occur during the afternoon and are attended preferentially by virgins, suggesting that congregating females are seeking mates (Jiggins *et al.* 2000). Larval and adult foodplants are generally absent from these sites, so females do not appear to congregate in order to feed or lay eggs. This behaviour can be contrasted with that of females in populations with *Wolbachia* at low prevalence. Females here do not congregate and are found mostly associated with the larval or adult foodplant. In these populations, males presumably seek out females dispersed throughout the habitat (Jiggins *et al.* 2000).

The characteristics of these female congregations (small area, receptive females, no resources) suggests they may be a sex role reversed form of the widespread lekking or swarming behaviour observed in other insects (Jiggins *et al.* 2000; Randerson *et al.* 2000). In classical vertebrate leks it is males that congregate, occupying micro-territories that do not contain significant resources (Emlen & Oring 1977; Ryan 1997). With numerous males within such a small area, these sites offer females an opportunity to choose between potential mates.

The roles of competing and chosen sex are defined by the operational sex ratio (OSR) in the population (Clutton-Brock & Vincent 1991; Kvarnemo & Ahnesjo 1996) (that is, the ratio of males to females who are ready to mate in a population at a given time (Emlen & Oring 1977)). In the majority of

species it is males that have the faster reproductive rate because they invest much less heavily in each reproductive attempt. However, a number of factors have been identified that can reverse this conventional position (*e.g.* male care, large nuptial gifts, biased sex ratio (Gwynne 1991; Kvarnemo 1997)).

There is some evidence that males, at the female congregations of *A. encedon*, might select mates based on their infection status. Jiggins *et al.* (2000) showed that uninfected females were more likely to have been mated than infected females. While this result is open to alternative explanations, there would be clear advantages to such a male choice strategy. Due to the extremely female-biased sex ratio, sons have roughly 20 times the reproductive success of daughters, so by Fisherian selection favouring production of the rare sex (Fisher 1930), one would expect a huge advantage to any gene allowing males to select uninfected females as mates. By mating with such females, a male would avoid the bacterial-killing of half his progeny (indeed, the most valuable half to him in terms of their reproductive success).

Randerson *et al.* (2000) investigated the theoretical plausibility of this suggestion using population genetic models. They found that such a modifier gene of male choice could spread under a broad set of conditions. However mate-selection mistakes by males are necessary for it, and the male-killer, to be maintained in the population. The model predicts that for the male-killer to be maintained at high prevalence in the population there must be frequent mistakes in male choice. Hence, if choice is occurring it must be fairly inaccurate (Randerson *et al.* 2000).

Although there are numerous examples of sex-role-reversal (see references in Kvarnemo 1997)), there are few convincing demonstrations of male mate choice in leks (but see Svensson & Petersson 1988; Gwynne 1991; Owens *et al.* 1994; Sandvik *et al.* 2000). Here, we test in the field whether *A. encedon* males select uninfected females.

Materials, Methods and Results

Estimating Wolbachia Prevalence

Sampling was carried out at three sites in Uganda, during January and February 2001. The sites (Makerere, Ggangu, Entebbe) were all in the vicinity of Kampala, no further than 22km apart. Makerere and Ggangu are both hill-top sites at which the butterflies form a 'lekking swarm' in the afternoons. Experiments involving the release of artificially reared males were also carried out at these two. The field sites are a sub-set of those described by Jiggins *et al.* (2000).

Sampling at Entebbe was undertaken (mostly during the morning) on 17 different days over a 20-day period. This population inhabits a lake-fringe habitat on the shores of Lake Victoria where the larval foodplant *Commelina benghalensis* is abundant. The population does not form afternoon mating congregations and males are more abundant than at the other two sites.

Initially, on successive afternoons, every effort was made to catch all *Acraea encedon* at the hilltops of the 'lekking' populations (*i.e.* Makerere and Ggangu). These samples were used to estimate the prevalence of the *Wolbachia* bacterium in the populations prior to release experiments. The daily intensive sampling had only a small effect on the numbers of *A. encedon* congregating at the sites. Any mating pairs were recorded as such. These were regarded as 'natural choices'. A further smaller

population sample was taken in the same way following release experiments. This was done in order to examine whether the bacterial prevalence had changed during the release period.

Infected individuals were identified using *Wolbachia*-specific polymerase chain reactions (PCR). The procedure for PCR and DNA extraction was identical to that described by Jiggins *et al.* (1999). A number of checks were carried out to verify the accuracy of this technique. Negative results were replicated twice to confirm the uninfected status of the individuals. Furthermore, insect specific primers were also used in order to check that the DNA extraction process had been successful. In 14 out of 1487 samples, DNA extraction was unsuccessful. Males in the sample acted as negative controls. The technique did not give a positive result for any of these ($N = 54$), suggesting no cross-contamination of bacterial DNA between samples. In addition, offspring were reared from some of the wild females, and those that produced all-female offspring acted as positive controls. The technique gave positive results for all of these ($N = 65$). The prevalence and sex ratio at the three sites is recorded in Table 1.

At Ggangu, the data support the notion that bacterial prevalence in females remained constant while release experiments were carried out (Uninfecteds: before, 14.9%; after, 12.2%. $\chi^2 = 0.161$, $DF = 1$, $P = 0.69$ (Table 1)). The population prevalence was therefore taken to be the combination of the before and after sample. However, at Makerere, bacterial prevalence appeared to decrease during the course of the release experiment (Uninfecteds: before, 2.8%; after, 9.2%. Fisher exact test, $DF = 1$, $P = 0.031$ (Table 1)). Hence, for the purposes of comparison with the batch of females chosen by males, the post-release experiment sample was used as the measure of bacterial prevalence being the most conservative way in which to treat the data when testing for choice against infecteds.

Experimental Male Releases

The males for release were artificially reared from lines collected previously at Entebbe. Although this population does not currently perform lekking behaviour, it has previously. When the infection was at higher frequency and males much more scarce, congregations of females were observed at a grassy area close to trees (Jiggins *et al.* 2000). Therefore, if there is a genetic component to lekking behaviour and choice then it is presumably present in the Entebbe population.

After emergence the males for release were kept in a large outdoor cage with females from the same lines. The males consequently had easy access to matings prior to their use in a release experiment. On the day of an experiment, males were fed on sugar solution and transported in paper envelopes to the field site. Males were released individually at the focal point of the lekking swarm (*i.e.* where there was the highest concentration of females) by placing them on vegetation. Prior to release, the males were marked with felt-tipped pen so that they could be recognised during the experiment and if they were recaptured on subsequent days. Behavioural data were recorded for a sub-set ($N = 21$) of the released males. The statistics that follow refer to this sub-set and are intended merely to give an impression of male behaviour.

After an initial inactive period, most males reacted to females flying overhead by taking off and giving chase. These mid-air chases were usually short (< 15 s). Males that mated spent a median of 3 minutes to do so from the time of their release. Of this, a median of 40 seconds was spent in the air and they engaged in a median of 4 mid-air chases. Some males did not take any interest in the congregating

females around them and flew directly upwards into the trees. Although initially lost to the experiment, we often recaptured these males either later the same day or on subsequent days. Almost invariably, when recaptured these males were in copula. These pairs were taken to represent genuine choices by males and were used in the analysis. In total, 172 males were released: 111 at the Makerere site and 61 at Ggangu. Of these, 144 were recaptured in copula. A further 2 were recaptured at the Makerere site away from the hill-top during a subsequent morning and one was observed to be predated by a flower mantid.

After a mid-air encounter with a female, some males broke off the chase and landed. Alternatively, some males would begin chasing another female while in mid-air. After a chase in which the male remained 'interested' (*i.e.* did not switch to a different female or land), the female would land and rest with a ventrally curved abdomen. The male would then land on top of her and position himself for mating. In a number of cases the male appeared to have grasped the female's abdomen with his claspers but subsequently broke off and began flying again. Hence, pairs were collected only after they had been in copula for a few minutes. The next phase involved the female taking-off with male attached and transporting him a few metres away from the focus of the lekking swarm. Every effort was made to collect pairs that reached this stage.

Are Males Choosing?

Does the bacterial prevalence in the chosen females differ from the bacterial prevalence in the population? At both sites, the proportion of uninfected females in the chosen sample was lower than that in the population as a whole (Table 1). At Ggangu (Uninfecteds: population, 14.2%; chosen, 1.8%. $\chi^2 = 5.61$, $DF = 1$, $P = 0.018$) this difference was statistically significant, but at Makerere it was not (Uninfecteds: population (post-release), 9.2%; chosen, 3.4%. $\chi^2 = 2.139$, $DF = 1$, $P = 0.14$; population (all data), 4.1%; chosen, 3.4%. Fisher exact test, $DF = 1$, $P = 0.90$). In addition, we captured 3 wild pairs in copula (2 at Makerere and 1 at Ggangu). In all of these 'natural choices', the female was infected.

Taken together, these data argue strongly against the existence of mate choice in favour of uninfecteds. Indeed, the trend for fewer uninfecteds in the chosen sample at both sites suggests that, if anything, males are selecting infected females. A more rigorous test of this hypothesis is possible by combining all the data from the two sites and using a technique developed by Fisher (Chapter 18 Sokal & Rohlf 1995) to combine the two p-values. This reveals a tendency for males to choose infected females significantly more often than uninfected females (combined p-value: $0.05 < P < 0.1$), although this trend is not significant. Hence, the data suggest, if anything, precisely the opposite situation to that predicted by theoretical modelling (Randerson *et al.* 2000). It appears that males mate more often with infected females (at least at Ggangu), either because of active male choice, or because infected females compete more effectively for males.

Do Uninfecteds Have a Mating Advantage?

In addition to working out bacterial prevalence, the mating status of females in all three populations was determined by dissecting their reproductive tracts and recording the presence/absence/number of spermatophores. It is known that males only transfer one spermatophore per mating (Jiggins *et al.* 2000). One check on the reliability of this technique was that all females that

Given that males appear not to be choosing uninfected females, is it possible to reproduce the previously reported finding that uninfected females at the congregations are more likely to have mated (Jiggins *et al.* 2000)? Firstly, the proportion of mated females at the congregations was low (Makerere; 11 of 157 females; Ggangu; 17 of 143 females). All females that were mated contained one spermatophore (except one at Ggangu which harboured two). These findings match previous results (Jiggins *et al.* 2000) and are to be expected given the scarcity of males at the populations with the bacteria at high frequency.

This situation contrasts sharply with that at Entebbe, where the majority of females contained at least one spermatophore (150 of 204 females dissected). Indeed many females here contained more than one; 14 females harboured two and 1 had three spermatophores. The likelihood that a female had been mated however did not differ between infecteds and uninfecteds ($\chi^2 = 0.1$, DF = 1, $P = 0.75$).

At Makerere, none of the uninfected females ($N = 8$) contained spermatophores, but amongst the infected females ($N = 136$), 11 were mated. This difference was not significant, however (Fisher exact test, DF = 1, $P > 0.9$). At Ggangu, out of 17 uninfecteds, 4 had mated, compared with 19 out of 122 infecteds. Again, these are not significantly different (Fisher exact test, DF = 1, $P = 0.5$). The data, therefore, strongly refute the hypothesis that uninfected females have a mating advantage. This runs contrary to data collected previously at the Makerere site (Jiggins *et al.* 2000).

Although the sample size in the previous study was larger ($N = 215$), one could argue that the data in the present study are more reliable because they were collected over a greater period of time. Sampling days were spread over about a month for both sites (Makerere: 7 days, Ggangu: 8 days). This compares with 2 days sampling in the previous study (Jiggins *et al.* 2000). Given that we find males apparently choosing infected females (see above), one might expect a mating advantage to infected females. We do not observe this, although the trend in the Makerere population was in this direction.

Discussion

The data presented here strongly reject the notion that male *Acraea encedon* choose to mate preferentially with uninfected females. In 'choice' experiments where captive males were released at female aggregation sites, the batch of females sampled by males in fact had a higher proportion of infecteds than the population as a whole. This goes against the predictions of previous theoretical models (Randerson *et al.* 2000).

Furthermore, we were unable to reproduce the previous result (Jiggins *et al.* 2000) that uninfected females have a mating advantage over infected females. In this study, sampling was spread over a greater period of time (see above), and so would be less vulnerable to any single migration event. By contrast, the previous result (Jiggins *et al.* 2000), of a mating advantage to uninfecteds, could have been due to a recent migration of females from a population with infection at low frequency. Such females would be more likely to have mated (because more males in that population) and also a smaller proportion would carry the infection. Although this explanation might account for the previous results, we note that Owen and Chanter (1969) found that migration between populations in *A. encedon* was generally very low.

Having established that males are not choosing uninfected mates (and indeed appear to be doing the opposite), we are left with the vexing question of what the mating congregations are for. It is possible

that males use the sites to choose some other aspect of female phenotype, but what that might be is not obvious from casual observation. Alternatively, the sites might facilitate the quick location of females by males, or may attract males from more distant populations where the bacterial prevalence is lower. Virgin females attending such sites might be at an advantage if the number of males attracted is disproportionately larger than the number of receptive females at the site. This hypothesis receives some support from the observation that the aggregations are always located at prominent landmarks such as on hilltops and beside tall trees. Another possibility is that the aggregations reduce predation on the butterflies. Warningly-coloured, distasteful insects are often thought to aggregate in order to reduce predation by 'naïve' predators (Gagliardo & Guilford 1993).

In other *Acraea* species, hilltopping behaviour (small congregations, typically of males) is widespread (pers obs). In *A. encedon*, males and females might search for mates at grassy areas near trees in all populations (whether *Wolbachia* is at high or low frequency). If pairs leave the sites once they have started mating (as we observe) then in a population with a 1:1 sex ratio, the numbers of butterflies at the congregation site will be low at any one time. Hence, a 'lekking swarm' might not be recognised as such. Conversely, in populations with the infection at high prevalence (and hence with scarce males), there will always be a large number of unmated females at the site who have failed to find a partner. This hypothesis would explain both the absence of congregations in populations with low infection frequency, and the previous result that unmated females are more likely to remain at mating congregations (Jiggins *et al.* 2000).

Why then, might males be mating preferentially with infected females? It has been suggested that, rather than imposing a metabolic cost on their host, the *Wolbachia* strain in *A. encedon* might confer some physiological benefit (Jiggins *et al.* 2000). Hence, males might mate with them more often not through choice, but simply because they are more likely to encounter them.

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Chapter 9. Conclusion

“...there had come the realisation that the genome wasn't the monolithic data bank plus executive team devoted to one project - keeping oneself alive, having babies - that I had hitherto imagined it to be. Instead, it was beginning to seem more a company boardroom, a theatre for a power struggle of egotists and factions. Emergent from the potential strife I was having to imagine a kind of parliament of genes, and the signs suggested a rowdy parliament at that.” (p. 133 Hamilton 1996)

Evolutionary conflict has clearly been an important force shaping genetic systems (Hurst *et al.* 1996), the most convincing examples being the evolution of mating types as a defence against cytoplasmic fast-replicators (Hurst 1995) and sex chromosome evolution in *Armadillidium vulgare* (Hurst 1995; Rigaud 1997). However, the work presented here suggests that one should exercise caution when assigning a role to conflict in particular cases.

This thesis includes three examples where, on closer analysis, conflict arguments have been found wanting. Whilst ESS/defence arguments have suggested a role for conflict in the evolution of meiosis (Haig & Grafen 1991; Haig 1993) and anisogamy (Grun 1976), more detailed modelling suggests that such an explanation is, at the very least, insufficient. Furthermore, the hypothesis that male mate choice has evolved in response to a male-killer infection has been refuted empirically in at least one species.

In the case of meiosis (Chapt. 2; Hurst & Randerson 2000), population genetics models show that selection for the necessary transitions (one-step meiosis → one-step with recombination and one→two step meiosis) is unlikely to have been imposed by the postulated selfish elements. This is particularly the case when such elements are unconstrained in their tolerance to their own killing action.

For cytoplasmic inheritance, it has been argued that anisogamy might represent a generalised defence against selfish cytoplasmic elements (Grun 1976; Cosmides & Tooby 1981; Hurst 1990), *i.e.* one that would encompass both errant organelles and symbionts. More detailed population genetic models supported this claim (Law & Hutson 1992). However, I have shown that the success of this argument is highly dependent on assumptions about the frequency and regularity of the invasion of 'selfish' cytoplasmic fast-replicators. The particular mechanism of uniparental inheritance implied by anisogamy (*i.e.* destroy your own gamete's cytoplasm prior to fusion) is unlikely to spread to high frequency in the face of a selfish cytoplasmic replicator unless the invasion of such replicators is regular and frequent. Such a model can work if the costs associated with isogamy arise directly from cytoplasmic mixing (Hurst 1990), but the evidence for such costs is limited (Randerson & Hurst 1999; Randerson & Hurst 2001b).

In light of these and other results (Randerson & Hurst 2001a), the solution to the anisogamy problem is far from clear. Whilst numerous models propose a variety of advantages to small sperm and large eggs (Sperm: increased motility, more numerous. Eggs: more resources, larger target, increased

pheromone production) none appear capable of providing a universal solution (Capt. 5 (Randerson & Hurst 2001b)). A central problem, identified by Parker, Baker and Smith (1972), is that any advantage to large eggs must be sufficient to counter the loss of fecundity associated with it. It is unclear what would give rise to such a disproportionate advantage to large eggs.

Perhaps the best candidate for a solution to this problem is provided by models proposing that larger eggs are favoured because they can produce more attraction pheromone (Dusenbery 2000). If the relationship between egg size and pheromone production rate is such that there is a greater than proportional increase in fertilisation chance per increase in size then such a model can work. This relationship is far from clear however. Furthermore, such a model fails to explain anisogamy in taxa where there is no pheromone production.

Returning to the theme of the wider consequences of selfish elements; we have shown that, in theory, the invasion of a male-killer produces the necessary selective conditions for the invasion of three types of resistance genes. In Chapter 6, I examine two; one acting in females to block bacterial transmission and one acting in males to block killing. Whilst it is not surprising that such genes can invade, the models also support the notion that stable maintenance of more than one male-killer is possible in the context of host resistance (Chapt. 6 (Randerson *et al.* 2000b)). Without resistance, theory expects that the 'best' male-killer will out compete all others. This depends on both its transmission efficiency and the metabolic cost it imposes (summed up in its 'Basic Rate of Increase' (Chapter 6)). The presence of host resistance might explain the co-existence of 4 male-killer variants in a single population of the 2 spot ladybird (*Adalia bipunctata*) (Majerus *et al.* 2000) and 2 such variants in a population of the butterfly *Acraea encedon* (Jiggins *et al.* 2001).

Observations of the natural history of *A. encedon* have suggested that high prevalence male-killer infection has had a profound effect on population mating system. In particular, it seemed that extremely female biased sex ratios might select for a sex role-reversal. In populations where such extreme biases exist, one observes a shift in the population mating system (Jiggins *et al.* 2000). Rather than males and females mating whilst dispersed amongst habitat containing the larval foodplant, one finds large female congregations that form during the afternoon at traditional sites (grassy, near trees and usually at hilltops). Here, females exhibit very strange behaviours (*e.g.* chasing and mounting other females, ventral curling of the abdomen). It has been proposed that these sites might act as role-reversed leks at which females congregate and males choose amongst them (Jiggins *et al.* 2000). Specifically, males might benefit by seeking out uninfected females. This proposition has some empirical support. Females appear to attend the congregations in order to mate (virgins are more likely to stay than mated females). Furthermore, there is evidence that uninfected females at the sites are more likely to have mated than infected females.

I show in Chapter 7 that this idea is theoretically plausible (Randerson *et al.* 2000a). The presence of a male-killer does set up selection for a resistance gene allowing male choice in favour of uninfecteds. However, unless there are frequent male mistakes, the male-killer will be chosen out of the population or reduced to a low level. The validity of this model in the *A. encedon* system however is strongly refuted by field data (Chapter 8). Not only do males not preferentially mate with uninfected females, but, if anything, they appear to do just the opposite. This puzzling result runs contrary to the

predictions of the model. Furthermore, I fail to recover the previous result (Jiggins *et al.* 2000), that uninfected females are more likely to have mated.

In conclusion, conflict has undoubtedly been an important force shaping genetic systems and has had a profound influence on evolutionary transitions from lower to higher levels of complexity. However, each example must be examined carefully (both theoretically and empirically), on a case by case basis. In particular, we must exercise the highest possible theoretical standards. 'Defence' arguments, which assume that just because a particular genetic system protects against a particular selfish element it must have evolved for this purpose, are not sufficient. I have shown that in two cases (meiosis and anisogamy) they can be misleading. Furthermore, Hammerstein (1996) argues that such modelling techniques are expected to be misleading in cases involving non-Mendelian genetics. It is vital, therefore, to model the dynamics of the transition itself.

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