



Dynamic Analysis of the Evolution of a Novel Genetic System: the Evolution of Ciliate Meiosis

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Many ciliates undergo a peculiar form of meiosis in which four haploid nuclei are produced, three are digested, and the single remaining nucleus undergoes mitosis. It is paradoxical that such a meiotic process occurs, since one could imagine several other less costly ways of producing two nuclei. Here we investigate a possible resolution of this paradox. It is shown that the spread of a selfish gene that kills the mate not containing it, provides the conditions for the spread of a costly modifier of the form of meiosis. We investigate the conditions under which the modifier can fixate.

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Introduction

Ciliates are unicellular eukaryotes that employ a curious form of meiosis. Ciliates typically have two types of nucleus, the macronucleus and the micronucleus. The micronucleus is a diploid nucleus that functions as the germ line, and the macronucleus is effectively somatic. Only the micronucleus undergoes meiosis, the macronucleus being destroyed just after this process. Prior to meiosis two cells will bond together, after which meiosis occurs in each cell. The typical meiosis is a strange process where four haploid nuclei are produced as in the classical meiosis of spermatogenesis, but three are digested (cf. polar bodies), and the remaining nucleus undergoes mitosis to leave a dikaryon. One haploid nucleus from each cell is then passed into the partner. Thus, the resulting pair of cells have two haploid nuclei. These nuclei fuse to produce a diploid nucleus. This nucleus undergoes mitosis (but without cell division) with one product becoming the new micronucleus and the other the macronucleus.

This novel meiotic process is paradoxical inasmuch as many simpler ways of performing a meiosis could be envisaged: a cell could extrude a product at meiosis I

and resolve the remaining nucleus through meiosis II to produce two nuclei, or a cell could produce four products through meiosis I and kill two in meiosis II. But why produce four, digest three and duplicate one?

It has been conjectured that paradoxical aspects of genetic systems may well be a response to the spread of selfish elements. Here we consider such a selfish genetic element and find that, at least in principle, such an element could have led to the evolution of this novel meiotic form.

Basic Model—Invasion of the Selfish Gene

GENETIC MODEL

We assume that the ancestral condition was that ciliates underwent a form of meiosis in which one nucleus would be destroyed after meiosis I and the remaining one would be resolved to two haploid nuclei in meiosis II. Crossing over occurs during meiosis I (Fig. 1). Following meiosis, two cells pair up side by side, and undergo the mutual sexual exchange of one haploid nucleus from each cell. The zygotic cells then separate.

Consider a population of ciliates that has an autosomal mate killing factor, a selfish gene to be referred to as allele “A”. The alternative allele at the same locus is assumed to be a null allele referred to

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as “a”. It is modelled as a single locus, but in fact represents two very closely linked loci held in an inversion so preventing recombination from separating them. One locus produces a toxin and the other locus produces the antitoxin. One might think of the system as being comparable to the *hok/sok* system of plasmids (see Gerdes *et al.*, 1990). Many, if not most, selfish genetic elements are of this nature (e.g. meiotic drive genes, see Lyttle, 1991). We will assume that the toxin is produced prior to nuclear exchange but after meiosis, and is held in the cell in which it is produced. The antitoxin locus, in contrast, is expressed after the exchange of nuclei and is also held in the cell in which it is produced.

Cells that contain the “A” allele following meiosis but lose it during nuclear exchange will die as a result of the presence of the toxin and absence of the antitoxin. It is for this reason that the selfish gene may spread: it only kills cells not containing it. The cells which mate with cells that are killed are assumed to have a competitive advantage g due to more nutrition and reduced competition. We shall also assume that cells containing the “A” allele will suffer a cost due to the stress of making a toxin and an antitoxin. This cost is allocated independently for toxin production and for antitoxin production. For the selfish gene (allele “A”) to function correctly it needs to produce the toxin before nuclei are exchanged, but produce the antitoxin after this exchange. Hence the toxin costs are applied to the cells after meiosis, but the antitoxin costs are applied to the cells after nuclear exchange.

If pre-exchange a cell has an “AA” genotype, then the cell suffers a cost U_t for producing the toxin. If a zygote (after exchange) is “AA” then it suffers a cost U_a for producing the antitoxin. These costs are reduced in heterozygotes, such that the cells suffer the

lesser costs $h_t U_t$ and $h_a U_a$ respectively. Fitnesses are assumed to be multiplicative.

MATHEMATICAL MODEL

There are three diploid genotypes in this population; “AA”, “Aa” and “aa”. Each of these is present in the population with a frequency given by x , y and z respectively, and $x + y + z = 1$. The frequencies of genotypes following meiosis may be determined and are labelled xm , ym and zm , respectively. Recombination affects the number of homozygotes produced from heterozygotes. If the locus is very close to the centromere ($r = 0$) then there will be no recombination, and there will be no heterozygotes in the population following meiosis. If the locus is far from the centromere and homology is high so that recombination is extremely likely ($r = 1$), then meiosis will not change the population frequencies. The post-meiotic frequencies are:

$$xm = x + 0.5(1 - r)y$$

$$ym = ry$$

$$zm = z + 0.5(1 - r)y.$$

There are six different mating combinations that may occur. The mating frequencies are determined by the genotype frequencies following meiosis, and are: AA–AA at xm^2 , AA–Aa at $2.xm ym$, AA–aa at $2.xm zm$, Aa–Aa at ym^2 , Aa–aa at $2.ym zm$, and aa–aa at zm^2 . (Note, we could have considered pairing to occur prior to meiosis, as actually occurs, but the above formulation is possibly clearer. It makes no difference to the analysis.)

Each cell contains two pronuclei since post-meiotic ciliates are dikaryons. When two such cells join together for mating, one nucleus will be expelled from each and pushed into the other. It is assumed that the expelled nucleus is randomly selected. There are three possible exchanges that may occur at the A–a locus: “A” swaps with “A”, “A” swaps with “a”, or “a” swaps with “a”. Each mating has four possible outcomes, and the genotypic outcomes must be worked out for all six mating possibilities. The frequencies of the genotypes resulting from each mating combination are shown in Table 1.

The new frequencies of each genotype are calculated by summing the frequency of cells resulting from each mating combination, each multiplied by the fitness of the cell and the frequency of the mating combination. This results in the following three equations for the frequency of each genotype in the next adult generation:

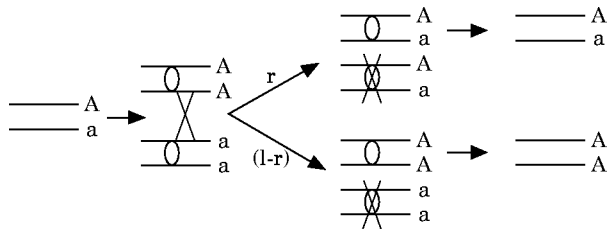


FIG. 1. The DNA replicates creating two pairs of sister chromatids, and recombination may occur. One pair of sister chromatids is digested, and the other pair of chromatids separates to leave a zygote ready for nuclei exchange. Recombination will result in a heterozygote, and no recombination will result in a homozygote (we show here only the production of an AA homozygote, but, aa homozygotes will be produced at equal frequency).

TABLE 1
Frequency of different genotypes resulting from the six mating possibilities

Type of Mating	Frequency of resultant cells				Fitness of resultant cells			
	AA	Aa	aa		AA	Aa	aa	
AA-AA	1				$(1 - U_t)(1 - U_a)$			
AA-Aa	0.5	0.5			$(1 - U_t)(1 - U_a)$	$(1 - h_t U_t)(1 - h_a U_a)$		
AA-aa		1			$(1 - U_t)(1 - h_t U_t)(1 - U_a)$	$(1 - h_t U_t)(1 - h_a U_a)$		
Aa-Aa	0.25	0.5	0.25		$(1 - h_t U_t)(1 - U_a)(1 + g)$	$(1 - h_t U_t)(1 - h_a U_a)$	$(1 - h_t U_t)(1 - h_a U_a)$	(0)
Aa-aa		0.5	0.5		$(1 - h_t U_t)(1 - U_a)(1 + g)$	$(1 - h_t U_t)(1 - h_a U_a)$	$(1 - h_t U_t)(1 - h_a U_a)$	$(1); (0)$
aa-aa			1					(1)

Frequency of “AA”

$$x' = (1 - U_a)[(1 - U_t)xm^2 - 0.5[(1 - U_t) + (1 - h_t U_t)]xm\ ym + 0.25(1 - h_t U_t)(1 + g)ym^2]/\bar{w}, \quad (1)$$

Frequency of “Aa”

$$y' = (1 - h_a U_a)[0.5[1 - U_t) + (1 - h_t U_t)]xm\ ym + [(1 - U_t) + 1]xm\ zm + 0.5(1 - h_t U_t)ym^2 + 0.5[(1 - h_t U_t) + (1 + g)]ym\ zm]/\bar{w}, \quad (2)$$

Frequency of “aa”

$$z' = [0.5\ ym\ zm + zm^2]/\bar{w}, \quad (3)$$

where \bar{w} is the sum of the numerators. An analytical solution for the invasion of the selfish gene was found by solving $dy'/dy = 1$, and setting $x = 0$, $y = 0$ and $z = 1$. This gave the condition for the advantage factor:

$$g > \frac{2h_a U_a + U_t - rU_t + rh_t U_t - h_a U_a U_t + rh_a U_a U_t - rh_a U_a h_t U_t}{r(1 - h_a U_a)}. \quad (4)$$

The equilibrium of the selfish gene was investigated by recursive simulations. The “A” allele was started at low frequency, and the model was run until all frequencies changed by less than 10^{-8} . This hence defined the equilibrium frequencies of the two alleles (E(A) and E(a)).

RESULTS OF THE BASIC MODEL

The invasion conditions for the selfish gene are dependent upon the advantage g and the recombination r , for given costs U_a and U_t (Fig. 2). If the A-a locus is very close to the centromere ($r = 0$), then there is no recombination during meiosis, hence all post-meiotic cells will be homokaryons. However, “AA” cells cannot kill, and “aa” cells cannot be killed during the chromosome exchange process, so this model is independent of the killing advantage g . Whilst the “A” allele is suffering costs it cannot spread through the population, so the “a” allele will fixate, and the selfish gene will be eliminated. If the A-a locus is further from the centromere and has, for example, a 50% chance of recombination ($r = 0.5$), then “Aa” post-meiotic cells will be present in the population. “Aa” cells may become “aa” cells after nuclear exchange, and will die. The surviving cell will be either “Aa” or “AA”, and the “A” allele will have killed two “a” alleles. Hence as $r \rightarrow 1$ the

invasion conditions broaden. The selfish “A” allele reaches an internal equilibrium, and as $r \rightarrow 1$ the equilibrium frequency increases for given costs and advantage.

It is unusual to find that the selfish gene invades more when it is far from the centromere, since similar selfish genes in other systems, such as the t-locus in mice (Lyon, 1992) or Segregation Distorter in *Drosophila* (Crow, 1991), are found close to the centromere. This is because high recombination allows more heterozygotes to be created in meiosis, and this selfish gene is able to do more killing in heterozygotes. In the absence of this benefit, recombination could destroy the selfish gene only by separating the toxin and the antitoxin loci. This type of recombination event has been assumed to be negligible in this model. Note however, that a two-locus selfish gene distant from the centromere is not without precedent, as such has been described on chromosome I in mice (Agulnik *et al.*, 1993).

One major point to note is that with no advantage ($g = 0$) or costs, the selfish gene does not invade in the analytical solution as “spite effects” limit its increase to $1/\infty$, hence it remains neutral. However, simulations confirmed that the selfish gene would invade when started at a finite frequency, and would spread to fixation providing the costs are sufficiently small. This is because cells such as “aa” are dying in every generation, so the relative frequency of “Aa” and “AA” cells is increasing in the population as a result of the death of “aa” cells. This “spite effect” is comparable to the effect of cytoplasmic incompatibility in arthropods (Hurst, 1991; Rousset & Raymond, 1991). A spiteful effect is one in which a trait spreads because of the harm done to others

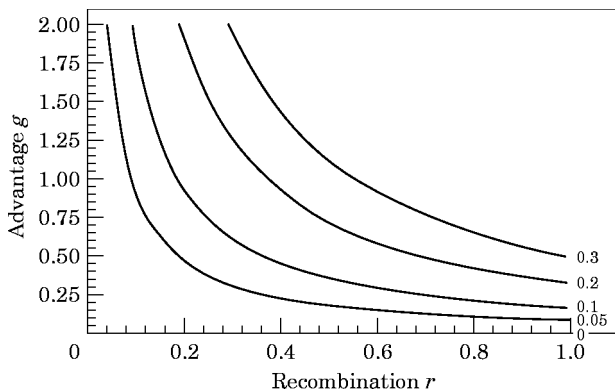


FIG. 2. Invasion conditions of the selfish gene “A” allele. For invasion, the relevant parameters must be above the curve for given costs ($U_a = U_t$, $h_a = h_t = 0.5$); g is the advantage and r the recombination (the costs shown are $U_a = U_t = 0, 0.05, 0.1, 0.2, 0.3$).

rather than as a direct advantage to self. If there are some costs to the “A” allele, then increasing the advantage factor allows the “A” allele to suffer greater costs whilst still being able to invade. It is a surprise, however, that the equilibrium frequency of the “A” allele will be slightly lower as the advantage increases.

Invasion is only possible if the costs are adequately low. If invasion is possible then the selfish gene typically goes to a high equilibrium frequency (cf. cytoplasmic incompatibility). Fixation is possible only if $U_a = U_t = 0$.

As would be expected, if the heterozygote suffers no cost ($h_a = h_t = 0$) then it is easier for the “A” allele to invade, but if the heterozygote suffers as much cost as the homozygote ($h_a = h_t = 1$) then it is harder for the “A” allele to invade. As h_a and h_t tend to 1 the equilibrium frequency of the selfish gene goes down, unless $U_a = U_t = 0$.

To conclude, the host-killing selfish gene “A” allele has broad invasion conditions in the ciliate population. The invasion conditions are most permissive when r is large, g is large, h_t and h_a are low, and the costs U_t and U_a are as small as possible. Fixation of the selfish gene is only achieved if no costs are suffered ($U_a = U_t = 0$).

Modifier Model—Invasion of the Meiosis Modifier

Could the spread of the selfish gene create the conditions needed for the invasion of a gene causing the peculiar form of meiosis? Let us consider an allele “M” entering the ciliate population with such a selfish gene. “M” is a dominant meiosis modifier allele (with respect to “m”), and causes the cell to undergo the form the meiosis found in present day ciliates (Fig. 3). The “M” allele, we assume, is neutral or deleterious, and therefore cannot normally invade. However, we show here that it can invade when the selfish gene considered above is present.

We examine three models. In the first model the meiosis modifier “M” allele is unlinked to the A-a locus, and in the second model the “M” allele is linked to the “a” allele. In the third model a possible mode of transition from unlinked loci to linked loci is analysed. It is a prerequisite of these models that the selfish “A” allele is able to invade in the absence of the “M” allele.

Bearers of the “M” allele, we assume, suffer a cost $(1 - U_s)$ in the homozygote, and $(1 - h_s U_s)$ in the heterozygote. However, cost-free modifiers ($U_s = 0$) were thoroughly investigated before costs were introduced.

TABLE 2

Parameters used in the basic model, and their numerical range

Function of parameter	Parameter	Range	Fixed value
Frequency of "AA"	x	0-1	—
Frequency of "Aa"	y	0-1	—
Frequency of "aa"	z	0-1	—
Post-meiotic frequency of "AA"	xm	0-1	—
Post-meiotic frequency of "Aa"	ym	0-1	—
Post-meiotic frequency of "aa"	zm	0-1	—
Linkage to centromere	r	0-1	0.25
Advantage of killing partner	g	0-1	0.05
Penetrance of cost antitoxin	h_a	0-1	0.5
Penetrance of cost toxin	h_i	0-1	0.5
Cost of making antitoxin	U_a	0-1	0.05
Cost of making toxin	U_i	0-1	0.05

RECURSION EQUATIONS IN THE MODIFIER MODEL

There are two components to the recursion equations involved in the model. The first set determines the post-meiotic zygote frequencies, and the second set determines the post-nuclear exchange cell frequencies. These frequencies are then normalized, and set as the starting values for the next generation. We assume that prior to meiosis, the population is made up of the following diploid genotypes at the following frequencies:

$$\begin{aligned}
 xa &= AAMM; \quad xb = AAMm; \quad xc = AAmM; \quad xd = \\
 &AaMM; \quad xe = AaMm; \quad xf = Aamm; \quad xg = aaMM; \\
 xh &= aaMm; \quad xi = aamm.
 \end{aligned}$$

These diploids will then undergo a form of meiosis dependent on whether or not they have the modifier. We can derive these post-meiotic frequencies of the above genotypes, which are labelled xma , xmb to xmi . If the action of the modifier is error-free then these frequencies are:

$$\begin{aligned}
 xma &= [xa + 0.5xb + 0.5xd + 0.25xe] \\
 &\quad \times (1 - U_i)(1 - U_s), \\
 xmb &= 0, \\
 xmc &= [0.5xb + xc + 0.25xe + 0.5(1 - r)xf] \\
 &\quad \times (1 - U_i), \\
 xmd &= 0, \\
 xme &= 0, \\
 xmf &= [rxf](1 - h_i U_i), \\
 xmg &= [0.5xd + 0.25xe + xg + 0.5xh](1 - U_s), \\
 xmh &= 0, \\
 xmi &= 0.25xe + 0.5(1 - r)xf + 0.5xf + xi.
 \end{aligned}$$

For completeness we consider the possibility that the modifier may not always operate. Hence there

exist nine possible genotypes. The possible cell types (genotypes) were tabulated into the 45 different mating combinations, with columns for every possible outcome (Table 3). From the frequencies given in Table 3 we can derive the equations governing the frequencies of the diploids following nuclear exchange. These are labelled $xmaa$, $xmbb$, to $xmii$, and are represented in Appendix A. Finally, after normalization the recursions can be represented as:

$$\begin{aligned}
 xa' &= xmaa/\bar{w} \\
 xb' &= xmbb/\bar{w} \\
 xc' &= xmcc/\bar{w} \\
 xd' &= xmdd/\bar{w} \\
 xe' &= xmee/\bar{w} \\
 xf' &= xmff/\bar{w} \\
 xg' &= xmgg/\bar{w} \\
 xh' &= xmhh/\bar{w} \\
 xi' &= xmii/\bar{w}
 \end{aligned}$$

where $\bar{w} = xmaa + xmbb + \dots + xmii$.

For the model with linked A-a and M-m loci, the equations may be derived as for the situation described above, making allowance for the new genotypes. These equations are presented in Appendix B.

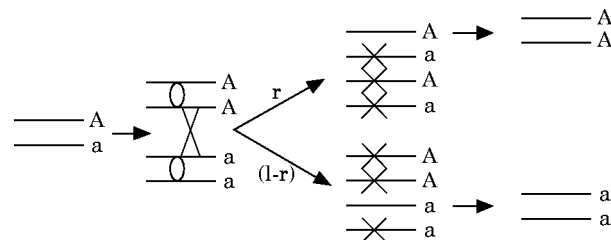


FIG. 3. The novel form of ciliate meiosis where three of the four chromatids are digested following recombination, and the remaining one is doubled by mitosis.

Aa-Aa									
AaMM-AaMM	<i>xm dx md</i>	0.25*	0.5	0.25	0.125**	0.25**	0.125**	0.125**	0.25**
AaMM-AaMm	2 <i>xm dx me</i>	0.125*	0.25	0.25	0.25*	0.125**	0.125**	0.125**	0.125**
AaMM-Aa mm	2 <i>xm dx m f</i>	0.25*	0.5	0.5	0.25*	0.0625**	0.25**	0.25**	0.25**
AaMm-AaMm	<i>xm ex me</i>	0.0625*	0.125	0.25	0.125*	0.125**	0.125**	0.125**	0.0625**
AaMm-Aa mm	2 <i>xm ex m f</i>	0.125*	0.25	0.25	0.125*	0.25	0.25	0.125**	0.125**
Aa mm-Aa mm	<i>xm f x m f</i>	0.25*	0.5	0.5	0.25*	0.5	0.5	0.25**	0.25**
Aa-aa									
AaMM-aaMM	2 <i>xm dx mg</i>		0.5***	0.25***		0.5***	0.25***	0.25***	0.5***
AaMM-aaMm	2 <i>xm dx mh</i>		0.25***	0.25***		0.25***	0.25***	0.25***	0.25***
AaMM-aa mm	2 <i>xm dx mi</i>		0.5***	0.5***		0.5***	0.5***	0.5***	0.5***
AaMm-aaMM	2 <i>xm ex mg</i>		0.25***	0.25***		0.25***	0.25***	0.25***	0.25***
AaMm-aaMm	2 <i>xm ex mh</i>		0.25***	0.25***		0.25***	0.25***	0.25***	0.25***
AaMm-aa mm	2 <i>xm ex mi</i>		0.125***	0.125***		0.125***	0.125***	0.125***	0.125***
Aa mm-aaMM	2 <i>xm f x mg</i>		0.25***	0.25***		0.25***	0.25***	0.25***	0.25***
Aa mm-aaMm	2 <i>xm f x mh</i>		0.5***	0.5***		0.5***	0.5***	0.5***	0.5***
Aa mm-aa mm	2 <i>xm f x mi</i>		0.25***	0.25***		0.25***	0.25***	0.25***	0.25***
aa-aa									
aaMM-aaMM	<i>xm g x mg</i>			1					
aaMM-aaMm	2 <i>xm g x mh</i>			0.5			0.5	0.5	0.5
aaMM-aa mm	2 <i>xm g x mi</i>			1			1	1	1
aaMm-aaMm	<i>xm h x mh</i>			0.25			0.25	0.25	0.25
aaMm-aa mm	2 <i>xm h x mi</i>			0.5			0.5	0.5	0.5
aa mm-aa mm	<i>xm i x mi</i>			1			1	1	1

This table is with the A/a locus and the M/m locus on separate chromosomes.

* These cells have an advantage due to their partner dying.

** These cells die because they have no anti-toxin to protect them against the toxin.

*** Half of these cells have an advantage due to their partner dying.

**** Half of these cells die because they have no anti-toxin, the other half survive because they do not have any toxin to kill them.

RESULTS OF THE MODIFIER MODEL

The invasion conditions of the “M” allele were investigated by recursive simulation within the limits of invasion of the selfish gene determined previously. Different parameters were varied to determine their effect on the invasion of the meiosis modifier allele (Table 4). The two most important parameters are the cost U_s , and the linkage of “M” to “a”. First, however, we examine the effect of U_i , U_a , and g on the invasion of “M”, and establish the equilibrium of “M”. For the purposes of this paper, we consider only error-free modifiers.

Invasion conditions for the unlinked meiosis modifier “M”

The cost-free meiosis modifier “M” allele will always invade, providing the initial population contains the selfish “A” allele (Fig. 4). Indeed, the “M” allele invades because the progeny of “AaMm” cells are never killed. The great advantage that “M” has is that under these circumstances it gets to be associated with the “aa” genotype in post-meiotic cells. These are neither killed nor suffer a cost.

When the “M” allele invades if does not fixate, but reaches an internal equilibrium. This equilibrium is influenced by the parameters g , U_i and U_a . The equilibrium of the “M” allele increases as the advantage g decreases, it also increases as the costs U_i and U_a decrease. The selfish gene is always eliminated. The equilibrium of the “M” allele is not changed if it is introduced when the “A” allele is not at equilibrium.

Once the “M” allele has been established to a high frequency, the invasion conditions for subsequent selfish genes are more restrictive. However, if they do invade, they are then eliminated, and the frequency of the “M” allele always increases. Some selfish genes are able to achieve high frequencies, but do not reach a stable equilibrium. This is because the frequency of the “M” allele also increases, and the “A” allele is no longer able to do any killing as nearly all post-meiotic cells are homozygotes. Therefore, cells with the “A” allele are suffering costs but have no advantage, and will be selected against (Fig. 5). As the frequency of the “M” allele increases with every attempted invasion by subsequent selfish genes, this is a possible mechanism by which the “M” allele could reach fixation.

To conclude, the invasion conditions of the cost-free unlinked meiosis modifier are as broad as the invasion conditions for the selfish gene “A” allele; the “M” allele is able to invade under all conditions in which the “A” allele has invaded. A potential

mechanism for the fixation of the “M” allele is by the invasion attempts of subsequent selfish genes, possibly followed by random genetic drift removing the “m” allele from the population.

The invasion conditions of the costly meiosis modifier are not as broad as the invasion conditions for the selfish “A” allele. The “M” allele can only invade if the costs on the “M” allele are less than the costs on the “A” allele. However, the system does not reach a stable internal equilibrium because the “A” allele is eliminated from the population, and then the costs on the “M” allele start to reduce its frequency until it becomes extinct. It is therefore difficult to see how an unlinked costly modifier could reach fixation. So how then is it reasonable to suppose that such modifiers could explain the evolution of ciliate meiosis?

Invasion conditions for the linked and transitional meiosis modifier

As noted before, the “M” allele spreads in the unlinked condition as it tends to associate with “aa” genotype more often than does the “m” allele. What if “a” and “M” were in tight linkage such that all “M” alleles were associated with “a”? Importantly, under this set of circumstances the “aaMM” genotype would reach fixation provided that there were costs on the selfish gene “A” and the costs on “M” were approximately less than the sum of the costs on the “A” allele (see Appendix B for recursion relations).

The above assumes that “M” appears next to “a”. But what if “M” was in the population (perhaps due to spread when initially unlinked to “A”) and a new version of “A” moved to be next to “m”. Following this subsequent meiosis and nuclear exchange there will be seven genotypes present in the population (see Appendix C). Initial investigations showed that when the “M” allele is costly, its frequency is initially reduced which allows a linked selfish gene to invade. This invasion in turn allows the “M” allele to increase in frequency again, and under these conditions, “M” will typically reach fixation (Fig. 6).

The overall conclusion is that the costly meiosis modifier “M” allele may invade and reach fixation under a reasonable range of conditions. It is able to reach a very high frequency by arising in a population containing an unlinked selfish gene. However, it too will be removed if costly. This elimination can be avoided if “M” is initially linked to “a”, or if further selfish genes invaded prior to elimination of “M”. Fixation can be reached if the new gene enters in linkage with “m”.

TABLE 4
Parameters used in the modifier model, and their numerical range

Function of parameter	Parameter	Range	Fixed value
Frequency of "AAMM"	xa	0-1	—
Frequency of "AAMm"	xb	0-1	—
Frequency of "AAmm"	xc	0-1	—
Frequency of "AaMM"	xd	0-1	—
Frequency of "AaMm"	xe	0-1	—
Frequency of "Aamm"	xf	0-1	—
Frequency of "aaMM"	xg	0-1	—
Frequency of "aaMm"	xh	0-1	—
Frequency of "aamm"	xi	0-1	—
Post-meiotic frequency of "AAMM"	xma	0-1	—
Post-meiotic frequency of "AAMm"	xmb	0-1	—
Post-meiotic frequency of "AAmm"	xmc	0-1	—
Post-meiotic frequency of "AaMM"	xmd	0-1	—
Post-meiotic frequency of "AaMm"	xme	0-1	—
Post-meiotic frequency of "Aamm"	xmf	0-1	—
Post-meiotic frequency of "aaMM"	xmg	0-1	—
Post-meiotic frequency of "aaMm"	xmh	0-1	—
Post-meiotic frequency of "aamm"	xmi	0-1	—
Linkage to centromere	r	0-0.5	0.25
Advantage of killing partner	g	0-1	0.05
Penetrance of cost anti-toxin	h_a	0-1	0.5
Penetrance of cost toxin	h_i	0-1	0.5
Penetrance of cost meiosis modifier	h_s	0-1	0.5
Cost of making anti-toxin	U_a	0-1	Below cut-off
Cost of making toxin	U_i	0-1	Below cut-off
Cost of modifier meiosis	U_s	0-1	0

Discussion

The hypothesis that the spread of a selfish gene, perhaps similar to a host-killing system of bacterial plasmids, allows the invasion or fixation of a costly gene causing the novel form of meiosis has been demonstrated under broad conditions. The paradox of why ciliates should carry out this form of meiosis

can hence be resolved by arguing that host-killing systems cannot function if all post-meiotic cells are homokaryons.

It is deliberate that the model we have constructed is possibly the least permissive for the selfish gene (and hence for the evolution of the system). For example, we assumed that "A" acts after meiosis. However, the toxin could be produced prior to

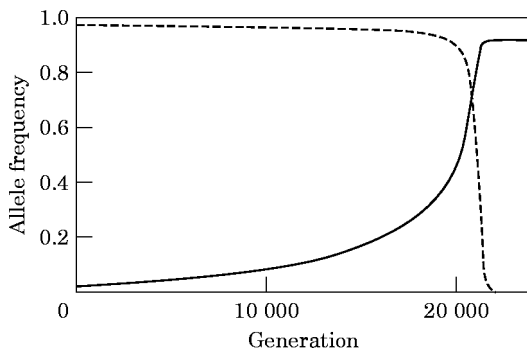


FIG. 4. Typical invasion of the unlinked cost-free "M" allele, and the elimination of the "A" allele ($r = 0.25$, $g = 0.01$, $U_a = 0.01$, $U_i = 0.012$, $U_s = 0.0028659$, $h_a = h_i = 0.5$, $f(M) = 0.025$, $f(A) = 0.97$). The change in frequency of the "A" and "M" alleles are shown ($E(M) = 0.913$, $E(A) = 0$). - - -, "A" allele; —, "M" allele.

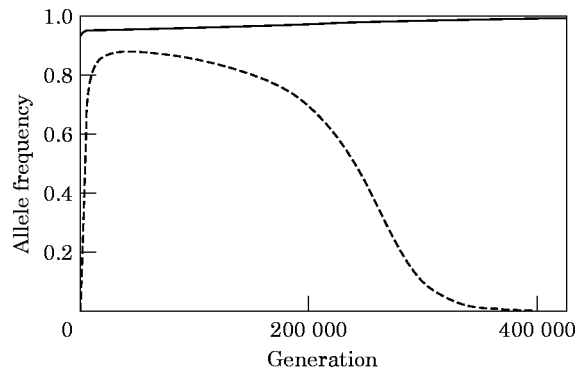


FIG. 5. The cost-free unlinked "M" allele increases in frequency from 94 to 99%, as a subsequent selfish element temporarily invades the population. The invasion conditions of the "A" allele are very restrictive because of the high frequency of the "M" allele, but temporary invasion is possible if the costs are sufficiently small ($U_a = U_i = 0.0001$, $g = 0.8$, $r = 0.25$). - - -, "A" allele; —, "M" allele.

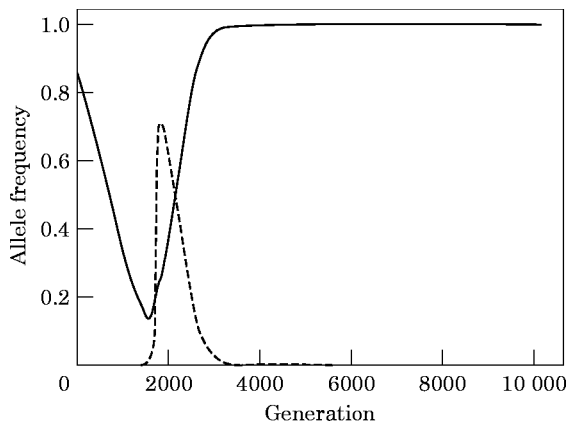


FIG. 6. The costly "M" allele fixates rapidly when it is tightly linked to an "a" allele. The changes in allele frequency are shown here. The high initial frequency of the "M" allele is rapidly reduced, hence permitting the invasion of the "A" allele. This allows the fixation of the "M" allele and causes the "A" allele to be eliminated (Start frequencies: 73.3% "MM", 24.6% "Mm", 2.1% "mm", $x_a = 0.733$, $x_b = 0.245999$, $x_c = 0.021$, $x_d = 0.0001$, $x_e = 0$, $x_f = 0$, $x_g = 0$, $r = 0.25$, $U_i = U_a = U_s = 0.005$, $h_i = h_a = h_s = 0.5$, $g = 0.15$. ---, "A" allele; —, "M" allele.

meiosis and hence affect cells that do not contain it following meiosis. Further, we considered that the toxin remains in the cell in which it is manufactured. We could alternatively have considered it to be diffusible. Such a diffusible toxin would be consistent with the action of ciliate cytoplasmic mate killers (Beale & Jurand, 1966). These factors behave as a two locus/anti-toxin selfish element. If a cell with such a factor mates with a cell without, then the latter is killed. Unlike nuclear genes, the cytoplasmic genes are not reciprocally transferred during conjugation. Hence, the toxic action must be transmissible to the partner cell. Note also that the existence of these cytoplasmic factors provides evidence that spiteful killing in ciliates is a viable strategy for a selfish element, as was supposed. Incorporation of either of the above alternatives would allow invasion of a selfish gene to be more trivial and would no doubt affect the dynamics of the process that we described above.

There are alternative mechanisms that could underlie the evolution of ciliate meiosis. If the nuclei are able to communicate and assess fitness, then a "choice" system could evolve where the fittest of the four nuclei is chosen to be the one that will be transmitted to the next generation. This mechanism is not unreasonable since a choice mechanism of the *In* gene results in non-Mendelian segregation ratios in a strain of Siberian mice. The choice seems to occur in oogenesis following fertilization (Agulnik *et al.*, 1993; Pomiankowski & Hurst, 1993).

However, there are two potential problems with this hypothesis. First, a choice of nucleus would require communication between the nuclei. In ciliates, the germinal micronucleus is under control of the somatic macronucleus. A study investigating intracellular communication found two factors emitted by the macronucleus that control the activation and inhibition of division in the micronucleus (Mikami, 1991). However, no evidence has been found of expression in the micronucleus, which is considered to be transcriptionally inert (Orias *et al.*, 1992). In order for the macronucleus to assess the fitness of the micronuclei, the micronuclei would need to emit a signal such as mRNA or a protein that is indicative of the fitness of the micronucleus. It seems unlikely that the micronucleus can communicate with the macronucleus, hence it is difficult to envisage how a choice mechanism could function in ciliates.

Even if communication between the micronucleus and the macronucleus is possible, the second problem is how the fitness of the micronucleus is assessed. The signals emitted by the micronucleus would need to contain information about its fitness. This assessment mechanism would be highly vulnerable to exploitation by other types of selfish genetic elements. For example, if a selfish gene arose that emitted a signal and is chosen irrespective of its genuine fitness, then such a cheating gene is likely to invade. A consequence of this is that the choice mechanism would no longer be functional, and all cells carrying the selfish genetic element are likely to be less fit than others due to its presence.

Notwithstanding the mechanistic difficulties of nuclear communication and fitness assessment in the choice hypothesis, another weakness is that of parsimony. It is very complex and costly for a cell to develop both of these mechanisms, and unlikely that they will evolve simultaneously. It is to be expected that intermediary stages are costly. Perhaps then the significant component of the above analysis is that even costly modifiers can reach fixation.

In order to test the model, it is necessary to be able to discriminate between these two evolutionary hypotheses of "choice" or of a costly meiosis modifier. The selfish gene/costly modifier argument would predict the existence of cytoplasmic killing systems as the spread of these would not be prevented by the novel form of meiosis. As noted previously, such factors do indeed occur in ciliates (Beale & Jurand, 1966). In addition, similar factors are found in yeast (Somers & Bevan, 1969) and in *Ustilago* (Puhalla, 1968). The diversity of such factors could be indicative of the possibility that spiteful killing may be a relatively common phenomenon within the protists.

Second, the selfish gene model would predict that other systems could evolve parallel means to protect against selfish elements similar to the ones examined here. Consider then the clamp connections of basidiomycete fungi: clamp connections are an effective but cumbersome method of mitosis which ensures that the progeny of a heterokaryon cell is also a heterokaryon with respect to mating type, and is an identical twin of the parent cell (Fincham *et al.*, 4th edn; cf. in ciliates the meiosis guarantees that the zygotic progeny are identical twins). If a choice of nucleus is a good idea, why did the fungi not evolve a choice mechanism, rather than one to guarantee perfect relatedness between cells?

A prediction of the selfish genetic element hypothesis, in the above formation, is that the selfish host-killing gene would be telomeric rather than centromeric. However, the selfish gene is always eliminated from the population and, therefore, could not be observed experimentally. Since one mechanism of fixation of the costly meiosis modifier is the integration of the selfish gene in tight linkage with the original meiosis gene, one could predict that the modifier meiosis locus would also be located in a telomeric region. The extent to which this is a robust prediction is unclear as firstly genes can move around genomes, and secondly alternative models of the process (those that are more permissive for the invasion of the selfish gene) may not require the same conditions. For example, if the ancestral form of meiosis were one in which four haploid nuclei were produced and two then randomly destroyed, the selfish gene need not be telomeric as in the above model (it could be anywhere in the genome). Likewise, the modifier could be anywhere in the genome.

Although we have presented a model here for the evolution of meiosis in ciliates, it should be noted that at least one ciliate, *Euplotes*, has a slightly different form (for example and references see Kuhlmann & Heckmann, 1991). In species of this genus, there is an extra duplication of the DNA resulting in two diploid nuclei prior to meiosis. Both of these nuclei then undergo the classical meiosis in parallel producing a total of eight haploid nuclei. As per the classical ciliate meiosis, three nuclei from each meiosis are destroyed leaving a total of two haploid products. However, the process does not stop there. These two then undergo the post-meiotic duplication producing four products. Two of these again are destroyed leaving just two pronuclei.

How can we interpret such a bizarre system? The incorporation of the final duplication and destruction ensures that this system could be equivalent to that of

other ciliates regarding the relatedness between post-exchange cells (and hence not greatly problematic to the theoretical outlook considered here). This would simply require that at the last stage the two destroyed nuclei were always sister nuclei (products of the same post-meiotic division). In contrast, however, were the digested pronuclei typically non-sister products, then *Euplotes* could be vulnerable to the form of selfish element that we envisage.

Whether they will be vulnerable depends, however, on the rate of inbreeding as the invasion conditions of a costly selfish gene become increasingly prohibitive as the frequency of selfing goes up (see Wade, 1985; Hickey & Benkel, 1986; Burt & Trivers, 1993). In highly inbred lineages costly selfish genes are not expected and so the conditions for the spread of modifiers will not be met. In general, inbreeding may lead to stabilization of unusual genetic systems that might otherwise be thought vulnerable to selfish elements (see Hurst, 1993, 1994). The model presented here would therefore be consistent with alternative meiotic forms in inbred ciliates.

This may explain to some extent why *Euplotes* is peculiar. Selfing in ciliates can take a number of forms. First, they could undergo conjugation between close relatives. Second, two cells may pair up but not exchange their pronuclei. The pronuclei of each cell may then fuse within each cell to regenerate the diploid condition (cytogamy). Alternatively, cells can simply allow the two pronuclei to fuse without ever meeting another partner (autogamy). The latter is quite commonly reported in *Euplotes* (see Luporini, 1970; Ito, 1971; Luporini & Dini, 1977; Dini, 1984; Kosaka, 1992), whilst it is claimed that autogamy is rare in ciliates as a whole (see Luporini & Dini, 1977). Selfing has also been found to be common in some populations (Heckmann, 1967) and can be induced by reducing the temperature (Heckmann, 1964).

The high frequency of autogamy and the evolution of the novel form of meiosis in *Euplotes* are probably related. It has been noted that autogamy with the novel form of meiosis might be advantageous as it could act as a means to ensure that a heterozygous locus is maintained heterozygous (note, this would be impossible with the classical ciliate meiosis; Luporini & Dini, 1977; Dini, 1984). The maintenance of heterozygosity would require that the pronuclei that fused were non-sisters.

This then provides parsimonious explanation for the evolution of this bizarre form of meiosis and for the apparent correspondence with frequent autogamy within this group. However, it leaves a quandary. Not all *Euplotes* are selfers or autogamous (for review see

Dini, 1984). For regularly outbred lineages it is predicted that the preferential retention of non-sister nuclei would provide the conditions for the invasion of the mate-killers that we envisage. We can then predict that an optimal solution might exist: in autogamous/selfing strains non-sister pronuclear recovery should be preferred (to main heterozygosity), but in regularly outcrossing strains sister-pronuclear recovery should be preferred (or at least should be found at a higher rate than in autogamous strains) so as to prevent nuclear mate killers from invading.

What evidence there is provides support for the prediction. In some strains meiosis is altered (Nobili & Luporini, 1967), in the expected direction, dependent upon whether the reproductive event is autogamous (preferential recovery of non-sister nuclei) or conjugative (higher rates of recovery of sister nuclei than in the autogamous case; see also Luporini & Dini, 1977).

In several instances in which autogamy is a regular event (this can mean instances where autogamous and non-autogamous isolates are in sympatry and can inter-mate) heterozygosity is usually maintained, hence the fusing pronuclei must more commonly be non-sisters (Luporini & Nobili, 1967; Nobili & Luporini, 1967; Luporini & Dini, 1977; Dini, 1981, 1984). In contrast, the same pattern is not found in numerous isolates of *Euplotes*, in which autogamy appears to be uncommon, and sister pro-nuclear recovery occurs at a higher rate than in autogamous strains and often with a preference for sister-pronuclear recovery (Katashima, 1960; Heckmann, 1963, 1964; Kuhlmann & Heckmann, 1991). It would be helpful to have a full phylogeny of these strains and species employed so that a full comparative analysis could be performed.

In broader view the analysis presented here adds to the growing theoretical literature that supposes that transitions between genetic systems may be mediated by the spread of a selfish gene and the concomitant spread of a modifier of this gene. Similar dynamical analyses have been presented to examine the possibility that recombination may have evolved in response to two locus meiotic drive genes (Haig & Grafen, 1991). Likewise, the evolution of chromosomes may have been mediated by group selection on cellular fitness when genes not attached to chromosomes could replicate at a faster rate (Maynard Smith & Száthmary, 1993). In particular, one body of theories has examined the possibility that uniparental inheritance of cytoplasmic genes may have evolved as a consequence of selection on nuclear modifiers to limit the spread of selfish cytoplasmic genomes

(Hoekstra, 1990; Hurst, 1990, 1994; Law & Hutson, 1992). In turn this analysis has been forwarded as the explanation for the evolution of sexes (Hoekstra, 1987; Hastings, 1992; Hurst & Hamilton, 1992; Law & Hutson, 1992; Hutson & Law, 1993). This latter theory receives considerable empirical support (Hurst, 1995). It remains to be seen whether the same can be said of the model presented here.

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xmii) to the post-meiotic frequencies (*xma*, *xmb*, . . . , to *xmi*). They are substituted into the recursion equations given in the body text to give the full model for unlinked loci. These equations can be derived using Table 3, and the parameters used are given in Table 4.

$$\begin{aligned} xma &= AAMM; & xmb &= AAMm; & xmc &= AAmm; \\ xmd &= AaMM; & xme &= AaMm; & xmf &= Aamm; \\ xmg &= aaMM; & xmh &= aaMm; & xmi &= aamm. \end{aligned}$$

$$\begin{aligned} xmaa &= (1 - U_a)[xma & xma + xma & xmb + 0.25 \\ xmb &+ xma & xmd + 0.5 & xma & xme + 0.5 & xmb \\ xmd &+ 0.25 & xmb & xme + (1 + g)[0.25 & xmd & xmd + 0.25 \\ xmd & xme + 0.0625 & xme & xme]] \end{aligned}$$

$$\begin{aligned} xmbb &= (1 - U_a)[xma & xmb + 2 & xma & xmc + 0.5 \\ xmb & xmb + xmb & xmc + 0.5 & xma & xme + xma \\ xmf &+ 0.5 & xmb & xmd + 0.5 & xmb & xme + 0.5 & xmb \\ xmf &+ xmc & xmd + 0.5 & xmc & xme + (1 + g)[0.25 & xmd \\ xme &+ 0.5 & xmd & xmf + 0.125 & xme & xme + 0.25 & xme \\ xmf &]] \end{aligned}$$

$$\begin{aligned} xmcc &= (1 - U_a)[0.25 & xmb & xmb + xmb & xmc + xmc \\ xmc &+ 0.25 & xmb & xme + 0.5 & xmb & xmf + 0.5 & xmc \\ xme &+ xmc & xmf + (1 + g)[0.0625 & xme & xme + 0.25 \\ xme & xmf + 0.25 & xmf & xmf]] \end{aligned}$$

$$\begin{aligned} xmdd &= (1 - h_a U_a)[xma & xmd + 0.5 & xma & xme + 0.5 \\ xmb & xmd + 0.25 & xmb & xme + 2 & xma & xmg + xma \\ xmh &+ xmb & xmg + 0.5 & xmb & xmh + 0.5 & xmd \\ xmd &+ 0.5 & xmd & xme + 0.125 & xme \\ xme &+ 0.5[1 + (1 + g)][xmd & xmg + 0.5 & xmd \\ xmh &+ 0.5 & xme & xmg + 0.25 & xme & xmh]] \end{aligned}$$

$$\begin{aligned} xmee &= (1 - h_a U_a)[0.5 & xma & xme + xma & xmf + 0.5 \\ xmb & xmd + 0.5 & xmb & xme + 0.5 & xmb & xmf + xmc \\ xmd &+ 0.5 & xmc & xme + xma & xmh + 2 & xma & xmi + xmb \\ xmg &+ xmb & xmh + xmb & xmi + 2 & xmc & xmg + xmc \\ xmh &+ 0.5 & xmd & xme + xmd & xmf + 0.25 & xme \\ xme &+ 0.5 & xme & xmf + 0.5[1 + (1 + g)][0.5 & xmd \\ xmh &+ xmd & xmi + 0.5 & xme & xmg + 0.5 & xme & xmh + 0.5 \\ xmf & xmg + xme & xmi + 0.5 & xmf & xmh]] \end{aligned}$$

$$\begin{aligned} xmff &= (1 - h_a U_a)[0.25 & xmb & xme + 0.5 & xmb & xmf \\ + 0.5 & xmc & xme + xmc & xmf + 0.5 & xmb & xmh + xmb \\ xmi &+ xmc & xmh + 2 & xmc & xmi + 0.125 & xme & xme + 0.5 \\ xme & xmf + 0.5 & xmf & xmf + 0.5[1 + (1 + g)][0.25 & xme \\ xmh &+ 0.5 & xme & xmi + 0.5 & xmf & xmh + xmf & xmi]] \end{aligned}$$

$$\begin{aligned} xmgg &= 0.5[1 + (1 + g)][0.5 & xmd & xmg + 0.25 & xmd \\ xmh &+ 0.25 & xme & xmg + 0.125 & xme & xmh] + xmg \\ xmg &+ xmg & xmh + 0.25 & xmh & xmh \end{aligned}$$

$$\begin{aligned} xmhh &= 0.5[1 + (1 + g)][0.25 & xmd & xmh + 0.5 & xmd \\ xmi &+ 0.25 & xme & xmg + 0.25 & xme & xmh + 0.25 & xme \\ xmi &+ 0.5 & xmf & xmg + 0.25 & xmf & xmh] + xmg & xmh + 2 \\ xmg & xmi + 0.5 & xmh & xmh + xmh & xmi. \end{aligned}$$

APPENDIX A

These equations relate the post-nuclear exchange frequencies of the nine genotypes (*xmaa*, *xmbb*, . . . , to

$$x_{mii} = 0.5[1 + (1 + g)][0.125 x_{me} x_{mh} + 0.25 x_{me} x_{mi} + 0.25 x_{mf} x_{mh} + 0.5 x_{mf} x_{mi}] + 0.25 x_{mh} x_{mh} + x_{mh} x_{mi} + x_{mi} x_{mi}$$

APPENDIX B

These equations relate the post-meiotic frequencies to the pre-meiotic cell frequencies, where the "M" allele is tightly linked to the "a" allele. The genotypes are as follows:

"aaMM" is x_{ma} ; "AamM" is x_{mb} ; "AAmm" is x_{mc} .

$$x_{ma} = x_a + 0.5 x_b,$$

$$x_{mb} = 0,$$

$$x_{mc} = 0.5 x_b + x_c.$$

The above equations can be substituted into the following recursions, which calculate the post-nuclear exchange frequencies in terms of the post-meiotic frequencies shown above:

$$x_{a'} = x_{ma} x_{ma} (1 - U_s)/\bar{w},$$

$$x_{b'} = x_{ma} x_{mc} ((1 - U_t)(1 - h_a U_a) + (1 - h_a U_a)(1 - U_s))/\bar{w},$$

$$x_{c'} = x_{mc} x_{mc} (1 - U_t)(1 - U_a)/\bar{w},$$

where \bar{w} = sum of the numerators.

APPENDIX C

These equations relate the post-meiotic frequencies to the pre-meiotic cell frequencies, where the "M" allele is initially only unlinked to the "a" allele, but subsequently comes into tight linkage. The genotypes are as follows:

"aM, aM" is x_{ma} ; "AM, am" is x_{mb} ; "am, am" is x_{mc} ; "a-M, A-m" is x_{md} ; "A-m, A-m" is x_{me} ; "a-M, a-M" is x_{mf} , and "A-m, a-m" is x_{mg} .

$$x_{ma} = x_a(1 - U_s) + 0.5 x_b(1 - h_s U_s),$$

$$x_{mb} = 0,$$

$$x_{mc} = 0.5 x_b(1 - h_s U_s) + x_c + 0.5(1 - r)x_g,$$

$$x_{md} = 0,$$

$$x_{me} = x_e(1 - U_t) + 0.5 x_d(1 - U_t)(1 - h_s U_s) + 0.5(1 - r)(1 - U_t) x_g,$$

$$x_{mf} = (1 - U_s) x_f + 0.5 x_d(1 - h_s U_s),$$

$$x_{mg} = x_g r(1 - h_t U_t).$$

The above mentioned equations can be substituted into the following recursions, which calculate the post-nuclear exchange frequencies in terms of the post-meiotic frequencies shown above:

$$x_{a'} = [x_{ma} x_{ma}]/\bar{w},$$

$$x_{b'} = [2 x_{ma} x_{mc} + 2 x_{mc} x_{mf} + 0.5 x_{mg} x_{ma} + 0.5 x_{mg} x_{mf}]/\bar{w},$$

$$x_{c'} = [x_{mc} x_{mc} + 0.5 x_{mg} x_{mc}]/\bar{w},$$

$$x_{d'} = [2 x_{ma} x_{me}(1 - h_a U_a) + 2 x_{me} x_{mf}(1 - h_a U_a) + 0.5 x_{mg} x_{ma}(1 - h_a U_a)[1 + (1 + g)] + 0.5 x_{mg} x_{mf}(1 - h_a U_a)[1 + (1 + g)]]/\bar{w},$$

$$x_{e'} = [x_{me} x_{me}(1 - h_a U_a) + x_{mg} x_{me}(1 - h_a U_a) + 0.25 x_{mg} x_{mg}(1 - U_a)(1 + g)]/\bar{w},$$

$$x_{f'} = [2 x_{ma} x_{mf} + x_{mf} x_{mf}]/\bar{w},$$

$$x_{g'} = [2 x_{mc} x_{me}(1 - h_a U_a) + 0.5 x_{mg} x_{mc}(1 - h_a U_a)[1 + (1 + g)] + x_{mg} x_{me}(1 - U_a) + 0.5 x_{mg} x_{mg}(1 - h_a U_a)]/\bar{w},$$

where \bar{w} = sum of the numerators.