

On the evolution of recombination and meiosis

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Summary

Theories on the evolution of recombination in regard to its ability to increase mean fitness require a consistent source of negative linkage disequilibrium among loci affecting fitness to show an advantage to recombination. Here we present evidence that, at least theoretically, genetic variation for recombination can spread in very large populations under a strictly multiplicative-fitness, deleterious-allele model. The model uses only Mendelian genetics in a multi-locus context to show that a dominant gene for recombination can spread when rare and resist invasion when common. In non-equilibrium populations driven by Muller's ratchet, the gene increases its probability of fixation by increasing the probability of being associated with the best individuals. This occurs at an optimal level of recombination. Its action results in both an immediate and a long-term advantage to recombination amongst the proto-meiotic organisms modelled. The genetic mechanism lends itself naturally to a model for the evolution of meiosis, where modern-day gametes are seen as derivative of ancient unicellular organisms.

1. Introduction

Sixty two years ago, Haldane (1937) showed that under independent gene action the equilibrium load in an infinite population is $e^{-\mu}$, where μ is the genome-wide mutation rate. This load is independent of either recombination or epistasis, but not of their combined action (Kimura & Maruyama, 1966). In terms of the benefits of recombination, there is nothing special about epistasis *per se*: epistatic selection works antagonistically with recombination to result in a consistent, non-zero level of linkage disequilibrium (Felsenstein, 1965). Because, by definition, epistasis means that mutations affect fitness as a function of the rest of the genome, the resultant equilibrium non-random distribution of mutations means that with recombination population mean fitness can deviate from the null expectation of $e^{-\mu}$. Alternatively, fluctuating selection or finite population size can also generate linkage disequilibrium, and thus they too can render an advantage or disadvantage to recombina-

tion. Under continual mutation pressure the advantage or disadvantage follows the sign of the disequilibrium, negative linkage disequilibrium rendering an advantage to mixis, positive linkage disequilibrium to linkage. This generalization can be indicative of the evolution of recombination if linkage is tight, though it does not guarantee the continued spread of a recombination modifier once linkage is loose (Barton, 1995; Feldman *et al.*, 1996; Otto & Feldman, 1997). In contrast to the situation under epistasis (where recombination can increase mean fitness), here, recombination helps the population maintain its determinist expectation of $e^{-\mu}$. The factors that generate linkage disequilibrium, and thus the factors that underlie an advantage to recombination, are sometimes categorized into deterministic or stochastic processes, for example as recently done by Kondrashov (1993), Barton (1995) and Antezana & Hudson (1997*a*).

Yet unfortunately the application of any of these above processes to the evolution of recombination has been hampered by the evidence. The evidence for epistasis in terms of physiological gene interactions is extensive (e.g. Barker, 1979; Cheverud & Routman, 1995), but evidence for the required nonlinear re-

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relationship between an organism's mutational load and log fitness is at best tentative (Kondrashov, 1988; Keightley, 1996; Elena & Lenski, 1997). Additionally, strong synergistic epistasis can decrease the variance in fitness so much that the decrease is not offset by a concomitant increase in the mean (Barton, 1995). In these cases, recombination is selected against – there being only a window where synergistic epistasis favours the spread of a recombination modifier (Otto & Feldman, 1997; Otto & Michalakis, 1998). Thus models on the evolution of recombination by means of its interaction with epistasis need not only empirical support for synergistic epistasis, but epistasis of the right magnitude.

Alternatively, one can examine finite population size as a generator of linkage disequilibrium. For example, in moderately sized populations computer simulations have shown that drift can generate sufficient linkage disequilibrium to yield an advantage to recombination (Felsenstein & Yokoyama, 1976). This advantage accrues because recombination stops Muller's ratchet and mitigates the Hill–Robertson effect (changes in alleles' probability of fixation because of non-random associations amongst selected loci over time: Hill & Robertson, 1966; Felsenstein, 1974). Yet once populations become large, long-standing, non-random associations between loci under multiplicative gene action are, at best, weak and transitive (Felsenstein, 1965). In the limit as $N \rightarrow \infty$ there should be no benefit to recombination at all (Maynard Smith, 1968). Thus classical analyses predict that all but the smallest of asexual populations should be in approximate mutation–selection balance and, for this reason, finite population size alone has often been thought to be unable to explain an advantage to recombination. These considerations, along with difficulties in quantifying Muller's ratchet (Charlesworth & Charlesworth, 1997), have hindered the ratchet's explanatory role for the evolution of recombination. This conceptualization has been perhaps further reinforced by D. Charlesworth *et al.* (1993) demonstration that with constant selection coefficients even small amounts of recombination can stop the ratchet.

One caveat on the above is that no matter how large a population is, new mutations are always rare, and thus experience stochasticity during their early sojourn. For beneficial mutations, even in large populations, this is a critical period (Haldane, 1927; Barton, 1994). Otto & Barton (1997) asked whether a modifier increasing recombination could hitch-hike with a nearby beneficial locus due to the modifier's effect in increasing the efficiency of selection. This is an aspect of the Hill–Robertson effect, where recombination acts to reduce not only the beneficial allele's initial negative linkage disequilibrium, but that of other segregating loci also (Fisher, 1930; Muller,

1932; Felsenstein, 1988). While Otto & Barton answer affirmatively – that is, modifiers that increase recombination can spread – the conditions are restrictive. The process works best with tight linkage, weak modifiers, and simultaneously segregating beneficial mutations – for example, due to rapidly changing environments (Otto & Michalakis, 1998). This is because of an inherent antagonism between hitch-hiking and recombination. In certain parameter spaces this can be partly overcome with the additional assumption that beneficial mutations pleiotropically alter the recombination rate (Hey, 1998). But, in general, considerations such as those above have been used to dismiss simple finite population size in a stable environment as singularly sufficient for the evolution of recombination.

The lack of empirical support for epistasis, and the lack of theoretical support for sufficient finite population size-induced linkage disequilibrium, means that there is currently no line of thought to support the simplest of hypotheses: the hypothesis that recombination could have evolved in very large populations of primitive eukaryotes under the null scenario of directional, multiplicative, steady selection; yet this is probably the easiest scenario to support evidentially.

There is, though, a way in which finite population size can render large and consistent levels of negative linkage disequilibrium in even extremely large populations. If mutation and selection are not in equilibrium, such that mutation pressure is stronger than selection, then disequilibrium is generated. A subsequent restoration of mutation–selection balance, for example by recombination, would therefore be advantageous. Classical theory shows no reason why mutation and selection should not attain equilibrium – at least in the statistical sense – but later work has demonstrated how this scenario can happen (Gessler, 1995).

Consider a large, asexual population. One can rank individuals by their relative fitness (for example, by the number of mutations they carry) and thereby create a distribution of the relative frequency of the number of mutations per individual. At an equilibrium between mutation and selection, this distribution is Poisson (Kimura & Maruyama, 1966; Haigh, 1978). For classes at the extreme ends of the distribution, the equilibrium number of individuals may be less than unity. In this case, the presence of even one individual in these classes exceeds their expectation. This is always true for classes in the far right-hand tail of the distribution (i.e. the worst classes). For the worst classes, any non-zero equilibrium number can be achieved as time-average, with only a slight generation of negative linkage disequilibrium (Felsenstein, 1988). But for the left-hand side of the distribution, any time-average that includes zero is manifest as the loss of the best class, and therefore a click of Muller's ratchet

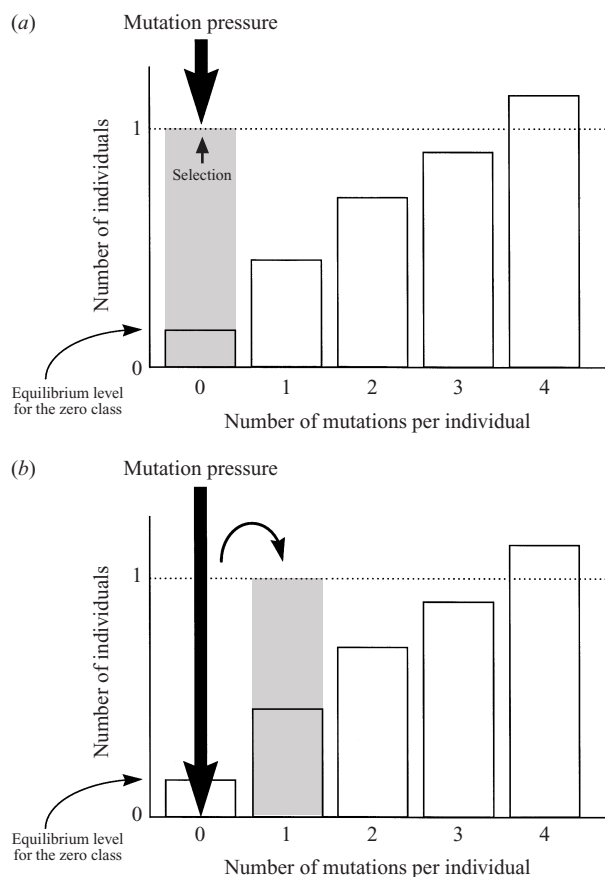


Fig. 1. A cartoon representation of a Poisson distribution, showing the equilibrium size of each class. The ordinate is marked in terms of the actual number of individuals, not the relative frequency. (a) If the equilibrium number ($Ne^{-\mu/\bar{s}}$) is less than unity, then the presence of even one individual in the zero class (the grey box) is too many. Mutation and selection attempt to equilibrate to the equilibrium level, in this case due to mutation being stronger than selection. (b) The attempted equilibration removes the class. When this happens to the best class, it simultaneously clicks Muller's ratchet. The distribution tries to re-equilibrate and the process repeats itself. The steady-state shape of the distribution under this situation can be markedly different from Poisson.

(Muller, 1964; Felsenstein, 1974). When the equilibrium number of individuals in a class is less than unity, the reason behind the loss of the class must include the effect of mutation and selection trying to equilibrate to that fractional number, the actual loss itself being a correlated result (Fig. 1). The long-term effect of such losses creates a chronic instability that is manifest as a hypodispersed, unstable distribution and is different from the stochastic loss of the best class as examined by Haigh (1978). The loss of the best class under hypodispersion is primarily due to the evolutionary force of mutation, and is conceptually distinct from that of drift. The extent of the hypodispersion is proportional to the number of unstable classes, and this number is extremely sensitive to the

ratio μ/\bar{s} (where \bar{s} is the arithmetic average segregating selection coefficient at equilibrium, i.e. the average selection coefficient over all mutant, non-fixed alleles). Because the steady-state shape of the distribution is no longer Poisson, the population fails to achieve mutation–selection balance. The variance in fitness of the population stabilizes, but it stabilizes at a level less than that necessary to counter mutation pressure. In the absence of epistasis, this condition is quantitatively demarcated by the inequality $Ne^{-\mu/\bar{s}} < 1$, where N is the census population size, and μ and \bar{s} are defined as above (Gessler, 1995). The exponential relation between N and μ/\bar{s} means that for biologically relevant values of μ and \bar{s} , even very large values of N can fail to guarantee $Ne^{-\mu/\bar{s}} \geq 1$. Recombination can regenerate the best class, and thus can help restore the population to mutation–selection balance.

In this paper we report on computer simulations that see whether, and how, a gene for recombination could spread through such a population. We follow a full trajectory, from invasion when rare, to ultimate fixation and resistance to counter-invasion. The process is demonstrated for the relatively small population size of 10^3 , and then analytical arguments are used to extend its applicability to populations of billions of individuals. A key appreciation of the model is that the primary benefit of recombination is to change a non-equilibrium population into one in approximate equilibrium. It is thus not inconsistent with the simultaneous operation of other plausible benefits of recombination (e.g. Fisher, 1930; Muller, 1932; Kimura & Maruyama, 1966) that operate on populations in approximate equilibrium. Lastly, we use this population genetic model and its subsequent quantification as an underlying model for the evolution of meiosis.

2. The model

The model is an extension of that of Gessler (1995), where haploid asexual individuals are now allowed to acquire mutations under the opportunity for the invasion of an allele conferring an exchange of genetic information. In computer simulations, individuals go through a process in discrete generations of (possibly recombination), reproduction, mutation and selection. Each individual has a genome of one chromosome, upon which deleterious mutations are introduced at random positions. The number of new mutations is drawn from a Poisson distribution. Selection coefficients are drawn from an approximate negative exponential distribution of mean 0.02 (Ohta, 1977; Gillespie, 1991; Lynch *et al.*, 1995). Selection is multiplicative; as mutations accrue, the strength of selection (as measured by the number of individuals before selection to the number after) is kept constant

at its expectation of e^{μ} . As mutations accrue, the simulations neither require an increasing number of individuals to be chosen before selection, nor do they allow population extinction via mutational meltdown (Lynch & Gabriel, 1990). As such, the analysis can be seen as conditional for a given strength of selection. Once the population reaches its steady-state relationship between mutation and selection, a random individual is chosen, and a neutral locus at a random position is allowed to mutate to a dominant allele for recombination. Individuals with this allele can recombine their genomes with another randomly picked individual. The allele codes for a mean number of chiasmata (the actual number being drawn from a Poisson distribution at each recombination event) and thus crossing-over is not mandated solely by the presence of the allele. This ‘conjugation phase’, where individuals may conjugate and recombine their genomes, is separate from, and precedes, reproduction. After conjugation, individuals separate and resume their place in the population. If the allele is lost from the population (a very likely occurrence), each successive generation has a probability of 0.1 of reintroducing the allele. The allele is only reintroduced if it is lost; the probability of 0.1 merely allowing some time (on average 10 generations) to elapse before a new introduction. On introduction, the allele is introduced as a single copy in a random individual. To control for the reintroduction rate, separate runs with an invading neutral, non-recombining allele are used to establish a control. Each of 25 independent simulations is continued for 50000 generations or until the allele is fixed. If the allele does not fix within 50000 generations, the run is discarded. With the exception of invading asexual mutants reported later, this is a rare occurrence and is reported in the accompanying tables. For each run that does fix the allele, all generations from the last introduction of the allele (the one that led to fixation) are used to generate the reported statistics. Theory predicts that the benefit of recombination should increase as $Ne^{-\mu/\bar{s}}$ falls below unity. Drake (1991) estimated the mutation rate for DNA microbes as $\mu = 0.0033$ per genome per generation: approximately 300-fold less than the mutation rate for some current-day metazoans (Crow, 1993a, b). This is used to establish a range of mutation rates from $\mu = 0.0033$ to 0.033 to 0.1. Because \bar{s} (the average selection coefficient of a mutation segregating in the population) is approximately 0.005 (verified from the simulations and analytically estimated in the Appendix), these rates correspond to $Ne^{-\mu/\bar{s}} \cong 517, 1.36$ and 2.06×10^{-6} respectively for $N = 10^3$. This represents a range in the expected strength of selection for recombination from null to weak to moderate.

3. Results

Fig. 2 shows the behaviour of a randomly chosen population as an invading recombination allele fixes. It shows how the decrease in the rate of decay is correlated with the spread and fixation of the recombination allele. Before the spread of the recombination allele the rate of Muller’s ratchet can be predicted. The observed rate of $0.0278 (\pm 8.77 \times 10^{-3}$ SD) mutations per genome per generation is not significantly different from the predicted rate of 0.0260 ($P = 0.3$; prediction uses \hat{s} from the Appendix and equations (8) and (10) from Gessler (1995)). Fig. 3 shows the rate of decrease in log mean fitness per generation as a function of the amount of recombination across all three genome-wide deleterious mutation rates. As expected, if $Ne^{-\mu/\bar{s}} \gg 1$ there is no advantage to recombination, while the advantage

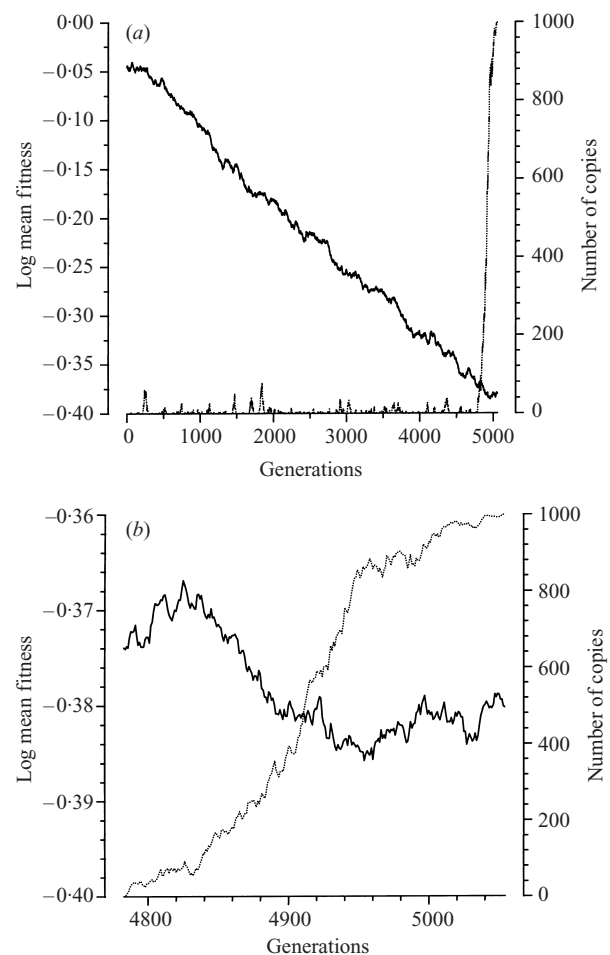


Fig. 2. (a) A sample simulation showing the linear rate of decline in log mean fitness (continuous line) as successive recombination alleles are introduced (dotted line). Most introductions are lost rapidly and play little role in population dynamics. (b) Exploded view of (a) during the final rise to fixation. When an allele does rise to fixation it mitigates the population’s decline in fitness (see supporting figures and tables for significance tests). ($N = 10^3$, $\mu = 0.1$, 0.2 chiasma).

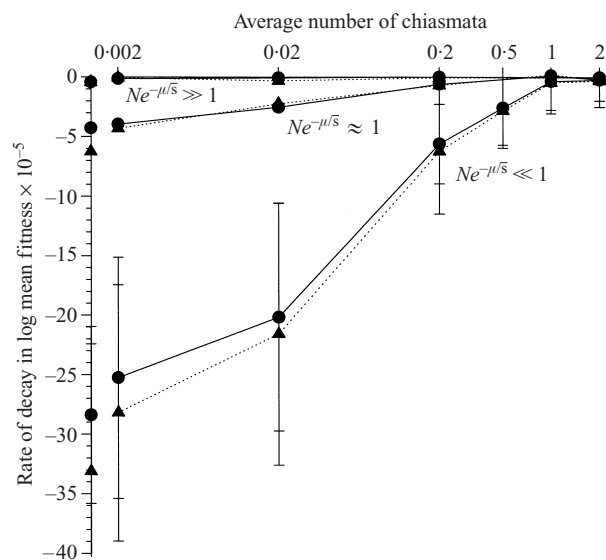


Fig. 3. Mean rate of decay in log fitness for $\mu = 0.0033, 0.033$ and 0.1 for various mean numbers of chiasmata as the upper two, middle two and lower two lines respectively. For each population, the rate of decay is measured from when the invading allele first appeared to when it fixed. In the graph, the rate is partitioned into that experienced by those individuals carrying the gene (continuous lines and filled circles) and those wild-type individuals without the gene (dotted lines and triangles). The non-recombining controls are plotted on the ordinate; standard deviations are included for $\mu = 0.1$. Each data point is the mean from a set of independent simulations for a specific mutation rate and mean number of chiasmata. ($N = 10^3$, mean incoming $s = 0.02$).

grows with the degree to which $Ne^{-\mu/s}$ is less than unity and the amount of recombination increases.

Table 1 reports results for the treatment where the benefit to recombination is predicted to be manifest ($Ne^{-\mu/s} \ll 1$). Under these parameters, an allele for recombination is 3 times as likely to fix as its non-recombining control: 0.00333 versus 0.00107 (Table 1, probability of fixation for 0.2 chiasma vs non-recombining control; $P = 6.12 \times 10^{-4}$ using Behrens–Fisher t -test). The benefit to recombination is reflected by the fact that the allele for 0.2 chiasma is more likely to fix than the non-recombining control, whether the control is invading an asexual population or a sexual population fixed for that level of recombination (neutral control from Table 3, $P = 5.1 \times 10^{-7}$). There is statistical support for an optimal amount of recombination by noting that the probability of fixation for 0.2 chiasma is significantly greater than for either 0.02 chiasma ($P = 0.038$) or 1 chiasma ($P = 0.0026$). There is no significant difference between the probability of fixation for 0.2 chiasma and 0.5 chiasma ($P = 0.47$). To simplify reporting the results, from here on we concentrate on the 0.2 chiasma runs as exemplary of an optimal amount of recombination, without inferring any prejudicial difference between 0.2 chiasma and 0.5 chiasma.

In asexual populations, the probability of fixation for an allele is strongly dependent on its association with the best class, since by expectation the best individuals leave the most descendents (Fisher, 1930). In the limit (with no mutation), the entire probability mass function for fixation is concentrated near $u \rightarrow 1$

Table 1. Performance of genes for different levels of recombination. $Ne^{-\mu/s} \ll 1$ ($\mu = 0.1$)

Mean No. of chiasmata	$\hat{\mu} \times 10^3$	No. of introductions until a successful invasion	No. of generations until fixation	Mean ratio in mean Fitness		n
				$\bar{w}_{\text{with}}/\bar{w}_{\text{w/o}}$ *** (SD) $\times 10^3$		
0	1.07	932 ^{NS} (766.53)	329 (99.47)	1.01357	(5.853)	25
0.002	1.37	732 ^{NS} (755.05)	358 (121.20)	1.01312	(5.953)	24
0.02	1.49	670 ^{NS} (773.90)	399 (156.32)	1.01143	(5.766)	25
0.2	3.33	300*** (339.17)	706 (392.93)	1.00670	(3.288)	25
0.5	2.65	378** (404.76)	1033 (461.92)	1.00258	(2.233)	25
1	1.39	717 ^{NS} (519.97)	1405 (711.32)	1.00154	(1.510)	25
2	1.54	651 ^{NS} (651.63)	1529 (655.04)	1.00081	(1.286)	23

‘ \hat{u} ’ is the probability of fixation estimated from the mean number of introductions. Because the number of introductions until fixation is a geometric random variate, the reciprocal of the mean is not the mean of the reciprocals (the mean of the reciprocal of geometric variates is $E[X^{-1}] = p[1-p]^{-1} \ln[p^{-1}] = 0.0069 \neq E[u]$). For this and other reasons, statistical tests are simpler if done directly on the raw number of introductions and then converted into a probability of fixation via the maximum likelihood estimator $\hat{u} = \bar{x}^{-1} = p = N^{-1} = 0.001$. The number of introductions for the neutral control is not significantly different from the expectation of 1000, while it is highly significantly different for both 0.2 and 0.5 chiasma ($P = 0.0007$ and $P = 0.003$ respectively, denoted by *** and **). ‘NS’ denotes ‘not significant’. ‘No. of generations until fixation’ applies to the final successful invasion. Conditional on fixation, mean fitness amongst non-fixed alleles (\bar{w}_{seg}) is partitioned according to those individuals with an invading gene (with) and those without (w/o). All entries in the column are significantly different from 1.0 at $P < 0.001$. See text for why these ratios decrease monotonically. ‘ n ’ is the number of runs included in the averages out of 25 attempted for each treatment. Statistical tests adjust for unequal variances. Standard deviations are in parentheses.

Table 2. Mean percentage of the best class with the invading allele.

$$Ne^{-\mu/s} \ll 1 (\mu = 0.1)$$

Mean no. of chiasmata	Amongst introductions that did not fix	<i>n</i>	Amongst introductions that did fix	<i>n</i>
0	0.252 (2.86)	132719	55.9 (26.8)	200
0.2	0.783 (5.31)	57119	65.1*** (14.4)	200
Free	2.37 (8.16)	135705	53.1 (10.0)	200

Additional 3×200 simulations that specifically measured the percentage of the best class each generation that had individuals with the invading allele. Free recombination controls segregated an allele that acted as though chiasmata were between every mutation. Amongst introductions that fixed, the 0.2 chiasma alleles were significantly more associated with the best class than either the non-recombining or freely recombining controls ($P = 2.26 \times 10^{-5}$ and 9.62×10^{-16} respectively). Simulations were run for up to 100000 generations; introductions were performed after selection. Standard deviations are in parentheses.

for the best class and $u \rightarrow 0$ for all other classes, where u is the probability of fixation. If an allele is already in the best class it should suppress recombination, since it is already amongst the most fit individuals. But for alleles in the less fit classes of a hypodispersed population, recombination can help them not only escape into the best class, but create an even better 'best' class. Recombination into worse classes is not symmetrically as deleterious, since the allele was essentially destined for extinction anyway. For the optimal recombination allele to increase its probability of fixation it needs to generate positive linkage disequilibrium with the best class. Additional simulations were performed specifically to measure the time-average percentage of individuals in the best class with the invading allele. Table 2 shows that, amongst those alleles that fix, on average 65% of the best class has the optimal recombination allele, compared with approximately 55% for non-recombining and freely recombining controls. These differences are highly significant ($P < 0.001$).

The role of background trapping (that is, the dependency of an allele's fate on the genome of its origin (Fisher, 1930; Peck 1994)) can be further monitored by comparing the mean fitness of each population's ancestral individual (the original individual with the allele that went to fixation) with the population average. For 0.2 chiasma the mean fitness of ancestral individuals across all populations at the time of introduction is significantly higher than the population average ($0.927 [\pm 3.23 \times 10^{-2} \text{ SD}]$ vs $e^{-\mu} = 0.901$ corrected for mutation accumulation; $P = 8.9 \times 10^{-6}$), while it is significantly lower than the non-recombining control ($0.949 [\pm 3.53 \times 10^{-2} \text{ SD}]$; $P = 0.030$). Thus at the optimal recombination rate the role of background trapping is decreased (alleles sometimes escape their ancestral genomes) though not eliminated (they maintain some positive linkage

disequilibrium). For two chiasmata there is no difference between the mean fitness of the ancestral individuals and the population expectation – there being little correlation between alleles that fix and the fitness of their ancestral individuals. Thus, non-recombining alleles, which are heavily influenced by background trapping, show the highest $\bar{w}_{\text{with}}/\bar{w}_{\text{w/o}}$ ratio when conditioned on fixation having occurred (Table 1), while recombining alleles, which find themselves in varied backgrounds and occasionally less fit individuals, show a reduced statistic.

If there is an advantage to recombination then a population with non-zero recombination should show resistance to invasion by alleles coding for zero recombination. Table 3 shows that the ability of asexual mutants to reinvade is strongly dependent on their underlying genetics. Recessive mutants, where the invading allele acts as a null mutation at the recombination locus, can drift into the population. This is because recombination can still be forced upon them by conjugating wild-types, so selection against them is weak (Table 3). Despite this, once invaded these populations decay at a significantly higher rate than their neutral control (mean rate of decay in log fitness amongst those with the invading allele: $-6.20 [\pm 5.08 \times 10^{-5} \text{ SD}]$ vs $-3.86 [\pm 4.07 \times 10^{-5} \text{ SD}]$; $P = 0.001$). Dominant asexual alleles fare worse. A dominant allele acts to unilaterally prohibit recombination in its lineage and thus causes permanent genetic isolation. Table 3 shows that genetic isolation is strongly selected against: of 100 attempts, only 63 resulted in fixation for the dominant mutant vs 92 for the neutral control. This is highly significant ($P < 10^{-4}$) using a conditional binomial exact test (Rice, 1988). Because only 63 of 100 runs showed fixation, the means in Table 3 are biased against rare events. Including runs that took more than 50000 generations would decrease the mean probability of fixation; thus

Table 3. Performance of genes for asexuality as they invade sexual populations. $Ne^{-\mu/s} \ll 1$ ($\mu = 0.1$, 0.2 chiasma)

Asexual mutant	$\hat{u} \times 10^3$	No. of introductions until a successful invasion	No. of generations until fixation	mean ratio in mean fitness		n
				$\bar{w}_{\text{with}}/\bar{w}_{\text{w/o}}$ ***	(SD) $\times 10^3$	
Neutral	1.17	856 ^{NS} (747-91)	814 (406-47)	1.00423	(2.634)	92
Recessive	1.08	929 ^{NS} (730-92)	834 (420-32)	1.00427	(3.124)	87
Dominant	0.745	1341 ^{***} (841-88)	313 (102-41)	1.01560	(5.811)	63

All individuals are haploid. ‘Recessive’ mutants can not conjugate with each other, but can have recombination forced upon them by non-mutant wild-types; ‘Dominant’ mutants are immune to recombination. Recombination is the default wild-type state. Because the dominant gene is unlikely to fix, 100 instead of 25 runs were attempted for each treatment. The number of introductions until a successful invasion is conditional on fixation occurring within 50000 generations, and thus is biased low when compared with the unconditional expectation. Neither the neutral control nor the recessive treatment is significantly different from each other or the expectation of 1000. The dominant mutant is significantly less likely to fix than the neutral control ($P = 4.61 \times 10^{-4}$). Both recessive and dominant alleles are significantly less likely to fix than the recombination gene when it was invading (number of introductions vs 300 from Table 1; $P = 2.41 \times 10^{-8}$ and $P = 4.61 \times 10^{-12}$ respectively). Note that although the dominant gene is unlikely to fix, when it does, it does so with comparative rapidity. This is because its likelihood of fixation is strongly dependent on it originating in a superior genetic background; these individuals cause rapid selective sweeps. Standard deviations are in parentheses.

re-invasion is even less likely than reported in the table.

4. Discussion

(i) Mechanism of evolution

What is the mechanism of evolution responsible for the fixation of the recombination allele? When the amount of recombination is low, an allele’s probability of fixation is strongly dependent on the individual it arose in: if it arises in any but the best individuals it is invariably destined for extinction (Fisher, 1930; Peck, 1994). The actual probability of such an event is a function of just how beneficial or deleterious the allele is (Barton, 1995). As the amount of recombination increases, the allele fares a better chance of recombining into other backgrounds before it is lost, and thus its fate is less dependent on its origin. For recombination alleles the process works antagonistically: the allele reduces negative linkage disequilibrium among its descendants (and thereby confers upon them a selective advantage (Maynard Smith, 1988)) but also helps any recipient conjugates that may not themselves carry the allele. This is exacerbated with high recombination, where a dominant recombination allele moves rapidly amongst genomes, and thereby distributes the benefit of sex promiscuously. To restrict its benefit to primarily its own offspring, the allele must maintain itself in positive linkage disequilibrium in these individuals long enough for selection to capitalize on their reduced load. Thus an allele that destroys linkage disequilibrium needs linkage disequilibrium to have any realized selective advantage. It is in part because of these considerations that Felsenstein & Yokoyama

(1976) thought that intuition on this process was ‘unduly risky’.

The results in Tables 1, 2 and Fig. 3 lead us beyond intuition and uncover some of the dynamics of the process. The optimal recombination rate reflects the ability of the allele to reduce the effect of background trapping, without entirely destroying the chance beneficial positive linkage disequilibrium of either its origin or its sojourn through its descendants. From the population’s perspective, the spread of recombination reduces the strength of the Hill–Robertson effect as it reduces the mean and variance in the amount of negative linkage disequilibrium. Before the allele’s invasion, even the mean was non-zero because $Ne^{-\mu/s} \ll 1$. Negative linkage disequilibrium is reduced because regeneration of rare types builds a more random distribution of associations. This generation of rare types slows the ratchet. From the population’s perspective the more recombination the better, until the attainment of approximate equilibrium. From the allele’s perspective the mitigation, though non-elimination, of background trapping is equivalent to a differential reduction in the Hill–Robertson effect among its descendants, in contrast to a smaller reduction experienced by its wild-type counterpart. Thus from the allele’s perspective an optimal amount of recombination is better. The process circumvents the group selection aspect of the Fisher–Muller hypothesis discussed by Felsenstein (1974) and is subtly distinct from the benefit associated with recombination *vis-à-vis* Muller’s ratchet identified in that paper. In that paper and its sequel (Felsenstein & Yokoyama, 1976) the identified benefit of recombination is due to the effect of recombination mitigating the Hill–Robertson effect in an otherwise approximate equilibrium population. While the Hill–

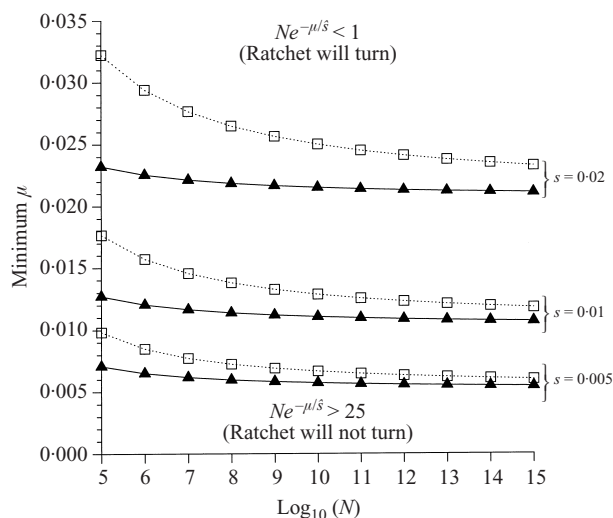


Fig. 4. The minimum mutation rate (μ) needed to ensure a deterministic drive to Muller's ratchet as a function of the population size (N). The dotted and continuous lines use \hat{s} from the Appendix and demarcate $Ne^{-\mu/\hat{s}} = 1$ and $Ne^{-\mu/\hat{s}} = 25$ for a mean incoming s of 0.02, 0.01 and 0.005 respectively. Since \hat{s} is a function of N , s and μ , this requires iterative root finding. For each mean incoming s the region above the triangles will have a drive to the ratchet. The region above the squares is where this drive will be due to the loss of mutation–selection balance ($Ne^{-\mu/\hat{s}} < 1$).

Robertson effect is still operational here – and is fundamental to the presence of the ratchet and the spread of the allele – it is the specific differential abatement of the hypodispersed, unstable distribution in this model that yields the largest benefit to recombination. This situation means that it is significantly more likely for an allele coding for the optimal recombination rate to invade an asexual population than it is for asexual mutants to counter-invade (Tables 1, 3). This results in a net directional pressure towards recombination. This directional pressure means that initially deleterious effects could have been associated with recombination as long as the net effect remained positive.

(ii) The evolution of recombination

A population size of 10^3 is too small to be evolutionarily interesting, while a mutation rate of 0.1 is too large to be deemed relevant for the initial evolution of recombination. But as N increases, \bar{s} decreases, and thus $Ne^{-\mu/\bar{s}} < 1$ will be satisfied for a lower and lower μ . We need, then, a quantitative estimate of \bar{s} in order to assess the model's relevance to the evolution of recombination. This is presented in the Appendix.

Fig. 4 uses \hat{s} from the Appendix to estimate \bar{s} and demarcate values of μ that satisfy the equalities $Ne^{-\mu/\hat{s}} = 1$ and $Ne^{-\mu/\hat{s}} = 25$. $Ne^{-\mu/\hat{s}} = 1$ is the critical condition for the mutational drive to Muller's ratchet, and $Ne^{-\mu/\hat{s}} = 25$ is an oft-accepted standard above which

there will be virtually no ratchet at all (Haigh, 1978). Note that in Fig. 4 the critical mutation rate is largely independent of population size, but that the slight right-hand skew means that populations grow *into* the ratchet: a qualitative result opposite of that derived from a constant s analysis. The figure shows large populations needing a mutation rate only slightly larger than the mean incoming s to ensure a drive to the ratchet. As the mutation rate increases further, increasingly strong conditions are created for the spread of recombination. The threshold character of Fig. 4 means that while strong arguments can be made for the process if μ is high, equally strong arguments exclude it as a reasonable hypothesis if it is found that μ is low.

One can envision early eukaryotic organisms as being under pressure to incorporate more and more genes as they fine-tuned their eukaryotic machinery. This creates an upward pressure on the genome-wide mutation rate and an antagonism between physiological constraints on error correction efficiency and selection for load-reducing mechanisms. While most error correction mechanisms were probably in place by the time early eukaryotes evolved, questions on their efficacy still leave us uncertain as to estimates of μ and \bar{s} . Current techniques tend to underestimate μ and overestimate \bar{s} , so organisms may have experienced a higher $E(\mu/\bar{s})$ than we expect from current estimates of $E(\mu)/E(\bar{s})$ (Deng & Fu, 1998).

As an example of the relationship between N , \bar{s} and μ , consider a conservatively small population of 10^9 (estimates of current-day marine microbes are about 10^6 bacteria ml^{-1} and 10^3 protozoa ml^{-1} : Fenchel, 1987, p. 109). With a mean incoming selection coefficient of 0.02 and $\mu = 0.03$, \hat{s} is equal to 1.2×10^{-3} . This leaves $Ne^{-\mu/\hat{s}} = 1.8 \times 10^{-2}$ individuals in the zero class. Because this is less than unity, the ratchet will turn under the force of mutation pressure at a rate of $R \cong 1.2 \times 10^{-3}$ (equation 8, Gessler, 1995). This rate may seem slow, but it translates into 1.2 million new mutations per generation for the population as a whole, and, assuming a generation time of 1 day, a click of the ratchet every $2\frac{1}{4}$ years. For individuals with genomes of even thousands of genes this drive will devastate the population. Yet $\mu = 0.03$ is still an order of magnitude higher than estimates for many current-day DNA microbes (Drake, 1991; Drake *et al.*, 1998).

Drake (1991) estimated $\mu = 0.0033$ for seven DNA microbes, four of which were bacteriophages. Restricted to eukaryotes, the mean mutation rate from his table 1 is 0.024. This is large enough to drive the above process with an incoming s of about 0.01. It must be noted, though, that this estimate includes two outliers that Drake claims are unrepresentative by 'both biological and statistical criteria'. Remove the outliers and eukaryotic and non-eukaryotic microbes have similar mutation rates. A re-analysis of the data

(excluding the outliers), along with other data, yields consistent estimates of 0.0034 mutations per genome per replication across a broad range of eukaryotes (Drake *et al.*, 1998). Drake *et al.*'s table 5 supports a slightly higher rate for higher eukaryotes (mean $\mu_{eg} = 0.00675$), with both numbers probably being underestimates. Underestimation of μ is concomitant with an overestimation of s (in part because we are failing to detect mutations of small effect), so estimates for proto-meiotic organisms of $\mu = 0.005$ and $s = 0.01$ may be reasonable; that is, μ may lie within a factor of 2 of s .

Numerical work with \hat{s} shows that as N becomes large, $Ne^{-\mu/\bar{s}} = 1$ is satisfied near $\mu \cong s$ (the mean incoming s), while \hat{s} (the estimated average segregating s) decreases slowly to zero. Thus the relation between theory and data is within a factor of 2, and less than our ability to resolve the issue using current empirical estimates of μ and s . A strict reading of the data shows that while the ratchet may be implicated, it is not proven. The resolution is somewhat complicated by the fact that the model *predicts* that current-day, non-obligatory sexual, unicellular organisms will have mutation rates close to the range where parameter values render the process weak. If mutation rates of proto-meiotic eukaryotes were actually closer to 0.01, or if the incoming s were closer to 0.005 (e.g. $\mu \cong s \cong 0.0075$), then a consequence of an increasing number of genes in eukaryotic genomes could have been a drive to the ratchet and an opportunity for the evolution of recombination as outlined here.

(iii) Caveats concerning the analysis

The analysis relies on the theoretical prediction that large, asexual populations can fail to attain mutation–selection balance. This relies on N , \bar{s} and μ and the basic assumptions of the Fisher–Wright model.

The analysis presupposes an absence of epistasis since we specifically wanted to demonstrate the process under a minimum of preconditions. Natural populations will most likely have some epistasis. Epistasis will mitigate the ratchet, but will not stop it (Butcher, 1995). It is unclear how an epistatically depressed ratchet will be offset by an advantage to recombination afforded by synergistic epistasis. This is further complicated by the fact that variance in epistatic effects tends to reduce its role re an advantage to recombination (Otto & Feldman, 1997).

In a similar fashion, caveats also apply to assumptions on the relationship between N and N_e , and this model's exclusion of beneficial mutations (Otto & Barton, 1997). \bar{s} will be affected by background selection (the process where strongly selected alleles affect the diversity, or effective population size, of weakly or non-selected, linked sites (B. Charlesworth *et al.*, 1993)). But since the process

predominantly affects only weakly selected alleles (Barton, 1994), their contribution to \bar{s} will be small. The combination of beneficial mutations decreasing \bar{s} and background selection increasing it means that there will be some variance in the conditions under which $Ne^{-\mu/\bar{s}} < 1$ will be satisfied. Perhaps importantly, if the mean incoming s is comprised of mildly beneficial and mildly deleterious mutations (even though the empirical mean is still 0.02), then the above relation between μ and s may become more favourable for the model.

Essentially, if the data show that $Ne^{-\mu/\bar{s}} \gtrsim 25$ then there exists no current theory to implicate the ratchet. If $1 \leq Ne^{-\mu/\bar{s}} \lesssim 25$ then the ratchet may play a role, but for the classic reasons of why recombination halts the ratchet (Felsenstein, 1974), not for the reasons outlined here. This characterization may be too generous in support of the status quo, since we do not fully understand mutation accumulation in when $1 \leq Ne^{-\mu/\bar{s}}$ (Charlesworth & Charlesworth, 1997). But if $Ne^{-\mu/\bar{s}} < 1$ then strong conditions favourable for recombination are generated as small increases in μ exponentially decrease $Ne^{-\mu/\bar{s}}$. These conditions are operating in the models of Antezana & Hudson (1997*a, b*), though Antezana & Hudson use a Poisson approximation to derive the number of individuals in the best class. We expect, though, that their qualitative conclusions, including the modelling of achiasmatic instead of chiasmatic recombination, could be robust to this assumption.

(iv) The evolution of meiosis

The feasibility of the foregoing must rest on data, not computer simulations: the use of a single gene for recombination is a computational motif, not a strict biological model. For the model to be biologically feasible it presupposes both an opportunity for recombination and its initial genetic variation. There is extensive evidence that both the former (Margulis, 1970; Margulis & Sagan, 1986; Maynard Smith & Szathmary, 1995) and latter (White, 1973; Baker *et al.*, 1976; Lichten & Goldman, 1995; Camerini-Otero & Hsieh, 1995) may have been present during the evolution of early eukaryotes.

The biological scenario of the modelling done here is shown in Fig. 5. The upper panels show the simple process of two primitive eukaryotic cells undergoing fission, i.e. mitosis. The lower panel shows the intermediate step of conjugation, followed by crossing-over and then a resolution of the conjugate. Note that after a renaming of the constitutive elements, the inclusion of the recombination step makes the whole process a canonical example of meiosis. Individual cells in the upper panel can be seen as ancestral to modern-day gametes. The shift in emphasis from the evolution of recombination to the evolution of meiosis

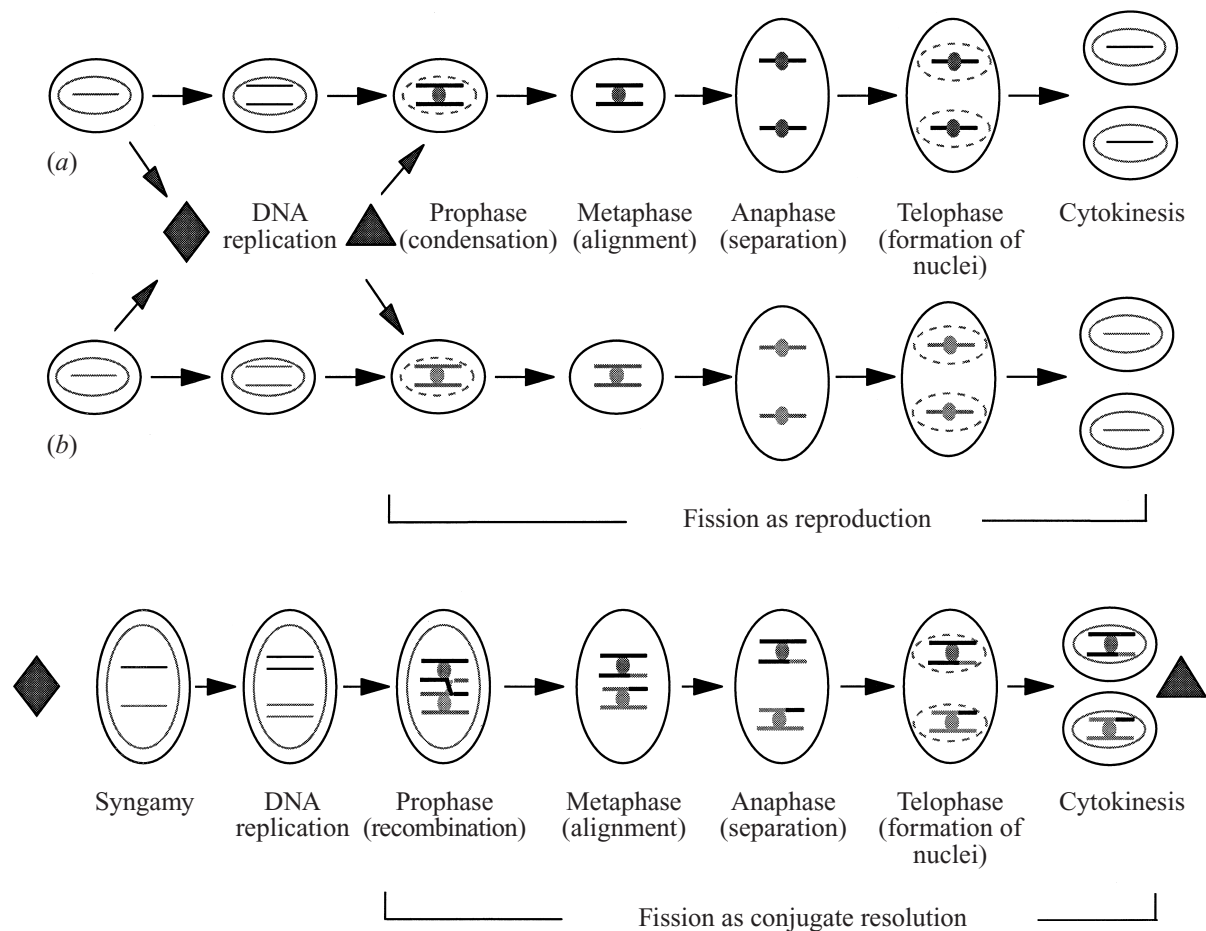


Fig. 5. Schematic diagram of meiosis similar to the computational model simulated. The upper panel shows two individuals (*a* and *b*) independently undergoing mitosis. The lower panel shows an earlier process of conjugation, recombination and fission hypothesized to take place between the diamond and the triangle. If one now considers the prototypal individuals (*a*) and (*b*) as current-day sperm and egg, the flow of genetic information is identical to meiosis. The figure makes the clear prediction that meiosis II is ancestral to meiosis I. As multicellularity evolved, the predominant stage of selection could shift, for example towards an extended diploid stage (marked 'syngamy') for most metazoa. The simulations differed slightly from the diagram in how they performed selection and conjugation. First, they used discrete parthenogenic generations; the products of the mitotic divisions in the upper panel being distinguished as 'parent' and 'offspring' generations. Second, the conjugation in the lower panel occurred in the parent generation as a process separate and independent from reproduction. As such it was done without chromosomal replication: two haploids recombined their genomes and then separated as haploids again. At a later phase they then underwent reproduction.

is contextual: the genetic mechanism is virtually unchanged. While Fig. 5 is devoid of any relation between N , s and μ the primary causative mechanism behind its evolution is not. We show that a realistic quantification of the model can yield its non-recombining component chronically unstable, and this creates selection for recombination that is far stronger than that experienced in approximate-equilibrium models.

The biological model assumes that the machinery for mitosis is already functional, and this is in accord with evidence that mitosis evolved before meiosis (Cleveland, 1947; Margulis & Sagan, 1986, p. 149; Maguire, 1992). The recruitment of the existing mitotic machinery for meiosis has been advanced by many authors with varying emphases (see, for example,

Stack & Brown, 1969; Halvorson & Monroy, 1985; Penny, 1985; Margulis & Sagan, 1986; Dyer & Obar, 1994; Maynard Smith & Szathmáry, 1995). For example, considerations such as DNA repair (Bernstein *et al.*, 1988) and gene conversion (Bengtsson, 1985) presumably could have played roles in allowing pressure on reducing the mutational load to exploit the existing mitotic machinery. Maguire (1992) summarizes evidence of how bound sister chromatids in meiosis I may be controlled by the inhibition of topoisomerase II activity; that is, even key distinguishing properties between meiosis and mitosis could have arisen by simple changes in the timing and target of existing gene products. The evolutionary history of meiosis I and meiosis II may be further revealed by noting that in some organisms

the nuclear membrane redevelops in telephase I only to dissolve again in prophase II. This is expected if the first fission event was to restore individuality – and thus can now be considered as a vestigial clue – yet remains curious if meiosis I and II are seen in the classical view of evolving as a mechanism to make gametes.

Some models have hypothesized that meiosis evolved as a way to rescue a cell from the diploidy resulting from fusion (Cleveland, 1947; Margulis & Sagan, 1986). This model neither confirms nor denies this, and does not mandate a causal explanation for recombination. Thus while diploids could have been under strong selective pressure to resolve their heterokaryotic state, it is also feasible that an advantage to recombination itself – that is, purely the consequences of the loss of mutation-selection balance on genetic variation for recombination in non-equilibrium populations – could have initiated a mitotic cascade, with the resulting fission proceeding relatively easily. The model of this paper differs from its predecessors by being essentially void of requiring an *individual* adaptationist explanation on the biological constituents of meiosis: the whole process proceeds under the simple laws of Mendelian inheritance in a finite population. It does so in the complete absence of epistasis, pleiotropy or fluctuating environments.

From the organismal level, a shift in emphasis to the dominant diploid component of the life cycle (the shaded step in fig. 5) creates a long, extended diploid phase – a phase that for some organisms would eventually include multicellularity, alternating generations or a sequestered germ line. This would in turn lead to the more difficult problem of the maintenance of sex, one that must address both the twofold cost of meiosis and the twofold cost of males. But the separation of conjugation and reproduction in this model yields these costs absent at this stage, and thus the origination and the maintenance of eukaryotic sex are considered as fundamentally different problems. This conceptual interpretation, and the distinction between the origin of fission in meiosis I and meiosis II, posits current two-step meiosis as being indicative, rather than enigmatic, of its history, and explains the ‘absurdity’ of a meiosis that doubles its DNA only to later halve it twice. This leads to the somewhat Dawkinsque (Dawkins, 1982) interpretation that meiosis reveals diploids as elaborate chemostats that are manipulated by, and have evolved entirely to maximize the fitness of, the haploid ‘organisms’ they carry: their gametes.

Appendix

We seek an estimate, \hat{s} , of \bar{s} (the arithmetic mean selection coefficient of alleles segregating in the population at equilibrium). One can show that in an

infinite population, $\hat{s} = E(S(x)^{-1})^{-1}$, where $S(x)$ is the probability density function of selection coefficients of incoming mutations; i.e. \hat{s} equals the harmonic mean of the incoming distribution. For distributions on support $[s_{\min}, 1]$, such as the negative exponential or even the uniform, as $s_{\min} \rightarrow 0$, \hat{s} also goes to zero.

For finite populations, one can follow Kimura (1969, 1983) and Ewens (1979) to solve for the equilibrium mean number of mutations per individual, $\hat{n} = 2S - H$, where S is the mean number of segregating sites and H is the mean number of heterozygous sites. Using

$$S = 2\theta(\alpha^{-1} - (e^{\alpha} - 1)^{-1}),$$

$$H = \frac{\theta}{-x} \left(\frac{1 - e^{\alpha/2N}}{1 - e^{\alpha}} - \frac{1}{2N} \right),$$

where $\theta = 4N\mu_{\text{haploid}}$ and $\alpha = 4N_e x$, we get

$$\hat{n} = \int_{s_{\min}}^1 (2S - H) S(x) dx$$

and

$$\hat{s} = \frac{\int_{s_{\min}}^1 x(2S - H) S(x) dx}{\int_{s_{\min}}^1 (2S - H) S(x) dx}$$

$$= \frac{1}{\hat{n}} \int_{s_{\min}}^1 x(2S - H) S(x) dx.$$

Amongst deleterious alleles, this expression is unlikely to fall below $\sim 5 \times 10^{-4}$ for biologically reasonable values of N , the average incoming s , and μ . The above expression applies to diploid populations of size N or haploid populations of size $2N$.

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