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POINT MUTATIONS, THE RATCHET, AND THE INITIAL SUCCESS OF EUKARYOTIC SEX: A SIMULATION STUDY.

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Received 14 January1997; 6 August 1997

ABSTRACT. It has often been argued that Muller's Ratchet limits the size of asexual genomes. This process should also limit the number of nucleotide sites at which optimal bases can be maintained in spite of point mutation. Here we study with simulations the ability of asexual populations and of sexual populations that lack crossing over to maintain optimal base occupancy, and some consequences thereof. Asexual populations initially free of deleterious point mutations often accumulate point mutations until they reach an equilibrium determined by drift, selection, and forward and backward point mutation. In contrast, comparable segregating diploids with a single pair of achiasmatic homologues accumulate lower numbers of such mutations and have much higher equilibrium fitness. These equilibria often develop with biologically plausible parameter values. We therefore studied two phenomena that may have favored the origin of eukaryotic sex in asexual populations carrying numerous deleterious point mutations. We show that a mutant lineage that selfes or ploidy cycles has a very high chance of invading such a population, if the population is diploid and free of recessive mutations. Indeed, such a lineage can fix the least mutated chromosome(s) carried by its founder and thereby become loaded with many fewer point mutations than even the least mutated asexual individuals in the population. However, if recessive mutations are frequent, such lineages should be at strong disadvantage because they make such mutations homozygous. Thus, eukaryotic sex may have originated in a haploid asexual population, if recessive mutations littered the genomes of most diploid asexual lineages at that time. We show that in the genomic genealogies of haploid asexuals loaded with substantial numbers of non-recessive point mutations, considerable genetic variance can be produced by neutral events consisting of a favorable and a deleterious mutation. We then show that when such haploids evolve syngamy and segregation, inter-chromosomal recombination exposes this variance to selection if the genome is segmented, resulting in large and often immediate fitness increases. These Ratchet-related advantages of sex involving reversible point mutations do not require crossing over and might have sufficed for eukaryotic sexuality to succeed before crossing over was evolved.

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INTRODUCTION

A classic population-genetical explanation of the advantage of sexual reproduction over asexuality is Muller's Ratchet (Muller 1964). The Ratchet is the irreversible accumulation of deleterious mutations in asexual populations that occurs when, in spite of purifying selection, genetic drift and/or mutational pressure result in the disappearance of the least mutated genotypes in the population. Once the asexual population loses such genotypes it can regain them only through back mutation, but this is considered very unlikely. The Ratchet might be a major factor disfavoring asexual forms relative to sexually reproducing organisms over evolutionary time (Crow 1988), since it should lead to an accumulation of deleterious mutations in asexuals. It is widely believed that recombining

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Evolutionary Theory 11: 209-235 (August, 1997)
The editors thank A. Kondrashov and another referee for help in evaluating this paper.

populations do not suffer from the Ratchet because recombination gives them the potential to produce mutation-free genomes out of the non-mutated genomic regions. Studies of the Ratchet to date have focussed mostly on how its pace is affected by the intensity of recombination, population size, the strength and mode of selection, and mutation and outcrossing rates (Felsenstein 1974; Haigh 1978; Maynard Smith 1978; Bell 1988; Pamilo *et al.* 1987; Gabriel *et al.* 1993; Lynch *et al.* 1993; Charlesworth *et al.* 1993a; Stephan *et al.* 1993, Kondrashov 1994a, Butcher 1995).

Muller's Ratchet is often cited as a major force constraining the size of asexual genomes (Maynard Smith 1978; 1988). The genome size of most phylogenetically well established asexual forms is in fact known to be small (Drake 1991) relative to that of ephemeral secondary asexuals derived from taxa that are normally sexual (but this does not imply that all sexual forms must have big genomes). Obviously the sheer genome size is not the directly constrained quantity but rather the genomic deleterious mutation rate which is one of the main determinants of the ratcheting process (Haigh 1978; Felsenstein 1974; Stephan et al. 1993, Antezana and Hudson, in press). Deleterious mutations can cause macroscopic damage to the genome, but a large share of them must be associated with damage to the informational content of genes, e.g. severe gene deactivation by insertion/deletions (indels) and de-optimization of gene sequences by point mutation. Thus the above considerations about constrained genome size under asexuality imply that in asexuals the number of nucleotides at which optimal bases can be maintained in spite of deleterious mutations is also constrained.

Drake (1991) showed that the bulk of the genomic deleterious mutation rate in the organisms he studied was due to indels. But we have seen above that point mutations, though reversible, can also participate in the Ratchet and that their rate of occurrence, together with the ability of a population to counter their accumulation, can constrain the informational content of asexual genomes. Virtually all workers who have studied the Ratchet have assumed that back mutation is negligible. This is a reasonable assumption when mutations are caused by indels which are indeed very unlikely to be exactly erased or balanced by subsequent indels. At the level of point mutations, however, the assumption that back-mutation is rare is not justified. For instance, when each of the four bases mutates without bias to each of other bases, whenever a point-mutation occurs, 1/3 of the time the event will be a back mutation. In this paper we will study how the introduction of back point-mutation modifies the dynamics of the ratcheting process in asexual populations and in comparable achiasmatic (not molecularly recombining) sexual populations with and without inter-chromosomal recombination.

We have shown elsewhere that segregation allows diploid sexual populations lacking both interchromosomal and molecular recombination to decelerate the Ratchet strongly in cases where asexuals would ratchet frequently, and also that segregation and outcrossing, through their side effect of inter-chromosomal recombination, free organisms with sufficiently segmented genomes from Ratchet-related constraints on genome size (Antezana and Hudson, in press). It is clear thus that a transition from asexuality to non-recombinational sexuality or, even more so, to achiasmatic sexuality with inter-chromosomal recombination would immediately free a population from the above-mentioned constraints on genome size typical of asexuality. (Obviously chiasmatic recombination would be even better, but it is an open question whether this process was immediately available to early eukaryotic sexual forms). Achiasmatic sexual populations with segmented genomes indeed are free to increase their genomic deleterious mutation rate. This could mean enlarging the genome by evolving new gene functions encoded by new genes derived from others through gene duplication and reorganization; but it could also mean increasing the density of information of the genome by beginning to maintain more nucleotide sites occupied by a specific base or class of bases. Obviously, a physically larger and more segmented genome would cause higher metabolic costs in terms of maintenance and result in more chromosome-number aberrations during mitosis and meiosis.

It is unlikely that a physical enlargement of the genome like that expected to occur through duplication and successive modification of existing genes would have occurred in ecological time after eukaryotic sex was evolved although, as the success of many polyploids indicates, polyploidization and other large-scale chromosomal aberrations can arise over a single generation and are sometimes adaptive. In contrast, an accumulation of optimal bases at already existing nucleotide sites occupied by non-optimal bases could have occurred rapidly, and it is difficult to see any disadvantages resulting from this process besides trade-offs with GC-content and other non-informational functions of the nucleic acids. Indeed, many nucleotide sites with non-optimal occupancy might have been present in the genome of the asexual ancestor(s) of eukaryotic sexual forms (e.g. thousands of third positions; see below). In such a case, even a moderate point-mutation rate per nucleotide site would have sufficed to produce large numbers of favorable mutations each generation in reasonably sized populations.

The Ratchet is often cited as a possible factor favoring the maintenance of eukaryotic sexual reproduction (Manning and Thompson 1984; Crow 1988; Kondrashov 1993; Kondrashov 1994a), but we have been unable to find references where it is postulated to play a role in the origin of eukaryotic sexual reproduction. This might be due to the seemingly inescapable conclusion that the ancestor of sexual forms could not have been on its way to a Ratchet-caused extinction and that the Ratchet is unlikely to produce substantial fitness advantages in ecological time. And indeed, save for works that focus on the intrinsic advantages of diploidy (Orr and Otto 1994, Orr 1995), much of the discourse about the advantages of eukaryotic sexual reproduction which favored its origin stresses asexual genetic loads (Crow and Kimura 1965; Kondrashov 1982; Kondrashov 1988; Kondrashov 1994b, 1994c) which are steady state burdens that should reach equilibrium in a relatively short time. In contrast, the fitness decays expected from the Ratchet are often thought to lead ultimately to extinction (Gabriel *et al.* 1993; Lynch *et al.* 1993).

In the following pages, however, we will show that Ratchet-related[1] events can have favored the origin of eukaryotic sex. The asexual ancestors of sexual eukaryotes, indeed, do not need to have been on their way to extinction for the Ratchet to have been constraining the size and the informational density of their genomes, i.e. making it impossible that every functionally important nucleotide site be occupied with an optimal base. We will show below that the presence of many such sites in these ancestors may have strongly favored the evolution of eukaryotic sexuality, often in ecological time.

In this paper, we present first a population-genetical model of a population in which both favorable and deleterious point mutations are allowed to take place. We have called this model "the favdel model" because it allows for both favorable and deleterious mutations to take place. We explore the dynamics of this model using Monte Carlo simulations and describe the parameter range where asexual populations accumulate deleterious point mutations above the level of the classic deterministic mutation-selection balance (dMSB; Haldane 1927; Kimura and Maruyama 1966) which is the state expected with free recombination in absence of epistasis in non-neutral or semi-neutral cases. We limited our exploration of the parameter range to biologically realistic values. In particular, we assumed that genomes have realistic numbers of selectively important nucleotide sites at which point-

[1] We will use the term Ratchet to indicate any accumulation of deleterious mutations that happens against the workings of selection and is reversible in the population in which it occurs only by back-mutation. Often the term is used to denote such accumulations, but only if they do not occur through the fixation of a deleterious mutation at a locus. This distinction is relevant when considering the effectiveness of introducing recombination to counter an occurred accumulation of deleterious mutations (fixations cannot be reversed by recombination). We consider this teleologically motivated restriction of the meaning of the word "Ratchet" not useful when describing the accumulation of deleterious mutations in asexuals, which will never undergo recombination. We will, nevertheless, honor this distinction by using the word "Rhatchet" when referring to the accumulation of deleterious mutations not by fixation.

mutation occurs at rates close to those of extant eukaryotes. Therefore the values of the favorable and the deleterious genomic mutations rates that participate in determining the dynamics and equilibria which we will present below are microscopically justified (Maynard Smith 1989).

The simulations of the favdel model showed that, very often, asexual populations evolving according to the favdel model accumulate deleterious point mutations because of the Ratchet until they reach an equilibrium between genetic drift and deleterious point mutation on the one hand, and favorable point mutation (back-mutation) and selection, on the other hand. Therefore such equilibria are qualitatively different from those under the classic dMSB in which neither drift nor favorable mutations need to be considered. Remarkably, even in very large populations in strongly non-neutral cases, deleterious point mutations accumulate readily whenever the strength of selection against them is small in absolute terms. We find that diploid segregating populations with genomes consisting of a single achiasmatic linkage group reach equilibria characterized by substantially lower numbers of deleterious point mutations than the number found in comparable asexuals and thus that their fitness can be much higher.

Since favdel equilibria in which the number of deleterious mutations per genome is above dMSB were often found to develop under combinations of parameter values that are biologically plausible, we looked for possible population-genetical consequences of the existence of such equilibria in the asexual populations in which the eukaryotic sexual cycle was first evolved. We identified and studied with simulations and analytical formulae two phenomena that might have strongly favored the origin of eukaryotic sex in asexual populations loaded with numerous deleterious point mutations.

We first show with analytical arguments that selfing or ploidy-cycling (Kondrashov 1994c) mutants have very high probability of invading such an asexual population, if this is diploid and many of its individuals carry no recessive deleterious mutations. In the presence of recessive mutations, selfing and ploidy-cycling produce inviable homozygotes and thus are unlikely to evolve. Given that asexual diploids can accumulate recessive mutations very rapidly (neutrally if recessivity is complete), if generalized recessivity was, at least initially, an immediate consequence of diploidy, eukaryotic sex most likely originated either in a very young diploid asexual population not yet carrying numerous recessives or directly in a haploid asexual population. The deleterious mutations that a haploid population can accumulate are indeed likely to show similar effects in the haploid and homozygous states. Thus, in presence of such mutations, homozygotes produced by syngamy and segregation are unlikely to show a reduced fitness. We therefore explored the fitness consequences of introducing syngamy and segregation into populations of haploid asexual organisms with genomes subdivided into multiple linkage groups and loaded with numerous point mutations. We show that cryptic variance in fitness can be maintained in asexual populations and that segregation and syngamy, through interchromosomal recombination, can expose this variance to selection and often result in large and even immediate fitness increases.

THE "FAVDEL" MODEL

The favdel model is completely determined by four parameters, which are the number of individuals, the deleterious effect of carrying a non-optimal base at a nucleotide site, the per-site per generation mutation rate from any one base to another specific base, the number of nucleotide sites under selection; and by the assumption of multiplicative cumulative effects of deleterious mutations.

We have chosen to assume a simple mutational scheme to allow for the occurrence of deleterious and favorable point mutations but more complex models are not excludable *a priori*. We assume that each individual has a single pair of homologous chromosomes, each with $L/2$ nucleotide sites involved in favdel dynamics (i.e. the model does not exclude the possibility that many other

nucleotide sites are under much stronger selection, have optimal base occupancy, and do not participate in favdel dynamics) and that at every site, one of the four nucleotides is optimal and the other three are equally deleterious. Mutations to deleterious base states occur according to a Poisson process with rate $3u$ at sites with optimal bases, u being the rate of mutation from any base to another specific one. Thus at sites occupied by non-optimal bases, favorable mutations occur with rate u. Therefore U_{max}, the maximum per genome per generation deleterious point-mutation rate, is equal to $L3u$, i.e. is realized only in genomes where every site is occupied by an optimal base. The maximum per generation favorable point-mutation rate F_{max} is equal to Lu and is realized only when non-optimal bases occupy all favdel-relevant sites. U_i is the deleterious point-mutation rate of a genome carrying i bad bases at as many nucleotide sites and is equal to $(L-i)3u$; F_i is the corresponding rate of favorable point-mutation for the same genome and is equal to iu. On occasion, we will replace i by a percent (as in $U_{60\%}$ and $F_{60\%}$) to denote the total favorable and deleterious point-mutation rates of a genome in which a given percent of its L favdel-relevant sites are occupied by non-optimal bases. Alex Kondrashov has kindly pointed out to us that similar models have been used elsewhere (Li 1987; Tachida 1990; Ohta 1992) although not for studying the effect of mating systems on their behavior; and also that Kimura, Maruyama, and Crow (1963) were the first to stress that in any finite population, back mutation is necessary to counter the fixation of bad mutations.

Given that the favdel model involves genetic drift, selection, the Ratchet, and both deleterious and favorable mutations, we resorted to Monte Carlo simulations of the model to study its dynamics and equilibria. To simulate favdel evolution in asexual populations, we have used a modified version of the approach followed by Haigh (1978) in simulating Muller's Ratchet: We assume a classic Wright-Fisher life cycle in which selection occurs before genetic drift and mutation. Like Haigh we assumed individuals carrying i mutations have a fitness of $(1-s)^i$, i.e. multiplicative cumulative fitness effects. The use of such a fitness scheme (i.e. one where fitness is a function of the number of mutations only) allows one to use haploid simulations without having to keep track of the genotypes at each single locus. In fact, since carrying i mutations results in a fitness of $(1-s)^i$ regardless of the allelism of the mutations, one can simply record the numbers of individuals that carry different numbers of deleterious mutations. This simplification speeds things up considerably and decreases memory requirements drastically. The multinomial sampling procedure used to produce each new generation in our program is based on Devroye's approach (Devroye 1986) and was written and kindly provided by J. Gillespie. Our simulations produced results that were consistent with neutral predictions when Ns values were close to zero, with previous studies of the Ratchet (Haigh 1978; Pamilo, *et al.* 1987; Stephan *et al.* 1993), and with predictions from diffusion approximations (Malecot 1952; Kimura 1957) whenever such were applicable.

To simulate favdel evolution in random-mating, segregating populations of N reproducing diploid individuals with genomes consisting of two achiasmatic homologous chromosomes, we simulated $2N$-sized Wright-Fisher populations of haploid asexual genomes each having half as many nucleotide sites as the diploid genome. Such a haploid population has exactly the same population-genetical behavior as a random mating segregating diploid population with a single pair of homologues, provided that mutations have multiplicative cumulative fitness effects, as assumed here, and that genetic drift occurs only after selection and before reproduction (Ewens 1979). This last condition is the standard assumption made in Wright Fisher models with selection (Ewens 1979). Adding stochastic fluctuations to the genotypic proportions among zygotes before selection can increase genetic drift and thus influence favdel dynamics, but here we will not attempt to study this violation of the Wright-Fisher life cycle. We have used a similar approach to study the effect of segregation on the Ratchet when only deleterious mutation can take place (Antezana and Hudson, in press).

The simulation approach that we have used to deal with segregating genomes subdivided into n achiasmatic linkage groups has been presented and justified elsewhere (Antezana and Hudson, in press). In a few words, we use haploid simulations like those above to study the behavior of a single linkage group. We assume that each of the $2n$ chromosomes in an individual has one $2n$-th of the favdel-relevant nucleotide sites of a whole diploid genome, and thus the maximum deleterious point-mutation rate per homologue is $U_{max}/2n$. We therefore run haploid simulations with $2N$, $U_{max}/2n$, and s as parameters. Results from such haploid simulations of single linkage groups can be extrapolated to the whole genome, if one can exclude epistatic interactions (Barton 1995, Antezana and Hudson, in press). In general, a simple multiplication or division by n is the only required scaling. Although we will reconsider the possibility that this assumption does not hold on a case-by-case basis below, the following *a priori* arguments can be made already. It is known that selection events at loci unlinked to a locus of interest lower the effective population size N_e applicable for that locus (Hill and Robertson 1966; Felsenstein 1974; Barton 1995). However, favdel equilibria and dynamics which, as we will show below, depend on drift, mutation, and selection, can be expected to be little influenced by the lowering of N_e caused by background selection: On the one hand we have shown elsewhere (Antezana and Hudson, in press) that the twofold increase in N_e due to segregation has little effect on the period of the Ratchet, and that correcting N_e as proposed by Barton (1995) does not lead to any substantial differences in assessing with haploid simulations the speed of Muller's Ratchet in segregating genomes with multiple linkage groups. On the other hand, Barton (1995) has shown that for biologically realistic parameters, the N_e-reduction due to selection events at loci unlinked to a locus of interest has negligible effects on the fixation probabilities of favorable mutations arising at that locus. On a similar note, Hudson and Kaplan (1995) have shown that the effect of selection events at unlinked loci on the level of neutral molecular polymorphism at a given locus are negligible compared to that of events at linked loci. Since among the population-genetical processes relevant for the dynamics of favorable variation, the initial stages of the fixation process are those most likely to be affected by drift, it can be argued that the N_e effect should be negligible also with respect to the dynamics of favorable mutations in the favdel model.

EVOLUTION UNDER THE "FAVDEL" MODEL

In studying with simulations the favdel model in asexual populations and in segregating outcrossing populations with one or more achiasmatic linkage groups, we aimed at determining in which region of the parameter space point mutations accumulate beyond the number expected under classic mutation-selection balance (dMSB) that is equal to U_{max}/s (Haldane 1927; Kimura and Maruyama 1966), but we restricted our exploration of the parameter space to biologically realistic values (see below). We then compared such equilibria in these types of populations in terms of potential adaptive differences. Afterwards we explored with simulations and/or analytical techniques two fitness consequences of favdel-related phenomena that might have occurred when asexual populations carrying substantial numbers of point mutations evolved either selfing/ploidy-cycling or both syngamy and segregation.

Reaching favdel equilibrium. Simulations of asexual populations evolving under the favdel model show that when U_{max} is high enough for the Ratchet to operate, steady-state situations are reached in which genetic drift and deleterious mutations on the one hand, and favorable mutations and selection on the other hand, balance each other when a certain average number of non-optimal bases over the L favdel-relevant sites has been accumulated. In Figure 1, we show an example of how i increases over time in a simulated asexual population started with genomes containing only optimal bases (i= 0) and where U_{max} is high enough to let the population accumulate deleterious point mutations beyond the

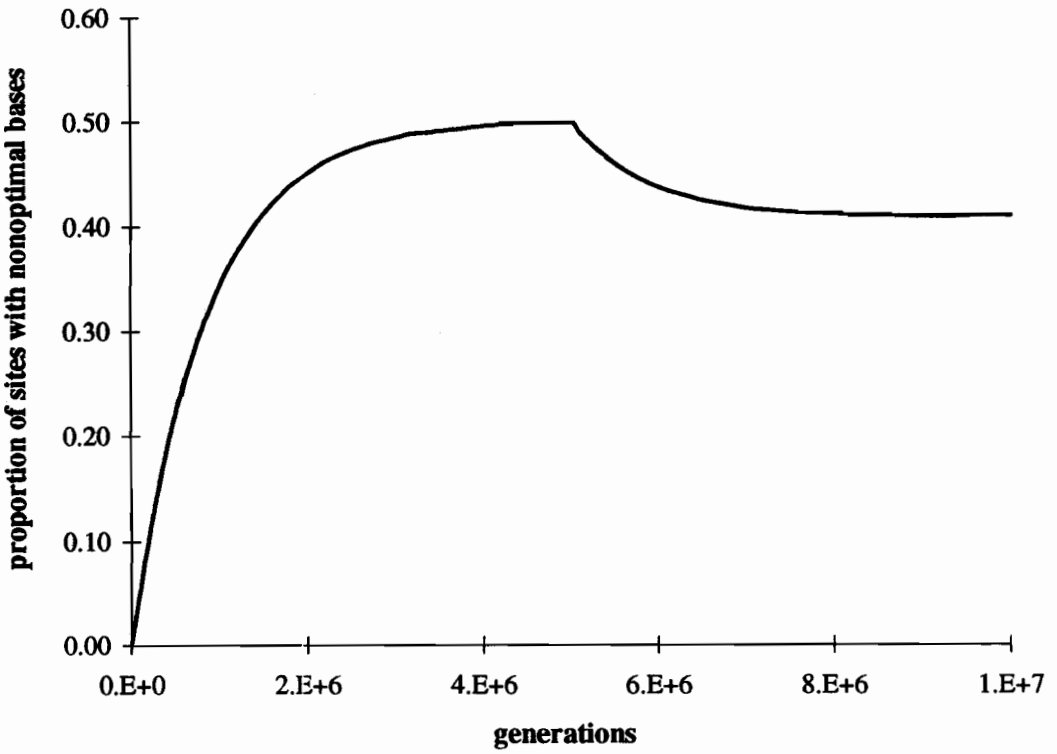

Figure 1. Accumulation and removal of deleterious mutations in the favdel model. The left side of the curve shows the increase in the average proportion of favdel-involved sites per genome that are occupied by non-optimal bases, in a diploid asexual population of one thousand individuals. U_{max} is the favdel-relevant per genome per generation deleterious point-mutation rate in genomes with only optimal bases. In the case shown, non-optimal bases were selected against with strength s of 0.01. L was equal to 10^6 nucleotide sites and u was $3.333\ 10^{-7}$ ($U_{max}= L3u$; see text). At the beginning of the simulation, genomes carried only optimal bases. Deleterious mutations accumulated due to the Ratchet until an equilibrium was reached after about five million generations. The trajectory after generation five million shows how non-optimal bases are replaced by optimal ones after the whole population begins undergoing segregation. At the onset of segregation, the mutations carried by each asexual genome, which was assumed to consist of two homologues, were assigned evenly to its two chromosomes (see text).

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dMSB expectation. Under such circumstances, mutations accumulate rapidly due to the Ratchet, thereby decreasing U and increasing F, until an equilibrium is reached and the ratcheting stops. When the population is started with non-optimal bases only, the same equilibrium is reached, but in such a case favorable mutations accumulate and increase the genomic deleterious rate until the Ratchet becomes strong enough to counter any further accumulation of favorable mutations.

The above-described processes of accumulation/removal of point mutations are the result of interactions between drift, mutation, and selection that are certainly more complex than the Ratchet itself. We attempted to predict the equilibrium with a simple deterministic model that includes forward and backward mutation as well as selection, but this was successful only when the number of sites was below ten (not shown). Note, however, that the fact that this attempt was successful with fewer sites implies that it might not be possible to explore the favdel dynamics of cases with many sites from results obtained with non-deterministic models with fewer sites.

Favdel equilibria under asexuality and segregation. We used simulations to explore the parameter space defined by N, s, and U_{max}, where the equilibrium favdel load in asexual diploids is higher than the minimum predicted for an infinite population under dMSB. For every such case we used simulations to determine the favdel load of otherwise identical segregating populations with genomes subdivided into a single linkage group. The chosen values of U_{max} were 1.0, 0.1, and 0.01 deleterious point mutations per genome per generation; the values of s were 0.1, 0.01, and 0.001; and the population sizes N were 1000, 10^4, 10^5, 10^6, and 10^7 individuals. Furthermore, we examined the effect of concomitant changes in L and u that give the just-mentioned values of U_{max}.

Figure 2 and Table 1 show when favdel equilibria above dMSB expectation were found. With no exceptions, the curves in Appendix 1 of Antezana and Hudson (in press) allowed us to predict whether a population with given values of N, U_{max}, and s would accumulate favdel loads above dMSB, but not which equilibrium favdel load would be reached. Therefore favdel loads above dMSB are expected to develop whenever the Ratchet can be expected to operate in a population of optimal genomes, i.e. when U_{max} is large and/or when Ns is small. A noteworthy trend that will become important below, is the tendency of point mutations selected against with a strength s that is small in absolute terms, to accumulate beyond the dMSB level even in very large populations, i.e. when Ns is much larger than 1.0, and even when U_{max} is relatively low. For instance when s= 0.001 and U_{max}= 0.01, any individual in an asexual population at equilibrium of size N= 10^7 has about 1% of its 10^6 favdel-relevant sites occupied by non-optimal bases (i.e. 10,000 sites), despite the moderately large Ns of 10,000. When s is large, however, increases in N have large effects on the equilibrium favdel load and thus larger U_{max} values are required for the load to increase beyond the dMSB level (see curves for s= 0.1 in Figure 2). In general when U_{max} is large, equilibrium levels are less sensitive to changes in N except when s is also large. Furthermore and importantly, Figure 2 and Table 1 show also that the equilibrium favdel load with segregation is always much lower than without segregation (i.e. in asexuals) and sometimes is as low as expected under dMSB, except in nearly neutral cases (e.g. N= 1000 and s= 0.001).

FAVDEL AND THE TRANSITION TO ACHIASMATIC SEXUALITY

In Figure 1, we showed that when an asexual diploid population at favdel equilibrium and with single-linkage-group genomes begins segregating, a dramatic increase in fitness can take place. For the transition to segregation shown in the same figure, we assumed that the two homologues of each individual in the population were identically loaded with deleterious point mutations (exact to ± one mutation). This minimized the variance in the number of mutations between the haploid genomes that began segregating. A more realistic procedure, given our assumption of multiplicative fitness effects, would have been to assign the mutations carried by an individual to each of its two homologous chromosomes as if the mutations had occurred randomly over all loci, i.e. according to an hypergeometric distribution. This however cannot be done for each individual independently since all the population genotypes must be related by a genealogy and thus must be identical by descent at many loci. For the simulation shown in Figure 1, we also assumed that the whole population began segregating and mating randomly as if a rapid transformation of the whole population (Rose 1983; Hickey and Rose 1988) had taken place.

The origin of eukaryotic sexuality is full of uncertainties not only about which steps were evolved first and which later but also, and possibly more importantly, about the population-genetical state in which the ancestors of sexual eukaryotes were at the moment of the origin as well as about the population-genetical constraints they were subjected to. In the following we will explore possible population-genetical consequences of favdel dynamics and equilibria and of shifts thereof which might

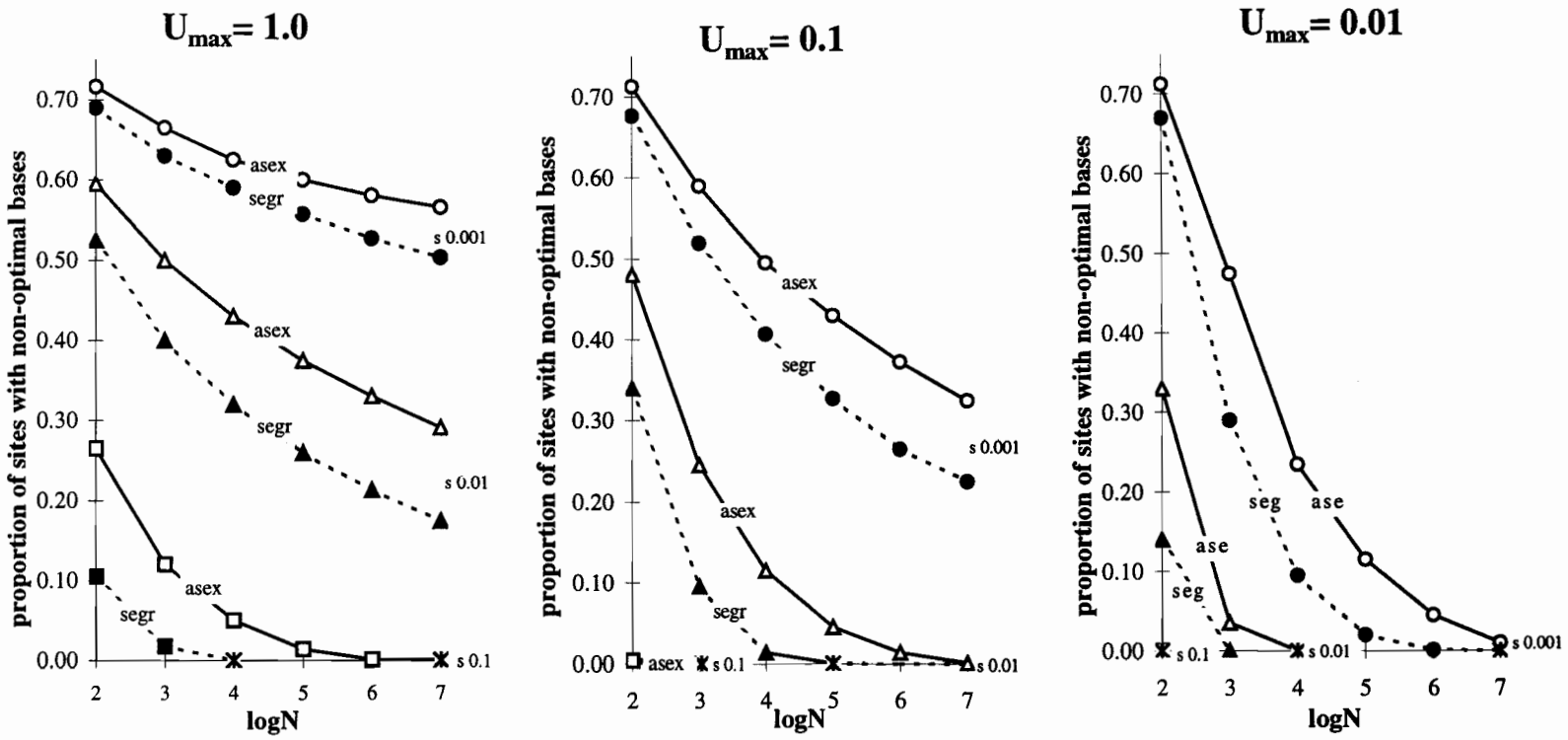

Figure 2. Equilibrium favdel loads in asexual and segregating populations. Plots of the average proportion of non-optimally occupied favdel-relevant sites in equilibrium asexual (**asex**) and diploid segregating (**segr**) populations with a single linkage group. Plots show the situation for U_{max} values of 1.0, 0.1, and 0.01, respectively. From left to right, u values were $3.333\ 10^{-7}$, $3.333\ 10^{-8}$, and $3.333\ 10^{-9}$, and L was 10^6 sites ($U_{max}= L3u$, see text). The favdel loads for many combinations of L and u which resulted in the U_{max}'s of the plots were identical to the plotted proportions whenever L was larger than 1000 sites. The proportions corresponding to mutation-selection balance (U_{max}/s) were plotted only in the smallest population that showed them (asterisks). Actual numbers of deleterious point-mutations per genome and their variances are listed in Table 1.

Table 1. The number of non-optimal bases in genomes at favdel equilibrium. Tabulation of the average and the variance of the number of non-optimal bases per genome in populations at favdel equilibrium.

U_{max}= 1.0

	s= 0.1				s= 0.01				s= 0.001			
	asexual		segregating		asexual		segregating		asexual		segregating	
logN	mean	var	mean	var	mean	var	mean	var	mean	var	mean	var
2	265,000	7.0	105,000	7.5	595,000	30	525,000	30	716,000	55	690,000	60
3	120,000	5.0	17,500	8.0	500,000	25	400,000	40	665,000	100	630,000	180
4	50,000	9.0	10	10	430,000	40	320,000	52	625,000	143	590,000	200
5	14,000	9.6	10	10	375,000	56	260,000	60	600,000	200	557,000	250
6	1,000	9.6	10	10	330,000	52	213,000	68	580,000	210	527,000	280
7	10	9.6	10	10	290,000	54	174,000	75	565,000	250	503,000	320

U_{max}= 0.1

	s= 0.1				s= 0.01				s= 0.001			
	asexual		segregating		asexual		segregating		asexual		segregating	
logN	mean	var	mean	var	mean	var	mean	var	mean	var	mean	var
2	4,000	1.1	1	1.1	480,000	3.06	340,000	5.8	712,500	7.2	676,000	8.5
3	1	1.0	1	1.0	245,000	7.4	95,000	9.9	590,000	26	520,000	32
4	1	1.0	1	1.0	115,000	8.3	13,500	10	495,000	38	407,500	45
5	1	1.0	1	1.0	45,000	8.4	10	10	430,000	43	327,000	56
6	1	1.0	1	1.0	13,500	9.94	10	10	372,500	49	265,000	62
7	1	1.0	1	1.0	700	9.94	10	10	325,000	55	225,000	64

U_{max}= 0.01

	s= 0.1				s= 0.01				s= 0.001			
	asexual		segregating		asexual		segregating		asexual		segregating	
logN	mean	var	mean	var	mean	var	mean	var	mean	var	mean	var
2	0.1	0.08	0.1	0.14	330,000	0.6	140,000	1.6	712,000	0.77	670,000	1.5
3	0.1	0.1	0.1	0.1	35,000	0.93	1	1.0	475,000	4.3	290,000	3.8
4	0.1	0.1	0.1	0.1	1	0.9	1	1.0	235,000	6.8	95,000	8.8
5	0.1	0.1	0.1	0.1	1	1.0	1	1.0	115,000	8.2	20,000	9.0
6	0.1	0.1	0.1	0.1	1	1.0	1	1.0	45,000	9.5	1,000	10
7	0.1	0.1	0.1	0.1	1	1.0	1	1.0	10,000	9.85	10	10

U_{max} is the favdel-relevant rate of deleterious point-mutation per genome per generation in genomes in which favdel-relevant sites are all occupied by optimal bases. U_{max} is then equal to $L3u$, where L is the number of sites participating in favdel dynamics and u is the per-nucleotide mutation rate from any base to another specific one. Here L was 10^6 nucleotide sites and u was, from top to bottom, $3.333\ 10^{-7}$, $3.333\ 10^{-8}$, and $3.333\ 10^{-9}$. However, the proportion of non-optimal sites was found to be identical for any combination of L and u that gives the U_{max}'s shown as long as $L > 1000$. Each haploid genome consists of a single chromosome.

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have favored the origin of eukaryotic sex in populations loaded with favdel loads above the dMSB level. Regardless of any advantages that segregation and outcrossing might produce, a single segregating mutant that produces outcrossing gametes cannot invade an asexual diploid population unless its gametes force others members of the population to undergo syngamy with them or to

segregate and produce gametes (see Rose 1983; Hickey and Rose 1988). This restriction does not apply to a selfing mutant, however, which could invade the asexual population if the costs of segregation and self-syngamy are not larger than the fitness advantages that might arise from selfing. We will show below that in diploid asexual populations that have accumulated point mutations beyond the dMSB level, i.e. beyond the minimal favdel load, a selfing mutant can often invade if it arises in an individual carrying almost no deleterious recessives simply because of the fitness advantages expected from fixing the least mutated of its two initial chromosomes (see below). As was just implied, segregation might have arisen in a population of asexual diploids with non-dMSB favdel load only if the point mutations accumulated in the population were neither recessive nor semi-recessive or, in the case of segregation being introduced together with outcrossing, if non-dominant mutations were at very low frequencies in the population. Otherwise, segregation and syngamy, with or without outcrossing, would have resulted in unfit individuals homozygous for multiple deleterious recessives.

Recessivity effects should not play any role in a transition to achiasmatic sexuality from asexual haploidy. In haploids, a mutation can be expected to be selected according to its homozygous effects, i.e. the mutation is likely to show deleterious fitness effects similar to those it would show when present in the homozygous state in a diploid. We therefore have studied a favdel-related advantage expected to occur in a transition from asexual haploidy to syngamy and segregation where the haploid asexual populations are assumed to have non-minimal favdel load, and genomes are assumed to be subdivided into multiple linkage groups. Under these conditions introducing syngamy and segregation would allow interchromosomal recombination to take place and expose to selection any variance in fitness that might have been present in the asexual population. We will see that this can result in decreases in favdel load that occur much faster than can be expected from a mere accumulation of new favorable mutations. Note that we will not deal with the way in which the asexual haploid population might have transformed itself into a segregating outcrossing one but take the transformation as given (see Rose and Hickey 1988 for speculations on these events).

From Asexual Diploidy to Achiasmatic Sexuality: The Invasion of the Selfers. A selfing lineage arising in a diploid asexual population where each individual genome has only two homologues will sooner or later become homozygous for one of the two homologues it had to begin with (this would occur immediately in a diploid individual that begins to undergo the asexual ploidy cycle, Kondrashov 1994c). If the number of mutations carried by each of the two homologues of an individual is not the same, then the selfing lineage can fix the one homologue with the least mutations with 50% probability. When the equilibrium favdel load is above dMSB, a selfing lineage that has fixed the least-mutated chromosome might have a good chance of invading because it might carry many fewer mutations than the average diploid asexual in the population.

In order to study the above process, we need to be able to describe the variance in the number of point mutations per homologue for all homologues in the population. We will show below that given the relative sizes of the mean and variance in the number of point mutations per individual that were observed in populations at favdel equilibrium (Table 1), this variance depends mainly on the average number of mutations carried by each individual and not so much on differences in this number among individual genomes.

If one assumes that the point mutations carried by an asexual individual in a population at favdel equilibrium are distributed randomly over the L favdel-relevant sites of its genome, the distribution of the number of deleterious mutations per chromosome is hypergeometrical and can be easily described on the basis of d, the observed number of deleterious point mutations carried by the individual.

Given an individual carrying d mutations, the probability that one of its two homologues carries k mutations ($k \leq d$) is therefore given by the following formula:

$$P(k \mid d) = \frac{\binom{d}{k}\binom{L-d}{L/2-k}}{\binom{L}{L/2}} \qquad (1)$$

The expectation and variance of an hypergeometric random variable are well known. With our notation they become *d/2* and *d(L-d)/4L*, respectively. The variance of *k* not conditional on *d*, i.e. for any individual in the population not only for those carrying exactly *d* mutations, depends on the average *d* per individual and on the variance of *d* across individuals and is given by

$$Var[k \mid E[d], Var[d]] = \frac{Var[d]\ (L-1) + E[d]\ (L - E[d])}{4L}\ , \qquad (2)$$

where *E[d]* is the expectation of *d* and *Var[d]* is the variance of *d* in the population. *E[d]* and *Var[d]* can be estimated by *D* and *VarD*, the empirically observed average and variance, respectively. The formula can be easily derived with the help of the well-known relationship

$$Var[k] = Var[E[k \mid d]] + E[Var[k \mid d]]\ . \qquad (3)$$

It is furthermore easy to show that these formulae also apply when the asexual diploid genome is subdivided into more than one linkage group (not shown).

Numerical evaluations of (2) show that *Var[d]* influences *Var[k]* only when *Var[d]* is similar in value to *E[d]*. In every favdel equilibrium that we have described so far, however, *VarD* is always very small relative to *D* except for the few cases where the favdel equilibrium is close to or at dMSB (see Table 1) so that we will ignore the contribution of *Var[d]* to *Var[k]* from now on.

More importantly, the numerical evaluations showed that large differences in *k* between the homologues of an asexual diploid individual can always arise except at dMSB, the largest being expected for cases where favdel load is 50%. For instance, when $N = 10^6$, $s = 0.01$, and $L = 10^6$, $u = 3.333 \cdot 10^{-8}$ (i.e. $U_{max} = 0.1$) the asexual favdel load was a low 1%, which gives a value of *D* of about 10,000 deleterious point mutations per diploid genome and a *VarD* of about ten. *Var[k]* becomes then 2,447 and thus the standard deviation is ±50 non-optimal bases per chromosome. If we change *s* to 0.001 in the above case, the equilibrium favdel load is 37%, *D* is about 370,000 sites per diploid genome, and *VarD* is about 50, which gives a *Var[k])* of 58,287 and a standard deviation of 241.

A newly arisen selfing lineage has a 50% chance of fixing the less mutated chromosome of the two carried initially by the lineage's founder. If we assign a fitness of 1.0 to a newly arisen selfer that happens to fix a chromosome with one standard deviation fewer mutations than the average chromosome found in either of the two asexual populations above, then the asexual diploids of those populations have an average fitness relative to the selfer of $(1-0.01)^{100} = 0.37$ and of $(1-0.001)^{482} = 0.62$ when *s is* 0.01 and 0.001, respectively, if we assume multiplicative effects of mutations. Note that since *varD* was around 10 and 50, respectively, an asexual with say two standard deviations fewer mutations than the average of the asexual population would have a fitness of $0.99^{100-6.6} = 0.39$ and $0.999^{482-14} = 0.63$ relative to the selfer, respectively, i.e. would be insignificantly better off relative to the

selfer than the average asexual individual. Exactly the same considerations can be applied to mutants that begin undergoing the ploidy cycle (Kondrashov 1994c).

We believe that these large fitness advantages should easily outweigh any possible costs of selfing, since it is hard to imagine that the metabolic cost of meiosis or of the ploidy cycle is very large. Thus we conclude that in an asexual diploid population burdened by a non-minimal favdel load, mutants undergoing selfing or the ploidy cycle will ultimately invade if they arise more than a few times over evolutionary time. In fact each of them will have a 50% chance of starting with much greater fitness than any of the asexual individuals in their populations of origin.

After invading, a selfer would increase its fitness even more because both its Ratchet and favdel dynamics depend on $U/2$. The only differences between a population of selfers and one with segregation, outcrossing, and achiasmatic genomes with a single linkage group are the N_e difference (asexuals have effective size $N/2$ and sexual have size N), which however has negligible consequences (Antezana and Hudson, in press); and the fact that in the selfer mutations are either quickly lost or quickly fixed in the homozygous state and thus begin being selected according to their fitness effects in homozygotes. Kondrashov (1994c) has found that diploids undergoing the asexual ploidy cycle every generation have genetic loads of $1-e^{-U/2}$. Also the speed of the Ratchet and thus favdel loads are $U/2$-dependent under the asexual ploidy cycle. A selfer could invade also a diploid population with obligate ploidy cycle because it would avoid the waste entailed by the destruction of half of its genome each generation that obligately ploidy-cycling diploids incur. Although it is obvious that the selfer should have good chances of invading an asexual diploid population directly, the ploidy cycle is likely to have preceded the rise of such a selfer because, unlike selfing, it does not require a preexisting cytological machinery for chromosome pairing, segregation, and syngamy and, in fact, it might have provided a context in which such a machinery evolved (Kondrashov 1994c). Outcrossing may have evolved later, perhaps through gametic leakage and fortuitous syngamy driven by genetic elements (Rose 1983; Hickey and Rose 1988), resulting in the delivery of all the advantages of interchromosomal recombination that were discussed earlier.

The establishment of selfing or of the ploidy cycle should result in the lowering of the classic genetic load predicted by Kondrashov (1994c) and in a decrease in favdel load as well. It is likely that the decrease in classic genetic load will take place quickly after the introduction of the ploidy cycle, but we do not know of any results about the speed of this decrease. To assess the speed of the reduction in favdel load which may have followed the evolution of selfing or of the ploidy cycle we carried out simulations. We found that favdel load decreases in ecological time when $U_{max} \geq 1.0$, $s \geq 0.1$, and $Ns > 1$, but more slowly otherwise.

Eukaryotic sexuality, however, can hardly have arisen in a population of ratchetwise old asexual diploids if recessivity was at that time an immediate, spontaneous consequence of diploidy. In the genomes of such diploids recessive deleterious mutations would have accumulated readily and thus in such populations, selfing or ploidy-cycling would have always resulted in strongly selected-against homozygotes. With segregation and outcrossing, at most 50% of the progeny are expected to be viable (i.e. would carry the heterozygous genotype) on average, if the genome consists of a single linkage group. Indeed, due to genetic drift, all genomes in an asexual population must have a common ancestor about $2N_e$ generations back into the past, and thus the genotype carried by this ancestor will be common to all individuals of the population. Thus in favdel populations that have been at equilibrium for more than $4N_e$ generations, most of the accumulated point mutations should be identically positioned across individual genotypes, i.e. a single heterozygous genotype should describe their locus position. On the other hand, some polymorphic sites with gene frequencies different from 0.5 would explain the extant variance in favdel load across individuals. With recombination between n linkage groups, the proportion of progeny not carrying multiple recessives in homozygous state decreases

quickly with increasing n according to the formula $(1/2)^n$ that gives the expected frequency of individuals heterozygous at every linkage group. With chiasmatic recombination the situation is worse, since then the only complementary gametes are those carrying non-recombinant chromosomes or chromosomes that recombined with each other. In such a case, the probability that a zygote can be produced with two haploid genomes that complement each other is very low if the recombination rate is one or more per genome. Thus it appears to be impossible to evolve segregation and syngamy or the ploidy cycle in an asexual diploid population that has accumulated many recessive deleterious mutations.

The Transition from Asexual Haploidy to Achiasmatic Sexuality. If strong recessivity was originally a spontaneous side-effect of diploidy, eukaryotic sexuality most likely arose in a haploid asexual population. In this case, favdel events could have favored the transformation of an asexual haploid population with segmented genomes into an achiasmatic segregating outcrossing population. The segmentation of the genome of the asexual population is required for interchromosomal recombination to be possible and outcrossing is necessary for such recombination to result in a lowering of the favdel load. In fact, the favdel load expected in haploid asexuals should to be identical to that of diploid asexuals with the same U_{max}, N, and s, if the assumption of multiplicative fitness holds and only a few or no mutations are ever selected against in homozygous state. The considerations we will make below about the increase in fitness expected in haploid asexual populations after they begin undergoing syngamy and segregation, apply also to diploid asexuals, as well as to selfing and ploidy-cycling organisms, provided that they have genomes with multiple linkage groups and undergo outcrossing and segregation. Here, however, we discuss only the haploid case.

The decrease in favdel load that should take place after a population of haploid asexuals with segmented genomes begins outcrossing and undergoing segregation must be caused by the elimination of deleterious mutations and the promotion of favorable mutations. Both of these selection-driven processes depend on the variance in fitness and on the point-mutation rate, and the second process also requires that favorable mutations can increase in frequency without interference of background selection against deleterious mutations (see Barton 1995).

The variance in fitness present after the haploid population begins recombining genomes at the linkage-group level can be very high. In any non-recombining population, each entire genome is related to all others by a tree-like genealogy. The longer this genealogy extends towards the past, i.e. the further back into the past the most recent common ancestor of all genomes in the population lies, the more time is available for neutral events consisting of a favorable and a deleterious point-mutation that occur in the same generation at any two sites of the same genome, to take place in the branches of the genealogy (Figure 3). Such double mutations cause polymorphism much like neutral mutations. An ancestral genome that experiences such a "doublet" becomes different at two sites from all other members of the genealogy but retains the same load of deleterious point mutations. Deleterious and favorable events in a doublet do not need to occur in the same chromosome. On the contrary, they rarely do if the number of linkage groups n is large: for instance with n= 20, this happens on average only 5% (= 1/20) of the time. Therefore the deleterious and the favorable event of a doublet can let two chromosomes of an individual carry different numbers of deleterious and favorable mutations from the numbers carried by the corresponding homologues in other individuals. Within each genome as a whole, however, a higher number of deleterious point mutations in a chromosome is exactly counter-balanced by lower numbers of mutations in chromosomes belonging to other linkage groups. Under outcrossing and segregation, however, the linkage between the favorable and deleterious parts of doublets should decay quickly due to interchromosomal recombination (50% each generation, seven generations for a 99% decay) and the genetic variance between homologues across individuals should be expressed fully.

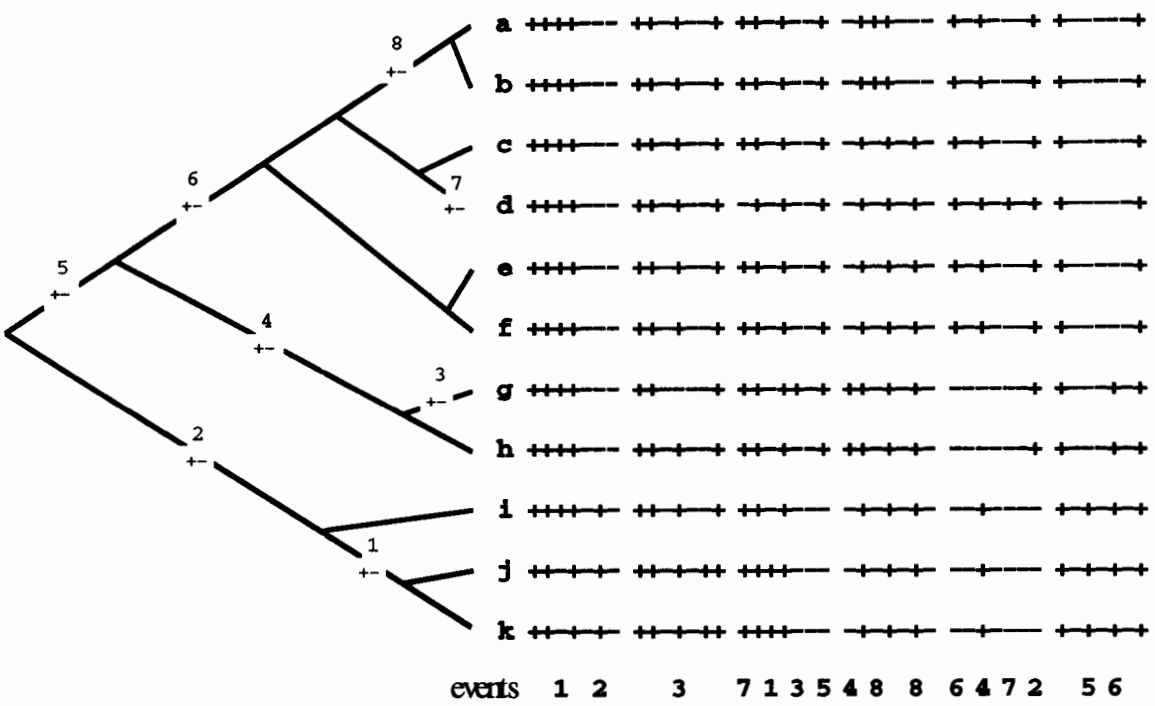

Figure 3. The origination of polymorphism hidden from selection at favdel-relevant sites. Graphic representation of the genealogical relationships among *k* haplotypes in an asexual haploid population and of the "doublet" events that produce polymorphism in the positioning of non-optimal bases within linkage groups across genomes. Doublets consist of a favorable and a deleterious mutation that take place in the same generation, are selectively neutral, can occur in any lineage of the genealogy, and create polymorphism leaving, however, unchanged the total number of deleterious mutations carried by the lineage affected. Flanking the genealogy are representations of the favdel-relevant sites in the genomes of the population; pluses indicate optimal bases and minuses indicate non-optimal bases. Numbers identify each doublet with respect to its occurrence in the tree and the sites it affected. If random syngamy and segregation were to be introduced in the population, thereby making interchromosomal recombination possible, an increase in the variance of the number of deleterious mutations per genome would take place. In the case of doublet number eight, both of its mutations occurred in the same linkage group and would not contribute to this variance (see also text).

The depth of the genealogies of haploid asexuals under favdel is not known, but their shape can be expected to be neutral since in each genealogy coalescent events occur between selectively identical lineages. We expect that these depths will be greater when deleterious mutations are less strongly selected against than with stronger selection. Ultimately, asexual genealogies should show the neutral depth (*2N* generations on average; Kingman 1982; Tajima 1983) as the value of *Ns* crosses the value one and goes toward zero. The depth of the genealogy of the zero class allows accurate predictions of neutral polymorphism in the presence of background mutations in the sexual eukaryote *D. melanogaster* (Charlesworth *et al.* 1993b) but it cannot be correct for the many favdel equilibria presented in Table 1 in which the zero class is well below one individual. We believe that only a computationally very exacting simulation can give more concrete indications about the depth of these genealogies.

Doublets can occur with appreciable frequency, for instance when U_{max}= 1 and favdel load is 60%, 10%, and 1%, respectively, doublets occur with frequency 0.08, 0.0333, and 0.00333 per genome per generation. With U_{max}= 0.1 and 0.01 the numbers are one and two orders of magnitude lower, respectively. Quadruplets can also contribute to such polymorphism, but they occur with a frequency at least one order of magnitude lower relative to that of corresponding doublets: for instance for U_{max}= 1 and the above favdel loads, quadruplets occur with frequency 0.0064, 0.00111, and 0.0000111, respectively. Short stays in a more mutated class followed by a return through a favorable mutation some generations later, or *vice versa*, can also occur in the genealogy and increase the polymorphism hidden from selection. Here we will deal with the expected variances due to single-generation doublets only.

To calculate the variance in the number of point mutations expected after interchromosomal recombination will have removed the linkage disequilibrium accumulated in the genealogy of an asexual population by the process just described, we have used the known relationship between the expected value of the square of all pairwise differences in a sample (in this case the whole finite haploid asexual population) and the empirical variance of the sample: $E[(X_i-X_j)^2]= 2var[X]$. In our case, X_i and X_j are the numbers of deleterious point mutations in two randomly sampled genomes (without replacement) and X is that in any randomly chosen genome. $E[(X_i-X_j)^2]$ is also equal to $Var[X_i-X_j]$ since $E[X_i-X_j]$ equals zero. When the time t to the common ancestor of X_i and X_j is known, X_i and X_j are independent and $Var[X_i-X_j]= Var[X_i \mid t] + Var[X_j \mid t]$ which, given that $Var[X_I \mid t]= Var[X_j \mid t]= tU_iF_i$, becomes $2tU_iF_i$, where U_i and F_i are the deleterious and the favorable mutation rates over a genome carrying i mutations (we ignore the asexual population's variance in U_i and F_i and use the average i for the whole population to calculate U_i and F_i). Using the above and the relationship shown in (3) with appropriate notation, one can show that $var[X]= U_iF_iE[t]$, i.e. NU_iF_i, where N is the neutral coalescence time for two genomes in an asexual population of size N (Kingman 1982, Tajima 1983). Since each doublet creates polymorphism through both a deleterious and a favorable mutation, the expected empirical $var[X]$ after linkage will have been erased, should be twice as large, i.e. $2NU_iF_i$.

In Table 2, we present the expected variances in the number of deleterious mutations per genome (assuming that linkage has already decayed to the minimum possible) for several values of the neutrally effective population size[2] N_n. We assumed that genomes have 30 linkage groups and decreased expected variances by 3% (1/30) because doublets that affect a single chromosome cannot be unlinked by interchromosomal recombination. We flanked these values with the actual variances observed in the asexual haploid population at favdel equilibrium in order to give the reader an idea of the size of the variances to be released by interchromosomal recombination.

Table 2 shows that the highest variances are expected to arise when U_{max} values are largest. When U_{max}= 0.01 variances are insignificantly higher than those shown by the asexual population at favdel equilibrium. They are intermediate when U_{max}= 0.1, and are very large when U_{max}= 1. The expected variances are larger in larger populations although sometimes the tendency of F_i to go to zero as N increases prevails. The table shows the effect of changing the depth of the assumed genealogy in that it tabulates the variance for every order of magnitude of N_n lower than N down to ten individuals. Obviously, expected variances decrease linearly with decreasing N_n.

In Table 3, we present the time $T_{50\%}$ for a haploid population with segmented genomes at the asexual favdel equilibrium to increase its fitness to the point that the fitness of the initial asexual population is 50% lower, after beginning to outcross and undergo segregation. Each value is from a

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[2] N_n is a parameter coined by us which we will use throughout the rest of the paper as the unit to measure the depth of the haploid asexual genealogy; it is equal to N_e when all mutations are neutral and smaller when mutations are selected.

Table 2. The hidden genetic variance that can arise in asexual genealogies. Part A U_{max}= 1.0.

U_{max}= 1.0 s= 0.1

	asexual equilibrium		logN$_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.120	5.0	73.1	11.8	5.7				
4	0.050	9.0	315.1	39.6	12.1	9.3			
5	0.014	9.6	899.2	98.6	18.5	10.5	9.7		
6	0.001	9.6	653.4	74.0	16.0	10.2	9.7	9.6	

U_{max}= 1.0 s= 0.01

	asexual equilibrium		logN$_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.500	25.0	186.1	41.1	26.6				
4	0.430	40.0	1619.5	198.0	55.8	41.6			
5	0.375	56.0	1.5E+4	1566.4	207.0	71.1	57.5		
6	0.330	52.0	1.4E+5	1.4E+4	1476.9	194.5	66.2	53.4	
7	0.290	54.0	1.3E+6	1.3E+5	1.3E+4	1380.9	186.7	67.3	55.3

U_{max}= 1.0 s= 0.001

	asexual equilibrium		logN$_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.665	100.0	243.6	114.4	101.4				
4	0.625	143.0	1653.4	294.0	158.1	144.5			
5	0.600	200.0	1.6E+4	1746.7	354.7	215.5	201.5		
6	0.580	210.0	1.6E+5	1.6E+4	1779.9	367.0	225.7	211.6	
7	0.565	250.0	1.6E+6	1.6E+5	1.6E+4	1833.9	408.4	265.8	251.6

The proportion of the L sites involved in favdel dynamics that is occupied by non-optimal bases (i/L) is denoted "**propdel.**" N_n is the neutrally effective population size of asexual populations of effective size N (see text). Genomes were assumed to have 30 linkage groups and the hidden variance was calculated as described in the text. For all orders of magnitude of N_n down to ten, we tabulate the sum of each maximum variance plus the observed variance at the asexual favdel equilibrium (**vardels**).

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simulation of an N-sized population of single chromosomes each carrying exactly the average favdel load. Genomes were again assumed to be subdivided into 30 linkage groups. The population was generated as follows: a population of N_n chromosomes was created with 1/30 of the average equilibrium asexual favdel load and 1/30 of the theoretically calculated, releasable doublet variance (= 29/30 of the total doublet variance) plus 1/30 of the actual variance observed in the asexual population across individuals. The deleterious point mutations were assigned to each chromosome in a

Table 2. The hidden genetic variance that can arise in asexual genealogies. Part B U_{max}= 0.1 and 0.01.

U_{max}= 0.1 s= 0.01

	asexual equilibrium		$logN_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.245	7.4	8.6	7.5	7.4				
4	0.115	8.3	14.9	9.0	8.4	8.3			
5	0.045	8.4	36.1	11.2	8.7	8.4	8.4		
6	0.014	9.9	95.8	18.5	10.8	10.0	9.9	9.9	
7	0.001	9.9	55.0	14.4	10.4	10.0	9.9	9.9	9.9

U_{max}= 0.1 s= 0.001

	asexual equilibrium		$logN_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.590	26.0	27.6	26.2	26.0				
4	0.495	38.0	54.1	39.6	38.2	38.0			
5	0.430	43.0	201.0	58.8	44.6	43.2	43.0		
6	0.373	49.0	1555.3	199.6	64.1	50.5	49.2	49.0	
7	0.325	55.0	1.4E+4	1468.8	196.4	69.1	56.4	55.1	55.0

U_{max}= 0.01 s= 0.01

	asexual equilibrium		$logN_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.035	0.93	0.9	0.9	0.9				

U_{max}= 0.01 s= 0.001

	asexual equilibrium		$logN_n$= logN -number given						
logN	propdel	vardels	0	-1	-2	-3	-4	-5	-6
3	0.475	4.3	4.3	4.3	4.3				
4	0.235	6.8	6.9	6.8	6.8	6.8			
5	0.115	8.2	8.9	8.3	8.2	8.2	8.2		
6	0.045	9.5	12.3	9.8	9.5	9.5	9.5	9.5	
7	0.01	9.9	16.2	10.5	9.9	9.9	9.9	9.9	9.9

way that the number per individual chromosome was normally distributed with average equal to the above fraction of the whole-genome favdel load and with variance equal to the just mentioned sum of fractional doublet and population variances. The total N for the simulation was reached by expanding multinomially to N the generated N_n-sized population. The times $T_{50\%}$ are relative to the initial average fitness produced but do not include the generations required for interchromosomal recombination to release the doublet variance (seven generations for 99% linkage equilibrium, but remember that

variation can be used by selection already in the first generation where it would already be 50% of the total possible). We list $T_{50\%}$ values for all possible orders of magnitude of N_n down to ten individuals.

Table 3 shows that $T_{50\%}$ values are extremely short when U_{max} is large and N_n is large, but are much longer when N_n or U_{max} become smaller. It is noteworthy that when s is 0.001, the times can be still quite short as long as $U_{max} \geq 0.1$. The size of N_n plays a very important role in determining the speed of fitness increase in the transition from asexual haploidy to syngamy and segregation under favdel (we plan to run simulations to estimate N_n). Our results show that depending on U_{max} and N_n, a transition from asexual haploidy to achiasmatic sexuality of any ploidy might lead to large and very fast fitness increases.

The evolution of segmented asexual genomes. The above described transition from asexual haploidy to achiasmatic sexuality depends on the segmentation of haploid asexual genomes. The evolution of segmented haploid genomes might have been a clear-cut process if recessivity was an spontaneous epiphenomenon of diploidy. Asexual diploids that arise from fusions of haploids (Figure 4) have a temporary fitness advantage since such asexual diploidy can cover "recessive loads" (Maynard Smith 1978). Such covering, however, will continuously decrease until every locus will be heterozygous for some recessive mutation. Functionally haploidized loci (see also Lokki 1976) would then progressively lose their mutated recessive alleles at the nucleic-acid level, perhaps even through deletions of whole chromosomal tracts containing contiguous recessives, until two functional chromosomes with non-homologous wild type genes would be left. In this manner, the diploid asexuals will become haploid again but will have a (more) segmented genome (note that the pairing of homologous chromosomes is not necessary for mitosis). After undergoing c such cycles of asexual diploidy, accumulation of recessives, and nucleic-acid haploidization, genomes should be subdivided into 2^c similarly large linkage groups.

THE REALISM OF THE "FAVDEL" MODEL

The biological plausibility of the conclusions drawn above about favdel equilibria and dynamics and of the favdel-related phenomena favoring the evolution of selfing/ploidy-cycling and/or segregation and outcrossing depends on two kinds of biological considerations. At the proximate population-genetical level, on the one hand, the question is the biological plausibility of the rates of point mutations per site, numbers of sites surveyed by selection, and values of s and N that are all required for the necessary favdel equilibria to arise and be maintained. It is also an open question what favdel dynamics and equilibria would be like when a mixture of mutations with disparate selective effects is present, in particular how insertion-deletions might affect favdel equilibria and dynamics. At the organismic level, on the other hand, the plausibility depends crucially on whether organisms carrying deleterious point mutations in numbers as large as those expected in non-dMSB favdel equilibria can be nevertheless biochemically, mechanically, and organismically functional.

Biological realism of the required parameter values. All the phenomena we have discussed above assumed point-mutation rates u between 10^{-7} to 10^{-9} per site per generation and a number L of favdel-relevant nucleotide sites equal to 10^6 sites. As we mentioned in the text, favdel loads and especially their consequences for the transition to sexuality from asexual haploidy depend heavily on the value of U_{max}, the genomic deleterious point-mutation rate. In fact, fitness increases in ecological time were found when $U_{\max} \geq 0.1$, i.e. when u and/or L are large (recall that $U_{max} = L3u$). Are these values of L and u biologically realistic? We will deal with this question below but separately for the transition from asexual diploidy to selfing and from asexual haploidy to syngamy and outcrossing.

Table 3. The time for fitness to increase twofold after syngamy and segregation are introduced into an asexual haploid population. Part A: U_{max}= 1.0. The average and the variance of the number of generations taken by haploid genomes at the asexual favdel equilibrium and subdivided into 30 linkage groups (see text) to increase their fitness twofold after they begin undergoing random syngamy and segregation.

U_{max}= 1.0 s= 0.1

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5	-6
3	0.120	5.0	2.2	0.2	23.6	35	39.6	100						
4	0.050	9.0	1.0	0.0	5.0	0.4	24.9	23	39.7	160				
5	0.014	9.6	1.0	0.0	2.0	0.0	15.0	2.7	30.0	36	47.6	314		
6	0.001	9.6	1.0	0.0	2.0	0.0	17.7	0.5	29.3	10	33.8	96	64.2 960	

U_{max}= 1.0 s= 0.01

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5	-6
3	0.500	25	64.5	865	209.8	4E+3	249.7	4E+3						
4	0.430	40	5.3	0.2	53.1	60	170.2	913	214.9	1E+3				
5	0.375	56	1.0	0.0	5.6	0.2	51.5	24	155.6	779	204.1	1E+3		
6	0.330	52	1.0	0.0	1.0	0.0	6.0	0.0	56.0	30	174.0	662	220.1 918	
7	0.290	54	1.0	0.0	1.0	0.0	1.0	0.0	6.0	0.0	61.4	45	182.7 818	230.5 913

U_{max}= 1.0 s= 0.001

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5	-6
4	0.625	143	1125	2E+5	2695	2E+5	2922	2E+5	3125	2E+5				
5	0.600	200	46.3	2.1	614.3	3E+4	2046	7E+4	2343	6E+4	2475	7E+4		
6	0.580	210	5.0	0.0	45.8	0.3	528.0	1E+4	835.3	2E+3	2079	3E+4	2238 4E+4	
7	0.565	250	1.0	0.0	5.0	0.0	45.4	0.2	536.8	1E+4	1734	9E+3	1879 4E+4	2035 2E+4

"**propdel**" and "**vardels**" are the average proportion of favdel sites occupied by deleterious bases selected with strength s and the variance in the number of such bases at the asexual favdel equilibrium, respectively. N_n is the "neutrally effective population size" (see text). Results are shown for N_n values down to ten individuals. The times do not include the few generations required to reach linkage equilibrium (ca. six generations for 99% equilibrium, see text) and are averages from 1000, 500, and 100 replicates for U_{max} values of 1.0, 0.1, and 0.01, respectively, except for N=10^7 when U_{max} was 0.1 and s was 0.001 (ten replicates).

With respect to the transition from asexual diploidy to selfing/ploidy-cycling, favdel gives noteworthy results with any U_{max} high enough to lead to non-minimal loads. In this transition, even a U_{max} of 0.01 suffices for a great many cases favorable for the invasion of selfers or ploidy-cycling forms. Such an U_{max} value that L and/or u be small. For instance, if we use a value of u close to that of unicellular eukaryotes (10^{-9}, Drake 1991) one can reach a U_{max} of 0.01 with L= 3.3 10^6 sites. To have such a number of sites being surveyed by selection of strength s= 0.001, is like having 33 third positions in each of 10^5 genes (or 330 in 10^4 genes) under such selection. This is not unrealistic.

Table 3. The time for fitness to increase twofold after the transition from asexual haploidy to random syngamy and segregation. Part B: U_{max}= 0.1 and 0.01. See Part A for explanations.

U_{max}= 0.1 s= 0.01

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5		-6	
3	0.245	7	602	2E+5	656	2E+5	835	2E+5								
4	0.115	8	168	2E+3	201	4E+3	285	2E+4	573	6E+4						
5	0.045	8	117	67	174	337	201	2E+3	330	3E+4	638	5E+4				
6	0.014	10	65	0.9	147	28	171	174	194	1E+3	364	4E+4	754	6E+4		
7	0.001	10	166	4.0	586	5E+3	1010	2E+5	1100	2E+5	1150	2E+5	1250	2E+5	1200	2E+5

U_{max}= 0.1 s= 0.001

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5		-6	
4	0.495	38	1E+4	4E+6	1E+4	4E+6	1E+4	4E+6	1E+4	4E+6						
5	0.430	43	8164	8E+5	9869	8E+5	1E+4	1E+6	9157	4E+6	10020	4E+6				
6	0.373	49	540	4E+3	6398	1E+6	8390	5E+5	8688	5E+5	8688	3E+5	8690	3E+5		
7	0.325	55	51	0.0	525	2E+3	6080	2E+5	7670	9E+5	1E+4	1E+6	1E+4	1E+6	1E+4	1E+6

U_{max}= 0.01 s= 0.01

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5		-6	
3	0.035	1	4E+4	5E+8	4E+4	3E+8	4E+4	4E+8								

U_{max}= 0.01 s= 0.001

	asexual equilibrium		$logN_n$= logN -number given											
logN	propdel	vardels	0		-1		-2		-3		-4		-5		-6	
4	0.235	7	1E+5	8E+7	1E+5	2E+8	1E+5	3E+8	1E+5	2E+8						
5	0.115	8	7E+4	7E+7	6E+4	3E+7	6E+4	4E+7	7E+4	3E+7	7E+4	4E+7				
6	0.045	10	6E+4	2E+7	6E+4	2E+7	6E+4	2E+7	6E+4	2E+7	6E+4	4E+7	6E+4	3E+7		
7	0.01	10	7E+4	6E+7	7E+4	4E+7	8E+4	2E+7	8E+4	3E+7	7E+4	5E+7	8E+4	3E+7	8E+4	4E+7

In the transition to achiasmatic sexuality from asexual haploidy, favdel-related events result in drastic fitness gains in ecological time when $U_{max} \geq 0.1$ and N_n is large. One cannot exclude that the relevant u values at the time of the origin of eukaryotic sex were higher than 10^{-9}, as could be expected from asexual organisms living under trophic stress, under pressure to reproduce faster, whose genomes must go through many mitotic divisions before producing propagules, or which must wait for long intervals between DNA replications. The first two possibilities are likely to be applicable to opportunistic life forms, as the ancestor of eukaryotic sexual forms possibly was. If u was between $3.333\ 10^{-7}$ and $3.333\ 10^{-8}$ and L about 10^6, i.e. if $U_{max} \geq 0.1$, then the transition from haploidy might

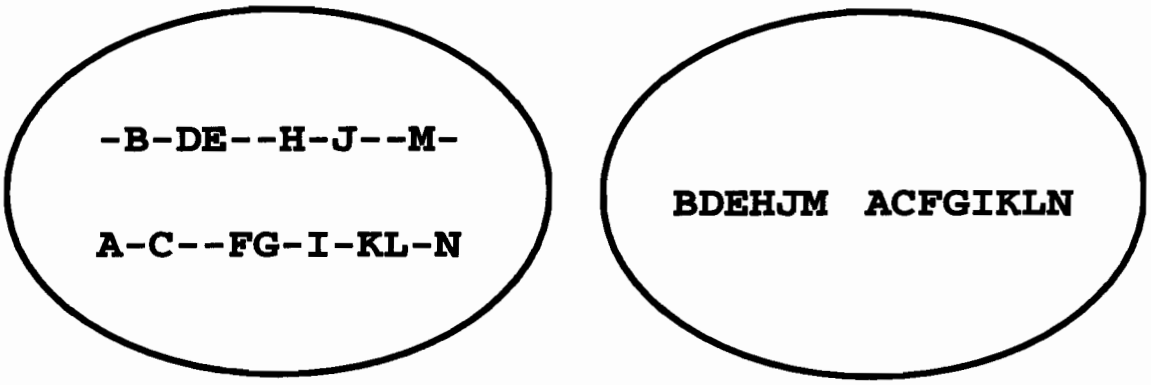

Figure 4. The evolution of genome segmentation through accumulation and removal of recessives. In the asexual diploid with two homologous chromosomes shown on the left, recessive alleles (minuses) have accumulated neutrally at loci initially homozygous for wild-type alleles (capital letters). Recessive alleles can then be eliminated at the nucleic-acid level, resulting in genetical and nucleic-acid haploidy and in a genome segmented into two non-homologous chromosomes, as shown on the right. After c cycles of diploidization and haploidization one expects to have a genome subdivided into 2^c more or less equally large segments.

have been strongly favored by immediate or at least very quick fitness gains due to favdel events. Furthermore, with such U_{max} values, evolving syngamy and segregation would have resulted in a much quicker reaching of the lower or minimal equilibrium favdel loads expected under achiasmatic sexuality in both the transition from asexual haploidy and that from asexual diploidy

Effect of background insertions-deletions. Insertion-deletions (indels) are the major part of the total deleterious mutation rate of eukaryotic and prokaryotic organisms (Drake 1991). In *Drosophila melanogaster*, for instance, the total genomic deleterious mutation rate has been estimated to be about 1.0 (Mukai *et al.* 1972; Crow and Simmons 1983; Houle *et al.* 1992; Keightley, 1994). If we assume that the *Drosophila* genome has 10^5 genes, each thousand nucleotides long, and that u is 10^{-9}, we reach a U_{max} of about 0.1 point mutations per generation as a likely generous estimate of the part of the genomic deleterious mutation rate in *Drosophila* due to point mutations. This value is only one tenth of the total deleterious rate, a proportion that is fully in accordance with direct estimates of the indel part of the deleterious mutation rate (Drake 1991). Selection against deleterious indel mutations decreases N_e (Hill and Robertson 1966; Barton 1995) which can increase favdel loads and make more likely that favdel equilibrium loads are above deterministic dMSB. Such selective events would thus make asexual diploid populations more likely to be invaded by selfing/ploidy cycling lineages as long as recessive indels are not present in large numbers in most asexual diploid individual. On the other hand, selection against indels might decrease N_n and thus make the fitness increases that we described for the transition from asexual haploidy to syngamy/outcrossing less drastic. Indel-related background selection can hinder the accumulation of new favorable mutations (Barton 1995) and thus can have slowed down the decrease in favdel load that possibly followed the origin of achiasmatic eukaryotic sex. Only full fledged simulations of whole populations of genomes with appropriate mutational tendencies can give an answer about the importance of these effects.

The biological realism of non-dMSB favdel equilibria. Under the assumption of multiplicative fitness effects, the differences in the number of non-optimal bases in asexual populations at non-dMSB favdel equilibrium from the number in otherwise identical segregating populations with genomes consisting of a single linkage group, translate into fitnesses that often differ by many orders of magnitude. In all cases shown in Figure 2, except when $Ns < 10$, moderate numbers of linkage groups

allow the segregating diploids to attain an equilibrium number of mutations equal to U_{max}/s (i.e. $L3u/s$; data not shown; see Antezana and Hudson, in press), which is the lowest possible without changing u, L, or the assumption of multiplicative fitness effects. This makes the potential fitness differences between asexuals and achiasmatic sexuals even more astonishing.

It might be difficult at first to envision organisms differing so immensely in fitness although in the laboratory, bottle-necked RNA viruses regularly reach fitnesses that are orders of magnitude lower than those of other viruses, but without losing their ability to infect and reproduce (A. Moya, pers. comm.). The relative and absolute numbers of sites occupied by non-optimal bases in some of the favdel equilibria shown in Figure 2 might also seem unrealistically high. We will argue below that neither the absurdly low absolute fitnesses predicted under the assumption of multiplicativity for some of the favdel equilibria described above, nor the numbers of sites expected to be occupied by non-optimal bases at such equilibria, are compelling evidence to argue that such equilibria are biologically implausible.

First of all it can be restated here that our model does not require that the whole genome be completely comprised in the L simulated sites in order for the favdel load to rise beyond dMSB levels. This implies that the genome-wide relative point-mutation load can very well be much lower if many additional nucleotide sites do not participate in the favdel dynamics and are kept optimized by much stronger selection. This is especially applicable when L is not already close to a maximum conceivable genome size (10^5 genes each 10^3 bases long would give an upper limit of 2 10^8 sites for L). For instance, when L= 10^6, U_{max}= 1.0, s= 0.001 and N= 10^5, on average 59% of the L sites should have non-optimal occupancy according to our simulations. Genome-wide, however, the percentage could very well be 5.9%, if we assume that there are 10^7 informationally important sites and that most of them are kept optimized by very strong selection. Such a number of sites under selection translates into a total deleterious mutation rate of about 10. Kondrashov (1988) has shown that such mutation rates can be withstood if selection is synergistic. It is thus unwarranted to argue that having 6% of all the sites surveyed by selection occupied with non-optimal bases must translate into inviability let alone into organismic non-functionality. In terms of organismic functioning, it is clear that genes with 6% non-optimal composition (e.g. with 60 third positions with non-optimal occupancy over 1000 coding nucleotides) are very likely to produce fully functional gene products that are perfectly able to participate in the development and maintenance of fully vigorous and fecund organisms.

According to the multiplicative fitness scheme used in our simulations, a favdel load like the one just mentioned translates into an infinitesimal absolute fitness. However, although this fitness schema makes favdel simulations more tractable, it is not essential to produce the dynamics shown by the favdel model under this assumption. Moreover, a fitness schema where fitness is relative to that of the average individual is biologically plausible. It suffices to think about situations in which one is selected for by virtue of being less maladapted than the average, not because one carries 10,000 point mutations more than a chimerical optimal genotype and one's competitors carry 10,002. Thus, scaling with respect to the chimerical optimum genotype is not only unnecessary but also can be biologically nonsensical. For instance, to lay one egg in a year can be considered a high fecundity when everybody else lays one every other year but it is dismal when competitors lay thousands of eggs per year. The extreme phenotypes mentioned in the previous pages are very unlikely to have ever coexisted in a population; and beyond saying that one is vastly superior to the other, attempts to further quantify their fitnesses relative to each other are useless exercises. Intermediate phenotypes, however, are not only possible but may even become comparable micro-evolutionarily. Such phenotypes could have connected lineages displaying the extreme phenotypes just mentioned.

The variances in fitness observed in populations at favdel equilibrium (Table 1) might also be relevant for judging the biological realism of the different favdel-equilibrium situations. The variances

observed in favdel runs were never larger than those expected under dMSB. The largest equilibrium variances in mutations per individual that we observed for s= 0.1, 0.01, and 0.001 were 10, 54, and 250 deleterious point mutations squared, respectively. Thus in these cases, individuals carrying three standard deviations more point mutations than the average had fitnesses of approximately 0.35, 0.80, and 0.95, respectively, relative to those of average individuals. These values are by no means unrealistic in that they do not require the best-adapted organisms in a population to have absurdly higher fitnesses relative to other members of the population.

Another issue concerning biological realism is the continuous production of deleterious and favorable mutations in each generation under favdel. In many equilibrium situations, such mutations should occur in relatively high numbers and could often result in different phenotypes. In the most extreme non-nearly-neutral case shown in Figure 2, U_{max} is 1.0 point mutations per genome per generation, N is 10^4 individuals, s is 0.001, and the equilibrium favdel load is about 64%. In this case $F_{64\%}$ is approximately 0.2 per genome (0.64/3) per generation. This might seem unrealistically high at first, especially if one believes that each carrier of a new favorable mutation must have an advantage of s over anybody else in the population. Such is likely to happen only rarely, however, because the mutation is likely to arise in a genome with average load, so that its carrier will simply become a member of the next less mutated class. Unfortunately, the frequency and phenotypic diversity of favorable mutations predicted when favdel loads are dMSB might be empirically not easily assessable because the favorable mutations should occur in many different genes in a background of frequent deleterious mutations.

Empirical work to test some of favdel assumptions. There are some experiments that could shed light on some of the favdel assumptions. One could measure for instance the cumulative fitness effects of various numbers of non-optimal degenerate codons by forcing an organism, say E. coli, to rely on artificially synthesized genes that carry different numbers of such codons. A first series of experiments would be to measure fitness relative to the wild-type sequence or to a gene with only optimal codons, only non-optimal codons, or half, or one quarter, etc. And then one should do this for two different genes at the same time to see how deleterious effects of a similar number of mutations accumulate across genes. Similarly one could produce recombinants between individuals of a long-lived asexual haploid species and test whether they have a higher fitness, but we cannot think of an actual biological system that would allow such an experiment.

CONCLUSIONS

We showed above that finite asexual diploid populations can accumulate deleterious point mutations in much larger numbers than comparable sexual populations. When such mutations are non-recessive, selfing or ploidy-cycling mutants are expected to have a high probability of invading such asexual populations. We also showed that a transition from asexual haploidy to achiasmatic sexuality can result in very quick or even immediate fitness advantages when asexual haploid populations with non-minimal numbers of deleterious point mutations become sexual by beginning to undergo random syngamy and achiasmatic segregation (Rose 1983, Hickey and Rose 1988).

Mutational deterministic processes (Crow 1970; Kondrashov 1982; Crow 1983) together with favdel-related events might have favored the evolution of achiasmatic sexuality if the mutational spectrum of the ancestors of the first eukaryotic sexual forms was composed of a mixture of synergistically and multiplicatively deleterious indels and point mutations. In fact, the mutational deterministic scenario is not necessarily an alternative to favdel, as it is possibly based on indel deleterious mutational pressures which are much more intense than those due to point mutations (Drake

1991). This scenario does not require the action of chiasmatic recombination either, since Kondrashov (1984) showed that interchromosomal recombination suffices to deliver most of the advantages of free recombination in the mutational deterministic scenario.

Thorough assessments of the deleterious mutational spectrum in early diverging eukaryotes, under fast growth, under stress, and with respect to recessivity, have become paramount for the reconstruction of the forces that favored the origin and rise of eukaryotic sexuality. Similarly important will be the study of the genetic basis of mitosis and meiosis, in order to evaluate the conjecture that the independent assortment of homologues at meiosis was an immediate side effect of the process of segregation and that therefore interchromosomal recombination might have begun taking place as soon as segregation and outcrossing were evolved.

More importantly, the above results are congruent with those of Kondrashov (1984), who showed that molecular recombination is not necessary to deliver the advantages of sexuality under the mutational deterministic hypothesis, and with results of ours (Antezana and Hudson, in press) that show that segregation and outcrossing, through their side of effect interchromosomal recombination, can deliver the advantages of crossing over with respect to Muller's Ratchet (Muller 1932), Fisher-Muller exclusions between concomitant favorable mutations, and the part of the Fisher effect (Fisher 1930) due to background selection against deleterious mutations. Taken together, these results strongly indicate that chiasmatic recombination was not required for the origin of syngamy and segregation and their initial success. This possibility is further supported by the existence of protozoans that undergo one-step meiosis and appear to be unable to undergo chiasmatic recombination (Raikow 1982).

Acknowledgments: We thank Steven Frank for computer time and comments on the manuscript, James Crow for advice and comments on the manuscript, Nick Barton for encouragement, Alex Kondrashov for comments and advice, Leigh Van Valen for comments and proof-reading, W.M. Fitch, Britta Serog, and Helene Van for computer time and/or programming help, Harry Mangalam and G. Gutman for computer time, Kostas Sophocleus and Domingos Begalli for computing help, and Gary Annij Gillis, Alistair B. Cullum, Paul N. Turner, Carlos Machado, and Corinne Antezana-Pernet for proof-reading and commenting on the manuscript. Marcos A. Antezana's work was made possible by a postdoctoral fellowship of the Swiss National Fonds. The first author is very grateful to Marty Kreitman for his support at Chicago and to R. L. Lenski for writing a crucial recommendation letter in 1994.

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