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The effect of linkage and population size on inbreeding depression due to mutational load

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Summary

Using a stochastic model of a finite population in which there is mutation to partially recessive detrimental alleles at many loci, we study the effects of population size and linkage between the loci on the population mean fitness and inbreeding depression values. Although linkage between the selected loci decreases the amount of inbreeding depression, neither population size nor recombination rate have strong effects on these quantities, unless extremely small values are assumed. We also investigate how partial linkage between the loci that determine fitness affects the invasion of populations by alleles at a modifier locus that controls the selfing rate. In most of the cases studied, the direction of selection on modifiers was consistent with that found in our previous deterministic calculations. However, there was some evidence that linkage between the modifier locus and the selected loci makes outcrossing less likely to evolve; more losses of alleles promoting outcrossing occurred in runs with linkage than in runs with free recombination. We also studied the fate of neutral alleles introduced into populations carrying detrimental mutations. The times to loss of neutral alleles introduced at low frequency were shorter than those predicted for alleles in the absence of selected loci, taking into account the reduction of the effective population size due to inbreeding. Previous studies have been confined to outbreeding populations, and to alleles at frequencies close to one-half, and have found an effect in the opposite direction. It therefore appears that associations between neutral and selected loci may produce effects that differ according to the initial frequencies of the neutral alleles.

1. Introduction

It is important for an understanding of the genetic and evolutionary properties of inbreeding depression to study systems of many loci subject to mutation. Deleterious mutation pressure appears to be the major cause of inbreeding depression, the decreased fitness of the progeny produced by inbreeding, in natural populations (recently reviewed by Charlesworth & Charlesworth, 1987). For the purposes of this paper, the magnitude of the inbreeding depression is defined as the reduction in fitness of the progeny of self-fertilisation, relative to the fitness of outcrossed progeny. While valuable analytical results can be obtained with simpler genetical models of fitness determination, such as single loci affecting fitness (Uyenoyama & Waller, 1991), realistic models must include many fitness-determining loci. Recent computer modelling studies of inbreeding depression have shown that mutation to partially recessive alleles with deleterious effects can produce inbreeding depression of a magnitude comparable with that estimated to occur in natural populations (Charlesworth, Morgan & Charlesworth, 1990, 1991).

The predictive value of inbreeding depression for breeding system evolution has been questioned (Holsinger, 1988). High selfing can sometimes evolve even when inbreeding depression due to mutational load is high (Holsinger, 1988; Charlesworth *et al.* 1990, 1991). When inbreeding depression is due to loci with heterozygote advantage, intermediate selfing rates can be evolutionarily stable (Holsinger, 1988; Charlesworth & Charlesworth, 1990). It has also been pointed out that pollen discounting, the loss of pollen available for outcrossing, reduces the advantage of selfing (Nagylaki, 1976), so that inbreeding depression levels alone do not allow one to predict the evolution of the breeding system of a population. However, inbreeding depression and pollen discounting parameters can be combined to yield such predictions (Charlesworth, 1980). The fact that discounting favours the evolution of outbreeding when levels of inbreeding depression

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are low does not, therefore, imply that inbreeding depression is unimportant.

In our previous work, we assumed the loci to be unlinked. This is biologically unrealistic, but allowed us to use the methods developed by Kondrashov (1985) for deterministic calculations of the frequencies in populations of genotypes with different numbers of mutations, and of alleles at modifier loci affecting the breeding system. Thus, not only could we study the inbreeding depression generated in some detail, without the difficulties of stochastic noise that might obscure slight differences, but we could also follow deterministic changes in the frequencies of modifier alleles. We showed that the inbreeding depression generated in these models could produce selection for outbreeding, with the necessary condition that the inbreeding depression be greater than one half, assuming no loss in male fertility to genotypes with higher selfing rates. The purpose of the present paper is to extend our previous work on genetic load and inbreeding depression due to multiple mutable loci, to the case of partial linkage.

Intuitively, one might expect that linkage would tend to reduce the magnitude of inbreeding depression, compared with the case when all the fitness determining loci are unlinked. If all loci are completely linked, i.e. in a genome with no recombination, it is evident that the inbreeding depression cannot exceed one-half. The situation with the greatest inbreeding depression would be that in which identity by descent for any particular chromosome is lethal, so that half of the progeny of selfing would die. But, assuming no identity between different gametes that unite to form outcrossed progeny, the outcrossed progeny would have a viability of one. Thus in this case the inbreeding depression would be one-half. This has been confirmed by computer calculations (Charlesworth, 1991).

The assumption of unlinked loci is also important in relation to the spread or loss of alleles at modifier loci affecting the breeding system. With linkage, associations between loci may be stronger than for unlinked loci. In partially inbreeding populations associations between loci with respect to homozygosity (identity disequilibrium) that are absent in outcrossing populations will also exist (Weir & Cockerham, 1973). Thus one might expect selection on modifier alleles to be affected by linkage with fitness determining loci, and this might affect either the rate or the direction of change of the modifier allele frequency. One of the main aims of the present paper is therefore to investigate the effects of partial linkage between the mutable loci that determine fitness, and also to study the effect of linkage between these loci and a modifier locus that controls the selfing rate.

Holsinger (1988) studied the evolution of the selfing rate in finite populations subject to recessive lethal mutations, using stochastic simulations. To understand the effects of linkage and of finite population size more fully, this type of study needs to be extended in the light of the insights into the behaviour of modifiers of the selfing rate that have been obtained from infinite population models (Charlesworth *et al.* Charlesworth, 1990). In the work to be described here, we investigate the effects of linkage and finite population size by using stochastic simulation to study partially inbreeding populations subject to mutation at many loci. Since the study of the effect of linkage involved stochastic simulations, we were also able to study the effects of finite population size on genetic loads.

2. Methods

(i) *Construction of the model*

The populations were simulated using the method of Fraser & Burnell (1960) in which loci can have two alleles and multi-locus genotypes are stored as computer words, i.e. as bit patterns of zeros and ones. With 32 bits per word, the genotype of an individual gamete at 32 loci is recorded using a single computer word. More loci per individual requires more than one word per gamete. A diploid individual's genotype is therefore emulated as two sets of words, each set representing a gamete. Mutation can be modelled by changing the state of individual bits from 0 to 1, or vice versa, and recombination by suitable logical operations using masks to combine parts of one gamete with the complementary parts of another (Fraser & Burnell, 1960).

The sequence of operations in each generation was the same as used by Kondrashov (1985) and in our previous modelling work on inbreeding depression (Charlesworth *et al.* 1990, 1991). Starting with a set of genotypes after selection, mutation was first assumed to occur. We assumed a Poisson distribution of numbers of mutation events, with mutations occurring at loci chosen at random, and the mutation rate for the whole diploid genome was denoted by U. In most of the work to be described, this value was held constant while the values of other parameters was varied. This meant that when the number of loci was changed, the mutation rate per locus was also changed.

After the mutations had been performed for all adult genotypes in the population, the mating process happened. In our work, we wished to include partial selfing. Therefore the method used to generate one new zygote was as follows. First a parent individual (pair of gametes) was chosen at random, and a gamete was generated as its contribution to the progeny individual. The process of generating each gamete involved using a random number to determine how many crossovers would be used, from a distribution of numbers of crossover events, and then performing that number of crossovers at locations randomly chosen among the between locus intervals in the genome. We assumed a Poisson distribution of numbers of recombination events, with a recombination fraction of r between adjacent loci, and

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without interference between the different events at different locations. Next, a random number was drawn to determine whether selfing or outcrossing was to occur, using a previously specified frequency of selfing (S) as the criterion. If selfing occurred, a second gamete was drawn at random from the same parental individual, but if outcrossing occurred a gamete was drawn at random from a different randomly chosen individual in the population. When two gametes had been generated in this way, the numbers of heterozygous and homozygous mutations in the resulting zygote were computed and its fitness was calculated, assuming the same selection regime at all loci. The selection parameters, and the models used, will be described below. Once the zygote fitness was calculated, a random number between zero and one was drawn, to determine whether or not the zygote would survive. If the fitness of the zygote was lower than the random number, the zygote did not survive. The process of generating a zygote was repeated independently until the number of surviving zygotes equalled the pre-determined population size, which will be referred to as N.

The events described here constitute a single generation. In the runs whose results are shown below, the population was initially run for 200 generations to allow mutations to build up and reach a steady state. After this initial stage, the state of the population was recorded every hundred generations for a further 2000 generations, and the results at each of these times were averaged to provide a value for such variables as the mean fitness and inbreeding depression for the set of parameters used for that run. In other words, for a given set of parameters, we did not do a set of replicate runs, but instead obtained a time average using successive time periods in a single run. The variables studied in this way included the means and variances of the numbers of heterozygous and homozygous mutations per individual in the population, the mean fitnesses of individuals produced by selfing and outcrossing (denoted by w_s and w_x, respectively), the population mean fitness $\bar{w}$, and the inbreeding depression (δ, defined as $1-w_s/w_x$). To get accurate values for the mean fitnesses and the amount of inbreeding depression, we generated ten zygotes per individual in the population, at each sampling time point. We also recorded the average linkage disequilibrium between all pairs of adjacent loci. Linkage disequilibrium values were expressed in terms of the squared correlation coefficient between pairs of loci $\overline{\rho^2}$ (see Sved, 1971), to correct for differences in allele frequencies.

(ii) *Fitness functions*

In the sets of runs whose results are to be described, two fitness models were studied. In one model, fitnesses are multiplicative, as previously assumed for some of our deterministic calculations (Charlesworth *et al.* 1990). In this model the fitness of a genotype is given by the expression:

$$w_{yz} = (1-s)^y(1-hs)^z, \tag{1}$$

where s is the selection coefficient against homozygotes for the mutant alleles, h is the dominance coefficient of these alleles, and y and z are the numbers of homozygous and heterozygous mutations in the genotype, respectively. Our second fitness model assumes synergistic epistasis (Kimura & Maruyama, 1966; Crow 1970). For this type of model, we used a generalisation of Crow's (1970) quadratic fitness model (see Charlesworth *et al.* 1991). This employs an 'effective number of mutations', n, which weights heterozygous mutations by the dominance coefficient (Sved & Wilton, 1989), so that $n = hz+y$. The fitness expression is then given by:

$$w_n = \exp\left[-\left(\alpha n+\frac{\beta n^2}{2}\right)\right]. \tag{2}$$

(iii) *Runs with modifiers of the selfing rate*

After the initial equilibration period of 200 generations, the mutational process reached a quasi-equilibrium state. Such a population will be referred to as a 'base population'. Alleles at a modifier locus could then be introduced into such a base population. We assumed that the locus designated as the modifier was not subject to mutation, and that the alleles at this locus did not affect fitness.

To monitor the fate of the modifier alleles, the frequency of the new allele at the modifier locus was calculated each generation after its introduction, and the time of its final fixation or loss was recorded. To get mean values and variances for the observed results of the runs, the same base population was used for a set of 40 independent introductions of the modifier allele, and 25 independent base populations were used for each parameter set studied. The total numbers of fixations and losses of the new allele and the mean times to fixation and loss, were recorded for each base population, and for each set of base populations with the same parameter values. The variances of the times to fixation and loss were calculated for each set of parameter values.

(iv) *Testing the program*

The program was checked in several ways. The mean fitness, inbreeding depression, and other statistics of the simulations, were compared with the results of previous deterministic calculations (Charlesworth *et al.* 1990, 1991). The results of the simulations are expected to approach those of deterministic calculations with the corresponding parameter values as the population sizes and numbers of loci become large, and this was checked for several cases, some of which are shown in the next section.

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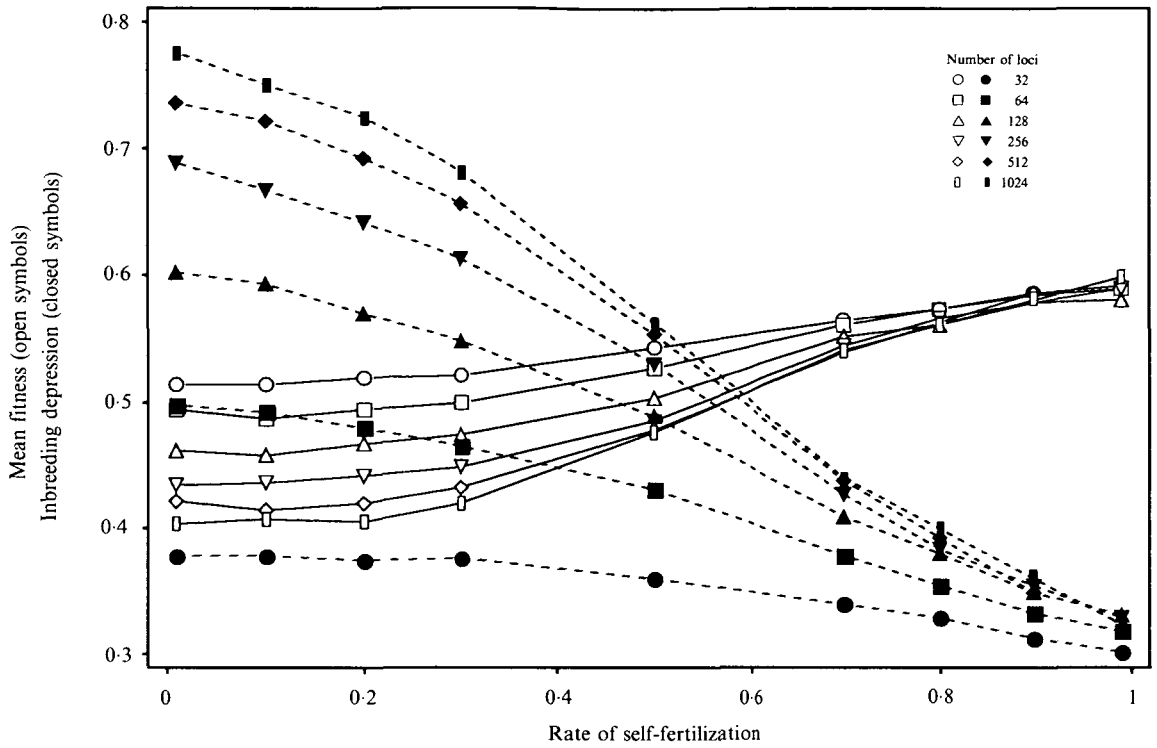

Fig. 1. Effect of the number of loci on the mean fitness (solid lines) and inbreeding depression values (dashed lines) in populations of 400 diploid individuals at quasi-equilibrium under the multiplicative fitness model with various selfing rates. The loci were unlinked. The mutation rate, U, was 1·0, the selection coefficient, s, was 0·2, and the dominance coefficient, h, was 0·1.

Correct behaviour of the modifier locus was tested by introducing a neutral allele in the same way as the modifier actually used in the runs (i.e. we introduced an allele assumed to have no effect on the selfing rate). The observed probability of loss, and the mean and variance of the times to loss or fixation, were compared with the expectations from neutral theory (Kimura & Ohta, 1969). These probabilities do not depend on the breeding system. For completely outcrossing populations, the times to loss or fixation can be compared with the expectations for neutral alleles at the same initial frequency (Kimura & Ohta, 1969). For partially selfing populations, the effective population size is given by $N_e = N/(1+F)$ (Pollak, 1987), and this can be used to obtain formulae for comparison with the results of our runs, using the equilibrium value of $F = S/(2-S)$. However, when selection occurs at some loci, associations between the modifier locus and the selected loci develop in partially selfing populations, and the times to loss or fixation of neutral alleles differ from the expectations based on the assumption of a neutral locus without selected loci present (see below). The times to loss or fixation were therefore checked by runs in which the mutation rate for the selected loci was set to zero. This means that no deleterious alleles were present in these runs. Neutral alleles were introduced into populations of 40 or 100 individuals, with 32 or 1024 loci, and with recombination fractions between adjacent selected loci of 0·5 and 0·005. In all cases, agreement between the simulations and the expectations were excellent; no statistically significant differences in either the frequencies of fixation, or the statistics of the distributions of times to fixation or loss were found. Runs were also done in which the selectively neutral locus was assumed to have an effect on the selfing rate, but the genetic background had no effect on fitness. The results from these runs agreed with those of single locus simulations of a modifier with the same effect on the selfing rate, in populations with the same degree of selfing.

3. Results

(i) *Effect of number of loci and of population size: unlinked loci*

Our main aims were to study the effects of finite population size and partial linkage, in relation to the results obtained previously for infinite populations, but since the method used for this also differs from our previous work in having fixed numbers of loci, we must first describe the effects on the populations of varying the number of loci. The effects seen with synergistic and multiplicative selection were similar, so we shall describe both together.

The number of loci affected the level of inbreeding depression markedly, with fewer loci producing lower values. Figure 1 shows the effects on inbreeding depression and mean fitness in populations with different numbers of loci, and with various selfing rates, with a population size of 400 and with

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Table 1. *Results for quasi-equilibrium outcrossing populations with different numbers of unlinked loci under the multiplicative and synergistic fitness models, in populations of various sizes (N). A mutation rate (U) of 1·0 per diploid genome per generation was assumed, and the dominance coefficient (h) was 0·2. The results of deterministic runs with the same parameter values are also shown at the bottom of the table, and for that case the numbers of heterozygous and homozygous mutations per individual are shown, instead of the proportions, as there is no fixed number of loci in the deterministic model*

	Multiplicative fitness: $s = 0{\cdot}1$				Synergistic fitness: $\alpha = 0{\cdot}01$, $\beta = 0{\cdot}02$			
			Average frequency per locus				Average frequency per locus	
Number of loci	Mean fitness	Inbreeding depression	Heterozygotes	Deleterious homozygotes	Mean fitness	Inbreeding depression	Heterozygotes	Deleterious homozygotes
$N = 100$								
128	0·407	0·339	0·2165	0·0257	0·592	0·415	0·1809	0·0158
256	0·396	0·380	0·1256	0·0105	0·567	0·469	0·1042	0·0063
512	0·365	0·424	0·0725	0·0049	0·573	0·483	0·0539	0·0026
1024	0·368	0·433	0·0373	0·0022	0·569	0·503	0·0282	0·0011
$N = 200$								
128	0·417	0·351	0·2267	0·0220	0·593	0·431	0·1898	0·0140
256	0·404	0·398	0·1339	0·0080	0·581	0·485	0·1079	0·0051
512	0·383	0·438	0·0757	0·0034	0·575	0·517	0·0579	0·0018
1024	0·379	0·455	0·0398	0·0014	0·576	0·528	0·0298	0·0008
$N = 400$								
128	0·424	0·357	0·2306	0·0200	0·600	0·437	0·1878	0·0119
256	0·396	0·417	0·1411	0·0075	0·588	0·493	0·1072	0·0040
512	0·395	0·446	0·0771	0·0025	0·579	0·525	0·0578	0·0014
1024	0·383	0·470	0·0414	0·0010	0·580	0·542	0·0299	0·0005
$N = 800$								
128	0·431	0·357	0·2313	0·0185	0·604	0·439	0·1892	0·0114
256	0·406	0·418	0·1413	0·0066	0·590	0·499	0·1086	0·0036
512	0·387	0·460	0·0805	0·0023	0·585	0·530	0·0583	0·0012
1024	0·383	0·481	0·0427	0·0008	0·576	0·556	0·0308	0·0004
$N = 1600$								
128	0·424	0·364	0·2364	0·0187	0·605	0·441	0·1958	0·0118
256	0·403	0·424	0·1438	0·0063	0·590	0·502	0·1129	0·0037
512	0·389	0·463	0·0811	0·0020	0·582	0·539	0·0612	0·0011
1024	0·380	0·490	0·0437	0·0007	0·581	0·556	0·0318	0·0003
Deterministic	0·369	0·525	48·37	0·053	0·572	0·588	33·74	0·0289

multiplicative interactions between loci. The effects of locus number are largest for outcrossing populations, but with high selfing all values are independent of locus number and converge on the approximate expected values of $\exp -\frac{1}{2}U (= 0{\cdot}606$ for the parameter values of Fig. 1) for the mean fitness of the population, and $1-\exp\{(0{\cdot}5-h)\,U\} (= 0{\cdot}329$ for the parameter values of Fig. 1) for the inbreeding depression [Charlesworth *et al.* 1991, eqns (12) and (13)].

Table 1 illustrates the effect of locus number on inbreeding depression, mean fitness, and the average numbers of mutant alleles per individual in highly outcrossing populations ($S = 0{\cdot}01$), for both the synergistic and multiplicative models. Various population sizes, $N = 100$ to 1600, are shown. In contrast to the marked effects of differences in the numbers of loci, the effects of population size differences (N) were slight on both mean fitness and inbreeding depression, over the whole range of selfing rate values and for a wide range of population sizes down to the lowest value we ran, with smaller population sizes yielding lower mean fitness and inbreeding depression than larger sizes, when models with the same numbers of loci are compared (Table 1). The effects of population size on mean fitness and inbreeding depression were greatest when the numbers of loci were large. In the multiplicative model with 1024 loci, when the population size was as low as 100 the inbreeding depression was reduced to only 0·88 of the value for a population sixteen times larger. The inbreeding depression values were lower with few than with many loci, and the mean fitness values somewhat higher. The convergence of the mean fitness and inbreeding depression values towards the values obtained by the deterministic calculations, assuming all loci unlinked, is also shown in Table 1. With the multiplicative selection model and 1024 loci, the inbreeding depression observed in our runs was 0·82 of the deterministic value, with a population size of 100, and 0·93 with a population size of 1600. For the synergistic model, the agreement was better.

The reason for the lower genetic load with smaller numbers of loci is probably that the mutation rate per locus is higher when the number of loci decreases, since the mutation rate per genome was assumed to be constant for all locus numbers. With small numbers of loci, the frequency of new mutant alleles will therefore be higher, and so there will be a higher frequency of homozygotes among the progeny generated by outcrossing. In contrast, in the method of Kondrashov (1985), which we used in our previous work on modelling inbreeding depression, an infinite number of loci is assumed, and homozygosity occurs only by inbreeding. This means that in the present models, mutations are eliminated in the homozygous state more often (especially when there are small numbers of loci) than in the infinite-locus models, for the same degree of inbreeding. We therefore expect lower average numbers of mutations per individual, and

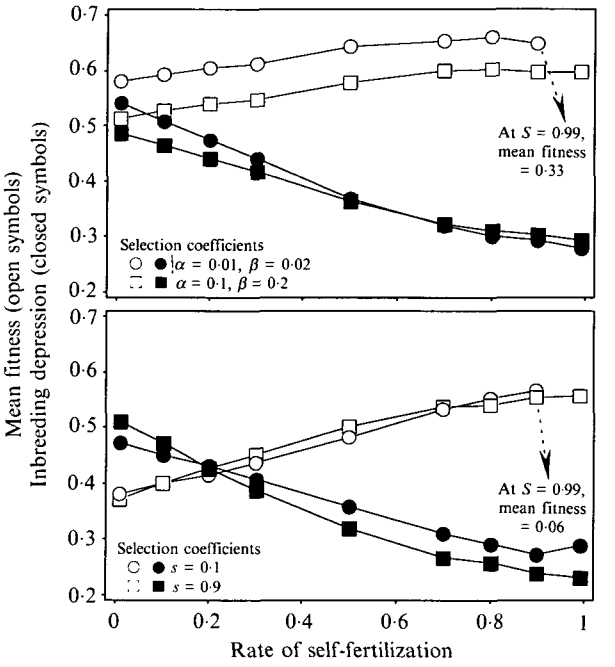

Fig. 2. Effect of the selection model on the mean fitness (open symbols) and inbreeding depression (filled symbols) values of populations of 400 diploid individuals at quasi-equilibrium under various selfing rates. The number of loci was 1024, and they were unlinked. The two fitness models, multiplicative (lower panel) and synergistic (upper panel), were run with a mutation rate, U, of 1·0, and the dominance coefficient, h, was 0·2. For each model, both strong and moderate selection runs were done, and the parameters are shown on the figure panels.

lower allele frequencies, at the fitness determining loci. Examination of the relative frequencies of homozygous and heterozygous mutations per locus per individual show that, as this explanation predicts, the proportion of homozygotes is higher when the locus number is small than when it is large (Table 1). Furthermore, the effect should disappear at high selfing rates, and this can be seen in Fig. 1, which shows that the effect of locus number is greatest in outbreeding populations. The effect of a finite number of loci is thus to give lower inbreeding depression values for populations with some degree of outcrossing, compared with those obtained for the same parameter values in our previous work, which assumed infinite locus number.

Figure 2 shows in detail the effects of selfing rate, for the case of 1024 unlinked loci, and population size $N = 400$, and for four selection models. The results are quite similar in all four cases and agree well with the infinite population size results. With complete selfing and 1024 loci, the inbreeding depression value was very close to the expected value of approximately 0·33.

With very high selfing, with small population sizes, some populations underwent a Muller's ratchet process of genetic deterioration, as expected from the theoretical results of Heller & Maynard Smith (1979), so that the mean fitness was greatly lowered. Examples can be seen in Figs 2 and 4 (see below).

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As can be seen from the results presented so far, we found effects of locus number and population size to be similar with the two different selection models. In our previous deterministic work on the synergistic model, we found that, in contrast to the multiplicative case, there was sometimes a maximum in the curve of mean fitness with increasing selfing rate. This difference from the multiplicative case was again observed in our stochastic runs (Fig. 2).

(ii) *Effect of linkage*

We next investigate the effect of linkage between the selected loci. Recombination fractions between adjacent loci from 0·5 to 0·005 were used. The effects of linkage on the equilibrium means and variances of the numbers of mutations per individual were slight and will not be shown. Figure 3 illustrates some of the linkage disequilibrium values obtained in our runs, for outcrossing populations and various frequencies of selfing, in populations of size 400 under the synergistic selection model. These were calculated as the mean squared correlation ($\overline{\rho^2}$) between adjacent loci, conditional on polymorphism for the loci. In all cases, the values were small. For tightly linked loci ($r = 0{\cdot}005$), the disequilibrium decreased with increasing selfing, but even with almost complete selfing the effect was only to halve the value found with outcrossing. The effects for loosely linked loci ($r = 0{\cdot}05$) were much smaller. An increase might have been expected intuitively, because selfing reduces the effective amount of recombination between loci, and so might be expected to increase the linkage disequilibrium. In a study of the effect of selfing on various measures of disequilibrium between neutral loci Golding & Strobeck (1980) found that increased selfing could either increase or decrease randomly generated disequilibrium, depending on the degree of linkage.

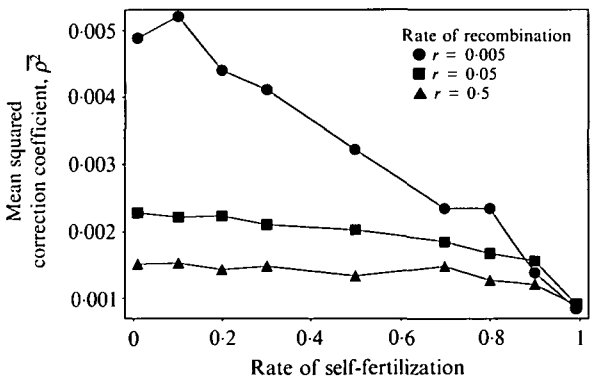

Fig. 3. Effects of the selfing rate and recombination fraction on linkage disequilibrium between adjacent loci in quasi-equilibrium populations of 400 individuals. There were 1024 loci, on a single chromosome. Linkage disequilibrium values were expressed as $\overline{\rho^2}$ (see text). The synergistic model with the standard parameter values ($\alpha = 0{\cdot}01$, $\beta = 0{\cdot}02$) was used, and the dominance coefficient, h, was 0·2.

In outcrossing populations with loose linkage, the values of $\overline{\rho^2}$ in our runs were similar to those expected for neutral loci [from the formula of Sved, 1971: $\overline{\rho^2} \approx 1/(1+4Nc)$, where c is the recombination fraction between the loci], suggesting that most of the disequilibrium is randomly generated, rather than due to selection. The magnitude of the effect due to selection can be assessed by comparing the observed values in runs with no linkage with the random expectation. The expected $\overline{\rho^2}$ value for outcrossing populations is $1{\cdot}25 \times 10^{-3}$, while that observed was just over 2×10^{-3}. As expected, tight linkage led to greater disequilibrium, though these values were in all cases small (Fig. 3). For tight linkage, however, the $\overline{\rho^2}$ values were lower than expected on the neutral theory, indicating an effect of selection on the correlation between loci. Synergistic selection is expected to produce negative linkage disequilibrium (see Charlesworth, 1990). There was also the expected effect of population size on linkage disequilibrium, with more linkage disequilibrium in smaller populations than in large ones (Sved, 1971).

Linkage also led to lower inbreeding depression, as we had expected (see introduction). Unless very close linkage was assumed, however, the effects were small. Examples of differences in inbreeding depression between the case of unlinked loci and loci with a recombination fraction of 0·005 can be seen in Table 2. We also studied the effect of organizing the selected loci into different numbers of chromosomes. The recombination fraction was then assumed to refer to adjacent loci within chromosomes, with independent segregation of different chromosomes. The effects are illustrated in Figure 4. In conformity with the results of reducing the recombination fraction described just above, this had the effect of reducing the inbreeding depression values somewhat. However, the effects were not large, and the differences between free recombination of all loci, and eight chromosomes with very tight linkage within each chromosome ($r = 0{\cdot}005$) was slight. Even with a single chromosome with all loci tightly linked, the reduction in inbreeding depression was minor. The effects on mean fitness were even less important (Fig. 4).

(iii) *Effects on modifiers of the selfing rate*

To study the effect of linkage on modifier alleles affecting the selfing rate, we compared results of stochastic runs with results previously obtained for the deterministic case assuming infinite population size and unlinked loci (Charlesworth *et al.* 1990, 1991). For the deterministic case, effects on modifier alleles increasing or decreasing the selfing rate were studied in terms of the asymptotic initial rates of increase ($\Delta p/p$) of the modifier allele frequency, p, which indicates the selection coefficient on a rare allele. For stochastic runs, this is no longer possible. We therefore studied the probabilities of fixation

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Table 2. *Results of introduction of modifier alleles into quasi-equilibrium base populations of 400 individuals previously run for 200 generations with mutation and selection. The mutation rate per diploid genome was assumed to be 1·0 per generation, and the synergistic selection model was used, with $\alpha = 0{\cdot}01$ and $\beta = 0{\cdot}02$. Three different values of the dominance coefficient, h, were used*

Selfing rate of initial genotype S_0	Number of runs	Recombination fraction	Inbreeding depression		Deterministic $\Delta p/p$	Frequency of loss of modifier	Time to loss		Time to fixation	
			Deterministic value	Mean of base populations			Mean	S.D.	Mean	S.D.
Neutral case (modifiers causing no change in selfing rate)										
$h = 0{\cdot}2$										
0·9	920	0·5	0·294	0·290	0	0·968	36·7	53·86	313·7	166·4
0·9	1000	0·005	0·294	0·265	0	0·976	32·8	40·88	269·5	157·3
0·5	1000	0·5	0·380	0·368	0	0·974	106·9	217·9	1044	595·7
0·5	1000	0·005	0·380	0·373	0	0·980	89·0	169·2	819·9	357·7
0·1	1000	0·5	0·582	0·507	0	0·987	136·7	258·2	1125	428·7
0·1	640	0·005	0·582	0·506	0	0·979	107·1	211·6	1240	531·3
Modifier increasing outcrossing ($S_1 = S_2 = 0{\cdot}01$)										
0·5	1000	0·5	0·380	0·368	−0·081	1·0	22·5	11·49	—	—
0·5	1000	0·005	0·380	0·373	−0·081	1·0	23·2	12·96	—	—
Modifiers increasing selfing ($S_1 = S_2 = 0{\cdot}99$)										
0·5	1000	0·5	0·380	0·368	0·0992	0·268	22·9	16·03	126·0	39·89
0·5	1000	0·005	0·380	0·373	0·0992	0·333	21·9	13·78	127·0	44.58
0·2	1000	0·5	0·507	0·479	0·0924	0·382	21·3	14·35	120·0	38·65
0·2	1000	0·005	0·507	0·467	0·0924	0·364	24·7	15·43	121·0	41·57
0·1	720	0·5	0·582	0·510	0·0672	0·507	23·8	15·23	128·9	36·41
0·1	1000	0·005	0·582	0·507	0·0672	0·485	22·0	14·35	118·2	37·35
Modifier increasing selfing ($S_1 = S_2 = 0{\cdot}3$)										
0·1	720	0·5	0·582	0·510	−0·0033	0·818	70·5	84·75	935·1	424·0
0·1	1000	0·005	0·582	0·507	−0·0033	0·767	58·8	65·15	720·0	304·7
Modifier increasing outcrossing ($S_1 = S_2 = 0{\cdot}01$)										
0·1	120	0·5	0·582	0·523	0·0017	0·983	169·0	294·1	855·0	(only 4 fixations)
0·1	640	0·005	0·582	0·508	0·0017	0·997	99·3	147·7	1714	56·60
Modifier increasing outcrossing ($S_1 = 0{\cdot}1$, $S_2 = 0{\cdot}01$)										
$h = 0{\cdot}15$										
0·2	1000	0·5	0·683	0·620	0·0177	0·663	67·2	55·87	488·1	139·2
0·2	1000	0·005	0·683	0·607	0·0177	0·730	80·2	81·65	582·1	185·7
Modifier increasing selfing ($S_1 = 0{\cdot}3$, $S_2 = 0{\cdot}4$)										
$h = 0{\cdot}1$										
0·2	1000	0·5	0·922	0·795	−0·0513	1·0	39·0	30·12	—	—
0·2	1000	0·005	0·922	0·769	−0·0513	1·0	41·2	32·28	—	—
Modifier increasing outcrossing ($S_1 = 0{\cdot}1$, $S_2 = 0{\cdot}01$)										
0·2	1000	0·5	0·922	0·799	0·0447	0·291	41·0	30·89	251·1	53·31
0·2	1000	0·005	0·922	0·777	0·0447	0·376	47·5	39·79	283·3	72·38

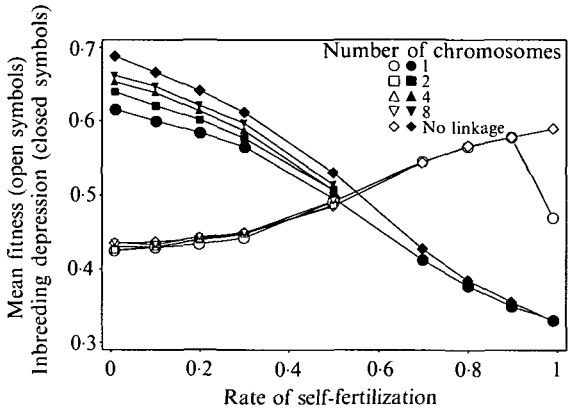

Fig. 4. Effects of the selfing rate and number of chromosomes on the mean fitness (open symbols) and inbreeding depression (filled symbols) for equilibrium populations of 400 individuals. There were 256 loci, either all unlinked, or with a recombination fraction of 0·005 between adjacent loci on the same chromosome. The synergistic model with the standard set of parameter values ($\alpha = 0{\cdot}01$, $\beta = 0{\cdot}02$) was used, and the dominance coefficient, h, was 0·2.

versus loss of alleles newly introduced at a low frequency (0·025 in most cases), for comparison with the neutral expectation. We also recorded the times to fixation and loss. Because of the possibility that the modifier locus will become associated with the selected loci in a partially selfing population, so that its behaviour will differ from that expected for a neutral locus in a population under selfing in which there are no selected loci, it is necessary to compare our results with those of a set of stochastic runs in which the modifier locus was assumed to have no effect on the selfing rate (i.e. was neutral), and in which we assumed the same selfing rates for the genotypes at this locus and the same population size as in the runs where the modifier alleles caused different selfing rates.

To have enough runs for statistical testing of the differences observed, each parameter set was studied by obtaining 40 base populations, and running 25 introductions of the modifier into each of these base populations. The runs for each parameter set were thus very time-consuming, and we therefore did few sets. The parameter sets were chosen because previous experience with the deterministic runs had shown them to provide situations in which selection for selfing or outcrossing could occur, depending on the selfing rate before the introduction of modifiers, and in which an inbreeding depression value exceeding one half appeared to be a necessary condition for the increase of modifier alleles increasing the outcrossing rate or for the elimination of modifiers causing small increases in selfing (see Charlesworth *et al.* 1990, 1991). To reduce the number of runs resulting in loss of modifiers, we introduced modifier alleles at a frequency of 0·025, instead of $1/2N$ as would be appropriate for a new mutation.

In most runs with modifiers, synergistic selection at 1024 loci was assumed, with the standard synergistic parameter set of Charlesworth (1990), i.e. $\alpha = 0{\cdot}01$ and $\beta = 0{\cdot}02$ in the notation of Charlesworth *et al.* (1991), and dominance coefficient 0·2. The mutation rate parameter, U, was set to 1 per diploid genome, and the population size, N, was 400. We compared the results of stochastic runs with all loci unlinked, to runs having the same number of selected loci arranged in a block of linked loci, with a recombination fraction of 0·005 between adjacent loci and the modifier locus in the centre of this block.

Some results for neutral modifiers are shown at the top of Table 2, for three selfing rates. None of the probabilities of loss is statistically significantly different from that expected for a neutral allele (0·975 for an allele introduced at a frequency of 0·025), but the times to loss were smaller than the neutral expectations. For example, with a selfing rate of 0·1, we observed a mean time of 107·1 for loss of the modifier in the linked case. The expected value for neutral alleles introduced into populations in which there are no selected loci, based on the appropriate effective population sizes under 10% selfing, is 143·8. Using the observed variance in time to loss, this is significantly different by a t test ($t = 4{\cdot}34$). With selfing rates of 0·5 or 0·9, for either linked or unlinked loci there were again significantly lower times to loss than the respective expected values of 113·5 and 83·2, but no significant effects of the recombination fraction.

Table 2 also shows results of runs when modifiers were introduced that changed the selfing rate. With an initial selfing rate of 0·5, the mean inbreeding depression value for the base populations was about 0·37 for either linked or unlinked selected loci, compared with a value of 0·38 for the corresponding deterministic case. For the deterministic case, the initial rate of increase was 0·099 for a modifier allele causing an increase in the selfing rate to 0·99; in other words, a high rate of selfing was strongly selected.

If the more realistic model studied here behaves similarly, we expect to find evidence that high selfing is selected. In accordance with this expectation, modifier alleles causing complete outcrossing were always eliminated (no fixations out of 1000 stochastic runs, for either unlinked or tightly linked loci). When dominant modifiers increasing selfing to a value of 0·99 were introduced, however, the probability of loss was 0·27 in the unlinked case and 0·33 in the linked case. This effect of linkage is statistically significant, and both probabilities of loss are also significantly lower than that expected for neutral alleles. These results indicate selection for high selfing in these base populations, in agreement with the deterministic results. The mean times to fixation and loss were closely similar in the linked and unlinked cases, and were considerably lower than for neutral modifiers, as expected if there is selection on the modifier (Table 2).

With the same parameters as above, but with base populations having lower selfing rates, modifiers increasing selfing to a very high level were more likely

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to be lost from the populations into which they were introduced, which is consistent with the higher inbreeding depression values in these populations. For example, with a selfing rate of 0·2, the mean inbreeding depression was about 0·47, compared with 0·50 for the deterministic case. In the deterministic case, selfing was again selected, with a rate of increase of 0·092 for an allele increasing S to 0·99. The stochastic runs yielded a frequency of loss of about 0·37, significantly higher than when the initial populations had a 50% selfing rate. The mean times to loss and fixation, however, were not much affected by the changed selfing rate. As for the case of initial $S = 0{\cdot}5$, there was no strong effect of the recombination fraction on either the probability of loss or the mean times to fixation or loss in the stochastic runs.

With initial selfing of 0·1, the rate of loss of the high-selfing allele was increased further, again with no significant effect of the recombination fraction, and with similar loss and fixation times. This set of runs involves an inbreeding depression value slightly greater than one-half, and might therefore be expected to produce selection for increased outcrossing, according to our previous deterministic runs. However, we have already shown that modifiers increasing selfing to very high levels may often be able to invade populations with inbreeding depression exceeding 0·5. For the present parameter set, this occurs in the deterministic runs (Charlesworth *et al.* 1991). When modifiers increasing the outcrossing rate are introduced, one can detect selection for outcrossing in the deterministic case. Alternatively, when modifiers causing slight increases in the selfing rate are introduced, they are eliminated. When stochastic runs were done with modifiers increasing the outcrossing rate, however, the result was that the modifier alleles were lost in about 99% of the runs. This is probably because of the fact that the inbreeding depression is very close to 0·5. Due to the low selfing rate in this case, there is also only a small phenotypic difference between the genotypes at the modifier locus. Even in the deterministic case, which is probably the most favourable for the evolution of outcrossing, the initial rate of increase of such a modifier allele was only 0·0017 (Table 2). The modifier alleles are therefore expected to behave as almost neutral alleles. This is supported by the long times to loss and fixation, which are similar to those for neutral alleles introduced into similar base populations with the same selfing rate (Table 2).

To investigate whether selection for increased outcrossing can occur in the stochastic runs in a similar way to that in deterministic runs, we have done a few runs with lower dominance coefficients. This has the effect of increasing the inbreeding depression to levels well above 0·5, and is thus expected to lead to selection for outcrossing. With $h = 0{\cdot}1$ and an initial selfing rate of 0·2, modifiers causing an increase in the selfing rate to 0·4 were eliminated in 100% of runs, whether the loci were linked or unlinked (Table 2), while modifiers producing almost complete outcrossing had a much lower frequency of loss, especially for unlinked modifiers (the difference between linked and unlinked cases is highly statistically significant). The mean times to loss were much lower than for neutral alleles. These results are therefore consistent with the deterministic runs, in which this type of base population leads to selection for outcrossing.

4. Discussion

(i) *Effects of finite population size*

The genetic load due to loci subject to mutation to deleterious alleles, with a very much higher frequency of forward- than back-mutation, is much greater in small than large populations because of a high frequency of fixation of the deleterious alleles (Kimura, Maruyama, & Crow, 1963). For sexually reproducing populations with a selection coefficient as high as 0·1, the population size must be below 100 for this effect to occur (see Kimura *et al.* 1963, Fig. 1). The situation in partially selfing populations, or other populations with inbreeding, has not previously been studied, but we have studied this case by a modification of the methods used by Kimura *et al.* (1963). We obtained results similar to those for outcrossing populations.

In our runs, we do not therefore expect this type of phenomenon to be seen, despite the fact that mutation was unidirectional, because the population sizes assumed were always above 100, moderately strong selection was assumed, and only 2000 generations were run. Of course, in the long term we would expect all loci to become fixed for deleterious alleles, but the rate of fixation with the parameters we have used is very low. Our results are thus nearly equivalent to those for a stationary distribution of allele frequencies maintained by a low rate of back-mutation or immigration of wild-type alleles from other populations. In such a state, the mean fitness of the population will be affected by two factors. The first is the fact that the mean frequency of deleterious alleles for the stationary distribution is lower than the equilibrium frequency for an equivalent infinite population (see Crow & Kimura, 1970, p. 449, for the case of recessive alleles), leading to a reduction in load. The second is the dispersion of allele frequencies around this mean, leading to an overall higher frequency of homozygosity for deleterious alleles than for an infinite population, where there is no dispersion around the mean gene frequency (Crow, 1970). This leads to an increase in load, compared with the infinite population case. The interaction of these two factors makes it hard to predict the effect of population size on mean fitness in our runs.

In fact, the mean fitness values in our runs generally showed only a slight decline as population size is

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reduced, except for a few cases in which the Muller's ratchet process operated (see below). The outcrossing case is shown in Table 1. The effect of population size differences on mean fitness values in partially selfing populations was also very slight. Table 1 also shows the low frequency of homozygosity for deleterious mutations, even in populations as small as 100. This also indicates that fixation of mutant alleles is rare, in these outcrossing populations. It does not therefore seem likely that the fixation of detrimental alleles will have significant effects on population fitness in sexually reproducing populations, despite the suggestion by Lynch & Gabriel (1990) that small sexually reproducing populations may experience a 'mutational melt-down' in fitness that causes their extinction. Effective neutrality of deleterious alleles requires $Ns < 1$; with the selection coefficient of 0·02 against homozygous deleterious mutations of minor effect suggested by the *Drosophila* data (Simmons & Crow, 1977), this requires $N < 50$. The rate of fixation of deleterious alleles under this condition (summed over all loci) is thus given by the rate of fixation of neutral alleles per genome, which is $\frac{1}{2}U$ in the present case. The net rate of deterioration in mean fitness is thus approximately $\frac{1}{2}Us$. With $U = 1$ and $s = 0{\cdot}02$, it would thus take of the order of 50 generations for the population mean fitness to be halved. But an isolated population as small as this is likely to be vulnerable to rapid extinction for purely demographic reasons (cf. Lande, 1988), so that this process is unlikely to be a significant factor in causing extinction.

Rapid deterioration of population mean fitness, can however, occur in populations of moderate size when the selfing rate is close to 1, as a result of the operation of Muller's ratchet (cf. Figs 2 and 4). This is in accord with the findings of Heller & Maynard Smith (1979), who showed that even unlinked loci are vulnerable to the operation of the ratchet in a fully selfing population, due to the effective absence of recombination. Our results demonstrate that the ratchet can operate even with a small amount of outcrossing (1 %) and with unlinked loci (cf. Fig. 2). Fig. 4 shows that close linkage greatly facilitates the operation of the ratchet when the selfing rate is 99 %, but that the ratchet does not appear to operate at a noticeable rate in more outcrossing populations of 400 individuals, even with a single chromosome with a recombination frequency of 0·005 for adjacent loci. Thus, the ratchet operates slowly or not at all in outcrossing populations when $Nr = 2$, with the selection parameters we have used. This appears to contradict the conclusion of Bell (1988) that much higher values of Nr are required to prevent the ratchet's operation.

(ii) *Behaviour of neutral alleles in populations with loci under selection*

Even for completely outcrossing populations, we did not find the mean times to fixation and loss of neutral alleles to agree with expectations based on alleles introduced at the same initial frequency. The expectations were calculated by the formulae of Kimura & Ohta (1969). For partially selfing populations, corresponding formulae were derived using the effective population sizes given by Pollak (1987). In our runs, the times were smaller than predicted. Ohta (1971, 1973) and Sved (1972) showed that the presence of selected loci with recessive deleterious alleles retards the fixation of alleles at neutral loci present at intermediate frequencies in finite outcrossing populations, due to associative overdominance caused by randomly generated linkage disequilibrium, and Ohta (1971) pointed out that this effect will be more pronounced in partially selfing populations. However, the case of allele frequencies close to zero or one has apparently not previously been studied. It appears from our results that the effect for low frequency alleles may be caused by chance associations with low-fitness genotypes. While such an association persists, it will cause a decline in frequency of the neutral alleles, and these alleles can thus be lost rapidly. For alleles at intermediate frequencies, however, occasional chance associations with selectively disadvantageous genotypes will not persist for long, but will usually be broken down by recombination before their frequency can reach levels close to fixation.

(iii) *Effects of linkage on inbreeding depression and on the selection of modifier alleles*

The effect of linkage on the level of inbreeding depression was slight unless the selected loci were very tightly linked indeed. A realistic case for many eukaryotes might be to assume 4–10 chromosomes with many loci subject to deleterious mutations occurring at intervals of 0·02 map units (based on a total map length of 1000, as appears reasonable for an organism with 10 chromosomes, and assuming 50000 loci in a genome). Assuming a mutation rate of 10^{-5} per locus per generation, this would yield inbreeding depression closely similar to the values we obtained previously for the multiplicative and synergistic selection models, for infinite numbers of unlinked loci (Charlesworth *et al.* 1990, 1991). Linkage disequilibrium was slight in all the cases we studied, and tended to decrease with the selfing rate. Since linkage tends to decrease the level of inbreeding depression, it will generally reduce selection for outcrossing, but the effects are slight with realistic levels of recombination between selected loci.

In studying the evolution of the selfing rate, it is important to distinguish this effect of linkage between the selected loci from effects caused by linkage between these loci and modifiers of the breeding system. Uyenoyama & Waller (1991) have shown that linkage affects the conditions for invasion of populations by modifiers of the selfing rate. They assumed a single selected locus, at which mutations lowering fitness

might occur. Recessive or partially recessive deleterious effects of mutations will mean that progeny produced by selfing will have lower fitness than outcrossed progeny. In models in which inbreeding depression is treated as a fixed parameter, if pollen discounting is complete [i.e. pollen contributions to the pollen pool fertilizing non-selfed ovules are decreased in proportion to the selfing rate (see Nagylaki, 1976)], any such fitness difference means that there will be selection for outbreeding (Charlesworth, 1980). By using a model with complete discounting, Uyenoyama & Waller (1991) were therefore able to investigate the effect of linkage between selected loci and modifiers of selfing, without the complications involved when there are multiple selected loci. This approach yields the result that linkage makes the evolution of selfing more likely, and hinders the evolution of outcrossing. An important conclusion from our runs is that the effects of both inbreeding depression and linkage of the modifier to the selected loci work in the same direction. Since this is so, it is not possible in the multi-locus case to distinguish how much of any effect observed is due to each of the two causes, but it seems likely that some of the comparisons between our runs with linked and unlinked loci may be mainly due to this effect of linkage between the modifier and the selected loci (Uyenoyama & Waller, 1991). We found that modifiers increasing outcrossing were more likely to be eliminated when linkage was assumed than when we assumed free recombination, though the differences in the inbreeding depression values were small (Table 2). For example, in the last two cases in Table 2, there is an increase of 29% in the probability of loss of the modifier allele with linkage, but only a 3% decrease in the inbreeding depression.

To test for effects of differences in the inbreeding depression in the base populations, we examined the relation between the probabilities of loss of the modifier and the initial inbreeding depression values. In the stochastic runs studied here, the inbreeding depression values in the different base populations are distributed around some average value. This differs from the situation in our previous deterministic calculations, when the modifier alleles were always introduced at the same frequency into each genotype present in the base population. In the present runs, the genotypes into which modifier alleles were introduced were chosen randomly. Whenever the inbreeding depression in the base population is high, a modifier allele causing high selfing has a high probability of being initially in a genotype whose inbred offspring will have low fitness, and thus a high chance of loss within a short period of time. This is true even though, if the modifier were not lost, the population would evolve to a different state, with a new, lower level of genetic load and inbreeding depression, which would be more favourable for establishment of the modifier. In an infinite population, this purging of the genetic load may enable the allele to spread (Lande & Schemske, 1985). In a finite population, however, this purging often may not occur in the small number of generations before the new allele is lost. The overall probability of loss must therefore depend on the distribution of inbreeding depression values in the base populations, not just on their mean value.

For the case with a dominance coefficient of 0·1, when the selection on a modifier increasing the outcrossing rate was examined (see Table 2), the inbreeding depression values and frequencies of loss of the modifier were statistically significantly correlated when the linked and unlinked runs were pooled, but there was no significant correlation within either of these sets taken alone. This again suggests that the inbreeding depression value of the base population is not itself responsible for the differences, but that linkage of the modifier to the selected loci makes selection for outcrossing less likely, as shown by Uyenoyama & Waller (1991) for the case of a single selected locus. This conclusion is supported by the case of $h = 0{\cdot}15$. For this case, there was also no significant effect of linkage on inbreeding depression, and no relationship between the inbreeding depression in the base population and the fate of the modifier allele, over the small range of inbreeding depression values among these populations.

In general, our results show that the similarities between runs with the same set of parameters but different degrees of linkage are much greater than the differences due to linkage, even with the extremely close linkage assumed in our runs. It therefore seems that linkage between loci is a factor of lesser importance, for selection on modifiers of the selfing rate, than differences in the initial selfing rate or the dominance coefficient. Differences in the values of these parameters of the models lead to differences in the inbreeding depression values generated, and our conclusion is thus that this is a valuable predictive parameter that can indicate the direction of selection on genetic variation for the selfing rate. The selective forces detected in the stochastic runs agree with those from the deterministic calculations, but one must also take into account the possibility that linkage between the selected loci and the modifiers may make outcrossing somewhat less likely to evolve. In our runs, we did not observe differences in the direction of selection due to linkage. Although this would presumably sometimes be found, the behaviour of the modifier alleles approaches that of neutral alleles when the inbreeding depression was close to one half, which made it difficult to determine the direction of selection on the modifier.

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Note added in proof. From results obtained since this paper was written, it appears that the cases of populations with very high selfing in which mean fitness declined to low levels (Figs 2 and 4) were not caused by Muller's ratchet, but by a high rate of fixation of mutant alleles. We are currently undertaking further detailed studies of the effects of close linkage and high levels of inbreeding on fixation of deleterious alleles.