Genet. Res., Camb. (2010), **92**, *pp.* 127–140. © Cambridge University Press 2010
doi:10.1017/S0016672310000133

Inbreeding depression and genetic load at partially linked loci in a metapopulation

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(*Received 12 December 2009 and in revised form 15 March 2010*)

Summary

Inbreeding depression has important implications for a wide range of biological phenomena, such as inbreeding avoidance, the evolution and maintenance of sexual systems and extinction rates of small populations. Previous investigations have asked how inbreeding depression evolves in single and subdivided populations through the fixation of deleterious mutations as a result of drift, as well as through the expression of deleterious mutations segregating in a population. These studies have focused on the effects of mutation and selection at single loci, or at unlinked loci. Here, we used simulations to investigate the evolution of genetic load and inbreeding depression due to multiple partially linked loci in metapopulations. Our results indicate that the effect of linkage depends largely on the kinds of deleterious alleles involved. For weakly deleterious and partially recessive mutations, the speed of mutation accumulation at segregating loci in a random-mating subdivided population of a given structure tends to be retarded by increased recombination between adjacent loci – although the highest numbers of fixation of slightly recessive mutant alleles were for low but finite recombination rates. Although linkage had a relatively minor effect on the evolution of metapopulations unless very low values of recombination were assumed, close linkage between adjacent loci tended to enhance population structure and population turnover. Finally, within-deme inbreeding depression, between-deme inbreeding depression and heterosis generally increased with decreased recombination rates. Moreover, increased selfing reduced the effective amount of recombination, and hence the effects of tight linkage on metapopulation genetic structure were decreased with increasing selfing. In contrast, linkage had little effect on the fate of lethal and highly recessive alleles. We compare our simulation results with predictions made by models that ignore the complexities of recombination.

1. Introduction

The genetic load of a population is the result of a balance between the loss of deleterious mutations via selection and the influx of new deleterious alleles by mutation (Muller, 1950). Large, randomly mating populations will tend to maintain a high genetic load because homozygosity is held at a minimum and recessive deleterious alleles remain unexpressed. In contrast, inbreeding populations are expected to purge their load as a result of selection against the alleles responsible as they become expressed in homozygotes (Crnokrak & Barrett, 2002; Swindell & Bouzat, 2006). This ability of populations to purge their load will, however, be compromised in small populations by the extent to which deleterious mutations become fixed by drift: once a mutation is fixed, it can no longer be purged (Kimular, 1962; Muller, 1964). Similarly, we expect the selective purging of deleterious recessive mutations to be compromised by genetic interference amongst closely linked loci (Hill & Robertson, 1966). On the one hand, deleterious mutations can be swept to fixation if they are linked to an advantageous mutation (Hill & Robertson, 1966; Felsenstein, 1974). On the other hand, selection will find it difficult to choose between deleterious

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https://doi.org/10.1017/S0016672310000133 Published online by Cambridge University Press

mutations affecting individuals at closely linked loci. This interference is the basis of predictions of the incremental decay of non-recombining genomes or chromosomes, such as the genomes of asexual organisms (Gordo & Charlesworth, 2000*a*, *b*), or sex chromosomes (Y or W chromosomes) in which recombination has been suppressed (Charlesworth, 1978, 1996).

In finite populations, mutations accumulate either as a result of the fixation of deleterious mutations, or through their accumulation at segregating loci. The effects of linkage on mutation accumulation and inbreeding depression due to these two different processes have been well studied (Charlesworth *et al*., 1992, 1993*a*, *b*). Charlesworth *et al*. (1992), for instance, used simulations to investigate the effect of linkage on the genetic load and inbreeding depression caused by deleterious mutations in finite undivided populations, and concluded that the effect of linkage on the level of inbreeding depression and genetic load should be modest, unless very small values of recombination are assumed. Here, the greatest rate of fixation of deleterious mutations occurs under very low recombination rates (though not when the recombination rate equals zero), whereas the rate of mutation accumulation at segregating loci tends to increase monotonically with a reduction in the recombination rate (Charlesworth *et al*., 1992, 1993*a*, *b*). The evolution of load and inbreeding depression in finite population would thus appear to depend on the relative importance of fixed versus segregating load.

Our understanding of the evolution of genetic load has come principally from studies of single undivided populations (Lande & Schemske, 1985; Schemske & Lande, 1985; Husband & Schemske, 1996; Bataillon & Kirkpatrick, 2000). In comparison, fewer studies have addressed the evolution of load under the more complex scenario of a subdivided population or a metapopulation. The added complexity in such situations begins with the very definition of inbreeding depression for populations subdivided into demes. Thus, for example, we may define inbreeding depression as the reduction in fitness of inbred individuals relative to outcrossed individuals from the same deme, or relative to individuals resulting from crosses between demes. Or we may compare individuals resulting from random mating in small local demes with those resulting from mating between individuals from different demes (Theodorou & Couvet, 2002; Roze & Rousset, 2004).

A second complication is that population subdivision, and the potential turnover of populations as a result of extinctions and recolonizations, alter the effective population size and should thus influence the extent to which load can accumulate in a population under the influence of genetic drift (Glémin *et al*., 2003). In a metapopulation of small demes, individuals are more likely to mate with relatives, which expose more deleterious recessive alleles to purifying selection, with direct implications for the level of inbreeding depression and genetic load in each deme. Several recent studies have addressed this issue (Theodorou & Couvet, 2002, 2006*a*, *b*; Whitlock, 2002; Glémin *et al*., 2003; Roze & Rousset, 2004); together, they point to the interactive effects of population structure, the mating system, the intensity of selection and the dominance coefficient.

Whitlock (2002) developed several approximate estimates of the genetic load and inbreeding depression on the basis of neutral F_{ST} in a large metapopulation under weak selection. He concluded that whether the efficiency of selection is increased or decreased with population subdivision depends on the dominance coefficient, the level of population subdivision (i.e. the size of F_{ST}), and on whether selection is hard or soft (Whitlock, 2002). Glémin *et al*. (2003) confirmed that the genetic load in a subdivided population should be due mainly to nearly neutral or slightly deleterious alleles, because mutations of larger effect are kept at low frequencies by selection. More recently, Theodorou & Couvet (2006*a*, *b*) conducted elasticity analysis to evaluate the relative importance of the various parameters of population structure (i.e. the number and size of demes, the migration rate, etc.). They concluded that the size of demes has the strongest influence on genetic load, and that the selection coefficient determines the magnitude of elasticity of genetic load in metapopulations. Roze & Rousset (2004) found that population structure helps to purge recessive deleterious mutations and to reduce the genetic load for some parameter values, but that structure makes selection against deleterious mutations less efficient when the selfing rate is high. In this context, and given the important feedbacks between the evolution of selfing and the accumulation or purging of genetic load (Lande & Schemske, 1985; Schemske & Lande, 1985), it is interesting that selfing will tend to be favoured in metapopulations linked by low gene flow, whereas intermediate levels of gene flow are more likely to promote mixed mating (Theodorou & Couvet 2002). All these studies deal with inbreeding depression and/or genetic load in a metapopulation caused by selection at only one locus, or at multiple loci with free recombination.

Given that reduced rates of recombination between loci should also compromise the efficacy of selection, it is not clear to what extent the conclusions from these studies remain valid in the more realistic situation in which there is more or less restricted recombination among loci. In this paper, we consider the joint effect of geographic population structure and reduced recombination amongst selected loci on the evolution of load and inbreeding depression. We consider two different kinds of mutations: weakly

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deleterious mutations that are partially recessive and lethal mutations that are highly recessive. We pay particular attention to the important distinction between the two different components of a population's genetic load highlighted above: the load due to the accumulation of deleterious alleles segregating in the population and the load due to deleterious alleles that have become fixed by drift. We begin our analysis by considering the extent to which Whitlock's (2002) approximations for load and inbreeding depression at single loci are valid for the domains of parameter space we explore. We then ask how restricted recombination between different loci should affect the genetic structure of a metapopulation under a range of different demographic scenarios. Given that self-fertilization reduces the effective recombination rate, we also investigated the effects of linkage under different selfing rates.

2. Model

Consider a metapopulation of D demes of N hermaphrodite diploid individuals. We assume migrant pool dispersal among demes. We characterize the genotype of each individual in terms of two haploid genomes, each with L loci. At each locus, the haplotype may be loaded with a deleterious mutation, or it may be 'clean'. At the beginning of each generation, each deme becomes extinct with probability e. Vacant habitat thus created is immediately recolonized by K diploid individuals drawn randomly from the rest of the metapopulation. At the same time, all other (occupied) demes receive a Poisson-distributed number of immigrants, with mean Nm, drawn randomly from the rest of the metapopulation; these immigrants replace the same number of individuals in the deme so that the population size N is maintained.

Mating and reproduction follow dispersal, which we modelled as follows: (1) we chose an individual from the (K or N) individuals in the deme with a probability proportional to its relative fitness (see below); (2) we then formed a haploid genome from the individual by allowing recombination with probability r between all adjacent loci, and by allowing a deleterious mutation to occur at each unloaded locus with probability μ; (3) with the selfing probability S we sampled the second haplotype from the same individual, following the same procedure; with probability $1-S$ we followed steps (1) and (2) for the second haplotype; The two haplotypes were combined into a single diploid offspring; (4) we continued to sample individuals until the population contained N new individuals. Note that we assumed only forward mutation to a deleterious state, and not reversal. $U=2\mu L$ is the genomic mutation rate.

In our simulations, the fitness of each individual (and thus the probability that it was sampled when generating the next generation) depended on its multilocus genotype. In particular, fitness for individual j was defined as

$$w=\prod_{i=1}^{L}(1-\lambda_i s), \tag{1}$$

where s is the selective coefficient, $\lambda_i=h$ if the locus is heterozygous, $\lambda_i=1$ if the locus is homozygous for the deleterious allele, $\lambda_i=0$ if the locus is homozygous for the non-deleterious allele and h is the dominance coefficient. Our model assumed that the selection and dominance coefficients were the same across all loci.

During our simulations, we recorded the mean fitness of all demes i and of the whole metapopulation, respectively, as

$$\bar{w}_i=\frac{1}{N}\sum_{l=1}^{N} w_{il}, \tag{2}$$

$$\bar{\bar{w}}=\frac{1}{D}\sum_{i=1}^{D} \bar{w}_i, \tag{3}$$

where w_{il} is the fitness of individual l in deme i, calculated using (1).

We used subscripts A, B, C and D to identify haplotype sampling for calculating inbreeding depression. Here, haplotypes A and B are both formed from the same individual following recombination and mutation (as in the mating procedure identified above); haplotypes A and C are drawn from different individuals in the same deme; and haplotypes A and D are drawn from individuals in different demes. With this terminology, we recorded inbreeding depression in three ways:

$$\delta_1=1-\frac{\bar{w}_{A/B}}{\bar{w}_{A/C}}, \tag{4a}$$

$$\delta_2=1-\frac{\bar{w}_{A/B}}{\bar{w}_{A/D}}, \tag{4b}$$

$$\delta_3=1-\frac{\bar{w}_{A/C}}{\bar{w}_{A/D}}. \tag{4c}$$

Here, δ_1 is the inbreeding depression within demes, which measures the mean fitness reduction of inbred individuals relative to outbred individuals within the same deme; δ_2, the inbreeding depression between demes, describes the mean reduction of fitness in inbred individuals relative to individuals outbred between different demes; finally δ_3 quantifies heterosis, defined as the excess in mean fitness of individuals produced by crosses among demes relative to the individuals outbred within a deme. We recorded the genetic load in two ways: (1) $L_1=1-\bar{\bar{w}}$ and (2) $L_2=1-\bar{w}_{A/D}$ (the latter denoting the genetic load caused by population structure).

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We ran simulations for two separate cases. First, we simulated the case for $L=1$ and recorded the genetic load and inbreeding depression every 100 generations over a period of 10 000 generations after equilibrium had been reached. Equilibrium was ascertained by running two simulations in parallel, one in which all individuals were started free of deleterious mutations, and the other in which all individuals began as heterozygotes; we assumed equilibrium to have been reached when measures of the genetic load in the two parallel simulations converged. For all simulations, we recorded F_{ST} at an additional, unlinked, neutral locus as $1-H_S/H_T$, where $H_T=2pq$ is the expected heterozygosity or gene diversity across the whole metapopulation at this locus, and $H_S=\sum_i 2p_iq_i/D$ is the mean within-deme heterozygosity averaged across all demes i. We also recorded the expected value of F_{ST} in a metapopulation according to the predictive formulae of Whitlock & McCauley (1990) for migrant-pool dispersal:

$$F_{ST}=\frac{(1-e)/2N+e/2K}{1-(1-m)^2(1-1/2N)(1-e)}. \quad (5)$$

Using both the measured and predicted values of F_{ST}, we calculated the predicted genetic load, L_1, with within-deme inbreeding depression, δ_1, and the between-deme inbreeding depression, δ_2, respectively, as given by Whitlock (2002), i.e.,

$$L_1=1-\bar{w}\cong\frac{\mu}{\vartheta}(2h(1-F_{ST})+F_{ST}),$$

$$\delta_1\equiv 1-E\left[\frac{w_{\text{inbred}}}{w_{\text{outbred, within}}}\right]\cong -sf(1-F_{ST})(1-2h)\hat{q},$$

$$\delta_2\equiv 1-\frac{\bar{w}_{\text{inbred}}}{\bar{w}_{\text{outbred}}}$$

$$\cong\frac{\mu(1+F_{ST})F_{TOT}(1-2h)}{(1-(1-2b)F_{ST})(F_{ST}+(1-F_{ST})h)}, \quad (6)$$

where $\vartheta\cong(1-F_{ST}+2bF_{ST})[F_{ST}+(1-F_{ST})h]/(1+F_{ST})$, $F_{TOT}=1-(1-F_{ST})(1-f)$, f is the inbreeding coefficient and $\bar{w}$ is the mean fitness of the metapopulation.

Second, we simulated the case for $L=100$ or $L=1000$, in each case for a range of r between zero and 0·5 (i.e. between no recombination and free recombination between adjacent loci). For mutations to accumulate in the metapopulation, we simulated two metapopulations in parallel, one in which all individuals were started free of deleterious mutations, and the other with 30% deleterious alleles distributed randomly across loci. We began simulations once the mean fitness of these two metapopulations converged. This method did not necessarily mean that the equilibrium was met (see results for lethals in Figs 2, 3, 5 and 6). We then ran the metapopulation model for 10 000 generations, recording the value of inbreeding depression, genetic load and number of fixations in the metapopulation every 2000 generations. For some simulations, equilibrium could not be found even for these longer runs, but the trajectories give an impression of the continued evolution of the metapopulation over periods of time almost certainly longer than one would expect for the life of any real metapopulation. Because we only assumed forward mutation from wild-type to deleterious alleles, the actual genomic mutation rate would be reduced once fixation occurred. To prevent the genomic mutation rate from decreasing with the accumulation of each new fixation, we reset a locus to wild-type state once it was fixed for deleterious alleles. However, fitness was still calculated to include the contribution made by the fixed locus (also see Charlesworth *et al.*, 1993*b*). All results reported below are averages across 100 independent runs of the model (or for 25 runs when $L=1000$).

3. Results

(i) *Comparison with Whitlock's (2002) expressions*

Our simulations for the case of $L=1$ indicate that Whitlock's (2002) approximations for the genetic load and, particularly, within-deme inbreeding depression are quite good if his simplifying assumptions are met, i.e. if selection is relatively weak ($s\leqslant 0{\cdot}01$) and the deleterious allele is maintained at a low frequency at equilibrium; note that between-deme inbreeding depression was less well predicted by Whitlock's (2002) approximations (Fig. 1). We calculated F_{ST} both as $1-H_S/H_T$ and using Whitlock & McCauley's (1990) formula; using these values of neutral F_{ST} in Whitlock's (2002) expressions for inbreeding depression and genetic load produced very similar results (not shown). Whitlock's (2002) approximations were also robust to differences in the migration rate, the dominance coefficient and the extinction rate. The approximations were also valid for inbreeding depression and genetic load in a metapopulation caused by selection at multiple independent loci experiencing free recombination ($r=0{\cdot}5$), assuming that fitness was multiplicative (results not shown). However, when loci were tightly linked, the approximations given by Whitlock (2002) were less satisfactory (see below).

(ii) *The effect of linkage between adjacent loci*

Figures 2–4 illustrate the effect of linkage between selected loci on inbreeding depression and genetic load for a metapopulation without extinction, with different number of loci (100 loci in Figs 2 and 3, 1000 loci in the Appendix), with various recombination rates ranging from $r=0{\cdot}5$ to $r=0$, and with one

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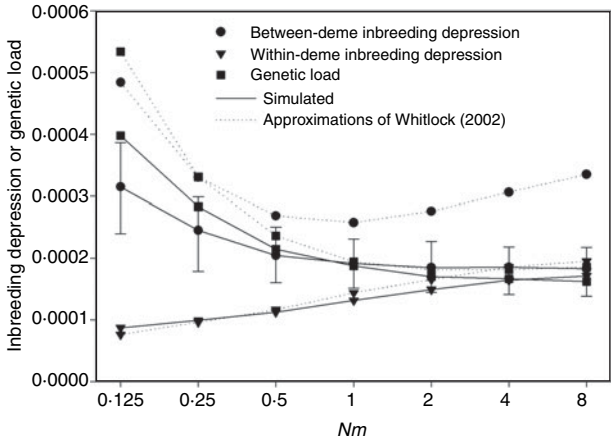

Fig. 1. The fitting of approximations of Whitlock (2002) (dotted lines) to the simulations results (solid lines) with different Nm. Parameter values: $D=100$, $N=100$, $S=0$, $e=0$, $s=0{\cdot}005$, $h=0{\cdot}1$, $\mu=0{\cdot}0001$, $Nm=1$ and $L=100$. Almost all of the predictions of within-deme inbreeding depression and genetic load by approximations of Whitlock (2002) fell within 95% confidence intervals of simulations; for clarity, we thus only provided 95% confidence intervals for between-deme inbreeding depression.

migrant entering each deme per generation. In all simulations, the two different kinds of genetic load, L_1 and L_2, showed similar patterns in our simulations; we thus cover them together here.

The effects of restricted linkage between adjacent loci on the genetic structure of a metapopulation depended heavily on the nature of the deleterious mutations. For weak selection ($s=0{\cdot}01$, $h=0{\cdot}1$; panels in the left columns in Figs 2 and 3, and in Fig. 4), a recombination rate higher than $r=0{\cdot}0001$ had a slight effect on the genetic structure of a metapopulation, which is consistent with the conclusion for undivided populations reached by Charlesworth *et al.* (1992). However, genetic interference between closely linked loci compromised the efficacy of selection against these slightly deleterious mutations, which were able to accumulate and resulted in increased genetic load with a decreasing recombination rate (Fig. 2*a* for 100 loci and Fig. 1 in electronic Appendix for 1000 loci). The accumulation of slightly deleterious mutations under restricted recombination was the result of both their fixation, which we rarely observed in simulations assuming free recombination ($r=0{\cdot}5$), and their rise to elevated frequency in general (with the loci remaining polymorphic). However, the effects of recombination on these two processes were different.

As illustrated in Figure 2*b* the effect of recombination on fixation was not monotonic. The largest number of deleterious fixations, and the corresponding load, occurred under very low recombination rates, rather than under complete linkage. This is consistent with results found for an undivided population (Charlesworth *et al.*, 1993*a*). In contrast to the effect of reduced recombination on the fixation process, the increase in load due to the accumulation of weakly deleterious mutations at segregating loci increased monotonically with reductions in r (Fig 2*b*). Moreover, in a metapopulation consisting of many small demes (i.e. $N=20$ in our simulations), the recombination rate at which fixation of deleterious mutations became important was lower than that at which load due to segregating loci became important.

Within-deme inbreeding depression (δ_1), between-deme inbreeding depression (δ_2) and heterosis (δ_3) were strongly affected by close linkage through the rate at which weakly deleterious and partially recessive alleles (e.g. $s=0{\cdot}01$ and $h=0{\cdot}1$) accumulated in the genome (Fig 3*a–c*). The rate of increase in inbreeding depression was increased with a fall in the recombination rate (Fig 3*a–c*), and the increase can be several times high. However, the change in within-deme inbreeding depression was the most dramatic, while heterosis remained the least affected.

Increased selfing reduced the effective amount of recombination, and so the effects of tight linkage on metapopulation genetic structure were decreased with increasing selfing (Fig. 4). This is consistent with predictions for a single undivided population (Charlesworth *et al.*, 1992).

In contrast to its effects on slightly deleterious and partially recessive mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$), very low recombination rates had little effect on the accumulation of lethal and highly recessive mutations ($s=1$ and $h=0{\cdot}01$) (right panels in Figs 2 and 3). Unsurprisingly, lethals could not be fixed even under complete linkage. Hence, the genetic load due to lethals always resulted from segregating loci. However, with the same mutation rate for these two types of mutations, lethals generally resulted in higher inbreeding depression, but much lower genetic load than weakly deleterious mutations (compare the panels on left and right of Figs 2 and 3).

The patterns reported above for weak selection remained unchanged for different numbers of loci in the genome with the same per locus mutation rate. The genomic mutation rate of 0·1 we assumed in our simulations is at the same order of those reported in real populations for 1000 loci (see Fig. 1 in the electronic Appendix), and the values of inbreeding depression and genetic load observed also similar to those observed in nature (Husband & Schemske, 1996).

(iii) *The joint effect of close linkage and gene flow*

The joint effect of close linkage and gene flow also differed for different classes of mutations. Although inbreeding depression and genetic load decreased slightly under close linkage, close linkage did not

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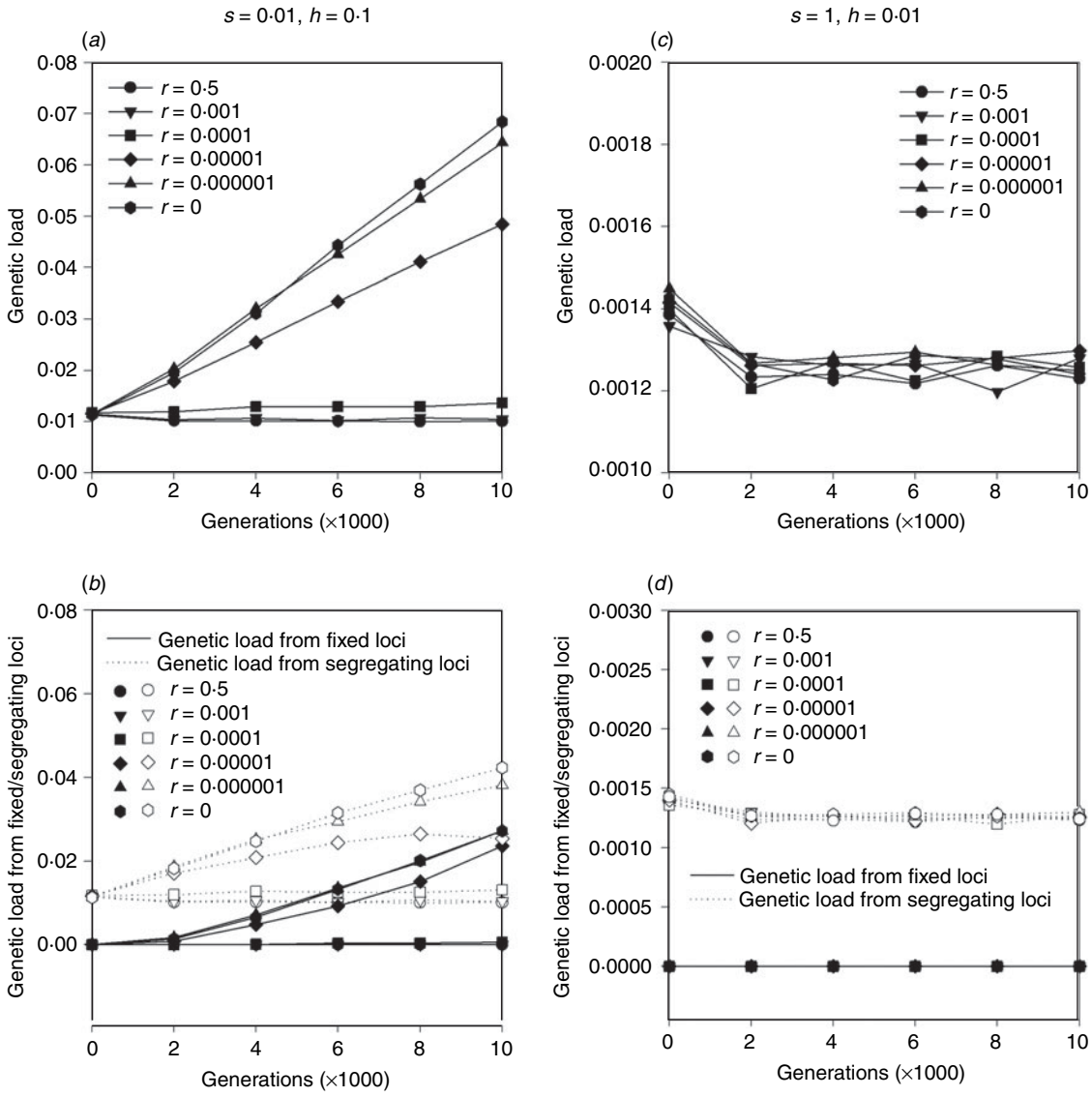

Fig. 2. The effects of recombination rate on genetic load for mildly deleterious mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$) and lethals ($s=1$ and $h=0{\cdot}01$) in a metapopulation under medium migration. Parameter values: $D=50$, $N=20$, $S=0$, $e=0$, $Nm=1$, $\mu=0{\cdot}00005$ and $L=100$.

change the effect of gene flow on accumulation of lethals, inbreeding depression and genetic load (right panels in Figs 5 and 6). However, the joint effect of close linkage and gene flow for weak selection was somewhat complex.

One effect of close linkage was to increase the effect of gene flow on the genetic structure of a metapopulation under weak selection (Figs 5*a*, *b* and 6*a–c*). Under close linkage, gene flow had opposite effects on the fixation of deleterious mutations compared with the accumulation of load at segregating loci. Slightly deleterious mutation could be more easily fixed in small and separated local populations ($Nm=0{\cdot}1$), while high migration rates allowed the operation of genetic rescue, preventing the fixation of these alleles ($Nm=1$ and $Nm=10$) (see also Richards, 2000; Ebert *et al.*, 2002). High migration rates among local populations helped to disperse slightly deleterious mutations at segregating loci. Hence, under close linkage, genetic load caused by the fixation of partially recessive deleterious mutations (e.g. $h=0{\cdot}1$) decreased monotonically, while genetic load caused by segregating partially recessive deleterious increased monotonically with enhanced gene flow.

Close linkage altered the effect of gene flow on patterns of inbreeding depression. As for single locus models, within-deme inbreeding depression increased, while heterosis decreased, with increasing gene flow (Fig. 6*a–c*). It should also be noted that tight linkage was able to reverse the effect of gene flow on between-deme inbreeding depression, resulting in high between-deme inbreeding depression under high

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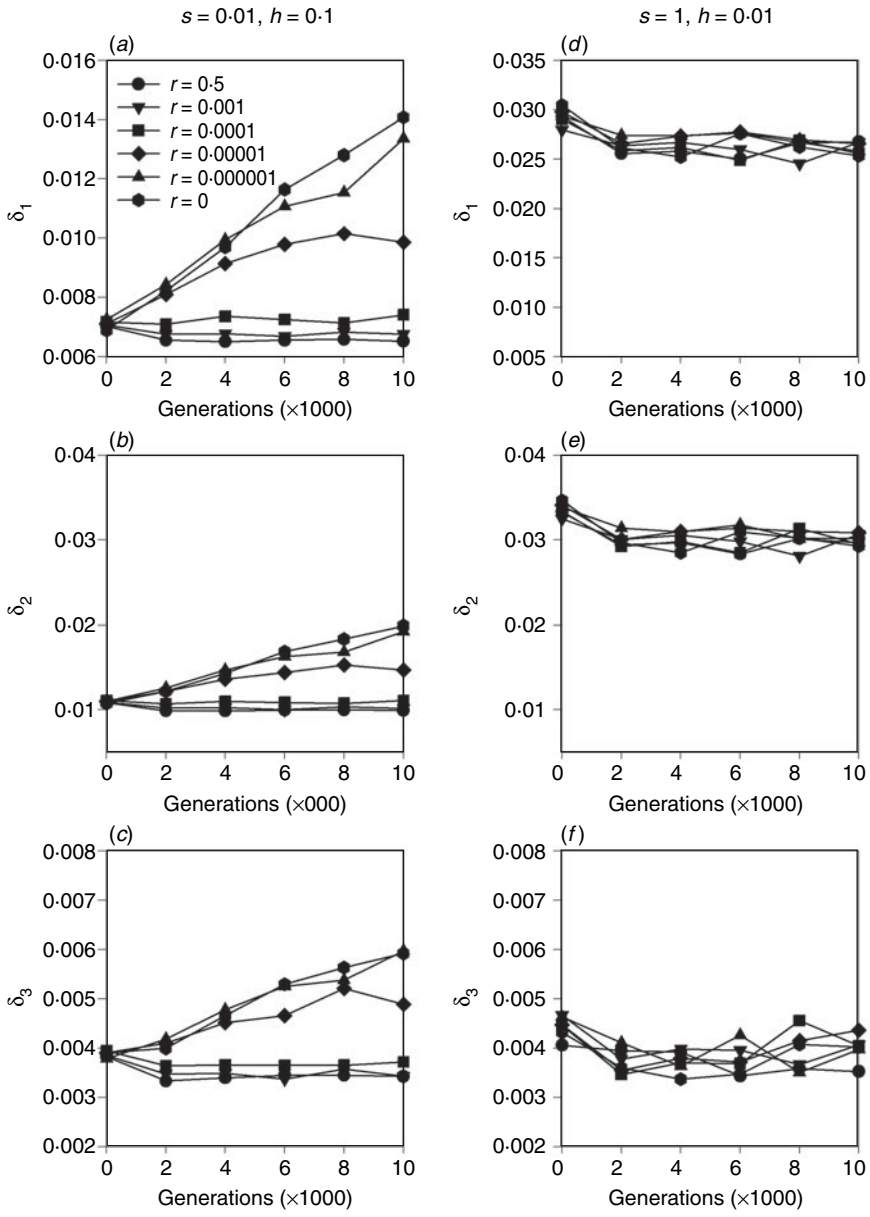

Fig. 3. The effects of recombination rate on the evolution of inbreeding depression (δ_1, δ_2 and δ_3) caused by mildly deleterious mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$) and lethals ($s=1$ and $h=0{\cdot}01$) in a metapopulation under medium migration. Parameter values: $D=50$, $N=20$, $S=0$, $e=0$, $Nm=1$, $\mu=0{\cdot}00005$ and $L=100$.

levels of gene flow. This might have been due to an enhanced rate of mutation accumulation at segregating loci under high migration rates among demes.

(iv) *The joint effect of close linkage and population turnover*

As expected for populations with reduced N_e, high rates of population extinction (e.g. $e=0{\cdot}1$) eased the fixation of deleterious alleles and the accumulation of mutations at segregating loci even with free recombination, especially when the number of colonists to the newly vacant habitat was low (Fig. 7*d*, *e*); reduced recombination enhanced these processes. Overall, frequent population turnover, low numbers of colonizing individuals and low recombination rates tended to result in the genetic deterioration of the metapopulation.

Close linkage increased the effect of population turnover on the level of inbreeding depression (δ_1, δ_2 and δ_3) in a metapopulation (Fig. 7*a–c*). Inbreeding depression was much greater under tight linkage than under free recombination, except in the case of a high local extinction rate, particularly when populations were established by small numbers of individuals. In the latter case, inbreeding depression somewhat decreased under tight linkage compared with that obtained under free recombination.

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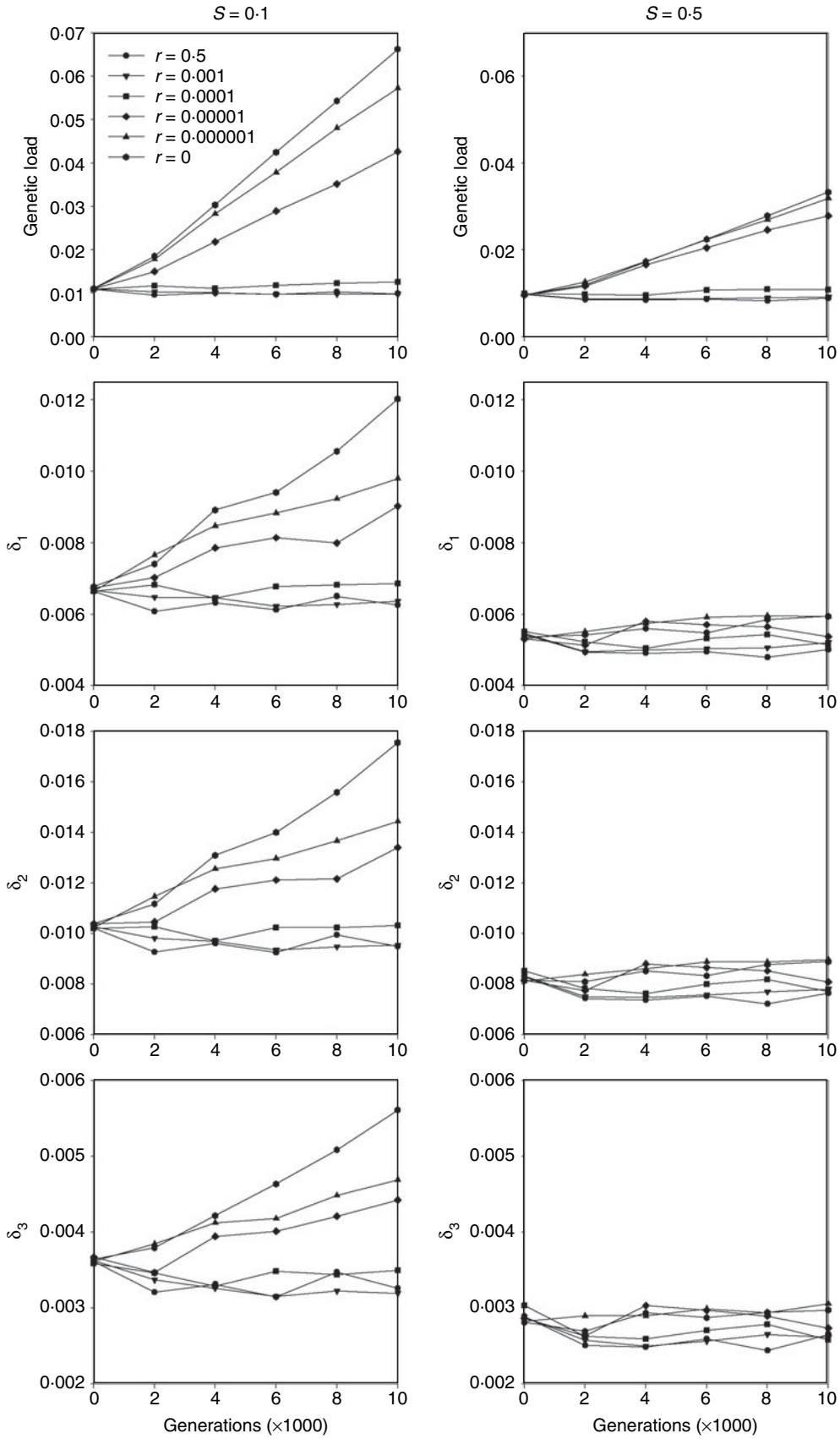

Fig. 4. The effects of recombination rate and selfing rate on the evolution of inbreeding depression (δ_1, δ_2 and δ_3), and genetic load caused by mildly deleterious mutations ($s = 0{\cdot}01$ and $h = 0{\cdot}1$) in a metapopulation under medium migration. Parameter values: $D = 50$, $N = 20$, $e = 0$, $Nm = 1$, $\mu = 0{\cdot}00005$ and $L = 100$.

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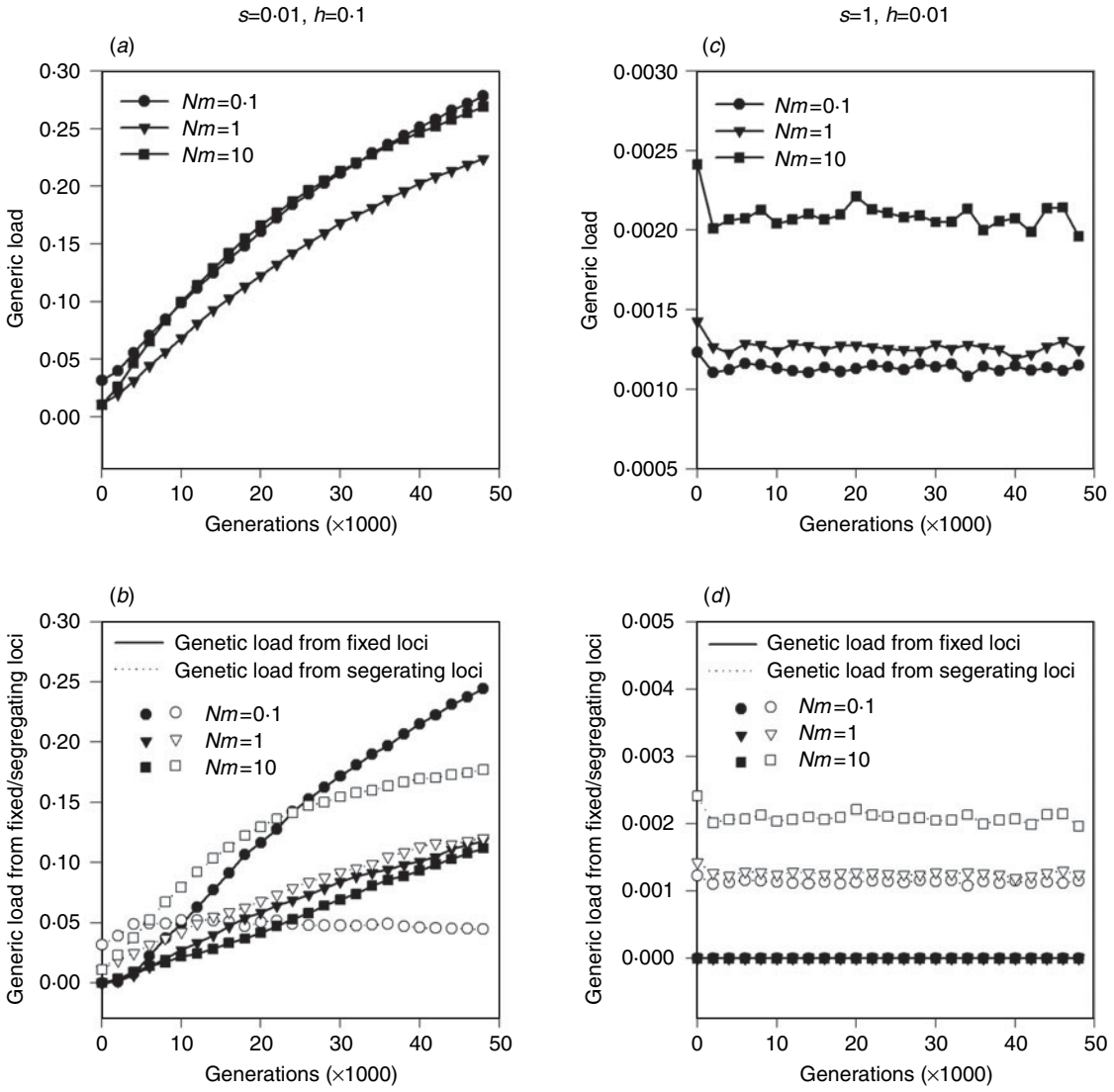

Fig. 5. The effects of migration on the genetic load for mildly deleterious mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$) and lethals ($s=1$ and $h=0{\cdot}01$) in a metapopulation without recombination between adjacent loci. Parameter values: $D=50$, $N=20$, $S=0$, $e=0$, $r=0$, $\mu=0{\cdot}00005$ and $L=100$.

4. Discussion

Our results confirmed that Whitlock's (2002) expressions provide good approximations for the level of inbreeding depression and genetic load maintained in a metapopulation with weak selection. Moreover, although Whitlock's (2002) analysis was explicit for inbreeding depression and genetic load at a single locus, the results hold for multiple independent (unlinked) loci. When loci are tightly linked, however, the approximations given by Whitlock (2002) are no longer valid; tight genetic linkage between adjacent loci can have an important effect on the rate of fixation and accumulation of mutations at segregating loci, and thus on inbreeding depression and genetic load in a metapopulation.

The effect of recombination limitation on mutation accumulation, genetic load and inbreeding depression depended on the types of mutations involved. It is expected that strongly deleterious mutations result in slower rates of accumulation than weakly deleterious mutations (Haigh, 1978; Lynch & Gabriel, 1990; Charlesworth *et al.*, 1993*b*). Our simulations confirmed this conclusion. We considered two extreme types of mutations in our simulations: lethal and recessive mutations ($s=1$ and $h=0{\cdot}01$), and weakly deleterious but partially recessive mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$). Even under close linkage, selection against lethals was still effective, and such lethal mutations hardly fixed or accumulated at segregating loci. For this reason, recombination limitation had little effect on genetic load and inbreeding depression caused by lethal and recessive mutations. This conclusion was also valid for moderate levels of selection (i.e. $s=0{\cdot}1$ and $h=0{\cdot}1$) (results not shown). However, low recombination between adjacent loci

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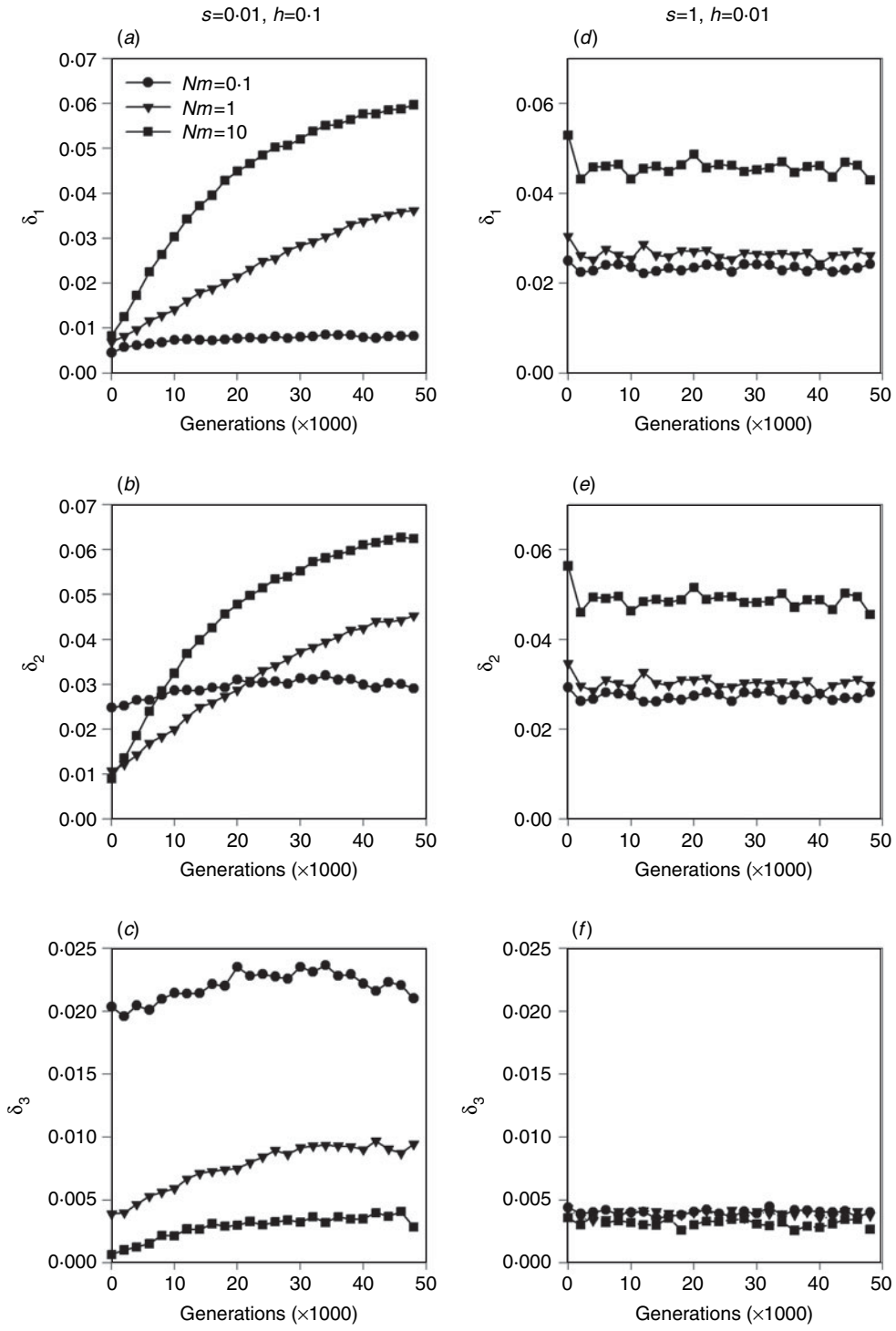

Fig. 6. The effects of migration on the evolution of inbreeding depression (δ_1, δ_2 and δ_3) caused by mildly deleterious mutations ($s=0{\cdot}01$ and $h=0{\cdot}1$) and lethals ($s=1$ and, $h=0{\cdot}01$) in a metapopulation without recombination between adjacent loci. Parameter values: $D=50$, $N=20$, $S=0$, $e=0$, $r=0$, $\mu=0{\cdot}00005$ and $L=100$.

had important effects on the accumulation of weakly deleterious and partially recessive mutations.

Highly restricted recombination had different effects on load due to segregating mutations compared with that due to their fixation. In our simulations, the accumulation of load at segregating loci was always slowed down by an increase in recombination between adjacent loci. This pattern is thus true for both subdivided and undivided populations (cf. Charlesworth *et al.*, 1993*a*). As a consequence, the genetic load due to segregating mutations and within-deme inbreeding depression tend to increase with a decrease in recombination rate. In contrast, the rate of fixation of weakly deleterious mutations was not a

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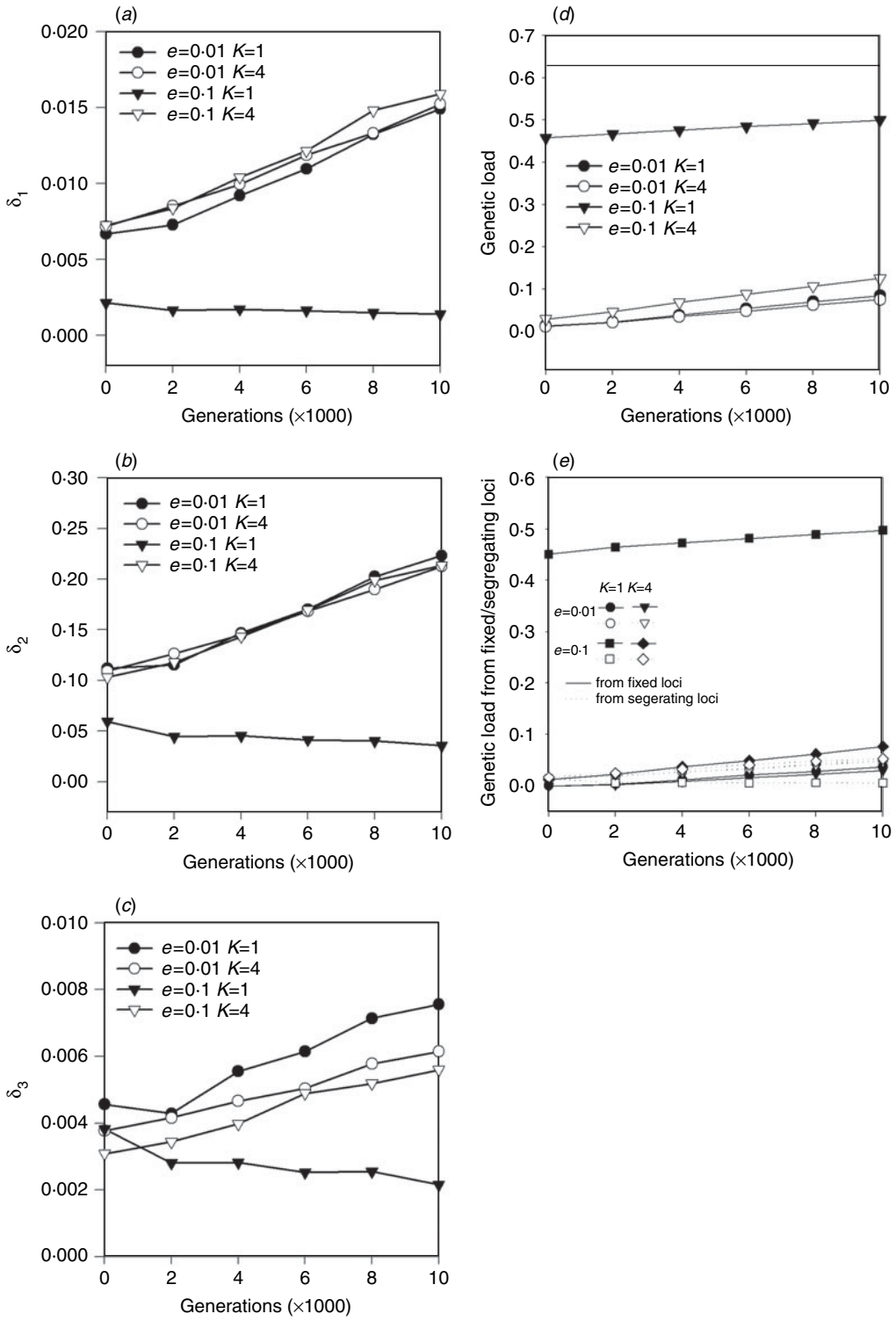

Fig. 7. The effects of population turnover on the evolution of inbreeding depression (δ_1, δ_2 and δ_3) and genetic load in a metapopulation without recombination between adjacent loci. Parameter values: $D=50$, $N=20$, $S=0$, $Nm=1$, $s=0{\cdot}01$, $h=0{\cdot}1$, $r=0$, $\mu=0{\cdot}00005$ and $L=100$.

monotonic function of the recombination rate. In particular, the largest number of fixations and the highest genetic load occurred with very low recombination rates, rather than under complete linkage. These results can be understood in part in terms of the Hill–Robertson effect and Muller's Ratchet (Muller, 1964; Hill & Robertson, 1966).

The Hill–Robertson effect, a reduction in the efficacy of selection at one locus by interference from other selected loci which it is linked (Hill &

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Robertson, 1966; Felsenstein, 1974), has long been invoked to explain reduced efficiency of selection with decreased recombination rates. For strong selection, the effect of interference is lower (Kimular & Maruyama, 1966; Haigh, 1978; McVean & Charlesworth, 2000), whereas selective interference can have considerable effects on the fate of weakly deleterious alleles (McVean & Charlesworth, 2000; Comeron & Kreitman, 2002). In our simulations, the Hill–Robertson effect provides a plausible explanation for the increased rate of fixation and the accumulation of weakly deleterious mutations with decreased recombination rates observed in our simulations. The association between new mutants and wild-type alleles at other loci may persist for a long time if they are closely linked, and selection against the new deleterious mutations will be weakened by selection in favour of wild-type alleles at other loci, thus promoting the accumulation and fixation of deleterious mutations under tight linkage (Li, 1987; Birky & Walsh, 1988).

In the complete absence of recombination, Muller's Ratchet may also contribute to the processes of the accumulation and fixation of deleterious mutations by the irreversible and successive loss of the least-loaded class by drift (Muller, 1964; Felsenstein, 1974; Gessler, 1995; Gordo & Charlesworth, 2000*a*, *b*; Combadão *et al*., 2007). However, under such conditions of tight linkage, gametes are likely to carry many deleterious alleles, so that the fixation of one mutant allele will often be accompanied by the fixation of other linked mutations on the same chromosome. This process should reduce fitness considerably, so that tight linkage might be expected to work against the chance fixation of mutant alleles and to result in a decreased rate of fixation of weakly deleterious mutations.

In our simulations, linkage increased both inbreeding depression and heterosis caused by weakly deleterious alleles (Fig. 3). This may seem non-intuitive at first glance, because genetic differences between local populations might decrease with increasing local genetic diversity. However, it can be understood in the context of selection. The purging of deleterious alleles through recombination was clearly retarded under tight linkage. Hence, mutations increasingly accumulated as the recombination rate between adjacent loci was reduced (Fig. 2). In this case, the combination of haploid genomes from different individuals through outcrossing, either within a deme or between demes, caused the loss of deleterious mutations and an increase in offspring fitness. Inbreeding depression, heterosis and genetic load are thus expected to increase with decreased recombination rate. Interestingly, genetic drift can bring about similar effects to those caused by reduced recombination. Thus, drift is expected to bring about increased genetic load in small populations. For instance, Bataillon & Kirkpatrick (2000) developed analytic approximations for inbreeding depression and load in very large and very small populations, and numerical values from simulation results were given for intermediate population sizes. Although they found that genetic load increased as population size became smaller, in contrast to our results they found that the drift caused a decrease in the inbreeding depression of the population. It would thus seem that reduced recombination has effects on the genetic load that cannot be summarized simply in terms of drift.

It is well known that patterns of inbreeding depression and genetic load in natural populations depend strongly on the kind of deleterious mutations involved. Weakly deleterious and moderately recessive mutations can be fixed in small populations or in strongly subdivided populations (Kimular *et al*., 1963; Whitlock *et al*., 2000). For lethal and very recessive alleles, population size and population structure can also affect their fates (Hedrick, 2002; Glémin, 2005). Our results demonstrate that weakly deleterious and moderately recessive mutations can be easily fixed under tight linkage in a metapopulation, but we found that recombination limitation between adjacent loci had little effect on the accumulation of lethal mutations and the corresponding genetic load and inbreeding depression (Figs 2, 3 and 5). The contrasting effects of linkage on weakly deleterious alleles and lethals suggest that the approximation and patterns of genetic load and inbreeding depression obtained in a metapopulation under free recombination may still be valid for lethals but not for tightly linked deleterious alleles of small effect.

We found that migration always affected within-deme inbreeding depression and heterosis in a similar way, i.e. inbreeding depression increases while heterosis decreases monotonically with enhanced gene flow among local demes. This is consistent with previous studies (Theodorou & Couvet, 2002; Whitlock, 2002; Glémin, *et al*., 2003; Roze & Rousset, 2004). However, the effects of migration on mutation load and between-deme inbreeding depression were relatively complicated. In our simulations, when recombination was not possible, mutation load from deleterious alleles at segregating loci increased with increased migration, while the patterns for mutation load from fixed loci depended on the types of mutations involved. Under tight linkage, mutation load from fixed weakly deleterious alleles decreased with the migration rate, while lethal mutations were never fixed (Fig. 5). The increase in between-deme inbreeding depression with gene flow was probably an outcome of a balance between these two effects (Fig. 6).

We found that the total mutation load reached a maximum value under medium migration rates for weak selection but increased monotonically with gene

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flow for lethals (Fig. 5). These effects of migration on the mutation load and on between-deme inbreeding depression also appeared under free recombination. Whitlock (2002) concluded that medium variance among local populations (i.e. intermediate migration rates) should result in the lowest mutation load under soft selection when mutations are mildly deleterious and partially recessive, whereas between-deme inbreeding depression due to the same class of mutations increases with decreased variance among demes. Roze & Rousset (2004) also found that medium migration rates could help to purge weakly deleterious and partially recessive mutations, thus minimizing mutation load.

In our simulations, we assumed the genomic mutation rate towards lethals to be 0·01, one-tenth of the mutation rate towards slightly deleterious alleles. As tight linkage largely increases inbreeding depression caused by weakly deleterious alleles, the level of inbreeding depression caused by non-lethal deleterious alleles may be an order of magnitude higher than that caused by lethals under close linkage (comparing Figs 2, 3 and Fig. 1 in electronic Appendix). Previous studies have suggested that lethal and non-lethal deleterious mutations probably contribute about equally to the amount of inbreeding depression (Simmon & Crow, 1977; Charlesworth & Charlesworth, 1987), but this may not be true for tightly linked loci. Restricted recombination appears to increase considerably the inbreeding depression caused by weakly deleterious mutations, but even complete linkage has little effect on the level of inbreeding depression caused by lethals (Fig. 3). Because of this, it seems that inbreeding depression caused by weakly deleterious mutations could be much higher than that caused by lethals under close linkage.

Our study has shown that the effect of linkage depends on the type of mutations involved, and that a recombination higher than $r = 0{\cdot}0001$ has little effect on the genetic structure of a metapopulation, whereas population structure and close linkage jointly affect the genetic structure of a metapopulation. These are exceptionally low recombination rates (e.g. the average realistic recombination rate estimated for human may be an order of magnitude higher; Graffelman *et al*., 2007), so that the effects we have identified are likely to apply mainly to genomic regions with restricted recombination rates, such as recombination cold spots (Arnheim *et al*., 2003; Graffelman *et al*., 2007), or to completely non-recombining elements such as Y chromosomes (Charlesworth, 1978, 1996). In this sense, our results suggest that genomic regions with low recombination rates could contribute more than other regions to inbreeding depression.

Finally, we also tested the robustness of our results for different population structures and found similar patterns for different population structures with the same total number of individuals and the same intensity of gene flow. These results are relevant for the evolution of inbreeding depression and genetic load in a metapopulation, with implications for the evolution of the mating system. On the one hand, reduced recombination could increase the level of inbreeding depression (Fig. 3), and thus decrease the likelihood for the evolution of increased selfing rates. On the other hand, however, self-fertilizing reduces the effective rate of recombination (see Fig. 4; Charlesworth *et al*., 1992). Hence, the evolution of the mating system probably depends on the joint effects of the recombination rate and initial selfing rate of the population. Further detailed investigation is clearly needed.

This work was supported by Royal Society China Fellowship, NSFC (30670314, 30970543), NCET and PCSIRT.

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