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Deleterious mutations in a hybrid zone: can mutational load decrease the barrier to gene flow?

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Summary

The aim of this paper is to investigate the effect of deleterious mutations in a hybrid zone maintained by selection against hybrids. In such zones, linkage disequilibria among hybrid depression loci, resulting from a balance between migration and selection, are crucial in maintaining the barrier because they allow each locus, in addition to its own selection coefficient, to cumulate indirect selective effects from other loci. Deleterious alleles produce heterosis and increase by this means the effective migration rate in structured populations. In a hybrid zone, they therefore contribute to decrease linkage disequilibria as well as the barrier to gene flow imposed by hybrid depression. However, deleterious mutations have no effect: (i) when selection against hybrids is weak, because linkage disequilibria are small even without heterosis in this case, or (ii) when selection against hybrids is so strong that it overwhelms heterosis. On the other hand, with moderate selection against hybrids, the decrease in the strength of the barrier due to heterosis may reach detectable levels, although it requires relatively small population sizes and/or migration rates. The effect is expected to be small and only within small genomes where loci are tightly linked can it become strong. Nevertheless, neglecting mutational load may to some extent obscure the estimations of selective parameters based either on artificial F1 crosses or on cline characteristics.

1. Introduction

Hybrid zones have often been shown to fit well the tension zone model (Barton & Hewitt, 1985, 1989). In this model, the homogenizing effect of migration and recombination is counterbalanced by selection against hybrids. This results in geographic clines at the loci causing the hybrid breakdown. The level of introgression at neutral loci depends primarily on the number and distribution of loci causing hybrid breakdown: it will be higher when selection is distributed on few loci of large effects than when selection is distributed on many loci of small effects.

There is a gradient of situations from independently selected loci to completely 'congealed' genomes (Kruuk *et al.*, 1999; Turner, 1967), well analysed in the multilocus cline theory (Barton, 1983; Kruuk *et al.*, 1999). Kruuk *et al.* (1999) have defined the 'summed coupling coefficient' $\phi = (L-1)s/r$, where L is the total number of loci under selection against hybrids, s is the relative strength of the selection acting on each locus and r is the rate of recombination. When ϕ is small (say $\phi < 1$), the effect of recombination is strong enough to eliminate associations between loci and there is no linkage disequilibrium. In this situation, evolution at a particular locus is independent of other loci and can be predicted knowing only its own selection coefficient by single locus theory. For example, neutral diffusion clines are expected for neutral molecular markers.

When ϕ is large (say $\phi > 4$), recombination is not strong enough to dissipate linkage disequilibrium created each generation. Linkage disequilibrium is therefore maximal and the whole genome behaves as a single locus under selection of magnitude Ls. Between these two states, as ϕ increases, linkage disequilibrium

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increases and the behaviour of each locus is increasingly affected by selection on other loci (see figure 4 in Kruuk *et al*., 1999).

Tension zone theory has been used to interpret clinal patterns observed in natural hybrid zones and to quantify the main parameters involved in this model: migration, selection and the number of loci involved in the hybrid breakdown (Barton, 1980; Barton & Hewitt, 1981; Szymura & Barton, 1986; Mallet *et al*., 1990). However, both the theory and its applications have been developed using several simplifying assumptions (equal strength of selection on each selected loci, equilibrium, uniform densities, etc.). Most of these assumptions have been discussed and their impact on expected patterns fairly well established theoretically. Moreover, empirical methods have been developed to test their validity (e.g. by comparing clines at several loci, at different times and in different places, respectively). The aim of the present study is to investigate an implicit assumption underlying the tension zone theory that has received very little attention so far: that the influence of deleterious mutations is negligible in a tension zone.

There are some empirical results in hybrid zones that show that genes involved in the reproductive isolation such as underdominants are not the only one implied. Reduction of fluctuating asymmetry in some central hybrid zone populations has been considered as evidence of heterotic effects (Alibert *et al*., 1994) and heterosis is often observed in the F1s even if hybrid breakdown appears in the F2s (Edmands, 1999). Models considering only loci involved in hybrid breakdown are indeed too simple. As soon as linkage disequilibrium exists, any kind of selection elsewhere in the genome (e.g. deleterious or favourable mutations, balancing selection) may affect the system or, reciprocally, the presence of a hybrid zone may affect other types of selection elsewhere in the genome. The presence of directional selection on a beneficial mutation in a tension zone has been studied (Barton, 1979; Pialek & Barton, 1997). The spread of a beneficial mutation, although delayed, is never stopped by the tension zone unless it is confined in its genomic background by underdominance. However, the reciprocal effect of a beneficial mutation on a genetic barrier has not been considered (probably because it is transient), neither has the presence of selection against partially recessive deleterious mutations.

In small structured populations at mutation–migration–selection-drift equilibrium, among-population variance in the frequency of partially recessive deleterious alleles may be sufficiently high to generate heterosis upon crossbreeding (Whitlock *et al*., 2000). Migrants therefore have an increased inclusive fitness over residents, resulting in an increased effective migration rate (Ingvarsson & Whitlock, 2000). Therefore, in hybrid zones, the effect of deleterious mutations may counteract selection against hybrids (Ingvarsson & Whitlock, 2000).

Barrier strength inferred from artificial hybridization could be underestimated if heterosis is neglected. At first approximation, if one imagines that two populations of equal size $N_e = 10^3$ are isolated by a barrier impermeable to deleterious mutations, the expected heterosis (following Whitlock *et al*., 2000) with usual genomic deleterious parameters (genomic mutation rate: $U = 1$, dominance: $h = 0{\cdot}1$, and selection against homozygotes: $s = 0{\cdot}1$) is expected to be approximately 8 % in F1 hybrids (and is still approximately 1 % when $N_e = 10^4$). Hybrid depression should therefore be corrected by this factor. Similarly, the use of expectations of the cline width or linkage disequilibria when deleterious mutations are neglected may lead to an underestimation of the selection coefficients.

We use a simulation approach to investigate the effect of deleterious mutations on underdominant loci. Although the range of parameters explored is of course restricted, the advantage of simulations is that it allows the combination of two phenomena already only tractable with difficulty in isolation. Heterosis operates only with finite population size (Kimura *et al*., 1963). In this situation, random drift decreases the efficiency of selection against hybrids (Felsenstein, 1975; Slatkin & Maruyama, 1975; Nagylaki, 1978) as well as against deleterious mutations (Li, 1978). We focus on linkage disequilibria between underdominant genes as a measure of the barrier. The advantage of this statistic is that it simultaneously informs about the strength of the barrier (as does cline width) and about the degree of associations among genes (Kruuk *et al*., 1999).

2. Methods

(i) *Model*

A Borland Delphi 4.0 program, adapted from the single population model described in Bierne *et al*. (2000), was written to simulate a stepping stone model of migration. In this model, a single chromosome with a map length of C morgans (M) was modelled. Positions of loci were not predefined but drawn from a uniform law. Two types of loci were considered: a fixed number (L) of underdominant loci, whose positions were drawn at random at the beginning of each simulation; and a variable number of loci with deleterious mutations. The latter were defined when deleterious mutations occurred (Poisson-distributed with rate U per generation per diploid genome) and their position was drawn at random at the same time. We were therefore under the genomic deleterious mutation framework with no constraint on the total number of mutable loci in the genome. In contrast, selection against hybrids was not equally distributed on an infinite number of loci along the genome (e.g. Baird, 1995)

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but restricted to a fixed number of loci, L (e.g. Barton & Gale, 1993; Kruuk *et al*., 1999).

We considered a secondary contact of two populations initially fixed for alternative alleles at the L underdominant loci. To model this situation, we used a classical one-dimensional stepping-stone model: two infinite populations were in contact via a chain of small demes (500 demes when $\phi<1$; 100 demes when $1<\phi<2$ and 50 demes when $\phi>2$), each of size N (Feldman & Christiansen, 1974). The migration rate between subpopulations was m (half in either direction). Migration was followed by mutation, reproduction and selection. Recombination rate, r, was related to the map distance between loci, d, using Haldane's mapping function or $r=0{\cdot}5$ between all the loci (be they deleterious or underdominants) for the model without linkage.

To account for selection, randomly drawn offspring survived until reproduction with a probability proportional to their fitness. The individual multilocus fitness was given multiplicatively by:

$$W=(1-s_{\mathrm{u}})^{x}(1-s_{\mathrm{d}})^{y}(1-hs_{\mathrm{d}})^{z}, \tag{1}$$

where s_{u} is the selection coefficient against heterozygotes at underdominant loci and x is the number of heterozygous underdominants ($0<x<L$), h is the dominance coefficient and s_{d} the selection coefficient against deleterious homozygotes, y and z are the numbers of deleterious mutations in homozygous and heterozygous state, respectively.

Simulations were checked as explained in Kruuk *et al*. (1999). We also have used two kinds of end demes: (i) free of deleterious mutations or (ii) at mutation–selection equilibrium using large-population approximations for the number of mutations per individual ($U/(hs_{\mathrm{d}})$; Crow & Kimura, 1970). We verified that a sufficient number of demes was used to obtain the same results in both cases (20 demes on each side of the cline was enough). This ensured that local mutation–migration–selection-drift equilibrium was reached within the tension zone, without edge effects due to the output or input of deleterious mutations from/to peripheral populations.

(ii) *Simulation*

For each set of parameters 10 pairs of runs were performed; within each pair, the first run was with, and the second without deleterious mutations ($U=0$). For each run, various measures were recorded after migration and reproduction every 200 generations between generations 1000 (which was enough to reach a steady state for all the range of parameters we have investigated, even with strong coupling, e.g. $\phi=40$) and 4000, resulting in 15 measures per run. They were averaged for each run. The measures recorded were as follows.

First, a hybrid depression coefficient was estimated as follows: the program first located the two demes of each 'subspecies' located at the opposite edges of the cline, i.e. the first demes, from the central deme outwards, where allele frequencies at underdominant loci reach 0 and 1 respectively. Using the genotypes from these two demes, hybrid depression was calculated as:

$$H=1-(W_{\mathrm{F1}}/W_{\mathrm{within}}), \tag{2}$$

where W_{within} is the mean fitness of offspring of an intrademe cross, and W_{F1} that for a F1 hybrid cross. Note that a series of simulations was also performed without a hybrid zone ($L=0$) to infer heterosis ($H=(W_{\mathrm{between}}/W_{\mathrm{within}})-1$) by drift load alone as a function of the distance between demes.

Second, pairwise linkage disequilibrium across underdominant loci, D, was estimated in the central deme (with allele frequencies at underdominant loci closest to 0·5). A simple estimate of D can be obtained from the variance in the hybrid index (see Barton & Gale, 1993), Var(Z), as described in Kruuk *et al*. (1999):

$$\bar{D}=\frac{1}{2L(L-1)}\left[\mathrm{Var}(Z)-\sum_{i=1}^{L}H_i\right], \tag{3}$$

where H_i is the heterozygote frequency under Hardy–Weinberg equilibrium at locus i. The differences between D when genomes were free of deleterious mutations and D when deleterious mutations segregated was termed ΔD. For each set of parameters (10 pairs of runs), D was averaged across the 10 runs of each type (with and without deleterious mutations), while ΔD was calculated for each pair of runs and then averaged over the 10 pairs.

Some parameters were fixed over all runs. The number of underdominant loci was $L=10$. The migration rate was $m=0{\cdot}2$. Deme size was $N=50$. These values of N and m ($Nm=10$) are not an optimal set of parameters for heterosis by drift load to occur (Whitlock *et al*., 2000). The variance in allele frequencies generating heterosis is therefore mostly created by the genetic barrier to gene flow due to underdominant loci (see Section 3). Genomic deleterious mutation parameters were fixed to $U=1$, $h=0{\cdot}1$ and $s=0{\cdot}1$. It is expected that the qualitative effect of genomic deleterious mutation parameters should be the same as described in length by Whitlock *et al*. (2000) and Ingvarsson & Whitlock (2000).

The strength of the barrier to gene flow is dependent on selection and recombination but these two parameters are not analogous. Selection is responsible for both the existence of clines and the level of interaction among loci whereas linkage only promotes interaction. In other words, linkage is not needed whereas selection is. To study the effect of selection alone, we first performed a set of runs without physical linkage ($r=0{\cdot}5$),

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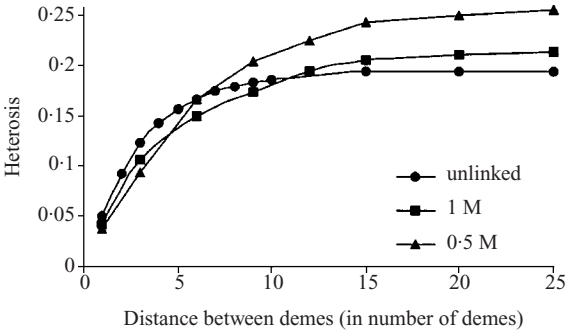

Fig. 1. Heterosis as a function of the distance between demes in the one-dimensional stepping stone model for various genome sizes (unlinked, 1 M, 0·5 M) in the absence of underdominant loci. Stepping stone parameters are $N=50$ and $m=0{\cdot}2$, genomic deleterious parameters are $U=1$, $h=0{\cdot}1$ and $s_d=0{\cdot}1$.

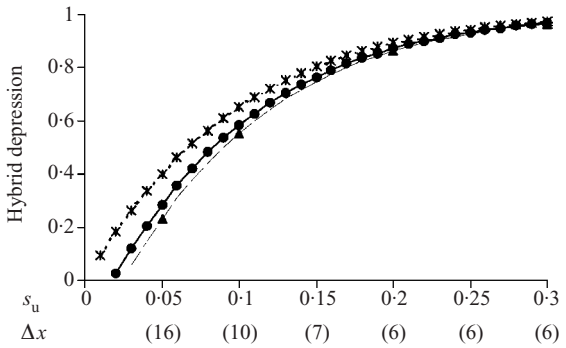

Fig. 2. Hybrid depression without deleterious mutations (stars), with deleterious mutations without physical linkage (circles) and with deleterious mutations and physical linkage (genome size of 0·5 M; triangles) as a function of underdominance, s_u. The rounded average number of demes, Δx, separating the closest two demes fixed for alternative alleles at underdominant loci is indicated in parentheses for information. The 95% CI are confounded with symbols and are not represented. The small dotted line represents expectations based on theoretical fitnesses without heterosis (equations 1 and 2 with $x=L$ for F1 hybrids and $x=0$ for the parental genotype). The other two lines are obtained by adding to hybrid fitnesses a fixed heterotic effect equal to the maximum possible with deleterious mutations only, from Fig. 1: +20% without linkage (continuous line) and +26% with linkage (large dotted line). Stepping stone parameters are $N=50$ and $m=0{\cdot}2$, the number of underdominants is $L=10$, genomic deleterious parameters are $U=1$, $h=0{\cdot}1$ and $s_d=0{\cdot}1$.

changing the selection coefficient against heterozygotes at underdominant loci, s_u, from 0·1 to 0·2 with 0·01 increments and from 0·2 to 0·3 with 0·05 increments ($0{\cdot}2<\phi<6$). To study the effect of physical linkage we analysed two reference situations: (i) a case where genomes did not congeal (i.e. weak linkage disequilibrium) in the absence of physical linkage, obtained by setting $s_u=0{\cdot}05$ ($\phi=1$ when $r=0{\cdot}5$); and (ii) a case where genomes were already partially congealed (moderate or large linkage disequilibrium) in the absence of physical linkage, obtained by setting $s_u=0{\cdot}1$ ($\phi=2$ with $r=0{\cdot}5$). For each of these two reference situations, the effect of linkage was studied by decreasing the genome length from 5 to 0·1 M.

3. Results and discussion

On both sides of the barrier to gene flow caused by the hybrid zone, different deleterious mutations segregate in the genetic background of each subspecies. These mutations tend to be in a heterozygous state in hybrids, resulting in heterosis which partially offsets the hybrid breakdown due to the underdominant loci. The two selection pressures counteract each other although they act on different loci. When all loci are in strong linkage disequilibrium, the two sources of selection tend to cancel out: hybrids are on average fitter than would be predicted by selection against hybrids alone.

(i) *Heterosis effect*

To better appreciate the effect of deleterious mutations in a hybrid zone, consider first the heterosis effect achieved in artificial crosses (i) between genitors from different demes of the same subspecies (i.e. in the absence of hybrid depression loci) and (ii) between subspecies. Fig. 1 presents the heterosis in crosses between conspecifics from increasingly distant demes (in number of demes) for the genomic deleterious parameters used throughout the present study. Heterosis increases with distance as expected, and converges to its maximum value (Glemin *et al.*, submitted). Linkage between deleterious mutations, neglected in analytical approximations (Whitlock *et al.*, 2000; Glemin *et al.*, submitted), tends to increase both the distance needed to reach the maximum value and the maximum value itself (Fig. 1). However, with the parameters we used in our simulations, approximately 15 demes are sufficient to reach the maximal heterosis. Fig. 2 presents the measured hybrid depression in artificial F1 crosses between genitors from peripheral demes of the hybrid zone (i.e. the closest two demes fixed for alternative alleles at underdominant loci) as a function of underdominance, s_u. This shows that, as expected, heterosis introduces a downward bias in the estimation of hybrid depression in the F1. This bias corresponds to an approximately 20% increase in the hybrid fitness for the unlinked model and approximately 25% for a map length of 0·5 M, with our parameters. This corresponds to the maximal heterosis obtained in the absence of underdominant loci (see Fig. 1). These values are expected as a simple effect of distance when the cline at underdominant loci is so large that the distance between peripheral demes exceeds approximately 15 demes (see Fig. 1). However, it is remarkable that in the presence of underdominants, heterosis between peripheral demes remain maximal even when their distance becomes fewer than 15 demes. This occurs in our

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simulations when underdominant selection is strong enough (say $s_u > 0{\cdot}1$). Therefore, for sufficiently strong deleterious selection ($N_e s_d > 1$), two populations taken outside the cline on either side do not effectively exchange deleterious mutations whatever the width of the cline. Indeed, it can be shown that the local genetic load in a one-dimensional stepping stone model is equivalent to the genetic load in an isolated single Wright–Fisher population with population size $N_e^s = N(1+2m/hs)^{1/2}$ (Glemin *et al.*, submitted). The fitness bonus obtained in F1s corresponds to the heterosis expected between two completely isolated populations of size N_e^s. The effect should be detectable in experimental crosses provided that N_e^s were sufficiently small (say $N_e^s < 10^3$) and that the non-genetic variance in the fitness traits measured was not too strong.

(ii) *Interaction between selection against hybrids and selection against deleterious mutations*

Within a cline, selection against deleterious mutations interacts with selection against hybrids in a complex manner. Differences in allele frequencies between adjacent demes are maintained by selection against the action of migration and recombination. Co-variation in allele frequencies among different loci results in linkage disequilibria (D) that tend to reinforce the effect of selection at each locus (Kruuk *et al.*, 1999). The phenomenon is to a certain extent self-sustained because strong selection enhances differences in allele frequency along the cline, and therefore favours linkage disequilibrium. On the other hand, differences in deleterious mutation frequencies produce a heterosis effect that tends to increase the effective migration rate and genetically homogenize adjacent demes. This should decrease D and reduce the impact of indirect selection.

ΔD, the decrease in D due to the deleterious mutational load, when all loci are unlinked, is presented as a function of ϕ in Fig. 3. The value of D without deleterious mutation is presented as a second axis to illustrate the link between ϕ and D. Linkage disequilibrium appears at $\phi = 1$ and is maximal at $\phi > 4$. The maximal reduction in D due to deleterious mutations is reached for intermediate values of linkage disequilibrium ($2 < \phi < 3$) and is rather small ($\Delta D_{max} \sim 0{\cdot}02$, i.e. 12% of the D value). When selection is very strong ($\phi > 3$), heterosis created by deleterious mutations is overwhelmed by selection against underdominants and the effect of deleterious mutations vanishes. The case of genomes completely congealed by selection against hybrids corresponds to such a strength of selection against hybrids that the effect of deleterious mutations is almost negligible. Contrary to the heterosis gained in artificial crosses, the effect of deleterious mutations on the barrier strength is not constant and does depend on the barrier itself.

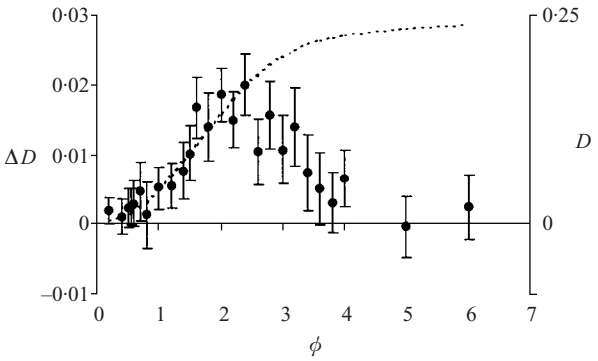

Fig. 3. Linkage disequilibrium without deleterious mutations (D; dotted line, second axis) and change in linkage disequilibrium, ΔD, due to the presence of deleterious mutations (dots; mean and 95% CI on 10 runs). Positive ΔD values correspond to a decrease in linkage disequilibrium. The values of ϕ are manipulated by changing s_u (see Section 2) with no physical linkage. See Fig. 2 and Section 2 for the values of other parameters.

(iii) *Effect of linkage*

ΔD is presented as a function of ϕ when linkage is modified, for $s_u = 0{\cdot}1$ (Fig. 4*A*) and $s_u = 0{\cdot}05$ (Fig. 4*B*). Fig. 4*A* represents a case in which underdominance is strong enough to maintain large linkage disequilibria even without physical linkage ($Ls_u = 1$). In this case increasing linkage has little effect, as expected (Fig. 4*A*). The effect of the mutational load remains approximately constant when map length is decreased, in contrast to its behaviour when selection is increased (compare Figs 2 and 4*A*). On the other hand, when underdominance is insufficient to maintain large D without physical linkage ($Ls_u = 0{\cdot}5$), increasing physical linkage does have a strong effect on the barrier to gene flow (Fig. 4*B*). In this case, deleterious mutations can have important effects ($\Delta D_{max} \approx 0{\cdot}1$, which means halving D compared with the situation without mutational load). As previously, the effect of deleterious mutations persists for maximal physical linkage. Therefore, if the tension relies on tight linkage and moderate selection against hybrids (rather than no linkage and strong selection), neglecting mutational load may strongly bias the estimations of selection coefficients.

However, when the barrier strength is manipulated by physical linkage rather than by selection intensity, maximal linkage disequilibria are obtained for far larger values of ϕ. Therefore the simple knowledge of ϕ seems insufficient to predict the behaviour of the hybrid zone, contrary to expectations (Barton, 1983). The reason is that the coupling coefficient, s_u/r, is assumed to be always small in Barton (1983). When this assumption is true, all the useful descriptive statistics of multilocus clines are functions of ϕ. This approximation works very well when $r = 0{\cdot}5$, even for strong selection (Barton & Gale, 1993; Kruuk *et al.*, 1999), but is much less accurate when linkage is tight (Baird,

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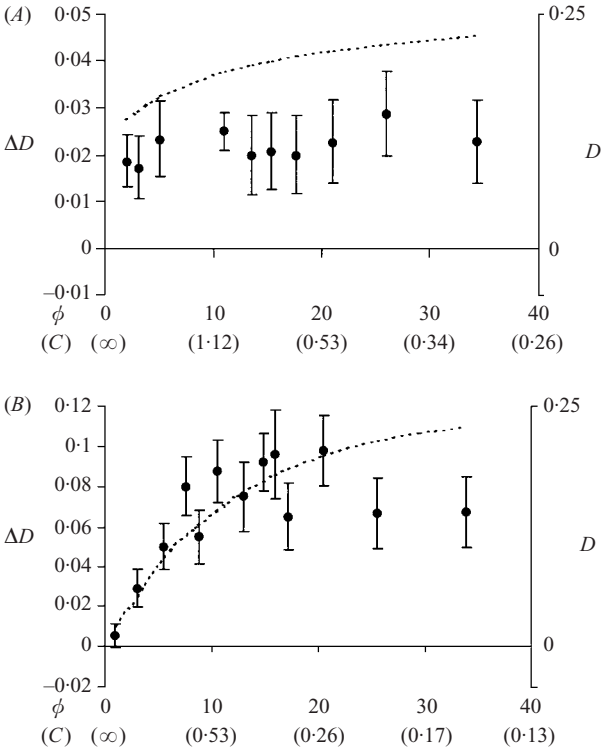

Fig. 4. Linkage disequilibrium without deleterious mutations (D; dotted line, second axis) and change in linkage disequilibrium, ΔD, due to the presence of deleterious mutations (dots; mean and 95% CI on 10 runs). The values of ϕ are manipulated by changing map length C (see Section 2) with $s_u = 0{\cdot}1$ (*A*), $s_u = 0{\cdot}05$ (*B*). See Fig. 2 and Section 2 for other parameter values.

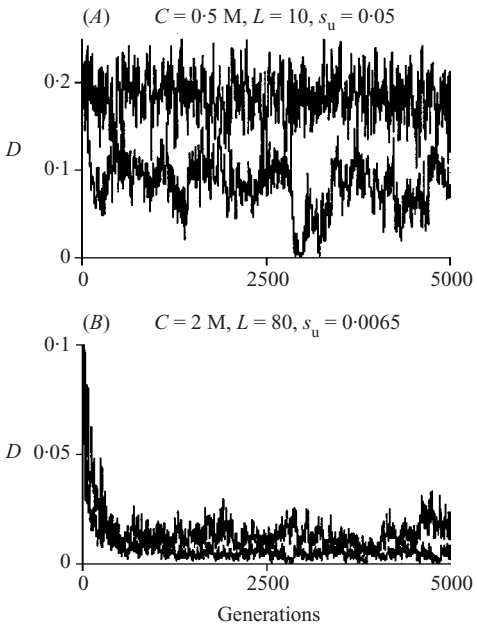

Fig. 5. Evolution of linkage disequilibria for two genome sizes with the same summed coupling coefficient ϕ. The 'small' genome is $C = 0{\cdot}25$ M with $L = 10$ underdominants and $s_u = 0{\cdot}05$ (*A*) and the 'larger' genome is $C = 2$ M with $L = 80$ underdominants and $s_u = 0{\cdot}0065$ (*B*). In each case, the top curve is without and the lower curve with deleterious mutations. Stepping stone parameters are $N = 50$ and $m = 0{\cdot}2$, genomic deleterious parameters are $U = 1$, $h = 0{\cdot}1$ and $s_d = 0{\cdot}1$.

1995; Kruuk *et al*., 1999). However, a closer look at Fig. 4 reveals that the map lengths needed to obtain large values of ϕ (say, $\phi > 10$) are very small (less than 0·5 M) with only $L = 10$ underdominants. Another effect of linkage is sometimes to strongly delay the time to reach a steady state (Baird, 1995). To further investigate these two effects (gene density and time to reach equilibrium), we have compared the evolution of D for two genome sizes with the same summed coupling coefficient ϕ: a chromosome of 0·25 M with $L = 10$ underdominants and $s_u = 0{\cdot}05$ and a chromosome of 2 M with $L = 80$ (i.e. same recombination rate between adjacent loci) and $s_u = 0{\cdot}0065$ (Fig. 5). For the small genome, a steady state is reached very quickly and large disequilibria are maintained. The time to reach the steady state is slightly delayed when deleterious mutations are segregating, simply because smaller disequilibria are maintained in this case (Fig. 5*A*). A different result is obtained when the same total amount of selection (Ls_u) is distributed into more loci in a bigger genome. Far smaller disequilibria are maintained at equilibrium with a slighter influence of the mutational load and a longer time to reach the steady state (Fig. 5*B*). The efficiency of selection against hybrids is more affected by random drift in this case. Therefore, for deleterious mutations to have a strong effect, not only should the linkage between loci be tight but the selection on each underdominant locus should be sufficiently strong to be efficient despite drift. All in all, it appears that the effect of deleterious mutations on a genetic barrier to gene flow can only be strong in small genomes.

(iv) *Is heterosis by drift load an important component of hybrid zones?*

Although it is clear that deleterious mutations do have an effect on hybrid zones, it also seems that the effect is very small with the parameters we have used. The deleterious mutation parameters used here ($h = 0{\cdot}1$, $s_d = 0{\cdot}1$) should already maximize heterosis (Whitlock *et al*., 2000). For heterosis by drift load to have a more pronounced effect, one should consider a smaller population size or migration rate. However the population size and migration rate we have used ($N = 50$, $m = 0{\cdot}2$) seem already very small for most organisms for which a hybrid zone has been studied (Barton & Hewitt, 1985). In addition, with smaller population sizes, drift overwhelms selection (be it deleterious or against hybrids) so much that we can hardly identify the hybrid zone any longer, especially since it may usually involve many loci of small effects (see results with 80 loci of very small effect in Fig. 5).

In our model, heterosis is due only to mutations newly arising within demes either side of the hybrid

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zone and drift. Much more heterosis could be expected if we consider a secondary contact between two historically isolated subspecies that have fixed alternative deleterious alleles in allopatry (given sufficient time and small population sizes). However, in this case the corresponding wild-type alleles provided by the other subspecies act as dominant favourable alleles and quickly invade all populations across the barrier (Pialek & Barton, 1997). In other words, 'historical' heterosis vanishes relatively quickly. It is therefore improbable, except for very young secondary contacts, that heterosis inherited from before the contact can still be observed today. For this reason, current mutations, although their effect is weak, seem more relevant to empirical studies.

Given the weakness of the observed effect, the present model cannot alone explain the fitness advantage sometimes seen in F1 hybrids (Dobzhansky, 1952; Arnold, 1997; Edmands, 1999). Our results indeed suggest that deleterious mutations cannot completely mask hybrid depression in the F1 (let alone generate F1 superiority) when underdominance is the sole source of the barrier. In this context, heterosis in the F1 still seems to be a paradox, as it is clear that genetic barriers must rely on some form of selection against hybrids (Barton & Hewitt, 1985, 1989; Barton, 1986, 2001). However, the debate on how this selection operates (Arnold & Hodges, 1995; Arnold, 1997) has tended to confound several classes of hybrids (e.g. F1s, F2s, backcrosses) (Barton, 2001). When F1s happen to be fitter than parental genotypes, the maintenance of a hybrid zone requires that other kinds of hybrids be counter-selected (Barton, 2001) and therefore lies with the genetic architecture of the barrier itself (Turelli & Orr, 2000). Although underdominance is a common shortcut to represent hybrid breakdown in models (but see Barton & Shpak, 2000), the lack of F1 disadvantage is only compatible with more complex architectures, such as Dobzhansky–Muller incompatibilities (Dobzhansky, 1937; Muller, 1940, 1942; Orr, 1996) and dominance by dominance epistasis (Turelli & Orr, 2000). However, although such a determinism can explain that hybrid breakdown appears in the F2s only (Turelli & Orr, 2000), it does not predict heterosis in F1s unless the fitness landscape was more pronouncedly distorted. The source of this heterosis should then be found in other genes not involved in the genetic barrier. Overdominant genes (or genes with positive epistatic interactions) could do the job, but very transiently, as they are supposed to quickly cross the barrier and be homogenized throughout the hybrid zone (Goodisman & Crozier, 2001). Invoking the mutational load to explain the fitness bonus in the F1s seems then completely plausible.

Finally, deleterious mutations are of course not the only selected genes that can decrease the strength of a genetic barrier. It is known that favourable mutations do cross such barriers very efficiently (Barton, 1979; Pialek & Barton, 1997) and each new favourable mutant is therefore expected to transiently increase the relative fitness of hybrids. Although favourable alleles may have detectable effects for a wider range of situations (e.g. large population size), their action is ephemeral while that of deleterious mutations is weak and permanent. In the long term, selection on genes not involved in reproductive isolation, due to the concomitant effect of the mutational load and recurrent cross of favourable alleles through the barrier, is expected to speed up the introgression of neutral alleles, but only for moderate barriers.

4. Conclusion

Tension zones are effective barriers not only to underdominants but also to gene flow of deleterious mutations and therefore allow the maintenance of differences in deleterious mutation frequencies resulting from a non-negligible, but small, heterosis effect even with a little drift (e.g. $N_e = 500$). It may therefore be useful to use intraspecific crosses between geographically well-separated populations as references to estimate the mutational load before interpreting interspecific crosses, at least when the effects detected in the latter are small. A combination of crosses from populations shared out along a grasshopper hybrid zone have proved useful to extract the genetic basis of hybrid inviability from other factors (Barton & Hewitt, 1981). Even if they do not effectively cross the tension zone, deleterious mutations decrease the strength of the barrier to gene flow and favour gene exchange between two subspecies. This effect is mainly significant when selection against hybrids is due to moderate selection coefficients on tightly linked loci. In practice, neglecting mutational load may obscure the estimations of selective parameters based (i) on artificial F1 crosses and (ii) on cline characteristics. Correction of biases requires independent estimations of the genetic load.

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