The evolution of strong reproductive isolation

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ABSTRACT

Felsenstein distinguished two ways by which selection can directly strengthen isolation. First, a modifier that strengthens prezygotic isolation can be favored everywhere. This fits with the traditional view of reinforcement as an adaptation to reduce deleterious hybridization by strengthening assortative mating. Second, selection can favor association between different incompatibilities, despite recombination. We generalise this "two allele" model to follow associations amongst any number of incompatibilities, which may include both assortment and hybrid inviability. Our key argument is that this process, of coupling between incompatibilities, may be quite different from the usual view of reinforcement: strong isolation can evolve through the coupling of *any* kind of incompatibility, whether prezygotic or postzygotic. Single locus incompatibilities become coupled because associations between them increase the variance in compatibility, which in turn increases mean fitness if there is positive epistasis. Multiple incompatibilities, each maintained by epistasis, can become coupled in the same way. In contrast, a single-locus incompatibility can become coupled with loci that reduce the viability of haploid hybrids because this reduces harmful recombination. We obtain simple approximations for the limits of tight linkage, and strong assortment, and show how assortment alleles can invade through associations with other components of reproductive isolation.

INTRODUCTION

Theoretical models of speciation have focussed on how individual incompatibilities might evolve, and on how incipient isolation might be reinforced by the evolution of adaptive mate preferences. For example, reproductive isolation can evolve through the random fixation of underdominant mutations (Wright, 1941; Lande, 1979), by random drift along ridges in the adaptive landscape (Nei, 1976; Gavrilets, 1999; 2004), or by the substitution of selectively favored alleles that prove incompatible with each other (Dobzhansky, 1936; Muller, 1940, 1942; Orr, 1997). Once some reproductive isolation has been established, selection then favours changes that reduce the loss of fitness due to mating between incipient species, thus reinforcing isolation (Wallace, 1889; Dobzhansky, 1940). This last possibility has received much attention, in the context of both parapatric and sympatric speciation (Turelli et al., 2001; Servedio and Noor, 2003; Bolnick and Fitzpatrick, 2007), in part because it gives a direct role for natural selection in speciation. In this paper, we draw attention to a different, and somewhat neglected, question: how do independent incompatibilities become coupled together, to build up a strong barrier to genetic exchange? This issue is implicit in the existing literature (in particular, in Felsenstein's influential (1981) analysis), but has not received an explicit and comprehensive treatment.

In allopatric speciation, different sets of alleles accumulate, and necessarily remain associated with each other as a simple consequence of geographic isolation. However, the coupling together of different components of reproductive isolation is an important part of speciation in both sympatry and parapatry. First, consider purely sympatric divergence. We can imagine that in a single, initially panmictic, population, balancing selection maintains multiple polymorphisms, and that some of these involve disruptive selection, favouring lower frequencies of heterozygotes and recombinants. As long as these different polymorphic systems remain independent of each other (i.e., in linkage equilibrium), the population may appear homogeneous, and there may be little variation in fitness between different mating pairs. However, once arbitrary sets of alleles become associated, the population may condense into two subpopulations that are largely incompatible. Under what circumstances will a single polymorphic population fragment in this way?

Similar issues are involved when divergence occurs in parapatry. Different incompatibilities may arise and spread independently of each other, and will not form a strong barrier to gene flow unless they are brought together, to separate two distinct populations. Even if divergent populations do meet in secondary contact, differences that initially coincide may scatter, leading to collapse of an initially strong barrier (e.g. Hatfield et al., 1992; Shuker et al., 2005). When will a strong barrier, which combines multiple

incompatibilities, be stable? This paper concentrates on the coupling of incompatibilities within an initially homogeneous population, as explained in the previous paragraph. However, the converse process, in which divergence collapses after secondary contact, is closely related.

In one of the first explicit models of speciation, Felsenstein (1981) distinguished between "one-allele" and "two-allele" mechanisms. In the former, one allele is favoured everywhere, because it reduces the formation of unfit genotypes. This reduction might arise through assortative mating (Felsenstein, 1981, Sanderson, 1989), reduced dispersal (Balkau and Feldman, 1972) or reduced recombination (Trickett and Butlin, 1994). Such models fit with the classical view of reinforcement as an adaptation to reduce hybridisation; the evolution of modifiers that strengthen isolation can be analysed in the same way as for modifiers of recombination or dispersal (De Cara et al., 2008, Otto et al., 2008). In two-allele models, in contrast, different alleles are favoured in different places or in different incipient species, so that recombination opposes divergence. In Felsenstein's (1981) model, alleles at one locus cause assortative mating; these may become associated with alleles at two other loci that cause postzygotic isolation by reducing the fitness of haploid recombinants.

Both one- and two-allele models are usually regarded as forms of reinforcement (e.g. Servedio and Noor, 2003), and indeed, in both cases selection to reduce the formation of unfit hybrids leads to stronger isolation. However, the processes involved are quite different. The classical view of reinforcement is that selection can only strengthen prezygotic isolation, not postzygotic, because selection cannot favor a *further* reduction in the fitness of hybrids (Wallace, 1889; Dobzhansky, 1940). (Selection can favour a reduced fitness of juveniles where these compete with siblings, but the principle is the same; Coyne, 1974, Cronin, 1991). This argument applies where a single allele strengthens isolation, but not when isolation is strengthened by an *association* between existing incompatibilities. As we show below, the two different incompatibilities then do not have to be at different stages of the life cycle: each may have the same status, and we cannot say that one evolves 'in order to' reinforce the other. The evolution of the association itself can be seen as adaptive, in the sense that (directly or indirectly) it raises the mean fitness of the population. However, it can involve incompatibilities at any stage of hybridisation.

Speciation has received surprisingly little theoretical attentione, despite its central importance to evolutionary biology. Even now, most theoretical studies are based on simulation, which limits their value (see reviews by Gavrilets, 2004; Kirkpatrick and Ravigné, 2002). Analysis of speciation is difficult, because many of the usual approximations do not hold when selection is strong, as is necessarily the case when substantial reproductive isolation evolves within a single population. However, the

development of associations between independent incompatibilities lends itself to a general analysis, because there is a fundamental symmetry: initially, the incompatibilities are in linkage equilibrium with each other, and the associations that develop are as likely to be positive as negative. Therefore, the key problem is to find the stability of the initial symmetrical state: do weak associations grow or shrink?

We begin by examining the simplest model of two loci, one interpreted as subject to selection against heterozygotes, and the other with assortment amongst haploid individuals. This can be seen as a simplified version of Udovic' (1980) model of coupling between a locus with underdominance, and one that causes assortment amongst *diploids*; it is equivalent to the symmetric viability model, which was originally developed to describe *overdominance* at multiple loci (Lewontin and Kojima, 1960; Franklin and Lewontin, 1970; Karlin and Feldman, 1978; Christiansen, 1999, Ch. 8). Whether we interpret the loci as inducing assortment among haploids or underdominance in diploids is arbitrary: once allele frequencies are taken as fixed, the population genetics are identical. When effects multiply across loci, there is a simple criterion for the stability of the symmetrical equilibrium. This result extends to many such loci, interacting in arbitrary ways; a simple analytical expression for the threshold still obtains. We then turn to Felsenstein's (1981) model of an assortment locus and a pair of epistatically interacting loci, and give an explicit solution. Finally, we generalise Felsenstein's model in two ways: to examine the coupling amongst multiple pairs of loci, and then the coupling of multiple genes with a single locus that causes assortative mating. In all these models, we allow for strong selection and assortment. Although our analysis is restricted to a single population, it suggests extensions to the parapatric case, where multiple incompatibilities meet across a cline.

METHODS

We derive our results in two ways. First, for general multilocus systems, we use the notation of Barton and Turelli (1991), to derive recursions for linkage disequilibria involving any number of genes. (See Kirkpatrick et al., 2002, for a more general account of this method). Second, for examples with up to 6 loci, we use *Mathematica* to give explicit recursions for the gamete frequencies. These are used for our numerical examples, but by starting these recursions with symbolic expressions for the gamete frequencies in terms of allele frequencies and linkage disequilibria, we have an independent check on our more general derivations. The *Mathematica* packages used to derive these recursions are available from http://www.biology.ed.ac.uk/research/institutes/evolution/software.php, and the code used to generate recursions by the two methods is available as Supplementary Information (SI 7-9).

Throughout, we will assume that some form of frequency dependence keeps the allele frequencies constant. Of course, the allele frequency dynamics may differ between assortment and disruptive selection. Following Felsenstein (1981), one might add frequency dependent selection to an underdominant locus to stabilise the polymorphism (e.g. Wilson and Turelli, 1986), and one might demand cost-free assortment, so that an assortment locus is neutrally stable. However, this does not alter the population genetics of linkage disequilibrium, given that there is a polymorphic equilibrium, and so does not affect the stability of the equilibrium towards growth of linkage disequilibrium. (Some care is needed here. Frequency dependent *epistasis* would affect the stability of linkage disequilibria. However, if the assortment and selected loci are independent in their effects on fitness, then it is reasonable to assume that there is no frequency dependent epistasis between these functionally independent sets of loci. We focus on the dynamics of linkage disequilibria, but not of allele frequencies).

We begin by briefly summarising the general recursions for allele frequencies and linkage disequilibria. We assume that selection and non-random mating act symmetrically across the two sexes. (This assumption is not restrictive: it would be straightforward to extend our results to cases where selection is asymmetric). There are two alleles at each of two loci, with frequencies $q_i,\ p_i$ at locus i, and similarly at locus j. Following Kirkpatrick et al. (2002), we define an indicator variable X_i which represents alternative alleles by 0 or 1, and mostly work with the deviation of X_i from its mean, $E[X_i] = p_i$. We denote this indicator by $\zeta_i = X_i - p_i$, which has mean zero, and takes values $-p_i,\ q_i$. The state of a haploid population is described by the allele frequencies, and by the linkage disequilibria $D_U \equiv E[\zeta_U]$, where $\zeta_U \equiv \prod_{i \in U} \zeta_i$, and U is a set of loci. The genotypes of the two haploid genomes that make up a diploid individual are indicated by the pair of vectors $\zeta_i,\ \zeta_i^*$, Throughout, we denote loci by lowercase letters, and sets of loci by uppercase letters: for example, U might denote a set $\{i,\ j,\ \ldots\}$ of loci. We refer to the complete set of all loci involved as Ω.

The fitness of a diploid genotype is defined as its contribution to the next generation, immediately after meiosis, relative to what would be expected from the product of haploid genotype frequencies. This includes viability selection on haploids, non-random mating amongst haploids, and viability selection on diploids. We can imagine that, at the beginning of the generation, a hypothetical diploid population is formed, with genotype frequencies assumed to equal the product of the haploid frequencies. Thus, $D_{U,V} \equiv E[\zeta_U\, \zeta_V^*] = D_U\, D_V$. The relative fitness of a diploid genotype is then written as a polynomial:

$$\frac{W}{\bar{W}} = 1 + \sum_{U,V \subseteq \Omega} a_{U,V}\, (\zeta_U\, \zeta_V^* - D_U\, D_V) \qquad (1)$$

where the coefficients $a_{U,V}$ represent the strength of selection on the set U of loci in one haploid genome and the set V in the other. Here and below, the sum is over non-empty subsets of Ω, denoted by $U,\ V \subseteq \Omega$. Non-random mating between haploid individuals is represented in exactly the same way as viability selection on diploids: both distort the contribution from sets of genes $U,\ V$ from the two haploid genomes. (We follow Kirkpatrick et al. (2002) in using this definition for the selection coefficients; it differs slightly from Barton and Turelli (1991), but leads to simpler recursions and is easier to generalise).

To simplify the dynamics, we begin with a hypothetical stage at which haploid gametes are randomly united. Assortative mating is followed by selection on diploids. Crucially, both these stages are described by a single set of selection coefficients, $a_{U,V}$, whether the frequency changes are due to non-random mating amongst the gametes or to selection in the diploid phase.

With these definitions, selection changes allele frequencies by:

$$\begin{aligned} p_i' &= p_i + \sum_{U\subseteq\Omega} \tilde{a}_U\, D_{iU} \quad \text{where } \tilde{a}_U \equiv \sum_{V\subseteq\Omega} a_{U,V}\, D_V \\ D_{U,V}' &= D_{U,V} + \sum_{X,Y\subseteq\Omega} a_{X,Y}\,\left(D_{XU}\, D_{YV} - D_X\, D_Y\, D_U\, D_V\right) \end{aligned} \tag{2}$$

(Eqs. 9, 10 in Kirkp[atrick et al., 2002). The leading terms often contain associations that involve repeated indices. With two alleles per locus, labelled 0 or 1, these can be reduced using the relation:

$$D_{iiU} = h_i\, D_U - \Delta_i\, D_{iU} \tag{3}$$

where $h_i \equiv p_i\, q_i$, $\Delta_i = p_i - q_i$; $D_\emptyset = 1,\ D_i = 0$, and $\emptyset$ is the empty set.

Recombination leaves allele frequencies unchanged and alters linkage disequilibria by:

$$D_U'' = \sum_{ST=U} r_{S,T}\, D_{S,T}' \tag{4}$$

where $r_{S,T}$ is the rate of recombination events in which the set of loci S is inherited from one parent, and T from the other. Note that non-random mating amongst haploids, and selection on diploids, leads to deviations from Hardy-Weinberg proportions (i.e., $D_{S,T} \neq D_S\, D_T$), which alter the effect of recombination. Since we assume throughout that allele frequencies are constant, we do not need to account for a final step, in which linkage disequilibria are defined relative to a new set of allele frequencies (Kirkpatrick et al., 2002).

UNDERDOMINANCE AND ASSORTMENT DUE TO THE EFFECTS OF SINGLE LOCI

Two loci that cause assortment or underdominance

The simplest model that addresses the coupling of assortment with disruptive selection involves two loci: one controlling assortment amongst haploid gametes, and the other with underdominance at the diploid zygote stage. In fact, assortment and selection are mathematically equivalent: both lead, at allele frequency equilibrium, to a reduced contribution of heterozygotes to the next generation. It is unusual to mix assortment amongst haploids with selection on diploids. However, such a model is biologically possible, and could apply (for example) to a marine organism in which haploid gametes disperse independently, and fertilize selectively, based on compatibility of their haploid genotypes.

We assume that allele frequencies are at equilibrium. If we look at one locus at a time, then from Eq. 2a, allele frequencies will stay constant provided that fitness does not depend on any terms involving the allelic effects $\zeta_i,\ \zeta_i^*$ alone. Therefore, the fitness contribution of a single locus is $(1 + s_i\, \zeta_i\, \zeta_i^*)$, where s_i measures the strength of underdominance. If fitnesses multiply across loci, and if the population is at linkage equilibrium (LE), then allele frequencies will remain unchanged under this form of selection. However, if there is linkage disequilibrium between loci, and if allele frequencies are asymmetric ($p_i \neq \frac{1}{2}$), then allele frequencies will change. Therefore, we can use this model to find whether the state of zero linkage disequilibrium is stable to changes in linkage disequilibrium for arbitrary allele frequencies. However, when we examine the full dynamics of linkage disequilibrium, we restrict attention to the symmetric case $p_i = \frac{1}{2}$. Analysis of the dynamics with asymmetric allele frequencies is possible, but more involved, because we must include terms involving $\zeta_i,\ \zeta_i^*$ alone in order to stabilise allele frequencies in the presence of linkage disequilibria. By analysing equal allele frequencies, we are focussing on the case most conducive to evolution of stronger isolation; we believe that polymorphisms with extreme allele frequencies will be much less likely to couple.

We begin by looking at associations between two loci, labelled $i,\ j$. The contribution of each diploid genotype at LE must be $W = (1 + s_i\, \zeta_i\, \zeta_i^*)\,(1 + s_j\, \zeta_j\, \zeta_j^*)$. The fitness contribution of the three genotypes at locus i is:

$$1 + s_i\, p_i^2 \;:\; 1 - s_i\, p_i\, q_i \;:\; 1 + s_i\, q_i^2 \qquad (5)$$

and similarly for locus j. Because fitnesses cannot be negative, $s_i\, p_i\, q_i \leq 1$; at the threshold $s_i\, p_i\, q_i = 1$, reproductive isolation is complete.

From Eq. 1, the selection coefficients are:

$$\bar{W}\, a_{i,i} = s_i \qquad \bar{W}\, a_{j,j} = s_j \qquad \bar{W}\, a_{ij,ij} = s_i\, s_j \qquad \text{where } \bar{W} = 1 + s_i\, s_j\, D_{ij}^2 \tag{6}$$

Here, $a_{i,i}$ denotes selection favouring an association between the two homologous alleles at locus i in a diploid, and $a_{ij,ij}$ denotes selection favouring an association between *pairs* of genes $i,\ j$ in the two genomes carried by a diploid. Where we consider the stability of the symmetrical equilibria ($D_{ij} = 0$), the mean fitness can be taken as $\overline{W} = 1$.

If a fraction α of individuals mate assortatively according to their genotype at some specific locus i, and the remainder mate at random, then we have $\alpha = s_i\, p_i\, q_i$. Under this 'cost-free' model of assortment, which was used by Felsenstein (1981), allele frequencies do not change. In the obvious extension to two loci, a fraction α would mate with the same haplotype. Then, the fitness of each haplotype would be the same, and linkage disequilibria would not build up. This is *not* the same as the multiplicative model used here: rather, it is a special case in which the relation between $a_{ij,ij}$ and the $a_{i,i},\ a_{j,j}$ is such that associations remain constant.

The mean fitness, $\overline{W}$, increases with the magnitude of linkage disequilibrium, whether it is positive or negative; this is still true when there is overdominance or disassortment at both loci ($s_i,\ s_j < 0$, rather than $s_i,\ s_j > 0$). This increase in mean fitness with linkage disequilibrium reflects the nonlinear dependence of fitness on the number of heterozygous loci: the arithmetic average fitness of a double heterozygote and a double homozygote is greater than the average of the single heterozygotes, and so mean fitness increases with the variance of the number of heterozygous loci (Fig. *1*). We will see that it is this selection for increased variance in heterozygosity that drives the increase of linkage disequilibrium that couples different components of reproductive isolation. At linkage equilibrium (LE), the definition of Eq. *5* ensures that $\overline{W} = 1$. With complete linkage disequilibrium, only two haplotypes are present, so that $p_i = p_j,\ D_{ij} = p_i\, q_i$. Three diploid genotypes are present; from Eq. *5*, these have fitnesses:

$$(1 + s_i\, p^2)\,(1 + s_j\, p^2) : (1 - s_i\, p\, q)\,(1 - s_j\, p\, q) : (1 + s_i\, q^2)\,(1 + s_j\, q^2) \tag{7}$$

and mean fitness is increased to $\overline{W} = 1 + s_i\, s_j\, p^2\, q^2$.

We denote the rate of recombination events that separate loci $i,\ j$ as $r_{i,j}$. From Eqs. 2, 4, linkage disequilibrium changes as:

$$D'_{ij} = (1 - r_{i,j})\, D_{ij} + r_{i,j}\, D_{ij}\,(a_{i,i}\, h_i + a_{j,j}\, h_j) + a_{ij,ij}\, D_{ij} \\ (r_{i,j}\, \triangle_i\, \triangle_j\, D_{ij} + (1 - r_{i,j})\,(h_i\, h_j + \triangle_i\, \triangle_j\, D_{ij} - D_{ij}^2)) \tag{8}$$

where $h_i = p_i\, q_i$, $\Delta_i = (p_i - q_i)$. An initial state of linkage equilibrium will be unstable to increases in D_{ij} in either direction if:.

$$(1 - r_{i,j})\,(1 + a_{ij,ij}\, h_i\, h_j) + r_{i,j}\,(a_{i,i}\, h_i + a_{j,j}\, h_j) > 1 \qquad (9)$$

(That is, $|D''_{ij}| > |D_{ij}|$ for small $|D_{ij}|$). Substituting for $a_{i,i} = s_i$, $a_{ij,ij} = s_i\, s_j$, and rewriting the threshold in terms of the recombination rate, we have instability if:

$$r_{i,j} < r^*_{i,j} = \frac{s_i\, s_j\, h_i\, h_j}{(1 - s_i\, h_i)\,(1 - s_j\, h_j)} \qquad (10)$$

Recall that $s_i\, h_i \le 1$ if fitnesses are to be non-negative; heterozygosity at locus i reduces fitness below the mean by a factor $(1\text{-}s_i\, h_i)$. This reduction in the fitness can be due to either underdominance, or to assortative mating amongst haploids: in the latter case, if a fraction α_i of individuals mate assortatively for alleles at locus i, then $\alpha_i = s_i\, h_i$.

With weak selection ($s_i\, h_i$, $s_j\, h_j << 1$) linkage must be very tight for coupling to evolve., but the threshold recombination rate can become arbitrarily high as selection approaches the maximum possible at each locus, and linkage disequilibrium can develop even with no linkage ($r_{i,j} = \frac{1}{2}$). Disequilibria will develop between unlinked loci ($r_{i,j} = \frac{1}{2}$) that have the same fitness effect if $s_i\, h_i = s_j\, h_j > \sqrt{2} - 1 = 0.414$ - that is, if heterozygosity at each locus reduces fitness below the mean by at least 41.4%.

Note that the stability results of Eqs. 9, 10 apply for arbitrary allele frequencies. If allele frequencies are asymmetric, then as linkage disequilibria build up, frequency-dependent selection must generate coefficients a_i, a_j to maintain allele-frequency equilibrium. However, by symmetry, these coefficients are $O(D^2)$, and so do not affect the stability analysis. If allele frequencies are symmetric, then even if coefficients of dominance and epistasis change with linkage disequilibrium, we must have $\partial a_U / \partial D_{ij} = 0$, by symmetry. Therefore, this kind of frequency-dependence does not alter the stability of the symmetric equilibrium. However, if allele frequencies are asymmetric, our conclusions about stability may depend on the assumption that epistasis and dominance do not vary with the strength of linkage disequilibrium.

Multiple loci with underdominance or assortment

Now, we extend the model to a set of underdominant or assorting loci, Ω.

$$\bar{W} = \prod_{k \in \Omega} (1 + s_k\, \xi_k\, \xi^*_k) = 1 + \sum_{U \subseteq \Omega} s_U\, \xi_U\, \xi^*_U \qquad (11)$$

where the sum over all sets of loci, U⊆Ω, does not include the empty set $U = \emptyset$, and where selection on a set, U, of loci is the product of the individual selection coefficients:

$s_U \equiv \prod_{i \in U} s_i$. (Note the distinction between sums over elements of the set Ω, denoted k∈ Ω, and sums over all subsets of Ω, denoted $U \subseteq \Omega$). The mean fitness is:

$$\bar{w} = 1 + \sum_{U \subseteq \Omega} s_U \, D_U^2 \tag{12}$$

Now, $\overline{W}\, a_{U,U} = s_U \; \forall \; U$ and all other coefficients are zero (that is, $a_{U,V} = 0 \; \forall \; U \neq V$). The pairwise linkage disequilibria follow the recursion:

$$D'_{ij} = (1 - r_{i,j})\, D_{ij} + \sum_{U \subseteq \Omega} a_{U,U} \left((1 - r_{i,j})\, D_U \, (D_{ijU} - D_{ij}\, D_U) + r_{i,j}\, D_{iU}\, D_{jU} \right) \tag{13}$$

where D_U represents the association between the set of alleles U. When linkage disequilibria are small and of the same order, this simplifies to:

$$D'_{ij} = D_{ij} \left((1 - r_{i,j}) \, (1 + a_{ij,ij}\, h_i\, h_j) + r_{i,j} \, (a_{i,i}\, h_i + a_{j,j}\, h_j) \right) + O(D^2) \tag{14}$$

So the criterion for instability of D_{ij} only involves $a_{ij,ij}$, $a_{i,i}$ and $a_{j,j}$, and for each pair of loci, we have exactly the same conditions as for the two-locus model (Eqs. 9, 10). (This is true even after using $\overline{W}\, a_{U,U} = s_U$ to substitute for the $a's$ in terms of the $s's$, because $\overline{W} = 1 + O(D^2)$). Because we are examining small perturbations from linkage equilibrium, the stability of associations between a pair of loci does not depend on any other loci.

So far, we have shown that $|D| > 0$ (i.e., coupling between incompatibilities) is favoured if $a_{ij,ij}$ is sufficiently strong relative to the opposing force of recombination. This term can arise with either under- or over-dominance if fitnesses multiply across loci, but will typically be weak, since it arises from the product of selection coefficients at the two loci.

The stability criterion extends in a surprisingly simple way to higher-order disequilibria. Making the same argument as for a pair of loci (Eq. 8), we see that the leading terms are:

$$D'_V \sim \left(r_{V,\emptyset} + \sum_{U \subseteq V} r_{U,V \setminus U}\, a_{U,U}\, h_U \right) D_V + O(D^2) \tag{15}$$

Here, $r_{V,\emptyset}$ is the probability that no recombination event breaks up the set V, and $r_{U,V\setminus U}$ is the rate of recombination events by which the set U is inherited from one parent of a haploid individual, and the remaining loci, V\U, from the other. We see that the condition for instability of pairwise D_{ij}, Eq. 9, is a special case of this formula. With equal allele frequencies ($p_i = \frac{1}{2}$), Eq. 15 is equivalent to the recursion above Eq. 8.7 of Christiansen (1999), with $2\, R_V(U) = r_{U,V\setminus U}$, and $e_U / e_\emptyset = a_{U,U}\, h_U$.

Interactions amongst multiple loci with underdominance or assortment

In the multiplicative model, we have $\overline{W}\, a_{U,U} = \prod_{i \in U} s_i$. However, the stability criterion, Eq. 15, applies to a much more general model. Suppose that fitness is an arbitrary function of which loci are heterozygous or homozygous:

$$W = 1 + \sum_{U \subseteq \Omega} s_U \, \zeta_U \, \zeta_U^* \qquad (16)$$

where the s_U are now *arbitrary* coefficients that describe interactions between heterozygosity at the set of loci U. (Note that this polynomial formula for relative fitness can describe *any* assignment of fitnesses to genotypes, provided that we only distinguish whether loci are homozygous or heterozygous). We see that the stability of each coefficient of linkage disequilibrium depends simply on the rates of recombination that break down that association, and the strength of selection favouring associations between the specific set of loci on the two homologous genomes ($a_{U,U}$). This is analogous to the result of Hastings (1986) and Barton (1986), who showed that close to linkage equilibrium, the association amongst a set of loci is directly proportional to the epistasis amongst precisely that set. Note that so far, we have not made any assumption about allele frequencies - these can be arbitrary.

The results described so far are largely contained in the extensive literature on the symmetric viability model, which is reviewed by Christiansen (1999, Ch. 8). The key difference is that previous analyses have assumed fixed genotypic fitnesses, and so required overdominance to maintain stable polymorphism. Here, we assume that stable polymorphisms are maintained despite underdominance, because fitnesses depend on allele frequencies. The symmetric viability model assumes that fitness depends only on which set of loci is heterozygous. It was introduced for two loci by Lewontin and Kojima (1960), who derived the threshold of Eq. 10 (their Eq. 20), and found that $D = \pm(1/4)\sqrt{1 - r_{i,j}/r_{i,j}^*}$ at the asymmetric equilibria (their Eq.19). Ewens (1968) found that symmetric ($D = 0$) and asymmetric ($D \neq 0$) equilibria could both be unstable towards changing allele frequency for intermediate rates of recombination. However, since we assume that allele frequencies are stabilised by frequency-dependent selection, this does not concern us here. Karlin and Feldman (1978) made a numerical investigation of the two-locus multiplicative model, and found that with unequal allele frequencies, equilibria with $D = 0$ and D≠0 could be simultaneously stable. Christiansen (1990; 1999) analyses the symmetric viability model, using a notation similar to ours, and derives Eq. 15 (Christiansen, 1999, above Eq. 8.7), assuming equal allele frequencies. The most general analytic results are for the generalised multiplicative model (Karlin and Liberman, 1979), which allows interactions amongst arbitrary sets of loci, but assumes that the effects of the

three genotypes at each locus have the same relative effects in all interactions - roughly speaking, fixing their dominance relations. This model includes the symmetric viability model as a special case, but extends to allow unequal allele frequencies. When a polymorphism at linkage equilibrium exists, its stability is given by an explicit (but complicated) formula (Christiansen, 1999, Eq. 8.9). Thus, the results summarised above are largely contained in previous analyses, though our main result (Eq. 15) applies for arbitrary allele frequencies, and so extends beyond the symmetric viability model.

The generalized model of Eq. 16 is intriguing, because we could in principle have instability of pairwise linkage disequilibria but not three-way - or vice versa. In SI 1, we show that with three loci, all possible stability regimes are possible, for appropriate choices of recombination rates, and of the fitness as a function of the number of heterozygous loci. These possibilities are illustrated in Fig. 2.

We can understand these outcomes by thinking of how selection acts on the distribution of numbers of heterozygotes. If this relationship is linear (i.e., if fitness depends additively on heterozygosity), then there is no selection on the variance of heterozygosity. If the relationship curves upwards, then selection favours a maximal variance of heterozygosity, and hence coexistence of two complementary genotypes (lower dots in Fig. 1). Conversely, if fitness is concave downwards, then selection favours a reduced variance in heterozygosity, which is achieved at linkage equilibrium (upper dots in Fig. 1). With more than two loci, we can have situations in which an intermediate variance in heterozygosity maximises mean fitness, so that intermediate numbers of genotypes are heterozygous. (Under recombination and selection, mean fitness is not maximised, and may decrease (Moran, 1964; Ewens, 2004, Ch. 2). However, the effect of selection alone is always to increase mean fitness, and so we can identify the direction of selection by using arguments based on mean fitness, as above).

As different incompatibilities become coupled with each other, their effect on yet other incompatibilities becomes stronger, so that further coupling evolves. Thus, a set may couple even if *most* pairs of loci, considered separately, would not. This positive feedback is illustrated in Fig. 3, for a five-locus example, with multiplicative fitnesses across loci. In this example, selection at each locus is strong, with each heterozygote reducing fitness by 54%. With weaker selection, this kind of feedback is unlikely, because it requires that linkage is only slightly looser than the critical value that would couple each pair, considered separately.

Note that throughout, we treat genotypic fitnesses as being fixed, independent of the pattern of linkage disequilibria, according to Eq. 16, with allele frequencies poised at a (possibly unstable) equilibrium. Dependence of fitness on allele frequencies can stabilise the allele frequencies, without altering the stability of the linkage disequilibria. However, a

generalised dependence of fitness on genotype frequencies (that is, on linkage disequilibria as well as on allele frequencies) would alter both the stability at linkage equilibrium, and the final outcome.

INCOMPATIBILITIES CAUSED BY EPISTASIS

An assortment locus and a pair of interacting loci: Felsenstein (1981)

The model of multiple single-locus incompatibilities shows that coupling can occur between incompatibilities that act at any stage - pre- or postzygotic; it also allows for arbitrary interactions amongst incompatibilities. However, assortment and selection act at different stages (amongst haploids, and on diploids, respectively); while biologically possible, this may seem unfamiliar. We therefore turn now to a model in which both assortment and selection acts on haploids. (Note, however, that our model of viaility selection on haploids is equivalent to one of viability selection in diploids, in the absence of dominance). In its simplest form, this is just the model introduced by Felsenstein (1981), and analysed by Gavrilets (2004, pp. 347-350). We first set out Felsenstein's model, expressing it in terms of selection coefficients and linkage disequilibria, and giving some explicit solutions. This will suggest a simpler and more general version, which extends to multiple loci.

Felsenstein's model includes one locus that causes cost-free assortment of strength α; a fraction α of haploid individuals mate assortatively, and 1-α mate at random. Two loci determine fitness in two niches (I, II) with (in the basic version) random mixing between them ($m = 0.5$) :

	I	II
00	1	$1 + 2s + \epsilon$
01	$1 + s$	$1 + s$
10	$1 + s$	$1 + s$
11	$1 + 2s + \epsilon$	1

Polymorphism is maintained, because the proportion from each niche is held fixed at 50% (i.e., 'soft' selection, as in Levene's (1953) model). We follow Felsenstein (1981) by labelling the assortment locus a, and the epistatically interacting loci b, c. (In the original version, d measured assortment and $\epsilon = ks^2$). Gavrilets (2004, p. 347) refers to these loci as CAB, respectively, and gives the analytical solutions for the symmetric state.

If there is any epistasis, then an association D_{bc} will be generated. The interesting question is whether or not associations D_{ab}, D_{ac} can build up, which associate assortment

with epistatic selection, and hence reduce gene flow. This analysis is relatively simple in the completely symmetrical case, $p_a = p_b = p_c = \frac{1}{2}$, $D_{abc} = 0$. (Note that when the population contains just two complementary genotypes - 000, 111 say - then $D_{abc} = 0$; a three-way association corresponds to an excess of *four* genotypes - 001, 010, 100, 111, say). We summarise this analysis of the completely symmetrical case in SI 2.

Because assortment is assumed cost-free, allele frequencies p_a could take any value. In SI 3, we show that although there is indeed a line of neutral equilibria with arbitrary allele frequencies at the assorting locus, a, perturbations away from this line tend to drive the allele frequency p_a towards $\frac{1}{2}$. This provides some justification for focussing on the simplest case, where all allele frequencies are symmetrical. We also show in SI 3 that the symmetrical equilibrium is stable towards fluctuations in the third-order association, D_{abc} away from zero.

Coupling between multiple pairs of interacting loci

We began our analysis by considering associations amongst multiple underdominant or assorting loci. We then considered Felsenstein's (1981) model, in which a single-locus incompatibility becomes coupled with a pair of loci that interact to determine haploid viability. We will extend Felsenstein's (1981) model in two ways. In the following section, we will see how a single locus for assortment or underdominance becomes associated with an arbitrary set of loci that interact to determine haploid viability. First, however, we will look at coupling between multiple pairs of interacting loci that influence haploid viability: that is, we assume random mating between haploids, but allow several pairs analogous to the pair of loci b, c in the Felsenstein (1981) model. This will show how results from our initial model of single-locus incompatibilities, acting at both haploid and diploid stages, carry over to the case where selection acts solely on haploids. In particular, this extension strengthens our argument that coupling can occur between incompatibilities at the same stage in the life cycle, in contrast to the standard view of reinforcement.

We label each pair by i, and the loci within pair i as i_1, i_2; thus, the association within pair i is $D_i = D_{i_1\, i_2}$. We denote a set of pairs by $D_U = \prod_{i \in U} D_i$. Similarly, $r_i = r_{i_1, i_2}$. The set of all pairs of loci is denoted Ω. We assume that there is epistasis between the loci within a pair, denoted by $a_{\{i_1\, i_2\}} = a_{\{i\}}$. There may also be epistasis between pairs, denoted by a_U, where U is a set of pairs of loci (e.g., $U = \{i_1, i_2, j_1, j_2\}$). This reflects the possibility that the cumulative effects of incompatibilities are not additive. In the special case where effects are multiplicative across pairs, we write haploid viability as a product of contributions $(1 + \gamma_i(\zeta_i - D_i))$ across pairs of loci; this greatly simplifies the recursions. Details of the derivations are given in SI 4.

At equilibrium, all associations involving pairs of interacting loci may exist. Clearly, we expect strong associations within pairs ($D_{i_1\, i_2} = D_i \neq 0$), generated by the epistasis between those loci. In general, however, there may also be associations *between* pairs (e.g. $D_{i_1\, i_2\, j_1\, j_2\, k_1\, k_2} = D_{ijk} \neq D_i\ D_j\ D_k$): unless the fitness effects of different incompatibilities multiply, selection will make multiple incompatibilities more or less frequent than expected from the frequencies of each separate incompatibility. In the special case where effects multiply across pairs, however, these cross-pair associations may reduce to the product of within-pair associations, so that there is an equilibrium with $D_U = \prod_{i \in U} D_i$. However, even in this multiplicative case, different pairs of incompatibilities will become associated with each other if the pairs overlap on the genetic map. This is because a recombination event that breaks up one pair may also break up another pair, and so the presence of an incompatibility involving one pair becomes correlated with incompatibility at the other. (For example, if loci are in the order $i_1\ j_1\ i_2\ j_2$, then a recombination between j_1 and j_2 will also cause recombination between i_1 and i_2). Note that there is a qualitative difference from the case of multiple underdominant loci, considered above, where even when the cumulative effects of multiple incompatibilities interact, the population can still settle to linkage equilibrium.

Even though all associations that involve complete pairs are non-zero in our model of epistasis, we assume that associations between *single* loci in different pairs are zero (i.e., $D_{i_a\, j_b} = 0$ for $i \neq j,\ \forall\ a, b \in \{1, 2\}$). That is, there can be an equilibrium in which all genotypes that give the same pattern of incompatibility are equally common. For example, the four haploid genotypes $\{X_{i_1}\ X_{i_2}\ X_{j_1}\ X_{j_2}\}$=$\{0,0,0,0\}$, $\{0,0,1,1\}$, $\{1,1,0,0\}$, $\{1,1,1,1\}$ have maximal fitness, and are equally common at the symmetrical equilibrium. Similarly, $\{0,1,0,0\}$, $\{0,1,1,1\}$, $\{1,0,0,0\}$, $\{1,0,1,1\}$ have a single incompatibility, at the first two loci ($i = \{i_1, i_2\}$), and are again equally common. The non-zero associations D_i, D_j and D_{ij}, which involve complete pairs, determine the frequencies of the four classes of genotype (i.e. with or without incompatibilities at loci i, j) but within those classes, genotypes are equally frequent. The question is whether, starting from this symmetrical state, pairwise associations such as $D_{i_a\, j_b}$ will grow, so that ultimately, the population is dominated by two complementary haplotypes.

The symmetrical equilibrium studied here is similar to that in the symmetrical model of Barton (1992), Doebeli (1996) and Shpak and Kondrashov (1998), who assumed equal frequencies of all haploid genotypes with the same value of an additive quantitative trait. This assumption drastically simplifies simulations, since with n loci, only n variables need be followed, rather than $2^n - 1$. However, although the symmetrical equilibrium exists when fitnesses show a corresponding symmetry, it may be unstable. Barton and Shpak

(2000) showed how the stability of an n locus system could be determined by examining an $(n+1)\times(n+1)$ matrix. In our model, we will be concerned with the stability of the symmetrical equilibrium in a similar way.

In the symmetrical equilibrium, there is high genotypic diversity, and little reproductive isolation. With m pairs of loci, each class includes 2^m different genotypes, which are equally frequent. Thus, even if selection were so extremely strong that only the most compatible class existed (i.e., genotypes 00 and 11 only at each pair of interacting loci), there would still be 2^m different genotypes in the population. In order for strong reproductive isolation to develop between two alternative genotypes, associations between pairs must develop, such that two complementary genotypes become common (e.g., {0000} and {1111} or {0011} and {1100}).

Recursions for this model are given in SI 4. We can find explicit results in several special cases. First, we consider maximal selection, where epistasis is so strong that recombinants {01, 10} at each pair are completely inviable. Then the leading eigenvalue is:

$$\lambda = \frac{(1-\bar{r})}{\left(1-\frac{r_i}{2}\right)\left(1-\frac{r_j}{2}\right)\bar{V}} \qquad (17)$$

where $\bar{r} = (r_{i_1,j_1} + r_{i_1,j_2} + r_{i_2,j_1} + r_{i_2,j_2})/4$ is the average rate of recombination between two loci in different pairs, and $\bar{V}$ is the average viability. We see that associations between alleles involved in different incompatibilities will grow if the average rate at which such associations are broken up ($\bar{r}$) is small, relative to the average rate at which each pair is broken up (r_i, r_j), causing loss of viability. In this case of complete selection, only four haploid genotypes are viable, and the model is equivalent to one of underdominance at two linked loci (see SI 4). Also, note that Eq. 17 does not depend on assuming multiplicative effects across loci: when selection is so strong that all within-pair recombinants die, there is no difference in viability between individuals that die because they carry one incompatibility and those that die because they carry two.

With no linkage, and when incompatibilities have multiplicative effects, there are no associations amongst incompatibilities (*i.e.*, $D_{ij} = D_i D_j$), and the leading eigenvalue is:

$$\lambda = \frac{1}{2}(1+\gamma_i(h_i - D_i))(1+\gamma_j(h_j - D_j)) \qquad (18)$$

The strongest possible selection is $\gamma_i = \gamma_j = \frac{8}{3}$, and $D_i = D_j = \frac{1}{8}$, in which case recombinants are completely inviable. Then, $\lambda = \frac{8}{9} < 1$, implying that unlinked incompatibilities cannot become coupled unless there are between-pair interactions, $\tilde{a}_{ij}$.

When loci are very tightly linked, so that recombination is much weaker than selection, the linkage disequilibria D_i, D_j are close to their maximum of $\frac{1}{4}$. Then, the leading eigenvalue simplifies to:

$$\lambda = 1 + \frac{r_i + r_j}{2} - \bar{r} - \frac{\chi}{4}\,\frac{(\gamma_j + \gamma_i - \gamma_i\,\gamma_j)}{(\gamma_i + \gamma_j - \frac{\gamma_i\,\gamma_j}{2})} > 1 \text{ for instability} \qquad (19)$$

where $\chi = r_{ij,\phi} + r_{i,j} - (1 - r_i)\,(1 - r_j)$. If the pairs of loci do not overlap, and there is no interference between crossovers, then χ=0. If χ=0, or if selection is weak (γ << 1), or if selection is as strong as possible (γ_i=2 for $D_i = \frac{1}{4}$), then the leading eigenvalue is independent of the selection coefficients, and depends only on the recombination rates. This is consistent with Eq. 17 above, which applies when selection is maximal, since Eq. 19 is the limit of Eq. 17 for tight linkage when γ_i, $\gamma_j = 2$ and recombination is rare.

Two pairs of interacting loci are much more likely to become associated if they overlap on the genetic map than if they are separate. To find whether alleles involved in separate incompatibilities will become associated (i.e., λ>1), we need to consider the three possible gene orders, assuming no interference between crossovers and tight linkage within pairs (r_i, r_j << 1):

$$\bar{r} = \frac{(r_i + r_j - r_i\,r_j)}{2} + r_{i_2\,j_1} \quad \chi = 0 \quad i_1\,i_2\,j_1\,j_2$$

$$\bar{r} = \frac{(r_i + r_j)}{2} - \frac{r_{i_2\,j_1}}{2} \quad \chi = r_{i_2\,j_1} \quad i_1\,j_1\,i_2\,j_2$$

$$\bar{r} = \frac{r_i}{2} \quad \chi = r_j \quad i_1\,j_1\,j_2\,i_2$$

where we keep only the leading terms in recombination rates. When the pairs do not overlap (order $i_1\ i_2\ j_1\ j_2$), they will become associated only if they are very tightly linked ($r_{i_2\,j_1} < \frac{r_i\,r_j}{2}$ for $r_{i_2\,j_1}$ small). If they do overlap (orders $i_1\ j_1\ i_2\ j_2$, $i_1\ j_1\ j_2\ i_2$), then they will become associated, because $\frac{r_i + r_j}{2} > \bar{r} + \frac{\chi}{4}$ for tight linkage. If selection is strong (γ_i, $\gamma_j \sim 1$), then the last term in Eq. 19 will be smaller, and so associations will still grow.

It is remarkable that the expressions for both maximal selection (Eq. 17) and for tight linkage (Eq. 19) depend only on the recombination rates, and not directly on selection. In both cases, pairwise associations will grow if the average rate of recombination between loci within pairs, ($\frac{r_i + r_j}{2}$), is greater than the average rate of recombination between two loci chosen randomly from different pairs ($\bar{r}$). However, in general the stability depends on both the strength of selection (γ_i, γ_j) as well as on the recombination rates. Figure 5 shows the conditions for instability for moderate selection and recombination rates.

We have found a simple criterion for when pairwise associations between two pairs of loci will grow. In this simple case where there are no interactions between pairs ($\tilde{a}_{ij}$), this

criterion depends only on the two pairs involved, and not on the full system. (We have not examined higher order associations: by analogy with the case of multiple underdominant loci, these might also grow). Thus, we can imagine that certain pairs of pairs, which satisfy the conditions on recombination rates derived above, may become associated. That will change the stability of the remaining pairs of loci. As with single-locus incompatibilities (Fig. 3), we might find a runaway process, in which all of the pairs become coupled.

Independent pairs of interacting loci couple together for the same reason that multiple underdominant loci will become associated; indeed, the two models become identical when within-pair recombinants are completely inviable. As argued above, alleles involved in independent incompatibilities will become associated because this leads to increased variance in viability, and hence, increased mean viability. In the extreme case, where two complementary genotypes segregate, then half of individuals will be completely homozygous, and their offspring will be substantially less fit as a result of recombination. Thus, the mean viability cannot be lower than $\frac{1}{2}$. In contrast, if many strong incompatibilities segregate independently, then each individual will be heterozygous for approximately half of these, and the average fitness of its offspring may be substantially reduced. One test of this interpretation is to ask whether coupling can occur if the effects of incompatibilities are strictly additive (i.e., if $a_U = 0$ for $|U| \geq 2$). With four loci, it is easy to show that the leading eigenvalue for the growth of pairwise associations $D_{i_x j_y}$ is precisely 1 in the absence of recombination, implying that coupling is indeed impossible. We believe that this is also true for arbitrarily many loci, but have not been able to prove it.

Coupling between an assortment locus and loci under arbitrary viability selection

Finally, we extend Felsenstein's (1981) model to find when a locus that causes assortative mating amongst haploids will become coupled with a set of loci that influence haploid viability. (Recall that this model of assortment amongst haploids is precisely equivalent to selection against diploid heterozygotes). We first consider pairwise epistasis for haploid viability, but with any number of selected loci. We then give a much more general result that allows for any kind of symmetrical epistasis. This gives approximations for the rate of growth of associations with an assortment locus.

We define fitness as:

$$W = \left(1 + \frac{\alpha}{h_a}\, \xi_a\, \xi_a^*\right) \left(1 + \sum_{U \subseteq \Omega} a_U\, (\xi_U - D_U)\right) \left(1 + \sum_{V \subseteq \Omega} a_V\, (\xi_V^* - D_V)\right) \qquad (21)$$

The first term represents assortative mating (α) at a single locus, labelled a, in the same way as in Felsenstein (1981). This has the same form as Eq. 5, if we identify the selection coefficient as $s_a = \alpha / h_a$. The second and third terms represent selection on haploid viability, which acts in the same way on males and females, and which involves a set of loci, Ω; the sums are over all possible sets of selected loci, $U \subseteq \Omega$. The selection coefficients a_U are arbitrary, and may generate strong associations, D_U. Viability selection is defined in such a way that when there is no association between the selected loci in the set Ω, and the assortment locus a, then $\overline{W} = 1$. We will be focussing on the growth of weak associations, ($D_{aU} \sim \epsilon$ for $U \subseteq \Omega$), in which case the deviation in mean fitness from 1 is negligible ($\overline{W} = 1 + O(\epsilon^2)$).

Throughout this section, we assume that genotype frequencies at the selected loci are at equilibrium, which sets a constraint on the a_U. Crucially, we make the further assumption that the frequencies of all selected alleles are symmetrical (i.e., $p_i = q_i = \frac{1}{2}\ \forall\ i \in \Omega$). This also implies that when the selected allele frequencies are at equilibrium, all coefficients a_i are zero. Thus, by assuming symmetrical allele frequencies, we avoid all terms involving coefficients of directional selection, a_i, and we can use the simple reduction formula $D_{iiU} = h_i\, D_U$, with $h_i = p_i\, q_i = \frac{1}{4}$(Eq. 3). The focus of this paper is on the growth of *associations* between polymorphic alleles (D_{aj} etc.), rather than on changes in allele frequencies; thus, we believe that it is reasonable to fix selected allele frequencies at one half.

We begin by finding the effect of assortative mating on the association between locus a and a selected locus $j \in \Omega$. Assortment, as defined in Eq. 21, is represented by a coefficient $a_{a,a} = \frac{\alpha}{h_a}$. From Eqs. 2, 4:

$$D_{aj}'' = (1 - r_{a,j})\, D_{aj}' + \alpha r_{a,j}\, D_{aj}' = (1 - \rho_j)\, D_{aj}' \qquad (22)$$

where the D_{aj}' are the associations after viability selection, and where $\rho_j = r_{a,j}(1 - \alpha)$ is the effective rate of recombination between a and j, allowing for its reduction by assortative mating, α. Equation 22 shows that recombination breaks down the association D_{aj} at a rate $(1 - \rho_j) = (1 - r_{a,j}(1 - \alpha))$, regardless of how selection acts on the other loci, or how they are linked. Whether this association will grow or shrink depends on whether viability selection increases D_{aj} faster than it is broken down by recombination.

We restrict attention to pairwise epistasis, a_{jk}, by assuming that $a_U = 0$ for $|U| \neq 2$. We make the further assumption that all the a_{jk} are positive. We can then focus on the case where all the D_{aj} have the same sign, and grow together. Although we have some freedom to change the a_{jk} by an arbitrary relabelling of the alleles, this assumption is still restrictive. It amounts to assuming that there is disruptive selection that favours two complementary genotypes, which we choose to label as 000… and 111…. This could represent disruptive selection on an additive trait, or selection for sets of alleles that are involved in adaptation to two alternative niches. (In the following section, we relax these assumptions)

From Eq. 2, the effect of pairwise epistasis is:

$$D'_{aj} = D_{aj} + \sum_{k \in \Omega \setminus j} a_{jk} \left(h_j D_{ak} - D_{jk} D_{aj} \right) + \sum_{k,l \in \Omega \setminus j} a_{kl} \left(D_{ajkl} - D_{kl} D_{aj} \right) \qquad (23)$$

This is a straightforward extension of Felsenstein's (1981) model, Eqs. 41, in which associations between a and j are amplified by contributions from other associations, D_{ak}, through the key terms $a_{jk} h_j D_{ak}$. The second term in Eq. 23 leads to a set of coupled linear equations in the pairwise associations D_{aj}. However, the third term introduces a contribution from four-way associations, due to selection on pairs of loci that do not include the focal locus j. In the previous section, we saw that these four-way associations are zero if the effects of different pairs of loci are multiplicative, *and* if interacting pairs do not overlap on the genetic map. On that model of non-overlapping pairs, with multiplicative effects, the problem reduces to Felsenstein's (1981) model. However, in this section we have assumed that the effects of different pairs are *additive* (Eq. 21), and so four-way associations will be generated. Even without this effect of selection, recombination would in general produce positive four-way associations whenever the same recombination event separates both a, j and k, l. We assume that the effect of recombination dominates, and that the signs of a_{kl}, D_{kl} are the same. With these assumptions, the last term in Eq. 23 is non-negative, so that we have a lower bound on the rate of increase in D_{aj}:

$$\triangle D_{aj} > -\rho_j D_{aj} + (1 - \rho_j) \sum_{k \in \Omega \setminus j} a_{jk} \left(h_j D_{ak} - D_{jk} D_{aj} \right) \qquad (24)$$

Summing over j, noting that $h_j = h = \frac{1}{4}$, and using the symmetry $a_{jk} = a_{kj}$:

$$\triangle \langle D_a \rangle > -\bar{\rho} \langle D_a \rangle + \sum_{j \neq k} a_{jk} \left(\frac{D_{aj} + D_{ak}}{2} \right) (h - D_{jk})$$

$$\text{where } \langle D_a \rangle \equiv \sum_{j \in \Omega} D_{aj}, \quad \bar{\rho} \equiv \left(\sum_{j \in \Omega} \rho_j D_{aj} \right) \Big/ \langle D_a \rangle \tag{25}$$

Because $h > D_{jk}$, and we assume that a_{jk}, $D_{aj} > 0$, the last term is positive. Therefore, if assortative mating is sufficiently strong ($\alpha \sim 1$), $\overline{\rho} = (1 - \alpha)\, \bar{r}$ will be small, and associations will grow: there must always be a critical strength of assortment above which coupling will develop. We can make a rough estimate of this by noting that when the population is at this critical point, $\overline{\rho}$ will be small, and so selection a_{jk} can be weak. The linkage disequilibria D_{jk} are then negligible, and the rate of increase is approximately $(nah - (1 - \alpha)\, \bar{r}_a)$, where there are n loci, with typical pairwise epistasis $\sim a$, and where the average linkage between the assortment and the selected loci is $\bar{r}_a$. Thus, the critical strength of assortment is $1 - \alpha^* = nah / \bar{r}_a$. It is tempting to argue that if linkage with the assortment locus, $\bar{r}_a$, is sufficiently tight, then $\overline{\rho}$ will also be small, and associations will grow. However, if all the selected loci are tightly linked to the assortment locus, then they must be tightly linked with each other, and therefore $h - D_{jk}$ will be small. The rate of growth of associations therefore depends on two opposing terms, both proportional to the recombination rates. (Recall Eq. 19 for the four-locus model). It does not seem possible to find an explicit expression, although the set of linear equations could readily be solved for any specific parameters.

We now generalise from pairwise epistasis to allow higher-order epistasis and linkage disequilibria. However, we maintain symmetry between alleles, such that $p_i = q_i = \frac{1}{2}$, and also, set all odd-order epistasis coefficients and linkage disequilibria to zero (i.e., a_U, $D_U = 0$ for $|U|$ odd, $U \subseteq \Omega$). We must now follow associations between the assortment locus, a, and sets of selected loci, D_{aU}. The symmetry assumptions imply that we follow associations with odd-sized sets of selected loci, such as D_{aj}, D_{ajkl}, etc. That is, we assume $D_{aU} = 0$ for $|U|$ even, and $U \subseteq \Omega$.

Equations 2, 4 yield a set of coupled linear equations for the D_{aU}, analogous to Eqs. 43. However, we can make a radical simplification by focussing on the sum of associations, $C_1 = \sum_{U \subseteq \Omega} 2^{|U|-n} D_{aU}$, where the sum is over all odd-sized sets of selected loci, U, and n is the number of selected loci. This measure has a simple interpretation: it is just the excess frequency of the assortment allele $X_a = 1$ within the genotype $\underline{1} = \{1, 1, 1 \ldots\}$ at the selected loci, multiplied by the frequency of that genotype. (By symmetry, it is minus the corresponding measure for genotype $\underline{0} = \{0, 0, 0 \ldots\}$; $C_{\underline{1}} = -C_{\underline{0}}$). We could relabel alleles to define the measure $C_{\underline{X}} = \sum_{U \subseteq \Omega} 2^{|U|-n} \left(\prod_{i \in U} (2 X_i - 1) \right) D_{aU}$, where the weight

$(2\,X_i - 1)$ is ±1, depending on the allelic state. This would correspond to the excess frequency of allele $X_a = 1$ within genotype $\underline{X}$.

In SI 5, we show that the measure $C_{\underline{1}}$ increases under viability selection as:

$$C_{\underline{1}}' = C_{\underline{1}} \left(1 + \sum_{U \subseteq \Omega} a_U \, (2^{-|U|} - D_U) \right) = C_{\underline{1}} \, \frac{\bar{W}_{\underline{1}}}{\bar{W}} \qquad (26)$$

where $W_{\underline{1}}$ is the mean fitness of genotype $\underline{1}$. (Note that since allele frequencies are assumed symmetrical, the maximum value of D_U is $2^{-|U|}$). This simple result arises just because the total frequency of genotype $\underline{1}$ increases as $(\overline{W}_{\underline{1}} / \overline{W})$, and the frequencies of neutral alleles at locus a within that genetic background stay the same. We choose to label the fittest pair of genotypes as $\underline{0}$, $\underline{1}$, so that $C_{\underline{1}} = -C_{\underline{0}}$ increase faster than any other association, $C_{\underline{X}}$.

With viability selection alone, the fittest pair of genotypes would fix, and the association $C_{\underline{1}}$ would increase to at most the initial excess frequency of allele $X_a = 1$ within that genotype. If that excess frequency were initially small, then it would remain small after any amount of viability selection. Yet, in Felsenstein's (1981) model, the association $C_{\underline{1}}$ increases to a high level, independent of its initial value, as a result of successive rounds of selection, assortment, and recombination. If selection and recombination acted alone, with random mating, then any associations with a neutral allele must decline to zero: the increase due to viability selection must be outweighed by the decrease due to recombination. Amplification of a small initial association depends on the combined action of the three processes of selection, assortment and recombination.

We now find the effects of assortment and recombination on associations with sets of selected loci, D_{aU}. In SI 5, we show that the change due to assortment and recombination is the sum of two components:

$$\triangle D_{aU} = D_{aU}'' - D_{aU}' = \sum_{ST=U} r_{s,T} \, (D_{aS}' \, D_T' - D_{aU}') - (1 - \alpha) \sum_{ST=U} r_{aT,S} \, D_{aS}' \, D_T' \qquad (27)$$

The first term is independent of assortment, α, and independent of the map position of the assortment locus, a. It arises from recombination within the set of selected loci, U, which replaces the association of a with U by a lower-order association, D_{aS}, $S \subseteq U$. This term is zero for pairwise associations, and is negligible in both the limits of strong assortment and of tight linkage, considered below. The second term is due to recombination involving the assortment locus, and is necessarily zero with complete assortment, α=1: then, the assortment locus is always homozygous and there is no effective recombination. The leading component of this second term is when T=$\emptyset$, giving $-(1-\alpha)\, r_{a,U}\, D_{aU}$; this gives a rate of decay proportional to the rate at which a separates from the set of selected loci, U.

This expression does not seem to simplify when we sum over all sets U, to give the increase in $C_{\underline{1}}$. If the covariance between the set of alleles aS, and the set T is always positive (i.e., $D'_{aU} > D'_{aS}\, D'_{T}\ \forall\ ST = U$), and if the associations D'_{aS}, D'_{T} are always positive, then we have the bound $\Delta D_{aU} \leq -(1-\alpha)\, r_{a,U}\, D_{aU}$. Hence, the decrease in $C_{\underline{1}}$ due to recombination is at least as fast as the average rate of recombination between a and selected sets, U. Including the increase due to viability selection (Eq. 26):

$$C_1'' \leq C_1\ (1 - (1-\alpha)\ \bar{r}_a)\ \frac{\bar{W}_1}{\bar{W}} \text{ where } \bar{r}_a = \frac{\sum_{U \subseteq \Omega} 2^{|U|-n}\, r_{a,U}}{\sum_{U \subseteq \Omega} 2^{|U|-n}} \qquad (28)$$

However, it is not clear what assumptions about epistasis and recombination are needed for this upper bound on the growth of associations to hold.

Although a completely general expression for the rate of growth in coupling does not seem possible, we can find simple results in two limiting cases: strong assortment with weak selection, and conversely, tight linkage. First, suppose that assortment is almost complete ($\alpha \sim 1$), Then, associations can grow even when epistasis is weak, and linkage is loose. Therefore, we can assume that $a_U \sim \epsilon << r_{s,T}$, so that associations amongst selected loci will be weak ($D_U \sim \epsilon$). In this limit, the quasi-linkage equilibrium approximation (QLE) for the linkage disequilibria holds (Barton and Turelli, 1991). Because products of linkage disequilibria are negligible, the first term in Eq. 27 simplifies to $-R_U\, D'_{aU}$, where R_U is the total rate of recombination amongst the selected loci, $R_U = \sum_{ST=U} r_{s,T}$; this is zero for single selected loci ($|U| = 1$), but large for more than one selected locus. The second term simplifies to $-(1-\alpha)\, r_{a,U}\, D_{aU}$, which under our assumption that $\alpha \sim 1$ is small. Therefore, we expect only pairwise associations such as D_{aj} to contribute, since higher-order associations will dissipate rapidly. The net increase in associations with locus a due to selection (Eq. 26) is just the relative fitness of the fittest genotype, which we write as $\overline{W}_{\underline{1}} / \overline{W} = 1 + S_{\underline{1}}$, where $\overline{W}$ is the mean fitness of a population that is approximately in linkage equilibrium. Overall, then:

$$C_1'' = C_1\ (1 + S_1 - (1 - \alpha)\ \bar{r}_a)$$
$$\text{where } \bar{r}_a = \frac{\sum_{j\in\Omega} D_{aj}\ r_{a,j}}{\sum_{j\in\Omega} D_{aj}} \qquad (29)$$

which is at the tentative bound of Eq. 28. Associations will grow if assortment is sufficiently strong that $(1-\alpha) < s_1/\bar{r}_a$. Since S_1 is assumed small relative to recombination rates, this is a stringent condition. (Eq. 29 is not a closed expression for the rate of increase of C_1, because the recombination rates are weighted by the associations, D_{aj}, which are determined by the leading eigenvector of the full matrix recursion. However, since we assume that the D_{aU} are positive, these averages are bounded by the $r_{a,U}$, and we can approximate $\bar{r}_a$ by the unweighted mean).

Next, suppose that linkage is tight, so that the population is dominated by the complementary genotypes $\underline{0}$, $\underline{1}$. We show in SI 5 that in this limit, associations grow as:

$$C_1'' = C_1 \left(1 + \frac{1}{2} R_\Omega\right) \left(1 - (1-\alpha) \left(\frac{R_\Omega}{2} + r_{a,\Omega}\right)\right) \qquad (30)$$

where R_Ω is the total rate of recombination amongst selected loci, and $r_{a,\Omega}$ is the rate of recombination events that precisely separate the assortment loci from all the selected loci. Equation 30 is similar to Eq. 19, which applies to the coupling between two pairs of interacting loci in the same limit of tight linkage. This expression is independent of the form of epistasis, and depends only on the relative rates of recombination. This is because one of the fittest genotypes ($\underline{0}$ or $\underline{1}$) has probability half of meeting its complement, in which case less fit recombinant offspring are produced at a rate R_Ω. The reduction in mean fitness (i.e., the recombination load; Crow, 1970; Charlesworth and Barton, 1996) is equal to the half the total rate of recombination, in the same way that the load due to deleterious mutations is equal to the total mutation rate.

Since we assume that $R_\Omega, 2\, r_{a,\Omega} \ll 1$, associations grow at a rate of approximately $\frac{1}{2}\alpha R_\Omega - r_{a,\Omega}(1-\alpha)$. When the assortment locus lies outside the set of selected loci, with recombination rate r_a^* to the nearest of them, we have that $r_{a,\Omega} \sim r_a^*$; if assortment is stronger than $\alpha^* = \frac{2 r_a^*}{R_\Omega + 2 r_a^*}$, then associations will increase. We see that even when the assortment locus is relatively far from the selected loci ($R_\Omega \ll r_a^*$) it can become coupled to them if assortment is very strong. When the assortment locus is embedded within the selected loci, at least two cross-overs are needed to separate it from them, and so $r_{a,\Omega}$ is very weak compared with the recombination amongst the selected loci ($r_{a,\Omega} \sim R_\Omega^2$). Therefore, very weak assortment ($\alpha \sim r$) is needed to induce coupling. The first-order approximation given in SI 5 cannot determine this value, and we expect that it will depend on the form of selection. Figure 6 illustrates these two cases, using a model of four loci: as predicted, the threshold strength of assortment is independent of selection when locus a lies

outside the selected loci (Fig. 6a, lower left), but decreases as selection becomes stronger when the assortment locus lies within the selected set (Fig. 6b, left).

The focus of this paper is on the evolution of coupling between different components of reproductive isolation, rather than on changes in allele frequency. However, we conclude our analysis by considering the invasion of an allele for which there is assortative mating - a generalisation of the analysis of Felsenstein's (1981) model (SI 2). This extension remains tractable, because we still assume symmetric allele frequencies at loci under viability selection ($p_i = \frac{1}{2}$ for $i \in \Omega$). The only change to the analysis of associations between the assortment and selected loci, which led to Eqs. 26, 27, is that associations with repeated indices D_{aaU} reduce to $p_a\, q_a\, D_U - (p_a - q_a)\, D_{aU}$ (Eq. 3), giving an extra term when $p_a \neq q_a$.

Because assortment is assumed to be cost-free, allele frequency at locus a is not affected by assortment or recombination, but changes only through associations with selected loci:

$$\triangle p_a = \sum_{U \subseteq \Omega} a_U\, D_{aU} \tag{31}$$

where we assume that selection acts only on even-sized sets of loci ($a_U = 0$ for $|U|$ odd). Thus, we must determine whether associations D_{aU} will grow, with $|U|$ even. To keep the analysis simple, we consider just pairwise epistasis ($|U| = 2$).

By analogy with $C_{\underline{1}}$, which is a sum over associations with odd-sized sets of selected loci (i.e., D_{aj} …), we define $B_{\underline{1}} = \sum_{U \subseteq \Omega} 2^{|U|-n}\, D_{aU}$, which is summed over even-sized sets. Provided that selection acts only on even-sized sets of loci, we find that $B_{\underline{1}}$ is inflated by viability selection in the same way, by a factor $\bar{w}_{\underline{1}} / \bar{w}$ (Eq. 26). In SI 5, we show that assortment and recombination changes the D_{aU} by:

$$\begin{aligned}\triangle D_{aU} &= \sum_{ST=U} r_{s,T}\, (D'_{aS}\, D'_T - D'_{aU}) - (1-\alpha) \sum_{ST=U} r_{aT,S}\, D'_{aS}\, D'_T \\ &-\alpha\, \frac{(p_a - q_a)}{p_a\, q_a} \sum_{ST=U} r_{S,T}\, D_{aS}\, D_{aT}\end{aligned} \tag{32}$$

As in Eq. 27, the first two terms give a set of coupled linear equations which represent the decay of associations, slowed by assortment α. The last term arises when assorting alleles deviate from equal frequency. We can understand its contribution most easily by considering three-way associations D_{ajk}:

$$\begin{aligned}\triangle D_{ajk} &= -r_{j,k}\, D'_{ajk} - \\ &(1-\alpha)\, r_{a,jk}\, D'_{ajk} - \alpha\, \frac{(p_a - q_a)}{p_a\, q_a}\, r_{j,k}\, D'_{aj}\, D'_{ak}\end{aligned} \tag{33}$$

We see that when D_{aj}, D_{ak} grow with the same sign (as they will if $a_{jk} > 0$), and when $p_a < \frac{1}{2}$, D_{ajk} will be driven to positive values by assortative mating. This in turn will cause p_a to increase, if (as assumed) $a_{jk} > 0$. More generally, if $\sum_U a_U \sum_{ST=U} r_{S,T} D_{aS} D_{aT}$ is positive, then the extra term in Eq. 32 causes an effective overdominance that drives the assortment locus towards maximum polymorphism. We can be slightly more specific if we assume that initially, the assortment allele is rare, and is only weakly associated with the selected loci. Then, the D_{aj}, D_{ak} will each increase at a rate λ, the leading eigenvalue of the linear equations for these associations. Both D_{ajk} and p_a will increase at the same rate as long as $p_a << 1$; as p_a approaches $\frac{1}{2}$, D_{ajk} declines to zero. Supplementary Information 6 shows a figure that illustrates these dynamics, with four selected loci.

When assortment is strong (α~1), D_{ajk} will tend to a quasi-equilibrium value of $-\alpha \frac{(p_a - q_a)}{p_a q_a} D_{aj} D_{ak}$, and so the effective selection on p_a is $s_a \sim -\alpha \frac{(p_a - q_a)}{p_a q_a} a_{j,k} D_{aj} D_{ak}$. This is proportional to the increase in mean fitness of offspring that is caused by associations D_{aj}, D_{ak}. However, if one follows the population from an initially symmetrical equilibrium with D_{aj}, D_{ak} small and D_{ajk} zero, then all these will increase together, and this QLE approximation may not be accurate. It may be possible to find more general results for the invasion of assortment alleles in the limits of tight linkage and of strong assortment; we leave this task for the future.

DISCUSSION

Felsenstein (1981) showed that gene flow could be reduced by the growth of associations between independent components of reproductive isolation - in his model, between a locus a that causes assortative mating between haploids, and a pair of epistatically interacting loci $\{b, c\}$ that cause disruptive selection on haploid viability. Assortment has no direct effects on individual fitness, and the selected loci are kept polymorphic by frequency-dependent selection. We extend Felsenstein's model in a variety of ways, in order to find more general conditions for when reproductive isolation can be strengthened in this way, and to find just what drives this process.

In the simplest model, we assume multiple loci that each cause assortment amongst haploids, or equivalently, selection against heterozygotes in diploids; each locus is analogous to locus a in Felsenstein (1981). We find a simple condition for when associations amongst any set of alleles will grow (Eq.15). This model of single-locus incompatibilities is just the symmetric viability model under disruptive selection (see Christiansen, 1999, Ch. 8). We extend this model to allow multiple pairs of interacting loci, each pair being analogous to $\{b, c\}$ in Felsenstein (1981), and find similar conditions

for when associations between these pairs will grow (see SI 4, and Eqs. 17 - 19). Finally, we extend Felsenstein's (1981) model to ask when a single assortment locus (analogous to a) will couple with an arbitrary set of loci that influence haploid viability ($\{b, c, \ldots\}$). We show that viability selection inflates the net pairwise association between a and the selected loci by a factor equal to the relative fitness of the fittest genotypes (Eq. 26). When linkage is tight, this increase equals the recombination load; associations increase if the total rate of recombination that breaks up fit gene combinations is greater than the average recombination between assortment locus and the selected loci, multiplied by $(1-\alpha)$, where α is the strength of assortment (Eq. 30). More generally, associations will grow if assortment is sufficiently strong ($\alpha \sim 1$), even if selection is weak and linkage loose. Finally, we consider the invasion of a rare assortment allele: if this allele becomes associated with the selected loci, as described above, then it will move towards a frequency of $\frac{1}{2}$, thus minimising gene flow.

Incompatibilities can become coupled for two distinct reasons. With multiple single-locus incompatibilities, or multiple pairs of interacting loci, coupling can occur when a population with a high variance in compatibility (in the limit, with just two complementary genotypes segregating) has higher mean fitness than a diverse population, in which most individuals have intermediate incompatibility. That is, coupling depends on the existence of disruptive selection on the degree of compatibility (Fig. 1). This is not consistent with the traditional view of reinforcement, in which an association with prezygotic isolation is favoured because it reduces the loss of fitness due to postzygotic isolation. In the models just described, incompatibilities occur at the same stage, and can be pre- or postzygotic.

We might ask why, on the usual view of reinforcement, it is impossible for a modifier that reduces heterozygote fitness to invade, whereas a modifier that causes assortative mating can do so: the population genetics of underdominance are the same as those of assortment amongst haploids. The difference is in the constraints that we implicitly assume. A cost-free modifier of mate choice reduces cross-mating, but this fitness loss is precisely compensated by increased assortative mating. (Recall that we define fitness as the contribution of a *pair* of haploid genotypes). An allele that reduced heterozygote fitness could be compensated by increased homozygote fitness in just the same way. However, we imagine that the fitness of homozygotes is fixed at its maximum, and so assume that alleles that reduce heterozygote fitness are necessarily selected against. This argument applies to the increase of a modifier allele (in Felsenstein's (1981) terminology, to "one allele" models). In this paper, we are concerned with the evolution of *associations* (i.e., linkage disequilibria). This is more straightforward to analyse, because we do not need to make any assumptions about the fitness effects of modifier alleles.

In Felsenstein's (1981) model, and in our generalisation to coupling of assortment with multiple loci affecting haploid viability, associations develop for a second, and different, reason. Here, alleles that cause assortment amongst haploids, or that reduce fitness of heterozygotes, become associated with alleles that affect haploid viability. In this case, associations develop because they reduce the production of unfit recombinants. This is clear from Eq. 27, which shows that the only effect of assortment (or underdominance) α is to reduce the effective rate of recombination by a factor (1-α). Moreover, the association is with an allele that acts *before* meiosis, and that reduces the production of haploid recombinants that would die at a later stage. It is still questionable whether this case fits with the traditional view of reinforcement: coupling with an underdominant locus would occur in just the same way as with an assorting locus. The point here is that although a modifier that killed heterozygotes could not invade, once selection against heterozygotes is established by some kind of frequency-dependence, a coupling with it can evolve without further fitness cost.

One way to demonstrate the distinction between the two mechanisms is to consider a model in which incompatibilities have additive, rather than multiplicative, effects:

$$\frac{\bar{W}}{\bar{W}} = 1 + \frac{\alpha}{p_a\, q_a}\, \zeta_a\, \zeta_a^* + a_{bc}\, (\zeta_b\, \zeta_c + \zeta_b^*\, \zeta_c^* - 2\, D_{bc}) \qquad (34)$$

Now, mean fitness is independent of associations D_{ab}, D_{ac}, because the effects of loci a, and $\{b,\ c\}$ add up. Yet, the analysis given above is almost unchanged: viability selection alone will inflate associations according to Eq. 26, and assortment will slow down the decay of associations according to Eq. 27. In models of this sort, the growth of associations does not depend on coefficients such as a_{abc}, which play a crucial role when incompatibilities that act at the same stage are involved.

When can we see the growth of linkage disequilibria as being adaptive? Most population genetic analysis of adaptation asks whether alleles that affect a trait will change in frequency. Indeed, Fisher (1930, Ch. 2) defined adaptation as requiring a change in allele frequency, and his "Fundamental Theorem" identifies the increase in mean fitness caused by selection on allele frequencies. Allele frequency change is emphasised because it is more or less permanent, whereas in a sexual population, changes in linkage disequilibria are transient, being broken down by recombination. However, this is a matter of degree: adaptive alleles will gradually be eliminated by mutation unless selection continues. Conversely, in an asexual organism, linkage disequilibria persist, since there is no recombination. However, for linkage disequilibria to remain permanently, the constituent alleles must remain polymorphic; this requires negative frequency-dependent selection, as envisaged here. Finally, we note that with assortative mating, changes in linkage

disequilibria can become permanent even with sexual reproduction, if they lead to complete speciation (e.g. upper curve in Fig. 4).

We can see the evolution of linkage disequilibria as adaptive, either because increased variance in compatibility increases mean fitness, or because associations with alleles that combine assortatively reduces the effective rate of recombination. It is true that mean fitness does not necessarily increase if there is recombination as well as selection (Moran, 1964). Nevertheless, selection alone increases mean fitness by an amount equal to the genotypic variance in fitness, and moreover, the rate of increase of linkage disequilibrium due to selection is proportional to the gradient in log mean fitness: $\Delta D_U = \sum_V (D_{UV} - D_U D_V)(\partial \log(\overline{W}) / \partial D_V)$ (Barton, 1986; Turelli and Barton, 1994). Thus, for example, in the model of single-locus incompatibilities, associations only grow if they lead to increased mean fitness; this growth is opposed by recombination, but nevertheless, the action of selection on linkage disequilibria is adaptive.

Coupling of independent components of reproductive isolation requires strong assortment, strong selection, or tight linkage. How likely is it that these requirements will be met? The symmetric viability model (the basis of our initial analysis) was developed in response to the discovery of extensive electrophoretic polymorphism in the 1960s, and showed that if this polymorphism were under strong balancing selection, then strong linkage disequilibrium should be seen (Franklin and Lewontin, 1970). It soon became clear that genetic polymorphism is close to linkage equilibrium, with the weak associations that are observed being attributable to random drift and population admixture, a pattern now confirmed in extraordinary detail by genome-wide data (HapMap Consortium, 2005). Thus, although it is plausible that a substantial fraction of polymorphism in protein sequence is subject to selection, this selection is unlikely to be strong enough to sustain linkage disequilibrium despite recombination.

However, we are concerned with those rare circumstances that may lead to speciation, rather than with polymorphism in general. The simplest scenario is that individuals can be adapted to one or other of two limiting resources, but not to both. If mating is associated with these resources, then linkage disequilibria will naturally be generated between the sets of alleles favoured on each resource, and coupling will be inevitable. (This would occur for herbivorous insects if mating occurs on different host plants (e.g. Via, 2002). Less obviously, adaptation to different ecological niches might lead to different body size, and hence assortment (e.g. Nagel and Schluter, 1998), or mimicry of different models might lead to different mating preferences; (e.g. Jiggins et al., 2001)). However, if mating is random, then there need not be any systematic pressure generating associations amongst different components of reproductive isolation, and our analysis applies.

To see this, think of the simple case where effects of different incompatibilities are additive. Felsenstein's (1981) model assumes that alternative alleles adapt to one or other of two niches (see above, and SI 2, 3). If their effects are additive (ϵ=0), then no linkage disequilibrium is generated. Now, imagine the extension to multiple pairs of interacting loci. Alleles 00 at two loci might add fitness s in environment I, alleles 11 might add s in environment II; and combinations 01 and 10 make no contribution in either environment. Thus, linkage disequilibrium would be maintained between these two loci. The existence of two resources could maintain similar polymorphisms at any number of other pairs, but as long as their effects are additive, there would be no systematic pressure favouring associations between pairs. However, introduction of alleles causing assortment at locus a could trigger associations between all these pairs, causing strong isolation between any pair of complementary genotypes (001100 and 110011, say). The point is that even if polymorphism is maintained by the existence of just two alternative niches, this does not necessarily select for associations amongst all the alleles best adapted to each niche (000000 and 111111, say). Thus, the mechanisms that we discuss may be important in the evolution of well-isolated species.

Associations between multiple selected loci and an assortment locus reduce the effective rate of recombination, and indeed, associations grow precisely because of that reduction. A modifier allele that directly reduced recombination is also favoured when selection maintains stable linkage disequilibria (the "reduction principle"; Feldman et al., 1996). Because associations with assortment loci are opposed by recombination, whereas recombination modifiers by definition are not opposed by any direct force, we expect that reduced recombination would evolve more readily than will linkage disequilibria - the key point from Felsenstein's (1981) distinction between "one allele" and "two allele" models. Now, reduced recombination makes it easier for selection to maintain polymorphism despite disruptive selection: for example, genes responsible for divergence of *Rhagoletis pomonella* into host races are held together in an inversion (Feder et al., 2003). However, reduced recombination only makes coupling between incompatibilities easier if the loss of hybrid fitness is due to heterozygosity rather than to recombination: in the latter case, the outcome depends on the *relative* rates of recombination (Eq. 30).

Although we consider only a single population,. strong disruptive selection on multiple polymorphisms is most likely to arise in parapatry, when continued migration maintains polymorphism. In his "shifting balance" theory of evolution, Wright (1931) thought of species as typically being subdivided into a patchwork of regions that carry different sets of alleles, which are somewhat incompatible with each other. These patches would be separated by narrow hybrid zones, maintained by disruptive selection towards different 'adaptive peaks'. In principle, the geographic patterns for different incompatibilities might be independent of each other, so that there would be no strong subdivision of the species.

However, various processes tend to bring independently evolved incompatibilities together - the most important being demographic fluctuations, and in the extreme, secondary contact between temporarily separated populations (Barton and Hewitt, 1985). Once a set of incompatibilities are more or less coincident, however, the processes modelled in this paper become relevant: selection may favour stronger or weaker coupling, depending on the sign of epistasis. If incompatibilities arose via a Dobzhansky-Muller process, via a set of fit intermediates, then selection will favour a *scattering* of clines, such that the fitter intermediates are reconstructed in the centre; there are several examples of this in nature (Searle, 1986; Shuker et al., 2005). When the effects of different incompatibilities multiply, we have shown that selection pulls clines together, via a weak interaction due to deviation from additivity. In addition, linkage disequilibrium caused by dispersal also pulls clines together; even with no linkage, this is a stronger effect (Slatkin, 1975; Charlesworth and Charlesworth, 1979). The analysis of selection and recombination in a single population given here needs to be extended to allow for the effects of gene flow, in order to determine when a set of independent isolating factors will pull together to form a strong barrier to gene flow.

Felsenstein's (1981) simulation model has been prominent as one of the first quantitative studies of speciation. The mathematical analysis here extends the model to a much wider range, which could not easily be studied by simulation alone. Moreover, our analysis shows that there are two distinct reasons why different components of reproductive isolation become coupled together: first, a locus that causes assortative mating may become associated with loci that affect haploid viability, if this reduces the production of unfit recombinants, and second, associations may develop if variance in compatibility increases mean fitness. The first is more or less consistent with the traditional view of reinforcement, but the second is not. As in many areas of evolutionary biology, understanding the cause of a phenomenon is not straightforward.

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FIGURES

Figure 1. The relation between fitness and the number of homozygous loci, k, determines whether selection favors increased linkage disequilibrium. Black dots shows this relation when fitness is multiplicative across ten loci ($W = (1-s)^{10-k}$), whilst the upper grey dots show the relation $W = (1-s)^{10\,(1-(k/10)^{0.1})}$; in both cases, s=0.5, and the extreme heterozygous and homozygous genotypes have fitness close to 0 and 1, respectively. The dark bars show the distribution of k for a population in complete linkage disequilibrium, in which there are just two complementary genotypes. Then, $\overline{W} = 0.5$ for both fitness functions. In contrast, a population in linkage equilibrium (grey bars) has lower mean fitness ($\overline{W} = 0.056$) if fitnesses are multiplicative across loci, but higher mean fitness ($\overline{W} = 0.623$) if fitnesses interact as shown by the upper dots. Selection favours increased variance in homozygosity in the first case, and decreased variance in the second case. (Note that in Eq. 5, it is convenient to define fitness so that the mean is always 1 at linkage equilibrium, rather than varying with allele frequency, as here).

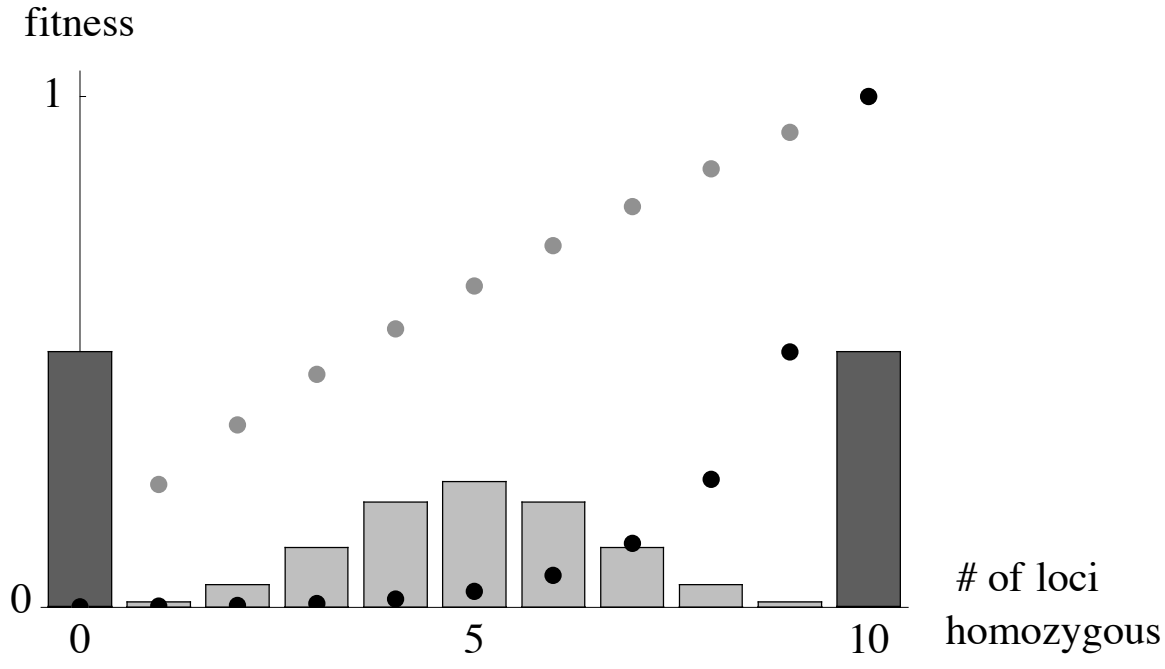

Figure 1

Figure 2. Examples of the varied behavior of three loci, with selection against heterozygotes. Each plot shows the pairwise disequilibria (D_{23}: blue; D_{13} green; D_{12}: black) and the three-way association (D_{123} : red). Initially, these associations are small and positive; allele frequencies remain at $\frac{1}{2}$ throughout. a) Fitness decreases with the number of heterozygous loci as {2.6, 0.9, 0.9, 0}. (See SI 1 for the relation between these fitnesses, and the coefficients $a_{U,V}$). Then, pairwise associations D_{ij} increase if $r_{i,j} < \frac{2-W_1-W_2}{W_2} = 0.222$ and the three-way association increases if $r_{123} < \frac{4-3\,W_1}{4\,W_2} = 0.361$. In this example, $r_{1,2} = 0.2$, $r_{2,3}$=0.3, and so $r_{1,3} = 0.38$, $r_{123} = 0.44$. (Loci are in order 1, 2, 3 and there is no interference between crossovers). Therefore, as predicted, D_{12} increases, and the other associations decrease, becoming yet closer to zero. After about 700 generations, genotypes 000, 001, 110, 111 are each at 0.164, and the other genotypes are rarer. However, this state is itself unstable: D_{13} and D_{23} increase, until the population contains only genotypes 000 and 111. Because the triple heterozygote has zero fitness, no recombinants survive. b) This shows the same, but with tighter linkage ($r_{1,2} = 0.1$, $r_{2,3} = 0.15$, and so `r1,3 = 0.22, r123 = 0.235`). Now, all the associations increase initially. Eventually, however, the pairwise associations decrease to zero, and an equilibrium is reached with a high value of D_{123}. Then, 001, 010, 100, 111 are each at 0.199, and the other four genotypes are rarer. Note that the mean fitness of a population at linkage equilibrium is defined as $\overline{W}_{\mathrm{LE}} = 1$. A population with only 000 and 111 produces half homozygotes and half triple heterozygotes, and so $\overline{W} = \frac{1}{2}(2.6 + 0) = 1.3$. In a population with 000, 001, 110, 111, each genotype has an equal chance of pairing to produce a zygote with 0, 1, 2, or 3 heterozygous loci. Therefore, mean fitness is 1.1. Finally, in a population with only 001, 010, 100, 111, each individual has a chance $\frac{1}{4}$ of pairing to produce a triple homozygote, and $\frac{3}{4}$ of producing a diploid with two heterozygous loci. So, mean fitness is $\overline{W} = \frac{1}{4} * 2.6 + \frac{3}{4} * 0.9 = 1.325$ - slightly higher than having two complementary genotypes. We see that the population does eventually evolve to the state with highest mean fitness when recombination is tighter, but does not do so when it is looser. c) If fitness decreases with the number of heterozygous loci as {2, 1, 1, 0}, then pairwise associations D_{ij} do not increase from zero, but the three-way association does if $r_{123} < \frac{1}{4}$. Below this threshold (for example, setting $r_{12} = r_{23} = 0.05$ so that. r_{123} =0.0975), we find that D_{123} increases to its maximal value, so that the four genotypes 001, 010, 100, 111 predominate..

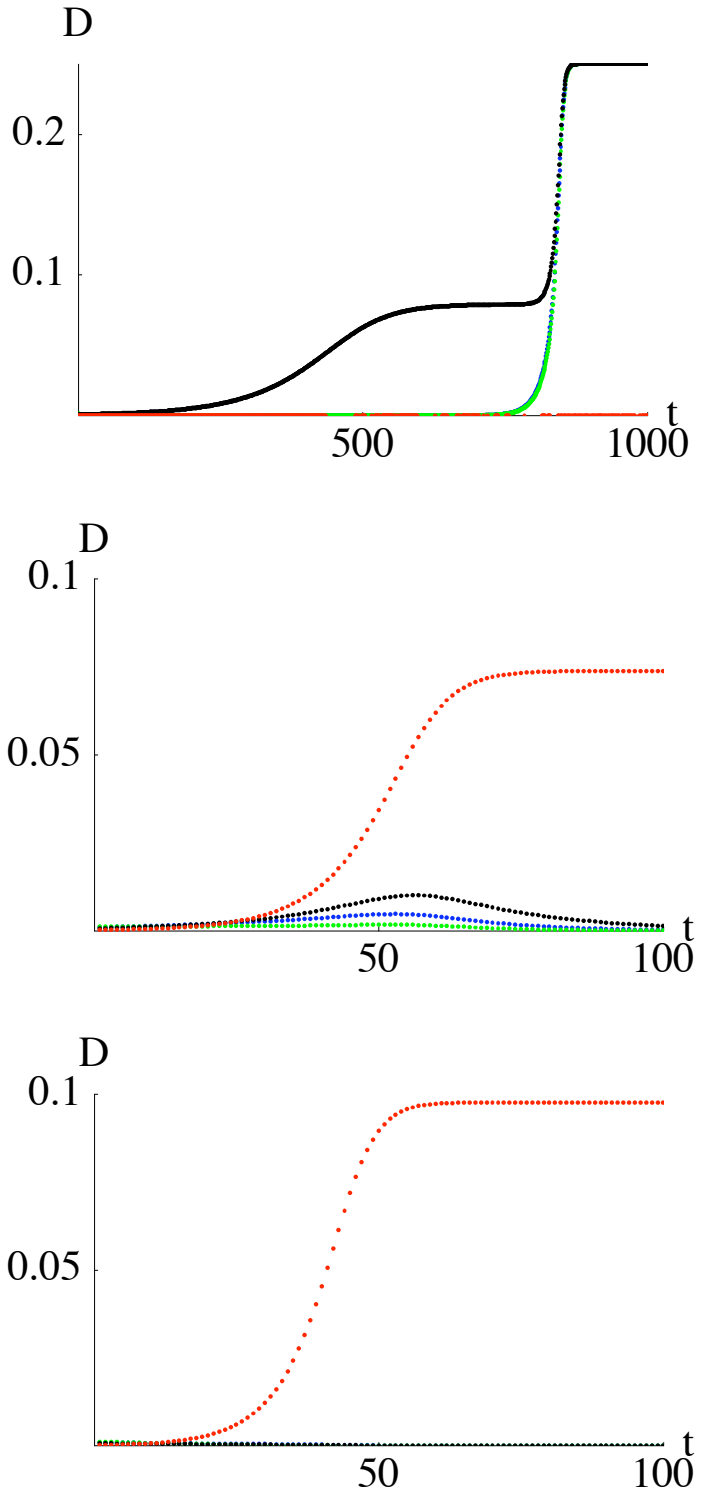

Figure 2

Figure 3. When two loci become associated, it is more likely that further loci will be associated in turn. This example shows the evolution of five loci, with underdominance $s = 1.2$ at each locus; this corresponds to a relative fitness of heterozygotes of $\frac{(1+s/4)}{(1-s/4)} = 0.538$, and fitness of the multiple heterozygote is $0.538^5 = 0.045$. In this instance, the threshold for pairwise coupling is $r^* = \frac{s^2}{(4-s)^2} = 0.184$. The population starts close to linkage equilibrium, with loci recombining at rates {0.3, 0.1, 0.3, 0.3} along the chromosome. The most closely linked pair couple first, followed by their neighbours, and finally, by the rightmost locus. There is a substantial increase in mean fitness, with the final population consisting of 92% of two complementary genotypes. The top panel shows the increase in mean fitness over time. The red bars show the mean fitness of successive populations at linkage equilibrium (left); with loci 2, 3 in complete LD and the other three at LE; with loci 1,2,3,4 in complete LD, and the fifth at LE; and finally, on the right, the mean fitness of a population containing just two complementary genotypes. The lower panel shows the distribution of numbers of heterozygous loci at times t=0, 300, 450, 600 (dark to light circles).

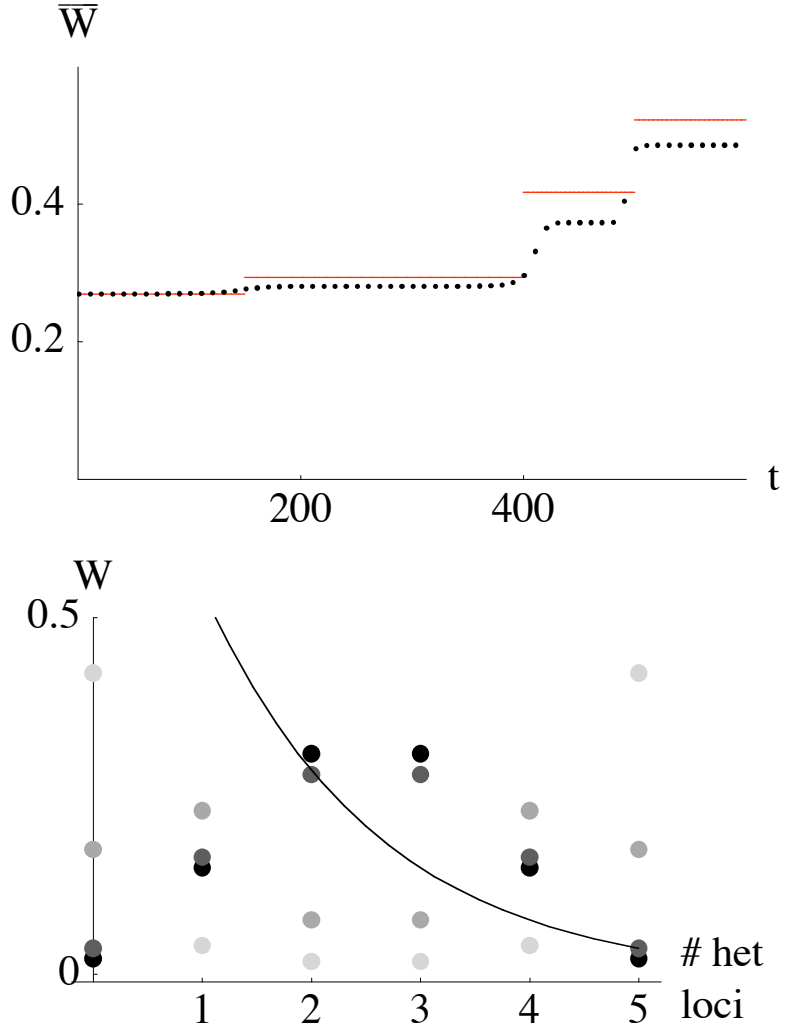

Figure 3

Figure 4. The threshold strengths of epistasis, γ, and assortment, α, above which associations D_{ab}, D_{ac} will increase, in Felsenstein's (1981) model. The rightmost solid curve shows the threshold for unlinked loci ($r_{b,c} = 1/2$); for α, γ above and to the right of this curve, associations will increase. The curves to the left are for linked loci $r_{b,c} = 0.05,\ 0.1,\ 0.2$ (left to right); the assortment locus is midway between b, c and there is no interference between crossovers. The upper dashed curve shows the maximum possible epistasis; on this curve, epistasis causes complete reproductive isolation. Values are calculated from Eq. 48, substituting $\rho = x(1-\alpha)$, $r_{b,c} = 2x(1-x)$, where $x = r_{a,b} = r_{a,c}$.

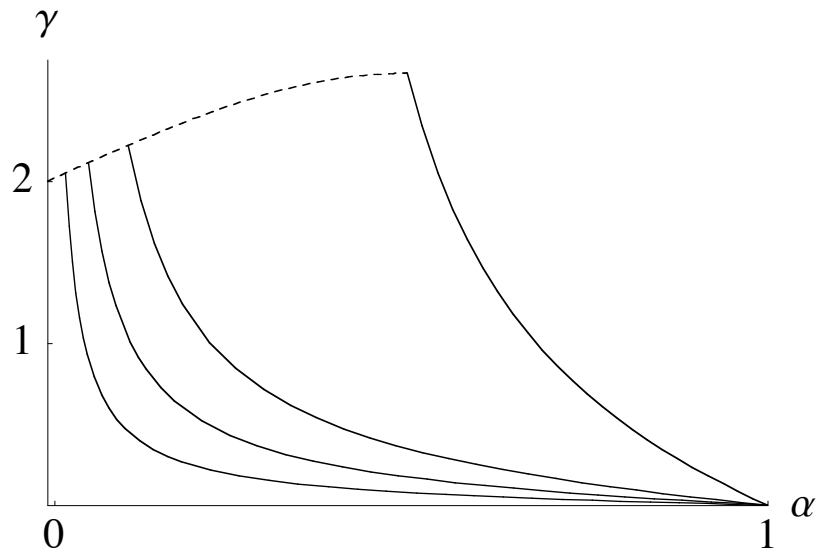

Figure 4

Figure 5. The conditions for growth of associations between two pairs of interacting loci. Each figure shows the critical strength of selection which is needed to give instability, plotted as a function of the linkage between pairs, $r_{i_2\, j_1}$. We assume $r_i = r_j$ and $\gamma_i = \gamma_j$; the curves show recombination rates $r_i = r_j = \frac{1}{16}, \frac{1}{8}, \frac{1}{4}, \frac{1}{2}$ (black, blue, purple, red). The top row is for non-overlapping pairs (gene order $i_1\ i_2\ j_1\ j_2$);. the right panel shows the region with small $r_{i_2\, j_1}$ in more detail. The lower row is for overlapping pairs, with gene order $i_1\ j_1\ i_2\ j_2$, in which case $r_{i_2\, j_1} < r_i = r_j$; for this case, γ_{crit} is plotted for $r_{i_2\, j_1} < \frac{r_i}{2}$, since the curve is symmetrical for $\frac{r_i}{2} < r_{i_2\, j_1} < r_i$. Note that the critical γ always increases with recombination between non-overlapping pairs, $r_{i_2\, j_1}$, and usually does so with overlapping pairs: instability generally requires stronger selection when recombination between pairs is higher. For large $r_{i_2\, j_1}$, instability requires less selection as recombination within non-overlapping pairs increases (black through to red curves, top left). However, the relationship is not simple when the pairs are tightly linked (top right). When pairs overlap, instability requires stronger selection when there is more recombination within pairs (black through to red curves, bottom row). This is because increasing r_i, r_j increases recombination between pairs as well as increasing recombination within, and this former effect outweighs the latter.

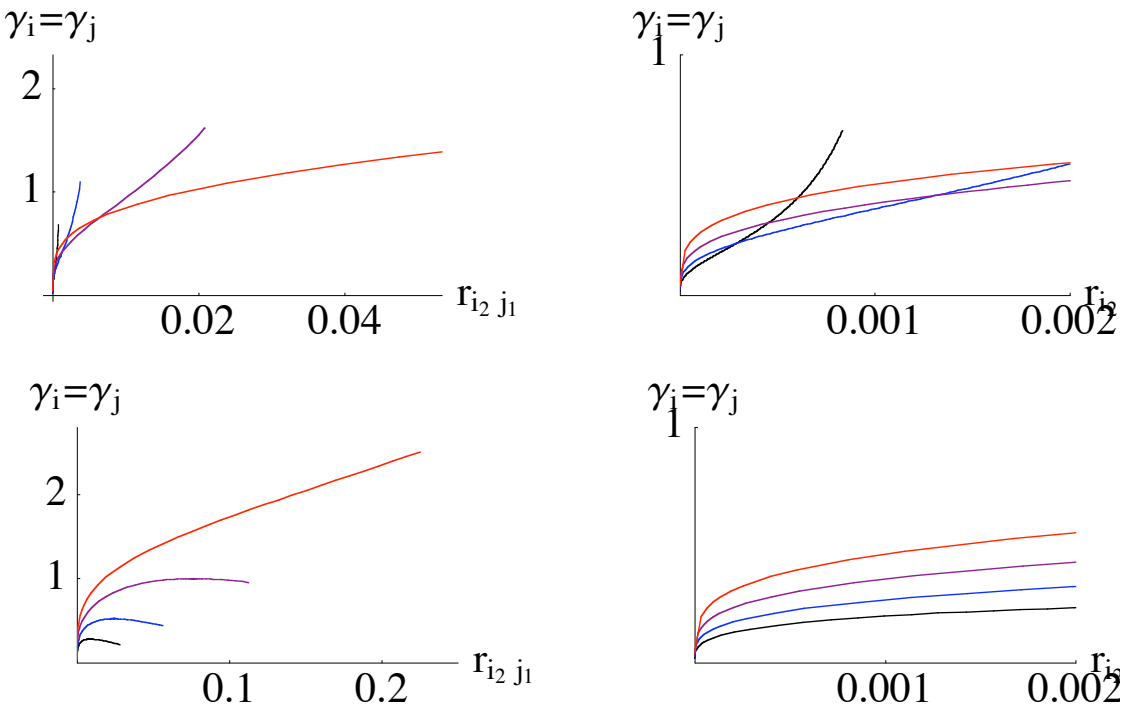

Figure 5

Figure 6. The critical strength of assortment, α, above which assortative mating becomes coupled with a set of four selected loci, which are evenly spaced on the genetic map. This is plotted against the recombination rate between adjacent selected loci, r. In the top panel, the assortment locus lies outside the selected set, and recombines at $\frac{r}{2}$ with the nearest of these. In the lower panel, the assortment locus lies mid-way between the first and second selected loci. (Note the different scales for α: assortment must be much stronger for coupling to occur when the assortment locus lies outwith the selected loci). Haploid viability is 1 for genotypes 0000, 1111; $1 - s$ for 0001, 0010,…,1101, 1110, and $1 - 5\,s$ for genotypes 0011, 0101, …, 1100. The critical α decreases as selection becomes stronger: the series of points are for s = 0.00625, 0.0125, 0.025, 0.05, 0.1, 0.2 (top to bottom). With the strongest selection, s = 0.2, genotypes such as 0011 have zero fitness. However, gene flow is still possible via genotypes 0111 etc. The thick lines at top right are the approximations for strong assortment (Eq. 29), while the thick line at lower left in the top panel is the approximation for tight linkage (Eq. 30). The critical threshold was calculated by a mixture of numerical calculation and computer algebra. The recursions for the four selected loci were iterated until equilibrium was approached (between 150 and 2000 generations, depending on the strength of selection). At this equilibrium, recursions for the D_{aU} were calculated, for the five-locus system. The strength of assortment, α, and the D_{aU}, were left as symbols. The recursions were then differentiated with respect to the D_{aU}, giving an 8×8 matrix that is a function of α. The critical α at which the leading eigenvalue equals one was then determined numerically.

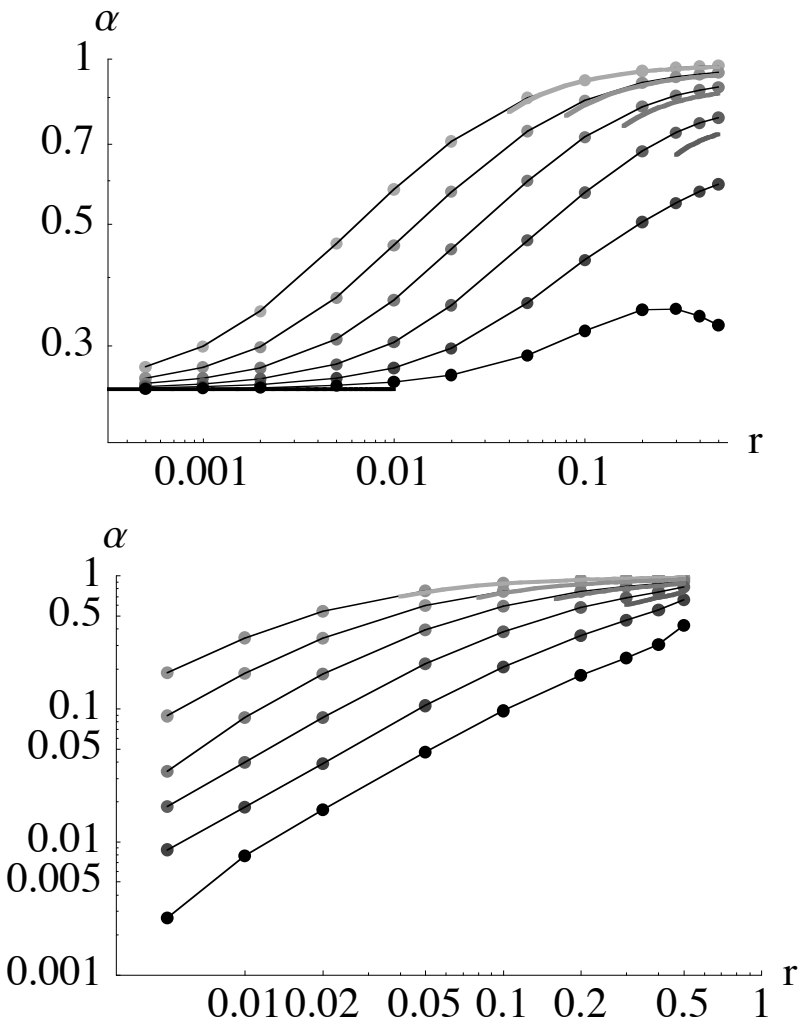
α
1
0.7
0.5
0.3
0.001
0.01
0.1
r
α
1
0.5
0.1
0.05
0.01
0.005
0.001
0.01
0.02
0.05
0.1
0.2
0.5
1
r

Figure 6

Supplementary Information

Supplementary Information 1: Stability of three-way associations with multilocus underdominance/assortment

Supplementary Information 2: Analysis of Felsenstein's (1981) model, assuming $p_b = p_c = \frac{1}{2}$

Supplementary Information 3: Stability of Felsenstein's (1981) three-locus model

Supplementary Information 4: Coupling between multiple pairs of interacting loci

Supplementary Information 5: Coupling between an assortment locus and multiple selected loci

Supplementary Information 6: Invasion of an assortment allele

Supplementary Information 7: Generating Figs. 1-6, 8

Supplementary Information 8: Generating Fig. 7

Supplementary Information 9: Mathematica definitions