

Genetic Analysis of Hybrid Zones

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When two distinct gene pools meet and produce fertile hybrids, the outcome varies from gene to gene. At some loci a universally favorable allele has been established on one side. Such alleles soon spread through the whole population and hence differences are rarely observed. At other loci different alleles may be favored in different environments or genetic backgrounds; selection maintains these differences in the face of random mixing. At other loci—perhaps at most of those we observe in molecular surveys—different alleles may have been established by chance and may have no appreciable effect on fitness. These differences gradually fade away, at a rate that depends on the strength of selection against introgression at the other loci with which they are associated.

The frequencies of the various genotypes found in a hybrid zone tell us about the overall strength of the selection, the number of genes involved, the rate of individual dispersal, and the ease with which alleles cross from one gene pool into the other. The aim of this chapter is to explain how data on discrete markers and on quantitative traits can be used to estimate such parameters. We illustrate the methods using examples from some of the hybrid zones that are discussed in more detail elsewhere in this book and use computer simulations to show that the estimates do not depend on exactly how selection maintains the differences between the hybridizing populations. Previous reviews have considered the wider questions of what hybrid zones can tell us about species and speciation and what role they themselves might play (Barton and Hewitt, 1985, 1989; Hewitt, 1988; Harrison and Rand, 1989; see also Ch. 1). We concentrate instead on the practical issues involved in the genetic analysis of hybrid zones.

A systematist, whose aim is to classify organisms, sees hybrid zones as boundaries between distinct types. A population geneticist, on the other hand, views them as sets of geographic gradients (i.e., of clines) in allele frequencies or quantitative traits. Both extreme views are misleading. Classification of individuals into parental, F_1 , F_2 , and backcross types wastes much information and, moreover, depends on which markers are used: an individual who is heterozygous for diagnostic alleles at five loci might be classified as an F_1 and yet be homozygous at the sixth locus. If even a small proportion of hybrids reproduce, all the individuals in the vicinity of the hybrid zone eventually carry introgressed alleles in some of their genes. However, describing a population

solely in terms of allele frequencies or the means of quantitative traits also throws away much information. The complete data set consists of the genotypes and phenotypes of each individual in the sample. How can this information be reduced to a manageable but informative state?

Hybrid zones can be described in several ways. We concentrate on the allele frequencies at each locus and the pairwise associations between loci ("linkage disequilibrium"). For continuously varying characters, the corresponding measures are the mean, variance, and covariances. Our aim is to explain how data presented in this way lead to estimates of selection and gene flow and to find how far these estimates depend on the details of how selection acts. This population genetic description may not always be the most appropriate. Where reproductive isolation is strong, the population may cluster around parental and F_1 genotypes, so that a classification into various recombinant types becomes more natural. One question which we consider is: At what point does selection become so strong that our methods break down? A third description becomes possible when one has sets of closely linked markers, for example, from DNA sequence data, so that the phylogenetic relation between the various genes sampled at a locus can be reconstructed. The extra information that might come from a set of such "gene trees" is discussed briefly at the end.

MECHANISMS FOR MAINTAINING CLINES

Clines can be maintained in two ways. There might simply be a balanced polymorphism, with an equilibrium that varies from place to place. Provided this equilibrium varies gradually enough, the shape of the cline directly reflects the local environment and has a shape that is independent of how far individuals move. For example, sickle cell anemia varies across Africa with the incidence of malaria: the frequency of the Hb^S allele tracks the relative fitnesses. A special case of such "dispersal-independent" clines has been suggested by Moore (1977), who argued that hybrid zones might be maintained by selection favoring hybrids within a narrow region of intermediate habitat.

Most hybrid zones cannot be explained in this way. First, dispersal is only negligible when clines are much wider than a characteristic scale, set by the ratio between the dispersal distance and the square root of the selection coefficient (Slatkin, 1973). Hybrid zones often consist of clines that are much narrower than likely environmental gradients, having widths approaching the individual dispersal range (see Figure 3 in Barton and Hewitt, 1985). Second, if cline shape were determined directly by local selective conditions, one would expect it to vary considerably from place to place. In fact, clines often have similar width and shape across different transects. For example, wherever the two races of the alpine grasshopper *Podisma pedestris* meet, the frequency of the Robertsonian fusion that distinguishes them changes in a sigmoid cline 500–900 meters wide (Barton and Hewitt, 1981a, 1989; Nichols and Hewitt, 1986); exceptions can be accounted for by barriers such as streams or scree. The fire-bellied toads *Bombina bombina* and *Bombina variegata* meet in a long hybrid zone that runs round the Carpathian Mountains and the Danube basin. Belly pattern and diagnostic allozymes change in almost exactly the same way across two transects 200 km apart in southern Poland (Szymura and Barton, 1991); the clines near Zagreb, in Croatia, are somewhat wider (9 km versus 6 km) but have the same form (Szymura, 1988; Ch. 10). Finally, if clines at each locus or for each phenotypic trait were maintained in

direct response to the environment, one would not expect them to change in the same way or at the same place: in contrast, almost all hybrid zones consist of a cluster of parallel clines, often involving characters with no obvious functional relation (Barton and Hewitt, 1985, 1989).

There are exceptions to this argument: some hybrid zones are so wide that dispersal can hardly be significant, for example, *Thomomys bottae* in the Sangre de Cristo mountains (Hafner et al., 1983). In some cases, genotype frequencies change together but do track the local environment, for example, the crickets *Gryllus firmus* and *Gryllus pennsylvanicus*, which are associated with different soil types (Rand and Harrison, 1989). The concordance of different characters in such "mosaic" hybrid zones (Harrison and Rand, 1989) may be a relic of secondary contact: one would expect that after prolonged hybridization, only those genes directly selected to fit the relevant environment (or that interact epistatically with directly selected genes) would be associated with that environment. In the absence of a sustained influx, differences in neutral traits would decay over a time inversely proportional to the rate of gene flow between habitats.

Their narrow width, consistent shape, and close concordance suggest that most hybrid zones are maintained by a balance between selection and dispersal: the sharp disjunction that would be produced by selection alone is blurred by the random movement of individuals. Selection could act in many ways. The primary distinction is between adaptation to the external environment (so that different alleles are favored in different places) and selection against hybrids (so that alleles are favored in their own genetic background or when at high frequency, regardless of location). The distinction is important, because it determines how the hybrid zone can move, or, in other words, how the sets of genes that distinguish the hybridizing populations compete with each other. If alleles are selected to fit their native habitat, the hybrid zone must lie at a particular point on an environmental gradient. If, on the other hand, selection acts against hybrids, the hybrid zone can move from place to place: it is then termed a "tension zone" (Key, 1968; Barton and Hewitt, 1985).

In reality, different kinds of selection act in the same hybrid zone. For example, the sharp boundaries between different warning patterns in *Heliconius* butterflies are maintained by Müllerian mimicry. This involves selection against heterozygotes, recombinants, and rare alleles, which all tend to produce patterns that are not recognized as distasteful by predators (Mallet, 1986; Mallet et al., 1990). Selection also acts to favor patterns that are common in other species in the same mimicry ring; it can be seen as an adaptation to the external environment, though the set of hybrid zones in all the mimetic species is still free to move as one.

Fortunately, the mechanism of selection has little effect on the shape of the clines; it is this factor that allows us to make inferences without needing to know just how selection is operating. Moreover, provided that selection is not too strong, cline shape does not depend on the local population structure: gene flow through both a continuous habitat and across a grid of demes or "stepping stones" can be approximated by diffusion (Nagylaki, 1975). The effect of gene flow then depends on a single parameter (σ) defined as the standard deviation of the distance between parent and offspring, measured along a linear axis; it does not depend on the whole distribution of dispersal distances, which would be much harder to measure accurately. (Note that in two dimensions, the standard deviation of the total distance moved is $\sqrt{2}\sigma$, as it includes

movements in two directions). Variations in σ from place to place can greatly affect cline shape (Karlin & Richter-Dyn, 1976); however, they might be detected by the presence of an obvious barrier to dispersal or by variation from transect to transect.

CLINE WIDTH

A variety of models of selection on single loci or quantitative traits produce clines with similar shapes and with width proportional to the ratio between dispersal and the square root of selection (σ/\sqrt{s}). It is convenient to define the width of a cline in allele frequencies as the inverse of the maximum slope; for a quantitative trait, the analogous definition is the ratio between the difference between the populations on either side and the maximum slope ($\Delta z/(\partial z/\partial x)$). (Explicit theoretical predictions are usually impossible with other definitions, such as the distance between the 20% and 80% points.) Cline widths can be calculated using the diffusion approximation, which holds when selection is weak; in the models considered below, the population is assumed to be diploid and to mate at random.

To compare the widths of clines maintained in different ways, we must use some common measure of selection. Let s^* be the difference in mean fitness between populations at the center (defined as the point where the slope is greatest) and the edge. The widths of the clines illustrated in Figure 2-1 are as follows: selection against heterozygotes (H): $2\sigma/\sqrt{s^*}$; selection favoring different alleles on either side of a sharp ecotone, with no dominance (E): $1.732\sigma/\sqrt{s^*}$; the same, but with dominance (D): $1.782\sigma/\sqrt{s^*}$. Thus for a given dispersal rate and a given reduction in mean fitness, the widths of clines maintained by a balance between dispersal and selection are similar. For the case of neutral mixing (N), the width t generations after the two populations met in an abrupt step is $2.51\sigma\sqrt{t}$, which is again similar if t is analogous to $1/s^*$, the characteristic time scale of selection.

Quantitative traits behave somewhat differently (Q in Fig. 2-1). Suppose that weak stabilizing selection favors one optimum on the left of a boundary and another on the right, and the genetic variance (V_g) is constant (a plausible assumption if many loci are involved). The cline width is then $(\Delta/2\sqrt{V_g})\sigma/\sqrt{s^*}$. It is proportional to the difference in optima (Δ), relative to the genetic standard deviation ($\sqrt{V_g}$) (Slatkin, 1978). In this case, the cline width is more simply related to the loss in mean fitness due to genetic variation around the optimum (L): $w = \sigma/\sqrt{L}$. Similar results hold for clines maintained by disruptive selection on a quantitative character (Rouhani and Barton, 1987). In both cases the width is still proportional to $\sigma/\sqrt{s^*}$.

This robust relation should allow one to infer selection pressures from cline widths and dispersal rates. However, it is not usually practicable. Barton and Hewitt (1985, Fig. 3) plotted cline widths against estimated dispersal rates for 26 hybrid zones. Though most clines had widths within one or two orders of magnitude of the estimated dispersal range, many were much wider, implying that they are maintained by unreasonably weak selection. Some of these clines may in fact be maintained by balancing selection alone and be independent of dispersal. However, a more likely explanation is that dispersal is often grossly underestimated. Many of the estimates used in that survey were based on limited mark-recapture studies, subject to great statistical inaccuracies, and inevitably missing long-distance migrants and juvenile dispersal; some

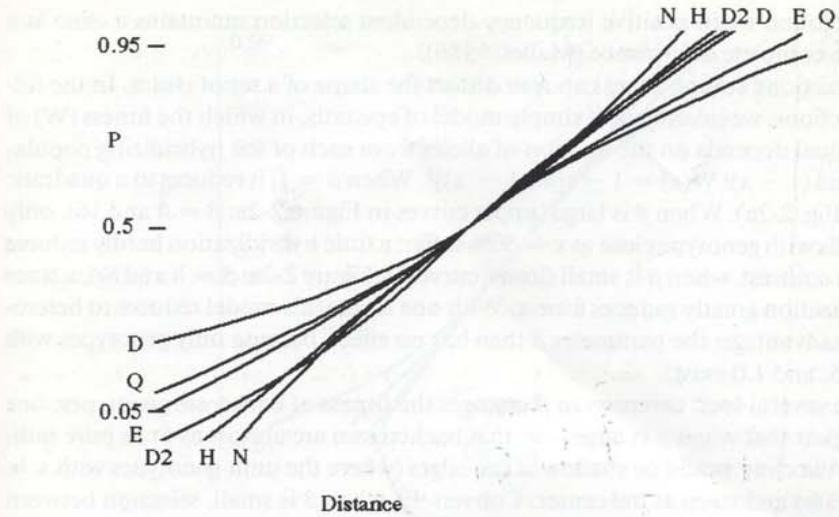


Figure 2-1. Clines maintained by a balance between dispersal and selection on a single locus have similar shapes. Allele frequencies are plotted against distance on a logit scale ($\log(p/q)$); the clines are scaled so all have the same position and width. Letters refer to different models. **N** = neutral introgression; **H** = heterozygote disadvantage; **E** = an ecotone, with fitnesses $1 + s:1:1 - s$ on the left, and $1 - s:1:1 + s$ on the right; **D** = an ecotone with dominance. Fitnesses are $1:1:1 - s$ on the left, and $1:1:1 + s$ on the right. **D2** refers to the same model, but now the frequency of the recessive homozygote is plotted. **Q** = stabilizing selection on a quantitative trait, with the optimum changing abruptly by Δ at an ecotone.

were not even derived from the same species (e.g., Hafner, 1982). In many examples of apparently wide clines, there is direct or indirect evidence of selection (e.g., compare Hafner, 1982, with Barton, 1982; see also next section).

CLINE SHAPE

The weak dependence of cline shape on the way selection acts is illustrated in Figure 2-1. Clines produced by a variety of models are scaled to the same width and plotted using a logit transformation ($z = \log(p/q)$), also known as a logistic transform. It has the advantage that it expands extreme gene frequencies, so a sigmoid curve appears as a straight line. A cline maintained by selection against heterozygotes follows a straight line with slope $(\partial z / \partial x) = 4/w$; other models give clines that approach a straight line on the logit scale. The same method could be used to represent clines in quantitative traits; however, the means of the two parental populations must be known accurately if the transformation is to succeed.

The clearest deviation from this pattern is produced by complete dominance (D). Because selection against rare recessives is ineffective, the frequency of the recessive allele can be high even in regions where it is not favored (Fig. 2-1, left). However, if the frequency of the recessive homozygote is plotted, instead of the frequency of the allele itself, the cline appears much more similar to the others (D2 in Fig. 2-1). A similar

pattern is found when positive frequency-dependent selection maintains a cline at a locus with complete dominance (Mallet, 1986).

Interactions among genes can also distort the shape of a set of clines. In the following sections, we investigate a simple model of epistasis, in which the fitness (W) of an individual depends on the fraction of alleles from each of the hybridizing populations, x and $(1 - x)$: $W(x) = 1 - s[4x(1 - x)]^\beta$. When $\beta = 1$, it reduces to a quadratic relation (Fig. 2-2a). When β is large (upper curves in Figure 2-2a: $\beta = 4$ and 16), only individuals with genotype close to $x = 50\%$ suffer: a little hybridization hardly reduces fitness. In contrast, when β is small (lower curves in Figure 2-2a: $\beta = \frac{1}{4}$ and $\frac{1}{16}$), a trace of hybridization greatly reduces fitness. With one locus, this model reduces to heterozygote disadvantage: the parameter β then has no effect, because only genotypes with $x = 0, 0.5$, and 1.0 exist.

With several loci, variation in β changes the fitness of backcross genotypes; one would expect that when β is large—so that backcrosses are almost as fit as pure individuals—the cline would be shallow at the edges (where the unfit genotypes with $x = 50\%$ are rare) and steep at the center. Conversely, when β is small, selection between pure and backcross genotypes is strong at the edges and relatively weak near the center, where all hybrids have low fitness. This pattern is seen when large numbers of loci interact (see Figure 2-2c, which shows results for eight loci), though large distortions are seen only when $\beta \gg 1$. However, the type of epistasis has remarkably little effect when just two loci interact (Fig. 2-2b).

The conclusion is that plausible forms of selection are unlikely to distort clines far from a sigmoid cline, which appear as a straight line when plotted on a logit scale. Strong distortions can be produced by epistasis, but only if a moderate to large number of loci interact in their effects on fitness, the effect is such that only individuals close to the central genotype have reduced fitness, and one is observing the loci that are actually under selection. One could produce models of frequency-dependent selection on a single locus, which would produce distorted clines; but, again, implausibly strong nonlinearity is needed.

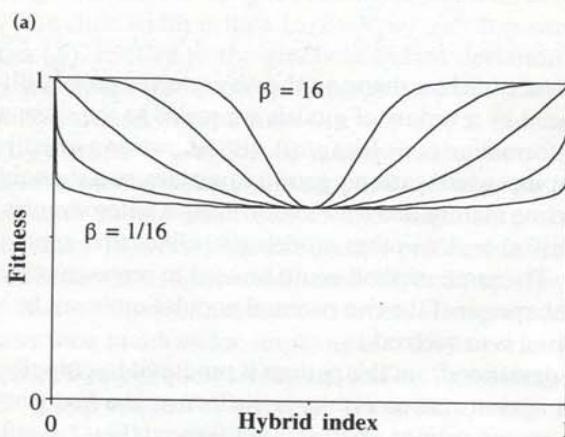


Figure 2-2. (a) In this model, individual fitness is $W(x) = 1 - s[4x(1 - x)]^\beta$, where x = the fraction of alleles derived from one of the hybridizing populations. The curves are for $\beta = \frac{1}{16}, \frac{1}{4}, 1$, and 16 .

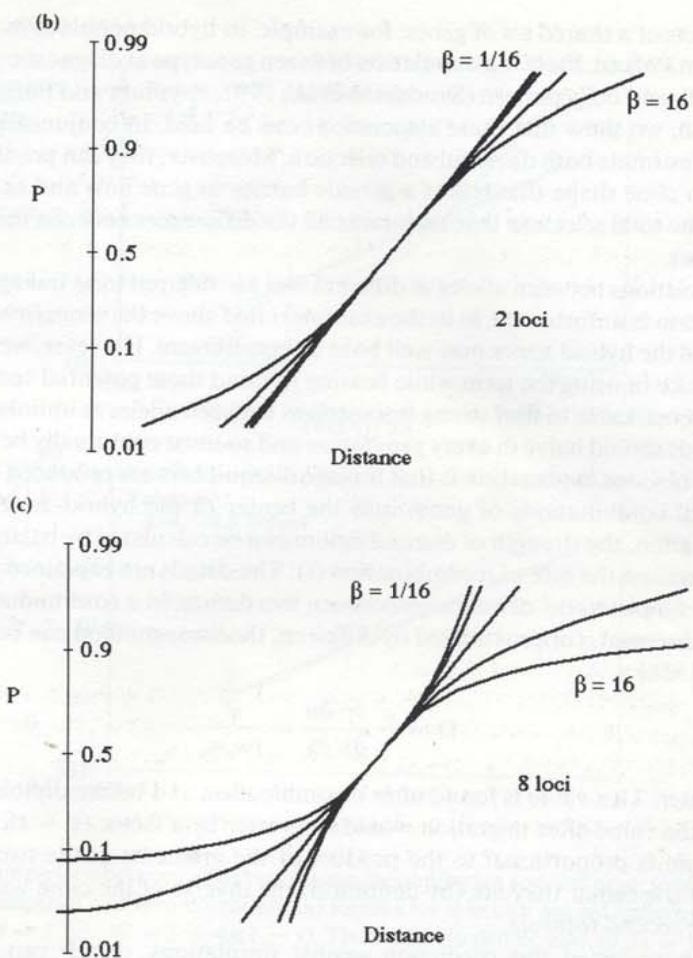


Figure 2-2. Continued (b and c) Note the shapes of clines with two and eight loci, respectively; each shows curves for $\beta = \frac{1}{16}, \frac{1}{4}, 1, 4, 16$ plotted using a logit transform and scaled to have the same width. With two loci (b), epistasis has little effect on cline shape. With eight loci (c), clines are stepped when $\beta > 1$; however, epistasis still has little effect when $\beta < 1$. Curves for large numbers are similar to those for $n = 8$. Cline shapes were calculated using the diffusion approximation and ignoring linkage disequilibrium (accurate when selection is weak).

LINKAGE DISEQUILIBRIA

The key assumption thus far has been that genes at different loci combine at random. In fact, strong associations are often found in hybrid zones: alleles derived from the same population tend to cluster together in the same individuals. Examples include *Heliconius* (Mallet et al., 1990), *Bombina* (Szymura and Barton, 1986, 1991), *Rana* (Kocher and Sage, 1986), *Uroderma* (Baker, 1981; Barton, 1982), *Gryllus* (Rand and Harrison, 1989), and *Caledia* (Shaw et al., 1985). Such associations are also revealed by correlations between unrelated traits that are unlikely to be explained by the pleio-

tropic effects of a shared set of genes: for example, in hybrid populations of *Bombina* in southern Poland, there is a correlation between genotype at diagnostic enzyme loci, mating call, and belly pattern (Sanderson et al., 1991; Szymura and Barton, 1991). In this section, we show that these associations can be used, in conjunction with cline widths, to estimate both dispersal and selection. Moreover, they can produce large distortions in cline shape that reflect a genetic barrier to gene flow and can be used to estimate the total selection that maintains all the differences between the hybridizing populations.

Associations between alleles at different loci are referred to as linkage disequilibria. This term is unfortunate, as in the examples cited above the genes involved are not linked, and the hybrid zones may well be in an equilibrium. However, we follow common practice in using the term while bearing in mind these potential confusions.

It is remarkable to find strong associations between alleles at unlinked loci. Such associations should halve in every generation and so must continually be replenished. The most obvious explanation is that linkage disequilibria are produced by the influx of parental combinations of genes into the center of the hybrid zone. To a good approximation, the strength of disequilibrium can be calculated by balancing the rate of influx against the rate of recombination (r). The details are explained in Appendix 1, using a simple model of exchange between two demes. In a continuous population, in which dispersal is approximated by diffusion, the same method can be used, giving (Barton, 1986)

$$D = \frac{\sigma^2}{r} \frac{\partial p}{\partial x} \frac{\partial u}{\partial x} = \frac{\sigma^2}{rw_p w_u} \quad (1)$$

at the center. This value is found after recombination and before diploid individuals migrate; the value after migration would be greater by a factor $(1 + r)$. Linkage disequilibrium is proportional to the product of the gradients at the two loci ($\partial p / \partial x$, $\partial u / \partial x$); at the center they are (by definition) the inverse of the cline widths (w_p , w_u), giving the second formula.

We have tested this prediction against simulations; details can be found in Appendix 2. Figure 2-3 shows results for hybrid zones maintained by heterozygote disadvantage or epistasis, with eight unlinked loci. The observed linkage disequilibrium is compared with the value expected from the cline width predicted by weak selection theory (straight line) and with that expected from the cline width actually seen in simulations (thinner curve). There is good agreement with the prediction from the observed width, even when selection is strong and even when there is epistasis: the values in the two graphs are similar. Thus dispersal rates could be estimated from observed linkage disequilibria, without the need to know just how the clines are maintained. When selection is strong, linkage disequilibria cause the clines to become narrower than expected and, in turn, to generate more disequilibrium. This phenomenon accounts for the discrepancy between the different predictions in Figure 2-3. When selection is weak, and the clines are maintained by heterozygote disadvantage, disequilibrium is somewhat weaker than expected. This may be because random drift scatters the clines to different locations, weakening their interaction. The effect is not seen with epistasis, as the latter tends to pull the clines together. Similar comparisons with just two loci show a similar pattern, though the confidence limits are wider, and there is less steepening of the clines with strong selection.

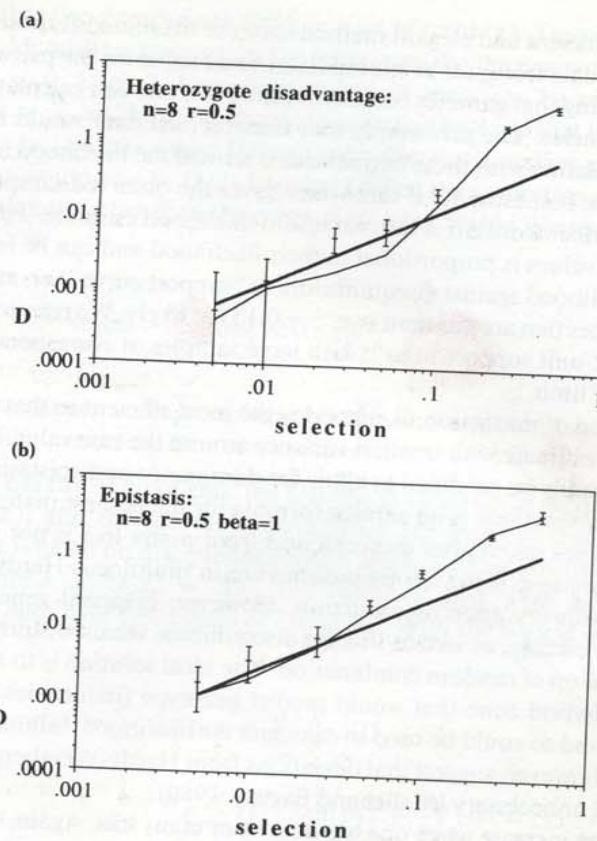


Figure 2-3. Comparison between observed linkage disequilibrium and that expected from a balance between dispersal and recombination. (a) Results for selection against heterozygotes, (b) Epistasis with $\beta = 1$, i.e., $W = 1 - 4x(1 - x)$. There were 40 demes, each of $2n = 50$ haploid individuals; selection acts on eight unlinked genes. The disequilibrium (D) at the center is plotted on a log scale, against the strength of selection at each locus (s). Haploid gametes migrate after meiosis and before selection; disequilibrium is measured immediately after meiosis, and so the predicted value is $m(l - m)/rw^2$ (see Appendix 1). The straight line shows the prediction, given the width calculated using a weak selection approximation $w = \sqrt{8m/s}$ for heterozygote disadvantage (a) and $w = \sqrt{64m/15s}$ for epistasis (b). The thinner line shows the prediction made from the actual width observed in the simulations. Deviations between the two lines are due to differences between observed and expected widths. Observed disequilibria are shown with 95% confidence intervals; data were accumulated from generations 100 to 400 in each run and recorded every 20 generations (apart from $s = 0.005$, for which generations 400 to 1000 were used).

Estimating Linkage Disequilibria: Maximum Likelihood. Estimation of linkage disequilibria is not as simple as estimation of allele frequencies. Because the double heterozygotes PU/QV and PV/QU cannot be distinguished, one cannot simply count up the contributions of different gametic types. Moreover, information from several loci must be combined to give reliable estimates, which is difficult when disequilibria are strong, as estimates from different pairs of loci are not then independent.

The most efficient and elegant method is to use likelihood (Edwards, 1972; Hill, 1974). Consider data from just two loci. Given some value for the pairwise disequilibrium and assuming that gametes combine at random, one can calculate the expected genotype frequencies. The probability that the observed data would have been produced by a population with these frequencies is termed the likelihood of the given disequilibrium. The best estimate is that which gives the observed sample with greatest probability. In other words, it is the maximum likelihood estimate. The relative plausibility of other values is proportional to their likelihood and can be represented by a graph of log likelihood against disequilibrium (a "support curve") or, more compactly, by rejecting values that are less than $\approx e^{-2} = 0.135$ as likely. We refer to this procedure as defining a "2-unit support limit"; with large samples, it corresponds to defining a 95% confidence limit.

The method of maximum likelihood is the most efficient in that with large samples it gives the estimate with smallest variance around the true value. It is also flexible in that it can readily be modified to allow for dominance and epistasis (e.g., Mallet et al., 1990). However, there is no explicit formula for the best estimate, and analysis of data from samples taken after dispersal and from many loci is not straightforward. With random mating, genotype frequencies are in multilocus Hardy-Weinberg proportions immediately after reproduction. However, dispersal generates deviations from Hardy-Weinberg, as well as linkage disequilibria, which distort estimates based on the assumption of random combination. The ideal solution is to set up an explicit model of the hybrid zone that would predict genotype frequencies as a function of dispersal rate and so could be used to calculate the likelihood. Simulations of a three-locus system, however, suggest that deviations from Hardy-Weinberg are small, making this model unnecessary (Mallet and Barton, 1989).

Difficulties increase when one has data from many loci. Again, the ideal solution would be to set up an explicit model, which would predict the complete frequencies of all multilocus genotypes: allele frequencies pairwise disequilibria, three-way disequilibria, and so on. This method would take an inordinate amount of computing; in practice, maximum-likelihood estimates from separate pairwise analyses must be combined. One cannot simply take the average of the separate estimates, as each may be substantially biased. The obvious approach is to use the total log likelihood, summed over all pairwise analyses, as a function of some common value of the pairwise linkage disequilibrium. There are two difficulties here. First, different pairwise estimates are not independent, so one cannot simply sum the log likelihoods. This problem may not be serious if disequilibria are weak, as with large samples from a population in linkage equilibrium estimates are uncorrelated. The second problem is that, if allele frequencies at different loci vary, one expects linkage disequilibria to vary. Indeed, the maximum possible disequilibrium depends on the allele frequencies. A rough solution is to estimate a standardized measure, $R = D/\sqrt{pquv}$. It must lie between -1 and +1 and can be thought of as the correlation between the states of the two loci.

Estimating Linkage Disequilibria: Variance in Hybrid Index. A simpler method, which is adequate in most cases, is to derive the average linkage disequilibrium from the variance in a "hybrid index." The variance is inflated if alleles that increase the hybrid index tend to be found together; when many loci are involved, the bulk of the variance may be caused by linkage disequilibria (Bulmer, 1980).

Suppose that two populations differ in a set of traits (z_i). They might be quantitative traits or Mendelian markers; in the latter case, the three genotypes in a diploid are labeled $z_i = 0, 1$, or 2 . Data from n loci or traits can be summarized by a hybrid index, $z = \sum_{i=1}^n \alpha_i z_i$. Here we choose to scale this index so that it runs from 0 for one population to 1 for the other. If there are n diagnostic marker genes, the appropriate weighting would be $\alpha_i = (1/2n)$, so that z is just the proportion of alleles derived from one population rather than the other. Assuming Hardy Weinberg proportions, the variance of z is:

$$\begin{aligned}\text{var}(z) &= \sum_{i,j=1}^n \alpha_i \alpha_j \text{cov}(z_i, z_j) \\ &= \sum_{i=1}^n \alpha_i^2 \text{var}(z_i) + \sum_{i \neq j} \alpha_i \alpha_j \text{cov}(z_i, z_j)\end{aligned}\quad (2a)$$

This expression has two components: the first due to variation in each contribution to the index [$\text{var}(z_i)$] and the second to covariance between different contributions. Where the index is based on discrete Mendelian markers, these covariances are due to linkage disequilibria: $\text{cov}(z_i, z_j) = 2D_{ij}$, and $\text{var}(z_i) = 2p_i q_i$. (The factor of 2 arises because z_i is the sum over two copies of i 'th gene). With the scaling $\alpha_i = (1/2n)$, we have:

$$\begin{aligned}\text{var}(z) &= \sum_{i=1}^n 2\alpha_i^2 p_i q_i + \sum_{i \neq j} 2\alpha_i \alpha_j D_{ij} \\ &= \frac{1}{2n} \left(\bar{z}(1 - \bar{z}) - \text{var}(p) \right) + \frac{1}{2} \left(1 - \frac{1}{n} \right) \bar{D}\end{aligned}\quad (2b)$$

Here, \bar{D} = average pairwise linkage disequilibrium; \bar{z} = average of the hybrid index; and $\text{var}(p) = 1/n \sum (p_i - \bar{p})^2$ = variance of allele frequency across the n loci. Because the variance of z and the individual allele frequencies can easily be calculated, Eq. 2b gives a straightforward way of estimating linkage disequilibria. Approximate confidence limits can be found by using the critical points of the $F_{n-1,\infty}$ distribution to set limits on $\text{var}(z)$ and ignoring uncertainty in the allele frequencies.

We illustrate this method with a sample of 351 *Bombina* taken from the center of the hybrid zone in southern Poland and scored for five diagnostic loci [sample Kopanka 2 (1981) in Szymura and Barton, 1986] (Fig. 2-4). The mean hybrid index is $\bar{z} = 0.486$, and the variance in allele frequency across loci is $\text{var}(p) = 0.005$. One can find the component due to heterozygosity at individual loci from the first term in Eq. 2b: $(1/10)(0.486 \times 0.514 - 0.005) = 0.0245$. The actual variance is 0.0397, which is significantly greater ($F_{356,\infty} = 1.62, P = 10^{-12}$). The excess variance (0.0397 - 0.0245 = 0.0152) is due to linkage disequilibria: $0.0152 = (1/2)(1-1/5)\bar{D}$, so that $\bar{D} = 0.038$. Because the critical points of the $F_{356,\infty}$ distribution are at 0.858 and 1.153, confidence limits on \bar{D} are from 0.025 to 0.054. This estimate compares with a maximum likelihood estimate of $\bar{D} = 0.037$, with support limits between 0.027 and 0.045. The maximum likelihood appears somewhat more powerful, though this may be because of correlations between the likelihoods calculated for each pair of loci.

It would be useful to know more about the statistical properties of estimators of multilocus linkage disequilibrium. Previous discussions have concentrated on just two

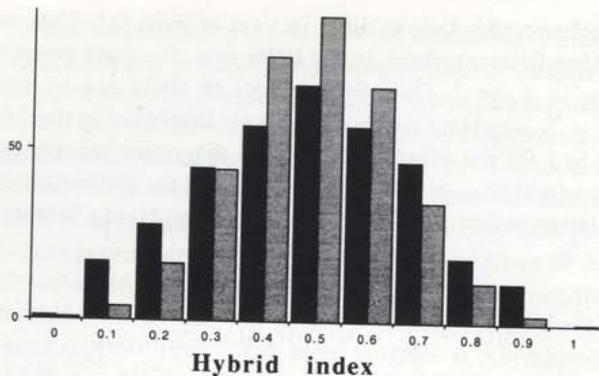


Figure 2-4. Distribution of the hybrid index in a sample of *Bombina* from the center of the hybrid zone in southern Poland (Kopanka 2, 1981; Szymura and Barton, 1986). The figure compares the observed distribution (solid bars) with the binomial distribution expected in the absence of linkage disequilibria (light bars). The index is based on five unlinked and diagnostic enzyme loci.

loci or on estimating the random disequilibrium generated by drift (Hill, 1974; Lewontin, 1988; Weir and Cockerham, 1989). A rough comparison of the two methods can be made by examining estimates from repeated subsamples from the actual data (C. MacCallum, pers. comm.). With 2000 replicate samples of 50 individuals (taken with replacement), the standard deviation of the maximum likelihood estimate around the true value is 0.014, which compares with 0.017 for estimates based on the variance in hybrid index. Bias is negligible, and the limits on the estimates are approximately correct; the true value falls within the support limits of the maximum likelihood estimate 93.0% of the time and within the confidence limits based on the hybrid index 97.3% of the time. As expected, the maximum likelihood estimate is more accurate, but the simpler estimate based on the hybrid index performs comparably. Because linkage disequilibria in hybrid zones are often strong and several loci can be sampled, samples of moderate size may suffice. For example, Barton (1982) detected significant disequilibria using data from 25 bats scored for three chromosome rearrangements.

Because analysis and simulations suggest that linkage disequilibrium is primarily maintained by a balance between dispersal and selection, we can work back from the maximum likelihood estimate of $\bar{D} = 0.037$ to find the dispersal rate. These five enzymes are unlinked (Szymura and Farana, 1978) and change across concordant clines with an average width of 6.05 km (Szymura and Barton, 1991). If the sample was taken before dispersal, Eq. 1 would apply and gives a dispersal rate of $\sigma = \sqrt{rDw^2} = 0.82 \text{ km gen}^{-1/2}$. If movement occurred before sampling, less dispersal would be required to explain the observed disequilibrium, giving a somewhat lower estimate of $\sigma = \sqrt{rDw^2/(1+r)} = 0.67 \text{ km gen}^{-1/2}$. (Note the units: σ^2 is the variance in distance moved along some axis per generation.) These values are rather lower than the figure of $\sigma = 0.99 \text{ km gen}^{-1/2}$ estimated by Szymura and Barton (1991), which was based on data from many samples across the hybrid zone. The main uncertainty in their estimate arose from random variation in disequilibria between samples, rather than from statistical errors in estimates from each sample.

COVARIANCE BETWEEN QUANTITATIVE TRAITS

Equation 2 can readily be extended to allow linkage disequilibria and hence dispersal rates, to be estimated from the covariance between quantitative traits. Suppose that two traits, z and z^* , are each determined by the sum of effects of a number of genes (n , n^* , respectively):

$$z = \sum_{i=1}^n \alpha_i z_i + E, z^* = \sum_{j=1}^{n^*} \alpha_j^* z_j^* + E^*$$

As before, z_i and z_j^* label the effects of the three genotypes at a locus, and take the values 0, 1, or 2. If these traits are determined by different sets of genes and the environmental components (E , E^*) are independent, any covariance must be due to linkage disequilibrium:

$$\begin{aligned} \text{cov}(z, z^*) &= \sum_{i=1}^n \sum_{j=1}^{n^*} \alpha_i \alpha_j^* \text{cov}(z_i, z_j^*) \\ &= 2 \sum_{i=1}^n \sum_{j=1}^{n^*} \alpha_i \alpha_j^* D_{ij} \end{aligned} \quad (3)$$

Now, if we assume that linkage disequilibria are due to a balance between migration and recombination, substitution from Eq. 1 gives:

$$\text{cov}(z, z^*) = 2 \sum_{i=1}^n \sum_{j=1}^{n^*} \alpha_i \alpha_j^* \frac{\sigma^2}{r} \frac{\partial p_i}{\partial x} \frac{\partial p_j}{\partial x} \quad (4a)$$

This equation can be rewritten in terms of the gradients of the traits themselves:

$$\text{cov}(z, z^*) = \frac{\sigma^2}{2r} \frac{\partial z}{\partial x} \frac{\partial z^*}{\partial x} \quad (4b)$$

Because we have defined the width of a cline in a quantitative trait as the ratio between the difference on either side and the gradient at the center ($w = [\Delta z / (\partial z / \partial x)]$), we have:

$$\text{cov}(z, z^*) = \frac{\sigma^2}{2r} \frac{\Delta z}{w} \frac{\Delta z^*}{w^*} \quad (4c)$$

Note that this derivation requires only that genetic variation be additive. Different loci might have effects that vary in strength and direction, and alleles need not be fixed on either side.

Fire-bellied and yellow-bellied toads differ in mating call as well as by biochemical markers: the natural logarithm of one component of call, the cycle length, differs by $\Delta z^* = 1.21$ (Sanderson et al., 1991). The cline in cycle length is not significantly different in width from the enzyme clines: $w^* \approx w = 6.05$ km. In the center of the hybrid zone, mating call is correlated with enzyme genotype: the covariance between $\ln(\text{cycle length})$ and a hybrid index (z) based on six diagnostic loci and scaled from 0 to 1 is $\text{cov}(z, z^*) = 0.021$. Because it is difficult to imagine that six arbitrarily chosen enzymes have a pleiotropic effect on call, this covariance is likely to be due to linkage disequilibrium. Assuming the loci to be unlinked ($r = 0.5$), the covariance can be explained by a dispersal rate $\sigma = \sqrt{2r \text{cov}(z, z^*) w w^* / \Delta z \Delta z^*} = 0.80 \text{ km gen}^{-1/2}$. This value is close

to the value estimated from covariances among the enzyme markers. Belly pattern also differs between the taxa and is correlated with both mating call and enzyme genotype to about the same extent. These covariances between unrelated characters support the idea that linkage disequilibria are built up primarily by dispersal, rather than by epistatic selection favoring particular combinations of alleles and traits.

VARIANCE OF QUANTITATIVE TRAITS

Both high heterozygosity and positive linkage disequilibria contribute to the variance in the hybrid index based on enzyme genotype—by definition, an additive and completely heritable trait (Fig. 2-4). In general, both these effects cause an increase in the variance of any quantitative trait in a hybrid zone. In addition, the nongenetic “environmental” variance may be greater if hybrids have reduced developmental stability. Consider a specific example: the variance in log(cycle length) in *Bombina*, which increases from an average of 0.029 outside the hybrid zone to 0.088 in the central Kopanka sample. Some of this increase can be ascribed to linkage disequilibria. To determine how much, suppose for the moment that the difference in trait between the two populations is entirely due to loci that are fixed for alternative alleles in the two races and that change in parallel.¹ Some variance is nongenetic (V_e), and some may be due to loci that are polymorphic in both races (V_{g0}); this amount remains constant across the hybrid zone. At the center, $p_i q_i = \frac{1}{4}$; if linkage disequilibria are generated by dispersal, for a given degree of linkage they are the same for all pairs of loci (\bar{D} on average). Then, from Eq. 2b:

$$\text{var}(z) = \sum_{i=1}^n 2\alpha_i^2 \left(\frac{1}{4} - \bar{D} \right) + \sum_{i,j=1}^n 2\alpha_i\alpha_j \bar{D} + V_{g0} + V_e \quad (5a)$$

Because the difference between the races is $\Delta z = 2\sum\alpha_i$, the variance at the center is:

$$\text{var}(z) = \frac{\Delta z^2}{2n_e} \left(\frac{1}{4} - \bar{D} \right) + \frac{\Delta z^2}{2} \bar{D} + V_{g0} + V_e \quad (5b)$$

The increase in variance is due partly to increased heterozygosity (first term) and partly to linkage disequilibria (second term). The effect of heterozygosity decreases inversely with the effective number of loci contributing to the difference between races, defined as $n_e = (\sum\alpha_i)^2 / (\sum\alpha_i^2)$.

In the *Bombina* example, the consistent covariances between different traits suggest that \bar{D} can be estimated from the disequilibrium between diagnostic enzyme markers: $\bar{D} = 0.037$. Hence $\Delta z^2 \bar{D} / 2 = 0.027$ of the increase in variance of 0.058 can be ascribed to linkage disequilibria. If the remaining increase of 0.032 is due to increased heterozygosity at diagnostic loci, the effective number of loci responsible for the difference in call must be small: $n_e \approx (0.25 - 0.037) / (2 \times 0.032) = 3.3$ loci (San-

1. The assumption that alternative alleles are fixed in the two races is not essential. If we take \bar{D} to be the average pairwise disequilibrium between alleles at *diagnostic* loci ($\Delta p = 1$), the disequilibrium between alleles at loci that contribute less to the difference is $D_{ij} = \Delta p_i \Delta p_j \bar{D}$; the second term in Eq. 5b still applies, but the effective number of genes contributing to the trait is now defined as $(\sum(\alpha_i \Delta p_i)^2) / (\sum(\alpha_i \Delta p_i)^2)$, and $(\frac{1}{4} - \bar{D})$ is replaced by the average of $[(pq/\Delta p^2) - \bar{D}]$ at the center (the average being weighted by $\alpha \Delta p$).

derson et al., 1991). This is an application of the Castle/Wright/Lande method of estimating gene numbers (Castle, 1921; Lande, 1981), which takes explicit account of linkage disequilibria. It suffers from the same disadvantages: primarily, sensitivity to dominance and epistasis, and poor statistical power. Moreover, this particular example should be taken only as an illustration, as the excess variance in mating call may be due to decreased stability of hybrids. Nevertheless, the calculation shows how measurements of genetic variance in quantitative traits across hybrid zones might give information about the genetic basis of quantitative variation. In the next sections, we see how the increase in genetic variance affects the shape of a hybrid zone and the rate of gene flow between the hybridizing taxa.

LONG-RANGE MIGRATION

If linkage disequilibrium is generated by the diffusion of genes from place to place, it should be proportional to the gradients in gene frequency [$D_{ij} \approx (\sigma^2/r)(\partial p_i/\partial x) (\partial p_j/\partial x)$]. It should therefore be lower at the edge than at the center. This pattern is seen with *Bombina*; even after linkage disequilibrium is standardized relative to allele frequencies ($R = D/\sqrt{pquv}$), it is lower at the edges (Fig. 2-5). However, there is much more linkage disequilibrium at the edges than would be expected from the shallow gradients there (dotted line in Fig. 2-5). The problem is that if genes move in a series of small steps, they would take many generations to diffuse from one side of the hybrid zone across to the other. Associations between unlinked alleles decrease by half each generation and so cannot be preserved for this long. These associations could be due to selection; epistasis produces linkage disequilibrium at a rate proportional to the product of allele frequencies ($pquv$) (Barton and Turelli, 1991), and so the standardized disequilibrium should be proportional to $pquv/\sqrt{pquv} = \sqrt{pquv} \approx pq$, as is observed. However, because linkage disequilibria at the edge of the zone are weak, this

(5a)

center is:

(5b)

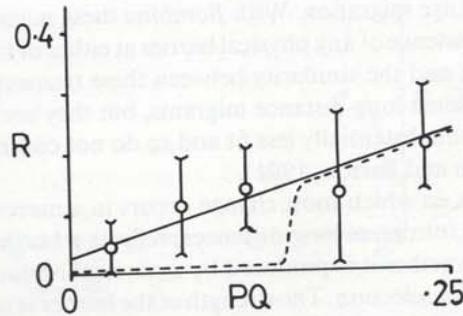


Figure 2-5. Average standardized linkage disequilibrium ($R = D/\sqrt{pquv}$), plotted against the product of average allele frequencies, $\bar{p}\bar{q}$, across the hybrid zone in *Bombina* in southern Poland. The edges of the hybrid zone are at the left, and the center is to the right. The dotted line shows the standardized linkage disequilibrium that would be generated solely by local dispersal; the solid line is the linear regression through the observed values. (From Szymura and Barton, 1991. With permission.)

selection would have to act directly on the enzyme loci, which seems unlikely. The most plausible explanation is that a few toads move long distances, thus taking intact sets of genes across the hybrid zone. The observed linkage disequilibria could be accounted for if about one per thousand toads at the edge comes from the other side of the hybrid zone. This hypothesis is consistent with the small fraction of foreign parental and F_1 genotypes: 4 of 1448 toads sampled between 6 and 40 km from the center (Szymura and Barton, 1991). Linkage disequilibrium can thus give information about the distribution of dispersal distances as well as the net rate.

CLINE SHAPE AND BARRIERS TO GENE FLOW

We argued above that selection on one gene, or one quantitative trait, should give a smooth sigmoid cline, which appears as a straight line when plotted on a logit scale (Fig. 2-1). Selection favoring particular combinations of genes can produce a distorted cline, but only with extreme interactions among several loci and with selection that acts directly on the observed loci (Fig. 2-2). However, these arguments neglect the linkage disequilibria that are generated by dispersal and are found in many hybrid zones. Because selection on one locus causes changes at all the loci in linkage disequilibrium with it, the "effective" selection experienced by each locus may be greatly increased; even neutral loci are affected by selection on loci with which they are associated. This increase in selection causes clines to become steeper, which in turn increases the linkage disequilibrium. There is a positive feedback, which produces a sharp step flanked by shallow tails of introgression (Barton, 1983).

This pattern is seen in many hybrid zones, for example, in *Bombina* (Fig. 2-6), *Ranidella* (Blackwell and Bull, 1978), *Uroderma* (Baker, 1981), *Caledia* (Moran et al., 1980), *Mus* (Hunt and Selander, 1973), and in some places *Podisma* (Currie, 1992; Jackson, 1992). Because a stepped pattern can be shown to be statistically significant only if there are many samples from both the center and edges of a continuous transect, it is difficult to judge its prevalence. It is also difficult to know whether stepped clines are caused by linkage disequilibria or, more simply, by either a physical obstacle to gene flow or long-range migration. With *Bombina* these possibilities can be distinguished. There is no evidence of any physical barrier at either of the two transects studied in detail (Fig. 2-6), and the similarity between these transects speaks against this possibility. There are some long-distance migrants, but they are rare and produce F_1 and F_2 progeny that are substantially less fit and so do not contribute significantly to introgression (Szymura and Barton, 1991).

A stepped pattern, in which most change occurs in a narrow cline at the center and yet foreign alleles introgress long distances, reflects a barrier to gene exchange. This situation is true whether it is produced by a physical obstacle or by linkage disequilibria with loci under selection. The strength of the barrier is measured by the ratio between the step in allele frequency and the gradient at the edge [$B = \Delta p / (\partial p / \partial x)$] (Nagylaki, 1976b). This ratio has the dimensions of a distance and can be thought of as the length of unimpeded habitat that would pose the same obstacle to the flow of a neutral allele. For *Bombina*, the barrier is substantial ($B = 51$ km for flow into *B. variegata*; support limits 22–81 km) and could delay neutral alleles for a few thousand generations ($T \approx (B/\sigma)^2$). However, local barriers of this sort cannot much delay the

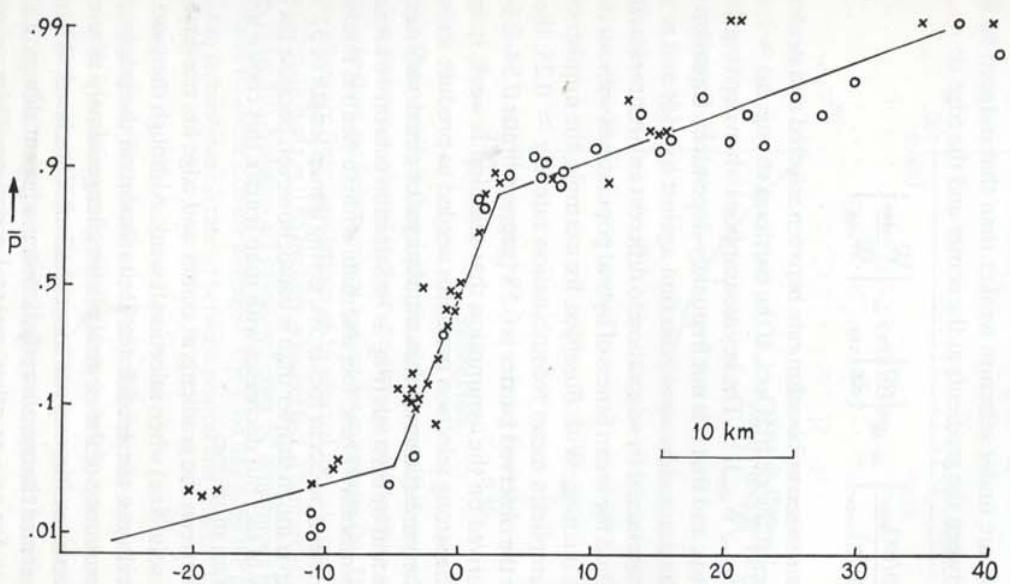


Figure 2-6. Stepped clines seen across two transects through the hybrid zone between *Bombina bombina* and *B. variegata* in southern Poland (circles = Przemysl; crosses = Krakow). The graph shows the average frequency of *B. variegata* alleles at six diagnostic enzyme loci, plotted on a logit scale. The strength of the barrier to gene flow into *B. variegata* is 51.2 km (2 unit support limits 22–81 km); the ratio between the gradients at the center and at the edge is 0.11 (limits 0.09–0.21). (From Szymura and Barton, 1991. With permission.)

spread of an advantageous allele because once a few copies recombine into the new genetic background they can spread rapidly ($T \approx \log[(B/\sigma)^2 \pi S/2]/2S$, where S = the selective advantage). The observed barrier cannot even account for the presence of fixed allozyme differences between *B. bombina* and *B. variegata*, suggesting that some weak selection must act to maintain these differences ($s \approx 0.37\%$) (Szymura and Barton, 1991).

There is a remarkably general relation between the barrier to gene flow and the net strength of selection maintaining the hybrid zone. Consider clines in loci or traits that are neutral or, at least, are under selection weaker than that induced by linkage disequilibria. The ratio between the gradients at the center and the edge is:

$$\left\{ \frac{(\partial p / \partial x)_{\text{edge}}}{(\partial p / \partial x)_{\text{center}}} \right\} = w \left\{ \frac{\partial p}{\partial x} \right\}_{\text{edge}} = \left\{ \frac{\bar{W}_{\text{center}}}{\bar{W}_{\text{edge}}} \right\}^{1/r} \quad (6)$$

where r = the harmonic mean recombination rate between selected and neutral loci; and w = the width of clines at the neutral loci. If the barrier is strong, $\Delta p \approx 1$, and so $B = \Delta p / (\partial p / \partial x)_{\text{edge}} \approx w(\bar{W}_{\text{center}} / \bar{W}_{\text{edge}})^{-1/r}$. The key assumptions when deriving this relation are that selection is weak and that it is not frequency-dependent. Equation 6 then applies exactly to clines maintained by weak selection against hybrids and to a good approximation to clines maintained by adaptation to different environments (Barton, 1986). It can be applied to find the mean fitness of hybrid populations without the need to know just how selection is acting. With *Bombina*, for example, the number of chromosomes and chiasmata implies a mean recombination rate of $r \approx 0.25$; the mean fitness required to explain the observed barrier is 0.58 (support limits 0.54–0.68).

Because Eq. 6 was derived on the assumption that selection is weak, it may not be safe to extrapolate to the strong selection pressures needed to produce strong barriers. We have checked the predictions against simulations of clines maintained by heterozygote disadvantage and by epistasis (Fig. 2-7). Selection acts on 16 loci, spaced evenly along a single chromosome. These loci alternate with 16 neutral marker loci; the recombination rate between adjacent loci is 5%, giving a map length of $31 \times 0.05 = 1.55$ Morgans. This map is much shorter than is usual; however, because the barrier increases with the number of loci but decreases with map length, this choice may give results typical of selection on more loci.

The observed ratio between the gradients at center and edge fits reasonably well with theory (shown by the solid line) when selection is weak. Although there are apparently some significant deviations, the confidence limits shown on the graphs may be too narrow because the frequency of the neutral alleles changes slowly as a result of random drift, so that successive estimates are somewhat autocorrelated. When selection is strong, allele frequencies change abruptly between adjacent demes, and so all demes are close to fixation for one or other parental genotype. Then, as selection increases further, the frequency of introgressing alleles decreases, so the net load on the population remains constant: the mean fitness never declines below about 0.5. Because migration has an effect similar to that of mutation, one would expect, by analogy with the mutation load, that mean fitness would be approximately $1 - m$ and to depend only weakly on the nature of selection. However, we have not been able to find a simple expression for the "migration load"; this lack is to be expected because with epistasis there is no analogous expression for the mutation load in a sexually repro-

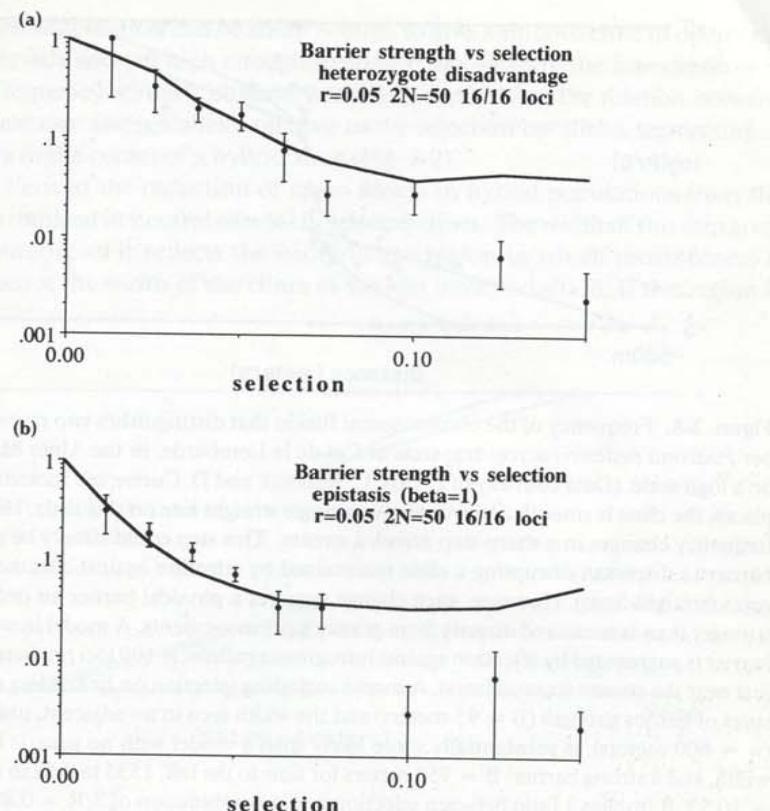


Figure 2-7. Ratio between the gradient in allele frequency at neutral loci at the edge of the hybrid zone and at the center, plotted against selection on each locus: a small ratio indicates a strong barrier to gene flow. (a) Selection acts against heterozygotes (b). There is epistasis, with $\beta = 1$. Observed values are from simulations of 16 selected loci, alternating with 16 neutral markers, with recombination of $r = 0.05$ between adjacent loci. There are 40 demes (except for $s = 0.025$, where there are 80). Statistics are calculated every 50 generations from generations 400 to 800; and the slopes on either side are averaged. The solid line gives the predicted ratio, $\bar{W}^{1/r}$; the harmonic mean recombination rate (r) between neutral and selected loci is here 0.1782.

ducing population (Kondrashov, 1988). The barrier to gene flow continues to become stronger with increasing selection against hybrids, even though the mean fitness does not decrease further. With *Bombina*, the observed ratio of gradients is 0.11 (limits 0.09–0.21); in this range, our simulations show that there is good agreement between simulations and theory.

Even where a barrier to gene flow is primarily caused by a physical obstacle, it may be augmented by selection. With the alpine grasshopper, *Podisma pedestris*, two chromosomal races are separated in most places by smooth sigmoid cline about 800 meters wide. However, at Lac Autier and near Col de la Lombarde, the pattern is sharply stepped (Currie, in prep.; Jackson, 1992) (Figs. 2-8 and 2-9a). Both steps are caused in part by small streams, which impede movement. However, the numbers of marked animals seen moving across the streams are too high to be consistent with the

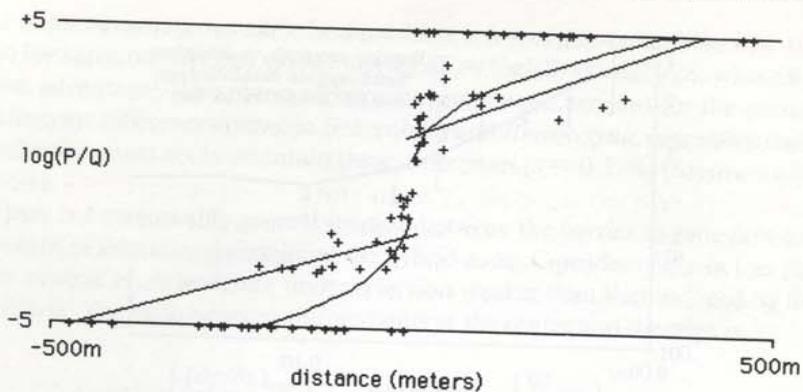


Figure 2-8. Frequency of the chromosomal fusion that distinguishes two races of the grasshopper *Podisma pedestris* across transects at Col de la Lombarde, in the Alpes Maritimes, plotted on a logit scale. (Data courtesy of F. Cox, C. Raboud, and D. Currie; see Jackson, 1991). In most places, the cline is smooth and would give a single straight line on this scale. Here, however, the frequency changes in a sharp step across a stream. This step could simply be due to a physical barrier to dispersal, disrupting a cline maintained by selection against chromosomal heterozygotes (straight lines). However, such change requires a physical barrier an order of magnitude stronger than is measured directly from grasshopper movements. A model in which the physical barrier is augmented by selection against introgressing alleles at 100 loci predicts a further change just near the stream (curved lines). A model including selection on linked loci using direct measures of barrier strength ($B = 95$ meters) and the width seen in an adjacent, unimpeded transect ($w = 600$ meters), is substantially more likely than a model with no genetic barrier, the same width, and a strong barrier: $B = 950$ meters for flow to the left, 1535 meters to the right; $\Delta \log(L) = 10.53$. It implies a ratio between selection and recombination of $S/R = 0.89$ (2-unit support limits 0.68–1.24) against introgression to the left and 0.55 (0.45–0.072) against introgression to the right; the net barrier would then be 7400 meters on the left, and 2360 meters on the right.

observed barrier: the strength of the physical barrier to movement is estimated to be 150 meters at Lac Autier, and 95 meters at Col de la Lombarde, whereas the barrier strengths estimated from cline shape are significantly stronger; averaging over movement in either direction, the estimates are 1.70 and 1.24 km, respectively.

The most likely explanation of this discrepancy is that selection against hybrids produces a genetic barrier to gene exchange, which augments the direct physical barrier. Hybrids from laboratory crosses and from nature have viability reduced by about 50% (Barton and Hewitt, 1981b). This finding is supported by a sharp change in frequency immediately adjacent to the stream of Col de la Lombarde (Fig. 2-8). When genes first cross the stream, they find themselves in unfit F_1 and F_2 hybrids, and so are rapidly eliminated. However, as genes recombine into the new genetic background, they experience much weaker selection, primarily due to their own effects on fitness. They can then penetrate well away from the central barrier. This sharp drop adjacent to the physical barrier is statistically significant and implies strong selection against sets of introgressing genes (for details, see Fig. 2-8). At Lac Autier there is no evidence of such a change next to the stream, perhaps because chromosome frequencies here are close to fixation and so give little information. The data are consistent with a weak physical barrier (albeit somewhat stronger than that observed), augmented by selection against hybrids (Fig. 2-9a). It is not yet clear, however, whether the ratio between

selection and recombination can be small enough to give a smooth cline in open habitat (as is observed) and yet high enough to reduce significantly the introgression of genes at low frequency across a physical barrier. It depends on the relation between selection against rare foreign alleles relative to the selection on alleles segregating at high frequency in the center of a hybrid zone (Fig. 2-2).

We have derived the reduction of mean fitness in hybrid populations from the size of the step induced in neutral or weakly selected clines. The width of this step gives further information, as it reflects the width of the region in which mean fitness is reduced and hence the width of the clines at the loci under selection. If this region is

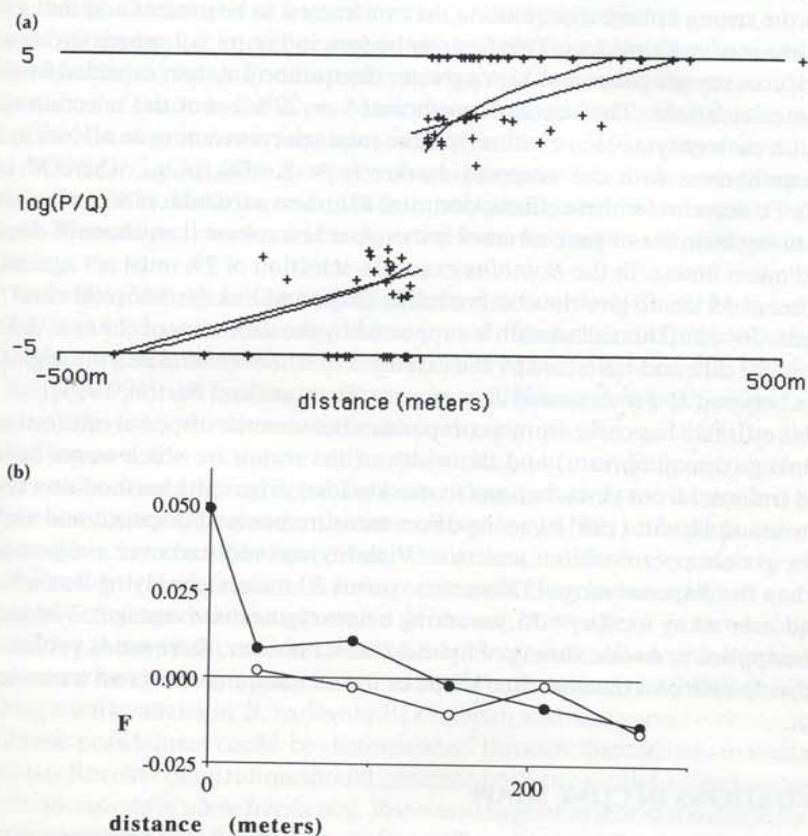


Figure 2-9. (a) Transect at Lac Autier shows a step similar to that shown in Figure 2-8. Here the best fit is to a simple model (straight lines). However, it again requires an unreasonably strong physical barrier ($B = 1390$ meters for flow to the left, 2050 meters to the right, versus 150 meters measured directly). The data are consistent with a weak physical barrier (lower limits $B = 280$ meters to the left, 130 meters to the right), augmented by a genetic barrier due to selection on 100 loci ($S/R = 0.26$ to the left, 0.61 to the right, giving net barriers of 1670 meters on the left and 3870 meters on the right). (b) Covariance between fluctuations in allele frequency at six polymorphic enzyme loci plotted against distance; data are from the hybrid zone in *Podisma pedestris* at Lac Autier. Closed circles = sites on the same side of the stream; open circles = sites on opposite sides. The net barrier estimated from the difference between the two lines is 1.5 km (limits 500 meters to ∞). This finding is consistent with the net barrier strength estimated from the cline in chromosomal fusion (see Figs. 2-8 and 2-9a).

wide relative to the dispersal rate, selection on each locus must be weak; and many loci must be responsible for maintaining the differences between the hybridizing taxa. Consider *Bombina*. The dispersal rate (estimated from linkage disequilibria at the center of the hybrid zone) is $\sigma = 0.99 \text{ km gen}^{-1/2}$, and the width of the enzyme clines averages $w = 6.05 \text{ km}$. If each cline were maintained by selection against heterozygotes, $w = \sigma \sqrt{8/s}$, and so $s = 22\%$. At the center of the hybrid zone, half the individuals would be heterozygous at any one of the n loci, and so the mean fitness would be $\bar{W} = (1 - s)^{n/2} \approx \exp(-ns/2)$. The cline shape gave an estimate of $\bar{W} = 0.58$, implying $ns = 1.09$, and hence $n \approx 5$ loci.

This calculation grossly underestimates the number of loci involved because it neglects the strong linkage disequilibria that are known to be present and that greatly steepen clines at multiple loci. This fact can be seen in Figure 2-3, where strong selection produces steeper clines and hence greater disequilibrium than expected from single-locus calculations. The selection coefficient $s = 22\%$ is not the selection acting directly on each enzyme locus; rather, it is the total selection acting on all loci in linkage disequilibrium with the observed marker ($s \approx \sum s_i D_{Mi}/p_M q_M$, where M is the marker). To account for these effects, one must assume a particular model of selection. The resulting estimate of gene number is therefore less robust than those of dispersal rate and mean fitness. In the *Bombina* example, selection of 2% must act against heterozygotes at 55 loci to give the observed cline shape and linkage disequilibrium (support limits 26–88). This calculation is supported by the similarity of clines at different genes and for different traits and by the extensive biochemical and morphological differences between *B. bombina* and *B. variegata* (Szymura and Barton, 1991).

This estimate has come from a comparison between the dispersal rate (estimated from linkage disequilibrium) and the width of the region in which mean fitness is reduced (inferred from perturbations to marker loci). The same method was applied by Barton and Hewitt (1981b), using direct measurements of dispersal and viability from the grasshopper *Podisma pedestris*. Viability was reduced over a region much wider than the dispersal range (330 meters, versus 20 meters), implying that selection is spread over many loci ($n \approx 75$, assuming heterozygote disadvantage). The method could be applied to a wide variety of hybrid zones; however, there are as yet few cases that provide sufficient data on cline shape or fitness components across a continuous transect.

FLUCTUATIONS IN CLINE SHAPE

Information can come from random fluctuations, as well as from the average patterns. Wright (1943) showed that in the "island model," in which each deme draws migrants from a common pool, the standardized variance of allele frequency ($F_{st} = \text{var}(p)/pq$) depends primarily on the number of migrants exchanged per generation (Nm). Populations spread over two dimensions are similar: F_{st} depends mainly on Nm (in a continuum, on neighborhood size, $4\pi\rho\sigma^2$, and only weakly on the selection or mutation

2. These parameters are often referred to as measures of "gene flow." This terminology is somewhat misleading, as they are the product of population size and dispersal rates and so describe the relative strengths of random drift and gene flow. The shapes of clines and the degree of linkage disequilibrium do not depend on N or ρ in a dense population and are determined primarily by the measures of gene flow, m and σ^2 .

that maintains the polymorphism (Wright, 1943; Slatkin and Barton, 1989). This is because random drift ($\approx 1/N$ or $1/\rho$) and gene flow ($\approx m$ or σ^2) quickly reach a balance, which determines the amount of spatial variation. Wright et al. (1942) applied these results to estimate Nm from the frequencies of recessive lethals in *Drosophila pseudoobscura*. Since that early work, much effort has gone into the estimation of Nm and neighborhood size from molecular data.

The same methods can be used to analyze random fluctuations in cline shape. The variance of allele frequency around a cline depends on the product of density and dispersal in the same way as does the variance around a spatially uniform polymorphism, which has been shown rigorously for a one-dimensional population (Nagylaki, 1978); though the pattern of fluctuations is qualitatively different in two dimensions, it should still be similar for a cline and a uniform polymorphism (Rouhani and Barton, 1987, unpublished data). For example, in a transect across the chromosomal cline of *Podisma*, near Tende in the Alpes Maritimes, the neighborhood size estimated from fluctuations in chromosome frequency is 135 (limits 70–310), compared with an estimate of 335 (265–440) from five polymorphic enzyme loci in the same area (Barton and Hewitt, 1981a; Halliday et al., 1983; unpublished data). There is rather more variation in cline shape than would be expected from fluctuations in uniform polymorphisms.

With *Bombina*, there are data from several concordant clines, and so allele frequency variances can be estimated either from differences in frequency between loci within sites or from fluctuations in the average frequency between sites (see Szymura and Barton, 1991). After allowing for consistent differences in position and width of clines at different loci, there is little residual variation ($F_{st} = 0.015$; limits 0.012–0.019). However, there is significantly more variation in average frequency from place to place than would be expected from the close concordance across loci. This is because some sites are consistently more *B. variegata*-like, and others are more *B. bombina*-like. Some correlation between deviations at different loci is to be expected as a result of drift alone, as there is linkage disequilibrium between all loci and covariance between diagnostic metric traits. However, the pattern is too strong to be explained in this way. It could be caused either by sporadic colonization (which might also account for the discrepancy in estimates from *Podisma*) or by selection favoring *B. variegata*-like alleles in *B. variegata*-like habitat, and vice versa.

These possibilities could be distinguished through fluctuations in linkage disequilibria. Recent colonization should generate both strong disequilibrium and concordant deviations in allele frequency. There is a suggestion that this is so for *Bombina*. Among the 33 central populations, linkage disequilibrium (measured by R/pq) (see Fig. 2-5) is correlated with fluctuations in the cline $[(p_{obs} - p_{exp})^2 / (p_{exp}q_{exp})]$, though not significantly (Kendall's rank correlation is 0.167; $P = 9\%$). A simpler way of explaining fluctuations would be to look for correlations with relevant environment variables. For example, within the hybrid zone between the crickets *Gryllus firmus* and *G. pennsylvanicus* there is a strong association between soil type and genetic and morphological traits, giving a "mosaic" distribution (Rand and Harrison, 1989).

Variation in allele frequency is usually described by a single statistic, F_{st} , which is then used to estimate Nm or neighborhood size. However, density and dispersal vary from place to place, and so a single measure may be misleading. For example, the original aim of estimating population structure was to determine if random drift could

overcome selection and gene flow, enabling a local shift from one adaptive peak to another (Wright, 1931). However, such shifts may be frequent even when the average neighborhood size is high, provided it is small in an appreciable fraction of the species' range. New adaptive peaks can spread if they are either at a strong advantage over the old peak or the sparse populations in which they arise occasionally expand.

Because heterogeneity in population structure can have important consequences, it would be useful to be able to measure it using spatial patterns at polymorphic markers. It would be particularly useful to find ways of detecting hybrid zones and barriers to gene flow. Sokal and his collaborators (1990) have developed a variety of statistical techniques for analyzing spatial patterns and have used them to reconstruct historical changes in European populations from the extensive genetic data available for humans. Sharp changes in gene frequency coincide, in most cases, with language boundaries in Europe.

A more detailed analysis is possible if one has a definite model of the processes responsible for spatial patterning. The variance in frequency of polymorphic alleles, and the covariance between frequencies in different places, depend on the balance between drift and gene flow (through Nm or neighborhood size) and on the balance between gene flow and the force maintaining the polymorphism (through $\sigma/\sqrt{2\mu}$ if the alleles are neutral and are maintained by recurrent mutation, μ). The effect of a barrier on this pattern can be detected: for a given geographic separation, the covariance between sites on the same side of the barrier is greater than that between sites on different sides (Pounds & Jackson, 1981). For *Podisma*, this pattern is found for allele frequencies at six polymorphic enzyme loci, across the hybrid zone at Lac Autier (Fig. 2-9b). Simulations show that a difference of this size is unlikely to arise by chance if there is in fact no barrier; the best estimate of the barrier strength is ≈ 1.5 km, which is consistent with the shape of the chromosomal cline and much stronger than indicated by direct observations of movement across the stream (Currie, in prep.).

ANALYZING GENE TREES

The examples we have used to illustrate our analytical methods have involved morphological, chromosomal, and electrophoretic variation. What more can new molecular techniques tell us about the evolution of hybrid zones? One advantage is that synonymous changes, or variation in noncoding regions, are likely to be neutral, or nearly so, and so provide a reliable baseline against which to compare traits of evolutionary significance. For example, we have argued that weak selection maintains the fixed allozyme differences that distinguish *B. bombina* from *B. variegata*; this hypothesis could be tested by comparison with sequences known to be neutral. However, genotype frequencies within hybrid zones may be little affected by weak selection, as is evidenced by the concordance across traits in *B. bombina*. Having large numbers of diagnostic markers does not help in such cases, as strong selection maintains linkage disequilibria among all the divergent loci. Analysis of any set of markers provides information about the net level of selection and gene flow over the whole genome but not about the markers themselves. If selection is weaker relative to recombination, associations between unlinked loci are weaker; and so more markers become valuable. Disequilibrium may then be detected between linked loci, allowing dispersal and selection to be

estimated; and the widths of clines may vary, reflecting different selection on different loci or traits.

There is a great deal of interest at present in mapping the loci responsible for quantitative variation through associations between Mendelian markers and polygenic traits. This approach cannot be applied directly to samples from hybrid zones, as associations may be caused by linkage disequilibrium. However, any associations seen *within* families must be due to linkage. Use of such families has the advantage that it requires only one generation of crossing, rather than two.

Whereas linkage disequilibria may be strong near the center of hybrid zones, making extra markers redundant, they should become weaker as sets of alleles introgress into a new genetic background. If closely linked markers are available, one could date introgressing alleles by finding whether they are still associated with neighboring alleles; the average length of an introgressing block of DNA is exponentially distributed with mean $1/t$ Morgans. Analysis would be most efficient if only those rare individuals found carrying foreign alleles at a chosen locus were sequenced.

Given enough closely linked markers, one can reconstruct the phylogeny that connects all the genes in a sample. If this "gene tree" can be estimated reliably, it is the most appropriate way of summarizing the data. To obtain the same information in the traditional way, one would need to include all the higher-order disequilibria. Techniques for estimating population genetic parameters from phylogenies of alleles are only just being developed. Slatkin and Maddison (1989, 1990) have shown that the numbers of migrants between demes (N_m) can be estimated by regarding spatial location simply as another character, which changes randomly, in the same way that mutation (μ) causes random changes in sequence. Just as the diversity of sequence within a population gives an estimate of $N\mu$, so the diversity of locations among related genes gives an estimate of N_m . Slatkin and Maddison (1989) showed that their method obtains results of accuracy comparable to the results obtained using the standardized variance of allele frequency (F_{st}). However, they argued that phylogenetic information might allow different kinds of gene flow to be distinguished, for example, sporadic extinction and recolonization versus steady dispersal.

In the context of hybrid zones, we can ask whether gene flow between hybridizing taxa could be detected more readily from gene trees. The best understood example is the introgression of mitochondrial DNA from *Mus musculus domesticus* into *M. m. musculus*. Though the morphological, enzyme, and nuclear DNA sequence all change together across the hybrid zone in Jutland, an mtDNA variant derived from *M. m. domesticus* is found in apparently *M. m. musculus* populations in southern Sweden (Ferris et al., 1983, Sage et al., 1990). Mitochondrial DNA does change across this hybrid zone, but this difference involves a more recently derived variant (Vanlerberghe et al., 1988). This pattern suggests that introgression has occurred through an ancient founder event, rather than through persistent introgression across the present hybrid zone.

This example, together with the general finding that mitochondrial DNA introgresses more readily than differences coded by the nuclear genome (Barton and Hewitt, 1989) shows that phylogenetic information from DNA sequences can tell us about the nature of barriers to gene flow. An objection to this approach is that it tells us about the history of only one or a few loci and so cannot give a good estimate of the general

rate of gene exchange. An allele that happened to cross the barrier in the distant past can leave many descendants, giving an inflated estimate of gene flow. Ideally, one would like good trees from many loci, together with a theory for interpreting the variation among these trees. A serious practical difficulty is that once one moves away from mitochondrial and chloroplast genomes to nuclear sequences, recombination may make it impossible to reconstruct accurate phylogenies.

The most fruitful application of phylogenetic information may be in disentangling the history of groups of many hybridizing taxa, such as the chromosomal races of mice and shrews in Europe, the warning color races of *Heliconius* butterflies in South America, or the diverse human races. There is a continuum here between taxa so distinct that introgression is negligible, through to populations where clines for different loci or traits are scattered independently. In the latter case, phylogenies of different loci vary, so that though one may be able to draw trees describing the relatedness of populations, these must be an average over many characters—as, for example, with human populations in Europe (Sokal et al., 1990). It is certainly possible to describe the history of such groups; what is not yet clear is whether it is feasible to go beyond that and describe the rates of the various processes responsible for the diversification of populations: the rate of establishment of new “adaptive peaks” and hybrid zones, their rates of expansion and contraction, and the rate at which hybridizing taxa develop into coexisting species.

In this chapter, we have shown how the study of hybridization between pairs of populations can tell us about the processes that keep them distinct and that presumably form the basis for separating full species. We hope that in the future comparable methods will be developed for analyzing data from parapatric groups of taxa, and that these methods will tell us about processes at a higher level.

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APPENDIX 1: GENERATION OF LINKAGE DISEQUILIBRIUM BY DISPERSAL

To illustrate how dispersal into a hybrid zone generates associations between alleles, consider two demes (labeled 1 and 2). Two loci segregate in each; one locus carries alleles P and Q at frequencies p and q, and the other carries alleles U and V at frequencies u and v. Associations between alleles are measured by the coefficient of linkage disequilibrium D, which is defined as the difference between the actual frequency of PU gametes, and the frequency expected if the genes combine at random (pu). Gamete frequencies in the two demes immediately after reproduction are as follows.

	<i>Deme 1</i>	<i>Deme 2</i>	
QV	$q_1v_1 + D_1$	$q_2v_2 + D_2$	
QU	$q_1u_1 - D_1$	$q_2u_2 - D_2$	
PV	$p_1v_1 - D_1$	$p_2v_2 - D_2$	
PU	$p_1u_1 + D_1$	$p_2u_2 + D_2$	

(A1)

We will see that the order of migration, random mating, and recombination have a significant effect on the linkage disequilibrium. The stage in the life cycle at which disequilibrium is measured is also important.

It is simplest to assume, in the first instance, that after meiosis a fraction (m) of the haploid gametes are exchanged between demes; they then combine at random to produce diploid zygotes, which go through meiosis to produce the next generation. The allele frequencies after migration are as follows.

$$p_1^* = (1 - m)p_1 + mp_2 \quad p_2^* = (1 - m)p_2 + mp_1 \quad (\text{A2a})$$

$$u_1^* = (1 - m)u_1 + mu_2 \quad u_2^* = (1 - m)u_2 + mu_1 \quad (\text{A2b})$$

The effect of migration on the frequency of PU gametes is similar:

$$(p_1^*u_1^* + D_1^*) = (1 - m)(p_1u_1 + D_1) + m(p_2u_2 + D_2) \quad (\text{A3a})$$

$$(p_2^*u_2^* + D_2^*) = (1 - m)(p_2u_2 + D_2) + m(p_1u_1 + D_1) \quad (\text{A3b})$$

Rearranging, we find the linkage disequilibrium immediately after migration.

$$D_1^* = (1 - m)D_1 + mD_2 + m(1 - m)(p_2 - p_1)(u_2 - u_1) \quad (\text{A4a})$$

$$D_2^* = (1 - m)D_2 + mD_1 + m(1 - m)(p_2 - p_1)(u_2 - u_1) \quad (\text{A4b})$$

Random mating, followed by recombination, reduces disequilibrium to $D_1^{**} = (1 - r)D_1^*$. The next change in disequilibrium is thus:

$$\begin{aligned} \Delta D_1 &= -rD_1 + m(1 - r)(D_2 - D_1) \\ &\quad + m(1 - m)(p_2 - p_1)(u_2 - u_1)(1 - r) \end{aligned} \quad (\text{A5a})$$

$$\begin{aligned} \Delta D_2 &= -rD_2 + m(1 - r)(D_1 - D_2) \\ &\quad + m(1 - m)(p_2 - p_1)(u_2 - u_1)(1 - r) \end{aligned} \quad (\text{A5b})$$

The first term describes the reduction in disequilibrium by recombination, the second term the averaging of linkage disequilibria between the two demes, and the third term (the most important) the generation of disequilibrium by the mixing of genomes from populations with different allele frequencies. This model is the same as that analyzed by Li and Nei (1974).

In most cases it is more plausible to assume that diploid individuals migrate, mate at random in their new location, and produce diploid offspring to found the new generation. Because the Wahlund effect causes a deficit in heterozygotes after migration, recombination is less effective at breaking down linkage disequilibrium. The change in disequilibrium (measured in the gamete pool immediately after meiosis) in this case is as follows.

$$\Delta D_1 = -rD_1 + m(1 - r)(D_2 - D_1) + m(1 - m)(p_2 - p_1)(u_2 - u_1) \quad (\text{A6a})$$

$$\Delta D_2 = -rD_2 + m(1 - r)(D_1 - D_2) + m(1 - m)(p_2 - p_1)(u_2 - u_1) \quad (\text{A6b})$$

The difference between diploid and haploid migration lies in the third term: with diploid migration, the disequilibrium generated by mixing of different populations is not broken down by recombination in the same generation.

Even in this example, which involves only migration and recombination, the full dynamics are complicated. However, a simple approximation is to assume that the population reaches a “quasiequilibrium” in which linkage disequilibrium generated by migration or other forces is balanced by its loss through recombination. This approach was developed by Nagylaki (1976a), and has been generalized by Barton and Turelli (1991). In this model, the disequilibria in each deme converge; and balancing the first and third terms in Eq. A6 gives:

$$D_1 = D_2 = \frac{m(1-m)(p_2 - p_1)(u_2 - u_1)}{r} \quad (A7)$$

This method is only approximate, as the population is not in fact in equilibrium. Migration continually erodes the differences between demes. In this case, Eq. A6 can be solved exactly. After the effects of initial conditions have decayed away, the linkage disequilibria converge to:

$$D_1 = D_2 = \frac{m(1-m)(p_2 - p_1)(u_2 - u_1)}{[r - 4m(1-m)]} \quad (r > 4m(1-m)) \quad (A8)$$

Thus the quasilinkage equilibrium (QLE) approximation of Eq. A7 is accurate if $m \ll r$, in other words, if the effect of recombination is much faster than changes caused by migration.

These values are measured immediately after recombination. If measurements are made on adults, after migration, disequilibria are higher. The QLE approximation is then:

$$D_1 = D_2 = \frac{m(1-m)(p_2 - p_1)(u_2 - u_1)(1+r)}{r} \quad (A9)$$

If loci are unlinked, $r = 0.5$, and so disequilibria are 50% greater if measured after migration instead of before. Formulas analogous to Eq. A7 were used in previous analyses (Barton, 1982; Szymura and Barton, 1986, 1991); they apply if measurements are made before dispersal.

Throughout most of this chapter we have concentrated on clines maintained in a stable balance between selection and dispersal. The QLE approximation can still be applied by balancing the disequilibrium generated by selection and dispersal against recombination: it is accurate when selection is weak, and disequilibria are small. [Strictly, results are accurate to order s^2 (Barton, 1986; Barton and Turelli, 1991)]. If one observes neutral markers embedded among selected loci, Eqs. A5 and A6 still apply. However, if the observed loci are themselves selected, and if this selection favors particular combinations of alleles, an additional term must be added. When a pair of clines is maintained purely by epistasis between two loci (i.e., favoring ++ and --) (Bazykin, 1973), numerical calculations show that when selection is weak Eqs. A5 and A6 can underestimate disequilibrium by a factor of two. However, the discrepancy is smaller with other forms of selection and when more loci are involved (Barton, 1983). Mallet and Barton (1989) used simulations to investigate the effect of the order of reproduction, selection, and migration. They found that the weak selection approximations are more accurate when selection follows reproduction, as linkage disequilibria and deviations from Hardy-Weinberg are then lower than they were immediately after migration.

APPENDIX 2: DETAILS OF COMPUTER SIMULATIONS

A fundamental difficulty when simulating multilocus clines is that although we are primarily interested in deterministic processes it is not practicable to simulate populations without including random sampling drift. In principle, one could iterate analytical recurrence relations for the gamete frequencies. However, even with as few as 10 loci, one would need to follow $2^{10} = 1024$ variables, which is barely feasible. If there were no linkage and if all loci had equivalent effects on selection, one could reduce the problem by following the frequencies of gametes carrying 0, 1, ..., n "1" alleles; in other words, one could follow the distribution of the hybrid index without needing to keep track of individual genotypes. However, we are interested in the effects of linkage and the interaction between selected and neutral loci. One must therefore make a direct simulation of selection, recombination, and gene flow in a finite population, following the genotypes of each individual. Large numbers of genes can be studied; however, random drift causes substantial fluctuations, necessitating a statistical analysis of the results. A particular difficulty is that we wish to compare simulations with theoretical predictions that are valid for weak selection: yet the effects of drift are most severe when selection is weak.

The simulation was written in Pascal and run on a Macintosh SE/30. A copy is available on request. Because each locus segregates for two alleles (labelled "0" and "1"), each haploid genome (or "haplotype") can be described by a binary number, represented in the computer by a set of 16-bit integers. This method allows us to save memory and time by using built-in binary operations (Crosby, 1970).

Events occur in the following order: movement of haploid gametes, selection, random union of gametes, and meiosis. The population is distributed across a linear chain of demes (Kimura, 1953); a proportion (m) of the gametes from each deme migrates in each generation, divided equally between the two neighbors. This proportion is fixed, and so must equal $0/2N, 1/2N, \dots$; in all the simulations reported here, we set $m = \frac{1}{2}$ to approximate a continuous spatial distribution. When selection is weak, gamete frequencies change smoothly from place to place. A variety of models of gene flow, including the stepping stone model used here, can then be approximated by diffusion. With this limit, the effect of gene flow depends only on the variance of parent-offspring distance, $\sigma^2 = m$. However, when selection is strong, the details of the dispersal process become important. A two-dimensional model would be more realistic. The effects of drift are qualitatively different in one and two dimensions (Malecot, 1948); however, this problem is not serious here, as we are mainly interested in deterministic processes, which do not depend on the number of dimensions if the hybrid zone is straight.

After migration, haploid gametes unite at random to produce diploids. Selection acts on these diploids and is followed by meiosis. For each haploid offspring, a diploid is chosen as the parent, with probability proportional to its fitness. Fitness may depend on only a subset of the loci, the rest being neutral markers. The offspring is derived from the two parental haplotypes by recombination; genes are arranged along a single chromosome, with probability (r) of crossing-over between adjacent genes.

Several tricks are used to speed up the simulations. Fitness depends only on the number of heterozygous loci, or on the number of "1" as opposed to "0" alleles. A table of fitnesses can therefore be compiled at the beginning of the run. Moreover, because the haplotype is stored as a set of integers, a table listing the number of "1"

alleles in each 16-bit integer can be compiled, so the number of "1" bits does not need to be counted for each individual in each generation. Selection of a diploid parent (i.e., a pair of haplotypes) is done by setting a table of cumulative fitnesses: for each deme in each generation, the fitness $w(i,j)$ of each possible diploid pair is calculated, and a table $[0, w(1,1), w(1,1) + w(1,2), \dots, \sum_{i,j=1}^{2N} w(i,j)]$ is set up. A random number is drawn from a uniform distribution between 0 and $\sum_{i,j=1}^{2N} w(i,j)$; the point in the table where this number lies gives the chosen parent.

The population is started with a sharp step. All demes to the left of the midpoint are fixed for "0" alleles, and those on the right are fixed for "1" alleles. Because it is a stochastic simulation, many estimates must be made. Strictly speaking, we should take these estimates from many independent replicates. However, because 100 or more generations must pass before the system settles down, this is not feasible: we therefore record statistics at set intervals, after a warm-up period. To the extent that successive estimates are autocorrelated, confidence intervals are underestimated.

The key statistics are based on the mean and variance of the "hybrid index" $x(0 < x < 1)$. The mean of x is just the mean allele frequency. The variance of x includes two components: the genic variance, due to heterozygosity at individual loci, and the remainder, due to linkage disequilibria (Eq. 2b). Because the genic variance can be calculated from individual allele frequencies, the average disequilibrium can be found without the need to calculate all $n(n - 1)$ pairwise associations.

To test the theory, we must estimate cline width, average linkage disequilibrium and mean fitness at the center, and the strength of the barrier to gene exchange. Because averages must be taken over many generations, they must be calculated automatically. Where selection is weak enough that there is no appreciable barrier effect, cline width is estimated by regressing $\ln(p/q)$ against distance, using the region between $\ln(p/q) = -2$ and $+2$. Here p is the average allele frequency; if the clines at different loci lie in different places, this method overestimates cline width. Where there is an appreciable barrier, the cline is divided into three regions. Because we expect gradients in allele frequency to be proportional to $W^{1/r}$, the center is defined as the region where mean fitness is reduced by at least 5% of its maximum drop. Three linear regressions of $z = \ln(p/q)$ against distance are then taken (excluding the edges, where demes are near fixation, and z is outside the range -5 to $+5$). The gradient in allele frequency is related to the gradients of these regressions by $(dp/dx) = pq(dz/dx)$; allele frequency gradients are calculated by extrapolation to the points where these regressions cross.

We expect linkage disequilibrium to be proportional to $(\partial p / \partial x)^2$; because in simple models the gradient $(\partial p / \partial x)$ is proportional to (pq) , the disequilibrium at the center can be estimated by fitting $D = \alpha(pq)^2$, using least-squares. This method gives a better estimate than relying on the maximum D , or the single value nearest the center. However, when the clines are narrow, no demes have $p \approx 0.5$, and so the disequilibrium estimated by regression may be greater than 0.25 (see Fig. 2-3). Mean fitness at the center is estimated in a slightly different way; because we expect cline shape to depend on the actual mean fitness, we use the actual minimum, averaged over all the sampled generations. Except where selection is strong, this method gives a value similar to that estimated by regression of mean fitness on (pq) .

The program was tested by comparison with standard population genetic results. The number of crossover events followed a Poisson distribution; the rate of increase

of gene frequency variance under drift was equal to $pq/2N$; linkage disequilibrium decayed at a rate $(1 - r)^t$; migration caused a step to collapse to a cline with width $\sqrt{2\pi\sigma^2 t}$; the decrease in correlation between gene frequency fluctuations under migration, heterozygote advantage, and drift was as predicted by Malecot (1948); and the width and shape of clines maintained by heterozygote disadvantage and epistasis was as expected when selection is weak. These tests give us confidence that, despite its complexity, the program is performing as it should.