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Abstract.—We study the strength of the genetic barrier to neutral gene flow in a general one-locus, two-allele model that includes viability selection as well as fertility selection and premating isolation. We have separately considered adult migration and pollen migration. Our theoretical results suggest that selection against hybrid formation in the form of fertility selection or assortative mating is more effective in preventing introgression of neutral alleles than is reduction in hybrid viability. We argue that in experimental studies of introgression testing of F_1 hybrids is as important as testing of parental forms. To illustrate the utility of this approach, we estimate the strength of the barrier to neutral gene flow between *Piriqueta caroliniana* and *P. viridis,* between *Iris hexagona* and *I. fulva*, and between *Chorthippus brunneus* and *C. biguttulus*.

Key words.—Gene flow, genetic barrier, hybrid zones, mathematical models.

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Analysis of natural zones of hybridization provides important information about the strength and mode of natural selection, the genetic architecture of species differences, and the dynamics of the speciation process (Endler 1977; Barton and Hewitt 1981, 1985, 1989; Harrison and Rand 1989; Harrison 1990; Barton and Gale 1993). Many hybrid zones are thought to be maintained by a balance between selection and dispersal. Mathematical models have proven to be very helpful in understanding complex processes leading to the formation and fate of such hybrid zones (e.g., Bazykin 1969, 1972a,b; Karlin and McGregor 1972; Slatkin 1973, 1985; Nagylaki 1975, 1976, 1994, 1996; Endler 1977; Moore 1977; Barton 1979a,b, 1983, 1986; Spirito et al. 1983; Bengtsson 1985; Barton and Bengtsson 1986; Mallet and Barton 1989; Barton and Gale 1993; Christiansen et al. 1995; Gavrilets 1997a,b).

Selection could act in many different ways, of which a very common one is selection against hybrid and recombinant phenotypes (Barton and Hewitt 1981, 1985, 1989; Barton and Gale 1993; but see Endler 1977; Moore 1977; Rand and Harrison 1989). In the simplest one-locus, two-allele model (Bazykin 1969; Karlin and McGregor 1972; Barton 1979a,b), individuals with genotypes **aa**, **aA**, and **AA** have relative fitnesses (viabilities) 1, $1 - s$, and 1, respectively ($s > 0$); that is, selection acts against heterozygotes at a single locus. Let us consider a system of two populations consisting of a main subpopulation (with constant genotype frequencies) and a series of smaller subpopulations each (see Fig. 1). (This is the stepping-stone cline model introduced by Feldman and Christiansen 1975; see also Gavrilets 1997b). Assume that initially all subpopulations of the first population have only **AA** individuals, whereas all subpopulations of the second population have only **aa** individuals. After individuals begin migrating between populations, a stable cline in the frequency of allele **A** is formed. This cline will reduce the ability of neutral genes to penetrate from one population to another. Let us consider a second neutral locus M with alleles **m** and **M**. Let allele **m** initially be absent in the first population, but fixed in the second population. After individuals begin migrating between the populations, the foreign neutral alleles

start to appear in each subpopulation, and a stable cline in the neutral allele frequency is expected to be formed. With no selection (i.e., with $s = 0$) and with equal migration rates between subpopulations, the equilibrium frequency of the neutral allele **m** will increase linearly from zero in the first main subpopulation to one in the second main subpopulation (Feldman and Christiansen 1975). Selection on locus A will act as a barrier to the neutral gene flow resulting in deviation of the equilibrium cline from the straight line. The strength of the genetic barrier to the flow of neutral genes from population 1 to population 2, $b_{1\to2}$, and from population 2 to population 1, $b_{2\to1}$, can be defined as (Gavrilets 1997b):

$$b_{1\to2} = \Delta u/\Delta u_2$$

and

$$b_{2\to1} = \Delta u/\Delta u_1, \qquad (1)$$

where Δu is the step in the neutral allele frequency between "peripheral" subpopulations and Δu_i is the step in the neutral allele frequency between a peripheral subpopulation and the adjacent subpopulation in population i ($i = 1,2$). This definition is a discrete-space analog of that for populations in continuous habitat (Nagylaki 1976; Barton 1979a). The barrier strength, b, can be thought of as the number of subpopulations one should put between two peripheral subpopulations that would present an equivalent obstacle to the flow of a neutral allele (see Figs. 1c and 1d in Barton and Bengtsson 1986). With no selection, the neutral allele frequency changes linearly, $\Delta u = \Delta u_1 = \Delta u_2$, and, thus, $b_{1\to2} = b_{2\to1} = 1$. In the case of symmetric underdominant selection on locus A, $b_{1\to2} = b_{2\to1} = b$, where

$$b = \frac{1 - (1 - r)(1 - s)}{r(1 - s)} \qquad (2a)$$

(Bengtsson 1985). Here r is the rate of recombination between the selected and neutral loci. The general expression (2a) reduces to

$$b = \frac{1 + s}{1 - s} \qquad (2b)$$

if a neutral locus is unlinked to locus A ($r = 1/2$), and to

$$K \xleftrightarrow{m_0} \cdots \xleftrightarrow{m_0} \bigcirc \xleftrightarrow{m_0} \bigcirc \xleftrightarrow{m} \bigcirc \xleftrightarrow{m_0} \bigcirc \xleftrightarrow{m_0} \cdots \xleftrightarrow{m_0} L$$
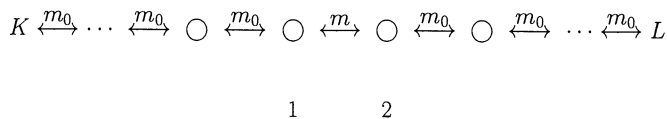
$$\qquad\qquad\qquad 1 \qquad\quad 2$$

FIG. 1. Stepping-stone cline model. Two populations each consisting of a main subpopulation (K and L) with constant genotype frequencies and a series of smaller subpopulations. The migration rate between subpopulations of the same population is m_0, whereas the migration rate between "peripheral" subpopulations (marked 1 and 2) of different populations is m.

$$b = \frac{s}{r(1 - s)} \tag{2c}$$

if a neutral locus is closely linked to locus A ($r \ll 1$). The strength of the barrier, b, increases with the intensity of selection against heterozygote (measured by s) and linkage (measured by r). The strength of the barrier to neutral gene flow in more realistic models including those with multiple selected loci has been analyzed by Barton (1979a 1983), Bengtsson (1985), Barton and Bengtsson (1986), Barton and Gale (1993), Gavrilets and Hastings (1996), Gavrilets (1997b), and Piálek and Barton (1997).

All these theoretical studies of hybrid zones maintained by a balance of dispersal and selection have only considered viability selection. Although viability selection against hybrid and recombinant phenotypes is a common type of selection, it is by no means the only manifestations of hybrid inferiority observed in nature and is not necessarily the most common (see table 1 in Barton and Hewitt 1985; table 2 in Harrison 1990). In many cases, hybrids and recombinants are equally viable as individuals from parent populations but have reduced (or zero) fertility. In some cases, hybrids are even more viable than individuals from parent populations (the phenomenon known as heterosis). Sometimes hybrids are perfectly viable and fertile but their formation in natural populations is prevented by some premating isolating mechanisms (Harrison 1990). All these different forms of reproductive isolation are expected to contribute to the strength of the genetic barrier between pairs of species; however, the relative importance of each of them in preventing introgression between taxa remains unclear. Most empirical analyses of hybrid zones have concentrated on patterns of selection on hybrid and parental genotypes (e.g., Anderson 1949; Harrison and Rand 1989; Arnold 1992; Cruzan and Arnold 1993; Scribner 1993; Nurnberger et al. 1995), although fewer cases with other forms of reproductive isolation between taxa have been characterized (e.g., Perdeck 1958; Carney et al. 1994, 1996; Rieseberg et al. 1995; Wang 1997; Wang and Cruzan 1998).

Recently, Gavrilets (1997a) has presented a general model describing the cline in the frequency of a selected locus arising when viability selection operates together with fertility selection and premating isolation mechanisms. Here, we study the strength of the barrier to neutral gene flow in this model. We compute the strength of the barrier to neutral gene flow between *Piriqueta caroliniana* and *P. viridis*, between *Iris hexagona* and *I. fulva*, and between *Chorthippus brunneus* and *C. biguttulus*.

THE MODEL

Selection

Let us consider a single population with nonoverlapping generations. Let v_1, v_h, and v_2 be viabilities (i.e., probabilities of survival to reproductive age) of genotypes **AA**, **Aa**, and **aa**, respectively. Let m_{ij} be the probability of mating between a male with genotype i and a female with genotype j given that they have met, and let f_{ij} be the fertility (i.e., the average number of offspring) of matings between males with genotype i and females with genotype j given that they mate. Indexes $i = 1,2,3$ correspond to genotypes **AA**, **Aa**, and **aa**, respectively. The average number of offspring of matings between males with genotype i and females with genotype j given that they have met is $f_{ij}m_{ij}$. Let $F_{ij} = (f_{ij}m_{ij} + f_{ji}m_{ji})/2$ be the average of these numbers for two reciprocal matings involving genotypes i and j. Note that $F_{ij} = F_{ji}$ for all i and j and, thus, the offspring matrix $\{F_{ij}\}$ can be represented as

$$\begin{pmatrix} \alpha_1 & \beta_1 & \gamma \\ \beta_1 & \delta & \beta_2 \\ \gamma & \beta_2 & \alpha_2 \end{pmatrix}, \tag{3}$$

where all parameters are non-negative. This model includes postmating reproductive isolation (in the form of differential viabilities v_i and fertilities f_{ij}) as well as premating reproductive isolation (in the form of differential probabilities of mating m_{ij}).

In general, the population will not be at Hardy-Weinberg proportions. Hence, one has to consider the dynamics of genotype frequencies. Let x, y, and z be the frequencies of genotypes **AA**, **Aa**, and **aa** at a certain generation. Then in the next generation

$$\phi x' = v_1(\alpha_1 x^2 + \beta_1 xy + 1/4 \, \delta y^2), \tag{4a}$$

$$\phi y' = v_h(\beta_1 xy + \beta_2 yz + 2\gamma xz + 1/2 \, \delta y^2), \tag{4b}$$

and

$$\phi z' = v_2(\alpha_2 z^2 + \beta_2 zy + 1/4 \, \delta y^2), \tag{4c}$$

where ϕ is a normalizing factor such as $x' + y' + z' = 1$ (Bodmer 1965; Gavrilets 1997a, 1998). Dynamic system (4) has always two monomorphic equilibria corresponding to fixation of one allele or another. The equilibrium with genotype **AA** fixed is stable if

$$v_h\beta_1 < v_1\alpha_1. \tag{5a}$$

The equilibrium with genotype **aa** fixed is stable if

$$v_h\beta_2 < v_2\alpha_2. \tag{5b}$$

We will assume that both these inequalities are satisfied.

Migration

We shall consider what happens after secondary contact of two populations initially fixed for alternative alleles. We assume that two very large populations contact via a chain of smaller subpopulations. To model this situation we use the stepping-stone cline model discussed above. There are $2l$ subpopulations of equal size arrayed along a line connecting two main subpopulations with constant genotype frequencies (see Fig. 1). The migration rate between subpopulations of

the same population is m_0, whereas the migration rate between "peripheral" subpopulations of different populations is m. We will assume that one of two main subpopulations, say that at left, consists of individuals with genotype **AA**, whereas the second main subpopulation consists of individuals with genotype **aa**. Initially all l subpopulations on the left have only **AA** individuals, whereas all l subpopulations on the right have only **aa** individuals. Let X_j be the frequency of a genotype in the jth subpopulation at the beginning of a generation, $j = 1, 2 \ldots 2l$. We consider two cases: adult migration and pollen migration.

Adult Migration.—Genotype frequency X_j' after selection is given by an equation in the form (4). The genotype frequencies after migration are

$$X_l'' = (1 - m - m_0)X_l' + mX_{l+1}' + m_0X_{l-1}', \quad (6a)$$

$$X_{l+1}'' = (1 - m - m_0)X_{l+1}' + mX_l' + m_0X_{l+2}', \quad (6b)$$

and

$$X_j'' = (1 - 2m_0)X_j' + m_0(X_{j-1}' + X_{j+1}'), \quad j \neq l, l + 1, \quad (6c)$$

with $j = 0$ and $j = 2l + 1$ corresponding to main subpopulations on the left and on the right, respectively. The case with $m_0 = 0$ and $l = 1$ describes direct contact of two populations.

Pollen Migration.—We assume that the pollen fertilizing the ovules in a subpopulation are coming only from this subpopulation and its left and right nearest neighbors. For example, the pollen coming to the first peripheral population ($i = l$) have originated in populations $l - 1, l$, and $l + 1$ and the corresponding proportions are $m_0 : (1 - m - m_0) : m$. The pollen coming to the second peripheral population ($i = l + 1$) have originated in populations $l, l + 1$, and $l + 2$ and the corresponding proportions are $m : (1 - m - m_0) : m_0$. (Moody [1979] and Nagylaki [1997] have considered similar models of pollen migration in the framework of viability selection.)

RESULTS

Cline in Selected Locus

Using methods outlined in the Appendix, one finds the following estimates of the frequency of selected allele **A** in the first and second peripheral populations. In the case of adult migration

$$p_1 = 1 - m\left(1 + \frac{\gamma v_h}{\alpha_1 v_1 - \beta_1 v_h}\right), \quad (7a)$$

and

$$p_2 = m\left(1 + \frac{\gamma v_h}{\alpha_2 v_2 - \beta_2 v_h}\right). \quad (7b)$$

In the case of pollen migration

$$p_1 = 1 - m\frac{\gamma v_h}{2(\alpha_1 v_1 - \beta_1 v_h)}, \quad (8a)$$

and

$$p_2 = m\frac{\gamma v_h}{2(\alpha_2 v_2 - \beta_2 v_h)}. \quad (8b)$$

These approximations are valid if $m, m_0 \ll 1$. Pollen migration maintains rare selected alleles at lower frequencies than adult migration.

The Strength of the Genetic Barrier

Unless the loci under selection are identified, allele frequencies p_1 and p_2 cannot be measured. However, what can usually be found is a neutral marker locus (or loci) at which the populations away from the hybrid zone have clearly different frequencies. Information on clines in neutral loci observed in natural populations is abundant. To use this information in making biological conclusions it is important to understand how neutral allele frequencies are expected to change across hybrid zones in different models.

Let us consider a second "neutral" locus M with alleles **m** and **M**. Let U and u be the frequency of allele **M** in the first and second peripheral subpopulations, respectively. Let r be the recombination rate between M and A. Let the neutral allele **m** initially be absent in the first population, but fixed in the second population (i.e., initially $u = 0, U = 1$). After individuals begin migrating between the populations, the foreign neutral alleles start to appear in each subpopulation, and a stable cline in the neutral allele frequency is expected to be formed.

Using methods outlined in the Appendix results in the following approximations for the strength of genetic barrier b:

$$b_{2\to1} \approx \frac{\alpha_1[\alpha_1 v_1 - (1 - r)\beta_1 v_h]}{\beta_1 \gamma v_h r}, \quad (9a)$$

and

$$b_{1\to2} \approx \frac{\alpha_2[\alpha_2 v_2 - (1 - r)\beta_2 v_h]}{\beta_2 \gamma v_h r}. \quad (9b)$$

These approximations are valid for both adult migration and pollen migration cases. The strength of genetic barrier increases with linkage (characterized by r) and selection against hybrids and recombinants (characterized by β, γ, and v_h). To compare efficiency of different factors in reducing neutral gene flow, let us consider three numerical examples. Let the neutral locus be unlinked to the selected locus ($r = 1/2$) and let $\alpha_1 = v_1 = 1$. Assume first that $v_h = 0.5, \beta_1 = \gamma = 1$, that is, there is 50% reduction in viability of hybrids with no premating isolation and/or fertility selection. In this case, $b = 3$. Next assume that $v_h = 1, \beta_1 = \gamma = 0.5$, that is, there is no viability selection against hybrids but there is 50% reduction in fertilities and/or probabilities of mating of hybrids and recombinants. In this case, $b = 6$. Thus, the barrier is twice as strong as with viability selection only. Finally, assume that $v_h = \beta_1 = \gamma = 0.5$, that is, there is 50% reduction in both viability and fertilities/probabilities of mating of hybrids and recombinants. For this parameter configuration, $b = 14$. Thus, "moderate" combined selection results in a "strong" barrier to gene flow. Note that to have $b = 14$ with viability selection only v_h should be extremely small (approximately 0.07). These numerical examples suggest that fertility selection and premating isolation are more efficient than viability selection in preventing gene flow and that combined action of several weak isolating factors can result in

TABLE 1. Offspring matrices F_{ij} for *Piriqueta caroliniana, P. viridis*, and their F_1 hybrid under conditions of excess (upper left corner of the matrix) and limited (lower right corner of the matrix) pollen present on stigmas. Barrier strength: $b_{v \to c} = 3.3$ (excess pollen), $b_{c \to v} = 1.7$ (limited pollen).

Donor	Recipient		
	P. caroliniana	F_1 hybrid	*P. viridis*
P. caroliniana	0.569	0.356	0.383/0.345
F_1 hybrid	0.356	0.332/0.270	0.290
P. viridis	0.383/0.345	0.290	0.373

strong genetic barrier. In the next section we analyze some real data.

Examples

Example I: Piriqueta caroliniana and P. viridis.—These two plant species form a broad hybrid zone in central Florida that extends for several hundred kilometers from lake Okeechobee north to Orlando (Ornduff 1970; Martin and Cruzan, unpubl. data). In this particular system the floral morphologies of each species appear to be very similar (Ornduff 1970; Cruzan, pers. obs.), so we assumed that probability of pollen transfer within and between genotypes is equal (i.e., $m_{ij} = 1.0$ for all three genotypes). Fertilities among genotypes (f_{ij}) were estimated using controlled crosses with mixtures of genetically marked pollen (Wang 1997; Wang and Cruzan 1998). For each cross, an equal mixture of pollen from two genotypes (P.c. + P.v., P.c. + F_1, or P.v. + F_1 pollen) was applied to recipients of all three genotypes in either limiting or excess amounts. Seeds from these crosses were assayed for their allozyme markers to determine their paternity and to estimate the relationship between pollen load size and the fertilization success of each pollen type (Cruzan and Barrett 1996). Regression equations and the average seed production by each recipient genotype were used to predict the expected number of seeds sired by each pollen type on each recipient under two different pollination conditions: (1) pollen limitation (20 grains); and (2) excess pollen (120 grains). Because this crossing design produced two siring success values for each donor-recipient combination, we took the mean of these to obtain the siring ability expected when all three pollen types were competing in the same style (f_{ij}) for each pollen load size. We then used these values to calculate the expected numbers of offspring for each donor-recipient combination to produce two offspring matrices F_{ij}. Inequality (5a) was not satisfied for *P. viridis* under limited pollen condition and for *P. caroliana* under excess pollen condition, indicating the absence of genetic barrier to gene flow. Genetic barriers $b_{v \to c}$ (under limited pollen condition) and $b_{c \to v}$ (under excess pollen condition) to neutral gene flow from *P. viridis* to *P. caroliniana* and in the opposite direction were estimated using data in Table 1 and assuming $v_i = 1.0$ and $r = 0.5$ for each level of pollination.

The genetic barriers between these two species appear to be very weak; however, the strength of each barrier is dependent on pollination conditions. When these species interact in nature and pollinator activity is low (small pollen loads), we would expect extensive introgression in both directions with a bias toward more introgression into *P. caroliana*. However, if large amount of pollen is applied to stigmas, the genetic barrier for *P. viridis* is virtually absent, indicating that introgression would be greater in the direction of the latter species if pollinator activity was high. The lack of a genetic barrier in *P. viridis* when pollen loads are large is a consequence of the weak competitive ability of this species' pollen (Wang and Cruzan 1998). Hence, pollen from *P. caroliana* and F_1 hybrid plants outcompete intraspecific pollen when there is an excess number of grains present on stigmas. For *P. caroliana*, on the other hand, the barrier strength increases with pollen load size as a result of the superior competitive ability of pollen in this species. These results suggest that the expected patterns of introgression between these species as a consequence of mating interactions would be largely dependent on average levels of pollinator activity. The observation of extensive introgression between these species is consistent with a lack of strong reproductive barriers. For a majority of diagnostic genetic and morphological markers there is an overall pattern of gradual change in frequency across the hybrid zone in central Florida, with nonhybrid populations of each species only occurring in geographically isolated areas of the southern and Panhandle regions (Martin and Cruzan, unpubl. data). Such a pattern of clinal variation after secondary contact between previously isolated species is indicative of migration-limited introgression and a lack of strong selection (Endler 1977; Barton and Hewitt 1985).

Example II: Hybridization in Louisiana Irises.—Populations derived from hybridization among three species of *Iris* can be found throughout the Mississippi Delta region of Louisiana (Viosca 1935; Riley 1938; Anderson 1949; Arnold 1994). In this area, hybrid populations as well as populations consisting of only one parental species are common (Arnold 1993, 1994). To make an estimation of the genetic barriers between two of these Louisiana iris species, we used previously published data on mating frequencies under field conditions (Arnold 1993; Hodges et al. 1996). Arnold (1993; Hamrick and Arnold, pers. comm.) introduced plants of *I. hexagona* into a population of *I. fulva* and tracked the formation of F_1 hybrid seeds by plants of both species that were in close proximity to each other. In a subsequent experiment, F_1 hybrid plants were introduced into the same population that now consisted of both species and the frequency of backcross and F_2 seed formation estimated for all three genotypes (Hodges et al. 1996). For our estimates of the genetic barriers between these two species, we used hybridization frequencies under field conditions for F_1 plants that were placed in close proximity to individuals of to each of the parental species (Hodges et al. 1996) in an effort to approximate a random distribution for intra- and interspecific mating opportunities. These data do not provide separate estimates on mating frequencies (m_{ij}) and fertilities (f_{ij}), however, results from other experiments with the two parental species indicate that both pollinator behavior and postpollination processes contribute to the genetic barriers (Carney et al. 1994, 1996; Cruzan and M. L. Arnold, unpubl. data). Because the mating data for each parental species were obtained from two separate experiments, the seed-siring frequencies given by Hodges et al. (1996; table 3) were first normalized by the intraspecific fre-

TABLE 2. Offspring matrix F_{ij} for *Iris hexagona, I. fulva*, and their F_1 hybrid. Barrier strength: $b_{f \to h} = 729$, $b_{h \to f} = 14{,}456$.

Donor	Recipient		
	I. hexagona	F_1 hybrid	*I. fulva*
I. hexagona	1.000	0.511	0.004
F_1 hybrid	0.511	0.900	0.034
I. fulva	0.004	0.034	1.000

TABLE 3. Offspring matrix F_{ij} for *Chorthippus brunneus, C. biguttulus*, and their F_1 hybrid. Barrier strength: $b_{bi \to br} = 446$, $b_{br \to bi} = 426$.

Donor	Recipient		
	C. brunneus	F_1 hybrid	*C. biguttulus*
C. brunneus	1.000	0.193	0.021
F_1 hybrid	0.193	0.210	0.201
C. biguttulus	0.021	0.201	1.000

quency. We used these normalized values to calculate the expected numbers of offspring for each donor-recipient combination to produce an offspring matrix F_{ij} (Table 2). The genetic barriers in each direction, $b_{f \to h}$ and $b_{h \to f}$, were calculated by assuming equal viabilities for all three genotypes ($v_i = 1.0$: M. L. Arnold, pers. comm.) and no linkage between the neutral marker and selected loci ($r = 0.5$).

The genetic barriers between these two species were relatively strong and asymmetrical, with the *I. fulva* barrier being about 20 times as strong as the *I. hexagona* barrier. The apparently stringent reproductive isolation between these two species is reflected by patterns of hybridization in Louisiana, where hybrid and parental populations form a mosaic (Riley 1938; Anderson 1949; Arnold 1994) rather than a broad-scale pattern of extensive introgression as in *Piriqueta*. Although the genetic barrier among parental and F_1 hybrids is strong, the isolation mechanisms are apparently weaker between parental species and advanced-generation hybrids, because populations consisting primarily of backcross hybrid individuals are common (Nason et al. 1992; Cruzan and Arnold 1993, 1994). However, a study of mating patterns in a hybrid *Iris* population indicated that the stronger genetic barrier between *I. fulva* and other *Iris* species is apparently effective at restricting matings with advanced-generation hybrids (Cruzan and Arnold 1994), suggesting that patterns of compatibility may not correspond to a linear relationship with phenotypic and genetic similarity among individuals. Further studies of the patterns of mating and compatibility in this and other hybrid zones may help elucidate the genetic basis of these reproductive isolation mechanisms.

Example III: Response of Females to Male Songs in Chorthippus brunneus and C. biguttulus.—These two species of grasshoppers are often found together in grassy fields in Northern and Eastern Europe from France and the Baltics north to Scandinavia (Perdeck 1958). There does not appear to be any habitat or temporal differences between these two species, but there are clear differences in their morphology and several characteristics of the male song. While there would appear to be ample opportunity for hybridization, observations made by Perdeck (1958) and his colleagues indicate that hybrid individuals are rare in nature. Our estimation of the genetic barrier between these two grasshoppers is based on fertility, viability, and behavioral data collected by Perdeck (1958). Similar numbers of eggs were produced by the females of each species after interspecific and intraspecific matings, so we will assume that fertilities are similar for all combinations of genotypes (all $f_{ij} = 1.0$; Table 3). We used observations of mating behavior in which the frequency at which the response of females (i.e., they produced a song) of each species to male songs were recorded to estimate mat-

ing probabilities for pairwise combinations of each species and their F_1 hybrid (m_{ij}). Because song responses for interspecific interactions and the response of each species to the F_1 hybrid were determined in two separate experiments, we standardized the results by the intraspecific response in each case. The viabilities of all three genotypes appear to be similar (Perdeck 1958), so we assume that all vs are equal to one.

The genetic barriers appear to be relatively strong and similar for each species, but are not as strong as those detected between *Iris* species. This difference is perhaps surprising given that the frequency of hybrids is much higher for irises than it is for these two species of *Chorthippus*. The estimates made for the grasshoppers may be conservative, however, because the frequency of copulations was not observed in these experiments and their mating behaviors and viabilities may be somewhat different under field conditions. The importance of song recognition as a mechanism for reproductive isolation in these grasshoppers is clear given that interspecific copulation frequency is two to three times greater in the presence of auditory stimulation from a singing male of the same species as the female (Perdeck 1958). In a more realistic scenario, females could have been given a choice between males of each species. Such an experimental design would probably result in lower frequencies of interspecific responses and much stronger barrier estimates for this pair of grasshopper species.

DISCUSSION

We have studied the strength of the genetic barrier to neutral gene flow in a general one-locus, two-allele model that includes viability selection as well as fertility selection and premating isolation. We have separately considered adult migration and pollen migration both resulting in the same expression for the strength of the genetic barrier b. Our main theoretical result—equation (9)—can be used for both animal and plant populations.

The results described here have several potentially important implications for both theoretical and experimental studies of gene flow between diverging taxa. Most of the theoretical work in this field (see references above) has been conducted in the context of standard population genetic models of viability selection. The model presented above indicates that selection against hybrid formation in the form of assortative mating or fertility selection is more effective at preventing introgression of neutral alleles than are reductions in hybrid viability. This, together with the fact that both assortative mating and fertility selection are widespread in natural populations (Grant 1963; Mayr 1963; Levin 1978;

Butlin 1987), suggest that additional theoretical work incorporating these factors is needed to understand patterns of introgression, the genetic structure of hybrid zones, and the processes affecting the shape of clines between taxa.

Our theoretical results can be used to characterize the efficiency of different types of reproductive isolation in preventing neutral gene flow between parapatric populations. The minimum set of data should include information about mating patterns and the relative fertility of crosses among parental and F_1 hybrid genotypes as well as their viabilities. Testing F_1 hybrids is as important as testing parental forms because mating interactions between the parental and first-generation hybrid genotypes will govern the formation of advanced-generation hybrids and patterns of introgression. For example, the expected asymmetry in gene flow between species of Louisiana irises is due to the differences in the frequency of successful pollination between the F_1 and parental genotypes. Further analysis of patterns of backcrossing and viability of advanced-generation hybrids would be necessary to fully characterize introgression. However, such an undertaking would be difficult from both mathematical and empirical standpoints. Assuming that the mating behavior of recombinant hybrids are within the range observed in the parental and F_1 generations, then the models described above should provide fairly reliable estimates of the strength of genetic barriers. Unfortunately, even with the minimal amount of data required for such estimates, it is difficult to find studies of interspecific mating that include the F_1 generation.

Empirical studies designed to estimate the parameters used to calculate the genetic barriers between species must be able to make accurate assessments of mating probabilities and fertilities while controlling for potentially confounding factors (e.g., opportunities for mating). Although laboratory studies of reproductive interactions in plants and animals may provide useful data, the estimates made may not be accurate if conditions present in natural populations are not considered. For example, the behavioral experiments on grasshoppers described above did not allow for choice among multiple males by females or for multiple matings. However, experiments conducted in populations may lack adequate control of other variables. In the *Iris* example, parental and F_1 hybrids were planted in close proximity to each other, but plants were not randomized by their position in the population, so there may not have been equal opportunities for mating among all genotypes. In such a case, the establishment of artificial populations (e.g., Kohn and Barrett 1992; Scribner 1993) may provide a more accurate estimate of reproductive barriers. Although it is possible to obtain relatively accurate estimates of mating and fertility parameters, the resulting values obtained for genetic barrier strength need to be interpreted within the limitations of the experimental design used.

In many cases it may be desirable to make separate assessments of mating probabilities and fertilities for different combinations of parental and F_1 genotypes, however the interpretation of these parameters may differ depending on the type of organism and the reproductive stage being studied. In the model presented here, factors affecting the probability of mating after an encounter (m_{ij}) include ethological and mechanical premating isolating mechanisms that affect the

chance of a successful copulation (Mayr 1963) or pollination (Levin 1978), but would exclude geographic, seasonal, or habitat isolation. Fertility differences among crosses (f_{ij}) could be affected by prezygotic factors such as pollen (Snow and Lewis 1993) or sperm (Smith 1984) competition, fertilization failure, or postzygotic factors such as differences in viability among classes of hybrid zygotes (Mayr 1963; Levin 1978). Although experiments that examine these components of the reproductive process are valuable, caution needs to be exercised when extrapolating results from single experiments. For example, in Louisiana irises, differences in pollen-tube growth are not predictive of siring patterns after pollination with mixtures of pollen from hybrid and parental genotypes (Carney et al. 1994). In the grasshopper example described above, differences in sperm competition after intra- and interspecific matings (Perdeck 1958) may modify estimates of genetic barriers based on behavioral interactions alone. Despite the potential limitations of experiments that assess individual reproductive components, knowledge of the contribution of individual stages to interspecific barriers allows for more accurate interpretations of the ecological and evolutionary factors affecting reproductive isolation.

Results from the models described provide empiricists with methods for making comparable assessments of the strength of genetic isolation within and among groups of similar or more distantly related taxa. Estimation of these statistics for pairs of hybridizing species may provide insights into patterns of hybridization and introgression and into the factors contributing to the maintenance and structure of hybrid zones (Harrison 1990; Arnold 1992). The methods described are particularly valuable for comparative studies because this measure of genetic barrier strength is not influenced by the demographic or life-history characteristics of individual taxa. Examination of patterns of variation in the strength of genetic barriers within and between taxa will provide insights into the evolution of reproductive isolation (e.g., Butlin 1987; Howard 1993) and processes of speciation and diversification.

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APPENDIX

With two alleles at each of the two loci there are 10 different genotypes. The vector of ten genotype frequencies characterizes the state of a subpopulation. Equations describing local dynamics under the joint action of viability and fertility selection as well as differential probabilities of mating are given by Nagylaki (1992). To gain insight into the properties of hybrid zones in this model, we have used a weak migration approximation and regular perturbations techniques that were introduced into selection-migration studies by Svirezhev (1968) and Karlin and McGregor (1972) and have proved very useful.

With two diallelic loci, the dynamics of the system with two peripheral subpopulations are described by 20 difference equations. With no migration between peripheral subpopulations (i.e., if $m = 0$), the dynamical system has a locally stable equilibrium with genotypes **AAMM** and **aamm** fixed in the first and second peripheral subpopulation, respectively. The idea of regular perturbation techniques (e.g., Holmes 1995) is to approximate equilibrium values, eigenvalues and other characteristics of the full system (with $m \neq 0$) as small deviations from those of the simplified system (with $m = 0$). Let indexes 1, 2, 3, and 4 correspond to gametes **AM, Am,**

aM, and **am,** respectively, and let x_{ij} be the frequency of a genotype formed by gametes i and j in the first peripheral subpopulation. Using these techniques and *Maple* gives the following first-order approximations for the genotype frequencies of genotypes in the first peripheral subpopulation. (*Maple* notebook used can be accessed at http://www.tiem.utk.edu/~gavrila)

Adult Migration

$$x_{44}^* = m, \tag{A1a}$$

$$x_{14}^* = m\frac{(1 - m_0)\gamma v_h}{\alpha_1 v_1 - (1 - m_0)(1 - r)\beta_1 v_h}, \tag{A1b}$$

$$x_{12}^* = m\frac{(1 - m_0)\beta_1 r}{\alpha_1 m_0}x_{1,4}, \tag{A1c}$$

$$x_{13}^* = m\frac{(1 - m_0)\beta_1 v_h r}{\alpha_1 v_1 - (1 - m_0)\beta_1 v_h}x_{1,4}. \tag{A1d}$$

All other genotype frequencies but that of **AAMM** are second order in m.

Pollen Migration

$$x_{14}^* = m\frac{\gamma v_h}{2\alpha_1 v_1 - (2 - m_0)(1 - r)\beta_1 v_h}, \tag{A2a}$$

$$x_{12}^* = m\frac{(2 - m_0)\beta_1 r}{\alpha_1 m_0}x_{1,4}, \tag{A2b}$$

$$x_{13}^* = m\frac{(2 - m_0)\beta_1 v_h r}{2\alpha_1 v_1 - (2 - m_0)\beta_1 v_h}x_{1,4}. \tag{A2c}$$

All other genotype frequencies but that of **AAMM** are second order in m.

The frequency of selected allele **a** is $p \approx x_{13} + x_{14} + x_{44}$, whereas the frequency of neutral allele **m** is $u \approx x_{12} + x_{14} + x_{44}$. Assuming that both m and m_0 are small and equal results in $b_{2\to1} \approx 1/u$, which simplifies to equation (8a). Equation (8b) can be found in a similar way.