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A GENETIC INTERPRETATION OF ECOLOGICALLY DEPENDENT ISOLATION

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Abstract.—Hybrids may suffer a reduced fitness both because they fall between ecological niches (ecologically dependent isolation) and as a result of intrinsic genetic incompatibilities between the parental genomes (ecologically independent isolation). Whereas genetic incompatibilities are common to all theories of speciation, ecologically dependent isolation is a unique prediction of the ecological model of speciation. This prediction can be tested using reciprocal transplants in which the fitness of various genotypes is evaluated in both parental habitats. Here we expand a quantitative genetic model of Lynch (1991) to include two parental environments. We ask whether a sufficient experimental design exists for detecting ecologically dependent isolation. Analysis of the model reveals that by using both backcrosses in both parental environments, environment-specific additive genetic effects can be estimated while correcting for any intrinsic genetic isolation. Environment-specific dominance effects can also be estimated by including the F_1 and F_2 in the reciprocal transplant. In contrast, a reciprocal transplant comparing only F_1s or F_2s to the parental species cannot separate ecologically dependent from intrinsic genetic isolation. Thus, a reduced fitness of F_1 or F_2 hybrids relative to the parental species is not sufficient to demonstrate ecological speciation. The model highlights the importance of determining the contribution of genetic and ecological mechanisms to hybrid fitness if inferences concerning speciation mechanisms are to be made.

Key words.—Ecological speciation, hybrid fitness, postzygotic isolation, reciprocal transplant.

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Determining the mechanisms by which new species arise is a fundamental problem in evolutionary biology. With the exception of the reinforcement of premating isolation, it is generally accepted that speciation occurs as a by-product of other factors, including divergent natural and sexual selection, polyploidization, and possibly genetic drift and founder events (Mayr 1963; Futuyma 1998). Although significant advances have been made in the laboratory testing the feasibility of these various models (Rice and Hostert 1993), mechanisms of speciation in the wild remain poorly understood (Coyne 1992; Schluter 1996a,b).

The study of speciation is made tractable by focusing on the evolution of reproductive isolation. Our focus here is on the evolution of postzygotic isolation, of which two types have been distinguished (Rice and Hostert 1993; Coyne and Orr 1998; Schluter 1998). Ecologically dependent isolation occurs when hybrids have a reduced fitness due to an interaction between their phenotype and their environment (termed ''environment-dependent'' postzygotic isolation by Rice and Hostert 1993). Hybrids, if intermediate in phenotype between the two parental forms, may not be fit in either of the parental environments. Intrinsic genetic (or ecologically independent) isolation, in contrast, occurs when hybrids have reduced viability and/or fertility largely independent of their environment (termed ''unconditional isolation'' by Rice and Hostert 1993). This fitness reduction is the result of incompatibilities between the parental genomes expressed when they are brought together in hybrids and/or the breakup of favorable gene combinations interacting epistatically in the parents.

This classification of postzygotic isolation is valuable because not every mechanism of speciation can produce every form of isolation. For example, ecologically dependent isolation can result only from divergent natural selection; thus, tests of the divergent selection speciation model (also referred to as by-product and ecological speciation, see below) focus on ecologically dependent isolation (Schluter 1998; Hatfield and Schluter 1999). However, intrinsic genetic incompatibilities can be produced by a number of speciation mechanisms (e.g., drift, founder-events, ecological speciation); thus, their presence yields little information about the mechanism by which they arose.

Ecological speciation occurs when reproductive isolation evolves ultimately as a result of divergent natural selection. Under this hypothesis, species reside on different adaptive peaks and intermediate phenotypes suffer reduced fitness due to ecological mechanisms, in effect falling between niches in the environment (Schluter 1996a,b). This prediction can be tested using reciprocal transplants in which the fitness of hybrids and parental types is evaluated in both parental habitats in nature (e.g., Hatfield and Schluter 1999). The use of hybrids (between divergent species or locally adapted populations) in reciprocal transplants has received much attention recently (e.g., Craig et al. 1997; Emms and Arnold 1997; Nagy 1997; Hatfield and Schluter 1999). However, because all mechanisms of speciation can also produce intrinsic genetic incompatibilities, the key to this approach lies in determining whether any reduction in hybrid fitness has an ecological basis, as opposed to an intrinsic genetic basis.

Here we ask whether there is a sufficient experimental design for detecting ecologically dependent isolation. Our goal is to detect the reasons for hybrid unfitness. A model of the expected phenotype of an individual under the influence of outcrossing, originally developed by Lynch (1991), is expanded to include two environments. We apply this model to measures of fitness of different genotypes in both an-

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cestral environments to determine the relative strengths and weaknesses of using various cross-types in reciprocal transplants.

Lynch's Model

The following model is a genetic interpretation of outcrossing, developed by Lynch (1991). It describes the expected mean phenotype of various crosses between two species or populations. For further details, consult Lynch (1991). Lynch made the following assumptions, which we will also make. Two parental populations (P_1 and P_2) are in gametic-phase equilibrium, with the major loci for the characters of interest unlinked and restricted to autosomal loci. Lynch (1991) also assumed that the phenotype of all individuals are evaluated in a common environment. Given these conditions, two coefficients can be defined that describe the genetic composition of any individual with any combination of the two parental genomes. The source index, θ_S, is a linear scale that ranges from -1 (when all an individual's genes come from P_2) to $+1$ (when they all come from P_1). The hybridity index, θ_H, is also a linear scale that ranges from -1 (when the individual contains genes from only one source population) to $+1$ (when the individual is crossbred at every locus).

The F_2 population, derived from random crossing of P_1 and P_2 and subsequent random crossing of the F_1s, is in Hardy-Weinberg equilibrium and is treated as the reference population (mean phenotype = μ_0), with all gene effects present in other crosses expressed as deviations from this mean. Additive and dominance effects are represented respectively by α_q and δ_q, with the subscript q indicating the number of loci involved (we drop Lynch's subscript $\times$ because it is required only when the model is expanded to include inbreeding). Interactions among loci of different genetic effects (i.e., additive and dominance) are represented by terms composed of the genetic effects involved at each locus; note that these are single terms and not products. For example, $\{\alpha_2\delta_1\}$ is the term representing the interaction of additive effects at two loci and dominance effects of another locus. Extracting the gene effects in the standard hierarchical order, the general expression for the expected phenotype of an individual, denoted μ, is

$$\mu = \mu_0 + \theta_S\{\alpha_1\} + \theta_H\{\delta_1\} + \theta_S^2\{\alpha_2\} + \theta_S\theta_H\{\alpha_1\delta_1\} + \theta_H^2\{\delta_2\} + \cdots. \quad (1)$$

Note that while only second-order epistatic terms are shown, the expression is easily expanded to include higher-order terms (see Lynch 1991).

Two Environments and Environment-Dependent Gene Effects

Here we change the assumption of a single, constant environment to explore the situation of two populations or species in different environments. Let parental population P_1 be native to environment A and P_2 to B. We will use subscripts A and B to indicate individuals grown in these two environments. The environmental coefficient, θ_E, is defined as $+1$ for environment A and -1 for environment B. The environmental effect, $\{\epsilon\}$, is one-half of the difference in phenotype

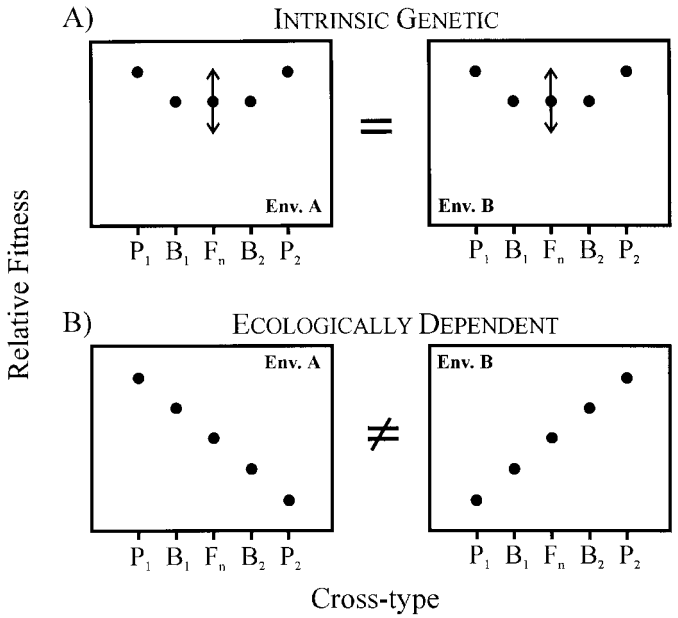

FIG. 1. Hypothetical examples of (A) ecologically independent and (B) ecologically dependent reproductive isolation. Reproductive isolation can be a function of either or both of these factors. Population P_1 is native to environment A and P_2 to B. (A) Reproductive isolation results only from intrinsic genetic incompatibilities between parental populations so the various genotypes have equal fitness in both environments. The relative fitness of F_n, where $n \geq 1$, may vary and depends on the extent of any heterosis, genetic incompatibilities, and the breakup of favorable gene combinations interacting epistatically in the parents. (B) Intrinsic genetic incompatibilities are absent, and reproductive isolation is solely the product of environment-dependent, single-locus, additive effects (i.e., a form of ecologically dependent isolation). Relative fitness increases as genotypes approach the native form in each habitat.

of the F_2s in environments A and B ($\{\epsilon\} = \frac{1}{2}[\mu(F_{2,A}) - \mu(F_{2,B})]$). The expanded model becomes

$$\mu = \mu_0 + \theta_S\{\alpha_1\} + \theta_H\{\delta_1\} + \theta_E\{\epsilon\} + \theta_S^2\{\alpha_2\} + \theta_S\theta_H\{\alpha_1\delta_1\} + \theta_H^2\{\delta_2\} + \theta_S\theta_E\{\alpha_1\epsilon\} + \theta_H\theta_E\{\delta_1\epsilon\} + \cdots, \quad (2)$$

where the reference μ_0 is now the average phenotype of the F_2 in both environments (μ_0 = mean $[\mu(F_{2,A}), \mu(F_{2,B})]$). In this expanded model, $\{\epsilon\}$ is the average effect of the environment on the phenotype, independent of gene action. Terms not including ϵ represent genetic effects that can contribute to reproductive isolation between populations independent of the environment (e.g., Fig. 1A). Interaction terms that include ϵ represent gene effects on the phenotype that act in an environment-dependent manner. For instance, $\{\alpha_1\epsilon\}$ represents the interaction between single-locus additive effects and the environment. The presence of these components, which include both ϵ and genetic terms, indicates ecologically dependent isolation (e.g., Fig. 1B). Again, only second-order interaction terms are shown in equation (2), but the extension to higher-order terms is straightforward. Coefficients are of the form $\theta_S^i\theta_H^j\theta_E^k$, where i and j refer to the number of additive and dominance effects involved and k is zero or one depending on whether the genetic effects are environmentally

TABLE 1. Coefficients for determining mean phenotype of various cross-types using equation (2). Coefficients for θ_S and θ_H are from Lynch (1991). B_1 and B_2 represent backcrosses of the F_1 to the parental forms P_1 and P_2, respectively.

			θ_E	
Cross-type	θ_S	θ_H	Environment A	Environment B
P_1	1	−1	1	−1
P_2	−1	−1	1	−1
F_1	0	1	1	−1
F_n ($n \geq 2$)	0	0	1	−1
B_1	½	0	1	−1
B_2	−½	0	1	−1

independent or dependent respectively. Coefficients for the common cross-types are presented in Table 1. Thus, for example, $\mu(F_{1,A}) = \mu_0 + \{\delta\}_1 + \{\epsilon\} + \{\delta_2\} + \{\delta_1\epsilon\} + \cdots$.

RESULTS

Ecologically dependent isolation is predicted by speciation via divergent selection, so our goal is to estimate the extent of ecologically dependent isolation when controlling for the effects of any intrinsic genetic isolation that may exist. The primary term of interest for ecologically dependent isolation is the additive-by-environment interaction $\{\alpha_1\epsilon\}$. Using Table 1 and equation (2), examination of the mean phenotype of various crosses reveals that

$$\frac{1}{2}[\mu(B_{1,A}) - \mu(B_{1,B})] = \{\epsilon\} + \frac{1}{2}\{\alpha_1\epsilon\} + \frac{1}{4}\{\alpha_2\epsilon\} + \cdots \quad (3)$$

and

$$\frac{1}{2}[\mu(B_{2,A}) - \mu(B_{2,B})] = \{\epsilon\} - \frac{1}{2}\{\alpha_1\epsilon\} + \frac{1}{4}\{\alpha_2\epsilon\} + \cdots, \quad (4)$$

where $\mu(B_{1,A})$ is the mean phenotype of backcrosses of F_1s to P_1 in environment A. Subtracting equation (3) from equation (4) gives an estimate of the additive-by-environment interaction:

$$\{\alpha_1\epsilon\} = \frac{1}{2}[\mu(B_{1,A}) - \mu(B_{1,B}) - \mu(B_{2,A}) + \mu(B_{2,B})]. \quad (5)$$

Extending the comparison to higher-order interactions, the difference between equations (3) and (4) estimates $AE = \Sigma\, 2^{1-q}\{\alpha_q\epsilon\}$, where $q = (1, 3, 5, 7, \ldots)$. If the higher-order terms are assumed small, then $AE \approx \{\alpha_1\epsilon\}$. The standard error of AE is $SE(AE) = \frac{1}{2}[SE(\bar{B}_{1,A})^2 + SE(\bar{B}_{1,B})^2 + SE(B_{2,A})^2 + SE(B_{2,B})^2]^{1/2}$, where, for example, $\bar{B}_{1,A}$ is the mean phenotype of all B_1 individuals in environment A and $SE(\bar{B}_{1,A})$ is the standard error of $\bar{B}_{1,A}$ (= standard deviation of $B_{1,A}$ divided by the square-root of the number of measurements of this particular genotype in this environment).

Another second-order interaction that may be relevant to ecologically dependent isolation is the dominance-by-environment interaction. Using Table 1 and equation (2) reveals that $\{\delta_1\epsilon\}$ can be estimated by measuring the F_1 and F_2 in both environments. A comparison of the F_1s yields:

$$\frac{1}{2}[\mu(F_{1,A}) - \mu(F_{1,B})] = \{\epsilon\} + \{\delta_1\epsilon\} + \{\delta_2\epsilon\} + \cdots. \quad (6)$$

Extending equation (6) to higher-order interactions reveals that this comparison estimates $\{\epsilon\}$ + DE, where $DE = \sum_{i=1}^{\infty}\{\delta_i\epsilon\}$. To isolate the environment-specific dominance effects (DE), we must be able to estimate $\{\epsilon\}$. This is done by subtracting: $\{\epsilon\} = \frac{1}{2}[\mu(F_{2,A}) - \mu(F_{2,B})]$ using F_2 data. Thus, $DE = \frac{1}{2}[\mu(F_{1,A}) - \mu(F_{1,B}) - \mu(F_{2,A}) + \mu(F_{2,B})]$. When estimated in this manner, the standard error of DE is $SE(DE) = \frac{1}{2}[SE(\bar{F}_{1,A})^2 + SE(\bar{F}_{1,B})^2 + SE(\bar{F}_{2,A})^2 + SE(\bar{F}_{2,B})^2]^{1/2}$. Alternately, if one is willing to assume that terms involving third-order and higher interactions are absent, equations (3), (4), and (6) can be used to estimate $\{\epsilon\}$, $\{\alpha_1\epsilon\}$, and $\{\delta_1\epsilon\}$ using only both backcrosses and the F_1s.

Examination of equation (2) also reveals that any isolation detected by a comparison of F_1 or F_2 hybrids to the native parental form in each habitat can involve contributions of both intrinsic genetic and environment-dependent gene effects. For instance,

$$\mu(P_{1,A}) - \mu(F_{1,A}) = \{\alpha_1\} - 2\{\delta_1\} + \{\alpha_2\} - \{\alpha_1\delta_1\} + \{\alpha_1\epsilon\} - 2\{\delta_1\epsilon\} + \cdots \quad (7)$$

and

$$\mu(P_{2,B}) - \mu(F_{1,B}) = -\{\alpha_1\} - 2\{\delta_1\} + \{\alpha_2\} + \{\alpha_1\delta_1\} + \{\alpha_1\epsilon\} + 2\{\delta_1\epsilon\} + \cdots. \quad (8)$$

When these cross-types alone are used, environment-independent gene effects cannot be controlled for and, if present, will contribute to any isolation detected. If these are the only data available, then one cannot infer the extent to which speciation was ecologically mediated.

DISCUSSION

For the ecological speciation hypothesis, it is critical to show that the fitness of hybrid genotypes is reduced relative to the parental forms in each habitat and that this reduction is the result of ecological mechanisms. An expansion of Lynch's (1991) model to two environments reveals that a comparison of the fitness of both backcrosses in both environments estimates environment-specific additive genetic effects. Including both the F_1 and F_2 in both habitats allows the additional estimation of ecological isolation attributable to dominance-by-environment genetic effects. This method is conservative because not all environment-dependent gene effects are estimated, and it does not require that the selective or genetic basis of the phenotypic differences between the species be known. It does assume that the present-day environments are representative of ancestral conditions that existed when the divergence occurred and, like all reciprocal transplants, that the environments used in the transplant are representative of the true environments of the two parental forms (e.g., enclosure effects are absent).

Ecological speciation requires that the fitness of hybrids be reduced relative to the parental forms. Because heterosis can increase the fitness of hybrids (Lynch 1991), the reciprocal transplant must include the native parental form in each habitat (P_1 in environment A and P_2 in B) to confirm that hybrid fitness is reduced. Reciprocal transplants of both parental forms alone would allow the inference of ecologically

dependent selection, but data on hybrids is required to infer the basis of postzygotic isolation.

Thus, to summarize, a powerful design for a reciprocal transplant to detect ecologically dependent isolation is a follows. Both backcrosses should be included in both environments to permit the estimation of environment-dependent, additive gene effects, independent of any positive or negative heterosis. The native parental form in each environment (i.e., P_1 in A and P_2 in B) should also be included as a benchmark to confirm that hybrid fitness is reduced. Finally, environment-specific dominance effects can also be estimated if the design permits the inclusion of the F_1 and F_2 in both environments. The decision to include the F_1 and F_2 must be balanced against any loss in replication of the backcrosses and parental forms that occurs by doing so.

Examination of the expanded model also reveals that any reduction in fitness detected using a comparison of the F_1 or F_2 to parental forms may result from a number of mechanisms. For instance, F_1 hybrids may be unfit as result of intrinsic genetic incompatibilities or because their intermediate phenotype is selected against ecologically. In other words, a reduction of F_1 or F_2 fitness relative to parental forms in a transplant experiment is not sufficient evidence for ecological speciation.

In addition to the method described above, there are at least two other ways to separate ecologically dependent from intrinsic genetic isolation. The first, used by Hatfield and Schluter (1999), is to compare the fitness of hybrids in the wild to that in a benign environment. The benign environment is one in which the environmental differences to which the divergent phenotypes are an adaptation have been removed or ameliorated. In effect, this method attempts to remove the hypothesized ecologically dependent isolation to allow the extent of the intrinsic genetic isolation to be estimated. Comparison of the two estimates can then yield the extent of ecologically dependent isolation. The second method involves artificially altering the phenotype of parental forms to resemble hybrids. Any reduction in fitness of these individuals in a reciprocal transplant, when properly controlled for the effects of the modification, can only be attributed to ecological mechanisms. An advantage of this approach is that it does not rely on the creation of hybrids and can thus be used even when reproductive isolation between parental species is complete.

Each of these methods has inherent strengths and weaknesses, and which of them is most useful in any situation will depend on the details of the species under study. For instance, the use of backcrosses in a reciprocal transplant does not require a detailed understanding of the selective basis of the phenotypic differences between the species but does require that any intrinsic genetic isolation is not so strong as to prevent hybrids from being made. Finally, it must be noted that, although useful, the separation of ecologically dependent and intrinsic genetic isolation may not be complete. The possibility exists that the fitness effect of some genetic incompatibilities may depend on the environment (e.g., along a benign-to-harsh axis), blurring the distinction between these two types of postzygotic isolation (see Kondrashov and Houle 1994). This is an area that deserves further attention.

Ecology has long been thought to play a role in speciation, but limited progress has been made in determining its contribution to natural speciation events. We must determine unique predictions of this model and design techniques to test these predictions in the wild. Ecologically dependent isolation between young species is one such prediction.

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