

A BIOGEOGRAPHIC GENETIC APPROACH FOR TESTING THE ROLE OF REINFORCEMENT: THE CASE OF *DROSOPHILA PSEUDOOBSCURA* AND *D. PERSIMILIS*

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Abstract.—The role of reinforcement in speciation can be explained by two distinct models. In model I, two diverged populations hybridize and produce fertile hybrids that successfully backcross (hybridization with gene flow). In model II, two populations hybridize but succeeding backcrosses are unproductive (hybridization without gene flow). Using *Drosophila persimilis* and *D. pseudoobscura*, we have tested model I by comparing the extent of heterospecific introgression in sympatric versus allopatric populations. We show that certain expectations of this particular model of reinforcement, which is based on hybridization and gene flow between divergent populations after secondary contact, are not realized in these two species. The evidence consists of the similarity of genetic distances as well as proportions of unique/rare alleles between sympatric and allopatric heterospecific populations and a *negative* correlation between genetic distance and geographical distance between heterospecific populations, which suggests ecological differentiation. This approach in quantifying differential gene flow has important consequences to studies that compare sympatric and allopatric isolation using genetic distance. Following model I, one would expect a pattern of higher prezygotic isolation in sympatric species compared to allopatric species of the same genetic distance simply as a result of an underestimation of genetic distance due to introgression between sympatric populations. We suggest more parsimonious explanations such as reinforcement without genetic exchange (model II) and ecological differentiation, which require high levels of preexisting reproductive isolation between populations.

Key words.—Allopatric, *Drosophila*, postzygotic, prezygotic, reinforcement, reproductive isolation, speciation, sympatric.

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In the reinforcement model of speciation, two populations that are physically isolated from one another and have reached a certain level of postzygotic reproductive isolation meet in sympatry. Because these populations would produce relatively unfit hybrids, prezygotic isolation mechanisms would be selectively “reinforced” to prevent further hybridization and consequently lead to the completion of reproductive isolation between two incipient species (Dobzhansky 1951). This model has been vigorously criticized (Templeton 1981; Paterson 1982; Spencer et al. 1986; Butlin 1987, 1989) for a variety of reasons (for an extensive review, see Howard 1993). However, a number of new theoretical analyses (Liou and Price 1994; Kelly and Noor 1996) as well as recent empirical evidence (Coyne and Orr 1989, 1997; Howard 1993; Butlin 1995; Noor 1995; Sætre et al. 1997) have furnished renewed support in its favor.

Gene flow between contacting populations has been a major argument against the reinforcement hypothesis (Moore 1957; Mayr 1963). Because hybridization is a necessary step in reinforcement, gene flow (in the form of allelic introgression) could play a preventative role in divergence even between populations that already possess a sufficient degree of differentiation at the time of secondary contact. We propose two mutually exclusive models based on whether interspecific hybridization leads to gene flow between species. In one model (model I, hybridization with gene flow), F₁ hybrids are not fully sterile (i.e., species pairs following Haldane’s rule or partial sterility), which enables successful backcrossing of hybrid genotypes to one or both of the parental species. Depending on the level of fertile offspring produced from such backcrossing, some gene flow will occur across the species barrier. In the second model (model II, hybridization without

gene flow), no successful backcrossing between hybrid and parental types takes place. This can occur through a variety of ways: complete sterility or inviability of the F₁ hybrids, complete hybrid breakdown over a number of generations, hybrids may only mate with other hybrids, or ethological problems in F₁ hybrids preventing backcrossing. The common result is the complete absence of gene flow between parental species.

This paper has two objectives. First, we test which model (model I or model II) best fits the two closely related species, *Drosophila pseudoobscura* and *D. persimilis*, by detecting the presence or absence of past gene flow between heterospecific sympatric versus heterospecific allopatric populations. These two species were employed for a variety of reasons: they do not possess high levels of postzygotic isolation in the laboratory (female F₁ hybrids are fertile, males are sterile), making introgression possible; their biogeography and species ranges are well documented; numerous studies have surveyed a large number of allozymes from these two species, thus creating a large dataset for this present analysis; and this species pair has become paradigmatic to the reinforcement hypothesis in *Drosophila* during the last half century (Mayr and Dobzhansky 1945; Koopman 1950; Kessler 1966; Noor 1995).

The second objective of the paper is to illustrate that such models of hybridization, based on gene flow levels, have an important bearing on the analyses of Coyne and Orr (1989, 1997), which have become a major source of evidence in favor of reinforcement. These authors compared the rate of isolation between particular species pairs in the genus *Drosophila* by regressing their genetic distance against their degree of reproductive isolation (both prezygotic and postzy-

gotic). The authors show that prezygotic (ethological) isolation evolves faster than postzygotic isolation (hybrid inviability and/or sterility). This difference was ascribed to the higher rate of development of prezygotic isolation in sympatric species pairs compared to allopatric species pairs. Coyne and Orr furthermore attribute this strengthened prezygotic isolation in sympatry to the reinforcement of ethological barriers. Following model I (hybridization leading to gene flow), we argue that the conclusion of reinforcement is not implicit from the data of Coyne and Orr (1989, 1997). In the presence of gene flow, any analysis that uses genetic distance measures to infer absolute time will overlook the corresponding deflation in genetic distances caused by allelic introgression between sympatric, but not allopatric, heterospecific species pairs. Consequently, the observation of greater prezygotic isolation in sympatry relative to allopatry is simply an artifact of higher sympatric gene flow. In fact, Coyne and Orr (1989) argued against higher sympatric gene flow following the observation that there was not a corresponding increase in postzygotic isolation among sympatric species pairs. We suggest that if there is *no* evidence of past gene flow between sympatric species pairs, this would be compatible with either reinforcement without gene flow (model II) or no reinforcement.

MATERIALS AND METHODS

Collection of Allozyme Data

Using electrophoretic data from the literature, we compiled allozyme frequencies from various natural populations of *D. persimilis* and *D. pseudoobscura*. Although there exist many more published accounts of allozyme variation from polymorphism studies of these two sibling species, the majority of studies could not be used. To be included, a particular study must have surveyed a large number of loci and/or populations of both sympatric and allopatric populations of *D. pseudoobscura*. In studies where this criterion could not be met, "compatible" studies from the same laboratory, using the same loci and the same allelic designations, were chosen to complete the dataset. No allopatric *D. persimilis* populations have been reportedly surveyed for allozymic variation and thus were not represented in this analysis.

To test whether loci harbored on third chromosome inversions resulted in greater distance values between species, we grouped loci according to their chromosomal location. The chromosomal origins of only 15 loci (of a total 48 surveyed loci) are known. For most loci, map position in *D. pseudoobscura* and *D. persimilis* was corroborated by corresponding Muller/Sturtevant/Novitski elements from other species, such as *D. melanogaster* and *D. obscura*, where the locus has also been mapped.

Biogeographical Information

Populations were categorized under one of three biogeographical headings, sympatric *D. persimilis*, sympatric *D. pseudoobscura*, and allopatric *D. pseudoobscura*, depending on their species type and the geographical location from which the population was sampled. Sympatric populations were defined as populations of either *D. persimilis* or *D. pseu-*

doobscura that are found within the range of their sibling species (Dobzhansky and Epling 1944). Populations that were classified as sympatric originate from the following California locations: Mather, Strawberry Canyon, White Wolf, Fish Creek, Salmon Creek, Weott, Wild Rose, Humbolt, McDonald, Santa Cruz Island, Saratoga, Corn Spring, and from Sisters, Oregon. Allopatric populations are located outside the range of their sibling species. Populations that were classified as allopatric (only *D. pseudoobscura*) originate from the following locations: Charleston Mountains, northeast; Cerbats Mountains, northeast; Flagstaff, Arizona; Mesa Verde, Colorado; Cimarron, Colorado; Hardin Ranch, Colorado; and Austin, Texas. Note that only populations sampled within the continental United States were included in this analysis. Geographic distances were calculated between locations using U.S. Census Bureau data retrieved from the website <http://www.indo.com/distance>.

Data Analysis

Two different approaches were undertaken in the comparison of genetic distance between interspecific sympatric and allopatric populations. The first approach averaged the allele frequencies of all the respective populations from each of the three biogeographical categories within and between species. Because most studies did not record population sizes, populations were equally weighted. From the category averages, the genetic distance as well as the proportion of unique and shared alleles were calculated for each between-group comparison. In the second approach, the genetic distance was calculated between all possible population pairs either within (intraspecific comparison) or between (interspecific comparison) each biogeographical category. The average of all pairwise comparisons (only those that compared more than 10 loci were used) was then calculated for each group comparison. The estimate of the standard genetic distance and the variance of its estimate was calculated as described by Nei (1972, 1987). For the regression analyses, correlation plots were made using Kaleidograph (vers. 2.1.3 for Mac). Regressions were performed separately on Minitab (vers. 10.5 for Mac).

RESULTS

Evidence of past gene flow was obtained by comparing the levels of unique or shared alleles and genetic distance between the sympatric versus allopatric populations of *D. persimilis* and *D. pseudoobscura*. The proportion of alleles unique to *D. persimilis* and absent in populations of *D. pseudoobscura* occupying the same geographical region (sympatric) was similar to the proportion of alleles unique to *D. persimilis* and absent in populations of *D. pseudoobscura* that are situated outside (allopatric) the *D. persimilis* species range (Table 1A). In fact, the proportion of shared alleles between allopatric populations (59%) was slightly larger than the proportion of shared alleles between sympatric populations (54%) of different species origin, but the difference was not significant ($Z = 0.82$, $P = 0.41$). The genetic distances calculated between the pooled sympatric and allopatric heterospecific populations, using average allele frequencies from

TABLE 1. Allozyme differences between sympatric and allopatric populations of *D. persimilis* and *D. pseudoobscura*. Populations are pooled into three groups according to their geographic location; all *D. persimilis* populations utilized are sympatric to the widespread species *D. pseudoobscura*, sympatric *D. pseudoobscura* populations are defined as geographically confined to the species range of *D. persimilis*, and allopatric *D. pseudoobscura* populations are defined as those populations found outside the range of *D. persimilis* but still remaining within the Southwest region of the United States. (A) The number of unique and shared alleles between sympatric and allopatric populations of *D. persimilis* and *D. pseudoobscura*. The fraction in parentheses is the actual number of unique or shared alleles (N_u) divided by the total amount of alleles in that particular group (N_t). (B) The standard genetic distance (D) between pooled sympatric and allopatric populations of *D. persimilis* and *D. pseudoobscura*. The variance of the estimate of the genetic distance is denoted in parentheses. *per*, *D. persimilis*; *pseu*, *D. pseudoobscura*; sym, sympatric; allo, allopatric. Data taken from (number of loci used): Lewontin and Hubby 1966 (13); Prakash and Lewontin 1968 (2); Prakash 1969 (24); Prakash et al. 1969 (24); Prakash 1977a (43); Prakash 1977b (22); Coyne and Felton 1977 (2).

	Heterospecific sympatric populations			Heterospecific allopatric populations			Conspecific allopatric populations of <i>D. pseudoobscura</i>		
	Unique to <i>per</i> % (N_u/N_t)	Unique to <i>pseu</i> % (N_u/N_t)	Shared % (N_s/N_t)	Unique to <i>per</i> % (N_u/N_t)	Unique to <i>pseu</i> % (N_u/N_t)	Shared % (N_s/N_t)	Unique to <i>per</i> % (N_u/N_t)	Unique to <i>pseu</i> % (N_u/N_t)	Shared % (N_s/N_t)
(A) Proportion of unique/shared alleles									
All surveyed loci: ($n = 48$)	0.21 (28/136)	0.25 (34/136)	0.54 (74/136)	0.19 (25/131)	0.22 (29/131)	0.59 (77/131)	0.12 (14/120)	0.10 (12/120)	0.78 (94/120)
Loci of known chromosomal origin:									
Chromosome I ($n = 5$)	0.31 (8/26)	0.35 (9/26)	0.35 (9/26)	0.23 (6/26)	0.35 (9/26)	0.42 (11/26)	0.48 (1/21)	0.14 (3/21)	0.81 (17/21)
Chromosome II ($n = 4$)	0.25 (4/16)	0.13 (2/16)	0.63 (10/16)	0.19 (3/16)	0.13 (2/16)	0.69 (11/16)	0.00 (0/13)	0.08 (1/13)	0.92 (12/13)
Chromosome III ($n = 4$)	0.31 (5/16)	0.25 (4/16)	0.44 (7/16)	0.47 (7/15)	0.20 (3/15)	0.33 (5/15)	0.27 (3/11)	0.00 (3/11)	0.73 (8/11)
Chromosome IV ($n = 2$)	0.32 (6/19)	0.32 (6/19)	0.37 (7/19)	0.28 (5/18)	0.28 (5/18)	0.44 (8/18)	0.24 (4/17)	0.24 (4/17)	0.53 (9/17)
All mapped loci ($n = 15$)	0.30 (23/77)	0.27 (21/77)	0.43 (33/77)	0.28 (21/75)	0.25 (19/75)	0.47 (35/75)	0.13 (8/62)	0.13 (8/62)	0.74 (46/62)
(B) Genetic Distance									
All surveyed loci: ($n = 48$)			0.060 (0.013)			0.067 (0.015)			0.005 (0.000)
Loci of known chromosomal origin:									
Chromosome I ($n = 5$)			0.181 (0.053)			0.246 (0.076)			0.019 (0.000)
Chromosome II ($n = 4$)			0.172 (0.060)			0.181 (0.063)			0.002 (0.000)
Chromosome III ($n = 4$)			0.074 (0.007)			0.076 (0.014)			0.014 (0.006)
Chromosome IV ($n = 2$)			0.013 (0.029)			0.004 (0.043)			0.010 (0.028)
All mapped loci ($n = 15$)			0.130 (0.016)			0.150 (0.025)			0.012 (0.000)

TABLE 2. Pairwise comparisons of genetic distance between two heterospecific sympatric populations and neighboring sympatric and allopatric populations of *D. persimilis* and *D. pseudoobscura*. The standard genetic distance (D) is calculated between a *D. persimilis* population from Mather, California, and five conspecific sympatric populations and four allopatric *D. pseudoobscura* populations. Pairwise comparisons are then repeated for a *D. pseudoobscura* population (Strawberry Canyon) in close proximity to the Mather site (38 km). N , the number of loci used in the calculation of the genetic distance, D .

Species Population location	<i>D. persimilis</i> Mather, CA		<i>D. pseudoobscura</i> Strawberry Canyon, CA	
	D	(N , $\text{Var}[D]$) ¹	D	(N , $\text{Var}[D]$) ¹
<i>D. persimilis</i>				
Mather, California	—	—	0.070	(48, 0.006)
White Wolf, California	0.010	(37, 0)	0.066	(37, 0.013)
Wild Rose, California	0.007	(43, 0)	0.073	(43, 0.002)
Salmon Creek, California	0.005	(43, 0)	0.071	(43, 0.010)
Fish Creek, California	0.008	(44, 0)	0.063	(44, 0.004)
Sisters, Oregon	0.006	(44, 0)	0.069	(44, 0.006)
Average over all populations (mean \pm SE)	0.007 \pm 0.001		0.069 \pm 0.001	
<i>D. pseudoobscura</i>				
Flagstaff, Arizona	0.101	(13, 0.020)	0.018	(13, 0)
Mesa Verde, Colorado	0.077	(47, 0.001)	0.009	(47, 0)
Cimmaron, Colorado	0.060	(13, 0.060)	0.009	(13, 0)
Austin, Texas	0.084	(47, 0.011)	0.017	(47, 0)
Average over all populations (mean \pm SE)	0.081 \pm 0.008		0.013 \pm 0.002	

¹ Because some of the genetic distances are very small, variances of the estimate of genetic distance are often negative. In such cases, $\text{Var}(D)$ is denoted as zero.

each geographical group, were also not significantly different (Table 1B; $t = 0.29$, $P > 0.5$).

Although there exists information on chromosomal location for less than one-third of the surveyed loci (15 of 48), these loci contained over half (81 of 143) of the alleles used in the analyses. As expected with the inclusion of mainly polymorphic loci in the mapped loci subset, the number of unique and shared alleles were respectively higher and lower on average compared to all surveyed loci (Table 1A), but these differences were not significant. However, differences were significantly higher in the genetic distance between all mapped loci compared to all surveyed loci (i.e., Table 2B; heterospecific sympatric populations, $t = 5.23$, $P < 0.01$). To evaluate the effects of interspecific gene flow on loci found in species-specific inversions harbored on the third chromosome, we compared the proportion of unique and shared third chromosomal alleles to loci found on other chromosomes. No significant differences were observed between chromosomal pair comparisons except for the proportion of shared alleles between heterospecific allopatric populations among loci on the second and third chromosomes (Table 1A; $Z = 1.97$, $P < 0.05$). The genetic distance between loci on the third chromosome and all mapped loci was significantly different from each other in both heterospecific sympatric (Table 1B; $t = 19.99$, $P < 0.01$) and allopatric populations. But the genetic distance of third chromosome loci was not significantly different between sympatric and allopatric heterospecific populations (Table 1B; $t = 0.03$, $P > 0.5$).

Additionally, pairwise comparisons of genetic distances between heterospecific populations were averaged for each particular geographic group comparison and compared to baseline distance values of pairwise population combinations within each conspecific group (i.e., mean genetic distance between sympatric population pairs of *D. persimilis* = 0.012 \pm 0.002). Again, no significant differences were found be-

tween the genetic distances of allopatric (mean = 0.066 \pm 0.006) and sympatric (mean = 0.070 \pm 0.005) heterospecific populations. The genetic distance between allopatric populations of *D. pseudoobscura* (mean = 0.020 \pm 0.003) was not significantly different from the mean genetic distances calculated for conspecific populations in the range of *D. persimilis* (mean = 0.015 \pm 0.003) and conspecific populations outside the range of *D. persimilis* (mean = 0.020 \pm 0.004). A three- to fivefold difference between the genetic distances of conspecific and heterospecific populations was observed.

The abundance of analyzable loci at two geographical sites (one for each species) that are in close proximity to each other (Mather, CA, *D. persimilis* and Strawberry Canyon, CA, *D. pseudoobscura*; approximately 40 km from each other; Table 2) presented an opportunity to calculate two sets of genetic distances in which one of the populations (i.e., Mather) is geographically located adjacent to the other heterospecific population (i.e., Strawberry Canyon). The average genetic distances of both sympatric and allopatric heterospecific populations, utilizing this site-defined sympatry approach (sympatry in this case being defined as two populations with overlapping ranges), are expected to be smaller than those using a range-defined sympatry approach (as used in the rest of this study). This expectation is based on the possible presence of isolated sympatric populations in the latter approach, which may obscure any apparent difference in genetic distance. In fact, slightly higher genetic distances were observed using site-defined sympatric populations, but these differences were not significant.

The geographic distance versus standard genetic distance for all pairs of populations, both within species (conspecific) and between species (heterospecific) is plotted in Figure 1A. Regressions of conspecific population pairs from *D. persimilis* and *D. pseudoobscura* revealed positive but nonsignificant correlations (*D. persimilis*: $r^2 = 0.17$, $F_{1,13} = 2.60$, P

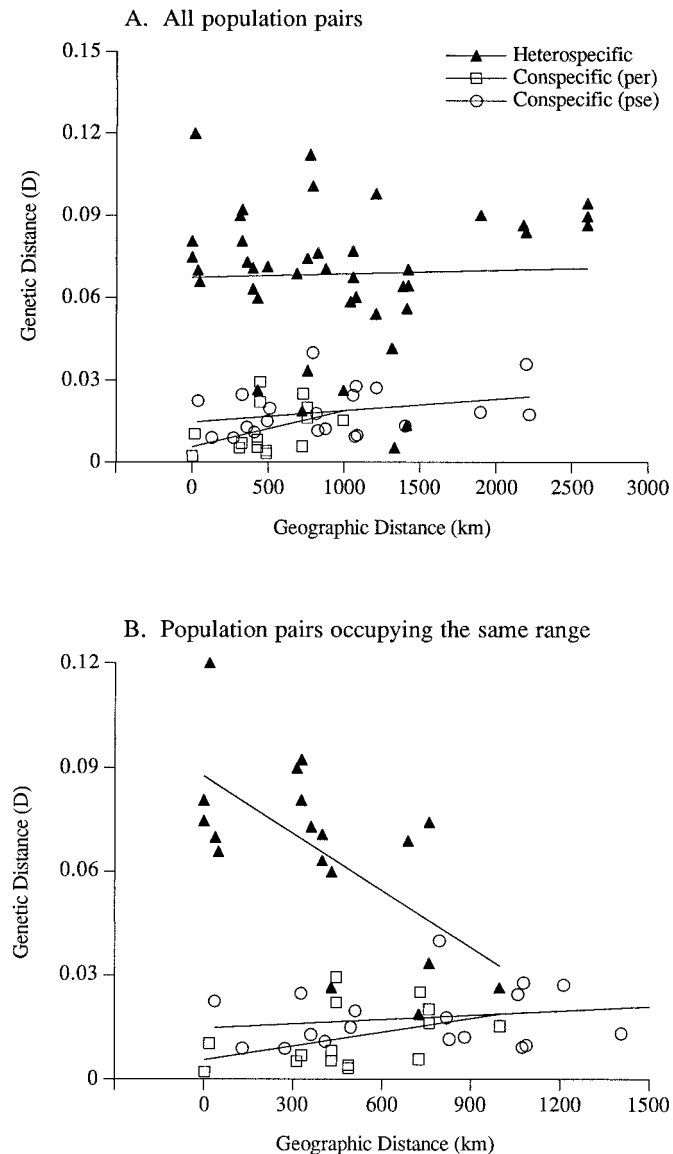


FIG. 1. The relationship between the standard genetic distance and the geographic distance between (A) both sympatric and allopatric heterospecific population pairs and (B) only sympatric heterospecific population pairs of *D. persimilis* (per) and *D. pseudoobscura* (pse; closed triangles). Comparisons between conspecific pairs of populations within each of the species, *D. persimilis* (open squares) and *D. pseudoobscura* (open circles) are also shown. Only pairwise combinations of populations that used greater than 10 common loci in the calculation of the genetic distance (D) are plotted. The slopes of the regression of both within species population pair comparisons, *D. pseudoobscura* and *D. persimilis*, are not significantly different from zero as is the slope of the regression for all (sympatric and allopatric) heterospecific population pairs. However, a significant negative slope of the regression is found for heterospecific sympatric population pairs ($r^2 = 0.41$, $F_{1,16} = 11.25$, $P = 0.004$).

$= 0.131$; *D. pseudoobscura*: $r^2 = 0.09$, $F_{1,19} = 1.82$, $P = 0.193$). Regression analysis between all heterospecific population pairs revealed the absence of any correlation ($r^2 = 0.001$, $F_{1,40} = 0.06$, $P = 0.809$). However, if populations from the most distant sampling site (Austin, TX) were excluded, therefore restricting the analysis to only population

pairs with geographic distances less than 1500 km, a negative correlation is observed ($r^2 = 0.15$, $F_{1,34} = 6.01$, $P = 0.020$). If we further limit the population pairs to only those which occupy the species range of *D. persimilis* (sympatric), an even stronger negative correlation is observed (Fig. 1B; $r^2 = 0.41$, $F_{1,16} = 11.25$, $P = 0.004$). Using a nonparametric measure of association, a rank correlation coefficient that is significantly different from zero ($r_s = -0.692$; $P < 0.005$) was obtained.

DISCUSSION

Rejection of Model I in Drosophila pseudoobscura and Drosophila persimilis

Over the last fifty years, *D. pseudoobscura* and *D. persimilis* have become a case study in reinforcement. Recently, Noor (1995) classified *D. pseudoobscura* populations as either sympatric or allopatric to its sibling species, *D. persimilis*, and showed that heterospecific matings were consistently less successful between sympatric pairs through the development of mating barriers. Noor attributed this pattern of reproductive character displacement to the process of reinforcement. In an attempt to quantify the extent of heterospecific allelic introgression, we have shown that the genetic distance and the proportion of unique and shared alleles between sympatric and allopatric interspecific populations of *D. pseudoobscura* and *D. persimilis* are not significantly different (Tables 1, 2) suggesting that gene flow through introgression has not occurred. These observations do not support model I, reinforcement with gene flow.

This result is in variance with a study by Powell (1983), who surveyed mtDNA restriction site variation in the same species pair and showed higher similarity between sympatric populations. However, Powell's results may reflect his choice of two fairly isolated allopatric populations of *D. pseudoobscura* (Sabinas, Mexico, is more distant than any of the populations used in our study and populations in Bogota, Colombia are well known for their partial reproductive isolation from other conspecific North American populations). In contrast, Hale and Beckenbach's (1985) extensive survey of mitochondrial haplotypes in the northern range of both species failed to support a difference in the amount of interspecific introgression between sympatric and allopatric populations. Unfortunately, only a few nuclear loci (in which natural selection has been shown to be acting) have been surveyed in other molecular studies employing these two species (Aquadro et al. 1991; Schaeffer and Miller 1991, 1992; Babcock and Anderson 1996; Wang et al. 1997). However, it seems evident from all of these studies that heterospecific gene flow has not been observed in sympatry to the extent that one would expect from reinforcement with gene flow.

The absence of greater sympatric allelic similarity is particularly surprising given the presumed more recent ancestry of present-day sympatric Californian populations. Loci thought to be locked in species-specific inversions, such as those found on the third chromosome, are not more differentiated than loci found in noninverted regions. There is no difference in genetic distance between pooled heterospecific sympatric (where both species share the "standard" inversion arrangement) and allopatric populations among third

chromosomal loci (Table 1B). Also, the number of unique/shared third chromosomal alleles is not significantly different from other chromosomes.

Using a second approach, we tested the prediction from model I of reinforcement that the more geographically proximate two heterospecific populations are in sympatry, the more genetically similar they will be. The expected positive correlation between geographic and genetic distances depends on the degree of dispersal between populations of the same species (the greater the *intraspecific* gene flow, the smaller the slope of the regression). We note that this positive correlation will also be expected, although to a lesser degree, between allopatric populations (in the absence of reinforcement).

The regression for *all* heterospecific population pairs (sympatric and allopatric) reveals a nonsignificant slope (Fig. 1A). However, by limiting the analysis to only sympatric species pairs, we observe a *negative* regression between genetic and geographic distances, in contradiction to the prediction (Fig. 1B). Even by extending our limit to all heterospecific populations within 1500 km of each other, both sympatric and allopatric to take into account all possibilities of dispersal overlap, a weaker yet significant negative correlation is found. This result is not expected under reinforcement with gene flow (model I).

Model II: Reinforcement without Genetic Exchange

Because rare hybrids of *D. persimilis*/*D. pseudoobscura* have been discovered in natural populations (Dobzhansky 1973; Powell 1983; Hale and Beckenbach 1985; Wang and Hey 1996), we can still entertain the hypothesis of hybridization-induced reinforcement, but without subsequent heterospecific gene flow (model II). Intermediate postzygotic isolation index values for this species pair may be an underestimate for a variety of reasons. For example, female hybrids may be less fit than parental types in mate acquisition (Coyne and Orr 1989; Davies et al. 1997) and there may be a systematic bias toward female fertility in the laboratory setting compared to the less serene natural environment. In addition, further genic incompatibilities in hybrids may further limit the amount of successful hybridizations. Orr (1987) has shown that interactions between the X-chromosome of *D. persimilis* and a hybrid or *D. pseudoobscura* cytoplasm drastically decrease the fertility of hybrid females, thus precluding female hybrid backcrossing to *D. persimilis* males.

This requirement for high levels of postzygotic isolation for reinforcement to proceed has been qualitatively stated in the past (Muller 1942; Mayr 1963) as well as in recent genetic models (Spencer et al. 1986; Liou and Price 1994; see fig. 1 in Kelly and Noor 1996). High levels of postzygotic isolation needed for reinforcement has also been recently shown empirically. Hostert (1997), demonstrated the development of prezygotic isolation in strains of *D. melanogaster* after removing all F₁ hybrids over a number of generations, similar to traditional demonstrations of reinforcement (see table 1, part C in Rice and Hostert 1993). However, when only a fraction of the hybrids was removed, thereby simulating incomplete postzygotic isolation, prezygotic isolation did not evolve.

In their comprehensive survey of the *Drosophila* genus, Coyne and Orr (1989, 1997) revealed higher prezygotic isolation in sympatric species pairs relative to allopatric species pairs with the same genetic distance. They concluded that reinforcement was the most likely explanation. Here, we equate their conclusion of reinforcement with model II, reinforcement without gene flow. In fact, Coyne and Orr (1989) themselves rejected any biased deflation of genetic distance due to sympatric gene flow on the grounds that, although prezygotic isolation was higher in sympatry, postzygotic isolation remained constant.

Alternatives to Models of Reinforcement

Both competitive exclusion and ecological character displacement can explain the observation of higher prezygotic isolation in sympatry as well as the absence of sympatric introgression and the negative correlation between genetic distance and geographic distance found in this study. Although these mechanisms are based on ecological interactions, there exists a correlation between ecological divergence and ethological isolation. Dodd (1989) showed ethological isolation as a pleiotropic by-product of adaptive divergence in experimental populations of *D. pseudoobscura*, and Markow (1981) observed behavioral isolation after selecting for geotactic and phototactic behavior in *D. melanogaster*.

Competitive exclusion, whereby one of two competing populations become extinct, would lead to a biased pattern of high prezygotic isolation in sympatry, akin to Templeton's (1981) fusion hypothesis. The negative correlation between geographic and genetic distances among sympatric population pairs (Fig. 1B) supports the extinction of less genetically differentiated proximate populations and the coexistence of populations with greater prezygotic isolation. A second mechanism, ecological character displacement, may also lead to prezygotic isolation and has been demonstrated in *Drosophila arizonensis* and *Drosophila mojavensis* (Wasserman and Koepfer 1977; Zouros and D'Entremont 1980), Darwin's finches (Lack 1947; Grant and Grant 1995), and most recently in the threespine stickleback (Schluter and McPhail 1992; Schluter 1994, 1996; Nagel and Schluter 1998; Rundle and Schluter 1998). Ecological competitive exclusion and character displacement may operate complementary to each other. If not ecologically differentiated enough at secondary contact, one of the two populations may be outcompeted and eventually become extinct. If there is ample ecological differentiation, competition between the populations for common resources may drive character displacement. We must stress that this situation is very different from reinforcing prezygotic barriers because hybridization and the production of hybrids is a necessary step in the reinforcement hypothesis. Explanations invoking ecological differentiation do not require any hybridization.

In summary, we reject model I (reinforcement with gene flow) in favor of more parsimonious mechanisms in *D. pseudoobscura* and *D. persimilis*. Future tests of model I would be best performed on species pairs in which each species have both a sympatric and allopatric geographical zone. In addition, organisms should be chosen with low intraspecific

gene flow (low dispersal) and low postzygotic isolation to clearly observe the effects of differential sympatric introgression.

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