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FLIES ACROSS THE WATER: GENETIC DIFFERENTIATION AND REPRODUCTIVE ISOLATION IN ALLOPATRIC DESERT *DROSOPHILA*

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Abstract.—Between sister species of *Drosophila*, both pre- and postzygotic reproductive isolation commonly appear by the time a Nei's genetic distance of 0.5 is observed. The degree of genetic differentiation present when allopatric populations of the same *Drosophila* species exhibit incipient reproductive isolation has not been systematically investigated. Here we compare the relationship between genetic differentiation and pre- and postzygotic isolation among allopatric populations of three cactophilic desert *Drosophila*: *D. mettleri*, *D. nigrospiracula*, and *D. mojavensis*. The range of all three is interrupted by the Gulf of California, while two species, *D. mettleri* and *D. mojavensis*, have additional allopatric populations residing on distant Santa Catalina Island, off the coast of southern California. Significant population structure exists within all three species, but only for allopatric populations of *D. mojavensis* is significant isolation at the prezygotic level observed. The genetic distances for the relevant populations of *D. mojavensis* were in the range of 0.12, similar to that for *D. mettleri* whose greatest D = 0.11 was unassociated with any form of isolation. These observations suggest further investigations of *Drosophila* populations with genetic distances in this range be undertaken to identify any potential patterns in the relationship between degree of genetic differentiation and the appearance of pre- and/or postzygotic isolation.

Key words.—Allozymes, *Drosophila mettleri*, *D. nigrospiracula*, population structure, reproductive isolation.

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Physical barriers to gene flow, coupled with differences in the environments experienced by geographically separated populations, are considered to be important factors in the allopatric divergence that precedes speciation (Lynch 1989). For populations of marine organisms, a classic example of a vicariant separation is the emergence of the Isthmus of Panama. The resulting isolation of Caribbean and eastern Pacific fauna is considered to be a major force in the evolution of closely related sister species (geminate species) on either side of the barrier (Knowlton et al. 1993; Bermingham et al. 1997).

For terrestrial organisms endemic to the Sonoran Desert of North America, the Gulf of California creates a 1100-km long separation of populations living in Baja California from those in the Mexican mainland. Although there are a number of large islands, including the midgulf islands in the upper Gulf, the average distance between the two land masses is about 120 km along its entire length. The impact of this body of water on gene flow between populations of terrestrial Sonoran Desert organisms on either side has yet to be systematically investigated, but it is predicted, especially for less vagile organisms, to have considerable potential to contribute to local differentiation.

Four species of cactophilic *Drosophila*, *D. nigrospiracula*, *D. mettleri*, *D. mojavensis*, and *D. pachea*, are endemic to the Sonoran Desert and are found both in Baja California and the Mexican mainland, as well as Arizona. Two of the species, *D. nigrospiracula* and *D. mettleri*, are typically associated with the same cactus host, either saguaro (*Carnegiea gigantea*) in Sonora and Arizona, or cardón (*Pachycereus pringlei*) in Baja California or coastal Sonora (Heed 1978). Adults of both species feed on necrotic cactus tissue, but *D. nigrospiracula* breeds in the tissue itself, whereas *D. mettleri* larvae utilize the soil soaked with the exudate flowing down from the necrotic tissue above. *Drosophila mettleri* is also found on Santa Catalina Island, off the coast of southern California, where it is associated with necrotic tissue of several different *Opuntia* species (Heed 1982). Not only is Santa Catalina Island separated from southern California by the San Pedro Channel, a distance of about 40 km, but no *D. mettleri* have been recovered in collections from coastal California, making it likely that the Santa Catalina populations of *D. mettleri* experience considerable geographic separation from their nearest neighbors. Furthermore, the land surrounding the upper Gulf of California is devoid of cactus hosts for the desert-endemic *Drosophila*, in effect extending northward on land the geographic isolation of cactophilic *Drosophila* created by the Gulf. The geographic separations outlined above, coupled with the host shifts in the physically separated populations, raise questions regarding the degree of genetic differentiation exhibited by each species.

Until the present study, genetic differentiation across the Gulf of California had only been examined for one of these four species, *D. mojavensis* (Zouros 1973; Hocutt 2000). *Drosophila mojavensis* has become a popular model system for speciation studies because it exhibits significant population genetic structure (Zouros 1973; Hocutt 2000) and several forms of reproductive isolation (Zouros and d'Entremont 1980; Krebs and Markow 1989; Krebs 1990; Markow 1991; Hocutt 2000; Knowles and Markow 2001). Despite the interest in the reproductive isolation observed among these genetically differentiated populations of *D. mojavensis*, no comparative genetic studies of peninsular and mainland populations of the other three species have been undertaken. Although local populations of *D. pachea* exhibit differentiation in chromosomal inversion frequencies (Duncan 1979), Pfeiler and Markow (2001a) found no evidence of genetic

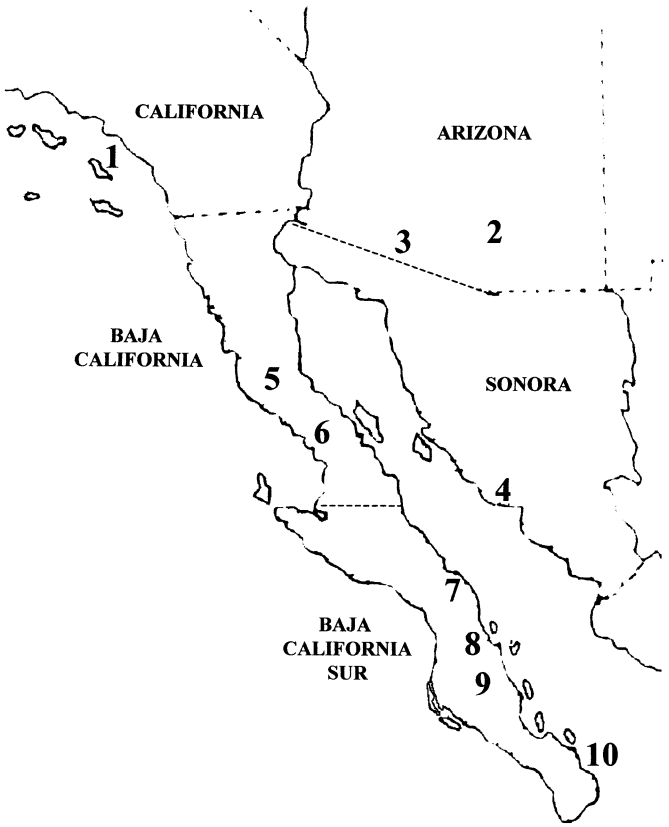

FIG. 1. Key to populations of desert *Drosophila* sampled: 1 = Santa Catalina Island, CA; 2 = Tucson, AZ; 3 = Organ Pipe Cactus National Monument, AZ; 4 = Guaymas, Sonora; 5 = Cataviña, BC; 6 = Kilometer 249, Mexico Highway 1, BC; 7 = Bahía Concepción, BCS; 8 = Nopolo, BCS; 9 = Puente Tevalle, BCS; 10 = Ensenada de los Muertos, BCS. *Drosophila mettleri* were obtained from all localities except for 8 and 9; *D. nigrospiracula* were obtained from all localities except for 1, 5, 6, and 10.

structure for mainland populations of *D. pachea*, *D. nigrospiracula*, or *D. mettleri*, across a continuous 475 km from southern Arizona to southern Sonora. Thus, within a continuous, large geographic area, these three species appear to be panmictic. With respect to the Gulf of California, Markow et al. (1983) failed to find any evidence of premating isolation between peninsular and mainland populations of *D. pachea*, *D. nigrospiracula*, or *D. mettleri*. The Santa Catalina Island population of *D. mettleri* was unknown at the time of that behavioral study, so it was not included. No studies of postzygotic isolation have been reported for any of these three species.

A survey of sister species of *Drosophila* (Coyne and Orr 1989, 1997) indicated that pre- and postzygotic isolating mechanisms commonly appear by the time two species exhibit a Nei's genetic distance of 0.5. One problem with comparisons of already distinct species is that it is impossible to distinguish differences that arose after the speciation event from those that actually served as the original isolating mechanisms. Thus, the question remains as to what patterns might be present with respect to the order of appearance of isolating mechanisms and their relationship to genetic distance when populations are at an earlier point on the divergence trajectory (Coyne and Orr 1998). The species of cactophilic *Drosophila* whose populations are separated by the Gulf of California are obvious groups in which to seek the existence of such patterns.

The present study was undertaken to examine the relationship between genetic distance among allopatric populations of the same species and the appearance of reproductive isolation. We selected two different Sonoran Desert-endemic *Drosophila* in which to address this question, *D. mettleri*, a member of the *eremophila* species complex, and *D. nigrospiracula*, a member of the *anceps* complex (Heed 1982). These two species complexes diverged approximately 40 million years ago (Pitnick et al. 1997) and *D. mettleri* and *D. nigrospiracula* are thought to have invaded the Sonoran Desert independently of each other (Heed 1982). Because the two species are associated with the same host resources, except on Santa Catalina Island where *D. nigrospiracula* is absent, they exhibit similar geographic distributions. As in the studies of species surveyed by Coyne and Orr (1989; 1997), we utilized allozymes to characterize genetic differentiation among populations. Despite the availability of molecular genetic approaches, Shoemaker and Jaenike (1997) and Castro et al. (1999) demonstrated that identical results are obtained in *Drosophila* population genetic studies when mitochondrial sequence and allozyme data are employed. We ask (1) what is the degree of genetic differentiation in allopatric populations of each of these two species, and (2) whether there is evidence that postzygotic isolation has evolved in allopatry. We then compare our observations to previous data on premating isolation for allopatric populations of both species, and to patterns known for another Sonoran Desert endemic, *D. mojavensis*, which has a similar geographic distribution.

MATERIALS AND METHODS

Collection of Flies

Adult *Drosophila* were collected from southern Arizona, southern California (Santa Catalina Island), and northwestern Mexico (Sonora and the Baja California Peninsula) (Fig. 1). *Drosophila mettleri* were sampled from eight populations: Guaymas, Sonora; Organ Pipe Cactus National Monument (OPNM), Arizona; Tucson, Arizona; Santa Catalina Island, California; Cataviña, Baja California (BC); Kilometer 249 (Mexico Highway 1, 70 km S of Cataviña), BC; the southern end of Bahía Concepción, Baja California Sur (BCS); and Ensenada de los Muertos (ENMU, 35 km SE of La Paz), BCS. Samples of *D. nigrospiracula* were obtained from six populations: Guaymas; OPNM; Tucson; Puente Tevalle (10 km S of bridge), BCS; Bahía Concepción, BCS; Nopolo (10 km S of Loreto), BCS. Flies from Sonora and Arizona were collected from March to May 1998 and February to June 2000; collections in Baja California and Santa Catalina Island were made during January and April 2001, respectively. Collections were made either at necrotic tissue of host cacti (for *D. mettleri*: saguaro at Tucson and OPNM; cardón at Guaymas and Bahía Concepción; senita [*Lophocereus schottii*] at Cataviña; *Opuntia* spp. at Santa Catalina Island; for *D. nigrospiracula*: saguaro at Tucson and OPNM; cardón at Guaymas and Bahía Concepción), or at artificial baits.

TABLE 1. Summary of genetic variability at eight enzyme loci in populations of *Drosophila mettleri* and *D. nigrospiracula*.[1]

Species	Locality[2]	H_o[3] (±SE)	H_e[4] (±SE)	Alleles per locus (mean ± SE)	P (95%)[5]
D. mettleri	Guaymas	0.125 (±0.069)	0.112 (±0.058)	2.63 (±0.63)	50.0
	OPNM	0.094 (±0.057)	0.086 (±0.050)	1.88 (±0.35)	12.5
	Tucson	0.104 (±0.050)	0.111 (±0.056)	2.63 (±0.46)	37.5
	Santa Catalina Island	0.119 (±0.066)	0.101 (±0.055)	1.63 (±0.26)	25.0
	Cataviña	0.078 (±0.050)	0.083 (±0.055)	1.63 (±0.32)	25.0
	BC km 249	0.086 (±0.086)	0.062 (±0.062)	1.13 (±0.13)	12.5
	Bahía Concepción	0.113 (±0.061)	0.126 (±0.060)	1.63 (±0.18)	37.5
	ENMU	0.082 (±0.074)	0.078 (±0.063)	1.25 (±0.16)	25.0
D. nigrospiracula	Guaymas	0.142 (±0.083)	0.160 (±0.086)	2.63 (±0.84)	37.5
	OPNM	0.141 (±0.069)	0.146 (±0.071)	2.75 (±0.65)	37.5
	Tucson	0.158 (±0.076)	0.170 (±0.086)	2.88 (±0.77)	37.5
	Puente Tevalle	0.117 (±0.050)	0.137 (±0.060)	1.75 (±0.31)	50.0
	Bahía Concepción	0.141 (±0.079)	0.124 (±0.067)	1.75 (±0.37)	50.0
	Nopolo	0.031 (±0.031)	0.028 (±0.028)	1.25 (±0.25)	12.5

[1] Data for Guaymas, OPNM, and Tucson from Pfeiler and Markow (2001b).
[2] Abbreviations: OPNM, Organ Pipe Cactus National Monument; BC, Baja California; ENMU, Ensenada de los Muertos.
[3] H_o, observed heterozygosity (direct count).
[4] H_e, Hardy-Weinberg expected heterozygosity, unbiased estimate (Nei 1978).
[5] Percent polymorphic loci (95% criterion).

Allozyme Electrophoresis

Male and female flies were separated and then homogenized individually in 25 μl of grinding buffer (Cleland et al. 1996). Homogenates were centrifuged for 5 min at 10,000 g and the supernatants analyzed by electrophoresis either on 12.5% starch gels (Starch Art Corp., Smithville, TX) or Titan III cellulose acetate plates (Helena Laboratories, Beaumont, TX). Starch gel electrophoresis was carried out at 4°C in a buffer system of 40 mM citrate adjusted to pH 6.0 with N-(3-aminopropyl)morpholine (diluted 1:20 in the gel). After electrophoresis, gel slices were stained for enzyme activity using standard recipes (Murphy et al. 1990). Cellulose acetate electrophoresis was performed for 20 min (22°C) at 200 V and using Tris-glycine buffer (pH 8.0); enzyme staining followed the recipes given in Hebert and Beaton (1989) with minor modification.

The eight enzymes (with abbreviations and EC numbers) analyzed in both species of *Drosophila* were phosphoglucomutase (PGM, 5.4.2.2); alcohol dehydrogenase (ADH, 1.1.1.1); malate dehydrogenase (MDH, 1.1.1.37); glycerol-3-phosphate dehydrogenase (NAD^+)(GPDH, 1.1.1.8); cytosol nonspecific dipeptidase (PEP-A, 3.4.13.18, glycylleucine substrate); tripeptide aminopeptidase (PEP-B, 3.4.11.4, leucylglycylglycine substrate); arginine kinase (ARGK, 2.7.3.3); and carboxylesterase (EST, 3.1.1.1, α-napthylacetate substrate). The loci coding for these enzymes are abbreviated with italics. There was no evidence for sex-linkage, or sex-specific suppression of enzyme activity (Pfeiler and Markow 2001b), for any of the loci examined. Therefore, for both species, allele frequency data for both sexes were combined for population analysis. With a few exceptions, the number of flies of each species from each locality analyzed ranged from 30 to 70.

Statistical Analyses

Estimates of genetic variation and Wright's *F*-statistics were performed with BIOSYS-1 (Swofford and Selander 1989). The calculation of significance of pairwise comparisons of F_{ST} for each species was performed with Arlequin version 2.000 (Schneider et al. 2000) using 1000 permutations of the data matrix.

Tests for Postmating Isolation

Males and females were separated as virgins and maintained in yeasted culture vials until sexually mature, four days of age for *D. mettleri* and eight days for *D. nigrospiracula*. Single pairs were aspirated into vials and observed until copulation took place. Pairs consisted of a male and a female from either the same strain (geographic location) or from different strains of the same species. For *D. mettleri*, strains from three localities were tested: Loreto, BCS; Guaymas, Sonora; and Santa Catalina Island, California. Twelve matings were conducted for each of nine *D. mettleri* pairing types. Two strains were used for *D. nigrospiracula*, one from Loreto and one from Guaymas, with 20 matings performed for each of four mating combinations. Mated females were transferred individually to fresh culture vials each day. Their male and female progeny were counted and sons were dissected to check for sperm immotility, the common measure of male sterility in *Drosophila*.

RESULTS

Frequencies of alleles at each locus obtained for *D. mettleri* and *D. nigrospiracula* (available from the authors upon request) were used to calculate the average genetic variabilities in allopatric populations of each species (Table 1). Allele frequencies for most polymorphic loci were in Hardy-Weinberg equilibrium (HWE). The few exceptions to HWE were due in part to the presence of one or two individuals in a population with genotypes containing rare alleles in heterozygous or homozygous combinations. For *D. mettleri*, however, a significant heterozygote excess for the *Adh* fast/slow polymorphism was observed in the Baja California km 249 population (Fixation Index = 0.403; D = 0.385). On the whole, *D. mettleri* exhibited less heterozygosity than *D. nigrospiracula*. Mean (±SE) observed heterozygosity (H_o) av-

TABLE 2. Summary of Wright's (1978) *F*-statistics in polymorphic loci in eight populations of *Drosophila mettleri* and six populations of *D. nigrospiracula.*

Species	Locus	F_{IS}	F_{IT}	F_{ST}
D. mettleri	*Pgm*	−0.137	0.520	0.578
	Adh	−0.123	−0.009	0.102
	Mdh-1	−0.011	−0.001	0.010
	Gpdh	0.415	0.443	0.048
	Pep-A	−0.085	−0.023	0.058
	Pep-B	0.188	0.206	0.021
	Est-2	−0.044	−0.022	0.021
	Mean	−0.073	0.136	0.194
D. nigrospiracula	*Pgm*	−0.050	−0.013	0.035
	Adh	−0.020	−0.009	0.011
	Mdh-1	−0.029	0.015	0.043
	Gpdh	−0.017	−0.005	0.012
	Pep-A	−0.083	−0.029	0.050
	Pep-B	0.139	0.183	0.052
	Est-2	0.043	0.113	0.072
	Mean	0.032	0.089	0.059

eraged across the eight populations of *D. mettleri* was 0.100 (±0.006); overall H_o was 0.121 (±0.019) for the six populations of *D. nigrospiracula*. One-way ANOVA indicated that the overall mean values for H_o were significantly different in the two species. For *D. nigrospiracula*, the Nopolo population showed much reduced variability (H_o = 0.031) compared with the other populations (Table 1). Excluding this outlying population increased the overall mean H_o in *D. nigrospiracula* to 0.140 (±0.007).

F_{ST}-values for both species are presented in Table 2. The mean F_{ST} for *D. mettleri* (0.194) is more than three times that of *D. nigrospiracula* (0.059), a difference primarily attributable to differences at the *Pgm* and *Adh* loci (see below). Pairwise comparisons of overall F_{ST} in *D. mettleri* (Table 3a) showed highly significant values for the Santa Catalina Island population compared with all other populations. In pairwise comparisons of the three mainland populations of *D. mettleri* (Guaymas, OPNM, and Tucson) with the four peninsular populations (Cataviña, km 249 BC, Bahía Concepción, and ENMU), lower, but significant, F_{ST}-values were found in seven of 12 comparisons. In *D. nigrospiracula*, pairwise comparisons of F_{ST} of the three mainland populations with the three peninsular populations (Puente Tevalle, Bahía Concepción, and Nopolo) showed that only four of the nine comparisons were significant (Table 3b). For both species, F_{ST}-values between the mainland Guaymas populations and the closest peninsular populations (Bahía Concepción), a distance of approximately 180 km, were not significant. On the other hand, in pairwise comparisons within the Baja California peninsula, significant F_{ST}-values were found in two of six comparisons in *D. mettleri* and all three comparisons in *D. nigrospiracula*.

F_{ST}-values in *D. mettleri* were particularly influenced by allele frequencies at two loci, *Pgm* and *Adh.* In the population of *D. mettleri* from Santa Catalina Island, the predominant *Pgm* allele (*155), was present at a frequency of 0.750. However, of the 245 individuals analyzed from all other populations, this allele appeared in only one individual (from Guaymas). The *Adh* locus in *D. mettleri* contains a fast (*-100) and slow (*-81) allele. In most populations, the frequencies of the two alleles were similar, or identical. In the population from Santa Catalina Island, however, the slow allele showed a much higher frequency (0.817) than in any other population.

The outcomes of crosses within and among geographic strains for both species are shown in Table 4. For *D. mettleri*, not all within-strain matings produced progeny and of those that did, the sex ratios were always somewhat female-biased. Of the male progeny, 18% in Loreto and 13% in Santa Catalina Island lacked motile sperm. In crosses among localities, there was no reduction in the number of matings producing

TABLE 3. (A) Pairwise comparisons of F_{ST} in populations of *Drosophila mettleri.* (Asterisk indicates statistically significant differences at the 5% level.)

	Guaymas 1	OPNM 2	Tucson 3	Catalina Island 4	Cataviña 5	km 249 BC 6	Bahía Concepción 7	ENMU 8
1	—							
2	0.005	—						
3	−0.003	0.007	—					
4	0.417*	0.525*	0.382*	—				
5	0.078*	0.167*	0.113*	0.448*	—			
6	−0.033	−0.014	−0.020	0.543*	0.058*	—		
7	0.013	0.045*	−0.012	0.409*	0.040*	−0.045	—	
8	0.030*	0.086*	0.059*	0.478*	0.020	0.000	0.015	—

(B) Pairwise comparisons of F_{ST} in populations of *Drosophila nigrospiracula.* (Asterisk indicates statistically significant differences at the 5% level.)

	Guaymas 1	OPNM 2	Tucson 3	Puente Tevalle 4	Bahía Concepción 5	Nopolo 6
1	—					
2	−0.041	—				
3	−0.059	−0.001	—			
4	−0.004	0.003	0.016	—		
5	0.009	0.019*	0.017	0.044*	—	
6	0.356*	0.126*	0.203*	0.084*	0.147*	—

TABLE 4. Test for postmating reproductive isolation between geographic populations of *Drosophila mettleri* and *D. nigrospiracula.*

Species	Female	Male	Productive matings (%)[1]	Total F_1	% Male F_1	% Male sterile[2]
D. mettleri	Guaymas	Guaymas	75	1477	48	0 (27)
	Loreto	Loreto	75	635	46	18 (27)
	Catalina Island	Catalina Island	83	368	44	13 (30)
	Guaymas	Loreto	75	1492	45	2 (27)
	Guaymas	Catalina Island	67	357	42	21 (24)
	Loreto	Guaymas	75	1489	45	0 (21)
	Loreto	Catalina Island	67	841	47	10 (30)
	Catalina Island	Guaymas	83	607	46	0 (30)
	Catalina Island	Loreto	67	514	41	0 (27)
D. nigrospiracula	Guaymas	Guaymas	80	1542	51	1 (129)
	Loreto	Loreto	90	1320	48	6 (89)
	Guaymas	Loreto	80	2025	47	6 (85)
	Loreto	Guaymas	80	1366	46	0 (100)

[1] Twelve matings were conducted for each of nine *D. mettleri* pairings; twenty matings conducted for each of four *D. nigrospiracula* pairings.
[2] Number of F_1 males examined from each pairing given in parentheses.

progeny or in the F_1 sex ratios. The only between-population F_1 male sterility observed was in those crosses in which fathers were from the Santa Catalina Island strain. But given that even the highest hybrid sterility observed (21%) was only slightly higher than that recorded within the Island strain itself, its relevance for isolation may be minimal. Crosses among strains of *D. nigrospiracula* were similar in success, sex ratio, and sperm motility of male progeny to those within geographic strains. Thus, in neither species is there evidence of postmating reproductive isolation.

DISCUSSION

We were interested in two related questions regarding allopatric populations of *D. mettleri* and of *D. nigrospiracula*. The first was to what degree allopatric populations of each exhibited genetic differentiation. Second, we sought evidence of postzygotic isolation between allopatric populations and whether its presence or absence was associated with the presence or absence of either genetic differentiation or previously determined patterns of prezygotic isolation.

With respect to our genetic characterizations of the two species, genetic variability was higher in *D. nigrospiracula*. Patterns of genetic differentiation among geographically separated populations of *D. nigrospiracula* and *D. mettleri* differ from what has been observed for *D. mojavensis*, where peninsular and mainland populations exhibit greater genetic differentiation (Zouros 1973; Hocutt 2000). If anything, differentiation within the Baja California peninsula may be greater than between the peninsula and the mainland for *D. nigrospiracula* and *D. mettleri*. The observed differentiation within Baja California for these two species could reflect the unique geological history of the Baja California peninsula recently shown to influence the phylogeographic population structure of terrestrial vertebrate species (Riddle et al. 2000).

As with *D. mojavensis* from Santa Catalina Island (Hocutt 2000), however, *D. mettleri* from this island do show significant genetic differentiation from both peninsular and mainland populations. In fact, the Santa Catalina Island *D. mettleri* are equally different from all other conspecific populations in the study. The lack of reduced genetic variability in the *D. mettleri* from Santa Catalina argues against a large bottleneck associated with the founding of this island population. An alternative, though less likely explanation, is that multiple colonization events have contributed to genetic variation in the Catalina Island population.

The relative importance of geographic isolation and host shifts for the observed differentiation in *D. mettleri* are not easily untangled. Populations from the peninsula and the mainland show the greatest differentiation from the Santa Catalina Island population, where flies have shifted from columnar to prickly pear (*Opuntia* spp.) cacti. Populations of *D. mettleri* from the mainland and from the peninsula, undergo their larval development in soil soaked with necrotic cactus juice. On Santa Catalina Island, however, they have been reared from prickly pear cactus pads (C. Ross, pers. comm.), suggesting that they may have shifted breeding site as well as host association.

The lack of significant genetic differentiation across the Gulf of California may have multiple explanations. Even if the host differences (cardón vs. saguaro) between the peninsula and the mainland, coupled with differences in climate, favored different genotypes, *D. nigrospiracula*, at least, is known to be a strong disperser (Johnston and Heed 1976; Markow and Castrezana 2000). Dispersing adults of both *D. nigrospiracula* and *D. mettleri* have been collected routinely from the necroses of a wide range of cacti and cactus fruits (Fellows and Heed 1972), such that they are not restricted, when dispersing, to their own breeding sites. The midgulf islands in the Gulf of California have large stands of host cacti with resident populations of these two *Drosophila* species (Heed 1978) and it is likely that these islands serve as corridors for gene flow. Furthermore, both species exhibit frequent female remating (Markow 1982), which, when coupled with dispersal, can serve to homogenize gene frequencies.

With respect to our second question regarding the relationships among prezygotic and postzygotic isolation and genetic distance, we constructed a summary of the relevant information for all of these variables for *D. mettleri* and *D. nigrospiracula*, as well as for *D. mojavensis* (Table 5). These three species have a similar geographic range, with the exception of *D. nigrospiracula*, which does not inhabit Santa

TABLE 5. Summary of genetic distances and reproductive isolation among geographically isolated populations of three cactophilic desert *Drosophila* species.

Species	Geographic area[1]	Genetic distance[2]	Prezygotic isolation	Postzygotic isolation
D. mettleri	Peninsular (4) Mainland (3)	0.005 (0.000 – 0.019)	No[4]	No[5]
	Peninsular (4) Catalina Is. (1)	0.094 (0.085 – 0.103)	No[6]	No[5]
	Catalina Is. (1) Mainland (3)	0.109 (0.098 – 0.119)	No[6]	No[5]
D. nigrospiracula	Peninsular (3) Mainland (3)	0.010 (0.002 – 0.027)	No[4]	No[5]
D. mojavensis	Peninsular (5) Mainland (5)	0.124 (0.076 – 0.169)[3]	Yes[7,8,9]	No[10]
	Peninsular (5) Catalina Is. (1)	0.069 (0.041 – 0.086)[3]	No[9]	?
	Catalina Is. (1) Mainland (5)	0.109 (0.080 – 0.138)[3]	Yes[8]	?

[1] Number of populations from each geographic area used for genetic distance calculations is shown in parentheses.
[2] Nei's (1978) unbiased genetic distance (range shown in parentheses).
[3] Genetic distances for *D. mojavensis* calculated from data given in Hocutt 2000.
[4] Markow et al. 1983.
[5] Present study.
[6] Castrezana and Markow, unpubl. ms.
[7] Zouros and d'Entremont 1981.
[8] Knowles and Markow 2001.
[9] Markow 1991.
[10] Etges and Heed 1987.

Catalina Island. Although experiments designed to test directly for evidence of postzygotic isolation between *D. mojavensis* populations from Santa Catalina Island and the other areas have not been performed, some crosses have been done in the course of other studies and they produce fertile progeny (T. A. Markow, unpubl. results). Castrezana and Markow (unpubl. ms.) found deviations from random mating between *D. mettleri* from Santa Catalina Island and the other localities, but unlike observations on the peninsular and mainland *D. mojavensis*, they were not consistent or strong enough to be interpreted as sexual isolation. The fact that the only among-population crosses of *D. mettleri* in which F_1 male sterility was observed involved the Santa Catalina Island strain may be an indicator of some early form of postzygotic isolation.

The only evidence of reproductive isolation is at the prezygotic level in *D. mojavensis*. In both cases (see Table 5), it is associated with a genetic distance of about 0.1. It is unclear if these observations indicate that prezygotic mechanisms are detectable before postzygotic ones. Prezygotic isolation observed in *D. mojavensis* may represent the influence of other factors, such as sympatry with its close relative *D. arizonae* in Sonora (Wasserman and Koepfer 1974; Zouros and d'Entremont 1980; Markow and Hocutt 1998). Furthermore, whether the appearance of different isolating mechanisms is a function of the scale at which these sorts of phenomena are measured (Markow and Hocutt 1998) remains an important but unanswered question. For example, post-mating-prezygotic interactions have recently received considerable attention for their role in reproductive isolation (Markow 1997; Knowles and Markow 2001; Price et al 2001), but because they are not typically examined in most studies, including the present one, the amount of genetic divergence required before they are observed is unknown. Also, factors controlling postzygotic isolation, especially when they are newly arisen, may not be fixed in a particular species, and thus may be underestimated (Ruiz et al. 1990). Finally, more species with allopatric distributions should be comprehensively examined for a relationship between early genetic differentiation and incipient reproductive isolation. The distribution of a number of other cactophilic *Drosophila* species, such as *D. pachea*, *D. eremophila*, *D. arizonae*, and *D. spenceri* on both sides of the Gulf of California presents an opportunity to expand the number of observations for these variables in species with similar distributions and ecologies.

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