

Sympatry, allopatry and sexual isolation between *Drosophila mojavensis* and *D. arizonae*

KATIE RENEE MASSIE and THERESE ANN MARKOW

Department of Ecology and Evolutionary Biology and Center for Insect Science, University of Arizona, Tucson, Arizona, USA

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Populations of the North American cactophilic fruitfly *Drosophila mojavensis* and its sibling species *D. arizonae* exist both in sympatry and in allopatry. Females of *D. arizonae*, regardless of their population of origin, are effectively completely isolated behaviorally from *D. mojavensis* males. On the other hand, females of *D. mojavensis* from the sympatric populations in Sonora, Mexico exhibit significantly stronger premating isolation from *D. arizonae* males than do *D. mojavensis* females from allopatric populations from the Baja California peninsula. Earlier studies interpreted these limited observations as support for reinforcement. Since the time of those studies, additional allopatric populations of *D. mojavensis* have been collected from southern California and from Santa Catalina Island, off the coast of southern California. Here, we tested the prediction that if sympatry is in fact associated with increased isolation in *D. mojavensis*, these additional allopatric populations also should show, relative to the sympatric ones, less isolation from *D. arizonae*. Our results are consistent with this prediction and suggest that isolation is in fact stronger in sympatry.

Therese A. Markow, Department of Ecology and Evolutionary Biology and Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA. E-mail: tmarkow@arizona.edu

For the genus *Drosophila*, sexual isolation appears to be increased when related species exist in sympatry, supporting the idea that reinforcement plays an important role in the speciation process. Because this apparent association was detected in an a posteriori meta-analysis of earlier studies (COYNE and ORR 1997), stronger support would be provided by studies designed, a priori, to look for greater sexual isolation in sympatric versus allopatric strains of any given pair of species.

Drosophila mojavensis and its sibling species *D. arizonae* have served as a popular model system for studies of speciation (MARKOW and HOCUTT 1998). Populations of these two species occur in sympatry as well as allopatry. Both species breed in the necrotic tissue of cacti, but *D. arizonae* is more of a generalist (FELLOWS and HEED 1972; RUIZ and HEED 1987) and is more widely distributed, occurring from Arizona to Guatemala (Fig. 1). *Drosophila mojavensis*, on the other hand, is found in four geographic regions, three of them deserts, and one an island off the coast of southern California (Fig. 1). *Drosophila mojavensis* utilizes four different cactus hosts in each of the four parts of its range (HEED 1978, 1982), forming, in effect, four geographic host races. In the Sonora – Arizona part of its range, organ pipe (*Stenocereus thurberi*) is the host, while in the Baja California peninsula, it utilizes agria (*S. gummosus*). Barrel cactus (*Ferrocactus cylindraceus*) is the host for

populations living in the deserts of southern California (Anza Borrego and Mohave) and those in the Grand Canyon. The isolated populations found on Santa Catalina Island, off the coast of southern California, breed in prickly pear cacti of the genus *Opuntia* (RUIZ et al. 1990). The two species are sympatric in the part of the Sonoran Desert that extends from southern Sonora to southern Arizona. In the area of sympatry, *D. arizonae* is associated with cina (*Stenocereus alamosensis*), another columnar cactus.

Drosophila mojavensis and *D. arizonae* are estimated to have diverged approximately 1.5 mya (MATZKIN 2004; MATZKIN and EANES 2003) and exhibit differences in the nature and degree of reproductive isolation, depending upon the geographic source of the *D. mojavensis* populations used in crosses. With respect to postzygotic isolation, there is an asymmetry in the production of sterile hybrid males (WASSERMAN 1982): when *D. arizonae* mothers are used, the hybrid sons are sterile, but in the reciprocal cross, hybrid males are only sterile when the *D. mojavensis* populations are from certain geographic host races (REED and MARKOW 2004). Sexual or behavioral isolation between *D. mojavensis* and *D. arizonae* also has been reported to vary with the strains used (WASSERMAN and KOEPFER 1977; MARKOW 1981). All populations of *D. arizonae* exhibit strong isolation from *D. mojavensis*, but

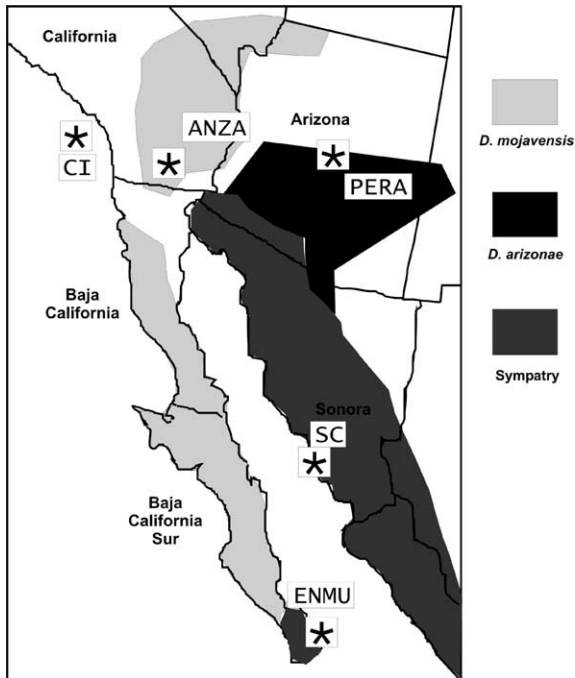


Fig. 1. Distributions of *Drosophila mojavensis* and *D. arizonae* and the collection localities of the strains utilized in the present study. For *D. mojavensis*, CI = Santa Catalina Island, SC = San Carlos, Sonora, ANZA = Anza Borrego Desert, California ENMU = Ensenada de los Muertos, Baja California Sur. For *D. arizonae*, PERA = Peralta Canyon, Arizona.

D. mojavensis from areas of sympatry are significantly more isolated behaviorally from *D. arizonae* compared to allopatric *D. mojavensis*. Earlier studies of sexual isolation in *D. mojavensis* focused upon strains of *D. mojavensis* from two parts of the *D. mojavensis* range (WASSERMAN and KOEPFER 1977; MARKOW 1981). One set of strains was from the Mexican mainland and southern Arizona, where *D. arizonae* is sympatric, the other set of strains was from the Baja California peninsula, where *D. arizonae* historically has been largely absent. Both sets of studies consistently observed that female *D. mojavensis* from the area of sympatry were significantly more isolated from *D. arizonae* males than were *D. mojavensis* females from Baja. Furthermore, the results of such studies do not change even after flies have been cultured for years in the laboratory or tested on different types of culture medium (MARKOW and HOCUTT 1998). These observations have been interpreted as support for reinforcement of reproductive isolation through selection on *D. mojavensis* in Sonora and southern Arizona where it is sympatric with its sibling species (WASSERMAN and KOEPFER 1977).

The interpretation of reinforcement would be more strongly supported if *D. mojavensis* females from other

areas of allopatry, in addition to just those from the Baja California peninsula, also showed less discrimination against *D. arizonae* males. In the present study, we tested for sexual isolation between these two species, but this time, included in the study *D. mojavensis* from all four geographic areas, including the Mohave Desert and Santa Catalina Island, both in California.

METHODS

Strains of flies

Four strains of *D. mojavensis* were used in this study, one from each of the geographic host races. All were formed from multifemale collections made in the field. The Sonoran strain was collected from San Carlos in 2001 (SC), the Baja strain from Ensenada de los Muertos in 1999 (ENMU), the southern California strain from the Anza Borrego Desert in 2002 (ANZA), and the Santa Catalina Island strain in 2002 (CI). The test strain of *D. arizonae* was collected from Peralta Canyon in the Superstition Mountains near Phoenix in 1997 (PERA). All strains were maintained in mass cultures on standard banana medium. Previous studies reveal that patterns of mating behavior in these species remain constant despite decades of laboratory culture (ZOUROS and D'ENTREMONT 1980; MARKOW 1981).

Mating experiments

Virgin males and females were separated under CO₂ and stored separately in yeasted food vials, 10 flies per vial, until used in mating experiments at nine days of age. Twenty-four hours prior to their use in experiments, flies were lightly colored with fluorescent dust (Radiant Corp., Richmond, CA) and allowed to clean themselves off overnight. This technique has been used in our laboratory for a long period, during which we have observed no effects of different pigments on the behavior of the flies. Experiments were conducted in the early mornings. Ten females and ten males were gently aspirated into plexiglas observation chambers (MARKOW 1991), observed for one hour or until all females had mated. Each chamber contained five virgin females and five virgin males of a given strain of *D. mojavensis* and five virgin females and five virgin males of the *D. arizonae* strain. The strains and species copulating were scored on the basis of their color. Chambers in which 50% or less of flies mated were excluded from the analysis. A minimum of four chambers per test was used in the analyses.

Statistical analyses

We analyzed the data in two ways. Deviations from random mating were detected using chi-square tests. We also employed the isolation index, I,

$$I = \frac{(\text{no. of homogametic matings}) - (\text{no. of heterogametic matings})}{\text{total no. of matings}}$$

(MALOGOLOWKIN-COHEN et al. 1965), calculated using the following formula. This index was used for joint isolation, as well as for measuring the strength of isolation of *D. mojavensis* females from different localities from the *D. arizonae* tester strain.

The standard error of the isolation index is estimated by the following formula (MALOGOLOWKIN-COHEN et al. 1965):

$$se = \sqrt{(1 - I^2)/N}$$

Isolation indices are considered to be statistically significant if the index is more than twice the standard error (MALOGOLOWKIN-COHEN et al. 1965, ZOUROS and D'ENTREMONTE 1980).

RESULTS

Total deviations from random mating for all combinations of *D. mojavensis* strains and *D. arizonae* are shown in Table 1. In all cases, there is a consistent

excess of homospecific matings, although the degree of positive assortative mating varies a bit among replications. Chi-square tests indicate significant deviations from random mating in most replications. Mating tests involving the *D. mojavensis* strain from San Carlos, however, are characterized by the largest and most significant joint isolation indices. In fact it is only in tests with the San Carlos *D. mojavensis* population that not one *D. mojavensis* female mated with a *D. arizonae* male.

By examining only matings by *D. mojavensis* females, the question of female isolation can be more directly addressed (Table 2). In this case, the isolation indices for every replication involving *D. mojavensis* from San Carlos are 1.0, suggesting isolation of these females from *D. arizonae* males is complete. In the experiment using females from the Baja population of

Table 1. Deviations from random mating in multiple choice tests.

Type of mating	<i>D. mojavensis</i> population	Rep. no.	Matings: females × males				No.	Chi-square (3 df)	Joint isolation index ± se
			M × M	M × A	A × M	A × A			
Sympatric	San Carlos	1	3	0	1	9	13	15.00**	0.84 ± 0.15*
		2	7	0	1	7	15	11.40**	0.86 ± 0.17*
		3	4	0	0	9	13	16.84**	1.00 ± 0.00*
		4	7	0	0	8	15	15.13**	1.00 ± 0.00*
		5	6	0	0	8	14	14.57**	1.00 ± 0.00*
Allopatric	Ensenada Muertos	1	8	2	1	8	19	10.80*	0.68 ± 0.17*
		2	7	2	0	7	16	10.27*	0.75 ± 0.16*
		3	4	6	0	9	19	21.86**	0.37 ± 0.21
		4	7	0	2	5	14	8.28*	0.71 ± 0.19*
		5	7	1	1	4	13	7.22	0.69 ± 0.20*
Allopatric	Anza Borrego	1	4	4	1	8	17	10.05*	0.41 ± 0.21
		2	2	7	1	5	15	16.18*	-0.07 ± 0.26
		3	7	2	0	4	13	8.11*	0.69 ± 0.20*
		4	6	5	0	7	18	6.88	0.44 ± 0.21*
		5	4	4	3	7	18	6.44	0.22 ± 0.23
Allopatric	Santa Catalina Island	1	5	2	0	10	17	13.35**	0.76 ± 0.16*
		2	6	1	0	7	14	10.57*	0.85 ± 0.14*
		3	9	1	0	9	19	15.31**	0.89 ± 0.10*
		4	6	3	1	6	16	4.50	0.50 ± 0.22*
		5	6	1	1	4	12	6.00	0.66 ± 0.22*
		6	6	4	1	4	15	3.40	0.33 ± 0.24*

Table 2. Female isolation indices derived from multiple choice tests.

Type of mating	<i>D. mojavensis</i> population	Rep. no.	Mating type (female × male)		Total	Chi-square *P < 0.05	Isolation index (± se) * P < 0.05
			M × M	M × A			
Sympatric	San Carlos	1	3	0	3	3.00	1.00 ± 0.00*
		2	7	0	7	7.00*	1.00 ± 0.00*
		3	4	0	4	4.00*	1.00 ± 0.00*
		4	7	0	7	7.00*	1.00 ± 0.00*
		5	6	0	6	6.00*	1.00 ± 0.00*
Allopatric	Ensenada Muertos	1	8	2	10	3.60	0.60 ± 0.25*
		2	7	2	9	2.78	0.56 ± 0.28
		3	4	6	10	0.40	-0.20 ± 0.31
		4	7	0	7	7.00*	1.00 ± 0.00*
		5	7	1	8	4.50	0.75 ± 0.23*
Allopatric	Anza Borrego	1	4	4	8	0.00	0.00 ± 0.35
		2	2	7	9	2.78	-0.56 ± 0.28*
		3	7	2	9	2.78	0.56 ± 0.28*
		4	4	4	8	0.00	0.00 ± 0.38
Allopatric	Santa Catalina Island	1	5	2	7	1.28	0.43 ± 0.34
		2	6	1	7	3.57	0.71 ± 0.26*
		3	9	1	10	6.76*	0.80 ± 0.19*
		4	6	3	9	1.25	0.33 ± 0.31
		5	6	1	7	4.33*	0.71 ± 0.26*
		6	6	4	10	1.37	0.20 ± 0.12

Ensenada de los Muertos, there was a clear tendency for females to mate with males of their own species, although it was only significant in some of the replications. Females of the two *D. mojavensis* strains which had not been tested before with *D. arizonae*, those from Anza Borrego and Santa Catalina Island, both mated occasionally with heterospecific males, and in the case of Anza Borrego females, there actually was little evidence of isolation. Although not the focus of the present study, our observations confirmed that, as in earlier studies with these two species, *D. arizonae* females, rarely mate with *D. mojavensis* males, regardless of their origin.

DISCUSSION

Three of the four *D. mojavensis* populations used in this study are considered to be allopatric with the sister species *D. arizonae*. Flies from two of these regions, Santa Catalina Island and the Anza Borrego Desert, had not been tested previously for isolation from *D. arizonae*. For the sympatric *D. mojavensis* from mainland Mexico and the allopatric *D. mojavensis* from Baja, we found identical patterns to those reported in earlier studies (WASSERMAN and KOEPFER 1977; MARKOW 1981). None of the newly tested allopatric populations showed greater isolation from

D. arizonae than those from Baja. Furthermore, our observations support earlier suggestions that the more extreme isolation between *D. arizonae* and populations of *D. mojavensis* from Sonora is a product of their sympatry in the Mexican mainland which has selected for greater discrimination by *D. mojavensis* females (WASSERMAN and KOEPFER 1977).

Drosophila mojavensis and *D. arizonae* have long been sympatric in the Mexican mainland, primarily in northern Sinaloa and all of Sonora. In these areas they are found feeding on their respective host plants, organ pipe (*Stenocereus thurberi*) and cina (*Stenocereus alamosensis*) which are often side by side in the desert (HEED 1978, 1982). Adults also have been reared from each other's host plants (FELLOWS and HEED 1972; RUIZ and HEED 1988), demonstrating considerable opportunity for encounters between males and females of the two species. Hybrids, however, never have been found in nature (HEED 1982), suggesting that behavioral isolation may be highly effective in keeping them apart. Recently, *D. arizonae*, have been collected in places where they previously had not been detected, such as in the Baja California peninsula, and in Riverside, California (Reed and Markow, unpubl.). The appearance of *D. arizonae* in Baja, a region historically occupied exclusively by *D. mojavensis*, is recent enough that the *D. mojavensis* from Baja can still be considered

allopatric. The *D. arizonae* recently found in southern Baja have not been found on the same local host plant with *D. mojavensis*. It is unlikely, given the frequent collections made in these areas without discovering, until recently, any predictable numbers of *D. arizonae*, that *D. arizonae* has been established there for any period of time. Rather, because it is a dietary generalist (MARKOW et al. 1999), it more likely is expanding its range into the Baja peninsula in response to recent anthropogenic disturbance in the form of habitat change. The impact of the spread of *D. arizonae* into habitats occupied previously only by *D. mojavensis* should provide a future opportunity, however, to study the responses of the populations of both species to evolving sympatry.

Mechanisms underlying sexual isolation between the two species have been the subject of genetic studies (MARKOW 1981; ZOUROS 1981), but these have focused upon *D. mojavensis* from Sonora and Baja. The finding that other allopatric *D. mojavensis*, in addition to those from Baja, also show less isolation from *D. arizonae* suggests that studies of genetic variability for sexual isolation be extended to these populations. It is interesting, for example, that female *D. mojavensis* from the Anza Borrego Desert show the least isolation from *D. arizonae* males. The role of ecology and population biology in female mating behavior differences among *D. mojavensis* populations has never been examined. Populations of *D. mojavensis* in the California deserts appear to exist in small numbers and are infrequently encountered. Female *D. mojavensis* typically remate daily and obtain only about 100 sperm from a given copulation. Perhaps low population densities in southern California underlie a need for females to be more receptive, in general, to courting males, in order to maintain their sperm supply. Indeed, females from the southern California strain exhibit faster mating speeds than do female *D. mojavensis* from other geographic host races (KREBS and MARKOW 1989). These two species of *Drosophila* offer the opportunity to test hypotheses about the speciation process in an ecological framework, a situation not yet possible with many other species in the genus.

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