Sexual Isolation and Mating Propensity Among Allopatric *Drosophila mettleri* Populations

Sergio J. Castrezana · Therese Ann Markow

Abstract *Drosophila mettleri* is found in deserts of North America breeding in soil soaked by the juices of necrotic cacti. Saguaro (*Carnegiea gigantea)* and cardón (*Pachycereus pringlei)* are the usual host cacti in Mexico and Arizona, while prickly pear (Opuntia spp.) is used by an isolated population on Santa Catalina Island off the southern California Coast. Populations of *D. mettleri* show significant local genetic differentiation, especially when geographical isolation is coupled with host shifts. We tested for evidence of sexual isolation among allopatric populations of *D. mettleri* using a variety of choice and no-choice tests. Populations exhibited significant differences in mating propensity, which translated into significant deviations from random mating. While in some cases these deviations were consistent with sexual isolation, in others, negative assortative mating was observed. No relationship between degree of genetic differentiation and the appearance of sexual isolation was detected.

Keywords Mating propensity · Sexual isolation · *Drosophila* · Speciation

Introduction

A major problem in evolutionary biology is to understand the way in which new species form. While there is renewed interest in defining the importance of sympatric speciation

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(Bush 1969, 1975; Berlocher and Feder 2002), speciation in allopatry appears to be most common (Dobzhansky 1941; Mayr 1963; Carson 1975). This being the case, the ability to study early events in speciation depends upon the identification of populations of the same species that are likely to be at early stages of divergence. The likelihood of divergence is increased if populations are geographically separated, utilize different resources, and/or are exposed to different environmental factors that could act as potential forces of selection and drive differentiation.

Drosophila mettleri is a cactophilic species found primarily in the Sonoran Desert of North America, where it breeds in the soil soaked by necrotic columnar cacti of several different species (Heed 1977, 1978; Markow et al. 1983). Their geographic range and primary local host cacti are shown in Fig. 1.

Larval *D. mettleri* most often are associated with necroses of saguaro or cardón in these regions (Heed 1977, 1982; Heed and Mangan 1986), because the larger cacti, such as cardón (*Pachycereus pringlei*) and saguaro (*Carnegiea gigantea*) provide greater quantities of necrotic juice than most organ pipe (*Stenocereus thurberi*) or senita cacti (*Lophocereus schottii*). On the other hand, *D. mettleri* also has been found on Santa Catalina Island (Heed 1989), off the coast of southern California, where there are no columnar cacti and adults have been reared from necrotic pads of *Opuntia littoralis* (Castrezana 2007), a prickly pear cactus found on the island.

Genetic studies of *D. mettleri* from across its range reveal significant differentiation only between the Santa Catalina Island population and the other localities (Hurtado et al. 2004; Markow et al. 2002). Populations from the Baja California peninsula and the Sonoran mainland or Arizona show minimal genetic differentiation, possibly due to the Sea of Cortez which serves to restrict gene flow and where
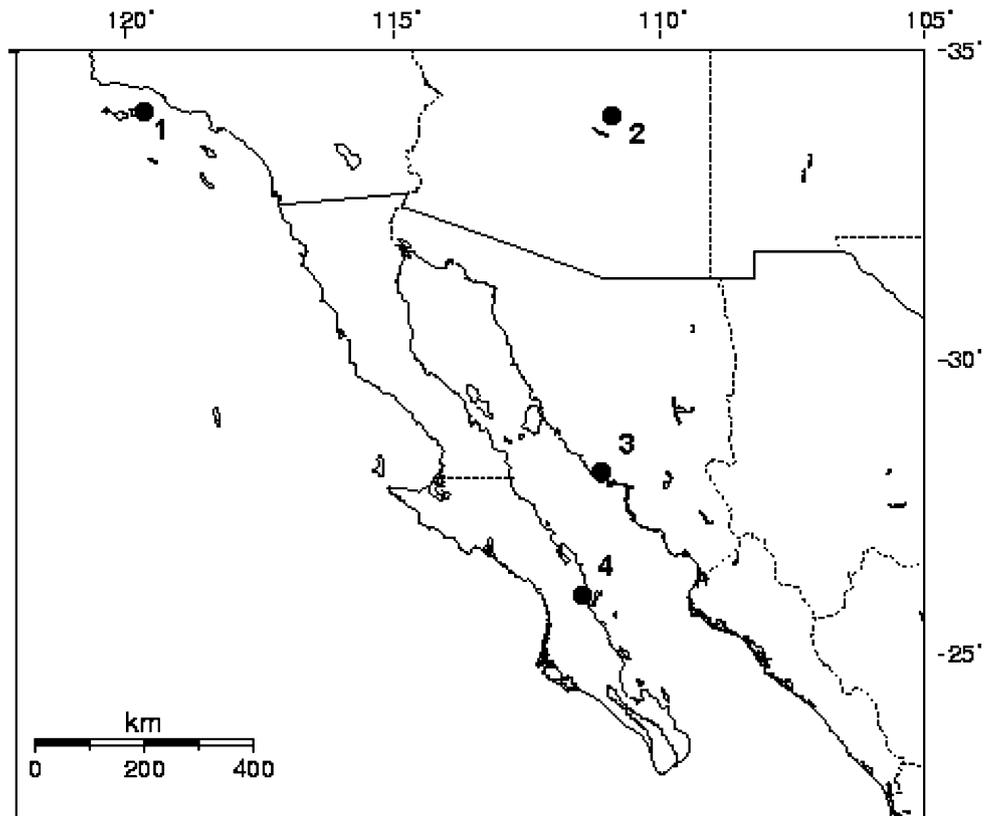
Fig. 1 Key to the *Drosophila mettleri* populations used in the study: 1 = Santa Catalina island, CA (CAT); 2 = Superstition Mountains, AZ (SUP); 3 = San Jose de Guaymas, Guaymas, Sonora, Mexico (GYM); 4 = Bahía Concepción, Loreto, Baja California South, Mexico (LO)

flies shift host plants and experience different climatic conditions on either side of the Sea. The population from Santa Catalina Island, however, exhibits significant genetic differentiation from all other *D. mettleri* populations (Hurtado et al. 2004). No evidence of postzygotic barriers promoting isolation among the different geographic host populations of *D. mettleri* has been detected (Markow et al. 2002). In another desert endemic cactophilic *Drosophila*, *D. mojavensis*, which occupies the same range as *D. mettleri*, the population from Santa Catalina Island does exhibit some prezygotic isolation from mainland populations (Markow and Hocutt 1998; Reed and Markow 2004), suggesting that Santa Catalina Island *D. mettleri* be examined for evidence of similar isolating mechanisms.

Coyne and Orr (1989, 1997) have examined patterns of reproductive isolation among *Drosophila* species and found evidence that premating isolation may precede postmating isolation during the speciation process. Because the studies they reviewed deal with species that already had formed, it is unclear, when considering populations that have not yet achieved the status of species, at what point during the differentiation process sexual isolation appears and whether it typically precedes postzygotic isolation. Populations of *Drosophila mettleri* provide an opportunity to examine for behavioral isolation and its possible relationship with the degree of genetic differentiation. In the current study, we search for the evidence of premating

behavioral isolation among populations of *D. mettleri*, specifically in the Catalina Island population since it exhibits the greatest degree of genetic differentiation. In addition, we investigate whether this type of behavioral isolation reflects male or female mating propensities in different populations.

Studies of sexual isolation in *Drosophila* have employed a wide range of experimental designs (Merrel 1950; Malogolowkin-Cohen et al. 1965; Markow 1980; Zouros and D'Entremont 1980). These tests typically are referred to as "choice tests" although the degree to which they actually measure "choice" has been questioned (Marin 1991; Casares et al. 1998). Nonetheless, they represent standard tests for such studies and provide accessible and useful measures of departures from random mating. In the "no-choice" design, males and females from different populations are placed together in a mating arena. In "female-choice" tests, females are placed with males from their own and another population. In the male-choice test males of one population are confined with females from two populations. In "multiple choice" tests, equal numbers of females and males from two populations are placed together. Random mating usually is tested for by direct observation, often using either wing clipping or colored dust to distinguish flies of different populations. In some cases, however, flies are left together for 24 h and departures from random mating are scored by progeny tests or

dissection of females to detect insemination. A potential problem with this last approach is the possibility of confounding premating isolation with postmating–prezygotic interactions, which may underestimate the actual number of matings that took place (Gilbert and Starmer 1985). Therefore, in the present study, courtship and mating were observed directly.

A difficulty with using choice tests alone to estimate isolation is the inability to infer anything about the processes leading to nonrandom mating, should it be detected. Male courtship propensity or vigor, exemplified by short courtship latencies, and female propensity, characterized by rapid receptivity, can conspire to create departures from random mating having nothing to do with choice or preference (Casares et al. 1998; Rolán-Alvarez and Caballero 2000). In our study we first characterize mating propensity in both sexes of all populations in order to detect differences that would predict specific vigor-based patterns of nonrandom mating in choice tests and to separate them from the effects of preferences. We then employ a series of choice tests (female choice, male choice, multiple choice) to evaluate departures for random mating as well as deviations from random mating predicted from differential mating propensity alone.

We examined sexual isolation in four populations of *D. mettleri*, derived from collections at geographically distant locations (Fig. 1) for which levels of genetic differentiation were determined previously (Hurtado et al. 2004; Markow et al. 2002). *Drosophila mettleri* from Santa Catalina Island, California, exhibits the greatest genetic differentiation from the other populations, leading to the prediction that sexual isolation, if observed, should be greatest between this and the other populations.

Materials and methods

Collection and handling of populations

Four populations of *D. mettleri* were selected to use in this experiment due to their degree of geographic separation. Collection sites and dates were as follows: (a) Santa Catalina Island, CA (3/97), designated CAT; (b) Superstition Mountains, AZ (3/97), designated SUP; (c) Loreto, Baja California, Mexico (11/96), designated LO; and (d) from Guaymas, Sonora, Mexico (11/96), designated GYM. Each population was founded from a multiple female collection ($n > 40$) and maintained in large numbers in half-pint bottles on potato cactus medium (Castrezana 1997). Experiments were conducted in 1997 such that no populations had been in the laboratory for very long before their use in the study.

Behavioral tests

Virgin, newly emerged adult flies were separated by sex under CO_2 and stored in standard banana food vials until five to 6 days old at which time they were used in behavioral tests. Four different types of behavioral tests were conducted: (1) no-choice tests of mating propensity, (2) male-choice tests, (3) female-choice tests, and (4) multiple-choice tests.

No-choice tests

The four populations and the 12 inter-population combinations were tested. A mature female and a male were aspirated into a vial and observed until copulation occurred or 15 min had elapsed. In the no-choice tests, we defined three parameters as follows:

(1) Male courtship latency, as the time elapsed from introduction of the flies in the mating arena until male started courtship; (2) female receptivity, as the time from male courtship initiation to female wing spreading to allow mating mounting and intromission; (3) overall mating speed, as the sum of male courtship latency and female receptivity.

At least 40 pairs of flies were observed for each combination. Also, data for pairs not courting or mating were recorded to obtain percent of successful courtship. The proportion of pairs not reaching a particular landmark also was determined.

Female-choice tests

The 12 possible combinations were tested. A virgin female was aspirated into a vial with two virgin males; one from her own and one from a different population. Males had been dusted lightly with radiant colors from Magruder Color Co (Alameda, CA) and allowed to clean themselves for 24 h before the experiment. Colored dusts were tested prior to the study and found to have no influence on the experimental outcomes. The population of the first male to court and the male to mate were recorded. Vials without mating activity were discarded after 15 min. At least 60 females were used for each combination.

Male-choice test

Twelve combinations were tested. A virgin male was aspirated into a vial with two virgin females, one from his own and one from a different population. Females were lightly treated with colored dust. The population of the first female courted and the female mating were recorded and analyzed using chi-square tests. Vials without mating

activity were discarded after 15 min. At least 60 males were used for each combination.

Multiple-choice test

The six possible combinations of the four populations were tested using a mating arena (400 cm^2) made of Plexiglas. For each population, five females and five males (previously dusted with radiant colors) were introduced simultaneously into the mating arena. Chambers were observed for 20 min and colors of copulating pairs were identified. No flies were removed from the chambers. At least 20 replicates were carried out for each combination.

Statistical analyses

Courtship latency, receptivity, and overall mating speed were subjected to factorial ANOVA with male and female population as the two factors. Departures from random mating in the choice tests were analyzed by Chi-square tests. The foregoing analyses were conducted using JMP software version 4.0.4 (A business unit of SAS, SAS Institute Inc., 2001). The Joint Isolation Index (I) of Merrel (1950) was calculated for the multiple-choice tests as follows:

$$I = ((\text{frequency of homospecific matings})$$
$$- (\text{frequency of heterospecific matings}))$$
$$/\text{total number of matings}$$

Standard errors were calculated according to:

$$\text{s.e.} = \sqrt{(1 - I^2)/N}$$

Results

No-choice tests

Of the total of pairs observed for each of the four populations, not all males courted before the cut-off point and not all courted females were receptive (Fig. 2). Male courtship, therefore, does not guarantee that mating eventually will occur, as females may not be receptive. For example, during the observation period, 90% of pairs of the GYM population exhibited courtship, and yet females were receptive to courting males in only 65% of pairs. Slightly less than 80% of courted females of the LO and SUP populations were receptive during the observation period.

Male courtship latency, female receptivity, and mating speed were recorded for all pairs in which these behavioral landmarks were observed (Fig. 3). An analysis of variance of cross effects in these behavioral landmarks showed

significant differences among crosses (Table 1). A two-way model II ANOVA revealed a significant effect of male but not female population on male courtship latency (females $F_{3,9} = 0.16$, ns; males $F_{3,9} = 4.8$, $P < 0.05$). Similar results were observed in female receptivity (females $F_{3,9} = 3.72$, ns; males $F_{3,9} = 3.90$, $P < 0.05$). On the other hand, the mating speed, which is the sum of previous behavioral landmarks, was not significantly influenced by the populations' sexes (females $F_{3,9} = 2.19$, ns; males $F_{3,9} = 1.14$, ns).

If the progress and success of between-population combinations are a function only of male courtship propensity, the above observations would predict that any interpopulation combination involving a GYM or LO males would be exhibit slower male courtship latencies and mating successes. If factors other than, or in addition to, male propensity, such as male discrimination between females based upon female population or preference for particular female type in order to initiate or continue courtship, different patterns, such as dependence on female type, are predicted.

The outcomes of no-choice pairings were measured in two ways: the proportion of pairs achieving a particular courtship landmark such as courtship or copulation, and the actual latencies recorded for male courtship and female receptivity. The proportions of interpopulation pairs reaching the courtship and copulation landmarks are shown in Fig. 2. In the three cases where CAT females were present, CAT × SUP, CAT × GYM, and CAT × LO, the proportion mating is intermediate between the proportions for the two within population combinations. In general, males and females from the GYM population tended to mate more readily with flies from populations other than their own. Most of the time, males from LO had the lower copulation success while CAT males exhibited the opposite tendency.

Male latency, female receptivity, and overall mating speed for between-population combinations are shown in Fig. 3. The most striking feature is the significant, exaggerated latency of LO males to initiate courtship when females are not from their population, which resulted in a significant delay in the overall mating speeds in these combinations. Although not statistically significant, LO females seemed to accept all males more quickly than did other females.
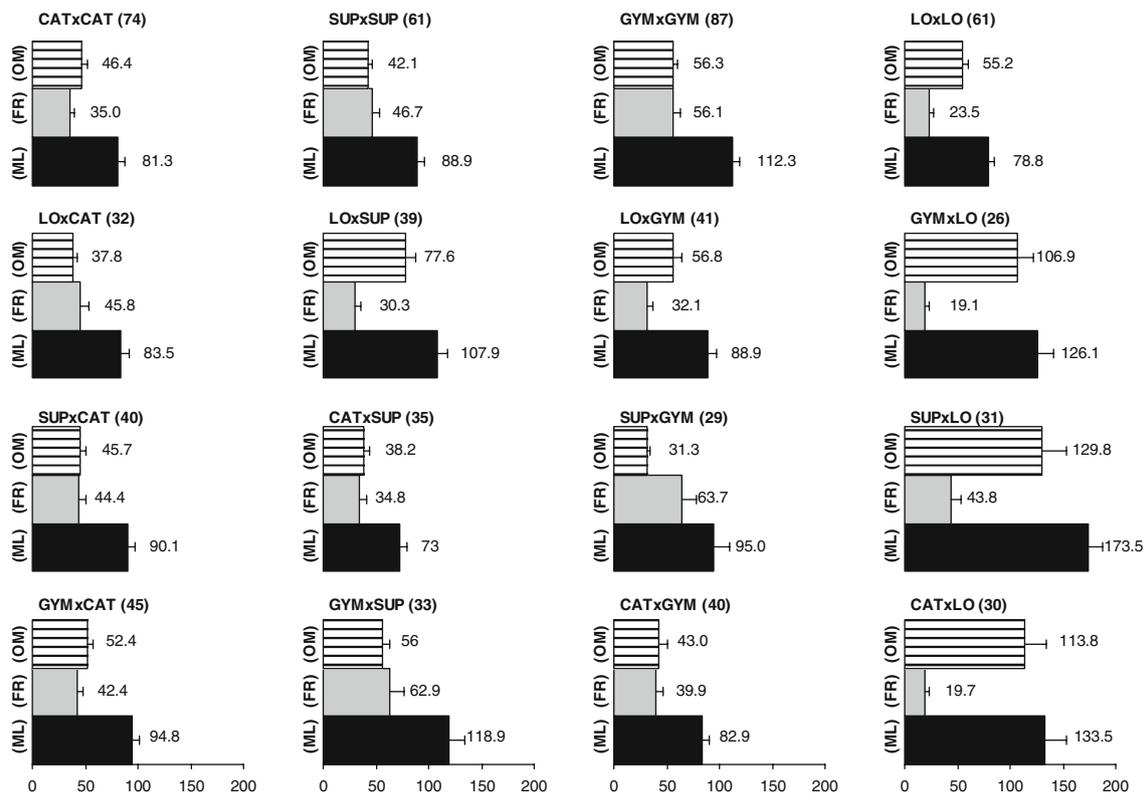
Choice tests

Male-choice test

During male choice tests, the first female courted by the male as well as the female that mated were recorded (Table 2). In partitioning the observations in this way, we
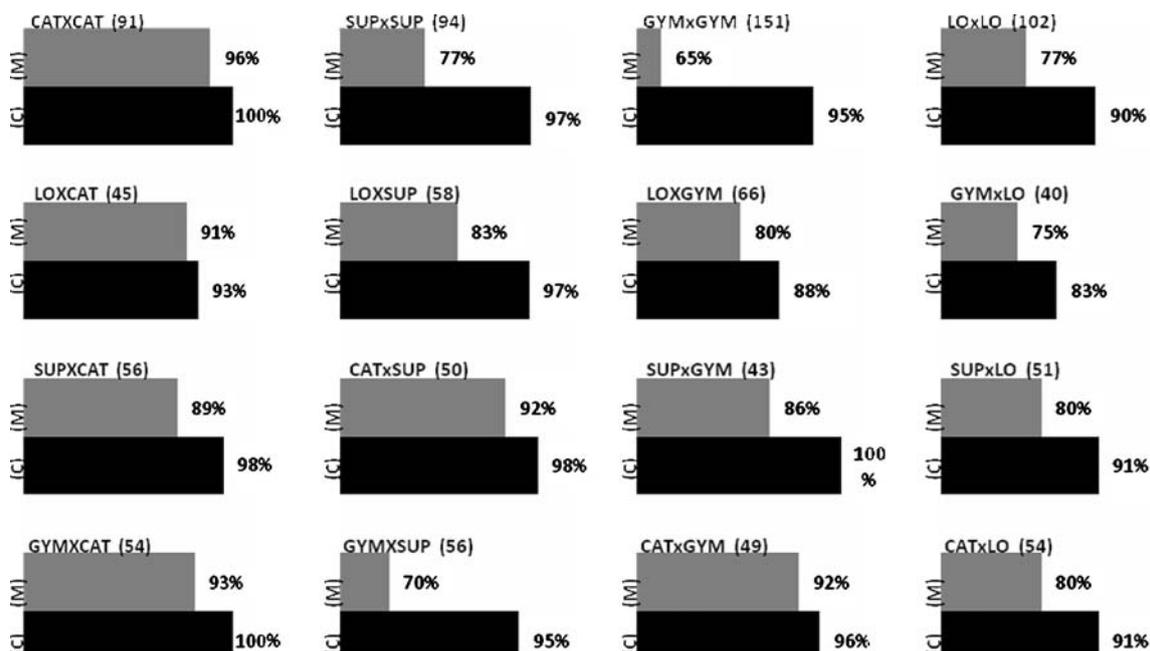
Fig. 2 No-choice test. Courtship (C = black bars) and mating (M = gray bars) frequencies for intrapopulation and interpopulation pairs in four populations of *Drosophila mettleri*. Female strain given first

Fig. 3 No-choice test. Male latency (ML = horizontal lines bars), female receptivity (FR = grey bars), and overall mating speed (OM = black bars) in four populations of *Drosophila mettleri*. Data in seconds. Results for intrapopulation and interpopulation combinations. Female strain given first

Table 1 Analysis of variance of cross effects in no-choice tests for four populations of *Drosophila mettleri*

Source	DF	Mean square	F ratio
(a) Male latency			
Model	15	28045.9	9.5020***
Female	3	3395.1	1.1503
Male	3	100038.2	33.8931***
Interaction (F × M)	9	20847.7	7.0632***
Error	688	2951.6	
(b) Female receptivity			
Model	15	7363.4	3.3211***
Female	3	11806.7	5.3252*
Male	3	12370.3	5.5795**
Interaction (F × M)	9	3170.2	0.1712
Error	688	2217.2	
(c) Overall mating speed			
Model	15	24217.3	5.4753***
Females	3	45718.6	10.3366*
Males	3	23841.8	5.3904**
Interaction (F × M)	9	20852.9	4.7147**
Error	688	4422.9	

Analyses between and within populations

*** $P < 0.0001$

** $P < 0.001$

* $P < 0.01$

could observe whether the first female courted was actually the female the male subsequently mated with. In no case was there a significant deviation from random courtship. Of those combinations that approached statistical significance with respect to first female courted (CAT males with SUP females, GYM males with CAT and with SUP females, and LO males with GYM females), the tendency was to court "strange" females first. With respect to actual copulation, on the other hand, three significant deviations from random mating were observed, as reflected in both Chi Square and isolation index values (Table 2). Males from CAT mated significantly more often with their own rather than with GYM females. The same was true of SUP males. Significant negative assortative mating, however, was seen between GYM males and SUP females. The deviations from random mating were not predicted on the basis of courtship for the tests using CAT males, but were consistent with the "first female courted" trends seen for SUP and GYM males. In the case of CAT males with GYM females, there was no initial bias toward courting their own females. In summary the outcome of the male choice tests indicate a lack of pre-courtship discrimination by males, but ultimately showed several significant departures from random mating. The departures are consistent with a role of female discrimination or preference in determining courtship outcomes.

Female-choice test

The degree to which female population contributed to these nonrandom mating patterns can be further examined by "female-choice" tests (Table 3). These observations, like those in the male choice tests, were broken down into which of the two males was the first to begin courting as well as type of male finally mating (Table 3). Four significant deviations in first male to court were observed. In three of the four, the first male to court was from the female's own population. These deviations are consistent with the slow courtship latencies of LO males when placed with females from other populations (Fig. 3). As predicted on the basis of courtship latencies, LO males rarely tended to be the first male to court, regardless of female type. There also was a perfect correspondence between the population of first male to court and the male to eventually mate. When females were from CAT, GYM, or the SUP mountains, the significant deviations from random mating were all positive, as were the isolation indices. When LO females displayed nonrandom mating, it was always negative assortative mating. Thus in these female choice tests, male propensity, rather than female choice, seemed to have the largest influence on deviations from random mating.

Multiple-choice tests

Results of multiple-choice tests are presented in Table 4. In only two cases were significant deviations from random mating observed, one involving the CAT × LO combination and the other in the CAT × SUP tests. Neither case was characterized by a significant isolation index.

Discussion

Drosophila mettleri from different geographic host populations exhibit significant differences in courtship behaviors such as male mating propensity as measured by courtship latency and overall mating speed. Furthermore, these differences in male behavior are influenced, to some degree, by genotypes of females presented to them. The differences in mating propensity revealed above enable us to make specific predictions about the outcomes of "choice" tests. If results of choice tests are a function of differences in general mating propensity rather than a true preference for, or discrimination against, potential mates from a given population, certain patterns should be observed in choice tests. For example, if mating propensity (male vigor or female receptivity) is the sole factor in determining any deviation from random mating among flies of two different populations, there should be a higher than expected number of matings between males of the

Table 2 Male choice test in four populations of *Drosophila mettleri*: first female-courted and female chosen

Male	Female			First female to be courted			Female mated			Isolation index
A	A	B	N	A (%)	B (%)	χ^2	A (%)	B (%)	χ^2	
CAT	CAT	SUP	41	36.6	63.4	3.0	51.2	48.3	0.0	0.02 ± 0.15
CAT	CAT	GYM	55	45.5	54.5	0.5	65.5	34.5	5.3*	0.31 ± 0.13*
CAT	CAT	LO	45	44.4	55.6	0.6	51.1	48.9	0.0	0.02 ± 0.15
SUP	SUP	CAT	41	43.9	56.1	0.6	46.3	53.7	0.2	−0.07 ± 0.16
SUP	SUP	GYM	42	61.9	38.1	2.4	66.7	33.3	4.7*	0.33 ± 0.15*
SUP	SUP	LO	43	55.8	44.2	0.6	53.5	46.5	0.2	0.07 ± 0.16
GYM	GYM	CAT	46	37.0	63.0	3.1	45.7	54.3	0.4	−0.9 ± 0.14
GYM	GYM	SUP	42	35.7	64.3	3.4	31.0	69.0	6.1*	−0.38 ± 0.14*
GYM	GYM	LO	52	42.3	57.7	1.2	42.3	57.3	1.2	−0.15 ± 0.14
LO	LO	CAT	43	55.8	44.2	0.6	39.5	60.5	1.9	0.21 ± 0.15
LO	LO	SUP	43	55.8	44.2	0.6	51.2	48.8	0.0	0.02 ± 0.15
LO	LO	GYM	40	37.5	62.5	2.5	37.5	62.5	2.5	−0.25 ± 0.15

Chi-square and isolation index are shown for each type of male combination

* $P < 0.05$

Table 3 Female choice test in four populations of *Drosophila mettleri*: first male to court and male accepted to mate

Female	Male			First male to court			Male accepted			Isolation index
A	A	B	N	A (%)	B (%)	χ^2	A (%)	B (%)	χ^2	
CAT	CAT	SUP	41	68.3	36.7	3.0	61.0	39.0	1.9	0.22 ± 0.15
CAT	CAT	GYM	42	52.4	47.6	0.1	47.6	52.4	0.1	−0.05 ± 0.15
CAT	CAT	LO	40	67.5	32.5	4.9*	67.5	32.5	4.9*	0.35 ± 0.15*
SUP	SUP	CAT	42	35.7	64.3	3.4	47.6	52.4	0.1	−0.05 ± 0.15
SUP	SUP	GYM	40	40.0	60.0	1.6	35.0	65.0	3.6*	−0.30 ± 0.15*
SUP	SUP	LO	40	77.5	22.5	12.1***	77.5	22.5	12.1***	0.55 ± 0.13*
GYM	GYM	CAT	42	54.8	45.2	0.4	54.8	45.2	0.4	0.10 ± 0.15
GYM	GYM	SUP	43	60.5	39.5	3.6	58.1	34.9	2.5	0.23 ± 0.15
GYM	GYM	LO	42	90.5	9.5	27.5***	90.5	9.5	27.6***	0.81 ± 0.09*
LO	LO	CAT	40	42.5	57.5	0.9	42.5	57.5	0.9	0.15 ± 0.16
LO	LO	SUP	45	26.7	73.3	9.8**	31.1	68.9	6.4*	−0.38 ± 0.14*
LO	LO	GYM	41	12.2	87.8	23.4***	12.2	87.8	23.4***	−0.25 ± 0.15

Chi-square and isolation index are shown for each type of male combination

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

population showing the shortest latencies to court and females of the population with the quickest receptivity. Males from populations showing slow courtship latencies and females from populations having low receptivity would not be mating as often as males that are quick to court and females that are quick to mate. The observation that LO and GYM males are slower than males of the other two populations to begin courting, predicts that they should be at a disadvantage in female choice tests, especially when females are from the somewhat more receptive CAT or LO populations (Fig. 3). In male choice tests, an excess of

matings is predicted to occur with the population of female that exhibits faster receptivity more frequently. While the differences found in propensity did, in fact, influence the outcome of various choice tests, the observed departures from random mating do not suggest strong or consistent sexual isolation among the populations.

Of the four populations used in the present study, flies from Santa Catalina Island exhibit the greatest genetic differentiation from others (Markow et al. 2001; Hurtado et al. 2004). It is the Loreto population, however, that exhibits the greatest difference from the others with respect

Table 4 Multiple choice tests between populations of *Drosophila mettleri*

Populations		Type (F × M)					χ^2	$♀\chi^2$	$♂\chi^2$	Isolation index
A	B	Matings	A × A	A × B	B × A	B × B	Df = 3	Df = 1	Df = 1	
SUP	LO	185	50	35	56	44	5.2	1.2	3.9*	0.02 ± 0.07
SUP	GYM	206	47	62	50	47	3.0	0.7	0.7	−0.09 ± 0.07
SUP	CAT	184	31	53	44	56	8.2*	1.4	6.3*	−0.05 ± 0.07
LO	CAT	203	31	64	40	68	19.3**	0.8	18.3**	−0.02 ± 0.07
CAT	GYM	195	58	39	49	49	5.7	0.0	1.9	0.05 ± 0.07
LO	GYM	190	46	43	46	55	1.7	0.8	0.2	0.06 ± 0.07

Each row represents data for two populations tested. Chi-square for all mating types, females, and males, and isolation index. $N = 240$ possible matings per combination

* $P < 0.05$

** $P < 0.001$

to sexual behavior. We found no evidence that the Santa Catalina Island population exhibits sexual isolation from the other populations.

The data suggest, however, that the outcome of inter-population combinations is not exclusively dependent upon male or female mating propensity. While it is often difficult to separate sexual vigor from discrimination and preference, the combination of tests used here is able to reveal evidence of mate selection. For example, Loreto males show significantly longer courtship latency when presented with females of populations other than their own. This was associated with longer times until mating as well. An obvious explanation could be the reduced ability of these females to stimulate courtship by Loreto males. If this is the case, it may represent some degree of incipient pre-mating or sexual isolation, but it clearly is not associated with greater genetic differentiation.

In an earlier study with *D. melanogaster,* Marin (1991) showed that mate choice results at the first 50% of pairs to mate differed significantly from the pattern for all matings. These results could be attributable to differential courtship vigor, or propensity. Following Marin (1991), we recalculated our Chi-Square tests to compare early (50%) versus all matings. For *D. mettleri,* one multiple choice test, between Santa Catalina Island and Guaymas, was the only combination that differed between early and overall mating. In this case, the significant result for early matings ($n = 120$; $\chi = 14.47$; $P < 0.01$) was caused by increased female receptivity. Santa Catalina Island females are essentially receptive to any type of male. On the other hand, Guaymas females are less receptive. When individuals from both populations were placed in a multiple-choice arena, Santa Catalina Island females thus were the first to copulate. By the end of the observation period, however, Guaymas females had mated and the overall Chi Square was not significant. The early-late mating difference is likely to assume a greater importance in species like

D. melanogaster in which copulation last approximately 20 min, taking most males out of circulation longer than in other species. In *D. mettleri,* for example, copulation last an average of three and a half minutes and most males are sufficiently vigorous to mate up to four times in the same period that one mating typically occurs for *D. melanogaster* (Castrezana, unpublished data).

Another Sonoran Desert Drosophilidae, *D. mojavensis,* also is associated with prickly pear cactus on Santa Catalina Island and exhibits significant genetic differentiation from mainland conspecifics (Ross and Markow 2006; Reed et al. 2007; Hocutt 2000) as seen for *D. mettleri* (Markow et al. 2002; Hurtado et al. 2004). Sexual isolation is observed between *D. mojavensis* from the Baja California peninsula and those from the Sonoran mainland, but not with flies from Santa Catalina (Markow 1991; Hocutt 2000). Taken together, the observations on *D. mettleri* and those for *D. mojavensis* suggest that at early stages of differentiation, no obvious relationship need exist between degree of genetic divergence and sexual isolation. These observations agree with studies of intraspecific other *Drosophila* species (Ehrman and Parsons 1980, 1981; Singh and Chatterjee 1985).

In the case of *D. mettleri,* it is clear that significant genetic differentiation does not create reproductive isolation in allopatric populations. Perhaps some ecological factors such as host shifts, resource distribution, or/and abundance of competitor species play an undetected role in the courtship behavior differences observed among *D. mettleri* populations. *Drosophila mettleri* has a closely related species, *D. eremophila* with which it is sympatric in the southern parts of its range in Baja California and Sonora, Mexico. Additional studies of sexual isolation in *D. mettleri,* from its closely related species, *D. eremophila,* similar to those of Anderson and Kim (2005), would be of interest in order to examine the influence of sympatry on sexual isolation between these two relatives.

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