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COURTSHIP BEHAVIOR AND CONTROL OF REPRODUCTIVE ISOLATION
BETWEEN *DROSOPHILA MOJAVENSIS* AND
DROSOPHILA ARIZONENSIS

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Wasserman and Koepfer (1977) reported a case of character displacement for reproductive isolation between *D. mojavensis* and its sibling species, *D. arizonensis*. *Drosophila mojavensis* is found in Baja California, the Mojave Desert of southern California, and the desert region of Sonora, Sinaloa and southern Arizona. *Drosophila arizonensis* is found in the Sonoran Desert of Arizona and Mexico. The two species are sympatric in northern Sinaloa, Sonora and in parts of southern Arizona. Low numbers of *D. arizonensis* have been collected from the tip of Baja California. It is among populations from the sympatric areas of western Sonora that Wasserman and Koepfer found the strongest reproductive isolation between the two species. While *D. arizonensis* and *D. mojavensis* utilize different rotting cacti species as primary substrates, they are occasionally found together on or reared from the same host plant (W. B. Heed, pers. comm.). In spite of this occasional niche overlap, hybrids in nature are apparently an uncommon occurrence, suggesting that behavioral isolating mechanisms are very successful even when ecological barriers break down.

Presumably *D. mojavensis* arrived in the area of sympatry after *D. arizonensis* (Wasserman and Koepfer, 1977; Zouros and D'Entremont, 1980) and the strong reproductive isolation between them is thought to have evolved as a result of selection acting on mating behavior of *D. mojavensis*. Since Wasserman and Koepfer obtained their findings by examining females for evidence of insemination, one cannot make inferences from their experiments about differences in the courtship behavior of the sympatric and allopatric *D. mojavensis*. The present study was undertaken to analyze the progress and structure of intra- and interspecific courtships in order to gain insight into particular behaviors that might be crucial to the observed isolation.

MATERIALS AND METHODS

This study employed strains of *D. mojavensis* and *D. arizonensis* derived from flies collected both from areas of sympatry and areas of allopatry and obtained from W. B. Heed at the University of Arizona. The allopatric strains (ALLO) of *D. mojavensis* came from collections at Las Flores (A606) and Laguna Chapala (A418), both from Baja California. Sympatric *D. mojavensis* strains (SYM) came from two Sonora localities, Altar Valley (A319) and Navajoa (A234.1). Sympatric *D. arizonensis* (SYM) also came from Altar (A650) and Navajoa (A657). Allopatric *D. arizonensis* (ALLO) were collected by the author near Tempe, Arizona (TM1). Since some of the strains had been in the laboratory a number of years, tests were conducted to see if any significant reproductive isolation existed between the two *mojavensis* allopatric strains, between the two *mojavensis* sympatric strains and between any of the *D. arizonensis* strains. All tests resulted in nonsignificant isolation indices.

All flies were reared in half-pint bottles containing standard cornmeal-molasses-agar medium with Tegosept. Virgin males and females were separated under CO_2 and stored separately in 8-dram vials, 10 flies/vial, until used in experiments at 10 days of age. Pairs of flies were aspirated into empty 8-dram vials, first the female then the male, and observed for one hour. The levels to which courtship progressed were scored. Courtship itself was scored when a male vi-

TABLE 1. *Inter- and intraspecific courtships of* D. mojavensis *and* D. arizonensis. *Pairs were scored for exhibiting male courtship, female receptivity, and eventual mating. The eight experimental and four control groups were compared by a Duncan multiple range test, $\alpha = 0.05$ and subset membership is designated by the vertical bars. The multiple range tests were performed independently for each of the three behavioral landmarks on arc-sin transformed values.*

	N	Courting		Receptive		Mating	
mojavensis females × *arizonensis* males							
ALLO × ALLO	108	83%		68%	b	50%	b
ALLO × SYM	187	82%	b	56%		35%	
SYM × ALLO	124	70%		33%	c	0	d
SYM × SYM	129	74%		36%		0	
arizonensis females × *mojavensis* males							
ALLO × ALLO	87	84%	b	16%	d	14%	c
SYM × ALLO	196	74%		20%		16%	
ALLO × SYM	135	54%	c	0	e	0	d
SYM × SYM	203	72%	b	2%		0	
Controls							
MO ALLO	70	98%		90%		90%	
MO SYM	113	96%	a	94%	a	93%	a
AZ ALLO	86	92%		86%		85%	
AZ SYM	83	90%		87%		84%	

a, b, c, d, e—Indicates membership in different subsets.

brated at a female or grasped or licked a female. Females of both species indicate their receptivity to a courting male by a characteristic spreading of the wings. Males respond to this gesture by an immediate copulation attempt. Rarely will a male attempt copulation in the absence of this female signal. Pseudocopulation can be distinguished from true copulation in that it only lasts a few seconds and true intromission is not achieved. Pseudocopulation is frequently observed in interspecific matings and therefore not all copulation attempts result in true copulation. Only true copulations were scored.

A series of pair combinations, based upon species and locality, was observed and courtship progress was recorded. *Drosophila mojavensis* females from allopatric and sympatric localities were placed with *D. arizonensis* sympatric and allopatric males. Likewise, sympatric and allopatric *D. arizonensis* females were paired with *D. mojavensis* males. Control groups were composed of observations on pairs within each species and locality. At least 3–4 replications of about 10 pairs each were observed for each type of combination.

RESULTS

The proportion of pairs showing male courtship, female receptivity, and copulation is shown in Table 1. Data from particular types of pairings were found to be homogeneous and therefore pooled. In all interspecific pairings the proportion of males performing any courtship is slightly lower than observed when males were with females of their own species (controls). The proportion of pairs having a receptive female and showing copulation showed statistical

groupings that always corresponded to whether or not the *D. mojavensis* member of the pair was ALLO or SYM. The receptivity of *D. mojavensis* females to *D. arizonensis* males is greatest among females from allopatric localities. While a number of these courtships resulted in matings, the majority of the copulations observed occurred only after multiple attempts and pseudocopulae by the males. *Drosophila mojavensis* females from sympatric localities were courted slightly less by *D. arizonensis* males than were allopatric females. An even smaller proportion of the sympatric *D. mojavensis* females indicated receptivity to these males. All copulation attempts resulted in only short pseudocopulae, giving zero values in the percent mating column.

Drosophila mojavensis males were seen to court *D. arizonensis* females, especially allopatric females, in slightly fewer numbers than seen in intraspecific control pairings. All of these courtships were extremely brief. *Drosophila mojavensis* males usually courted *D. arizonensis* females less than three seconds before they turned abruptly away and became immobile somewhere in the vial. A very low proportion of *D. arizonensis* females were receptive to allopatric *D. mojavensis* males and almost none were receptive to sympatric males. Interestingly, however, all observed matings between *D. arizonensis* females and *D. mojavensis* males occurred on the first attempt, with no pseudocopulae.

The large differences in receptivity and mating seen between allopatric and sympatric *D. mojavensis* suggested that a genetic analysis be conducted. Reciprocal hybridizations were carried out between *D. mojavensis* strains from Navajoa (SYM) and Laguna Chapala (ALLO) and between strains from

TABLE 2. *Courtship of hybrid* D. mojavensis *paired with* D. arizonensis. *Hybrid* D. mojavensis *came from crosses between flies of the following allopatric and symnpatric localities: Navajoa with Laguna chapala (SYM-ALLO¹) and Altar with Los Flores (SYM-ALLO²). Hybrid* D. mojavensis *were compared to parental* D. mojavensis *of the same sex by performing a Duncan multiple range test* ($\alpha = 0.05$) *on arc-sin transformed values.*

	N	Courting		Receptive		Mating	
F₁ *D.* mojavensis females × *D.* arizonensis males							
SYM-ALLO¹ × ALLO	82	88%		78%		51%	
SYM-ALLO¹ × SYM	65	96%	a	66%	b	42%	b
SYM-ALLO² × ALLO	50	86%		82%		49%	
SYM-ALLO² × SYM	62	74%		63%		41%	
D. arizonensis females × F₁*D.* mojavensis males							
SYM × SYM-ALLO¹	79	82%	a	0	c	0	c
SYM × SYM-ALLO²	66	80%		0		0	
ALLO × SYM-ALLO¹	91	88%		0		0	
ALLO × SYM-ALLO²	68	34%		3%		3%	

a—in same subset with both parental types.
b—in same subset with *mojavensis* ALLO females.
c—in same subset with *mojavensis* SYM males

Altar (SYM) and Las Flores (ALLO) and the F₁ females and males were paired with *D. arizonensis* of the opposite sex from Navajoa or Tempe (Table 2). Duncan's multiple range test was used to compare the behavior of hybrids to males or females from allopatric and sympatric localities. All hybrids and parental flies were in the same subset for percent initiating courtship. Hybrid females grouped with females from allopatric localities for receptivity and mating while hybrid males were like males from sympatric populations for these two behaviors.

In hybrids of a given sex the same parental pattern is seen for both receptivity and suggests that these two behaviors could be under the influence of a single gene or set of genes. Since F₁ females are most similar to females from allopatric strains, the courtship system of allopatric females may be due to a dominant gene or genes. On the other hand F₁ males behave more like sympatric males, suggesting that sympatric male behavior is dominant in these crosses. Either male and female courtship behaviors are under the control of separate genetic systems or they are controlled by the same gene or genes acting in a sex-influenced manner. In all interspecific pairings the percent of the males that courted was lower than observed for control pairs, implying some sort of precourtship isolation due to male disinterest. To investigate this possibility, single males from either species were placed with two females, one from each species and the type of female courted first was noted. From the results in Table 3, a significant degree of male precourtship discrimination is apparent. Furthermore, while *D. arizonensis* males from both sympatric and allopatric localities seem to exhibit similar levels of precourtship discrimination, only sympatric *D. mojavensis* males appear to correctly discriminate the species of the female prior to initiating courtship.

DISCUSSION

Quantitative aspects of courtship behavior in *D. mojavensis* and *D. arizonensis* are, superficially at least, indistinguishable by conventional laboratory methods (Markow and Hanson, 1981). Males court close behind females and their courtships consist of extended periods of licking and grasping the females' abdomen and occasional wing vibration (Spieth, 1952). The amount of time spent performing these behavior components and their sequential relationships are both effectively identical in these two species (Markow, unpubl.). Then how do the flies distinguish each other during courtship?

This question was approached in the above experiments by scoring the proportion of pairings reaching any of three different temporal landmarks: 1) proportion of pairs showing courtship, 2) proportion of pairs showing a receptive female and 3) pro-

TABLE 3. *First female courted.*

Male	Courting female of own species		χ^2 (1 1)
	N	%	
D. mojavensis (sympatric)	66/85	77.65%	25.984*
D. mojavensis (allopatric)	48/94	51.06%	0.004
D. arizonensis (sympatric)	53/81	65.43%	7.716*
D. arizonensis (allopatric)	57/79	72.15%	15.50*

* $P < .01$.
Females were marked by wing clipping, alternated between replications. Overall clipping $\chi^2 = 0.196$ (N S).

portion of pairs mating. The first parameter is scored on the basis of observed male behavior and the second is based on an observable female behavior. However, this does not mean that courtship only measures male behavior or that receptivity is only a measure of female behavior. Females differ in their ability to stimulate males to court them. Whether or not a courtship takes place depends upon the attributes of the females as well as upon the males' ability to perceive those attributes as being conspecific. The same kind of interaction exists for female receptivity. Receptivity is scored by watching for female wing spreading but it is dependent upon the male giving proper information. Within a population, male and female courtship behavior coevolve as a specific mate recognition system (Paterson, 1980; Templeton, 1980). Using their own data as well as data from Wasserman and Koepfer (1977), Zouros and d'Entremont (1980) hypothesized that sympatric *D. mojavensis* females have been under selection to accept males whose mating behaviors don't overlap with *D. arizonensis* behaviors. This consitutes, in effect, selection on the entire mate recognition system of both sexes of sympatric *D. mojavensis*.

Discrimination of males appears to be a function of both sexes and occurs at more than one point during the courtship process. Males seem to be somewhat particular about the females they court, and in *D. mojavensis,* the ability to discriminate a mate prior to courtship appears to show character displacement. It is commonly thought that since *Drosophila* males are promiscuous, they are not discriminating about the flies they court. Males of most *Drosophila* species, including *D. mojavensis* and *D. arizonensis,* court other males as well as females. But given a choice, the above data suggest a tendency to invest courtship time in an individual who is most likely to be a suitable mate. Other data from this laboratory support this idea. For example, *D. mojavensis* males have been found to initiate more courtships with virgin females than with recently inseminated females in a laboratory choice situation (Markow and Richmond, 1981). Since members of these two species are visually very similar and since wing vibration doesn't begin until after courtship is initiated, it is unlikely that visual or auditory cues are important in precourtship discrimination. On the other hand, chemical communication, mediated by tapping or by airborne molecules, appears to be the most likely means by which precourtship identification of conspecifics occurs.

Sympatric *D. mojavensis* females are much less receptive than allopatric females to courtship of *D. arizonensis* males, supporting the idea that selection on sympatric *D. mojavensis* has caused the range of acceptable male mating behaviors to move away from the range of *D. arizonensis* behaviors (Wasserman and Koepfer, 1980; Zouros and d'Entremont, 1980). *Drosophila arizonensis* males persist in courting sympatric *D. mojavensis* females even though they are unable to elicit an acceptance gesture. Interestingly, courtships of *D. arizonensis* females by *D. mojavensis* males are very short, terminating

when the male abruptly leaves the female, as if he had received an offensive rejection. Either sympatric *D. mojavensis* females are not actively rejecting *D. arizonensis* males or they are, and the males are not able to perceive the rejection signal.

The nature of the information that females of either species require to become receptive remains unclear. Visual cues are of minimal importance to the female since the male tends to remain behind her during courtship. Females may receive tactile stimulation through the continual licking and grasping of the abdomen performed by the males. At the same time, males may be obtaining information from the females. The importance of courtship song has been shown to be important in isolation between other *Drosophila* species (Ewing, 1969; Von Schilcher and Manning, 1975) and is currently under investigation in *D. mojavensis* and *D. arizonensis*.

Even though a female may indicate acceptance of a courting male, copulation still may not result. Such is the case between sympatric *D. mojavensis* females and *D. arizonensis* males. It would be interesting to know if this is behavioral, i.e., if some last minute event doesn't "feel right" to the female so she closes her genital plates, or if, instead, some mechanical impediment to copulation exists.

There are a number of examples in *D. melanogaster* of single mutant genes (yellow, scabrous, white) in which there is a tendency toward asymmetrical isolation with wild type (Sturtevant, 1915; Bastock, 1956; McKenzie and Parsons, 1971). In each case, the same gene which causes reduced mating success in males causes an increased receptivity in females. It is unlikely that the isolation seen between *D. mojavensis* and *D. arizonensis,* and the character displacement of that isolation, has such a simple genetic basis. The fact that in *D. mojavensis,* sympatric male behavior is dominant and female behavior is recessive argues against the action of a single locus. Recent findings by Zouros (1980) that genes controlling isolation between male and female *D. mojavensis* and *D. arizonensis* are on different chromosomes support the existence of a more complex genetic situation.

It is highly likely that those courtship behaviors which are showing character displacement are precisely those behaviors which are most important for intraspecific mate recognition. Evidence suggests that these behaviors come into play before courtship begins as well as at several critical points afterwards. If the nature of these behavioral interactions can be elucidated in some quantitative way, the question of the loss or addition of courtship elements during speciation (Watanabe and Kawanishi, 1979; Kaneshiro, 1980) can be meaningfully addressed.

SUMMARY

Behavioral control of interspecific courtships between *D. mojavensis* and its sibling species *D. arizonensis* was analyzed in the laboratory using flies from regions where the two species occur allopatrically and sympatrically. The asymmetry seen for sex-

ual isolation between *D. arizonensis* and *D. moja-vensis* can be traced to behavioral interactions at several stages of courtship. Evidence is presented suggesting that males exercise precourtship discrimination under laboratory conditions. The existence of factors influencing male courtship "interest" and female receptivity is also implicated. Character displacement appears to be occurring for behaviors operating before as well as during courtship when flies are from sympatric populations.

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