# CHARACTER DISPLACEMENT FOR SEXUAL ISOLATION BETWEEN
## *DROSOPHILA MOJAVENSIS* AND *DROSOPHILA ARIZONENSIS*

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According to Brown and Wilson (1956) character displacement exists between two closely related species when their allopatric populations are very similar and their sympatric populations are quite distinct in one or more characters. The disparate characters could be either morphological, physiological, ecological, or behavioral. They viewed the phenomenon as a result of species interaction in sympatry, where divergence would decrease competition or reduce hybridization, and thus be at a selective advantage. They gave several instances which have since been widely cited as classical, textbook examples. More recently, Grant (1972, 1975) reexamined the phenomenon of interspecific interaction, including convergent character displacement as well as the divergent character displacement described by Brown and Wilson. Restricting himself to morphological attributes, Grant reviewed the literature and concluded that there really was no good example of character displacement. Where divergent character displacement had been proposed to exist, Grant argued, the data were incomplete or else the reason for the divergence was not a result of interspecific competition but rather other ecological factors. We feel that many of these problems could be avoided if one could demonstrate character displacement for sexual isolation. Although ethological barriers to interspecific mating are expected to be developed in allopatric populations (Muller, 1939, 1942), their reinforcement in sympatric populations can only be attributed to interspecific interaction and not to other correlated factors. Dobzhansky (1940) proposed on theoretical grounds

that sexual isolation between closely related species should be greater in sympatric than in allopatric populations. His arguments essentially are that if there is selection against the hybrids, those individuals which are involved in interspecific crosses would be wasting their gametes. Any gene which improved the ability of the species to discriminate would be advantageous and would increase in frequency, or be fixed, in the region of sympatry. Reinforcement of sexual isolation by means of artificial selection against the hybrids can sometimes be accomplished in the laboratory (Koopman, 1950; Thoday and Gibson, 1962; Kessler, 1966; Ehrman, 1971, 1973; Soans et al., 1974). However, despite its theoretical importance and despite the moderate degree of success obtained in the laboratory, evidence for reproductive character displacement in natural populations is poor and controversial (see e.g., Loftus-Hills, 1975 and Jones, 1975). It has only been shown to exist in some anurans (Fouquette, Jr., 1975; Littlejohn and Loftus-Hills, 1968), some grasshoppers (Cohn and Cantrall, 1974), possibly in two species of damselfly (Waage, 1975), and between races of *Drosophila paulistorum* (Ehrman, 1965).

In 1973 we undertook an investigation on the sexual isolation between natural populations of *Drosophila mojavensis* and *Drosophila arizonensis* species. The advantages of using *Drosophila* for this purpose are manifest in the availability of techniques which would not only enable us to detect reinforcement if it exists, but also to measure its strength and perhaps determine its genetic basis. *D. mojavensis*

and *D. arizonensis*, moreover, are ideal for such a study.

*D. arizonensis* is known from the Cape region of Baja California, and from southern Arizona and the southwestern tip of New Mexico south into southern Sonora. We have examined strains of *arizonensis* from the state of Hidalgo in eastern Mexico and from Guatemala, kindly sent to us by Drs. R. Richardson, University of Texas, and W. B. Heed, University of Arizona. There are also some early unconfirmed reports of *arizonensis* in Tamaulipas and southern Mexico. No chromosomal variability has ever been detected in *arizonensis*. *D. mojavensis* occurs in the Mojave and Sonoran Deserts of southern California (U.S.), in Baja California (Mex.) and along the west coast of Sonora and Sinoloa where it is sympatric with *arizonensis*. Extensive collections in the sympatric region by the University of Arizona group has shown that hybridization in nature is either non-existent or extremely rare. Dr. Heed (pers. comm.) reared individuals of both species from saguaro fruit collected in the sympatric region. One of these specimens, he believes, might have been a hybrid male. However an intensive cytological investigation of *mojavensis* by Mr. W. Johnson (1977) failed to produce any evidence for hybridization. The two species differ from each other by inversions in three of the six chromosomes. Hybrids, or their offspring, carrying chromosomes from both species, would be easy to recognize cytologically.

Unlike *arizonensis*, *mojavensis* shows some morphological and genetic geographical variability. Mettler (1961) divided the species into two subspecies, a light colored *D. mojavensis mojavensis* and *D. mojavensis baja*, a darker form which he said could only be distinguished from *arizonensis* with difficulty. The former had been known only from a single strain collected in the U.S. Californian Chocolate Mountains in 1941 or 1942, and from some earlier collection records from the general area. We have recently (summer of 1975) collected new strains from the Sonoran deserts of the U.S. All of the strains of *D. m. mojavensis* are cytologically monomorphic and differ from *D. arizonensis* by seven inversions: *Xe; 2i,q,r,s; 3a,d*. Although these species show such extensive cytological divergence, *D. m. mojavensis* and *arizonensis* hybridize easily in the lab and produce fertile offspring. Hybrid swarms have in fact been produced in laboratory cage experiments and chromosome replacement, heterosis and genetic loads have been investigated (Mettler, 1957, 1962; Nagle and Mettler, 1969; Nagle, 1969). *D. m. baja*, on the other hand, is polymorphic for several newer inversions in chromosome 2 and one new one in chromosome 3 (Mettler, 1961; William Johnson, 1977). When placed in population cages with *arizonensis*, very little hybridization takes place and *baja* tends to replace the *arizonensis* (Nagle, 1965; Mettler and Nagle, 1966). Since the distribution of *baja* overlaps that of *arizonensis* Mettler and Nagle (1966) stated, and cage data strongly suggested, that reproductive isolation is stronger between the two species where they are sympatric. They were never able to test this critically since the strains they used actually came from populations of *baja* and *arizonensis* which are allopatric. However the two species clearly offered a likely system for character displacement of sexual isolation.

## MATERIALS AND METHODS

Four populations for each species were tested. (a) *D. arizonensis* from two localities allopatric to *mojavensis*: ALLO strain 1, Venados, Hidalgo from eastern Mexico; and ALLO strain 2, Tucson, Arizona; (b) *D. arizonensis* from two localities sympatric with *mojavensis*: SYM strain 3, Navojoa, Sonora; and SYM strain 4, Caborca, Sonora; (c) *D. mojavensis baja* from two localities allopatric to *arizonensis*: ALLO strain 5, San Ignacio, Baja California; and ALLO strain 6, La Presa, Baja California; and (d) *D. mojavensis baja* from the same two localities as the

SYM *arizonensis* strains: SYM strain 7, Navojoa, Sonora; and SYM strain 8, Caborca, Sonora.

The two allopatric strains of *mojavensis* (ALLO strains 5 and 6) were collected by Mr. William Johnson, University of Arizona, in March 1972. Dr. W. B. Heed sent us strains 2, 4 and 8, which he had collected himself. Strain 2 was collected in April 1974; strains 4 and 8 were collected in August 1965 and originated from approximately 40 *arizonensis* wild flies and approximately 30 *mojavensis* wild flies, respectively. Strains 1, 3 and 7 were collected and sent to us by Dr. R. Richardson; the sympatric strains from Navojoa, strains 3 and 7, were "derived from flies which emerged from separate rots of *Rathbunia* collected within a few hundred meters of each other."

The mating behavior of the flies is influenced by a number of factors which may interact with each other (Spiess, 1970). In our experimental design reasonable care was taken to avoid, as much as possible, the effect of environmental and genetic factors which are not involved in the sexual isolation, *per se*, but which might influence mating ability and thereby confound the results obtained from the experiments. The experiments were begun in October, 1974, and completed in May, 1976. Five independent experiments were run (Exp. A,B,C,D and E). In each experiment a different single isofemale strain was established from each of the four localities for each of the two species. All tests were run between strains within an experiment, e.g., A with A, or B with B, but never A with B. The "male choice" method was used to determine the amount of sexual isolation present. Males from each of the eight locality strains were given a "choice" between their own females and an equal number of females from each of the seven other species locality-strains, there being a total of fifty-six combinations. Male and female virgins were collected, aged five to seven days, reexamined immediately before testing to assure that

they were in good physical condition, and then tested: two males from a single locality-strain were confined in a shell vial (95 mm × 25 mm) with three females from their own strain (homogamic) and three females from a single foreign strain (heterogamic). After approximately 40 h under constant light and at approximately 23 C, the flies were separated and the females examined for the presence of sperm in their ventral receptacle. Four replicas were run. In two of the replicas, for identification purposes, the left wings of the homogamic females were notched, while in the other two, the left wings of the heterogamic females were notched. Since there were three homogamic and three heterogamic females in each of the four replicas, a total of twelve homogamic and twelve heterogamic females were tested for each of the fifty-six combinations, yielding a total of 1,344 females dissected per experiment and a total of 6,720 females in the five experiments.

## RESULTS

A summary of the male choice tests is given in Table 1. The left value of the number pairs is the number of inseminated homogamic females, while the right is the number of inseminated heterogamic females. These values are the totals of the five experiments, each consisting of four replicas. Each of the values, therefore, represents the results of the exposure of 40 males to 60 homogamic and 60 heterogamic females.

Several different indices have been proposed for the measurement of sexual isolation in male choice tests. The isolation index of Stalker (1942) is equal to (the proportion of homogamic matings − the proportion of heterogamic matings)/(the proportion of total matings). Levene (Malogolowkin-Cohen, Simmons and Levene, 1965) developed an index, $I$, which under our experimental conditions turns out to be numerically identical to Stalker's index but has the added property of a standard error. In our experiments, if $Hm$

TABLE 1. *Results of "male choice" tests. The left value of the number pair is the number of homogamic females inseminated. The right value is the number of heterogamic females inseminated. There was a total of sixty females of each kind of females exposed to the forty males for each number pair. For allopatric and sympatric derivation see Materials and Methods.*

| Strain of males and homogamic females | D. arizonensis | | | | D. mojavensis | | | |
|---|---|---|---|---|---|---|---|---|
| | Allopatric | | Sympatric | | Allopatric | | Sympatric | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Allopatric 1 | – | 47–41 | 47–44 | 51–48 | 40–14 | 51–15 | 59–3 | 41–1 |
| Allopatric 2 | 43–32 | – | 40–52 | 28–28 | 41–30 | 45–45 | 44–4 | 52–0 |
| Sympatric 3 | 51–45 | 50–32 | – | 46–40 | 43–38 | 51–24 | 44–1 | 49–2 |
| Sympatric 4 | 56–38 | 36–34 | 50–48 | – | 52–22 | 53–11 | 52–0 | 49–1 |
| Allopatric 5 | 50–8 | 39–5 | 49–14 | 50–6 | – | 47–48 | 50–14 | 46–37 |
| Allopatric 6 | 33–1 | 55–1 | 43–8 | 44–2 | 29–33 | – | 41–24 | 41–25 |
| Sympatric 7 | 54–1 | 56–1 | 44–4 | 50–0 | 44–41 | 44–41 | – | 43–42 |
| Sympatric 8 | 42–3 | 47–0 | 41–6 | 34–0 | 55–45 | 35–32 | 39–21 | – |

= number of homogamic matings; $Ht$ = number of heterogamic matings; $N$ = total number of matings; and SE = the Standard Error of $I$,

$$I = \frac{Hm - Ht}{N}, \text{ and}$$

$$SE = [(1 - I^2)/N]^{1/2}$$

$I$ varies from 1.0 (no heterogamic matings due to complete isolation) through 0.0 (random matings) to –1.0 (only heterogamic matings).

Table 2 gives a summary of the isolation indices obtained in our experiments. There are three broad categories: the *arizonensis* intraspecific tests, the *mojavensis* intraspecific tests, and the interspecific tests. Each is organized so as to show the overall isolation index ($I$) from tests between strains which are from allopatric populations (ALLO × ALLO), tests from strains which are from sympatric populations (SYM × SYM), and tests involving an allopatric strain and a sympatric strain (ALLO × SYM). Our summary data show virtually no isolation between localities within the species *arizonensis*: approximately 46.9% of all matings involved interstrain heterogamic crosses. None of the isolation indices, $I$, is significantly greater than 0.0. The frequency of the heterogamic matings in the *mojavensis* intraspecific tests is somewhat lower, 44.0%.

A small, but significant level of isolation is found between the allopatric populations from Baja California and the sympatric populations from Sonora ($I = .158 \pm .040$). Zouros and d'Entremont (1974) have also found a low level of isolation in *mojavensis* between strains from Baja California and from Sonora. They have suggested that the two populations be considered separate subraces. However, in order to gauge the importance of these levels of isolation it is necessary to compare these values with values obtained by others. The $I$ index has been used by Malogolowkin-Cohen et al. (1965) and Dobzhansky et al. (1969) for strains within semi-species of *D. paulistorum* ($I$ varied from 0.0 to 0.4); by Wasserman et al. (1971) for male discrimination tests between geographical populations in *D. pegasa* ($I$ varied from .33 to .43); and by Wasserman and Koepfer (unpubl.) for discrimination tests between strains from different cytological races in *D. nigricruria* ($I$ varied from .2 to .4). Thus, the level of isolation found in our tests between the Baja California *mojavensis* and the Sonoran *mojavensis* is on the same order of magnitude as those often found between populations in other species.

The critical information, for our purposes, is to be found in the interspecific tests. When both strains come from localities which are allopatric with the other

TABLE 2. *Summary of "male choice" data: For ALLO and SYM Derivations see Materials and Methods.*

| Type of cross | | Available females of each type | Inseminated females | | % of (Heterogamic matings) (Total matings) | $I \pm SE$ |
| Species | Region | | Homogamic | Heterogamic | | |
|---|---|---|---|---|---|---|
| *ARIZONENSIS* × *ARIZONENSIS* | ALLO × ALLO | 120 | 90 | 73 | 44.78 | .104 ± .078 |
| | ALLO × SYM | 480 | 359 | 321 | 47.20 | .056 ± .038 |
| | SYM × SYM | 120 | 96 | 88 | 47.83 | .043 ± .074 |
| | TOTAL | 720 | 545 | 482 | 46.93 | .061 ± .031 |
| *MOJAVENSIS* × *MOJAVENSIS* | ALLO × ALLO | 120 | 76 | 81 | 51.59 | −.032 ± .080 |
| | ALLO × SYM | 480 | 356 | 259 | 42.11 | .158 ± .040 |
| | SYM × SYM | 120 | 82 | 63 | 43.45 | .131 ± .082 |
| | TOTAL | 720 | 514 | 403 | 43.95 | .121 ± .033 |
| INTERSPECIFIC | ALLO × ALLO | 480 | 354 | 119 | 25.16 | .497 ± .040 |
| | ALLO × SYM | 960 | 780 | 138 | 15.03 | .699 ± .024 |
| | SYM × SYM | 480 | 363 | 14 | 3.71 | .926 ± .019 |
| | TOTAL | 1920 | 1497 | 271 | 15.33 | .693 ± .017 |

species (ALLO × ALLO), the percentage of heterogamic matings is 25.2 and the two species show an isolation index, $I$, of .497 ± .040. If one of the two strains comes from a locality sympatric with the other species (ALLO × SYM), the frequency of heterogamic matings drops to 15.0% and $I$ rises to .699 ± .024. Tests involving both strains from the sympatric region (SYM × SYM) yield few heterogamic matings, 3.7%, and a high isolation index, $I = .926 ± .019$. The degree of isolation between the two species is greatly influenced by the geographical origin of the strains. Those populations which are sympatric with the other species show a higher degree of isolation than do those which are allopatric. This is clearly an example of character displacement for sexual isolation.

It is possible to analyze further the data to determine the contribution of each individual locality to the degree of isolation present between localities and between regions both within and between species. This can be done for males and females separately. The "male choice test" calls for males of type A to be present with females of type A (homogamic) and females of type B (heterogamic). The isolation index is a function of the frequencies of the homogamic and heterogamic matings. The index may be thought of as indicating the choice the males are making between the two types of females, and therefore, to be due exclusively to male discrimination. On the other hand the index may reflect the relative reluctance of the heterogamic female (B) to mate with the foreign male (A) as compared to the willingness of the homogamic female (A). The isolation indices obtained in the "male choice tests" may, therefore, actually be due to the behavior of the females rather than that of the males. Indeed there is a great deal of evidence showing that females can discriminate among males of different species, different localities and different genotypes (Spieth, 1968; Ehrman, 1969; Spiess, 1970). Unequivocal evidence for male discrimination is rarer (Wasserman et al., 1971). It is most likely that the reality is somewhere between these two extremes, and that both male and female behavior are contributing to the indices obtained. However by analyzing the data separately for the males and the females we hope to find consistencies which will clarify the results. These analyses are given in Tables 3–5.

Let us consider the intraspecific crosses first. We wish to ascertain the relative degree of isolation occurring between two localities within a region (Intraregion Tests) and between regions (Interregion Tests). The results of both the *arizonensis* and the *mojavensis* crosses are given in Table 3. The data for the males and females are treated separately. For example, strain 1 (Venados) *arizonensis* fe-

males from the Allopatric Region have a mean isolation index ($I$) of .126 ± .072 when exposed to Interregion males (strains 3 + 4 from the Sympatric Region); whereas their mean index ($I$) is .147 ± .114 when exposed to males from the other allopatric locality (strain 2). The mean isolation index ($I$) for strain 1 females when exposed to all foreign conspecific males is .132 ± .061; while the mean $I$ for the allopatric region (strains 1 and 2) is .121 ± .044. Although most of the indices listed in the table are positive, they are all low. Asterisks denote those few indices which are greater than twice their standard error. In the *arizonensis* tests one finds few significant isolation indices and no real differences between the behavior of individuals with flies from the intraregional locality and those from the interregional localities.

The intraspecific *mojavensis* tests (Table 3) show a greater number of significant, but still low, isolation indices. We had previously seen, (Table 2) that a small, but significant, isolation exists between the sympatric and allopatric populations of *mojavensis*. In Table 3, we observe that interregional significant values are due to the behavior of the sympatric females and the allopatric males.

We can summarize the results of the intraspecific tests by stating that the level of isolation between strains within both *arizonensis* and *mojavensis* is low and comparable to that found between strains of other species. There is, however, a tendency for the sympatric *mojavensis* females to be isolated from the allopatric *mojavensis* males.

The interspecific isolation indices resulting from the crosses involving *mojavensis* females and *arizonensis* males are given in Table 4. The data are organized to show the mean isolation index and its standard error of each locality when tested against the allopatric populations of the sister species (column 4), the sympatric populations of the sister species (column 5), and all sister species populations (column

TABLE 3. *Mean isolation indices ± standard errors (I ± SE) for intraspecific tests.*

| Species | Sex | Region | Locality | Interregion $I \pm SE$ for locality | Intraregion $I \pm SE$ for locality | Total $I \pm SE$ for locality | Total $I \pm SE$ for region |
|---|---|---|---|---|---|---|---|
| *arizonensis* | Females | Allopatric | 1 | .126 ± .072 | .147 ± .114 | * .132 ± .061 | * .121 ± .044 |
| | | | 2 | .132 ± .080 | .068 ± .106 | .108 ± .064 | |
| | | Sympatric | 3 | −.049 ± .074 | .020 ± .101 | −.025 ± .060 | .004 ± .044 |
| | | | 4 | .019 ± .080 | .070 ± .108 | .037 ± .064 | |
| | Males | Allopatric | 1 | .032 ± .073 | .068 ± .106 | .043 ± .060 | .022 ± .045 |
| | | | 2 | −.081 ± .082 | .147 ± .114 | −.004 ± .067 | |
| | | Sympatric | 3 | .135 ± .074 | .070 ± .108 | .114 ± .061 | * .099 ± .043 |
| | | | 4 | .122 ± .078 | .020 ± .101 | .084 ± .062 | |
| *mojavensis* | Females | Allopatric | 5 | .070 ± .073 | −.065 ± .127 | .036 ± .064 | .028 ± .045 |
| | | | 6 | .039 ± .081 | −.011 ± .103 | .020 ± .064 | |
| | | Sympatric | 7 | * .411 ± .080 | * .300 ± .123 | * .376 ± .067 | * .229 ± .047 |
| | | | 8 | * .168 ± .081 | .012 ± .108 | .111 ± .065 | |
| | Males | Allopatric | 5 | * .306 ± .079 | −.011 ± .103 | * .182 ± .063 | * .168 ± .047 |
| | | | 6 | * .252 ± .085 | −.065 ± .127 | * .150 ± .071 | |
| | | Sympatric | 7 | .035 ± .077 | .012 ± .108 | .027 ± .063 | .079 ± .045 |
| | | | 8 | .078 ± .077 | * .300 ± .123 | .137 ± .066 | |

\* = Significant Isolation

6). Column 7 gives the mean isolation index for all the interspecific tests involving both localities from the allopatric or sympatric regions. The Mann-Whitney *U*-test and Wilcoxon two-sample tests were run on the isolation indices (Sokal and Rohlf, 1969). These nonparametric methods are useful because our experimental design lends itself to comparisons within pairs (see Appendix for details). With the *mojavensis* female data, no significant differences were observed between allopatric and sympatric tests within a locality (comparisons made between columns 4 and 5), or between localities within a region (comparisons made between localities 5 and 6, and between localities 7 and 8). However, there is a marked difference between the regions: the sympatric *mo-javensis* females are much more isolated from *arizonensis* males than are the allopatric females ($I = .940 \pm .017$; $I = .308 \pm .040$; $P < .001$). The male *arizonensis* data complement and confirm the female *mojavensis* analysis. First, and most important, each strain of *arizonensis* males, regardless of its geographical origin is more isolated from the sympatric *mojavensis* females than from the allopatric females (comparisons made between columns 4 and 5; $P < .01$ for strain 1, $P < .001$ for strains 2, 3 and 4). There are differences between *arizonensis* strains within a region in their behavior with allopatric *mojavensis* females (compare strain 1 with 2 and strain 3 with 4 in column 4; $P < .01$); however in their overall behavior (column 6) there are no significant dif-

TABLE 4. *Mean isolation indices ± standard errors (I ± SE) for interspecific tests*: D. mojavensis *females with* D. arizonensis *males*.

| Species | Region | Locality | Allopatric I ± SE for locality | Sympatric I ± SE for locality | Total I ± SE for locality | Total I ± SE for region |
|---|---|---|---|---|---|---|
| mojavensis females | Allopatric | 5 | .296 ± .085 | .226 ± .078 | .257 ± .058 | .308 ± .040 |
| | | 6 | .231 ± .078 | .496 ± .074 | .356 ± .054 | |
| | Sympatric | 7 | .873 ± .047 | .979 ± .021 | .923 ± .027 | .940 ± .017 |
| | | 8 | .979 ± .021 | .941 ± .034 | .959 ± .020 | |
| arizonensis males | Allopatric | 1 | .517 ± .078 **→ .923 ± .038 | | .705 ± .047 | .538 ± .038 |
| | | 2 | .068 ± .079 ***→ .920 ± .039 | | .395 ± .057 | |
| | Sympatric | 3 | .205 ± .078 ***→ .938 ± .036 | | .484 ± .055 | .598 ± .036 |
| | | 4 | .522 ± .073 ***→ .980 ± .020 | | .717 ± .045 | |

——** → P < .01
——*** → P < .001

ferences, nor are allopatric males, as a whole, different from the sympatric males (column 7).

The results of the reciprocal crosses are given in Table 5. Only three comparisons yielded significant differences. Two are relatively unimportant: strain 4 of *arizonensis* females is more isolated from *mojavensis* males than is strain 3 (column 5; P < .01); and strain 6 of *mojavensis* males is more isolated from *arizonensis* females than is strain 5 (column 5; P < .05). However character displacement is found in the *mojavensis* males: the sympatric males are more isolated from all *arizonensis* females than are the allopatric males (P < .01; column 7).

The data were further analyzed to de-

TABLE 5 *Mean isolation indices ± standard errors (I ± SE) for interspecific tests* D arizonensis *females with* D. mojavensis *males*.

| Species | Region | Locality | Allopatric I ± SE for locality | Sympatric I ± SE for locality | Total I ± SE for locality | Total I ± SE for region |
|---|---|---|---|---|---|---|
| arizonensis females | Allopatric | 1 | .804 ± .062 | .920 ± .039 | .865 ± .036 | .899 ± .022 |
| | | 2 | .880 ± .047 | .981 ± .019 | .931 ± .025 | |
| | Sympatric | 3 | .614 ± .074 | .789 ± .063 | .694 ± .050 | .797 ± .030 |
| | | 4 | .843 ± .053 | 1.000 ± .000 | .914 ± .030 | |
| mojavensis males | Allopatric | 5 | .745 ± .066 | .664 ± .069 | .701 ± .048 | .779 ± .031 |
| | | 6 | .956 ± .031 | .794 ± .062 | .872 ± .036 | |
| | Sympatric | 7 | .964 ± .025 | .918 ± .040 | .943 ± .023 | .922 ± .020 |
| | | 8 | .935 ± .037 | .852 ± .058 | .896 ± .034 | |

——* → P < 05
——** → P < 01

termine whether there was any interregion interaction, e.g., perhaps sympatric *mojavensis* males are more isolated from sympatric *arizonensis* females than they are from allopatric *arizonensis* females. No evidence for such interactions was found. We can summarize the results of the interspecific tests and thereby explain the basis of the character displacement. Sympatric *mojavensis* shows a high level of isolation from all *arizonensis* regardless of the origin of the *arizonensis*. Allopatric *mojavensis* shows a relatively low level of isolation from all *arizonensis* localities. The corollary is, of course, true: the behavior of *arizonensis* individuals is independent of their site of origin and dependent upon the site of origin of the *mojavensis* individuals.

## DISCUSSION

Despite a certain level of heterogeneity in the behavior of flies from different localities, the overall picture is clear. Character displacement for sexual isolation exists between *D. mojavensis* and *D. arizonensis*. The reduction in the frequency of interspecific mating when sympatric strains are used can be ascribed primarily to the behavior of *mojavensis*. Sympatric *mojavensis* females are much more isolated from all *arizonensis* males ($I = .940 \pm .017$) than are the allopatric *mojavensis* females ($I = .308 \pm .040$), $P < .001$. Similarly sympatric *mojavensis* males are more isolated from all *arizonensis* females ($I = .922 \pm 0.20$) than are the allopatric *mojavensis* males ($I = .779 \pm .031$), $P < .01$. There are no significant differences between the sympatric ($I = .598 \pm .036$) and the allopatric ($I = .538 \pm .038$) *arizonensis* males, nor are the sympatric *arizonensis* females ($I = .797 \pm .030$) significantly different from the allopatric ($I = .899 \pm .022$) *arizonensis* females. The results shown in Table 2 become understandable. Interspecific ALLO × ALLO crosses show a relatively low level of isolation ($I = .497 \pm .040$). Interspecific SYM × SYM show a high level of isolation ($I = .926 \pm .019$) because every cross involves a SYM strain of *mojavensis*. Interspecific ALLO × SYM show an intermediate level of isolation ($I = .699 \pm .024$) because half of these tests utilize the SYM *mojavensis* while the other half have the ALLO *mojavensis*.

*D. mojavensis* and *D. arizonensis* are cytologically, morphologically and genetically more similar to each other than either is to any other known species. Their cytological evolution as seen in the changes that have occurred in the salivary gland chromosomes show that these two daughter species evolved from contiguous populations which were cytologically differentiated (Wasserman, 1960, 1962). W. Johnson (1977), who made an exhaustive study of the chromosomal polymorphism of *mojavensis*, stated that the cytology supported Baja California as the site of origin of *mojavensis* and the mainland, more specifically, Sonora, as the site of origin of *arizonensis*. He proposed that the Gulf of California was the main barrier that allowed the two populations to differentiate cytologically and ecologically. There is a minimum of seven inversional differences between the two species. Heed et al. (1968) and Fellows and Heed (1972) have shown that although the two species share certain larval habitats, they are essentially ecologically distinct. Despite these differences, sexual isolation between the allopatric *mojavensis* and *arizonensis* is not well developed. We feel that a subsequent invasion of Sonora by *mojavensis* from Baja California led to a renewed contact between the two forms and the reinforcement of sexual isolation in the area of sympatry. It is not known what roles, if any, the prior cytological and ecological differentiation played in allowing for the enhancement of the sexual isolation. The character displacement for sexual isolation in the area of sympatry was accomplished by a change in only one of the two species. Perhaps being the smaller, migrant, population isolated from

its parental populations, the Sonoran *mojavensis* was able to change more quickly than *arizonensis*, the species with the much larger distribution extending well beyond the area of sympatry. Changes in the behavior of the single species, *mojavensis*, in the area of sympatry were enough to accomplish the reproductive isolation between the two forms and complete the speciation event.

An alternative, but we think much less likely, possibility is that the speciation event had taken place at an early date and that the two species, behaviorally and ecologically isolated, were coexisting in Sonora. Only the species *mojavensis* was then able to invade and colonize Baja California and southern U.S. California, where it lost its ability to discriminate against *arizonensis*.

## SUMMARY

1. Character displacement for reproductive isolation between *Drosophila mojavensis* and *Drosophila arizonensis* has been demonstrated to exist in their region of sympatry in Sonora.

2. This increased level of sexual isolation is due to the behavior of the sympatric *mojavensis* flies, which are more isolated from all strains of *arizonensis* than are the allopatric *mojavensis*. No differences were found in the behavior of *arizonensis* from the different regions.

3. Intraspecific tests showed that the level of isolation between strains and regions within both *arizonensis* and *mojavensis* is low and comparable to that found between strains of other species.

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## APPENDIX

The general method used for the Wilcoxon two-sample test is given in Sokal and Rohlf (1969, p. 391–395). Each of the eight strains was crossed to the seven other strains, giving a total of 56 tests. There were five replicas per test, thus 280 values of $I$ were calculated. The 280 values were ranked from the lowest to the highest, ties being given average ranks. A $U$ value was calculated for those specific values needed in the particular comparison to be made. An example follows. In the cross between *D. mojavensis* females and *D. arizonensis* males (Table 4), a comparison was made between the $I$ values of *arizonensis* males from Venados (strain 1) when tested with the allopatric *mojavensis* females (strains 5 and 6), mean $I = .517$ (Table 4, column 4) as compared to the $I$ values of the same males when tested with the sympatric *mojavensis* females (strains 7 and 8), mean $I = .923$ (Table 4, column 5). Each of these mean $I$s consists of ten $I$ values, 0.11, 0.28, 0.4, 0.4, 0.56, 0.57, 0.6, 0.78, 0.85, and 1.0 for the allopatric tests; and 0.71, 0.71, 0.84, 1.0, 1.0, 1.0, 1.0, 1.0, 1.0 and 1.0 for the sympatric tests. These ranked 83, 118.5, 135, 135, 150, 154, 158.5, 180, 196 and 239 for the allopatric tests, and 171, 171, 196, 239, 239, 239, 239, 239, 239 and 239 for the sympatric tests. A $U$ value of 89 was calculated using the method described by Sokal and Rohlf. Their Table CC indicates that

a $U$ of 89 has a $.001 < P < .01$. The other three values of $U$ calculated for *arizonensis* males were each larger and yielded probabilities of less than .001. Where the number of $I$ values was greater than 20 by 20 (e.g., comparisons between Regions, Table 4, column 7), a $t$ value was calculated:

$$t = \frac{(U - (n_1 n_2 / 2))}{((n_1 n_2) \ (n_1 + n_2 + 1) \ (1/12))^{1/2}}$$

$t$ is approximately normally distributed and critical values of $t$ are then 1.96 ($P = .05$); 2.58 ($P = .01$) and 3.29 ($P = .001$).