Evolution of the *Mojavensis* Cluster of Cactophilic *Drosophila* with Descriptions of Two New Species

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The *mojavensis* cluster of the *repleta* species group of *Drosophila* (Drosophilidae: Diptera) consists of three species. One is newly described as *D. navojoa*. A second species, described here as *D. arizonae*, replaces *D. arizonensis*, which has become a junior subjective synonym for *D. mojavensis*, the third species in the cluster. A phylogeny of the three species is presented, based on chromosomal inversions, morphology, and the ability to produce hybrids. Breakage points are assigned for all inversions, and male genitalia are figured; 186 crosses were made from 225 possible combinations among 15 geographic strains from the southwestern United States, Mexico, and Guatemala. It is confirmed that *D. mojavensis* and *D. arizonae* are very closely related and shown that *D. navojoa* is more distantly related in regard to all criteria. This relationship is supported by the geographical positions of the ancestral gene sequences in each species, which show a sequential northwest movement (*D. navojoa* → *D. arizonae* → *D. mojavensis*) from southern Mexico to southern California and northern Arizona. The relationship is also supported by the fact that *D. navojoa* breeds in *Opuntia* cactus, an ancestral behavior, whereas the other two species breed chiefly in *Stenocereus* cacti, a derived behavior. The possible role of this host plant shift in speciation is discussed.

Despite the development of several molecular techniques during the last 20 years,[17] inversion analysis remains one of the most powerful and reliable methods for inferring phylogenetic relationships among *Drosophila* species. The method is based on the cladistic principle that species sharing derived states of a given character (synapomorphies) are phylogenetically related.[51] In this case, however, the derived features are of a very special kind: chromosome rearrangements. Because the creation of a given inversion is a unique event in the history of a species group, two species that share the same inversion must be descendants of the same ancestor.[34,44] Although chromosome phylogenies are intrinsically bidirectional, the phylogenetic tree can be given a polarity through the use of additional information. For instance, Wasserman[42,45] determined that the ancestral sequence for the *D. repleta* species group probably differs from the *D. repleta* standard by six inversions: Xa, Xb, Xc, 2a, 2b, and 3b. This PRIMITIVE I sequence connects the *D. repleta* group species with those of the *D. castanea, D. dreyfusi,* and *D. canalinea* groups, which are their closest relatives.[37,38] PRIMITIVE I is also the standard sequence of three

species in the *D. hydei* subgroup, which, Throckmorton[38] concluded on the basis of internal anatomy, split off early during the evolution of the *D. repleta* group.

The *D. mojavensis* cluster consists of three species: *D. mojavensis,*[26] a form that has been widely known by the name *D. arizonensis,*[27] and an undescribed form originally collected from Navojoa (Sonora, Mexico). The three species belong to the *D. mulleri* complex of the *D. repleta* species group[45] and inhabit chiefly the deserts of the southwestern United States and northwestern Mexico, breeding on the necrotic tissues of a number of cactus species.[12] In the last decade, they have increasingly attracted the interest of investigators who have used them as a model to study speciation.[8,12,18–20,39,46,47,52–54,56,57] As the validity of these studies depends on knowledge of the phylogenetic relationships among the three species and among populations within each species, it is important to determine these relationships as accurately as possible. Wasserman[43,45] presented a tentative picture of the chromosome evolution in the cluster, but his cytogenetic work was not published in detail. The reproductive relationships among the three species have been worked out but have
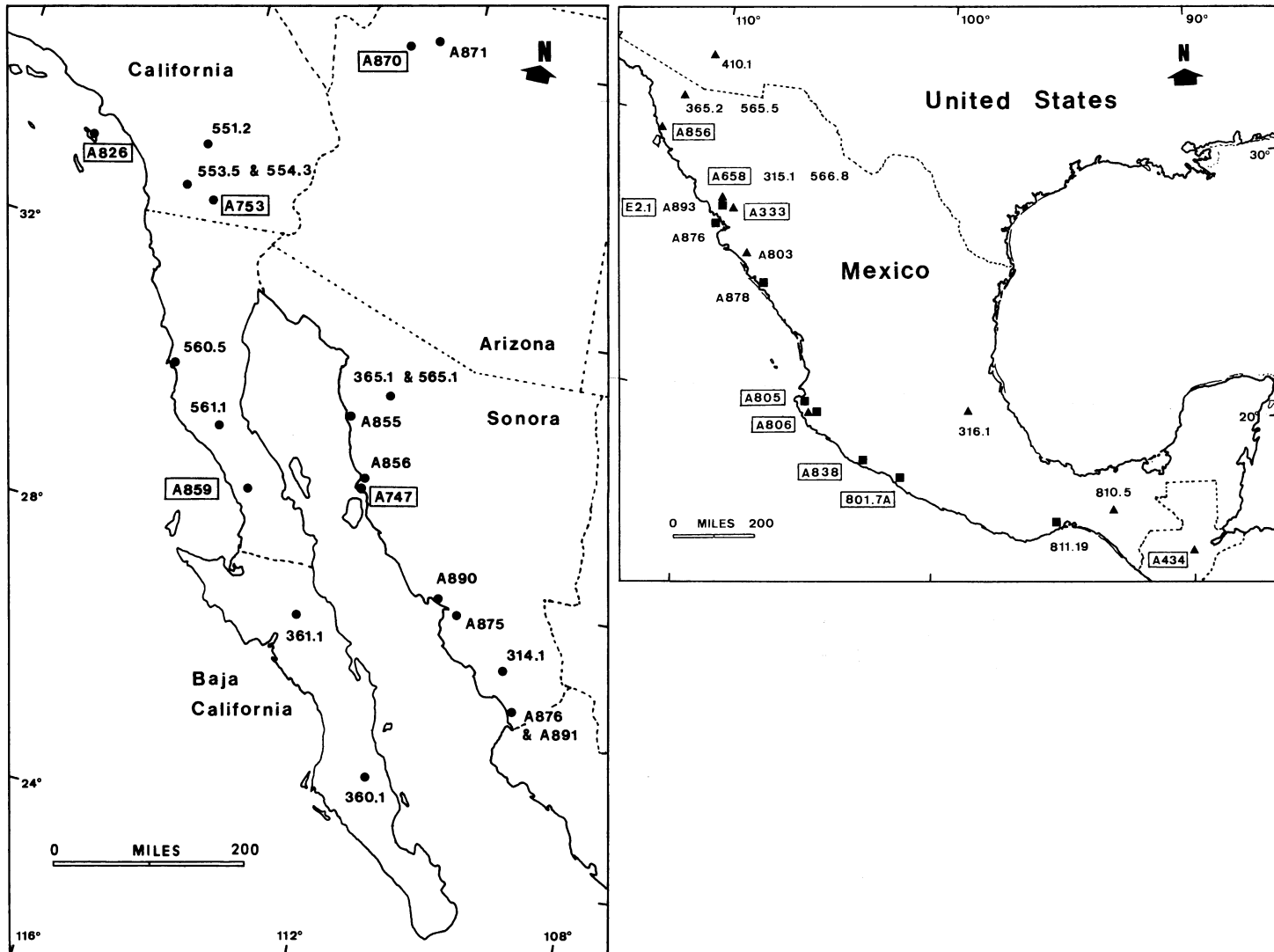
Figure 1. Geographical origin of all the stocks involved in our study. The collection numbers of the strains used in the hybridization tests are framed. Left: locations for *D. mojavensis* (●). The six populations located above the U.S. border belong to the *D. mojavensis mojavensis* subspecies; those in Baja California and Sonora, Mexico, pertain to the *D. mojavensis baja* subspecies. Right: locations for *D. arizonae* (▲) and *D. navojoa* (■). See Table 1 and text for names of locations.

been described only partially.[45] Moreover, *D. arizonensis* has been synonymized to *D. mojavensis* by Vilela.[40] Thus, only one of the three species of this interesting species cluster has a valid name.

We provide detailed cytogenetic and hybridization data showing that the three forms are genetically isolated but closely related species. All three species are polymorphic for inversions on the second and/or third chromosomes, and *D. mojavensis* exhibits considerable cytological differentiation among geographically isolated populations. We determined the phylogenetic relationships and combined them with available biogeographical and ecological information[9,11,12,30] to achieve a plausible reconstruction of their evolutionary history. A revised taxonomy of the cluster, including a description of *D. na-*

vojoa and the species formerly known as *D. arizonensis* under the name of *D. arizonae,* is given in the Appendix.

Materials and Methods

The geographical distribution of the three *D. mojavensis* cluster species has been reviewed by Heed[12] and Heed and Mangan.[13] A summary is given below (see Discussion). We used the following stocks of the three species in the hybridization tests. *D. mojavensis mojavensis*: A753, Vallecito, California; A826, Santa Catalina Island, California; and A870, Deubendorf, Arizona. *D. mojavensis baja*: A859, Punta Prieta, Baja California Norte, and A747; Punta Onah, Sonora. *D. arizonae*: A856, Desemboque, Sonora; A658, Navojoa, Sonora; A333, Alamos, Sonora; A806, Tomatlán, Jalisco; and

A434, Santa Cruz, Guatemala. *D. navojoa*: E2.1, Navojoa, Sonora; A805, Nahuapa, Jalisco; A806, Tomatlán, Jalisco; A838, Chahiapan, Michoacán; and 801.7A, Zihuatanejo, Guerrero. The geographical position of all the localities is shown in Figure 1. The *D. mojavensis* population from Punta Onah (A747) was the only one that was found to be sympatric to *D. arizonae*; the remaining four *D. mojavensis* populations were allopatric. As with the *D. arizonae* populations, those from Desemboque (A856), Navojoa (A658), and Alamos (A333) were found to be sympatric to *D. mojavensis,* whereas the other two were allopatric. On the other hand, *D. navojoa* is sympatric with *D. arizonae* over the major part of its geographic range, and both species co-occur with *D. mojavensis* in a very small area near Navojoa (E2.1).

Of the 225 possible combinations among the 15 strains, we tested 186, including 61 intraspecific crosses that we used as controls. Crosses were performed in 8-dram shell vials with regular banana food. We placed eight pairs in each vial and allowed them to lay eggs for 8 to 10 days. They were transferred to a new vial with fresh food; after a new period of 8 to 10 days, the adults that were still alive were discarded. All the adult offspring emerging from both vials of the same parents were counted, and the data were pooled. One to four replicates were set up for each combination. The fertility of the hybrid progeny was tested by intercrossing the F_1 adults and backcrossing hybrid females with males from the parental stocks when no F_2 generation was obtained.

We obtained metaphase chromosomes from 14 stocks of the three species (the same used in the hybridization tests except *D. arizonae* A856 from Desemboque, Sonora). Slides (4 to 6 per stock) were prepared staining the larval cerebral ganglia with acetic-lactic orcein (1%) and lactopropionic orcein (4%).

We prepared salivary gland chromosomes using the technique described by Wasserman.[41] A list of the localities investigated for *D. mojavensis* is shown in Table 1. For the other two species, the following populations were analyzed. *D. arizonae*: 410.1, Tucson, Arizona; 365.2 and 565.5, Caborca, Sonora; 316.1, Venados, Hidalgo; 315.1 and 566.8, Navojoa, Sonora; A803, Guamúchil, Sinaloa; A806, Tomatlán, Jalisco; 810.5, Tuxtla Gutiérrez, Chiapas; and A434, Santa Cruz, Guatemala. *D. navojoa*: E2.1 and A893, Navojoa, Sonora; A876, Las Bocas, Sonora; A878, El Dorado, Sinaloa; A805, Nahuapa, Jalisco; A806, Tomatlán, Jalisco; 801.7A, Zihuatanejo, Guerrero; and 811.19, Tehuantepec, Oaxaca. The geographical origin of all the stocks is shown in Figure 1.

Results

Metaphase Chromosomes

The three species showed similar metaphase plates consisting of five pairs of rods and a pair of dots. The four pairs of autosomes were about the same length, whereas the X chromosome was approximately 1.5 times longer. The Y chromosome was approximately half the length of the X chromosome in *D. mojavensis* and *D. navojoa* and two-thirds the length of the X chromosome in *D. arizonae*. The length of the Y chromosome for *D. arizonae* and *D. mojavensis* was reported to be very short

Table 1. Chromosomal constitution of the *Drosophila mojavensis* populations arranged according to subspecies and geographic origin (see Figure 1)

Collection no. and origin	Inversions				
	Xabce	2abcfghqrs	3abd	4	5
D. mojavensis mojavensis: California and northern Arizona					
551.2: Palm Desert, California	St	St	St	St	St
553.5 and 554.3: Julian, California	St	St	St	St	St
A826: Santa Catalina Island, California	St	St	St	St	St
A870: Deubendorf, Arizona	St	St	St	St	St
A871: Mohawk Canyon, Arizona	St	St	St	St	St
D. mojavensis baja: Baja California					
560.5: San Quintín, Baja California Norte	St	q^5, q^5 r^5	St, f^2	St	St
561.1: Cataviña, Baja California Norte	St	q^5	St, f^2	St	St
A859: Punta Prieta, Baja California Norte	St	St, q^5, q^5 r^5	St, f^2	St	St
361.1: San Ignacio, Baja California Sur	St	St, q^{5a}	f^2	St	St
360.1: La Presa, Baja California Sur	St	St, q^5	St	St	St
D. mojavensis baja: Sonora					
365.1 and 565.1: Caborca, Sonora	St	q^5	St	St	St
A855: Puerto Libertad, Sonora	St	q^5	St, f^2	St	St
A856: Desemboque, Sonora	St	St, q^5	St, f^2	St	St
A890: Playa Cochorit, Sonora	St	q^5	St	St	St
A875: Peon, Sonora	St	q^5	St, f^2	St	St
314.1: Navojoa, Sonora	St	q^5	St	St	St
A876 and A891: Las Bocas, Sonora	St	q^5	St, f^2	St	St

[a] 2abcfghqrs^{7}t^{7}u^7 arrangement.

by Wharton.[48] None of our stocks of either species matched Wharton's description. We detected no conspicuous geographic variation in metaphase pattern among the strains of any of the three species.

Salivary Gland Chromosomes

The banding pattern in the polytene chromosomes of the three species was compared with that of *D. repleta*,[48] which is the reference karyotype for the *D. repleta* species group.[45] All three species have inversions Xa, Xb, Xc, 2a, 2b, and 3b, which make up the PRIMITIVE I sequence, Xabc,2ab,3b. In addition, they are homozygous for inversions 2c, 2f, 2g, and 3a, which are shared by several other *D. mulleri* complex species.[45]

One inversion, Xe, has been fixed in *D. mojavensis*. It overlaps Xa and includes Xb, encompassing regions from about D2f to C4g, D4c to F1a, and F3a to F1a. These breakage points (BPs) are approximate.

The evolution of chromosome 2 is shown in Figures 2 and 3. Figure 2A, which depicts the ancestral 2ab chromosome, shows the BPs of 2c, 2f, and 2g, three inversions that are found in all the *D. mojavensis* cluster species. Figure 2B, which depicts the 2abcfg chromosome, the standard chromosome of *D. navojoa* and the *D. mulleri* cluster, shows the approximate BPs of 2h. Figure 2C, which depicts the 2abcfgh chromosome, is the most primitive sequence found in *D. arizonae*. Thus far, this sequence has been found only in Tomatlán, Jalisco, where the 2i sequence also is

found. All the other strains are homozygous for the 2i inversion shown in Figure 2C. In view of the apparent restricted distribution of the more primitive chromosome, the advanced 2abcfghi chromosome will continue to be designated as the standard of *D. arizonae*. The approximate BPs of the 2q and 2r (which overlaps 2q) inversions, which were incorporated during the evolution of *D. mojavensis*, are shown in Figure 2C. The 2abcfghqr chromosome, shown in Figure 2D, has not been found. A cytological divergence occurred at this level, leading either to 2s or to 2t^7. The BPs of inversions 2s and 2t^7 are shown.

Figure 3A shows the 2abcfghqrs chromosome, the standard (ST) chromosome of *D. mojavensis*. The approximate BPs of the polymorphic inversions 2q^5, 2r^5, and 2v^7 (LP, BA, and SL, respectively, of Mettler[21] and Johnson[15]) are shown. Figure 3B shows the approximate BPs of 2s^7 and 2u^7 on the 2t^7 chromosome, producing the 2abcfghqrs^{7}t^{7}u^7 (SI of Johnson[15]), a chromosome, found thus far only at a low frequency in San Ignacio and San Lucas, Baja California Sur (Figure 3C).

Figure 4 shows the evolution of chromosome 3. Figure 4A depicts the 3ab sequence and shows the approximate BPs of these two inversions. This chromosome is the primitive chromosome of the *D. mojavensis* cluster. Wasserman[43,45] had previously reported that *D. arizonae* lacks the 3a inversion. However, a close reexamination of this chromosome by A. R. revealed that there has been a shift of ap-
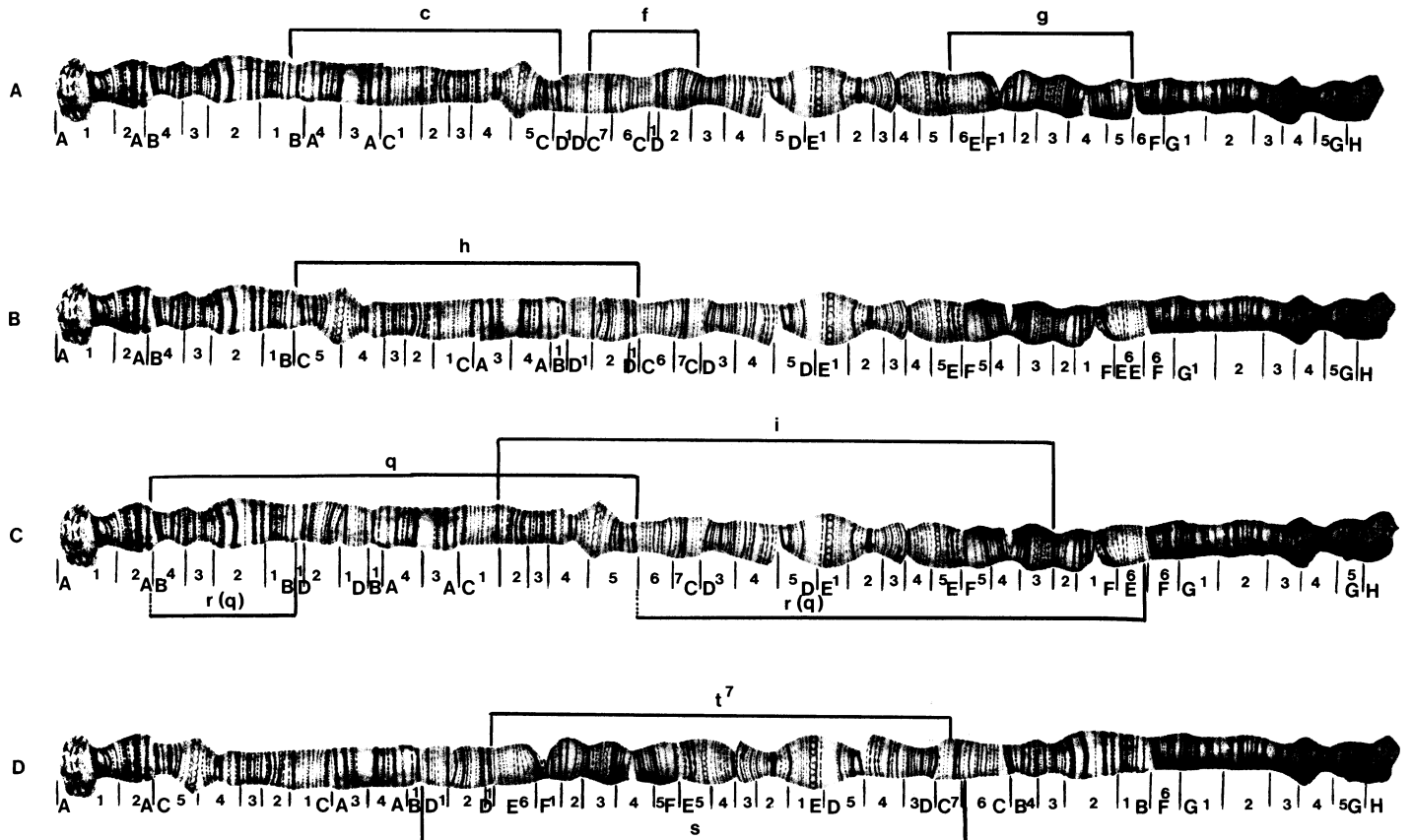
Figure 2. The salivary gland chromosome map, showing the evolution of chromosome 2 in the *D. mojavensis* cluster species. (A) The ancestral 2ab chromosome showing the BPs of 2c, 2f, and 2g, three inversions found in all *D. mojavensis* cluster species. (B) The 2abcfg arrangement, the standard chromosome of *D. navojoa* and the *D. mulleri* cluster. The BPs of inversion 2h, fixed in *D. mojavensis* and *D. arizonae,* are shown. (C) The 2abcfgh chromosome, primitive sequence found in *D. arizonae,* showing the BPs of inversions 2i found in *D. arizonae* and 2q and 2r (which overlaps 2q) found in *D. mojavensis.* (D) The 2abcfghqr chromosome, primitive arrangement of *D. mojavensis,* showing the BPs of inversions 2s and 2t[7].
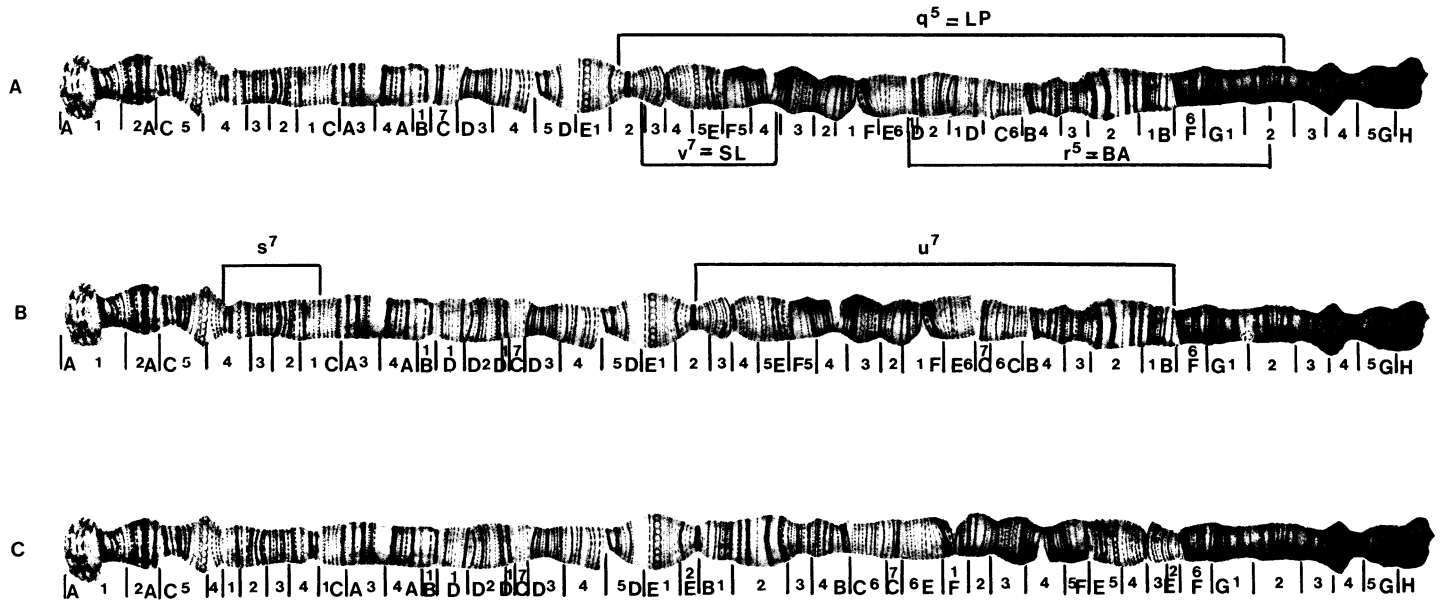
Figure 3. Cytological map of chromosome 2 showing the inversions found to be polymorphic in the *D. mojavensis* populations. (A) The 2abcfghqrs chromosome, standard sequence of *D. mojavensis.* The BPs of inversions 2q[5], 2r[5], and 2v[7] are shown. (B) The 2t[7] chromosome, not found in natural populations, showing the BPs of inversions 2s[7] and 2u[7]. (C) The 2abcfghqrs[7]t[7]u[7] chromosome, found only in two localities of Baja California (San Ignacio and San Lucas).

proximately four or five bands from the C4-5 region distally to the distal BP of 3a, yielding the chromosome shown in Figure 4B. The most likely interpretation of this banding pattern is a sequence of two inversions, the first of which is 3a. The second, 3p[2], as shown in Figure 4A, includes 3a and shares with it one of the BPs, thus almost restoring the ancestral sequence (Figure 4B).

D. mojavensis is homozygous for the 3d inversion (Figure 4C), whose BPs are

Figure 4. Salivary gland chromosome map showing the evolution of chromosome 3 in the *D. mojavensis* cluster species. (A) The 3ab chromosome, primitive sequence of the *D. mojavensis* cluster, showing the approximate BP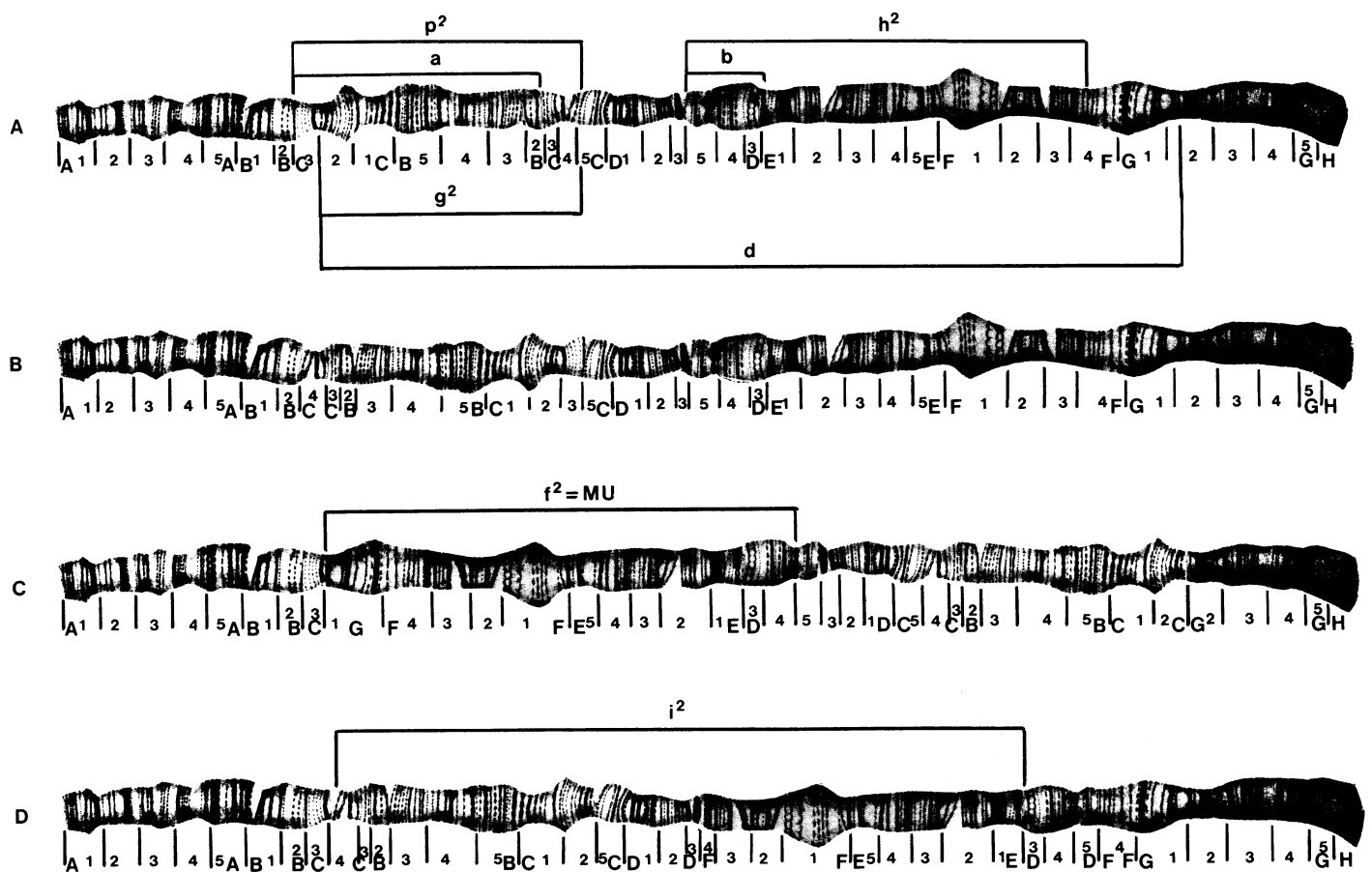s of these two inversions. The BPs of inversions $3g^2$ and $2h^2$, fixed in *D. navojoa*; $3p^2$, fixed in *D. arizonae*; and 3d, fixed in *D. mojavensis,* are also shown. (B) The $3abp^2$ chromosome, standard sequence of *D. arizonae*. (C) The 3abd chromosome, standard sequence of *D. mojavensis,* showing the BPs of the polymorphic inversion $3f^2$. (D) The $3abg^2h^2$ chromosome, standard sequence of *D. navojoa,* showing the BPs of the polymorphic inversion $3i^2$.

shown in Figure 4A. The species is polymorphic for inversion $3f^2$ (MU of Mettler[21] and Johnson[15]) shown in Figure 4C.

D. navojoa, in addition to 3a and 3b, is homozygous for $3g^2$ and $3h^2$ and polymorphic for $3i^2$. The $3g^2$ inversion, whose BPs are shown in Figure 4A, has approximately the same size and position as 3a and $3p^2$. It also seems to share a BP with the *D. mojavensis* 3d inversion. The $3h^2$ inversion shares a BP with 3b. Figure 4D shows the standard gene order, $3abg^2h^2$, of *D. navojoa* and indicates the approximate BP of $3i^2$. This inversion has a north–south cline in frequency. Northern populations from Navojoa and Las Bocas, Sonora, are homozygous for this inversion; intermediate populations from El Dorado, Sinaloa, Tomatlán, and Nahuapa, Jalisco, are heterozygous; and southern populations from Zihuatanejo, Guerrero, and Tehuantepec, Oaxaca, are homozygous for the standard sequence. Therefore, the northern populations are cytologically derived. The *D. mojavensis* cluster species

all appear to be homozygous for the standard *D. repleta* chromosomes 4 and 5.

The polymorphic inversions in *D. mojavensis* show a remarkable geographical differentiation (Table 1 and Figure 1). All populations of the subspecies *D. mojavensis mojavensis,* which in this study include populations from the Palm Desert region (551.2) and the Anza-Borrego Desert (553.5 and 554.3) of southern California, are cytologically monomorphic for the standard arrangements on both the second and third chromosomes. Samples from Santa Catalina Island (A826) and two localities (A870 and A871) along the Colorado River in northern Arizona had the same chromosomal constitution, indicating that they belong to the *D. m. mojavensis* subspecies as well. The Baja California populations of the subspecies *D. mojavensis baja* are quite polymorphic, with all the inversions described above occurring in them (Table 1). In contrast, most of the mainland Sonora populations of *D. mojavensis baja* are fixed for arrangements $2q^5$ and 3 ST. None-

theless, inversion $3f^2$ is present in low frequency (less than 5%) along the Sonora coast from Puerto Libertad (A855) south to Las Bocas (A876 and A891), and the second chromosome is also polymorphic in the vicinity of Desemboque (A856).

Reproductive Relationships

All intraspecific crosses yielded abundant and fertile progeny. The mean number of offspring per replica was 224.3 for *D. mojavensis,* 242.8 for *D. arizonae,* and 277.5 for *D. navojoa.* No evidence of isolation among conspecific populations, as indicated by decreased productivity, was found in any of the three species.

An examination of the results of the crosses between *D. mojavensis* and *D. arizonae* (Table 2) revealed the following.

1. Interspecific crosses between *D. mojavensis* and *D. arizonae* yielded relatively abundant offspring in most combinations, but the numbers were significantly lower than those in intraspecific controls. The

Table 2. Total number of offspring produced in interspecific crosses between *D. mojavensis* and *D. arizonae*

Cross[a]	N[b]	F₁	Adults[c]
♀♀ *D. mojavensis* × ♂♂ *D. arizonae*			
A753 × A856	2	181	FM FF
A753 × A658	2	149	FM FF
A753 × A333	2	253	FM FF
A753 × A806	2	123	FM FF
A753 × A434	2	14	FM FF
A870 × A333	1	61	FM FF
A870 × A806	2	191	FM FF
A826 × A856	1	15	SM FF
A826 × A658	2	193	SM FF
A826 × A333	1	26	SM FF
A826 × A434	1	5	SM FF
A859 × A856	2	60	FM FF
A859 × A658	2	120	FM FF
A859 × A333	3	277	FM FF
A859 × A434	2	28	FM FF
A747 × A856	2	0	
A747 × A658	2	0	
A747 × A333	2	16	FM FF
A747 × A806	2	0	
A747 × A434	2	0	
Total	37	1,712	
♀♀ *D. arizonae* × ♂♂ *D. mojavensis*			
A856 × A753	2	317	SM FF
A658 × A753	1	156	SM FF
A333 × A753	2	449	SM FF
A806 × A753	2	470	SM FF
A434 × A753	2	391	SM FF
A333 × A870	2	568	SM FF
A806 × A870	2	385	SM FF
A856 × A826	1	60	SM FF
A658 × A826	1	162	SM FF
A333 × A826	2	357	SM FF
A434 × A826	2	478	SM FF
A856 × A859	2	307	SM FF
A658 × A859	1	148	SM FF
A333 × A859	3	475	SM FF
A434 × A859	3	122	SM FF
A856 × A747	2	247	SM FF
A333 × A747	2	174	SM FF
A806 × A747	2	167	SM FF
A434 × A747	3	179	SM FF
Total	37	5,612	

[a] See Materials and Methods and Figure 1 for stock numbers and localities.
[b] Number of replicated cultures.
[c] SM = sterile males; FM = males at least partially fertile; FF = fertile females.

mean progeny per replica, averaged over all combinations, was 97.5, which is less than half the production of the intraspecific crosses.

2. Interspecific crosses between *D. mojavensis* males and *D. arizonae* females always produced far more progeny than did the reciprocal crosses. The mean number of progeny per replica in the first case was 154.7, compared with 43.2 in the second. This asymmetry in productivity between reciprocal crosses cannot be accounted for by asymmetry in sexual isolation. Wasserman and Koepfer[46,47] observed asymmetrical sexual isolation between *D. arizonae* and the allopatric populations of *D. mojavensis baja* from Baja California. It was,

however, in the opposite direction to our results: *D. mojavensis* males were more isolated from *D. arizonae* females (isolation index $I_m = 0.779$) than *D. mojavensis* females were from *D. arizonae* males ($I_f = 0.308$). When sympatric *D. mojavensis baja* from Sonora were tested, a much higher isolation was found, but no asymmetry was observed (the isolation indices, $I_m = 0.922$ and $I_f = 0.940$, were not significantly different). Comparable data for the *D. mojavensis mojavensis* populations from southern California are not available, but a reversal of the isolation indices seems unlikely. The most probable explanation of this paradoxical situation is derived from the insemination reaction,[25] in which sperm is destroyed in the vagina. Baker,[1] who examined the genital tracts of females after interspecific copulations, noted that as a result of the intensity of the insemination reaction in the cross between *D. mojavensis* females and *D. arizonae* males, only 3% of the inseminated females produced offspring, whereas in the reciprocal cross 74% of the inseminated females produced progeny. This is an example of selection against the gametes of a foreign species.

3. There was a large variation among *D. mojavensis* populations in terms of the number of hybrids they produced when crossed to *D. arizonae* (Table 2). In contrast, the differences among the *D. arizonae* populations were not statistically significant. When *D. mojavensis* provided the female parent, the mean number of offspring per replica was 72.0 for Vallecito (A753), 78.2 for Deubendorf (A870), 35.6 for Santa Catalina Island (A826), 49.1 for Punta Prieta (A859), and 1.6 for Punta Onah (A747). The variation among populations was highly significant ($F = 6.85$, df = 4,18, $P = .002$). The mean progeny produced by the same five *D. mojavensis* populations in the intraspecific crosses varied from 191.1 to 251.2, but the differences were not statistically significant ($F = 1.00$, df = 4,16, $P > .05$). Thus, the variation in productivity among *D. mojavensis* populations when crossed to *D. arizonae* cannot be accounted for by differences in fecundity and viability among the parental strains. We obtained similar results in the reciprocal crosses. When *D. mojavensis* males were crossed to *D. arizonae* females, the mean number of offspring per replica was 193.9 for Vallecito (A753), 238.2 for Deubendorf (A870), 159.9 for Santa Catalina Island (A826), 125.1 for Punta Prieta (A859), and 88.4 for Punta Onah (A747). The variation again was highly significant ($F = 15.20$, df = 4,21, $P < .001$). Comparable results for

the intraspecific crosses ranged between 195.2 and 262.7; again, the differences were not significant ($F = 1.55$, df = 4,16, $P > .05$). Thus, the variation in the interspecific crosses was not due to differences in fecundity and viability among the parental strains in this case. In summary, the overall pattern for *D. mojavensis* seems clear (Figure 1). In both reciprocal crosses, strains from Vallecito (A753) in California and Deubendorf (A870) in northern Arizona produced more offspring than did any of the other stocks. On the other hand, the strain from Punta Onah (A747) in Sonora, the only one sympatric to *D. arizonae*, produced the lowest number of progeny. The populations from Santa Catalina Island (A826) in California and Punta Prieta (A859) in Baja California behaved in an intermediate way between these two extremes. These differences are most easily interpreted as being due to variation in sexual behavior (see Discussion).

4. Hybrid females from crosses between *D. mojavensis* and *D. arizonae* were always at least partially fertile (Table 2). Hybrid males from crosses between *D. mojavensis* males and *D. arizonae* females were always sterile. Hybrid males from crosses between *D. arizonae* males and *D. mojavensis* females were at least partially fertile, as some F₂ progeny were usually obtained. However, very few F₂ progeny resulted when the *D. mojavensis* females came from Deubendorf (A870), and virtually none resulted when the Santa Catalina Island stock (A826) was involved. This implies that hybrid males were practically sterile in this case, as hybrid females proved to be fertile in backcrosses.

The results of the crosses between *D. navojoa* and both *D. mojavensis* and *D. arizonae* (Tables 3 and 4) were as follows.

1. In all types of crosses, there was some evidence of hybrid inviability, at least in the form of pupal lethality and the abnormal sex ratios, in which the male offspring occurred at lower frequency than did the female offspring. Interspecific crosses between *D. navojoa* and either *D. mojavensis* or *D. arizonae* were considerably less productive than were those between *D. mojavensis* and *D. arizonae* (Tables 3 and 4). This low productivity can be attributed at least in part to the high pupal mortality (around 80%) observed in these crosses. This is clear from Tables 3 and 4, in which the number of pupae and the number of emerging adults are given. Sex ratios also were skewed among these hybrids. A total

of 20 males and 58 females was obtained from the crosses between *D. mojavensis* females and *D. navojoa* males, whereas 73 males and 148 females resulted from the reciprocal crosses. *D. arizonae* females crossed to *D. navojoa* males yielded 24 males and 377 females, whereas 12 males and 2 females resulted from the reciprocal crosses.

2. In all cases, hybrid individuals, both males and females, had abnormal traits such as thin and short bristles, altered abdominal pattern, and reduced eyes (in some individuals). They were completely sterile.

3. As in the crosses with *D. arizonae*, there was a remarkable variation among *D. mojavensis* strains in regard to their crossability with *D. navojoa* (Table 3). Vallecito (A753) and Deubendorf (A870) produced more hybrids, pupae, and adults in both reciprocal crosses than did any of the other stocks. On the other hand, none of the 15 crosses performed with the strain from Punta Onah (A747) yielded any offspring.

Discussion

Phylogenetic Relationships in the *Mojavensis* Cluster

The species *D. mojavensis*, *D. arizonae*, and *D. navojoa* belong to the *D. mulleri* species complex, a cytologically remarkable taxon that includes nearly 30 cactophilic species inhabiting the American deserts and xerophytic woodlands.[45] They are externally quite similar, and more important, they share the hooklike shape of the aedeagus, a trait that is not found elsewhere in the *D. repleta* species group (see Appendix). Furthermore, although the *D. mojavensis* cluster does not possess any unique, key inversions that can be used to identify the species as members of the cluster, the species do share a set of inversions that places them as a unit within the *D. mulleri* complex: 1) They are homozygous for inversions 2c, 2f, 2g, and 3a, which are shared by other members of the *D. mulleri* complex and are found nowhere else. 2) They lack inversion 3c, which is found in all other North American *D. mulleri* complex species. 3) *D. arizonae* and *D. mojavensis* are both homozygous for a unique inversion, 2h.

All the evidence indicates a closer relationship between *D. mojavensis* and *D. arizonae* than exists between either of these species and *D. navojoa*. The morphological differences between *D. arizo-*

nae and sympatric *D. mojavensis* are slight, and they can be considered sibling species.[21] On the other hand, *D. navojoa* is morphologically somewhat dissimilar (see Appendix). Crosses between *D. mojavensis* and *D. arizonae* produced far more progeny in both directions than did any of the interspecific crosses involving *D. navojoa*. In addition, all crosses with *D. navojoa* produced weak and abnormal adults in unbalanced sex ratios, whereas crosses between *D. mojavensis* and *D. arizonae* gave rise to fully viable and some-

times fertile offspring. These results are consistent with Zouros's relatively low estimate (0.212) for Nei's genetic distance between *D. mojavensis* and *D. arizonae*.[52] Unfortunately, no estimates have been obtained for the genetic distances between the latter two species and *D. navojoa*.

A different concern is the phylogenetic relationships of the three species. On the basis of the available information, *D. navojoa* must have split off early from the
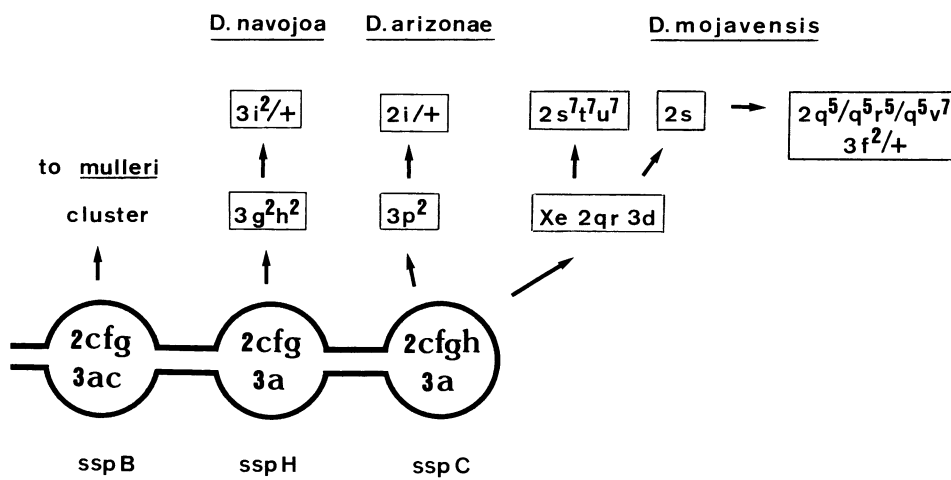
Table 3. Total number of offspring produced in interspecific crosses between *D. mojavensis* and *D. navojoa*

Cross[a]	N[b]	P[c]	A[d]
♀♀ *D. mojavensis* × ♂♂ *D. navojoa*			
A753 × E2.1	2	78	3
A753 × A805	2	24	7
A753 × A806	2	10	1
A753 × A838	2	6	0
A753 × 801.7A	1	0	0
A870 × E2.1	2	120[e]	18
A870 × A806	3	191	47
A826 × E2.1	1	0	0
A826 × A805	1	0	0
A826 × A806	1	1	1
A826 × A838	2	0	0
A826 × 801.7A	2	0	0
A859 × E2.1	2	3	1
A859 × A805	2	0	0
A859 × A806	2	0	0
A859 × A838	2	0	0
A859 × 801.7A	1	0	0
A747 × E2.1	1	0	0
A747 × A805	2	0	0
A747 × A806	2	0	0
A747 × A838	1	0	0
A747 × 801.7A	1	0	0
Total	37	433[e]	78
♀♀ *D. navojoa* × ♂♂ *D. mojavensis*			
E2.1 × A753	1	2	1
A805 × A753	2	200[e]	152
A806 × A753	2	54	17
A838 × A753	2	62	14
801.7A × A753	2	88	21
E2.1 × A870	4	33	4
A806 × A870	3	10	7
E2.1 × A826	1	0	0
A805 × A826	2	6	4
A806 × A826	2	7	1
E2.1 × A859	2	21	0
A805 × A859	3	2	0
A806 × A859	2	0	0
A838 × A859	2	0	0
801.7A × A859	1	0	0
E2.1 × A747	1	0	0
A805 × A747	2	0	0
A806 × A747	2	0	0
A838 × A747	2	0	0
801.7A × A747	1	0	0
Total	39	485[e]	221

[a] See Materials and Methods and Figure 1 for stock numbers and localities.
[b] Number of replicated cultures.
[c] F_1 pupae.
[d] F_1 adults.
[e] Minimum estimate.

Table 4. Total number of offspring produced in interspecific crosses between *D. arizonae* and *D. navojoa*

Cross[a]	N[b]	P[c]	A[d]
♀♀ *D. arizonae* × ♂♂ *D. navojoa*			
A856 × E2.1	1	1	0
A856 × A805	2	40	4
A856 × A806	2	25	0
A856 × A838	1	40	0
A856 × 801.7A	1	30	1
A658 × E2.1	1	0	0
A658 × A805	2	46	6
A658 × A806	2	9	0
A658 × A838	1	45	5
A658 × 801.7A	1	40	9
A333 × E2.1	3	135[e]	2
A333 × A805	3	89	19
A333 × A806	3	121[e]	23
A333 × A838	2	87	9
A333 × 801.7A	1	27	2
A806 × E2.1	2	144	5
A806 × A806	2	132	7
A434 × E2.1	2	49	0
A434 × A805	2	200[e]	64
A434 × A806	2	51	0
A434 × A838	2	200[e]	160
A434 × 801.7A	1	100[e]	85
Total	39	1,611[e]	401
♀♀ *D. navojoa* × ♂♂ *D. arizonae*			
E2.1 × A856	1	0	0
A805 × A856	2	0	0
A806 × A856	1	4	0
A838 × A856	2	5	0
801.7A × A856	2	1	0
E2.1 × A658	1	0	0
A805 × A658	3	16	0
A806 × A658	2	0	0
A838 × A658	2	51	0
801.7A × A658	2	24	3
E2.1 × A333	3	0	0
A805 × A333	3	18	3
A806 × A333	4	46	1
A838 × A333	2	28	7
801.7A × A333	1	0	0
E2.1 × A806	3	0	0
A806 × A806	4	1	0
E2.1 × A434	2	0	0
A805 × A434	2	0	0
A806 × A434	2	0	0
A838 × A434	2	2	0
801.7A × A434	2	0	0
Total	48	196	14

[a] See Materials and Methods and Figure 1 for stock numbers and localities.
[b] Number of replicated cultures.
[c] F_1 pupae.
[d] F_1 adults.
[e] Minimum estimate.

Figure 5. Cytological evolution of the *D. mojavensis* cluster species. The three species arose from Ancestor II, the putative species that gave rise to the *D. mulleri* complex, whose chromosomal constitution is shown at the bottom.
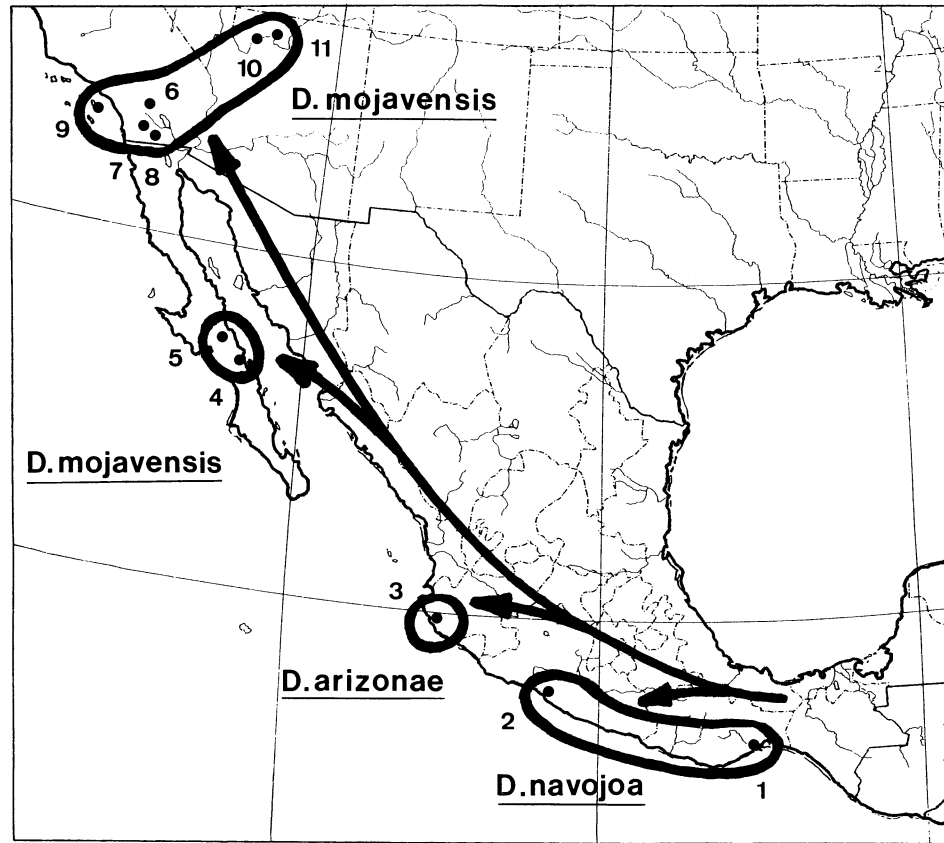

Figure 6. Localities with the more ancestral gene sequences in each branch of the phylogeny shown in Figure 5. Inversions Xabc, 2ab, and 3b make up the PRIMITIVE 1 sequence and are not indicated. The primitive gene sequence for *D. navojoa* (2cfg, 3ag²h²) is found homozygous near Tehuantepec, Oaxaca (1), and Zihuatanejo, Guerrero (2). The primitive sequence for *D. arizonae* (2cfgh, 3ap²) is heterozygous with 2i near Tomatlán, Jalisco (3). The sequence for the first branch of *D. mojavensis* (Xe, 2cfghqrs⁷t⁷u⁷, 3ad) is found heterozygous in low frequencies near San Lucas (4) and San Ignacio (5), Baja California Sur. The sequence for the second branch of *D. mojavensis* (Xe, 2cfghqrs, 3ad) is found homozygous near Palm Desert (6), Julian (7), Vallecito (8), and Santa Catalina Island (9) in California and near Deubendorf (10) and Mohwak Canyon (11) in Arizona.

populations that eventually gave rise to the cytologically more advanced *D. mojavensis* and *D. arizonae*. *D. navojoa* arose from subspecies H of Ancestor II, the putative species that gave rise to the *D. mulleri* complex, and it has two unique fixed inversions (Figure 5). The *D. mulleri* cluster species—*D. mulleri, D. aldrichi, D.*

wheeleri, D. mayaguana, and several related, undescribed forms—arose from a contiguous population of the Ancestor II—namely, subspecies B—and therefore are the closest known relatives of the *D. mojavensis* cluster.[45] *D. navojoa* shares with them its standard second chromosome (2cfg) and is thus the link between *D. mojavensis* and *D. arizonae* and the rest of the *D. mulleri* complex. *D. mojavensis* and *D. arizonae* originated from subspecies C, which is homozygous for one more inversion (2h) than is subspecies H and hence is cytologically more derived (Figure 5). *D. mojavensis* also has four fixed inversion differences, whereas *D. arizonae* bears only one (Figure 5). This suggests that *D. mojavensis* may be the most derived of the three species in other characters as well.

The derived status of *D. arizonae* and *D. mojavensis* is further confirmed by molecular studies of the *Adh* duplication. Both species have a DNA sequence of about 700 base pairs inserted between their two *Adh* genes, and this sequence is not present in *D. mulleri* and *D. navojoa*.[22] Furthermore, *D. arizonae* and *D. mojavensis* are the only two North American *D. mulleri* complex species that are known to express *Adh* in eggs and ovaries.[2] Further evidence that *D. navojoa* may be considered ancestral to the other two species comes from the geographic position of its primitive gene sequence (discussed in the next section) and from its breeding ecology (discussed in the concluding section).

Inversion Geography and Regions with Ancestral Sequences

The three *D. mojavensis* cluster species are polymorphic for inversions on the second and/or third chromosomes and show interpopulation cytological differentiation. *D. navojoa* has a rather restricted geographical distribution. It has been collected along a narrow strip on the Pacific coast of western Mexico from Navojoa (Sonora) to the Isthmus of Tehuantepec.[12,30] The area where its cytological characteristics are more ancestral includes Guerrero and Oaxaca (Figure 6, points 1 and 2), where the species is homozygous for the standard arrangement on the third chromosome. *D. arizonae* has the widest distribution of the three species, ranging from Phoenix (Arizona) and Ruidosa (New Mexico) south to central Guatemala through the west coast of Mexico. It is also found in Taumalipas, San Luis de Potosí, and Hidalgo in eastern Mexico and has been collected in low numbers in the Cape

Region of Baja California.[12] *D. arizonae* was found to be heterozygous for the primitive arrangement of the second chromosome only at Tomatlán, Jalisco (Figure 6, point 3), whereas it is fixed elsewhere for the derived standard sequence carrying the 2i inversion.

D. mojavensis is a polytypic species that consists of two subspecies.[21,49] *D. m. mojavensis* occurs in the Mojave and Anza-Borrego Deserts of southern California and is chromosomally monomorphic for the standard arrangements on the second and third chromosomes. On the basis of their chromosomal constitution, the populations recently discovered on Santa Catalina Island, California, and in the Grand Canyon in northern Arizona belong to this subspecies as well. *D. m. baja* has two distinct geographical entities: Baja California, including most of the islands in the Gulf of California, and southern Arizona, Sonora, and northern Sinaloa, Mexico.[11,13] Our cytological data for the Baja California populations agree qualitatively with those of the more extensive survey done by Johnson,[15] who showed that there is a close coincidence of inversion frequencies with the phytogeographical provinces of the peninsula. The Vizcaino province and the central Gulf coast are the most polymorphic areas, whereas San Pedro Mártir, Magdalena, and the Cape Region are less cytologically variable. All Sonora populations, except those around Desemboque, are homozygous for inversion 2q[5] and thus are cytologically derived.

The unusual disjunctive distribution of *D. mojavensis* suggests that the ancestral populations of this species were present in Baja California, separated from the mainland populations that were to give rise to *D. arizonae* (Figure 6, points 4 and 5). After fixing inversions Xe, 2qr, 3d, and later 2s, the precursor of *D. mojavensis* migrated north along the peninsula. The southern California and northern Arizona localities (Figure 6, points 7 through 11) that have no heterozygosity for the most recent inversions may be considered as marginal isolates that lost contact with the remainder of the population at an earlier time. *D. mojavensis* subsequently migrated across the midriff islands into Sonora, where it came into contact with *D. arizonae,* which also had arisen from subspecies C, possibly in Jalisco. The coincidence of the inversion phylogeny shown in Figure 5 with the localities that have the more ancestral gene sequences for the three *mojavensis* cluster species (Figure 6) is evident on the basis of the assumption that the pattern of migration of the original populations was generally northwestward.

Genetic Isolation, Ecology, and Speciation

D. mojavensis, D. arizonae, and *D. navojoa* are morphologically distinct and genetically isolated entities. Despite potential gene exchange under laboratory conditions described below, no noticeable degree of hybridization between *D. mojavensis* and *D. arizonae* has been revealed in areas of sympatry during an extensive chromosomal survey. Also, no gene flow is possible between *D. navojoa* and either of the other two species. Furthermore, since the three forms are sympatric in southern Sonora and northern Sinaloa and maintain their identity there, they must be considered biologically valid species.

Almost all degrees of reproductive isolation can be found among the various populations of the three *D. mojavensis* cluster species. This situation is not uncommon in many other *Drosophila* groups.[5] However, in the *D. mojavensis* cluster, there is a distinct and important difference between isolating mechanisms caused by mating behavior, such as sexual isolation, and isolating mechanisms subsequent to mating.

In our crosses between *D. mojavensis* and *D. arizonae,* the sympatric *D. mojavensis* strain from Punta Onah, Sonora, always yielded the lowest number of offspring, most likely because of its high level of sexual isolation. This is consistent with previously published results showing that interspecific sexual isolation between sympatric populations is usually equal to or greater than sexual isolation between allopatric populations.[8] Populations of *D. mojavensis* that are sympatric with those of *D. arizonae* are more isolated from *D. arizonae* than are allopatric populations.[18,46,47] Incidentally, Sonoran *D. mojavensis* females have greatly increased their discrimination against *D. arizonae* males. Sexual isolation is also greater between sympatric populations of *D. mojavensis* and *D. navojoa* than it is between allopatric populations.[20] Thus, character displacement for sexual isolation has played a systematic and important role in the final steps of speciation in this cluster of species.

Postmating isolation can arise while the two forms are geographically isolated or after they become sympatric and are behaviorally isolated. It can consist of selection against gametes, hybrid inviability, hybrid sterility, and genetic imbalance in succeeding generations. There is evidence that all these postmating isolation mechanisms have occurred in this species cluster.

D. arizonae females crossed to *D. mojavensis* males always yielded sterile F_1 male offspring. To our knowledge, this result has been obtained by every researcher who has made this cross. The genetic basis of this sterility is probably complex and has not been elucidated.[57] However, since all *D. mojavensis* strains behave in the same way, it probably arose very early after the two species separated geographically and before the populations of *D. mojavensis* diverged from each other. The reciprocal crosses produced variable results. In most cases, partially fertile males were obtained, but when *D. mojavensis* from Santa Catalina Island was involved, hybrid males were practically sterile. Vigneault and Zouros[39] and Zouros et al.[57] showed that when these partially fertile males are backcrossed to females of the parental species, some sterile males are recovered among the offspring and that several incompatibilities between sex chromosomes and autosomes lead to this sterility. They suggested that these kinds of interactions, which are quite common between closely related species of *Drosophila,* may represent the early stage of development of postzygotic isolation.

The available evidence indicates that speciation in the *D. mojavensis* cluster has proceeded through the geographical isolation model.[7,35] Under this model, initial reproductive isolation arises as a by-product of adaptation to various environments during the phase of geographical isolation. Ringo et al.[29] recently reviewed the experimental evidence, and Zouros[55] provided a specific theoretical model that postulates the existence of autosomal genes with a pleiotropic effect on both male fertility and adaptation to the ambient environment. Therefore, one can ask what ecological factors brought about the adaptive divergence that eventually led to speciation in this cluster.

D. navojoa is an ecologically restricted species that breeds exclusively on the decaying pads and fruits of *Opuntia wilcoxii.*[12,30] On the other hand, populations of *D. mojavensis* and *D. arizonae,* although occasionally found on *Opuntia,* are supported in the southwestern United States and western Mexico chiefly by several species of columnar cacti of the genera *Stenocereus* and *Ferocactus.*[9,11-13,30] The three closest relatives of the *D. mojavensis* cluster—*D. mulleri, D. aldrichi,* and *D.*

wheeleri—breed in *Opuntia,* which is considered the ancestral niche of the North American *D. mulleri* complex.[32] Therefore, at least one host plant shift from *Opuntia* to columnar cacti must have occurred during the evolution of the *D. mojavensis* cluster.

In an attempt to ascertain such a host plant effect, Ruiz and Heed[30] measured the three fitness components—viability, development time, and thorax size—for *D. mojavensis, D. arizonae, D. navojoa,* and *D. aldrichi* (a member of the *D. mulleri* cluster) on their various host plants. The results showed a significant decrease in larval performance (up to 50%) when the two *Opuntia* breeders—*D. navojoa* and *D. aldrichi*—were grown on *Stenocereus.* In contrast, the two columnar breeders—*D. mojavensis* and *D. arizonae*—suffered only a slight decrease in fitness when raised on *Opuntia.* The reason for this asymmetrical response is probably the greater chemical complexity of the *Stenocereus* cacti compared with *Opuntia.*[10,16] These observations suggest that adaptation to *Stenocereus* played a significant role in the origin of *D. mojavensis* and *D. arizonae.* Ruiz and Heed[30] also discussed the stressful environmental conditions of low humidity that might have been present when host shifting occurred. Strong selection is thought to hasten genetic divergence,[35] and is one of the requirements of Zouros's model.[55] A similar role of host plant shifts in the allopatric speciation of phytophagous insects has been suggested by other authors.[31,33]

Appendix

The *D. mojavensis* cluster includes three species.[45] *D. mojavensis* was first described by Patterson and Crow[26] as a subspecies of *D. mulleri* from a culture that originated from a collection by Warren P. Spencer at Mesquite Springs, Death Valley, California. Raised to the specific status by Patterson and Wheeler,[27] it has more recently been considered a polytypic species consisting of two subspecies: *D. m. mojavensis* and *D. m. baja.*[21,49]

D. arizonensis also was first listed as a subspecies of *D. mulleri.*[6] *D. arizonensis* was then described by Patterson and Wheeler,[27] presumably in a culture that originated from a collection by Gordon B. Mainland near Tucson, Arizona.[23] Recently, Carlos R. Vilela,[40] working on a taxonomic revision of the *D. repleta* species group, reexamined the type material of this species and concluded that it does not differ from that of *D. mojavensis.* Previously, Vilela had urged Marshall R. Wheeler to synonymize *D. arizonensis* with *D. mojavensis* in a review paper of Wheeler's in 1981, and this was done.[50] Accordingly, the name *D. arizonensis* has become a junior subjective synonym, and a new name is therefore required. This species, so extensively studied by the American authors, is described here as *D. arizonae.* The new description, of course, closely matches the description and illustrations already published,[24,27,36] because it was the type material rather than the original description that became illegitimate.

The third species in the *D. mojavensis* cluster is an undescribed form that originally was collected from Navojoa, Sonora, and Los Mochis, Sinaloa (Mexico) by W. B. Heed and J. S. Johnston in January 1969. It is described here as *D. navojoa* from a more recent culture from Navojoa, Sonora. It is known in the literature as "from Navojoa"[30,45] or *D.* sp. N.[12,13,20] Furthermore, the name *D. navojoa* has been used by Bicudo[3,4] and Richardson.[28] *D. navojoa* is considered a *nomen nudum* in each case. In Richardson,[28] it is misspelled *D. navajoa.*

Drosophila arizonae, New Species
External characters of imagines. Arista with 3 branches above and 2 branches below in addition to the terminal fork. Third antennal joint brownish black, darker at base. Carina present and sulcate. Face tan. One strong oral bristle. Palps with three stronger bristles on the anterior lateral margin and numerous short hairs. Frons orange-brown, pollinose, contrasted with light gray areas at base of orbitals and around the ocelli. Each ocellus arises from a black area, and the posterior orbital, anterior vertical, and postverticals also arise from a black spot. The ocellar bristles, the anterior and middle orbitals, and the posterior verticals do not have a black spot at the base. Eye bright red. Cheek light tan. Cheek width ⅛ to ⅙ the long diameter of the eye. Proboscis light tan.

Ground color of mesonotum is light gray. Midline of mesonotum and area between dorsocentrals is darker gray. Mesonotal hairs arise from dark brown spots that fuse into an irregular pattern. Acrostichal hairs in 8 rows. No prescutellars. Scutellum ground color same as that of mesonotum. Midregion of scutellum is an X-shaped darker area. Scutellar bristles also arise from brown spots. Anterior scutellar convergent. Pleurae light gray with two indistinct darker stripes. Anterior sternopleural about ⅘ the length of the posterior. Midsternopleural minute. Legs yellow-brown with dark apical band on the second and third femora and a basal band on the second and third tibiae. Abdomen is light tan. Tergites with dark brown posterior bands that are interrupted in the middorsal line. On the angle of the tergites, the band extends anteriorly and becomes wider. There is a separate dark triangular spot in the posterior lateral margin of the second, third, fourth, and fifth tergites in both sexes. Wing clear; veins brown; apex of first costal section darker, with two heavy bristles. Costal index about 2.5; fourth vein index about 1.9; 5x index about 1.4; 4c index about 1.3; third costal section with heavy bristles on basal ¼.

Body length (freshly etherized): 2.5 to 2.8 mm (male) and 2.6 to 3.0 mm (female). Wing length: 2.0 to 2.2 mm (male) and 2.2 to 2.5 mm (female).

Internal characters of imagines and male genitalia. Male testes with 2.5 bright yellow outer coils and 3 orange-yellow inner coils. Paragonia and ejaculatory bulb very similar to that pictured for *D. arizonensis* by Throckmorton[36] using strain 2156.4 from Fairbanks, Arizona. Ventral receptacle with 12 to 16 loose coils. Ovipositor with 13 to 16 teeth on the margins and 2 to 3 teeth on the outer surface. Spermathecae minute and precisely as pictured for *D. arizonensis* by Throckmorton[36] in strain 2156.4 from Fairbanks, Arizona.

Epandrium with 8 to 9 short lower bristles and 3 upper bristles. Cerci fused at lower half. Surstylus with 10 to 11 primary teeth and 8 to 9 prominent marginal bristles (Figure 7B). Hypandrium as long as epandrium. Concha bearing one anterior bristle (Figure 7E). Aedeagus ventrally expanded with a pair of subapical, sclerotized short spurs (Figure 7H). Dorsal cleft about ¾ of length. Gonopod with one sensillum. Ventral rod ⅔ the length of gonopod. Phallosomal index 2.3.

Egg. With 4 equally long thin filaments, longer than the egg.

Puparia. Golden yellow. Anterior spiracles with 11 to 12 branches. Horn index approximately 3.5.

Chromosomes, relationship, distribution, and ecology. As described in text.

Type material. Holotype male and 8 paratypes from collection A21, Tucson, Arizona, by W. B. Heed in October 1960. Also 11 paratypes from collection A806, Tomatlán, Jalisco, Mexico, by W. B. Heed and R. H. Thomas in July 1981. All material deposited in American Museum of Natural History, New York, New York.
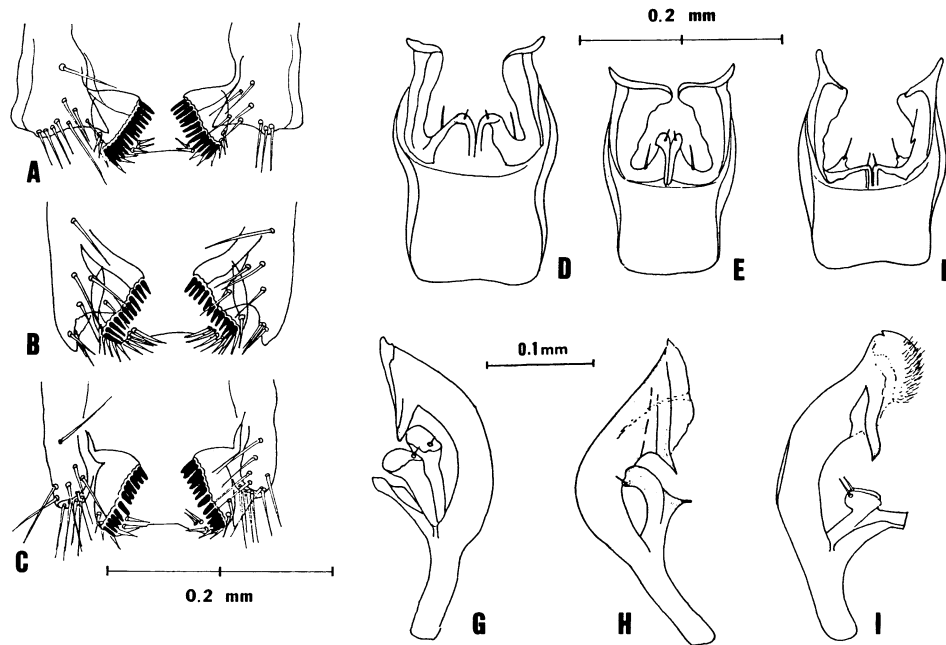
Figure 7. External genitalia (A–C), hypandrium (D–F), and aedeagus (G–I) of the *D. mojavensis* cluster species: *D. mojavensis* (A, D, and G), *D. arizonae* (B, E, and H), and *D. navojoa* (C, F, and I).

Table 5. External features of the three *mojavensis* cluster species

Feature	D. mojavensis	D. arizonae	D. navojoa
Eyes	Medium bright red	Medium bright red	Bright orange-red
Thorax	Dark brown or gray	Dark brown or gray	Light brown
Lateral margin of tergites	Very slight bar	Triangular spot in posterior corner	Medium heavy bar
Testes	Orange-yellow	Yellow	Bright yellow

Drosophila navojoa, New Species

External characters of imagines. Arista with 7 branches including terminal fork. Antennae brown; third antennal joint dark. Frons tan with two darker brown stripes forming a "V" pattern with the base pointed anteriorly. Anterior reclinate bristle even with anterior proclinate and about half its length. Face tan; carina not sulcate. Palps with one strong apical bristle and one subapical bristle; the remainder evenly covered with short erect hairs. Eye bright orange-red with black piling on upper half. Cheek width ⅛ to ⅙ the long diameter of eye.

Ground color of mesonotum is pollinose brown-gray with dark brown spots at the base of each hair, tending to fuse into two stripes along middorsal line in anterior half. The spots also fuse on each side of the transverse suture. Acrostichal hairs in 8 rows. No prescutellars. Anterior scutellar convergent. Pleurae with three irregular darker stripes. Sterno-index 0.7 to 0.8; midsternopleural bristle short. Abdomen pale brown with darker posterior banding on each tergite, interrupted on middorsal line. Banding on second, third, and fourth tergites (and fifth in females) comes forward at the angle to preceding tergite. Dark spot at the edge of each tergite with no connection to the banding. In southern populations, the gap between the angle and the edge is not as distinct and the pattern is more similar to *D. arizonae.* Legs light tan with slight darkening at the base of all tibiae and apical section of second and third femora. Wings clear with slightly darkened posterior cross-vein. Costal index about 2.5. Heavy bristles on basal ⅓ of third costal section; fourth vein index about 2.5; 5x index about 1.3.

Body length (freshly etherized): 2.3 to 2.4 mm (male) and 2.8 to 3.0 mm (female). Wing length: 1.9 to 2.2 mm (male) and 2.0 to 2.3 mm (female).

Internal characters of imagines and male genitalia. Male testes sulfur-yellow turning light orange with age, with 1.5 outer and 2.5 to 3 inner coils. Spermathecae weakly chitinized and bell-shaped. Ventral receptacle with 8 to 10 basal thicker coils and 5 to 10 distal thinner coils. Ovipositor rounded with 11 to 15 teeth on the margin, usually 13, and 2 to 3 teeth on the outside surface. In southern populations, the number of teeth on the margin seems to be slightly more, 12 to 16. One prominent, long and curved apical bristle.

Epandrium with 11 to 12 lower and 3 upper bristles. Cerci fused at lower half. Surstylus with about 11 primary teeth and 7 marginal bristles (Figure 7C). Hypandrium as long as epandrium. Concha bearing one anterior bristle (Figure 7F). Aedeagus ventrally expanded and micropubescent at posterior end, with a pair of subapical, ventrally fused, strongly sclerotized spurs (Figure 7I). Dorsal cleft about ⅔ of length. Aedeagal apodeme slightly bent. Ventral rod longer than gonopod. Gonopod with one sensillum. Phallosomal index approximately 2.5.

Egg. Two pairs of filaments. Anterior filaments approximately the length of egg; posterior filaments somewhat longer.

Puparia. Pale tan color, rather transparent. Horn with 6 short filaments and 8 long filaments. Horn index approximately 3.

Chromosomes, relationship, distribution, and ecology. As described in text.

Type material. Holotype male and 10 paratypes from collection A893, 5 km north of Navojoa, Sonora, Mexico, by A. Ruiz and W. J. Etges in March 1985. Also 11 paratypes from collection A236, 23 km north of Los Mochis, Sinaloa, Mexico, by W. B. Heed and J. S. Johnston in January 1969. All material deposited in American Museum of Natural History, New York, New York.

Distinguishing Characters

Color and pattern. In areas of sympatry, the three *D. mojavensis* cluster species, although externally quite similar, differ in a number of characteristics. The most distinct and easily recognizable species is *D. navojoa,* which is lighter in color and has bright orange-red eyes. *D. mojavensis* and *D. arizonae* differ mostly in abdominal pattern and male genitalia (see below). Flies of the *D. m. mojavensis* subspecies from southern California are lighter in color than those of the *D. m. baja* subspecies from Baja California and Sonora.[21] Table 5 is a summary intended to help with identification using a binocular microscope.

Male genitalia. They are similar in the three species (Figure 7) and rather different from those of other *D. mulleri* complex
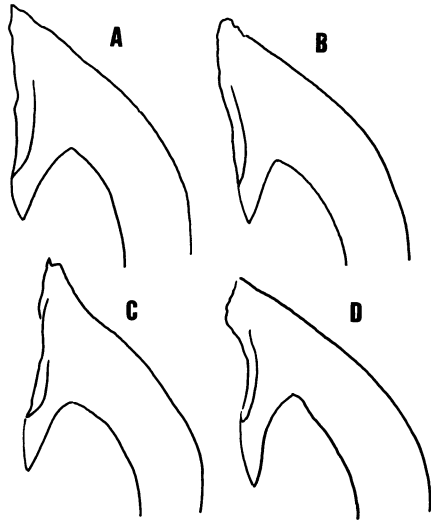
Figure 8. Tip of aedeagus in *D. mojavensis* from various locations: (A) A753 Vallecito, California; (B) A826 Santa Catalina Island, California; (C) A859 Punta Prieta, Baja California del Norte; and (D) A747 Punta Onah, Sonora.

species. In particular, the sclerotized subapical spurs of the aedeagus are found only in this cluster.[40] There are, however, many specific traits that distinguish the three species in both the external and internal genitalia. The undermargin of genital arch, for instance, is remarkably different: The toe is pointed in *D. mojavensis* and *D. arizonae,* but it appears much more rounded in *D. navojoa* (Figure 7, A–C). On the other hand, *D. mojavensis* and *D. arizonae* differ in heel shape and in the number, length, and location of the bristles in this part of the genital arch. *D. mojavensis* has 10 to 11 lower bristles on the epandrium, whereas *D. arizonae* has 8 to 9 and *D. navojoa* has 11 to 12 (all these figures are median values). The feasibility of using the shape of the heel, undermargin, and toe to distinguish species in the *D. repleta* group was pointed out by Hsu,[14] but he apparently failed to find such differences between *D. mojavensis* and *D. arizonae.* The three species differ also in the shape of the surstylus and the number and location of primary teeth and marginal bristles (Figure 7, A–C). The shape of the tooth row in the surstylus is visible in live specimens under low magnification and can be used to identify the males of the three species. The hypandrium and aedeagus show a closer similarity between *D. mojavensis* and *D. arizonae,* but in this case too the two species can be readily distinguished (Figure 7, D–I). Furthermore, we found a slight but consistent difference in the shape of the spurs of the aedeagus among several *D. mojavensis* populations (Figure 8). Mean values for the ratio of

length to width ± standard deviation for the four studied strains was 1.64 ± 0.18 for Vallecito (A753), 1.34 ± 0.18 for Santa Catalina Island (A826), 1.96 ± 0.18 for Punta Prieta (A859), and 2.31 ± 0.05 for Punta Onah (A747). Thus, males from the *D. m. baja* subspecies have longer spurs than do those from the *D. m. mojavensis* subspecies.

References

1. Baker WK. A study of the isolating mechanisms found in *Drosophila arizonensis* and *Drosophila mojavensis.* Austin: University of Texas Publication No. 4752. 1947.

2. Batterham P, Chambers GK, Starmer WT, and Sullivan DT. Origin and expression of an alcohol dehydrogenase gene duplication in the genus *Drosophila.* Evolution 1984; 38:644–657.

3. Bicudo HEMC. Nucleolar organizer activity and its regulatory mechanisms in *Drosophila* species of the *mulleri* complex and their hybrids. Caryologia 1981; 34:231–253.

4. Bicudo HEMC. Silver staining and the nucleolar organizing activity in *Drosophila* species of the *mulleri* complex and their hybrids. Rev Bras Genet 1982; 5: 31–50.

5. Bock IR. Interspecific hybridization in the genus *Drosophila.* Evol Biol 1984; 18:41–70.

6. Crow JF. Studies in *Drosophila* speciation: I. The *Drosophila mulleri* group. Genetics 1941; 26:146.

7. Dobzhansky TH. Genetics of the evolutionary process. New York: Columbia University Press; 1970.

8. Ehrman L, and Wasserman M. The significance of asymmetrical sexual isolation. In: Evolutionary biology, vol 21 (Hecht MK, Wallace B, and Prance GT, eds). New York: Plenum Press; 1987:1–20.

9. Fellows DP, and Heed WB. Factors affecting host selection in desert-adapted cactiphilic *Drosophila.* Ecology 1972; 53:850–858.

10. Gibson AC, and Nobel PS. The cactus primer. Cambridge, Massachusetts: Harvard University Press; 1986.

11. Heed WB. Ecology and genetics of desert *Drosophila.* In: Ecological genetics: the interfase (Brussard PF, ed). New York: Springer-Verlag; 1978:109–126.

12. Heed WB. The origin of *Drosophila* in the Sonoran Desert. In: Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system (Barker JSF, and Starmer WT, eds). Sydney, Australia: Academic Press; 1982:65–80.

13. Heed WB, and Mangan RL. Community ecology of Sonoran desert *Drosophila.* In: The genetics and biology of *Drosophila,* vol 3e (Ashburner M, Carson HL, and Thompson JN, eds). London: Academic Press; 1986: 311–345.

14. Hsu TC. The external genital apparatus of male Drosophilidae in relation to systematics. Austin: University of Texas Publication No. 4920, 1949.

15. Johnson WR. Chromosomal polymorphism in natural populations of the desert-adapted species *Drosophila mojavensis* (Ph.D. thesis). Tucson: University of Arizona; 1980.

16. Kircher HW. Chemical composition of cacti and its relationship to Sonoran Desert *Drosophila.* In: Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system (Barker JSF, and Starmer WT, eds). Sydney, Australia: Academic Press; 1982:143–158.

17. MacIntyre RJ, and Collier GE. Protein evolution in the genus *Drosophila.* In: The genetics and biology of *Drosophila,* vol 3e (Ashburner M, Carson HL, and Thompson JN, eds). London: Academic Press; 1986: 39–146.

18. Markow TA. Courtship behavior and control of reproductive isolation between *Drosophila mojavensis* and *D. arizonensis.* Evolution 1981; 35:1022–1027.

19. Markow TA, Fogleman JC, and Heed WB. Reproductive isolation in Sonoran Desert *Drosophila.* Evolution 1983; 37:649–652.

20. Markow TA, and Maveety N. More character displacement for reproductive isolation in the *mulleri* complex. Dros Infor Serv 1985; 61:115.

21. Mettler LE. *D. mojavensis baja,* a new form in the *mulleri* complex. Dros Infor Serv 1963; 38:57–58.

22. Mills LE, Batterham P, Alegre J, Starmer WT, and Sullivan DT. Molecular genetic characterization of a locus that contains duplicate Adh genes in *Drosophila mojavensis* and related species. Genetics 1986; 112: 295–310.

23. Patterson JT. Interspecific hybridization in the genus *Drosophila.* Austin: University of Texas Publication No. 4228. 1942.

24. Patterson JT. The *Drosophilidae* of the Southwest. Austin: University of Texas Publication No. 4313. 1943.

25. Patterson JT. A new type of isolating mechanism in *Drosophila.* Proc Natl Acad Sci USA 1946; 32:202–208.

26. Patterson JT, and Crow JF. Hybridization in the *mulleri* group of *Drosophila.* Austin: University of Texas Publication No. 4032. 1940.

27. Patterson JT, and Wheeler MR. Description of new species of the subgenera *Hirtodrosophila* and *Drosophila.* Austin: University of Texas Publication No. 4213. 1942.

28. Richardson RH. Phyletic species packing and the formation of sibling (cryptic) species clusters. In: Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system (Barker JSF, and Starmer WT, eds). Sydney, Australia: Academic Press; 1982:107–123.

29. Ringo J, Wood D, Rockwell R, and Dowse H. An experimental testing of two hypotheses of speciation. Am Nat 1985; 126:642–661.

30. Ruiz A, and Heed WB. Host plant specificity in the *Drosophila mulleri* species complex. J Anim Ecol 1988; 57:237–249.

31. Scriber JM. Evolution of feeding specialization, physiological efficiency and host races in selected *Papilionidae* and *Saturnidae.* In: Variable plants and herbivores in natural and managed systems (Denno RF, and McClure MS, eds). New York: Academic Press; 1983:373–412.

32. Starmer WT. A comparison of *Drosophila* habitats according to the physiological attributes of the associated yeast communities. Evolution 1981; 35:38–52.

33. Strong DR, Lawton JH, and Southwood R. Insects on plants: community patterns and mechanisms. Cambridge, Massachusetts: Harvard University Press; 1984.

34. Sturtevant AH, and Dobzhansky TH. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura,* and their use in the study of the history of the species. Proc Natl Acad Sci USA 1936; 22:448–450.

35. Templeton AR. Mechanisms of speciation: a population genetics approach. Annu Rev Ecol Syst 1981; 12:23–48.

36. Throckmorton LH. The problem of phylogeny in the genus *Drosophila.* Austin: University of Texas Publication No. 6205. 1962.

37. Throckmorton LH. The phylogeny, ecology and geography of *Drosophila.* In: Handbook of genetics, vol 3 (King RC, ed). New York: Plenum Press; 1975:421–469.

38. Throckmorton LH. Pathways of evolution in the genus *Drosophila* and the founding of the *repleta* group. In: Ecological genetics and evolution: the cactus-yeast-*Drosophila* model system (Barker JSF, and Starmer WT, eds). Sydney, Australia: Academic Press; 1982:33–47.

39. Vigneault G, and Zouros E. The genetics of asym-

metrical male sterility in *Drosophila mojavensis* and *Drosophila arizonensis* hybrids: interactions between the Y-chromosome and autosomes. Evolution 1986; 40: 1160–1170.

40. Vilela CR. A revision of the *Drosophila repleta* species group (*Diptera, Drosophilidae*). Rev Bras Entomol 1983; 27:1–114.

41. Wasserman M. Cytological studies of the *repleta* group. Austin: University of Texas Publication No. 5422. 1954.

42. Wasserman M. Cytological and phylogenetic relationships in the *repleta* group of the genus *Drosophila*. Proc Natl Acad Sci USA 1960; 46:842–859.

43. Wasserman M. Cytological studies of the *repleta* group of the genus *Drosophila*: V. The *mulleri* subgroup. Austin: University of Texas Publication No. 6205. 1962.

44. Wasserman M. Cytology and phylogeny of *Drosophila*. Am Nat 1963; 97:333–352.

45. Wasserman M. Evolution of the *repleta* group. In: The genetics and biology of *Drosophila*, vol 3b (Ashburner M, Carson HL, and Thompson JN, eds). London: Academic Press; 1982:61–139.

46. Wasserman M, and Koepfer HR. Character displacement for sexual isolation between *Drosophila mojavensis* and *Drosophila arizonensis*. Evolution 1977; 31: 812–832.

47. Wasserman M, and Koepfer HR. Does asymmetrical mating preference show the direction of evolution? Evolution 1980; 34:1116–1124.

48. Wharton LP. Analysis of the *repleta* group of *Drosophila*. Austin: University of Texas Publication No. 4228. 1942.

49. Wheeler MR. Are the new species described in DIS validly published? Dros Infor Serv 1981; 56:157–158.

50. Wheeler MR. The *Drosophilidae*: a taxonomic overview. In: The genetics and biology of *Drosophila*, vol 3a (Ashburner M, Carson HL, and Thompson JN, eds). London: Academic Press; 1981:1–97.

51. Wiley EO. Phylogenetics: the theory and practice of phylogenetic systematics. New York: Wiley-Interscience; 1981.

52. Zouros E. Genic differentiation associated with the early stages of speciation in the *mulleri* subgroup of *Drosophila*. Evolution 1973; 27:601–621.

53. Zouros E. The chromosomal basis of viability in interspecific hybrids between *Drosophila arizonensis* and *Drosophila mojavensis*. Can J Genet Cytol 1981; 23: 65–72.

54. Zouros E. The chromosomal basis of sexual isolation in two sibling species of *Drosophila*: *D. arizonensis* and *D. mojavensis*. Genetics 1981; 97:703–718.

55. Zouros E. A model for the evolution of asymmetrical male hybrid sterility and its implications for speciation. Evolution 1986; 40:1171–1184.

56. Zouros E, and D'Entremont CJ. Sexual isolation among populations of *Drosophila mojavensis*: a response to pressure from a related species. Evolution 1980; 34:421–430.

57. Zouros E, Lofdahl K, and Martin PA. Male hybrid sterility in *Drosophila*: interactions between autosomes and sex chromosomes in crosses of *D. mojavensis* and *D. arizonensis*. Evolution 1988; 42:1321–1331.