Description and Evolutionary Relationships of Two Species of the *Drosophila mulleri* Cluster (Diptera: Drosophilidae)

ANTONIO FONTDEVILA,[1] MARVIN WASSERMAN,[2] CARLES PLÁ,[1,3] LUISA PILARES,[4] ROSA DE ARMENGOL,[1] MARIA DEL PILAR SUYO,[4] ARMAND SANCHEZ,[1] JAIME VASQUEZ,[4] ALFREDO RUIZ,[1] AND JOSE LUIS GARCIA[1,3]

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ABSTRACT *Drosophila huaylasi* Plà & Fontdevila, n. sp., and *Drosophila nigrodumosa* Wasserman & Fontdevila, n. sp., are members of the *D. mulleri* cluster (*D. mulleri* complex) of the *D. repleta* species group. They are described here using morphological, reproductive, chromosomal, and genetic (allozymic) characters. Morphology of the male genitalia is a distinctive, but not unique, characteristic. All the described *D. mulleri* cluster species are homosequential, there being no variability in the polytene chromosomes. The metaphase chromosomes show minor interspecific differentiation in the length of the sex chromosomes. Allozyme differentiation is more informative and shows that *D. huaylasi* and *D. nigrodumosa* are closely related to each other. They are much closer to *D. mulleri* than to *D. aldrichi*. Postzygotic reproductive isolation is complete between either *D. huaylasi* or *D. nigrodumosa* and *D. aldrichi*, which accords with their genetic differentiation. On the other hand, in laboratory crosses with no choice, some interspecific gene exchange is possible among *D. mulleri*, *D. huaylasi*, and *D. nigrodumosa*, through backcrosses of fertile F_1 hybrid females. However, the three species appear to be allopatric and presumably no gene flow exists in nature.

KEY WORDS Insecta, *Drosophila*, genetic distance, reproductive isolation

THE *Drosophila mulleri* complex of the *D. repleta* species group contains several species that share and are homozygous for one or more unique inversions (Wasserman 1954). The distribution of these particular inversions among the species cannot be explained by simple allopatry. Rather, it was suggested (Wasserman 1982) that the ancestral species consisted of several geographically and cytologically defined subspecies which gave rise to clusters of species. One of these, the *D. mulleri* cluster, is comprised of four described species, *D. mulleri* Sturtevant, *D. aldrichi* Patterson & Crow, *D. wheeleri* Patterson & Alexander (see Wheeler [1959] for references to descriptions), and *D. mayaguana* Vilela (Vilela 1983).

A study of the cytology of these species has shown that they are closely related to each other, distinct from other species clusters, but has not yielded information upon which relationships among the species could be based. The species are homosequential (i.e., they appear to have identical polytene chromosomes). In addition, no variability of chromosomal rearrangements has been detected within the species (Wasserman 1982). The only cytological variability present is found in some minor interspecific differences in metaphase karyotypes. Neither these differences nor the degree of reproductive isolation has been sufficiently informative to construct even a tentative phylogeny within the *mulleri* cluster.

We have collected two new members of this cluster in South America. One was called "from Venezuela" (Wasserman 1982); the other occurs in Peru. Here we describe these species, *Drosophila nigrodumosa* (="from Venezuela") and *Drosophila huaylasi*, and present morphological, genetic, reproductive, and cytological evidence upon which we base a tentative phylogeny of these species. Fig. 1 shows the geographical distribution of *D. nigrodumosa*, *D. huaylasi*, *D. mulleri*, and *D. aldrichi*. The last two species also will be discussed in this paper.

Drosophila huaylasi Plà and Fontdevila, new species

Adult: external. *Male, female:* Arista with 5 branches, 3 dorsals and 2 ventrals, plus terminal fork. Antennae brown, slightly yellowish; base of second, third segments dark brown. Frons dark brown; orbits slightly dark with light areas in superior margin; ocellar triangle slightly lighter, with few hairs; ocellus slightly reddish with basal dark brown spot. Posterior orbital, postvertical, anterior

[1] Departamento de Genetica y Microbiologia, Universidad Autonoma de Barcelona, Bellaterra, Spain.
[2] Department of Biology, Queens College, Flushing, N.Y. 11367.
[3] Colegio Universitario de Girona, Universidad Autonoma de Barcelona, Girona, Spain.
[4] Departamento de Biologia, Universidad Mayor de San Marcos, Lima, Peru.

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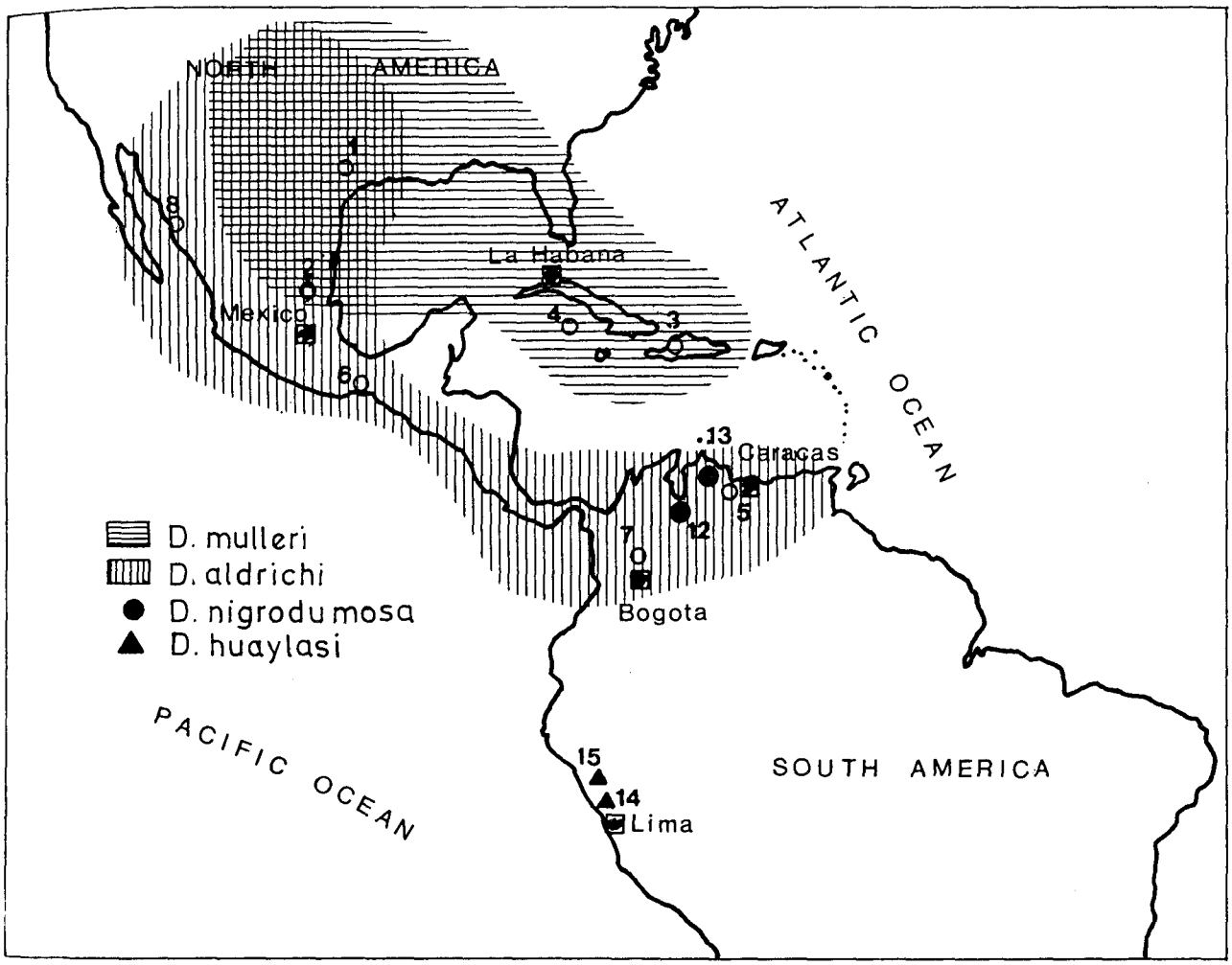

Fig. 1. Geographical distribution of *D. nigrodumosa, D. huaylasi, D. aldrichi,* and *D. mulleri.* Strains used in Nei's genetic distance are: (1) Lake Travis (*D. mulleri*); (2) Guayalejo (*D. mulleri*); (3) Petionville (*D. mulleri*); (4) Cayman Brac (*D. mulleri*); (5) Zuata (*D. aldrichi*); (6) Tehuantepec (*D. aldrichi*); (7) Tricolandia (*D. aldrichi*); (8) Las Bocas (*D. aldrichi*); (12) El Anis (*D. nigrodumosa*); (13) Quiragüe (*D. nigrodumosa*); (14) Quives (*D. huaylasi*); (15) Caraz (*D. huaylasi*). Strains 3, 7, 12, and 15 have been used for the reproductive isolation analysis.

vertical bristles arising from dark brown spots; ocellar, anterior, middle orbital and posterior vertical arising from lighter areas. Middle orbital about ½ length of the other two. One prominent oral bristle, second oral bristle weak, about ⅓ length of the first. Face yellowish brown, lighter than frons. Carina broadened below, sulcate, yellowish brown with dark spots along surface. Proboscis, palpi pale yellowish; maxillary palpus with one stout terminal bristle, two lateral bristles, few smaller weaker bristles; labial palpus with few long thin bristles. Cheeks yellowish gray with dark area in lowest margin of eye, their greatest width about ⅛ greatest diameter of eyes. Eyes red metallic with short black piles. Acrostical hairs in eight irregular rows. No prescutellars. Anterior scutellars slightly convergent. Mesonotum yellowish gray, pollinose, bristles, hairs arising from dark brown spots with tendency to fuse in the upper and in the middle parts, except immediately anterior to scutellum. Scutellum yellowish gray with dark brown X-shaped mark; bristles with basal dark brown spot. Pleurae yellowish gray with indistinct longitudinal dark band from propleurum to base of halteres, and with paler area near sternopleurals. Sterno index (i.e., ratio of length of anterior sternopleural bristle divided by length of posterior sternopleural bristle) about 0.8; middle sternopleural minute. Haltere pale yellow with dark spot laterally of the segments. Legs light yellowish without black spots; apical bristles on 1st, 2nd tibiae, preapicals on all three. Abdomen yellowish, 2nd to 6th tergites with medially interrupted apical dark brown bands which extend forward at interruption, lateral margins and angles of tergites. Last forward extensions widening at anterior margins connecting laterally with lateral extensions, thus enclosing irregular yellow area; in males, yellow lateral area often incompletely enclosed due to interruptions at both anterior and posterior angles of tergite. Wings clear, veins dark; apex of first costal section darker. Costal index about 2.9; fourth vein index about 1.9; 5X index about 1.3; 4c index about 0.9; M index about 0.5. Two well-developed bristles at apex of first costal section; third costal section

with heavy bristles on basal 0.29. Wing length of female, 2.4–2.6 mm; of male, 2.3–2.5 mm. Body length of female, 3.0–3.5 mm; of male, 3.0–3.2 mm.

Adult: Internal and genitalia. *Males:* Testes light yellow, becoming dark orange with age, with 2 inner and 2 outer coils. Penis apparatus as in Fig. 2. Genital arch of male with 2–3 bristles in upper third, 0–2 bristles in middle, and 12–16 in lower third. Surstylus with about 13 primary teeth, 8 secondary teeth, 8 marginal bristles (Fig. $3a_1$–$3a_4$). *Females:* Ventral receptable with about 20 loose coils.

Puparia. Light brown; each anterior spiracle with about 10–11 branches; horn index about 2.8.

Eggs. Four thin filaments.

Chromosomes. Autosomes consisting of 4 pairs of rods, 1 pair of dots, larger than in *D. nigrodumosa*. X chromosome a rod about twice as long as autosomes. Y chromosome a rod slightly shorter than autosomes.

Relationship, Distribution, and Ecology. *D. huaylasi* belongs to the *D. mulleri* cluster of the *D. mulleri* complex of the *D. repleta* species group. It is closely related to *D. mulleri* and *D. nigrodumosa* (see below), but differs considerably in distribution and ecology. So far, it has only been collected in Quives and Caraz, Peru. Both localities differ markedly in their ecological and topographic characterization. Quives is located at kilometer 69 on the road from Lima to Canta, at altitude about 1,100 m in the yunga region. This is a semiarid zone with sparse vegetation; several columnar cacti predominate, particularly *Neoraimondia* sp. and *Armathocereus* sp.

Seventeen samples of decaying cactus stems, rots (5 of *Armathocereus* and 12 of *Neoraimondia*) were brought to the laboratory. *D. huaylasi* emerged from two *Armathocereus* rots and one *Neoraimondia* rot. Previously, all *D. mulleri* cluster species were believed to breed on *Opuntia* exclusively (Patterson 1943, Patterson & Alexander 1952). *D. huaylasi* is an exception.

D. huaylasi has also been collected in Caraz, a high (altitude about 2,800 m) locality in a large fertile valley, "Callejón de Huaylas," (hence the species name) 60 km from Huaraz and 400 km north of Lima. This isolated valley belongs to the "Quechua country," in the middle of the Peruvian Andes and is flanked by two cordilleras (Negra and Blanca). Vegetation in this valley is diverse and luxurious, and cacti are well represented. Both columnar cacti and *Platyopuntia* species are present. Flies were collected here using conventional banana traps.

Type Material. The type locality is Caraz (Perú) (Fig. 1). HOLOTYPE: ♂, CARAZ: Callejon de Huaylas (Perú), 19-V-1984, A. Fontdevila, M. P. Suyo, and J. Vasquez. PARATYPES: Same data as holotype. The holotype and five paratypes (2 ♂♂, 3 ♀♀) are deposited in the American Museum of Natural History in New York. Other paratypes are in the National Drosophila Species Resource Center at Bowling Green State University.

Drosophila nigrodumosa Wasserman and Fontdevila, new species

Adult: external. *Male, female:* Arista with 8 branches; antennae dark tan. Frons chocolate brown, orbits and ocellar triangle lighter, pollinose; bristles arising from black spots. Middle orbital about ½ length of the other two. Second oral bristles weak, <½ length of the first. Carina broad below, sulcate. Palpae pale with two prominent bristles and several smaller ones. Face gray. Cheeks gray, their greatest width about ⅓ greatest diameter of eyes. Eyes red with short black pile. Acrostical hairs in eight rows; no prescutellars. Anterior scutellars convergent. Mesonotum gray, pollinose, bristles arising from dark brown spots, these spots partially fused to form two narrow irregular dark stripes, one on either side of dorsocentral line, stripes tending to fade posteriorly. Some fusion of spots at humerus. Scutellum dark brown; dark yellow margin divided into 5 areas by dark brown spots at base of the scutellar bristles. Pleurae gray with indistinct dark bands going from wing base to humerus, from base of halteres to fore coxae, and near sternopleurals. Sterno index about 0.9. Middle sternopleural about ¼ length of posterior. Legs brownish gray with indistinct dark band at distal ends of femora and near base of tibiae; apical bristles on first, second tibiae, preapical on all 3. Abdominal segments dark yellow, 2nd to 6th tergites with medially interrupted apical black band about ½ width of each tergite, with forward extensions at angle of tergites, forming solid lateral areas.

Wings clear, veins yellowish brown. Costal index about 2.6; 4th vein index ca. 2.0; 5X index about 1.3; 4c index about 1.1. Heavy bristles on basal 0.22 of 3rd section of costal vein. Body length of male, 2.4–3.1 mm; of female, 2.6–3.2 mm.

Adults: internal and genitalia. *Males:* Testes yellowish orange, turning darker with age, with two outer and two inner coils. Fig. 2 shows the penis apparatus. Genital arch of male with 2–4 bristles in upper third section, 1–2 bristles in the middle, and 10–12 in lower third; toe elongated and pointed. Surstylus with 10–11 teeth in primary row; about 24 teeth in irregular secondary rows; with 8 strong bristles; teeth and bristles, black giving the males the appearance of having black bushy genitalia; thus, the name, *Drosophila nigrodumosa,* black, bushy (Fig. $3b_1$–$3b_4$). *Females:* Ventral receptacle with about 15 loose coils.

Puparia. Tan; each anterior spiracle with 7–8 branches; horn index 2.6–3.0.

Eggs. Four thin filaments.

Chromosomes. Autosomes consisting of 4 pairs of rods and 1 pair of dots. X chromosome a rod about 1.3- to 1.5-times longer than autosomes. Y chromosome with 2 constrictions, dividing it into

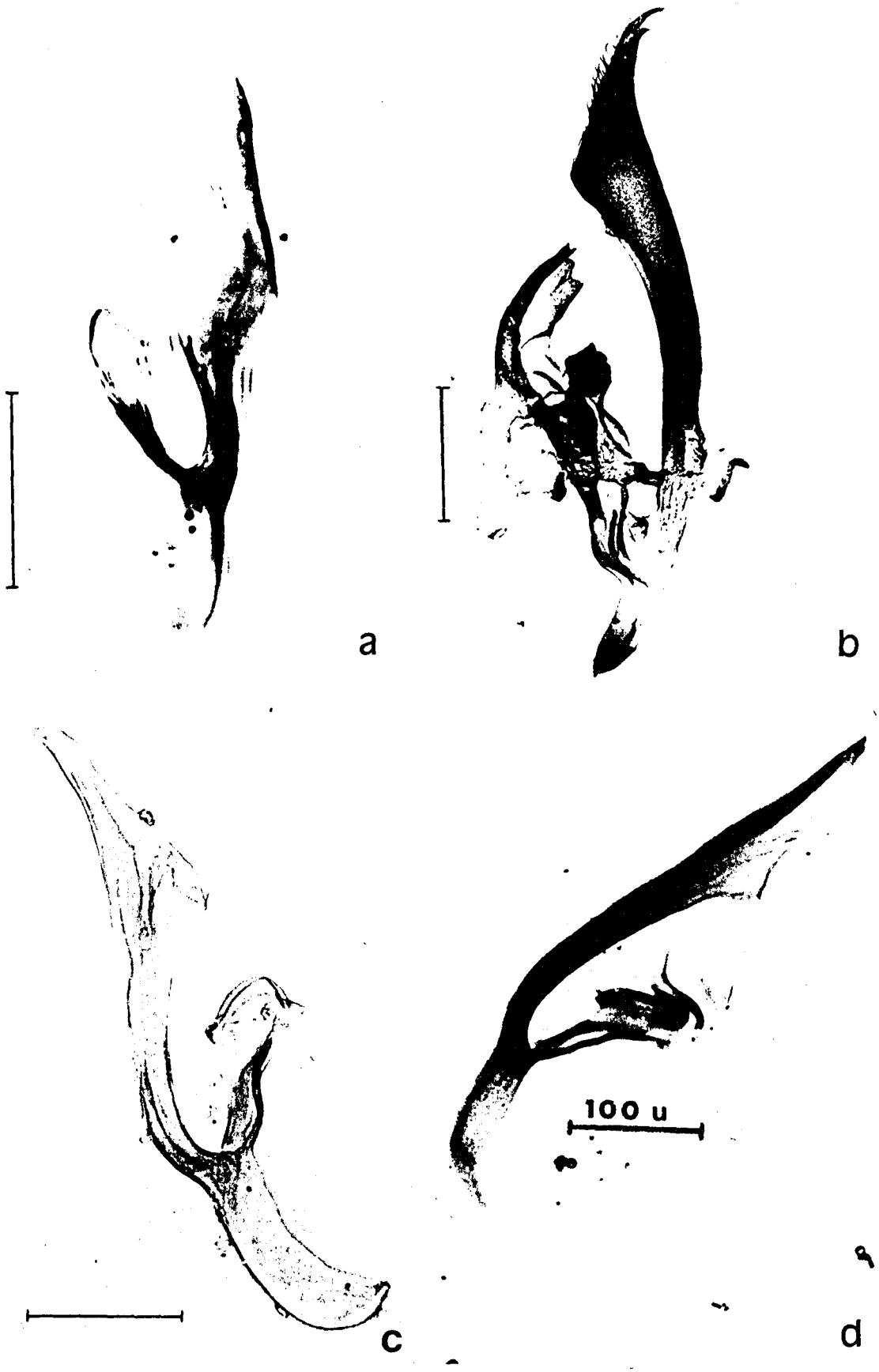

Fig. 2. Aedeagus morphology of *D. mulleri* (a), *D. aldrichi* (b), *D. huaylasi* (c), and *D. nigrodumosa* (d). Bar represents 100 μm. Notice that *D. huaylasi*, *D. nigrodumosa*, and *D. aldrichi* penis apparatuses are similar to each other, but different from that of *D. mulleri*.

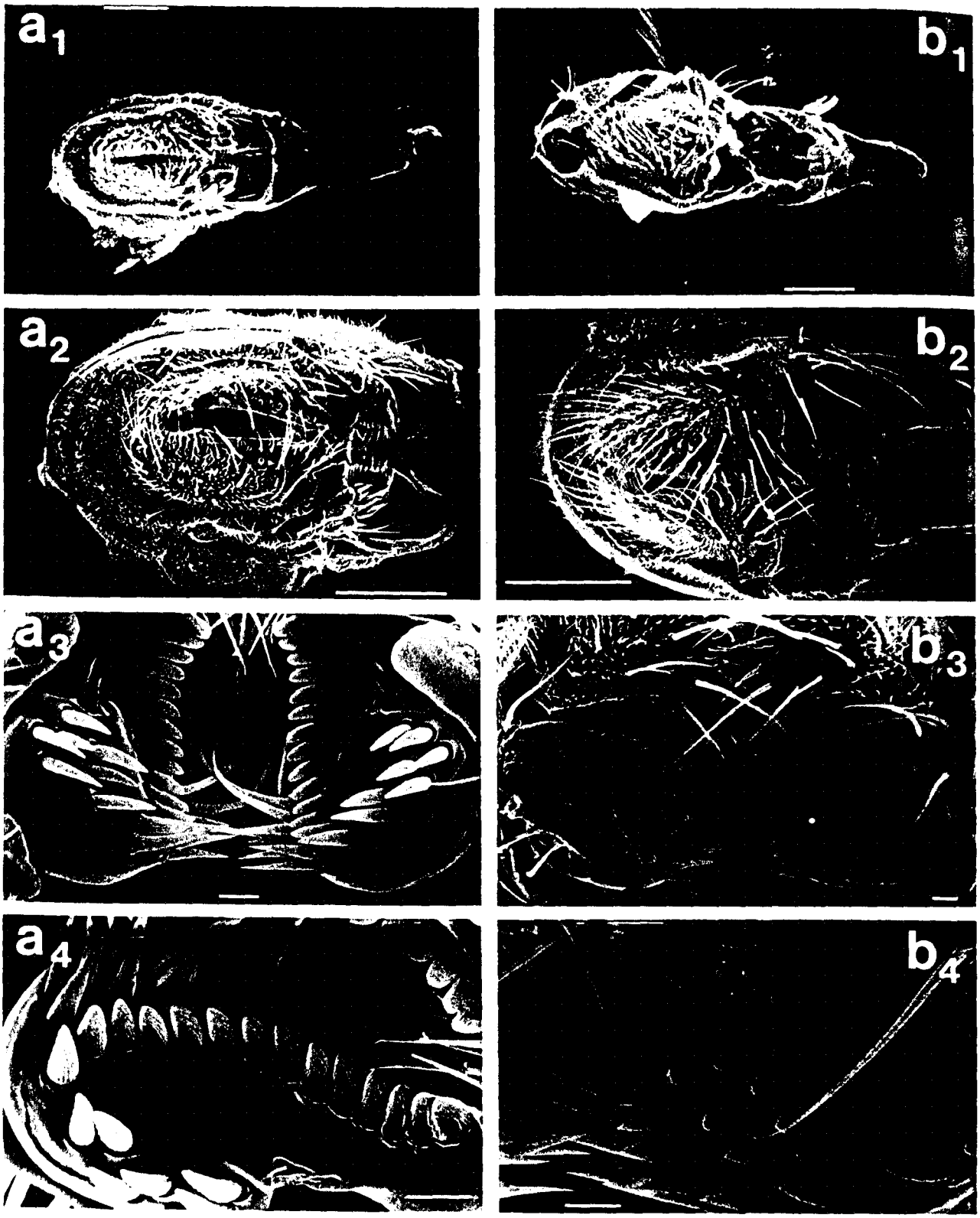

Fig. 3. Scanning electron micrographs of male genital morphology of *D. huaylasi* (a) and *D. nigrodumosa* (b). Epandrium general (a_1 and b_1) and close (a_2 and b_2) views. Surstylus general (a_3 and b_3) and close (a_4 and b_4) views. Notice the extreme difference in number of secondary teeth between the species. Bar in a_1, a_2, b_1, and b_2 represents 100 μm. Bar in a_3, a_4, b_3, and b_4 represents 10 μm.

3 more or less equal parts; total length of Y equal to that of autosomes.

Relationship, Distribution, and Ecology. *D. nigrodumosa*, originally cited as "from Venezuela" by Wasserman (1982), belongs to the *D. mulleri* cluster of the *D. mulleri* complex of the *D. repleta* species group. It is distributed in Venezuela and has been collected in El Anis, Quirague, and Sonare. El Anis is located south of Merida, 13.6 km south of Lagunillas, at kilometer 34.4 on the road

from Merida to El Vigia, in an arid pocket in the Sierra de Merida, where cacti of the genera *Ritterocereus* Backeberg, *Mammillaria* Haworth, *Melocactus* Link & Otto, and *Opuntia* are present. *O. wentiana* Britten & Rose and *O. elatior* Miller are present, and *D. nigrodumosa* was observed to emerge from the former. Quirague is situated at 1,100 m altitude in the Sierra de San Luis, close to kilometer 98 on the road from Coro to Churuguara. Cacti present are *Ritterocereus griseus* Haworth, *Subpilocereus repandus* Backeberg, *Pilosocereus lanuginosus* Byl. & Rowl., *Opuntia elatior*, and *O. wentiana*. *D. nigrodumosa* emerged from these two *Opuntia* species. Sonare is situated in the Estado Lara ranges, at 1,350 m altitude and 60 km southwest of Barquisimeto. At this locality *D. nigrodumosa* emerges from *O. elatior*.

Type Material. Type locality about 25 km south of Merida (Venezuela) on road to San Cristobal. M. Wasserman, type strain number 514.8. HOLOTYPE: 1 ♂. PARATYPES: 2 ♂♂, 3 ♀♀ (American Museum of Natural History, New York). Other paratypes are in the National *Drosophila* Species Resource Center at Bowling Green State University.

Morphological Differentiation

Male genitalia of *D. aldrichi*, *D. mulleri*, *D. huaylasi*, and *D. nigrodumosa* were studied by means of optical and electron microscopy. Although *D. wheeleri* and *D. aldrichi* differ in a number of characters of the adult external morphology (Patterson & Alexander 1952), their genitalia are reported to be identical (Vilela 1983). Therefore, *D. wheeleri* was not included in this study.

D. mulleri and *D. aldrichi* aedeagi are quite different from each other, whereas those of *D. huaylasi* and *D. nigrodumosa* are similar to each other, being intermediate between those of *D. mulleri* and *D. aldrichi*, yet more similar to those of the latter species (Fig. 2). The aedeagus of *D. huaylasi* is somewhat more robust than that of *D. nigrodumosa*.

The external genitalia are most informative (Fig. 3 and 4). The four species differ in the number of bristles in the lower part of the epandrium and in the size, shape, and number of secondary teeth on the surstylus. *D. aldrichi* has three bristlelike secondary teeth, whereas the other three species show a higher number of short and heavy teeth. Those of *D. nigrodumosa* being both black and numerous give the appearance of black, bushy genitalia, unique in the *D. repleta* species group (Fig. $3b_1$–$3b_4$, Table 1).

Genetic Differentiation

All described members of the *D. mulleri* cluster are homosequential, being fixed for the identical gene sequences in the polytene chromosomes of the larval salivary glands. This includes the four species discussed here, *D. mulleri*, *D. aldrichi*, *D. huaylasi*, and *D. nigrodumosa*, and also *D. wheeleri* (Wasserman 1982) and *D. mayaguana* (unpublished data). The metaphase chromosomes of the cluster species also are essentially the same, being four pairs of autosomal rods, a pair of dots, and a pair of sex chromosomes. There are minor differences in the relative lengths of the X and Y chromosomes. Thus, among these six species, there are no cytologically distinguishing characteristics that could help us determine relationships within the species cluster. On the other hand, allozyme differentiation does show relationships. Recently, Armengol et al. (unpublished data) have computed Nei's (1972) genetic distances using 22 allozyme loci from several strains of *D. mulleri*, *D. aldrichi*, *D. huaylasi*, and *D. nigrodumosa*. Table 2 shows a summary of these results. *D. huaylasi*, *D. nigrodumosa*, and *D. mulleri* are genetically close to each other and are much differentiated from *D. aldrichi*.

Reproductive Isolation

Interspecific crosses were performed using some of the species strains shown in Fig. 1. Crosses were set up in cultures of 10 males and 10 females of each strain except in the crosses between *D. mulleri* and *D. nigrodumosa*, where single pairs were tested. Hybrid progenies were scored for number and sex. In those crosses yielding abundant F_1 progenies, the F_1 progenies were both backcrossed and crossed among themselves in an attempt to obtain an F_2. Where F_1 progeny were few in numbers, only backcrosses to the parental species were attempted. *D. aldrichi* is genetically completely isolated from the other species: females yield no offspring when exposed to males of *D. mulleri* (Wasserman 1982), *D. huaylasi*, or *D. nigrodumosa* (Table 3). Reciprocal crosses yield only sterile offspring: both males and females when crossed to *D. mulleri* (Wasserman 1982); only males when crossed to *D. huaylasi* or *D. nigrodumosa*. However, *D. mulleri*, *D. nigrodumosa*, and *D. huaylasi* are potentially capable of exchanging genes. Although *D. huaylasi* males produce no offspring when crossed to females of the other two species, the reciprocal crosses yield fertile F_1 females, many in the cross with *D. mulleri*. Reciprocal crosses between *D. mulleri* and *D. nigrodumosa* produce few viable offspring, but those F_1 females resulting from the *D. mulleri* female × *D. nigrodumosa* male cross are fertile.

Discussion

The *D. mulleri* complex, which includes the *D. mulleri* cluster, originally consisted of species whose distribution appeared to be centered in the North American deserts of Mexico and the southwestern United States (Wasserman 1954). This was true of the original species of the cluster, *D. mulleri*, *D. aldrichi*, and *D. wheeleri*. More recent collections

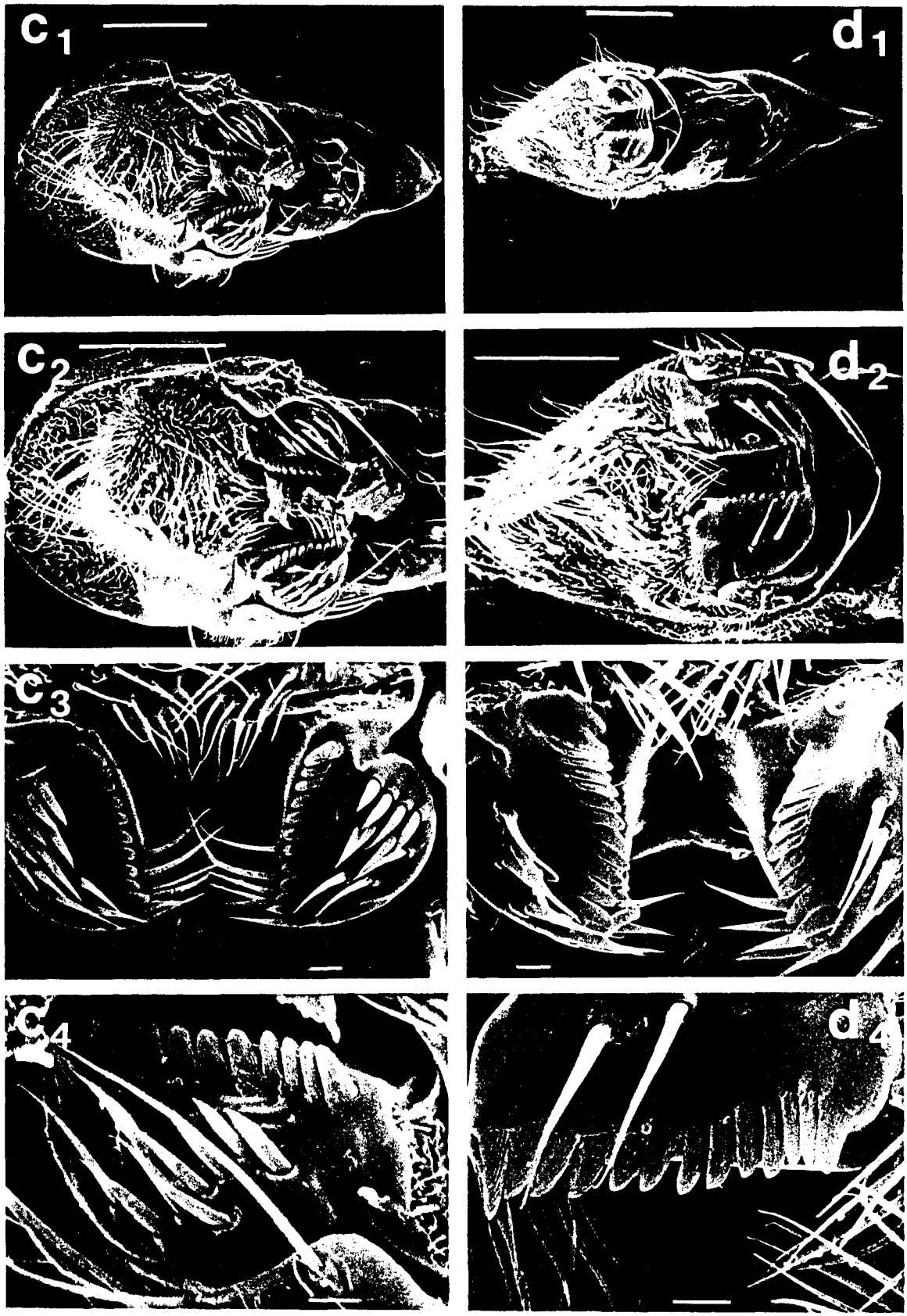

Fig. 4. Scanning electron micrographs of male genitalia morphology of *D. mulleri* (c) and *D. aldrichi* (d). Epandrium general (c_1 and d_1) and close (c_2 and d_2) views. Surstylus general (c_3 and d_3) and close (c_4 and d_4) views. Notice the differences in secondary teeth among all *D. mulleri* cluster species. Bar in c_1, c_2, d_1, and d_2 represents 100 μm. Bar in c_3, c_4, d_3, and d_4 represents 10 μm.

Table 1. Tooth and bristle number in the external genitalia of several species of the *D. mulleri* cluster

Species	Epandrium		Surstylus		
	Lower	Upper	Primary teeth	Secondary teeth	Marginal bristles
D. aldrichi[a]	9	2	12	3	9
D. mulleri[a]	8	1	10	10	8
D. huaylasi	12–16	2–3	13	8	8
D. nigrodumosa	10–12	2–4	10–11	24	8

[a] Data from Vilela (1983).

have both extended their distributions and increased the number of species in the cluster. *D. wheeleri* is still limited to a relatively small region in Baja California, Mexico, and the southern part of California, U.S.A. *D. aldrichi* not only occurs in Texas and throughout the lowlands of Mexico, but has been collected in El Salvador, Colombia, Brazil, and Australia (Wasserman 1982; Ruiz & Fontdevila 1981). *D. mulleri*, which occurs from Nebraska south to the northeastern part of Mexico, also ranges widely throughout the Bahamas and the Greater Antilles (Wasserman 1982; Wasserman & Heed, unpublished data). Both *D. nigrodumosa* and *D. huaylasi*, described here, are South American species. Three other species in this cluster, not discussed here, are found in the Caribbean: *D. mayaguana*, which is found in the Bahamas and Cuba; and two new undescribed species, which are found on Jamaica, Hispaniola, and Cuba (Wasserman & Heed, unpublished data). Thus, these recent findings have not only expanded the number of species in the cluster, but also its range, which strongly suggests that the site of origin of the species cluster may be in the Caribbean or in South America.

In their abdominal color pattern and in the shape of their aedeagus, *D. nigrodumosa* and *D. huaylasi* appear to be closer to *D. aldrichi* than to *D. mulleri*. These superficial similarities are not corroborated in the male genitalia, where characters of epandrium and surstylus morphology (such as the number and shape of surstylus secondary teeth) separate *D. aldrichi* from the other three species (Table 1, Fig. 3 and 4). This latter division is corroborated by the crosses, which showed that *D. aldrichi* is completely incompatible with the other three species, while they, in turn, are capable of producing at least some viable, fertile offspring among themselves under laboratory conditions. Thus, although the three species show enough reproductive isolation to be considered true species, they can exchange genes. Nevertheless, these species are allopatric and their disjunct distribution suggests that there is no interspecific interbreeding in nature.

The allozyme differentiation among the *D. mulleri* cluster species is in the range between semispecies and sibling species according to most *Drosophila* studies (MacIntyre & Collier 1986).

Table 2. Nei's mean genetic distances and standard deviations (D ± SD) among closely related *D. mulleri* cluster species[a]

Species comparison	D ± SD
D. mulleri versus *D. huaylasi*	0.362 ± 0.029
D. mulleri versus *D. nigrodumosa*	0.538 ± 0.046
D. huaylasi versus *D. nigrodumosa*	0.321 ± 0.023
D. aldrichi versus *D. huaylasi*	1.080 ± 0.069
D. aldrichi versus *D. nigrodumosa*	1.171 ± 0.113
D. aldrichi versus *D. mulleri*	1.051 ± 0.082

[a] Data from Armengol et al. (unpublished).

However, the few studies on allozyme differentiation in the *D. repleta* group that have been carried out show contrasting ranges in the values of genetic distance between species. Zouros (1973), using 11 allozyme loci, found a genetic distance of only 0.124 between *D. mulleri* and *D. aldrichi*, a level of differentiation which he pointed out was remarkably small. Moreover, it contrasts with the value of 1.051 in our study where 20 loci were examined (Armengol et al., unpublished data). Richardson & Smouse (1976), using average relative mobilities instead of mobility frequencies, found great differences between *D. mulleri* and *D. aldrichi*, a result in agreement with our data. Sanchez (1986), studying the *D. martensis* complex of the *D. mulleri* subgroup, and using the same set of allozyme loci that Armengol et al. used, found Nei's genetic distances very similar to those obtained in other *Drosophila* groups such as *D. willistoni* (Ayala 1975), although there appears to be variability among species groups (MacIntyre & Collier 1986). This difference in absolute distance values between our results and those of Zouros may be attributed to the use of different sets of allozyme loci in the two studies. Whatever the reasons for the discrepancy between the absolute values obtained by Zouros and Armengol et al., the relative values within the Armengol et al. study clearly show that *D. aldrichi* is genetically distinct from the other three species.

Zouros (1973), Richardson & Smouse (1976), and Richardson et al. (1977) have argued that host plant

Table 3. Offspring numbers in crosses between *D. huaylasi*, *D. nigrodumosa*, *D. mulleri*, and *D. aldrichi*, members of the *D. mulleri* cluster

Cross		Adult offspring		
♀	♂	*n*	♀	♂
D. huaylasi	*D. nigrodumosa*	5	16F	13S
D. nigrodumosa	*D. huaylasi*	5	0	0
D. aldrichi	*D. huaylasi*	5	0	0
D. huaylasi	*D. aldrichi*	5	0	36 S
D. aldrichi	*D. nigrodumosa*	5	0	0
D. nigrodumosa	*D. aldrichi*	5	0	78 S
D. mulleri	*D. nigrodumosa*	63*	4 F	7 S
D. nigrodumosa	*D. mulleri*	56*	4 S	3 S
D. mulleri	*D. huaylasi*	5	0	0
D. huaylasi	*D. mulleri*	5	216 F	243 S

n, number of mass replicas of 10 pairs each or the number of replicas of single pairs (*). F and S, fertile and sterile, respectively.

specificity is a selective agent for allozyme genetic variation. Using different allozyme systems, they examined 10 North American *D. mulleri* complex species. Eight of these, members of three *D. mulleri* clusters, but all *Platyopuntia* breeders, showed little differentiation. In contrast, the two members of the *D. mojavensis* cluster, *D. mojavensis* and *D. arizonensis*, both columnar cactus breeders, were distinct from the *Platyopuntia* breeders. Moreover, *D. mulleri* and *D. aldrichi*, which produce only sterile offspring but are found together and can be reared from the same *Platyopuntia* fruit, are electrophoretically more similar to each other than are *D. mojavensis* and *D. arizonensis*, which produce fertile offspring but are ecologically distinct. Our data, however, do not support this as a universal phenomenon. Among the four species listed in Table 2, *D. huaylasi* breeds in columnar cacti, and the other three are *Platyopuntia* breeders. Yet, *D. huaylasi* is not more distant from *D. aldrichi* than are the other two *Platyopuntia* breeders. Perhaps more interesting, *D. huaylasi* is closer to *D. mulleri* and to *D. nigrodumosa* than these two *Platyopuntia* breeders are to each other.

Taken together, genetic (allozyme), reproductive, and morphological data indicate that *D. huaylasi* and *D. nigrodumosa* are distinct species closely related to *D. mulleri*, with which they form a phylogenetic unit distinct from *D. aldrichi*.

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