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REPRODUCTIVE RELATIONSHIPS AMONG TEN SPECIES OF THE *DROSOPHILA REPLETA* GROUP FROM SOUTH AMERICA AND THE WEST INDIES

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In a comprehensive review of the genus *Drosophila,* Bock (1984) listed 266 cases of interspecific hybridization in the laboratory and also 8 reported cases of hybridization in nature. These data show that interspecific hybridization in this genus is not at all a rare phenomenon, at least under the artificial conditions of the laboratory. But this review also shows clearly that the production of viable hybrids is restricted to the most closely related species. When large species groups have been subdivided into subgroups using classical taxonomic criteria, crosses have in most cases been accomplished only within subgroups, and in some cases only within complexes within subgroups. This makes sense because the ability to produce hybrids must require that the two species have similar genetic constitutions. In this study, however, we will show that frequent successful interspecific crosses occur among several species of the *Drosophila repleta* group that are distantly related according to their current phylogenetic classification.

The phylogenetic relationships among the species of the *D. repleta* species group have been traced back by a combination of morphological and cytological analyses (Wasserman 1982 and many earlier references). Among the six main subgroups, the most complex evolutionary situation is that of the *mulleri* subgroup, which comprises 40 species grouped into five species complexes plus four miscellaneous forms. The *stalkeri* complex comprises two species inhabiting Florida and the West Indies: *D. stalkeri* and *D. richardsoni* (Wasserman 1982; Vilela 1983). The *mulleri* complex, the largest of the *mulleri* subgroup, has been further subdivided into six clusters of closely related species. Two of the six clusters are endemic to South America (except for the colonizing species *D. buzzatii,* which is subcosmopolitan). The *martensis* cluster consists of four species found in the deserts of northern Colombia and Venezuela: *D. martensis, D. uniseta, D. starmeri,* and *D. venezolana* (Wasserman 1982; Wasserman et al. 1983). Another four species have been grouped into the *buzzatii* cluster: The Brazilian *D. serido* and *D. borborema* (Sene et al. 1982), and *D. buzzatii* and *D. koepferae,* which are found chiefly in Argentina and

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TABLE 1. List of the *Drosophila* species and stocks used in this study. The field collectors (except for the Bowling Green stocks) were A. Ruiz and A. Fontdevila for the *buzzatii* cluster species; H. Cerda, M. Benado, and A. Fontdevila for the *martensis* cluster species; W. B. Heed and M. Wasserman for the *stalkeri* complex species.

Taxon	Species	Geographical origin of stocks
buzzatii cluster	*D. borborema*	Cafarnaum, Bahia, Brazil (Bowling Green stock no. 15081-1281.0).
	D. buzzatii	San Luis, Argentina; San Lorenzo, Argentina; Los Negros, Bolivia; Adeje, Canary Islands, Spain.
	D. koepferae	San Luis, Argentina (two stocks); San Isidro, Bolivia.
	D. serido	Cafarnaum, Bahia, Brazil (Bowling Green stock no. 15081-1431.4).
martensis cluster	*D. martensis*	Guaca, Venezuela.
	D. uniseta	Oricao, Venezuela; La Boca, Venezuela.
	D. starmeri	Guaca, Venezuela; Oricao, Venezuela (two stocks); El Anís, Venezuela; Mal País, Curaçao, Netherlands Antilles.
	D. venezolana	Guaca, Venezuela; Oricao, Venezuela; Piritu, Venezuela; Prudencio, Venezuela; Mal País, Curaçao, Netherlands Antilles.
stalkeri complex	*D. stalkeri*	St. Petersburg, Florida (Bowling Green stock no. 15081-1451.0); Discovery Bay, Jamaica; Little Cayman, Cayman Islands; Grand Cayman, Cayman Islands.
	D. richardsoni	Fox's Bay, Montserrat; Spanish Point, Montserrat; Beef Island, Tortola; Biras Creek, Virgin Gorda.

Bolivia (Wasserman 1982; Wasserman and Richardson 1987; Fontdevila et al. 1988). With the extant information, the species in the *stalkeri* complex are not closely related to those in the *martensis* and *buzzatii* clusters (Wasserman 1982). However, their apparent morphological similarities in the male genitalia (Vilela 1983) suggested that they could be closer relatives. Their frequent interspecific hybridization, as shown in this study, reinforces this observation. The data presented here show that 41 of the 90 possible interspecific crosses (45.6%) attempted among the 10 species of the *stalkeri* complex and the *buzzatii* and *martensis* clusters produced hybrid progeny. Among the successful combinations, 12 of 26 (46.2%) took place between species within the same cluster, 13 of 32 (40.6%) between species in different clusters, and 16 of 32 (50%) between species in different complexes. These results are discussed in relation to the proposed phylogenetic relationships among the 10 species.

MATERIALS AND METHODS

Thirty strains of the 10 species were used in the hybridization tests (table 1). Their geographical origin is shown in figure 1. Several stocks of *Drosophila starmeri, D. buzzatii,* and *D. koepferae* (formerly known as *D. serido* from Argentina; Fontdevila et al. 1988) were used because each of these three species is highly polymorphic for chromosome inversions, and there is evidence for a certain degree of genetic differentiation among populations of different geographical origin (Ruiz and Fontdevila 1981; Fontdevila et al. 1988). Although the Brazilian species *D. serido* probably consists of several partially iso-

FIG. 1. Geographical origin of the stocks used in this study. (a) St. Petersburg; (b) Grand Cayman; (c) Little Cayman; (d) Jamaica; (e) Tórtola; (f) Virgin Gorda; (g) Montserrat; (h) El Anís; (i) Prudencio; (j) Curaçao; (k) La Boca; (l) Oricao; (m) Piritu; (n) Guaca; (o) San Isidro; (p) Los Negros; (q) San Lorenzo; (r) San Luis; (s) Cafarnaum.

lated subspecific taxa (Sene et al. 1988), only one strain was available to us and was included in the study.

We tested 372 out of the 900 possible crosses among the 30 strains, including 28 intrastrain crosses as controls. Two to 17 replicates (usually 5) were set up for each strain combination as follows. Ten pairs of virgin flies, 4 to 9 d old, were placed in a vial with 25 cc of fresh culture medium (David 1962). After 6 d, they were transferred to a new vial with fresh food where they remained for 10 more days, and they were discarded thereafter. Vials were carefully inspected for the presence of larvae and pupae, and all the emerged adults were sexed and males and females separately counted.

The fertility of the F_1 adults was tested in several ways. When several were available, they were mass crossed to test for F_2 yield. When the number of F_1 individuals was very limited or no F_2 progeny was produced, males and females were separately studied. A sample of F_1 females were backcrossed in mass with males of at least one, and frequently both, parental strains. They were scored as fertile if any offspring, no matter how few, was obtained. However, males were studied by combining morphological analysis and crossability. Mature males were dissected and regarded as sterile if they showed noticeably reduced testes and absence of germinal tissue. Only when the size or microscopic appearance of the testes was normal or nearly normal were they backcrossed in mass with virgin females from the parental stocks.

Results

Intraspecific Crosses.—All intraspecific crosses produced abundant (usually more than 100 adults per vial) and fertile progeny. The only single exception was found in *Drosophila starmeri.* When the strains from Oricao and El Anís in western Venezuela (see fig. 1) were crossed to that from Guaca in eastern Venezuela, very few or no F_2 adults were obtained, no matter the direction of the original cross. These results corroborate those of Ruiz and Fontdevila (1981), which indicated the existence of two geographical races (western and eastern) in this species.

Interspecific Crosses within the martensis Cluster.—In general, interspecific crosses within the *martensis* cluster did not produce hybrids (table 2A). Only the cross between *D. starmeri* males and *D. venezolana* females yielded some progeny, although the number of F_1 adults was usually quite low. The reciprocal cross produced only larvae but Ruiz and Fontdevila (1981) have reported sterile males and fertile females in the F_1 of this cross. When crossed to *D. starmeri* males, *D. venezolana* females of the strain from Piritu in eastern Venezuela produced more hybrids than those of the strains from western Venezuela. Ruiz and Fontdevila (1981) also reported a high degree of hybridization in similar crosses using another *D. venezolana* strain from eastern Venezuela (La Esmeralda, Sucre). Thus, there seem to be genetical differences among the *D. venezolana* strains in relation to their geographical origin.

Although we have not studied *D. martensis* and *D. uniseta* as extensively as *D. starmeri* or *D. venezolana,* absence of F_1 hybrids in crosses among *D. martensis, D. uniseta,* and *D. starmeri* have also been reported by other authors (Wasserman et al. 1973; Wasserman and Koepfer 1979). Thus, the low crossability within the cluster does not seem to be a consequence of the particular strains used in this study.

Interspecific Crosses within the buzzatii Cluster.—A very different situation is found within the *buzzatii* cluster where 10 of 12 interspecific combinations produced hybrids and in five cases F_1 females were partially fertile (table 2B). The species that hybridized most readily was *D. koepferae*: all five cases producing fertile F_1 females involved this species. In both reciprocal crosses between this species and *D. serido,* the strain from San Isidro in Bolivia produced many more hybrids than those from Argentina, in good agreement with the observations of Fontdevila et al. (1988). The species that offered the most difficulties was *D. buzzatii.* Relatively few F_1 hybrids were obtained when *D. buzzatii* males were crossed with females of the other three species, and virtually no adult offspring were recovered when *D. buzzatii* females were tested. In general, the results were similar to those previously obtained by other authors (Wasserman et al. 1983; Fontdevila et al. 1988). It is worth noting, however, the production of sterile males and females in two combinations with negative results in these previous studies: *D. buzzatii* males × *D. borborema* females and *D. buzzatii* males × *D. serido* females.

Interspecific Crosses within the stalkeri Complex.—No hybrids were produced in either of the two reciprocal crosses between *D. stalkeri* and

TABLE 2. Summary of the results obtained in the hybridization tests among 10 species of the *Drosophila mulleri* subgroup from South America and the Caribbean. N^+, number of vials producing progeny. *N*, number of vials set up. C (%), crossability, percentage of vials with offspring. M, F, males and females; their total number and fertility (F) or sterility (S) are given. *P*, productivity, number of F_1 hybrids per crossed female.

Cross	♀♀ × ♂♂					♂♂ × ♀♀				
	N^+/N	C (%)	M	F	*P*	N^+/N	C (%)	M	F	*P*
A. Interspecific crosses within the *martensis* cluster										
martensis × *uniseta*	0/10	—	—	—	—	0/9	—	—	—	—
martensis × *starmeri*	0/5	—	—	—	—	0/10	—	—	—	—
martensis × *venezolana*	0/5	—	—	—	—	0/5	—	—	—	—
uniseta × *starmeri*	0/5	—	—	—	—	0/5	—	—	—	—
uniseta × *venezolana*	0/5	—	—	—	—	0/5	—	—	—	—
starmeri × *venezolana*	4/50	8.0	LARVAE		—	22/66	33.3	347S	182F	0.8
B. Interspecific crosses within the *buzzatii* cluster										
borborema × *buzzatii*	3/19	15.8	12S	16S	0.1	1/20	5.0	—	1	<0.1
borborema × *koepferae*	9/11	81.8	177S	218F	3.6	11/16	68.8	223S	246F	2.9
borborema × *serido*	1/12	8.3	LARVAE		—	0/13	—	—	—	—
buzzatii × *koepferae*	1/48	2.1	1 LARVA		—	20/45	44.4	149S	254F	0.9
buzzatii × *serido*	0/30	—	—	—	—	6/23	26.1	29S	16S	0.2
koepferae × *serido*	16/24	66.6	706S	899F	6.7	20/21	95.2	1147S	943F	10.0
C. Interspecific crosses within the *stalkeri* complex										
stalkeri × *richardsoni*	0/22	—	—	—	—	0/18	—	—	—	—
D. Interspecific crosses between the *martensis* and *buzzatii* clusters										
borborema × *martensis*	0/5	—	—	—	—	0/6	—	—	—	—
borborema × *uniseta*	0/5	—	—	—	—	0/5	—	—	—	—
borborema × *starmeri*	6/18	33.3	82S	105S	1.0	0/20	—	—	—	—
borborema × *venezolana*	9/22	40.9	19S	20S	0.2	0/16	—	—	—	—
buzzatii × *martensis*	1/10	10.0	—	1	<0.1	0/14	—	—	—	—
buzzatii × *uniseta*	0/11	—	—	—	—	0/13	—	—	—	—
buzzatii × *starmeri*	0/66	—	—	—	—	0/50	—	—	—	—
buzzatii × *venezolana*	3/52	5.8	7S	11F	<0.1	0/56	—	—	—	—
koepferae × *martensis*	2/10	20.0	4S	3S	<0.1	0/7	—	—	—	—
koepferae × *uniseta*	1/10	10.0	2S	2S	<0.1	0/10	—	—	—	—
koepferae × *starmeri*	37/37	100	1672S	2060F	10.1	13/40	32.5	62S	81F	0.4
koepferae × *venezolana*	35/53	66.0	254S	284F	1.0	3/52	5.8	7S	6F	<0.1
serido × *martensis*	0/12	—	—	—	—	0/10	—	—	—	—
serido × *uniseta*	0/5	—	—	—	—	0/7	—	—	—	—
serido × *starmeri*	10/10	100	660S	873F	15.3	1/26	3.8	1S	—	<0.1
serido × *venezolana*	6/18	33.3	7S	12S	0.1	0/23	—	—	—	—
E. Interspecific crosses between the *stalkeri* complex and the *martensis* and *buzzatii* clusters										
borborema × *stalkeri*	0/10	—	—	—	—	8/13	61.5	42S	41S	0.6
borborema × *richardsoni*	0/9	—	—	—	—	2/10	20.0	5S	2S	0.1
buzzatii × *stalkeri*	0/30	—	—	—	—	1/30	3.3	LARVAE		—
buzzatii × *richardsoni*	2/19	10.5	2S	1S	<0.1	17/30	56.7	92S	129F	0.7
koepferae × *stalkeri*	0/19	—	—	—	—	3/20	15.0	4S	5S	<0.1
koepferae × *richardsoni*	3/13	23.0	8S	5F	0.1	4/21	19.0	2S	6S	<0.1
serido × *stalkeri*	0/6	—	—	—	—	3/8	37.5	—	5S	0.1
serido × *richardsoni*	1/4	25.0	8S	9F	0.4	1/5	20.0	—	1S	<0.1
martensis × *stalkeri*	0/10	—	—	—	—	0/10	—	—	—	—
martensis × *richardsoni*	7/10	70.0	6S	34S	0.4	0/10	—	—	—	—
uniseta × *stalkeri*	0/14	—	—	—	—	0/10	—	—	—	—
uniseta × *richardsoni*	0/12	—	—	—	—	0/10	—	—	—	—
starmeri × *stalkeri*	0/28	—	—	—	—	6/36	16.7	10S	9S	0.1
starmeri × *richardsoni*	0/26	—	—	—	—	6/24	25.0	6S	9F	0.1
venezolana × *stalkeri*	0/31	—	—	—	—	5/36	13.9	2S	7S	<0.1
venezolana × *richardsoni*	0/22	—	—	—	—	18/30	60.0	206S	213S	1.4

D. richardsoni (table 2C). This confirms previous information on this pair of species (Wasserman 1982).

Interspecific Crosses between the martensis and buzzatii Clusters.—The species of the *martensis* cluster yielded progeny often when crossed with those of the *buzzatii* cluster, but there was a clear asymmetry in the production of hybrid offspring (table 2D). Ten of 16 interspecific combinations rendered F_1 when males from a *martensis* cluster species were crossed with females from a *buzzatii* cluster species and in four cases F_1 females were partially fertile. However, when the *martensis* cluster species provided the female parent, only 3 of 16 interspecific combinations produced hybrids, and F_1 females were fertile in two of them. Clear differences in crossability among the four species in each cluster were observed. *D. koepferae* produced more hybrids than any of the other three *buzzatii* cluster species, an observation that parallels the results of the intracluster crosses. In addition, *D. starmeri* and *D. venezolana* produced more hybrids than *D. martensis* or *D. uniseta*. It is very significant, however, that even the latter two species, which did not yield any hybrids in crosses within their own cluster, were able to produce a few hybrids in crosses with the *buzzatii* cluster species. Finally, it is worth mentioning that males of the two *D. starmeri* races did not behave in an identical manner when crossed to females of the *buzzatii* cluster species. Thus, in crosses with *D. borborema* females, the strain from Guaca in eastern Venezuela was the only one to produce adults, whereas in crosses with *D. koepferae* or *D. serido* females, the stocks from western Venezuela were usually the most productive.

Interspecific Crosses between the stalkeri Complex and the martensis and buzzatii Clusters.—Females of the *stalkeri* complex produced hybrids in all four combinations with males of *D. starmeri* and *D. venezolana,* and at least in one case F_1 females were partially fertile (table 2E). In contrast, they did not produce progeny with males of either *D. martensis* or *D. uniseta*. Only one of the reciprocal crosses yielded progeny.

Eleven of 16 interspecific crosses between the *buzzatii* cluster and the *stalkeri* complex produced hybrids (table 2E). The most successful crosses were those involving females of *D. richardsoni* or *D. stalkeri* and males of the *buzzatii* cluster species. Another important point is that *D. stalkeri* produced only sterile adults of both sexes in the F_1, whereas three crosses with *D. richardsoni* yielded fertile F_1 females. In fact, their fertility was low, only a few individuals being recovered in the backcross, but nevertheless this fact indicates a high genetic affinity among *D. richardsoni* and the *buzzatii* cluster species.

Discussion

The most striking result of this study is the high level of experimental hybridization found among these 10 *Drosophila* species. This result was relatively unexpected given that the species are not phylogenetically very close (see below). The results of intraspecific hybridization tests depend to a certain extent upon two factors: the experimental method and the amount of effort invested in the crosses and the geographical origin of the stocks used. The amount of genetic variability in the strains used in the crosses (which in turn depend on the number of founders and the time elapsed in the laboratory), the number of flies allowed to mate in each vial, and the number of replicates set up may have an influence in the results of the hybridization tests and explain at least in part the different results sometimes obtained by different authors. We do not think, however, that these factors explain our unexpected results. The experimental method used here was comparable to those found in the literature. The effort in this work was considerable (more than 35,000 flies were used as parents in the interspecific crosses alone) but was not concentrated particularly on the successful crosses. In addition, in those cases where information was already available, our results agreed in general with those previously reported.

In our study, there was usually a good correlation among the crossabilities of the different strains of each species. Hence, in table 2 only the pooled results have been presented. A few exceptions, however, have been already noted. *Drosophila starmeri* consists of two geographical races, western and eastern, which are partially isolated (Ruiz and Fontdevila 1981) and do not behave in an identical manner in crosses with *D. borborema, D. serido,* and *D. koepferae*. In addition, *D. koepferae* populations in Bolivia are genetically differentiated from those in Argentina as shown by the results of crosses with *D. serido* (Fontdevila et al. 1988). It may be worth emphasizing that the artificial no-choice conditions employed in the laboratory are very different from those in the field and that the results reported here therefore do not imply in any sense that hybridization is taking place in nature. They may

reveal, nevertheless, genetic affinities among the species, and this was the purpose here.

Isolation mechanisms are usually classified in two groups, prezygotic and postzygotic, and evidence shows that both are operative in this group of species. Prezygotic isolation mechanisms include ethological isolation and insemination reaction and probably explain most of the unsuccessful crosses. Male-choice experiments (Spieth and Ringo 1983) performed with some of the species show that interspecific matings are rare. For example, when males of *D. stalkeri* from Grand Cayman (Cayman Islands) were essayed with a mixture of females from the same stock and females of *D. richardsoni* from Beef Island (Tortola) only 1 of the 64 *D. richardsoni* females was inseminated, whereas 56 of the 64 control females were inseminated. Similar results were obtained when males of *D. stalkeri* were tested with females of *D. venezolana* and *D. buzzatii,* and when *D. borborema* males were confronted with *D. starmeri* females (unpubl. data). Interspecific matings, when observed, occurred with a low frequency compared with the intraspecific controls. Ethological isolation may be important even between species that render relatively abundant F_1 adult progeny. For instance, Fontdevila et al. (1988) using a multiple-choice test observed a fairly strong sexual isolation between *D. koepferae* and *D. serido.* The insemination reaction, described for the first time in the *repleta* species group (Patterson 1947), is also a significant isolation mechanism in these species. The only *D. richardsoni* female inseminated by a *D. stalkeri* male in the experiment cited above showed a dense, crystalline reaction that probably precluded oviposition. Moreover, when *D. buzzatii* females were crossed with *D. starmeri* males, a cross that should yield F_1 offspring according to the characteristic asymmetry found between the *martensis* and *buzzatii* clusters and to the great crossability of *D. starmeri,* they died shortly after copulation, and a dense aggregation was observed in their vagina; it can be said without exaggeration that they were killed by the foreign males. Similar cases have been previously described by Patterson and Stone (1952).

Coyne and Orr (1989) observed a greater prezygotic isolation in sympatric than in allopatric closely related species of *Drosophila* and concluded that the reinforcement of mating discrimination by natural selection is the most likely explanation for this pattern. In fact, there is direct evidence for this hypothesis within the *D. repleta* group (Wasserman and Koepfer 1977). Therefore, we may expect that the geographical distribution of the species may be one of the factors affecting their ability to produce hybrids. Of the 45 species pairs studied here, 10 are sympatric: the 4 *martensis* cluster species are sympatric over much of their range, *D. buzzatii* is sympatric with *D. serido* and *D. borborema* in Brazil and with *D. koepferae* in Argentina and Bolivia. The remaining 35 species pairs are all allopatric. *D. stalkeri* is found in Florida, Cuba, Jamaica, Bahamas, and the Cayman Islands. *D. richardsoni* inhabits Puerto Rico and the Lesser Antilles. As far as we know they are allopatric to each other and to the other eight species. The *martensis* cluster species are allopatric to those in the *buzzatii* cluster species. Finally, *D. koepferae,* which is found in Argentina and Bolivia, do not coexist with *D. serido* and *D. borborema,* which are found in Brazil. A crude evaluation of the prezygotic isolation in a given species pair can be obtained from our data (table 2) as the proportion of vials that did not give progeny averaged over the two reciprocal crosses. This measure confounds sexual isolation with the effect of the insemination reaction. However, the latter is not expected to show any geographical pattern, thus it can only obscure whatever trend might exist. Even so, prezygotic isolation measured in this way was, as expected, higher for the sympatric species pairs (average 93%) than for the allopatric species pairs (average 82%). Most significantly, the minimum prezygotic isolation in the 10 sympatric species pairs was 77%, whereas 9 of the 35 allopatric species pairs (about one-fourth) showed a lower prezygotic isolation. Obviously, these calculations are only suggestive, and more work is needed. Other factors can also influence the outcome of interspecific crosses. Among them, correlated results caused by phylogenetic relationships among species must be considered (Felsenstein 1985). For example, the asymmetry of hybrid offspring production in the crosses between the species of the *martensis* and *buzzatii* clusters could be explained by differentiation in the courtship behavior of the females ancestral to the *martensis* cluster, before any cladogenetic event occurred in this cluster.

Because it can be so greatly affected by evolutionary history, hybridization is not per se a good character to establish phylogenetic proximity between a determined pair of species. However, finding several productive interspecific crosses between two groups of species estab-

lishes that they have strong genetic affinities. A good correlation between reproductive isolation and phylogenetic relationships is found when groups of species are considered. The subdivision, based on morphological and cytological evidence, of the *D. repleta* group into subgroups and complexes within subgroups has been confirmed by extensive reproductive isolation testing (Crow 1942; Wharton 1944; Patterson 1947; Wasserman 1962, 1982). Thus far, no hybrids between species assigned to different subgroups have been reported, although successful copulations have been observed in some cases (Patterson 1947). Conversely, there is only one described case of intercomplex hybrids, which is found in the *hydei* subgroup: the cross *D. eohydei* females × *D. hydeoides* males (Wasserman 1982). However, the two species complexes in the *hydei* subgroup differ by a single fixed inversion on the second chromosome and do not seem comparable to those in the *mulleri* subgroup. Within the latter subgroup, females of *D. mulleri,* a species which belongs to the *mulleri* complex, produce sterile males and females when crossed to males of *D. hamatofila,* a species not formally assigned to any complex yet (Wasserman 1962, 1982). The subdivision of the *mulleri* complex in clusters also seems consistent with the results of the hybridization tests performed thus far: 14 of 31 (45%) intracluster crosses yield fertile females in the progeny, whereas only 1 of 47 (2%) intercluster crosses does (Crow 1942; Wharton 1944; Patterson 1947; Wasserman 1962, 1982; Fontdevila et al. 1990).

Our results using 10 species of the *mulleri* subgroup from South America and the West Indies were quite different from what would be predicted based on the data obtained in the rest of the *repleta* group. Ten of 12 crosses within the *buzzatii* cluster and 2 of 12 crosses within *martensis* cluster produced progeny, whereas *D. stalkeri* did not hybridize with its closest relative *D. richardsoni.* However, 13 of 32 intercluster crosses and 16 of 32 intercomplex crosses produced hybrids. Furthermore, quite often intercomplex or intercluster hybrid females were at least partially fertile. These results seem hardly compatible with the current phylogenetic classification of the three taxa, the *stalkeri* complex and the *martensis* and *buzzatii* clusters, which are depicted in figure 2. The eight species in the *martensis* and the *buzzatii* clusters share a minimum of two chromosome inversions (2 d^2s^6) and are thought to come from cytologically differentiated populations of Primitive II, the putative polytypic species that gave rise to the *mulleri* complex (Wasserman 1982). The close relationship of the species within these two clusters is corroborated by the results of the hybridization tests reported here (table 2). Particularly, several of the crosses yield fertile females, which is very unusual for species in different clusters (see table XIII in Wasserman 1982). Interestingly, some intercluster results can be explained assuming that some species have retained a genetic constitution closer to the ancestral state, while their chromosomes have evolved. The four species in the *buzzatii* cluster are equally differentiated from their ancestor's sequence: each of them shows a single fixed inversion. However, the interspecific crosses show that only *D. koepferae* can produce fertile hybrid females with the other three species (as well as with *D. starmeri, D. venezolana,* and *D. richardsoni*). The cytological phylogeny also indicates that *D. martensis,* among the four species in the *martensis* cluster, is the one closest to the *buzzatii* cluster (fig. 2). Yet, *D. starmeri* and *D. venezolana* can produce many more hybrids than *D. martensis* when crossed with the *buzzatii* cluster species.

Drosophila stalkeri and *D. richardsoni* arose from Primitive I, the ancestral sequence of the entire *repleta* group and do not share apparently any inversion with the *martensis* or *buzzatii* cluster species (fig. 2). They seem therefore distantly related to the eight species in these two clusters. In contrast, our results show that *D. stalkeri* and *D. richardsoni* are closely related to the *buzzatii* cluster species and somewhat less closely related to the *martensis* cluster species. It is also clear that of the two species in the *stalkeri* complex, *D. richardsoni* is the one that shows a closer relationship with the South American species. *D. richardsoni* produced fertile hybrid females with *D. buzzatii, D. koepferae, D. serido,* and *D. starmeri,* whereas *D. stalkeri* produced only sterile hybrids. These results agree with the similarities observed in the male genitalia, in particular the shape of the aedeagus, among these species (Vilela 1983), and suggest that a reexamination of the polytene chromosomes of these species is needed. This reexamination may be facilitated by the fact that many interspecific combinations produce hybrid larvae whose salivary gland chromosomes can be observed, allowing a direct test of the cytological phylogeny. As a matter of fact, cytogenetic work in progress (Ruiz and Wasserman, unpubl. data) shows that the second chro-

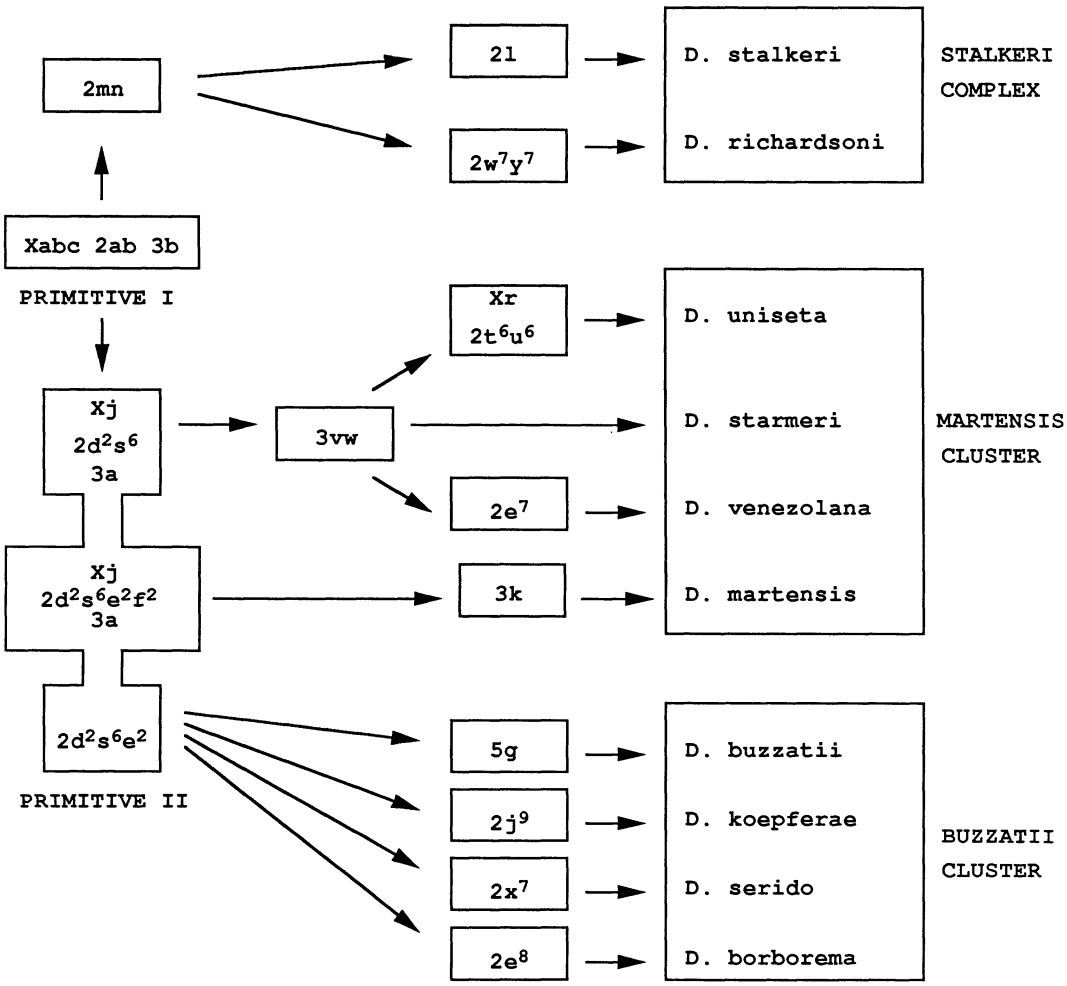

FIG. 2. Proposed chromosomal relationships among the species of the *stalkeri* complex and the *martensis* and *buzzatii* clusters (Wasserman 1982).

mosome of *D. stalkeri* and *D. richardsoni* is directly related to that of the *D. buzzatii* cluster species and all the 10 species may be accommodated in the same phylogenetic tree.

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