Drosophila koepferae: A New Member of the *Drosophila serido* (Diptera: Drosophilidae) Superspecies Taxon[1]

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ABSTRACT *Drosophila koepferae* Fontdevila et al., n. sp., is a member of the *D. buzzatii* cluster (*D. mulleri* complex) of the *D. repleta* species group of the genus *Drosophila*. It is distinguished from its sister species, *D. serido*, by morphological, genetic, ecological, and reproductive criteria. Chromosomal differentiation has led to standard sequences and inversion polymorphisms characteristic for each species. Genetic distance between both species measured by allozyme loci polymorphisms is in the range of true species and by itself justifies species status. In laboratory tests involving mass cultures with no choice, interspecific matings take place. Gene exchange between the two species is theoretically possible, for fertile hybrid females as well as sterile hybrid males are produced. However, both species have developed a strong premating isolation and also appear to be allopatric. In view of the marked differences between the two species, it is very improbable that any significant gene flow could occur in an area of sympatry, if such a region does exist. The extreme biological diversity of known *D. serido* populations suggests that the species may qualify as a superspecies.

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

THE *D. buzzatii* cluster consists of several species of the *Drosophila mulleri* complex inhabiting South America and sharing a common chromosomal phylogeny (Fontdevila 1982, Ruiz et al. 1982). At present, three species (*D. buzzatii* Patterson & Wheeler, *D. serido* Vilela & Sene, and *D. borborema* Vilela & Sene) are included in this cluster (Wasserman 1982). Although these three species can exchange genetic material under laboratory conditions, sympatric populations are reproductively isolated.

D. serido was described from specimens collected in northeastern Brazil (Vilela & Sene 1977). Later it was found to range from Brazil to Argentina and Paraguay (Vilela et al. 1980, Ruiz et al. 1982). Flies from different geographical areas differ morphologically, (e.g., male genitalia), and in their karyotypes and inversion polymorphisms, showing that, at least, *D. serido* is a polytypic species, consisting of several geographical races or subspecies (Sene et al. 1982, Baimai et al. 1983, Wasserman et al. 1983). The taxonomic status of these races is at present unclear, for no detailed reproductive studies among populations of diverse origin have been reported. In our view, the status of *D. serido* deserves close scrutiny, which could shed light on the evolution not only of the *D. buzzatii* cluster but also of the entire *D. mulleri* complex.

We report here some results of recent collections in northwestern Argentina and central Bolivia. Genetic analysis of these samples has revealed a remarkably high degree of divergence between these populations and those from Brazil, from which the type material of *D. serido* was derived (Vilela 1983). In addition, reproductive tests performed in the laboratory using strains from both areas show an almost complete prezygotic isolation under artificial conditions of sympatry. These data, coupled with previous information, lead to the conclusion that these Argentinian and Bolivian populations should be considered a separate species from *D. serido*. A formal description of the new species, which we are naming *Drosophila koepferae*, follows.

Drosophila koepferae Fontdevila & Wasserman, n. sp.

External Characteristics of Imagines. Male, female: Arista with 7 branches, antennae yellowish brown, third segment slightly darker. Frons dark brown, orbits, small median area pollinose; bristles arising from blackish spots. Middle orbital about ⅓ length of other two. Second oral about ⅛ length of first. Palpus pale yellow, with several bristles. Face yellowish brown. Cheeks yellowish, their

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...st width about ⅓ greatest diameter of eyes. ... orangish-vermilion with short black pile.

... ostical hairs in 8 rows; no prescutellars. Anterior scutellars convergent, Sterno index ca. 0.9. Middle sternopleural ca. ¼ length of posterior. Mesonotum pollinose, bristles arising from brown spots, these not tending to fuse. Scutellum pollinose, with wide brown X-shaped mark; scutellar bristles arising from darker brown spots. Pleura light yellowish gray with indistinct fuscous band from base of wing to humerus, from base of halter to forecoxa, ... across the sternopleurals. Legs yellowish gray, ... arrow dark bands on distal ends of femora ... ar tibial bases. Wings clear, veins brown, crossveins darker, apex of 1st costal section darker. Costal index ca. 3.0; 4th vein index ca. 2.0; 5× index ca. 1.2; 4c index ca. 1.0. Two well-developed bristles at apex of 1st costal segment; 3rd segment with heavy bristles on basal ¼.

Abdominal segments yellowish pollinose; 4th–6th tergites with interrupted grayish-brown band with forward extensions at interruption, lateral ... and angle of tergites; last extensions wid... at anterior margin, connecting laterally with lateral extensions and enclosing irregular yellowish area; 2nd and 3rd tergites as above but band often interrupted at posterior part of angle of tergites.

Body length of female 2.8 mm, that of male 2.7 mm.

Internal Characters of Imagines. Testes yellow, turning orange with age, with 2½ inner and 2½ outer coils. Ventral receptacle with ca. 8 loose coils proximally, ca. 10 tight coils distally. Penis appa... Fig. 1.

Puparia. Each anterior spiracle with ca. 12 branches; horn index ca. 2.5.

Chromosomes. Metaphase plate showing 5 pairs of rods, one pair of dots. X chromosome approximately 40% longer than autosomes. Y chromosome a metacentric, total length ca. equal to that of an autosome.

Relationship. Distribution, and Ecology. *D. koepferae* belongs to the *D. buzzatii* cluster of the *D. ...elleri* complex of the *D. repleta* species group. ... summarizes the collecting sites in Argentina and Bolivia, including those reported previously as *D. serido* by Ruiz et al. (1982). The known distribution extends from Sierra de San Luis in Argentina to Comarapa in Bolivia.

Data on the breeding and feeding niches of *D. koepferae* are fragmentary but indicate that it lives primarily, if not exclusively, on columnar cacti. Decayed portions of *Trichocereus terschekii* Parmentier and *Neocardenasia herzogiana* Backeb... columnar cacti collected in localities 4 ... respectively, were taken to the laboratory and yielded 25 and about 100 adults of *D. koepferae*, respectively. Fontdevila and Ruiz (reported in Wasserman et al. 1983) collected rotting cladodes of *Opuntia quimilo* Schumann in Vipos, Argentina, where *D. koepferae* occurs, but obtained only adults of *D. buzzatii*. More recently, Hasson and Naveira

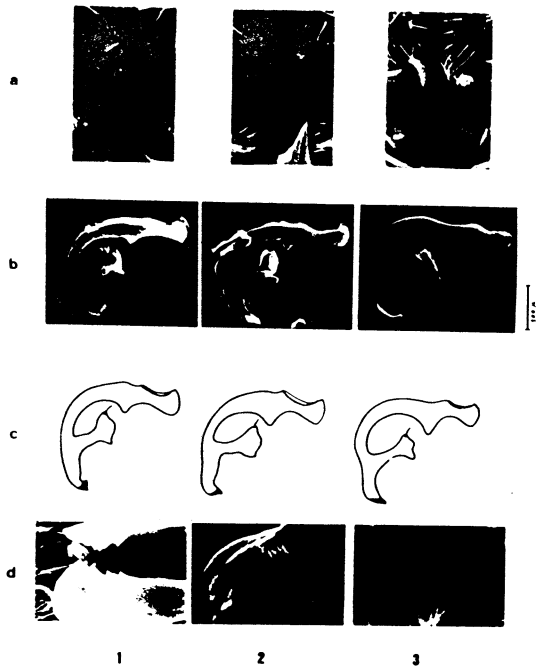

Fig. 1. Male genitalia of *D. koepferae* (1, Argentina; 2, Bolivia) and 3, *D. serido:* (a) Frontal view of epandrium primary teeth at the scanning electron microscope (SEM), 900×; (b) Lateral aspect of aedeagus (SEM), 230×; (c) Lateral drawings of aedeagus at the light microscope (LM), 100×; (d) View of tip and ventral margins of aedeagus (SEM)(1, 2,000×; 2, 1,500×; 3, 1,000×).

(unpublished) collected decayed portions of *Cereus validus* Haworth and *O. quimilo* in Vipos. Cladodes of *O. quimilo* produced *D. buzzatii* almost exclusively, thus confirming previous observations. On the other hand, *C. validus* produced 4:1 *D. koepferae*/*D. buzzatii*, respectively. *D. serido*, the Brazilian sibling species of *D. koepferae*, also appears to live primarily on columnar cacti (*Cereus sp.* Miller and *Cephalocereus piauhyensis* Gürke), although a few adults have been reared from rotting cladodes of *Opuntia ficus-indica* Linné (Pereira et al. 1983). It seems that *Opuntia* species are not the common hosts of either of the two sibling species, which both prefer columnar cacti of their natural substrates.

Morphological Differentiation. Lighter in color than *D. serido* and *D. borborema*, *D. koepferae* also differs from its sibling species, *D. serido*, from northeastern Brazil, by having 7 branches in the arista instead of 6, by having an X-shaped mark on the scutellum (lacking in *D. serido*), and by having the spots at the base of the 8 rows of acrostical hairs more clearly defined than they are in *D. serido*. In general, *D. koepferae* shows a tergite pattern more similar to *D. buzzatii* than to *D. serido*. However, the penis apparatus is quite distinct from *D. buzzatii* and differs from *D. serido* (Fig. 1). The posterior end of the aedeagus of *D. koep-*

Fig. 2. Map of South America showing the known distributions of *D. koepferae* and *D. serido*. Description of sampled localities Comarapa (1), San Isidro (2), Los Negros (3), Quilmes (4), Vipos (5), Mazán (6), Palo Labrado (7), El Diquecito (8) and San Luis (9). Localities 5, 7, 8, and 9 are described in Ruiz et al. 1982. Localities 1, 2, and 3 are situated in a broad valley between the Central and the Oriental Andean ranges of Bolivia, about 200 km west of Santa Cruz. The most abundant cacti are: *Cereus dayamii* Spegazzini, *Cereus comarapanus* Cárdenas, *Neocardenasia herzogiana* Backeberg, *Roseocereus tephracanthus* (Lab.) Backeberg, *Opuntia sulphurea* G. Don, *Cleistocactus fusiflorum* Cárdenas, *Gymnocalicium zegarrae* Cárdenas, and *Echinopsis obrepanda* (Salm-Dyck) Schumann. Locality 4 is in the ruins of Quilmes, an old Indian fort in the Santa María Valley, 90 km west of San Miguel de Tucumán. Cactus species present are: *Trichocereus terschecki* Parmentier and *Opuntia sulphurea* G. Don. Locality 6 is on the western slopes of the Sierra de Ambato, just at the entry of the passage named Quebrada de la Cébila, about 20 km east of Villa Mazán. Cactus species as in locality 5.

ferae differs from that of *D. serido* in shape, length (Fig. 1, part c), and teeth size (Fig. 1, part d). Moreover, the epandrium teeth are different in the two species (Fig. 1, part a).

Type Material. Holotype male: ARGENTINA, Tucumán, 2 km south of Vipos, old road to Tucumán, collected by A. Fontdevila and A. Ruiz, 22-XI-1979 (Fig. 2). Paratypes: same data as holotype. The holotype and five paratypes (2 ♂♂ and 3 ♀♀) will be deposited in the National *Drosophila* Species Resource Center at Bowling Green State University. Four additional paratypes (2 ♂♂ and 2 ♀♀) will be deposited in the Museo Entomológico del Instituto Miguel Lillo, Tucumán, Argentina.

Genetic Differentiation

Two kinds of genetic markers have been used to study genetic differentiation between *D. koepferae* and *D. serido;* i.e., chromosomal rearrangements and allozymes.

This study has been performed by using strains from *D. koepferae* populations 1, 2, 3, 4, 6, and 7 (Fig. 2) and *D. serido* strains 1431.1 (Rio Paraguaçu), 1431.2 (Milagres), and 1431.4 (Cafarnaum), all from the State of Bahia, northeastern Brazil.

Chromosomal Rearrangements. Both species stem from the standard karyotype of the *buzzatii* cluster (Xabc;2abd^{2}s^{6}e^2;3b;4;5) (Ruiz et al. 1982). *D. serido* has a fixed inversion (2x^7) and is polymorphic for 4 other inversions, 2a^8, 2b^8, 2c^8, and 2d^5 on the second chromosome (Wasserman & Richardson, personal communication). On the other hand, the phylad of *D. koepferae* has a different standard sequence on the second chromosome, with 2j^9 inversion fixed (2d^{2}s^{6}e^{2}j^9), and is polymorphic for eleven inversions not found in *D. serido*. Four of these inversions (2u^9, 2v^9, 2x^9, 2w^9) are characteristic of the Bolivian populations, four (2n^9, 3k^2, 4m, 5w) are found only in the Argentinian populations, and three (2l^9, 2k^9, 2m^9) are common in both areas.

Allozymic Differentiation. Twenty-two allozyme loci were studied for the cited populations and strains of *D. serido* and *D. koepferae* (Sanchez & Fontdevila, in preparation). Genetic distances and identities (Nei 1972) are given in Table 1. *D. serido* shows a high degree of genetic differentiation from *D. koepferae*, being 0.850 from Argentinian *D. koepferae* and 0.778 from Bolivian *D. koepferae*. Comparison between Argentinian and Bolivian *D. koepferae* gives a small degree of divergence (0.131), yet higher than that obtained in most intrapopulational studies.

Reproductive Isolation

Two tests were performed to evaluate degree of reproductive isolation between *D. koepferae* and *D. serido;* i.e., postzygotic and prezygotic.

Postzygotic Isolation. Interspecific crosses were performed in mass cultures, each containing 10 males and 10 females (Table 2). Both species can exchange genes, because although the male hybrids are sterile, the female hybrids are fertile. Offspring production may depend on the sex of the parents and also on the geographical origin of *D. koepferae*. Thus, fewer offspring are produced when the parental males are *D. serido*, regardless of the geographical origin of the strains, and also when Argentinian *D. koepferae* are used, regardless of whether *D. koepferae* is the male or female parent.

Prezygotic Isolation. In multiple-choice mating tests, replicates of 20 males and 20 virgin females each of *D. serido* and *D. koepferae* were placed together and allowed to mate for 72 h. Each female was then placed in its own vial and allowed to oviposit. Electrophoretic analysis of the offspring

Table 1. Nei's genetic identity (I) and distance (D) and their standard errors between *D. koepferae* and *D. serido*

Comparison	N[a]	I	D
D. serido versus *D. koepferae* (Argentina)	9	0.429 ± 0.012	0.850 ± 0.030
D. serido versus *D. koepferae* (Bolivia)	9	0.460 ± 0.010	0.778 ± 0.022
D. koepferae (A) versus *D. koepferae* (B)	9	0.878 ± 0.016	0.131 ± 0.019

[a] N, number of population pairs in each comparison.

identified the male(s) which inseminated the female—*D. serido* strain 1431.4 is homozygous for Pgm 87 and Idh 106 electromorphs and *D. koepferae* strains from S. Luis (Argentina) and Los Negros (Bolivia) are homozygous for Pgm 95 and Idh 102 electromorphs.

Table 3 gives the results of four replicates of the multiple-choice tests. The tests involving *D. serido* with *D. koepferae* from Argentina show total isolation; all offspring were the result from homogamic matings. *D. koepferae* from Bolivia, on the other hand, is only partially isolated from *D. serido*. *D. koepferae* females from Bolivia do not accept *D. serido* males, but *D. serido* females produce three kinds of offspring: pure *D. serido*, hybrids, and a mixture of both. The latter case results from multiple inseminations.

Discussion

Early studies by Vilela (1983) showed that *D. serido* is morphologically polytypic. In addition, Baimai et al. (1983) found six different types of intraspecific karyotypes within *D. serido*. This species has, in fact, prove to be a superspecies, consisting of several allopatric species, the geographical distributions of which are presently not known. Here data are presented which clearly demonstrate that the Argentinian and Bolivian populations are specifically distinct from the remainder of the *D. serido* forms.

D. serido and *D. koepferae* are quite distinct cytologically. They represent two independent phyla[ds] from the second chromosome standard sequence (2abd[3]e[2]), *D. koepferae* being homozygous for 2j, whereas *D. serido* is homozygous for 2x. In addition, each has its own unique inversion polymorphism.

A great deal of information on genetic distances based on protein polymorphisms is available. Thorpe (1982), in a comprehensive review of Nei's genetic identity (I) distributions among congeneric species and conspecific populations, concludes that if two allopatric populations have a genetic identity below 0.85 (D > 0.16) it is very improbable ($P = 0.02$) that they would be conspecific. We found values of I between *D. serido* and *D. koepferae* much smaller than 0.85 and, following Thorpe's reasoning, their probability of being conspecific is still much lower than 0.02. However, this conclusion is based mostly on vertebrate taxa, excluding birds, with very few data from *Drosophila* studies. So, the applicability of Thorpe's review rests in the universality of the molecular clock.

Avise and Aquadro (1982) have pointed out the extreme heterogeneity in mean interspecific genetic distances among vertebrate genera and have challenged the idea of a unique and universal molecular clock. This is certainly true for birds, whose molecular evolution seems to be much slower than that of other vertebrate classes.

In *Drosophila*, mean D heterogeneity among species groups is lower than among vertebrate classes, but there are group differences (MacIntyre & Collier 1986). Whether these differences are due to different rates of molecular evolution among groups is open to discussion. Carson (1976), combining electrophoretic and biogeographic data, claimed that Hawaiian *Drosophila (planitibia* subgroup) show an accelerated rate of protein evolution. However, this idea has recently been challenged by Beverley & Wilson (1985), who used immunological distances for a *Drosophila* larval hemolymph protein (LHP). Moreover, these authors claim not only that LHP evolves at the same rate in continental and in Hawaiian *Drosophila*,

Table 2. Offspring numbers in crosses between *D. koepferae* and *D. serido*

♀	Interspecific crosses ♂	N[a]	Offspring ♀[b]	Offspring ♂[b]
D. [illegible]	*D. serido*	5	147 (F)	111 (S)
D. koepferae (Bolivia)	*D. serido*	5	621 (F)	476 (S)
D. serido	*D. koepferae* (Argentina)	5	947 (F)	981 (S)
D. serido	*D. koepferae* (Bolivia)	5	1,097 (F)	1,023 (S)

[a] N, number of replicates.
[b] F, fertile; S, sterile.

Table 3. Number of females per species that give a certain offspring type in interspecific crosses

Type of cross	Type of offspring[a] D.s.	D.s. D.k.	D.s. + D.s. D.k.	D.k.
D. serido × *D. koepferae* (Argentina)				
D. serido females	50	0	—	—
D. koepferae females	—	0	—	57
D. serido × *D. koepferae* (Bolivia)				
D. koepferae females	—	0	—	60
D. serido females	40	5	18	—

[a] D.s., *D. serido*; D.k., *D. koepferae*.

but that this protein changes at a rate similar to that of other secreted proteins in mammals (Beverley & Wilson 1982, 1984).

The few studies on the *D. repleta* group show some of the smallest D values. Thus, Zouros (1973), working with four species of the *mulleri* subgroup, found maximal D values of interspecific differentiation ranging from 0.27 (sibling species) to 0.32 (nonsibling species). In contrast, recent work by Sánchez (1986) with eight species of the *mulleri* subgroup has shown that mean D values for interspecies comparisons range from 0.59 (siblings) to 0.97 (nonsiblings). These figures are similar to those reported for other species groups (Ayala 1975). In the superspecies *D. serido*, genetic distances between allopatric populations from northeastern Brazil and northwestern Argentina (D = 0.85) or Bolivia (D = 0.78) fall in the range of true different species.

These comparisons are based on the premise that the rate of protein evolution is steady, mostly independent of ecological conditions. The low D values in the *D. repleta* group led Zouros (1973) to assign the small niche differentiation among these cactiphilic species as the main cause for their low protein divergence. Richardson et al. (1977), working with species of the *mulleri* subgroup, reached similar conclusions—that molecular divergence is correlated with ecological differentiation. Several independent authors (Sene & Carson 1977, Cabrera et al. 1983, González et al. 1983) have also pointed out certain correlations between genetic distances and similarities in ecological conditions. However, an exhaustive study of the cactophilic niche in the *D. martensis* cluster (*mulleri* subgroup) (Benado et al., unpublished) shows that ecological differentiation is unrelated to protein evolution.

At the moment we have no basis in *Drosophila* to believe that protein evolutionary rates are changed by ecological or demographic causes, and the hypothesis of genetic distances as evolutionary clock seems workable. Therefore, the degree of protein divergence between *D. koepferae* and *D. serido* is large enough to justify their definition as true, separate species.

The true test of species differentiation is reproductive isolation, and this may not be correlated with the degree of genetic divergence. Thus, Zouros (1973) showed that in some species of the *mulleri* subgroup this correlation is significant for hybrid viability (developmental factors) but not for hybrid sterility, an equally important mechanism for isolating species. These considerations point to the danger inherent in the use of the degree of genetic divergence as the major criterion for species definition.

Our data on reproductive isolation relate directly to the question of the validity of the species. The sterility of the hybrid males obtained in all our crosses between *D. serido* and *D. koepferae* is a common postmating isolation mechanism found in many other sibling species and semispecies. Premating isolation, if present, is readily observable in sympatric species, but it becomes an experimental problem when species are allopatric. In our experiments we have been able to show a highly developed prezygotic isolation between both species. Ethological isolation between Argentinian populations of *D. koepferae* and *D. serido* is complete, whereas Bolivian *D. koepferae* is only partially isolated from *D. serido*. In this latter case we have calculated the Levene index (Magolowkin-Coehn et al. 1965), considering that mixed offsprings (Ds/Dk + Ds) are the result of double matings—one homogamic plus one heterogamic mating. The index value (0.67 ± 0.06) is in the order of magnitude found in allopatric semispecies of the *D. paulistorum* Dobzhansky & Pavan complex (Ehrman 1965) or between allopatric populations of *D. mojavensis* Patterson & Crown and *D. arizonensis* Patterson & Wheeler (Wasserman & Koepfer 1977). It is uncertain whether our sibling species are sufficiently ecologically or ethologically distinct to allow them to coexist sympatrically, but it seems most likely that here, strong premating and postmating isolation arose in allopatry, there being no reason to believe that there has ever been any secondary contact between the two diverging populations. Other similar cases unveiled by electrophoretic studies have been reported in *Aedes* and *Culex* mosquitoes (Bullini 1983).

Within the species *D. koepferae*, a certain level of differentiation has arisen. Geographical cytological differentiation between Argentinian and Bolivian populations of *D. koepferae* is present. Both populations are polymorphic for $2k^9$, $2l^9$, and $2m^9$. The Bolivian populations are polymorphic for $2u^9$, $2v^9$, $2w^9$, and $2x^9$, and the Argentinian populations are polymorphic for $2m^9$, $3k^2$, 4m, and 5w (Ruiz et al. 1982). The data thus far indicate that all of the polymorphism in the Bolivian populations is limited to chromosome 2, whereas that of the Argentinian populations is spread throughout the genome. If true, this probably indicates there is a fundamental difference in the selective basis of the polymorphisms between the two populations. The genetic distance between the Argentinian and Bolivian populations of *D. koepferae* also indicates a degree of divergence (D = 0.131), being within the range often found when geographical races are compared.

Although chromosomal and allozymic data suggest an incipient racial differentiation among *D. koepferae* populations, this is not sustained by reproductive isolation tests. In fact, populations of both geographic areas can be crossed and produce abundant F_1 and F_2 offspring similar to crosses between populations belonging to the same region (unpublished results).

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