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DIFFERENTIATION OF *Drosophila serido* (ISOFEMALE LINE A95F3) AND *D. koepferae* (ISOFEMALE LINE B20D2): REPRODUCTIVE ISOLATION, DEVELOPMENT TIME AND POLYTENE CHROMOSOME BANDING PATTERNS

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ABSTRACT

Drosophila serido is considered to be a superspecies consisting of two species: *D. serido*, from Brazil and *D. koepferae* from Argentina and Bolivia. However this probably does not express the entire evolutionary complexity of its populations. Isofemale lines A95F3 (from Brazil) and B20D2 (from Argentina), at present representing, respectively, the first and second species, were analyzed for fertility and fecundity in pair-mating intracrosses and intercrosses, as well as for development time, banding patterns and asynapsis of polytene chromosomes in the isofemale lines and their hybrids.

Although variations in experimental conditions resulted in some variability in the results, in general A95F3 fertility and fecundity were lower than in B20D2. Intercrosses of A95F3 females and B20D2 males showed lower fertility and fecundity than the reciprocal crosses, following more closely characteristics of the mother strains. This is in contrast to the results obtained by Fontdevilla *et al.* (*An. Entomol. Soc. Amer. 81*: 380-385, 1988) and may be due to the different geographic origin of *D. serido* strains they used in crosses to B20D2. This difference and others cited in the literature relative to aedeagus morphology, karyotype characteristics, inversion polymorphisms and reproductive isolation strongly indicate that A95F3 and *D. serido* from the State of Bahia, Brazil are not a single evolutionary entity, reinforcing the idea of greater complexity of the superspecies *D. serido* than is known today.

The reproductive isolation mechanisms found operating between A95F3 and B20D2 were prezygotic and postzygotic, the latter included mortality at the larval stage in both directions of crosses and sterility of male hybrids in intercrosses involving B20D2 females and A95F3 males. The two isofemale lines differed in egg-adult development time, which was also differently affected by culture medium composition.

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A95F3 and B20D2 also showed differences in the banding patterns of proximal regions of polytene chromosomes 2, 3 and X, a fixed inversion in chromosome 3 (here named 3t), apparently not described previously, and a high degree of asynapsis in hybrids.

These observations, especially those related to reproductive isolation and chromosomal differentiation (including the karyotype, previously described, and the differentiation of banding patterns, described in this paper), as well as the extensive asynapsis observed in hybrids reinforces the distinct species status of A95F3 and B20D2 isofemale lines.

INTRODUCTION

When geographic Brazilian populations of *D. serido* Vilela and Sene (1977), were first analyzed, marked differences among them came to light. These differences involved karyotypes (Baimai *et al.*, 1983), chromosomal polymorphisms (Tosi, 1982; Ruiz *et al.*, 1982; Wasserman and Richardson, 1987; Tosi and Sene, 1989), aedeagus morphology (Silva, 1983; Silva and Sene, 1991) and reproductive isolation (Bizzo, 1983). This species, which was collected in Brazil, Argentina, Bolivia and Paraguai, was soon considered to be a species complex or at least a polytypic species (Tosi, 1982; Sene *et al.*, 1982; Baimai *et al.*, 1983; Wasserman and Richardson, 1987; Fontdevila *et al.*, 1988; Sene *et al.*, 1988).

At present, although the entire evolutionary situation of *D. serido* is far from being understood, it is considered to be a superspecies consisting of two species: *D. serido*, from Brazil and the eastern part of the Argentinian chaco and *D. koepferae*, from the slopes of the Andes in Argentina and Bolivia (Fontdevila *et al.*, 1988; Tosi and Sene, 1989).

We deal mainly with reproductive isolation and differences in development time and banding patterns of polytene chromosomes of two isofemale lines: A95F3 from Brazil and B20D2 from Argentina.

A95F3 and B20D2 are respectively characterized as presenting type C and E aedeagus morphology (Silva and Sene, 1991), type II and IV metaphase plates (Baimai *et al.*, 1983) and types IV and II polytene chromosomes (Tosi and Sene, 1989). Data on reproductive isolation showed that they are isolated from other populations analyzed (Bizzo, 1983). At present, however, in the literature (and also in the present paper) A95F3 continues being named *D. serido* while B20D2 has been described as a new species, *D. koepferae* (Fontdevilla *et al.*, 1988). *D. serido* strains used by Fontdevilla *et al.* (1988) are from Milagres, BA (*D. serido* type locality) and from Argoin, BA. In the studies mentioned above, these lines (including A95F3 and B20D2) were classified as aedeagus type A, metaphase plate type I, polytene chromosomes type I, and they exhibited a complex pattern of reproductive isolation relative to other lines, differing in those parameters.

MATERIALS AND METHODS

An isofemale line A95F3 from Serra do Cipó, MG, Brazil and another B20D2 from Tapia, Argentina were used. They were captured in 1978 by Sene and Vilela and have been kept in our laboratory since 1983 on a 1:1 mixture of cactus and banana culture medium, at 20 C ± 1. Our experiments were performed in 1985, 1986 and part of 1987.

Cactus culture medium was prepared with agar-agar (12 g), Fleischmann's yeast (100 g), 3 squashed bananas, propionic acid (10 ml), sugar-cane syrup (50 ml, prepared by boiling 100 g of sugar-cane in 100 ml of water), 7% nipagin solution (10 ml), cactus pulp (500 ml) and water (1000 ml). The cactus pulp was obtained by cooking the cactus in water, followed by liquefaction and sterilization. We used the cactus *Opuntia ficus-indica* for maintaining the stocks. Banana culture medium was as usual.

Reproductive isolation was studied in two experiments. In each, pair-mating crosses were performed in tubes containing the banana-cactus medium. Intracrosses and intercrosses in both directions were prepared. Fifty crosses were made in every case, using five day old flies (first experiment) or 10 day old flies (second experiment). One couple was put into each tube and transferred to new medium at six day intervals (first experiment) or seven day intervals (second experiment) (Table I).

Table I - Age interval and mean (in parentheses) of the mated flies, in the sequence of tubes (transferences to new medium) in each reproductive isolation experiment.

Sequence of tubes	Age of the flies (days)	
	Experiment I	Experiment II
1	5-11 (8)	10-17 (13.5)
2	11-17 (14)	17-24 (20.5)
3	17-23 (20)	24-31 (27.5)
4		31-38 (34.5)

Data on fertility (percentage of crosses yielding progeny) and fecundity (number of progeny) were obtained. In both experiments, number and sex of progeny were computed daily from the beginning of adult emergence. F1 fertility was analyzed in mass crosses using descendants of intercrosses. Seven day old couples were put into 30 tubes (five couples per tube) and transferred three times to new medium, at five day intervals.

In order to study the development time of the two isofemale lines, three different culture mediums (named M1, M2 and M3) were used. Culture medium M1 was that already described for maintaining the strains. Culture medium M2 differred from M1 only in the species of cactus used, *Cereus jamacuru* instead of *Opuntia ficus-indica*. M3 was prepared with the *Opuntia* pulp alone, that is, without banana medium.

Development time was also studied in two experiments, using population cages which had Petri dishes with the mentioned culture mediums. One hundred 7 to 10 day old couples were put into each cage. Petri dishes were changed at 24 hour intervals. When the eggs started to eclode in the Petri dishes, larvae born during one hour were collected and transferred to tubes containing the same culture medium. In each of two experiments 10 tubes with 10 larvae each for every type of culture medium were prepared. Development was followed daily until the complete emergence of the adults, whose number and sex were computed.

Preference for egg laying was also observed when the different mediums were placed together in population cages for developmental studies.

Cytological preparations of brains and salivary glands from larvae of both parental isofemale lines and hybrids were made for chromosome analysis. Larvae from the early third instar and from the late third instar were used for brain and salivary gland preparations, respectively. For brain preparations the Guimarães and Bicudo (1982) procedure was followed. Larvae were put in Fleischmann's yeast suspension for about 15 minutes, washed and dissected in Demerec solution (Demerec and Kaufmann, 1945). Brains were then put on slides containing a drop of 45% acetic acid (2 minutes), transferred to 2% lacto-acetic orcein (3 minutes), to 50% lactic acid (8 minutes), covered with a coverslip and squashed following the technique of Atherton and Gall (1972). The slides were sealed with colorless nail polish.

For salivary gland preparations, after dissection of larvae in Demerec solution and isolation of the salivary glands, the same steps described for brains were followed except that the time in 2% lacto-acetic orcein and in 50% lactic acid was 5 minutes.

RESULTS AND DISCUSSION

Bizzo (1983) stated that strains of *D. serido* from Tapia and Famatina (Argentinian Chaco) behaved as "an entity", yielding sterile male progeny in crosses with all the remaining strains. Bizzo also emphasized the singularity of those strains in terms of aedeagus morphology (Silva, 1981, 1983), inversion polymorphism (Tosi, 1982) and metaphase karyotype (Baimai *et al.*, 1983).

As shown in Tables II and III, in the pair-mating crosses the fertility (percentage of fertile crosses) of *D. serido* (A95F3 strain) intraspecific crosses was lower than that of Argentinian *D. koepferae* (B20D2 strains); interspecific crosses involving both strains

showed a lower fertility when the females were A95F3 (MSD = 0.10; $P < 0.05$, Table IV). The fertility of interstrain crosses followed that of the mother strain. The fertility of intercrosses between B20D2 females and A95F3 males was even greater than that of A95F3 intrastrain crosses.

The fertility was the same in the two experiments performed, despite different ages, numbers and intervals of transferences of couples to new media.

Table II - Experiment I. Number and percentage of intra and intercrosses yielding progeny, and distribution of crosses according to the beginning of offspring production, in the sequence of tubes. Numbers in parentheses are the mean ages of the flies. F: females; M: males; T: total crosses prepared; t1-t3: sequence of tubes; +: fertile crosses.

Type of crosses		Number of crosses		Beginning of fertility of the couple in sequence of tubes		
F	M	T	+	t1(8)	t2(14)	t3(20)
B20D2	B20D2	50	49	49	0	0
A95	A95	42	27	6	15	6
B20D2	A95	50	39	31	8	0
A95	B20D2	50	17	5	10	2

Table III - Experiment II. Number and percentage of intra and intercrosses yielding progeny, and distribution of crosses according to the beginning of offspring production, in the sequence of tubes. Numbers in parentheses are the mean ages. Symbols as in Table II.

Type of crosses		Number of crosses		Beginning of fertility of the couple in sequence of tubes			
F	M	T	+	t1 (13.5)	t2 (20.5)	t3 (27.5)	t4 (34.5)
B20D2	B20D2	50	46	45	1	0	0
A95	A95	42	29	28	1	0	0
B20D2	A95	50	43	29	14	0	0
A95	B20D2	50	15	9	2	2	2

However, variations in experimental conditions affected the beginning of adult progeny production. In the first experiment, all the B20D2 intracrosses already produced progeny in the first tube while most of the A95F3 intracrosses did so only in the second tube (first transfer to new medium) or in the third. In the second experiment, however, most of the A95F3 intracrosses yielded progeny in the first tube. In the intercrosses of B20D2 females with A95F3 males, progeny started to be produced, in both experiments, mostly in the first tube. In the intercrosses involving A95F3 females and B20D2 males, descendants started to be produced, in experiment I, predominantly in the second tube while in experiment II, most of the couples produced progeny in the first tube but some did so in the second, third and even the fourth tube.

The number of couples which yielded larvae and imagoes, in the intracrosses and intercrosses was also affected by experimental conditions (Tables V and VI). In each type of cross in experiment I in some vials there was complete mortality of flies at the larval stage. Significant differences among crosses were found in the first and second series of tubes (χ^2 for homogeneity, first series: = 62.32; $P < 0.05$); second series: = 37.71; $P < 0.05$). In experiment II, the percentage of couples with complete mortality of progeny at the larval stage was smaller than that in the first experiment. Significant differences were found only in the second series of tubes (χ^2 for homogeneity = 8.97; $P < 0.05$). In both cases the intercrosses of A95F3 females and B20D2 males were different from the others, that is, in the second experiment, as in the first, these intercrosses showed higher percentages of complete larval mortality than the intracrosses. Equality of larvae and imagoes productivity proportions of intracrosses and intercrosses was calculated by homogeneity χ^2 and multiple comparisons. Significance of two by two comparisons are in Table VII (Experiment I: first and second series of tubes) and in Table VIII (Experiment II: second series of tubes).

Table IV - Experiments I and II. Significance of two by two comparisons for fertility of the 4 types of crosses. Minimum significant difference (MSD) = 0.10 (Experiment I and Experiment II); * = $P < 0.05$.

	Types of crosses					
	F A95F3 x M A95F3		F B20D2 x M B20D2		F A95F3 x M B20D2	
	I	*II*	*I*	*II*	*I*	*II*
F B20D2 x M B20D2	0.34*	0.23*	0.20	0.06	0.64*	0.62*
F A95F3 x M A95F3			-0.14	-0.17	0.30*	0.39*
F B20D2 x M A95F3					0.44*	0.56*

Table V - Experiment I. Total number of couples which produced larvae and among them, the percentage of those which yielded imagoes in the sequence of tubes of the four types of crosses. Numbers in parentheses are the mean ages of the flies in days.

Crosses		Tube 1 (8)		Tube 2 (14)		Tube 3 (20)	
F	M	Larvae (number of couples)	Imago (%)	Larvae (number of couples)	Imago (%)	Larvae (number of couples)	Imago (%)
B20D2	B20D2	49	100	46	78	43	77
A95	A95	6	100	18	100	23	96
B20D2	A95	31	93	34	85	21	86
A95	B20D2	5	0	12	8	4	75

In the pair-mating crosses (Tables IX and X), fecundity (number of progeny) of B20D2 was greater than that of A95F3, in both experiments. Also in both experiments, the fecundity of intercrosses involving B20D2 females and A95F3 males was greater than that of reciprocal crosses and even greater than that of A95F3 intracrosses. Such differences were stronger in the first experiment (F for homogeneity of total means of the four types of crosses was significant).

Table VI - Experiment II. Number of couples which produced larvae and the percentage of those among them which yielded imagoes, in the sequence of tubes of the four types of crosses. Numbers in parentheses are the mean ages of the flies in days.

Crosses		Tube 1 (13.5)		Tube 2 (20.5)		Tube 3 (27.5)		Tube 4 (34.5)	
F	M	NCPL	Imago (%)	NCPL	Imago (%)	NCPL	Imago (%)	NCPL	Imago (%)
B20D2	B20D2	45	98	42	100	14	100	0	-
A95	A95	28	93	24	100	8	100	0	-
B20D2	A95	34	100	41	100	34	94	15	80
A95	B20D2	9	100	7	88	7	86	4	75

NCPL = Number of couples which produced larvae.

Table VII - Experiment I. Significance of two by two comparisons for productivity of larvae and imagoes of the four types of crosses, in the first (t1) and second (2) series of tubes. MSD = 0.03 (first series of tubes) and 0.08 (second series of tubes); * = $P < 0.05$.

	Types of crosses					
	F A95F3 x M A95F3		F B20D2 x M B20D2		F A95F3 x M B20D2	
	t1	t2	t1	t2	t1	t2
F B20D2 x M B20D2	0.00	-0.22	0.07*	-0.07	1.00*	0.70*
F A95F3 x M A95F3			0.07*	0.15*	1.00*	0.92*
F B20D2 x M A95F3					0.93*	0.77*

Significant differences were also found in the first experiment, for the first tubes of A95F3 intracrosses, B20D2 intracrosses and intercrosses involving B20D2 females and A95F3 males, and, in a comparison of the sequence of tubes, for A95F3 intracrosses and B20D2 females x A95F3 males intercrosses. The F value could not be calculated for the intercrosses involving A95F3 females and B20D2 males, due to the low number of descendants.

The second experiment (in which 10 day old flies were used and three transfers to new medium were made) gave better information on the fecundity of interspecific crosses. As mentioned, there was a delay in the beginning of adult progeny production in interspecific crosses relative to the intracrosses, probably reflecting difficulties in the acceptance of foreign males by females. Apparently such difficulties decrease when mated flies are older (second experiment) but they still remained, to some extent. Thus, in the fourth group of vials (third transfer), in the second experiment, the interspecific crosses still yielded progeny while the intraspecific crosses did so only till the third group of vials (second transfer). In this case the total mean number of progeny of both types of interspecific crosses increased, attaining nearly identical values which were also close to that of the total mean number of progeny of intraspecific crosses (F for homogeneity of means was not significant).

F for mean progeny of experiment II, in the sequence of tubes, gave significant values for B20D2 intracrosses, for intercrosses involving B20D2 females and A95F3 males and for intercrosses involving A95F3 females and B20D2 males. F values were also significant for mean fecundity in the second and third groups of tubes in the four types of crosses.

The present results on fecundity using pair-mating crosses differed from those in mass crosses performed by Fontdevilla *et al.* (1988) in which intercrosses involving B20D2 females from Argentina and A95F3 males yielded much fewer offspring than the reciprocal intercrosses. It is important to point out again the different geographic origins of the strains used by Fontdevilla *et al.* (1988) and the isofemale lines used in the present paper, and also the several differences these strains already showed when other characteristics were analyzed (both mentioned in the introduction of this paper). Besides, Bizzo (1983) showed that mass intercrosses between isofemale line A95C3 (from the same locality as A95F3) and isofemale line B53Q5 (from Milagres, Bahia) yield sterile males and fertile females in both crossing directions. We used a different isofemale line from Bahia, which shows reproductive isolation relative to A95F3, which reinforces the contention that A95F3 and A95C3 isofemale lines and isofemale lines from Bahia are not a single evolutionary entity.

When five day old flies were used, fecundity of A95F3 intracrosses and of B20D2 females x A95F3 males intercrosses increased slowly till the 20th day (Figure 1). When 10 day old flies were mated, intracrosses exhibited greater fecundity at 13.5-14 day parental age, decreasing after that. Both types of intercrosses, however, showed a fecundity increase between 13.5-14 day and 20-20.5 day parental age. As shown in the second experiment, all four types of crosses exhibited a fecundity decrease after 20-20.5 day parental age.

The mechanisms of reproductive isolation operating between B20D2 and A95F3 include prezygotic mechanisms which decrease fertility of intercrosses and also postzygotic ones, including mortality at the larval stage in both directions of crosses and sterility of male hybrids in intercrosses involving *D. koepferae* (B20D2 strain) females and *D. serido* (A95F3 strain) males. Among the 30 mass crosses prepared in each direction of intercrosses for testing fecundity and fertility of F1 hybrids one, a B20D2 female x A95F3 male, yielded F2 larvae and 25 pupae from which 12 females and 11 males were produced.

Bizzo (1983) had already observed sterility of male hybrids in crosses of strains from Argentina (B26D2 - *D. koepferae*, Província de La Rioja and B31D6 - *D. serido*, Província del Chaco, Argentina) with the A95F3 strain. Male hybrid sterility and prezygotic mechanisms preventing intercrosses were also detected by Fontdevilla *et al.* (1988), involving *D. serido* strains from the State of Bahia, Brazil and *D. koepferae*.

Egg-imago cycle length was influenced by culture medium composition. On M1 and M2, prepared with different species of cactus (respectively *Puntia ficus-indica* and *Cereus jamacuru*) the life cycle of A95F3 was longer than that of B20D2 while on M3 (*Opuntia* pulp alone) the length of the A95F3 life cycle decreased to very close to that of B20D2 (Table XI, Figures 2 and 3).

The stocks and experiments in this study were maintained on M1 medium on which the egg-imago span was approximately 27 days for A95F3 and 22 days for B20D2,

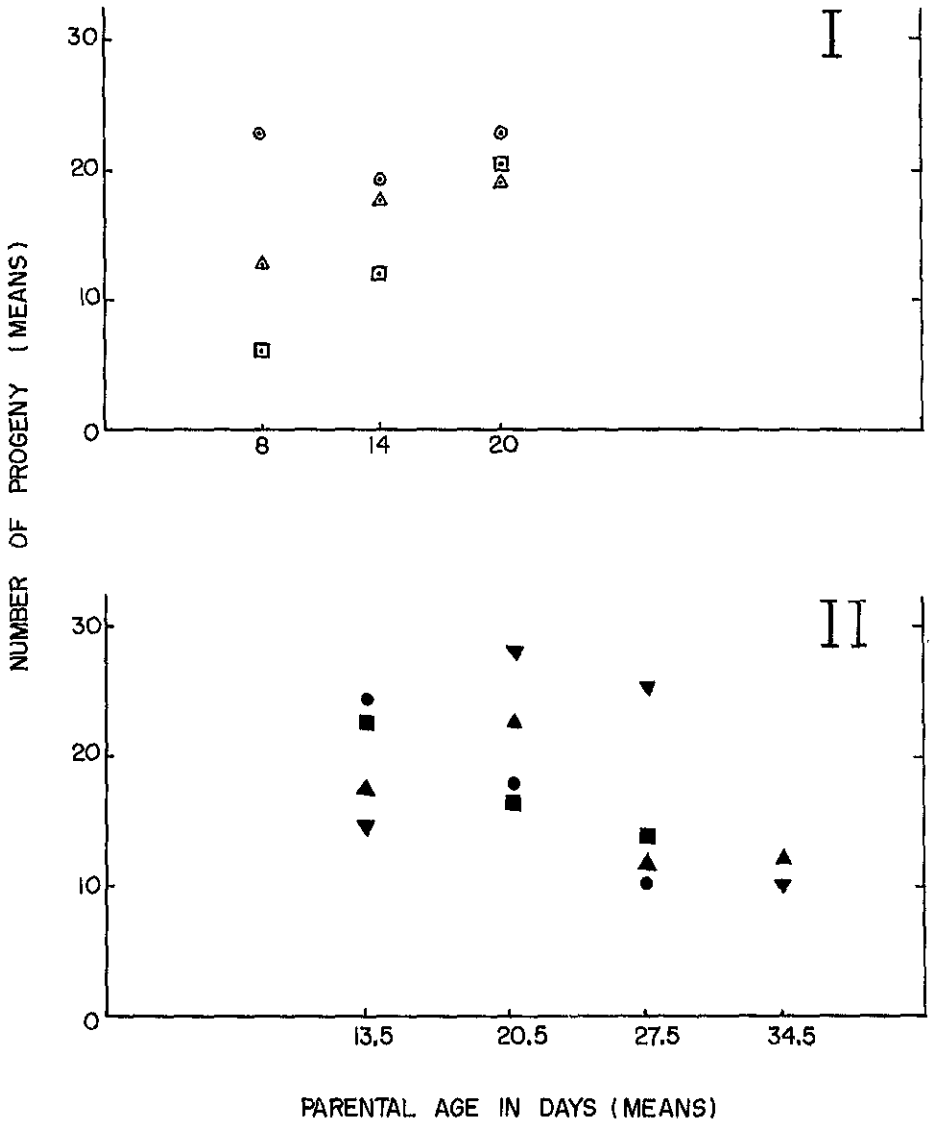

Figure 1 - Fecundity in the four types of crosses performed in experiments I and II. Symbols used: circles = B20D2 intracrosses; squares = A95F3 intracrosses; upper arrowhead = B20D2 females x A95F3 males; lower arrowhead = A95F3 females x B20D2 males.

Table VIII - Experiment II. Significance of two by two comparisons for productivity of larvae and imagoes of the four types of crosses, in the second series of tubes. MSD = 0.04; * = P < 0.05.

	Types of crosses		
	F A95F3 x M A95F3	F B20D2 x M B20D2	F A95F3 x M B20D2
F B20D2 x M B20D2	0.00	0.00	0.12*
F A95F3 x M A95F3		0.00	0.12*
F B20D2 x M A95F3			0.12*

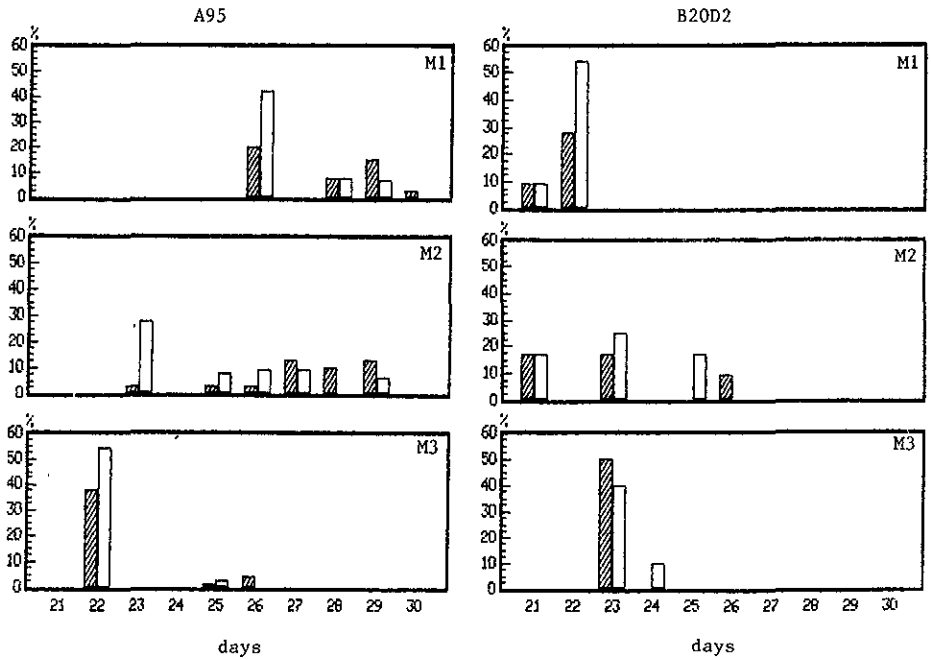

Figure 2 - Development time of A95F3 and B20D2. Experiment I. Percentage distribution of emergence of flies in days, in culture mediums M1, M2 and M3.

in the first experiment. No adults developed in the second experiment. A95F3 development time differed between sexes on the mediums used in both experiments, being longer for females than for males, but in B20D2 there was a very slight difference

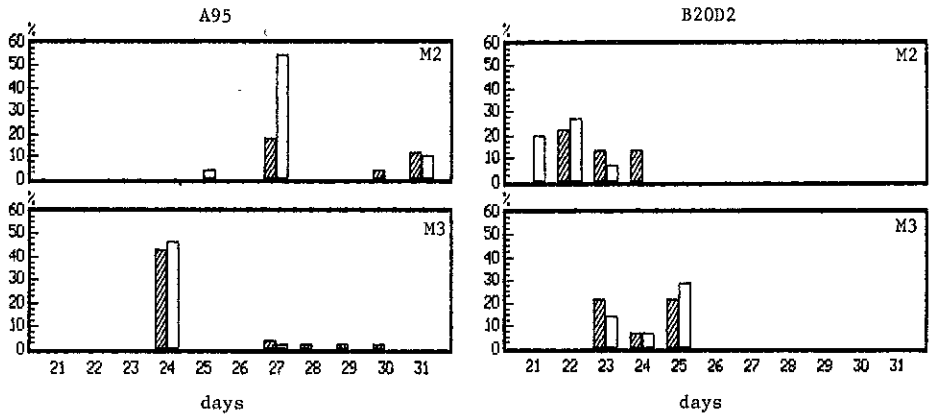

Figure 3 - Development time of A95F3 and B20D2. Experiment II. Percentage distribution of emergence of flies in days, in culture mediums M1, M2 and M3.

between sexes on every medium and, in both experiments, a shortened development time of females relative to males.

Figures 2 and 3 present the distribution of emergence of A95F3 and B20D2 imagoes, respectively in experiments I and II. In the first experiment, 77, 81 and 74 A95F3 flies were computed respectively on the M1, M2 and M3 mediums, while 11, 12 and 10 B20D2 flies were computed on the same mediums. The greatest distribution intervals in development time for both strains were found in M2 culture medium (8 days for A95F3 and 6 days for B20D2); in M1 and M3 these intervals were greater for A95F3 (5 days in both mediums) than for B20D2 (2 days in both mediums).

In the second experiment 61 and 54 A95F3 flies were analyzed respectively on M2 and M3 culture mediums, and 15 and 14 B20D2 flies were analyzed on the same mediums. The same intervals were obtained in M2 and M3 for A95F3 (7 days) while for B20D2 the interval was one day longer on M2 than on M3 (respectively 4 and 3 days).

A95F3 showed a preference for laying eggs in M2 and M3 when the two medium types were present, while B20D2 preferred M3 under the same conditions. Such behaviour might be indicative of their preferences in nature. According to Sene *et al.* (1982), *D. serido* is absent in collections from *O. ficus-indica* plantations. However, Pereira *et al.* (1983) reported that the species has been reared from *O. ficus-indica* and other cactus species such as *O. vulgaris, Cephalocereus piauhyensis, Cereus pernambucensis* and *Cereus* sp.

In a study of differential survivorship on *Opuntia* of *D. serido* and the closely related *D. buzzatti*, Ruiz *et al.* (1985) found that adults of *D. serido* and *D. buzzatii* put into population cages and fed with *Opuntia ficus-indica* roots died within a few days.

Table IX - Experiment I. Fecundity ($\overline{X} \pm S$) of the four types of crosses, in the sequence of tubes. N = number of crosses; F = values for homogeneity of means; * = $P < 0.05$; ** = $P < 0.01$. For A95 x B20D2 intercrosses, numbers of descendants instead of means are given.

Type of crosses		Fecundity								
		Tube 1		Tube 2		Tube 3			Total	
F	M	$\overline{X} \pm S$	N	$\overline{X} \pm S$	N	$\overline{X} \pm S$	N	F	$\overline{X} \pm S$	N
B20D2	B20D2	22.4 ± 11.4	(49)	19.2 ± 11.0	(36)	22.5 ± 10.3	(33)	1.07	51.7 ± 25.1	(49)
A95	A95	6.8 ± 5.8	(6)	12.0 ± 13.7	(18)	21.7 ± 17.2	(22)	3.28*	27.2 ± 24.4	(27)
B20D2	A95	12.8 ± 7.0	(29)	17.8 ± 9.7	(29)	19.2 ± 11.4	(18)	3.34*	33.4 ± 16.8	(37)
A95	B20D2	-		1	(1)	14	(3)	-	15	(4)
F		12.79**		2.55		0.37			12.46**	

Table X - Experiment II. Fecundity ($\overline{X} \pm S$) of the 4 types of crosses, in the sequence of tubes. Symbols as in Table 9.

Type of crosses		Fecundity										
		Tube 1		Tube 2		Tube 3		Tube 4			Total	
F	M	$\overline{X} \pm S$	N	$\overline{X} \pm S$	N	$\overline{X} \pm S$	N	$\overline{X} \pm S$	N	F	$\overline{X} \pm S$	N
B20D2	B20D2	23.9 ± 14.1	(44)	17.7 ± 10.6	(42)	10.1 ± 6.2	(4)	-	-	7.91**	42.1 ± 23 3	(46)
A95	A95	20.2 ± 11.5	(26)	16.6 ± 11.7	(23)	13.6 ± 7 5	(8)	-	-	1.27	35.1 ± 19.9	(29)
B20D2	A95	17.6 ± 8.7	(34)	23 0 ± 14.9	(41)	11.1 ± 7.7	(32)	11.4 ± 9 2	(12)	8 02**	45.2 ± 28.0	(45)
A95	B20D2	14.7 ± 11.5	(9)	28.6 ± 9.6	(7)	25 3 ± 14.9	(6)	9 7 ± 7.6	(3)	3 09*	39.5 ± 36 1	(13)
F		2.58		2.88*		5 56**		0 09			0.94	

The populations which originated from them, however, behaved differently: in the case of *D. serido* they became extinguished after the first generation and in the case of *D. buzzatii* they survived and were maintained with the same food for more than two years. According to these same authors, these results are reasonable, since at least in Argentina

Table XI - Mean development time (egg-imago) of females (F) and males (M) in the three culture mediums (M1, M2 and M3), for the two strains and experiments. In parentheses are differences of means.

Experiment	Strain	M1		M2		M3	
		F	M	F	M	F	M
I	A95	27.5	26.6 (0.9)	27.4	24.9 (2.5)	22.4	22.1 (0.3)
	B20D2	21.7	21.8 (0.1)	22.8	23.0 (0.2)	23.0	23.2 (0.2)
		(5.8)	(4.8)	(4.6)	(1.9)	(0.6)	(1.1)
II	A95			28.7	27.3 (1.4)	24.7	24.1 (0.6)
	B20D2			22.8	22.7 (0.1)	24.0	24.2 (0.2)
				(5.9)	(4.6)	(0.7)	(0.1)

where both species were collected, *D. buzzatii* is associated with many *Opuntia* species while *D. serido* utilizes mainly columnar cacti. On the basis of present knowledge we conclude that the authors studied *D. koepferae*. Ruiz *et al.* (1985) also mentioned the preliminary studies of F. Peris who showed that when *D. serido* adults were put on *Opuntia* culture medium they died very early.

Our data with *Opuntia* culture medium did not show the same effects. In M1 and M3 (first experiment) and M3 (second experiment), prepared with *O. ficus-indica*, A95F3 and B20D2 survived and yielded progeny. As mentioned, they have been maintained for several years in our laboratory, using M1. But, the high larval mortality and low productivity detected in intraspecific crosses of A95F3 might be due to inadequate food rather than to genetic constitution. Kircher (1982) and Ruiz *et al.* (1985) found toxic components in small or significant amounts in pads of *Opuntia*, probably produced by microorganisms acting in decay, which also could be involved with those problems. These observations indicate the need to be careful in speciation studies in relation to data on fertility.

According to Baimai *et al.* (1983), strain A95F3 has a large submetacentric 6th chromosome, differring from the basic karyotype of *D. serido* which has a pair of small dot autosomes. In turn, B20D2 differs from the basic type in the Y chromosome which is metacentric in this strain and telocentric in the basic type, being almost entirely heterochromatic.

Figure 4 shows the metaphase chromosomes of hybrids and parental strains for comparison, observed in brain preparations. Differences of size and intensity of staining

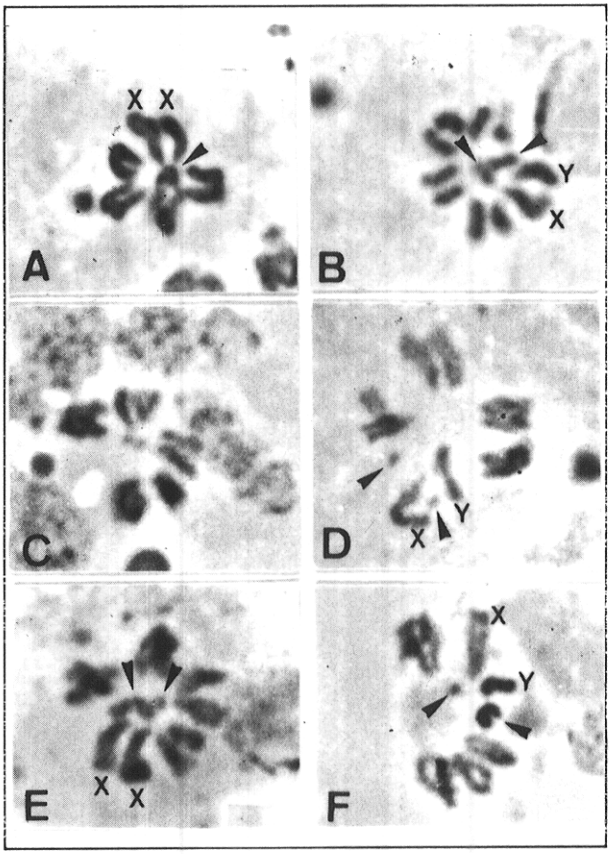

Figure 4 - Metaphase chromosomes. A, B. A95F3 (A) female, (B) male. C, D. B20D2 (C) female, (D) male. E, F. Hybrids of B20D2 females x A95F3 males (E) female, (F) male. Arrowheads point to the microchromosomes.

of the 6th chromosome between strains and homologous 6th chromosomes in hybrids are very clear.

We also observed asynapsis between homologous polytene chromosomes in hybrids A95F3/B20D2 (Figure 5). Such asynapsis is as extensive as detected normally in interspecific hybrids of other *Drosophila* species. Asynapsis is a characteristic considered so constant in *Drosophila* interspecific hybrids that it has been used as a marker in studies of chromosomal introgression (e.g. Tadei, 1977; Naveira and Fontdevilla, 1985).

As shown by Riede and Renz (1983) the degree of pairing of polytene chromosomes decreases following the degree of DNA homology between homologous chromosome bands. They also showed that inversions disturb the process of somatic pairing, but they are not the primary cause of asynapsis in hybrids.

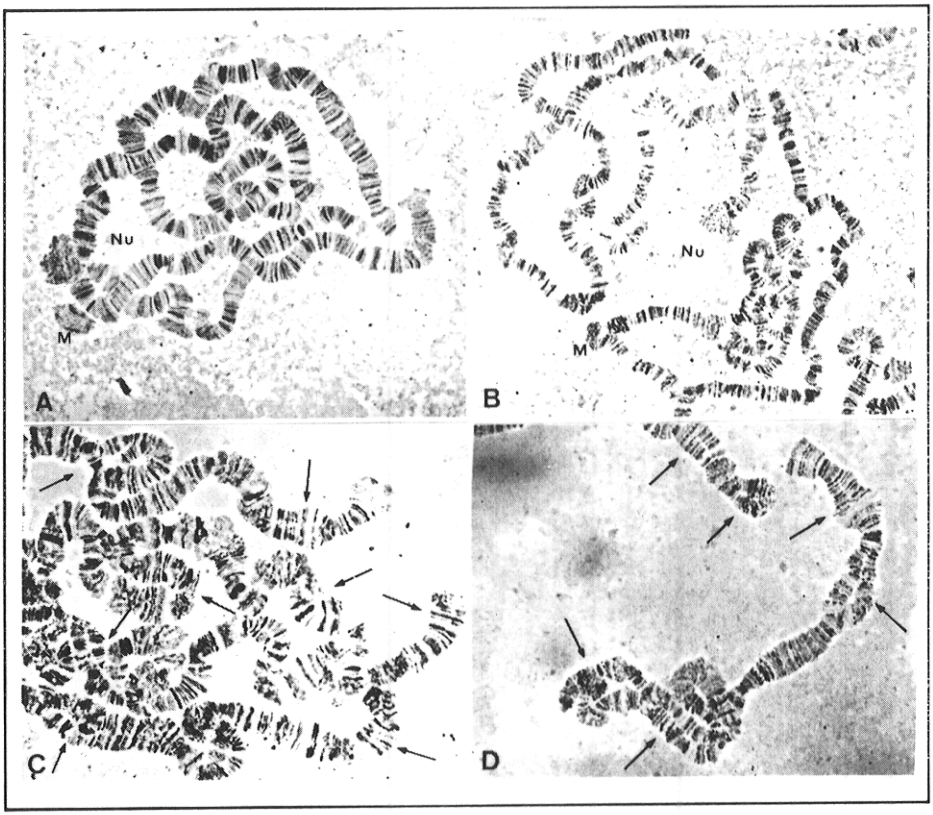

Figure 5 - Synapsis in salivary gland chromosomes of the strains and their hybrids. (A) A95F3; (B) B20D2; (C, D) hybrids of B20D2 females and A95F3 males. Arrows in C and D point to asynaptic chromosomal regions.

A95F3 strain did not show intraspecific inversions, but B20D2 strain showed heterozygous inversions in chromosomes 2, 3 and 5. In hybrids, interspecific inversions were detected in every chromosome except chromosome X. Chromosome 2 shows a complex figure of pairing due to the polymorphic inversions $2k^9$, $2l^9$ and $2m^9$, present in B20D2, and the inversions $2e^9$ and $2j^9$ respectively fixed in A95F3 and B20D2 (Ruiz *et al.*, 1982; Wasserman and Richardson, 1987; Tosi and Sene, 1989). Besides, another subterminal and independent inversion described in B20D2 and named $2n^9$ by Ruiz *et al.* (1982) was sometimes present in hybrids (Figure 6A).

In chromosome 3 of hybrids, two inversions were detected: one of these, 3a, was described by Tosi (1982) and renamed $3k^2$ by Tosi and Sene (1989), being polymorphic in strains from Tapia, Argentina, as is the case of B20D2 strain. The second inversion is present in every hybrid, thus being a fixed inversion in one strain relative to

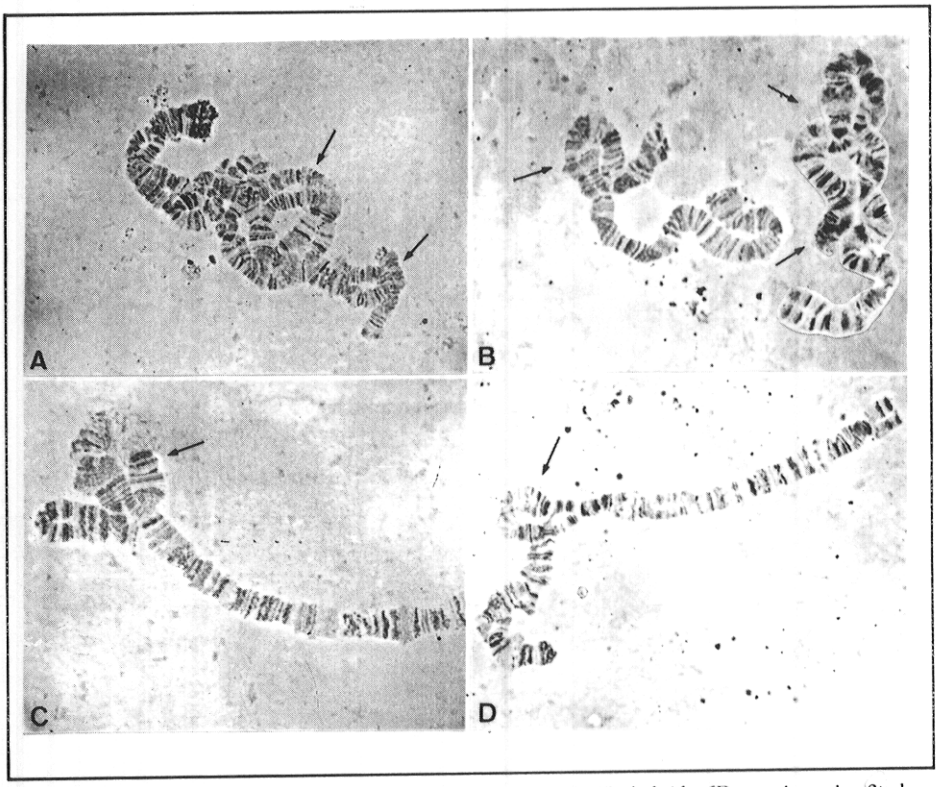

Figure 6 - Inversions in chromosomes 2 (6A), 3 (6B), 4 (6C) and 5 (6D) hybrids. 6B: new inversion 3t alone (6B left) and included in 3k (6B right).

the other (Figure 6B left). When both are present, the fixed inversion is included in the polymorphic one (Figure 6B right). This inversion has apparently not been described previously and thus it is named 3t in the present paper.

In chromosomes 4 and 5, the inversions are simple and single. They were previously described by Tosi (1982) and respectively named 4a and 5b. Tosi and Sene (1989) changed their designation to 4m and 5w, following Wasserman's nomenclature (Figure 6C,D). Polymorphic inversions also occur, according to Tosi (1982), in strains from Tapia, Argentina.

In the present study, differences between A95F3 and B20D2 in the banding pattern of proximal regions of chromosomes 2, 3 and X were also detected (Figures 7 and 8). These differences involved five bands in chromosomes 2 and 3, but in the X chromosome, one apparently longer non-homologous and heterochromatic segment was observed. In each chromosome the differential bands are present in *D. serido* (A95F3)

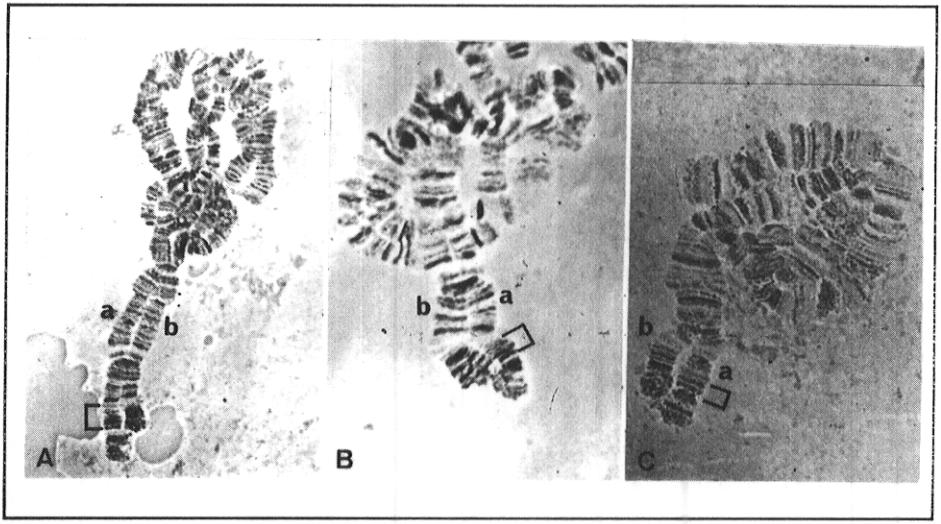

Figure 7 - Comparison of banding pattern of the proximal regions of polytene chromosomes 2 (A, B) and 3 (C) in hybrids of B20D2 females and A95F3 males. Brackets denote bands present in A95F3 (chromosome a) and absent in B20D2 (chromosome b).

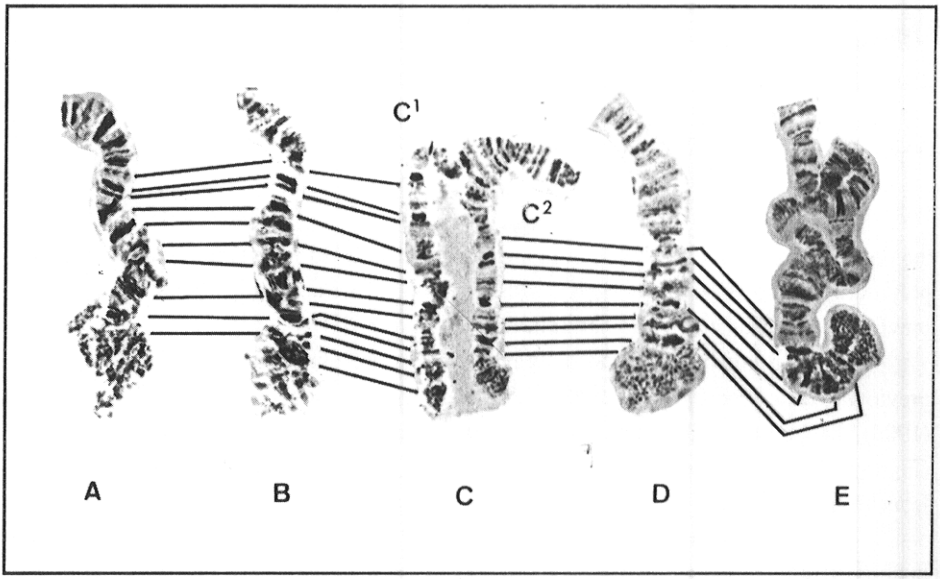

Figure 8 - Comparison of the banding pattern of the proximal region of chromosome X in salivary glands of both species. (A, B) A95F3 males; (C) hybrid female (C1 = A95F3 X, C2 = B20D2 X); (D) B20D2 male. (E) B20D2 female.

and absent in *D. koepferae* (B20D2) and consequently chromosomes 2, 3 and X of the first species are longer than those of the second. Such differences however are difficult to detect in metaphase chromosomes in which greater differences involving chromosome Y and the microchromosomes of A95F3 and B20D2 strains were detected by Baimai *et al.* (1983), already mentioned.

Taken together, the differences observed between isofemale lines A95F3 and B20D2 reinforce their status as distinct species. However, as already mentioned, several kinds of differences, including male sterility in intercrosses, strongly indicate that A95F3 is an evolutionary entity separate from *D. serido* from the State of Bahia (type locality). This strengthens the idea that the complete evolutionary complexity of *D. serido* superspecies remains to be understood. Data obtained by previously mentioned authors, as well as those obtained in a detailed study on reproductive isolation and isozymes of several geographic strains which is being performed in our laboratory, are already suggesting that the cluster is also formed by species in *status nascendi*, similar to *D. paulistorum* (e.g. Dobzhansky and Spassky, 1959; Dobzhansky and Powell, 1975; Richmond and Dobshansky, 1976) and *D. prosaltans* (Bicudo, 1978).

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RESUMO

Drosophila serido é hoje considerada uma superespécie consistindo das espécies *D. serido* (Brasil) e *D. koepfarae* (Argentina e Bolívia). Contudo, há um consenso entre vários autores de que isto não retrata sua verdadeira complexidade evolutiva. No presente trabalho, as linhagens A95F3 (Brasil) e B20D2 (Argentina), portanto, consideradas, respectivamente, representantes da primeira e segunda espécies, foram analisadas quanto à fertilidade e fecundidade em cruzamentos por casal intra e interlinhagem, quanto ao tempo de desenvolvimento, ao padrão de bandas e ao pareamento dos cromossomos politênicos nas linhagens e híbridos. Embora variações nas condições do experimento tenham levado a algumas variações dos resultados, de modo geral os estudos intralinhagem mostraram que a fertilidade e fecundidade de A95F3 são menores do que de B20D2. Intercruzamentos de fêmeas A95F3 com machos B20D2 apresentaram menor fertilidade e fecundidade que os recíprocos, deste modo seguindo as condições da linhagem materna. Estes resultados diferem dos obtidos por Fontdevilla *et al.* (*An. Entomol. Soc. Amer. 81*: 380-385, 1988), o que pode ser atribuído ao fato de que, aqueles autores utilizaram moscas de outra procedência (Estado da Bahia) nos cruzamentos com *D. koepferae*. Essa diferença, somada a outras mostradas na literatura, quanto à morfologia do edeago, às características

cariotípicas, ao polimorfismo de inversões e ao isolamento reprodutivo são fortemente indicativas de que A95F3 (Estado de Minas Gerais) e *D. serido* do Estado da Bahia não são uma mesma entidade evolutiva, reforçando a idéia corrente de maior complexidade da superespécie *D. serido* do que aquela conhecida hoje.

Os mecanismos de isolamento detectados operando entre essas linhagens são pré e pós-zigóticos, incluindo, entre os últimos, mortalidade larval em ambas as direções de intercruzamento e esterilidade dos machos híbridos nos cruzamentos de fêmeas B20D2 com machos A95F3.

As duas linhagens diferiram no tempo de desenvolvimento ovo-adulto que se mostrou também influenciado diferentemente pela composição do meio de cultura em que as moscas foram criadas.

Além disso, mostraram diferenças no padrão de bandas da região proximal dos cromossomos politênicos 2, 3 e X, uma inversão fixa no cromossomo 3 (3t), aparentemente não descrita antes, e um alto grau de assinapse nos híbridos.

A soma das observações, principalmente as referentes ao isolamento reprodutivo, à diferenciação cromossômica (abrangendo o cariótipo, analisado em trabalho anterior, de outros autores, e a diferenciação de bandas, descrita neste trabalho), mais a extensa assinapse verificada nos híbridos dessas linhagens, reforçam o "status" de espécies distintas a elas atribuído.

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