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REPRODUCTIVE ISOLATION IN *Drosophila prosaltans* (*saltans* GROUP)

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ABSTRACT

Reproductive isolation among 18 strains of *D. prosaltans* was studied in mass mating crosses. On the basis of the results, 3 isolation sets of strains were distinguished: set A, including strains from Central America; set B, including strains from South America, north of the Amazon River; and set C, including Brazilian strains, south of the Amazon River. Sexual isolation and hybrid sterility were isolating mechanisms found to operate between the 3 sets, mainly between A and B, and A and C. The results are to some extent comparable to those of *D. paulistorum* semispecies and in this way the 3 sets could also be classified as 3 semispecies. Analysis of chromosomal polymorphism of strains in every set indicated that sets B and C are ancestral to set A, thus suggesting that *D. prosaltans* originated in South America and dispersed to Central America.

INTRODUCTION

In spite of the many biological and environmental aspects involved in speciation, the whole process may be summarized in two basic events: adaptation and selection for reproductive isolation. The multiple aspects of these events and their interplay in speciation allow us to consider as probably unique the evolutionary history of each group of populations of a given species or the evolutionary history of each group of related species. Thus new lights on the knowledge of the speciation process may be expected from each of these newly studied groups.

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The purpose of the present work was to study the speciation process in *Drosophila prosaltans*. Since this species has a large distribution area (the largest among species in the saltans subgroup) which extends from Costa Rica to the south of Brazil and Paraguay, its populations are exposed to many different environments, among them geographic barriers such as mountains, rivers and forests. Under these conditions, genetic differences are expected to accumulate as the populations adapt to their environments. The development of reproductive isolation would then lead to the formation of independent gene pools.

Genetic differences in *Drosophila prosaltans* strains due to paracentric inversions have already been described (Cavalcanti, 1948; Bicudo, 1967, 1973; Bicudo, Hosaki, Machado and Marques, 1978). Data on hybridization tests are presented in this study. They show that a variable degree of reproductive isolation also exists between *D. prosaltans* strains. The evolutionary divergence within *D. prosaltans*, analyzed on the basis of both cytological and isolational pictures, is to some extent comparable to that of *D. paulistorum*, considered as a cluster species in "statu nascendi" (Dobzhansky and Spassky, 1959).

MATERIAL AND METHODS

Geographical origins and stock references of the strains used are in Table I. Eighteen strains from several localities including the northern and southern limits of the species distribution area were analyzed for reproductive isolation.

The experiments involved reciprocal mass crosses of ten pairs per vial (250 ml) using flies aged from 5 to 7 days. Two mass crosses were made in each direction and changed to new vials on the fifth day. The analysis was performed 25 days later. On the basis of the results, the crosses were classified into sterile, high productivity, or low productivity. This last class included crosses which yielded from just one larva up to about 20 adults. Females of sterile crosses were dissected and examined for the presence of spermatozoa in their reproductive tracts.

Depending on the number of available flies, the fertility of F_1 progeny was verified using pair mating or mass endocrosses which were classified, at the time of analysis, like the parental crosses. When F_1 endocrosses did not yield progeny, backcrosses were prepared in order to detect the sterile sex.

Banana culture medium was used and the stocks and tests were maintained at the temperature of 25° C $\pm$ 1° C.

Table I - Geographical origins and stock references of the strains used.

Origin		Reference
Costa Rica	Palmar	P_{1a}
	Piedras Blancas	P_{2a}
	San Isidro	P_{2}
Panamá		P_{26}
Trinidad	Sangre Grande	P_{4}
Colombia	Bucaramanga	P_{5}
Venezuela	Barquisimeto	P_{25}
Guiana	Apoteri	P_{22}
		P_{23}
Brazil	Macapá	P_{12}
	Belém (PA)	P_{6}
	Várzea, Belém (PA)	P_{13}
	(CE)	P_{10}
	Boa Viagem (CE)	P_{16}
	Salvador (BA)	P_{17}
	Campo Grande	P_{18}
	Leste (PA)	P_{20}
	(RS)	P_{21}

RESULTS

The results of intercrosses between 3 strains from Costa Rica (P_{1a}, P_{2a}, P_{2}), one strain from Panama (P_{26}), Trinidad (P_{4}), Colombia (P_{5}), Venezuela (P_{25}), and 2 strains from Guiana (P_{23} and P_{22}) are shown in Figure 1. This Figure is arranged in such a way that the large triangle above the diagonal (where the strain symbols are presented) contains the results of fertility and fecundity of the parental crosses, and the large triangle below the diagonal contains the results of F_1 endocrosses.

The 2 triangles in each small square are related to both directions of crosses of every comination: the triangle below the diagonal refers to the crosses between females from the left strain and males from the strain below (which are respectively above and right strains considering F_1 endocrosses); the triangle above the diagonal is indicative of the reciprocal crosses.

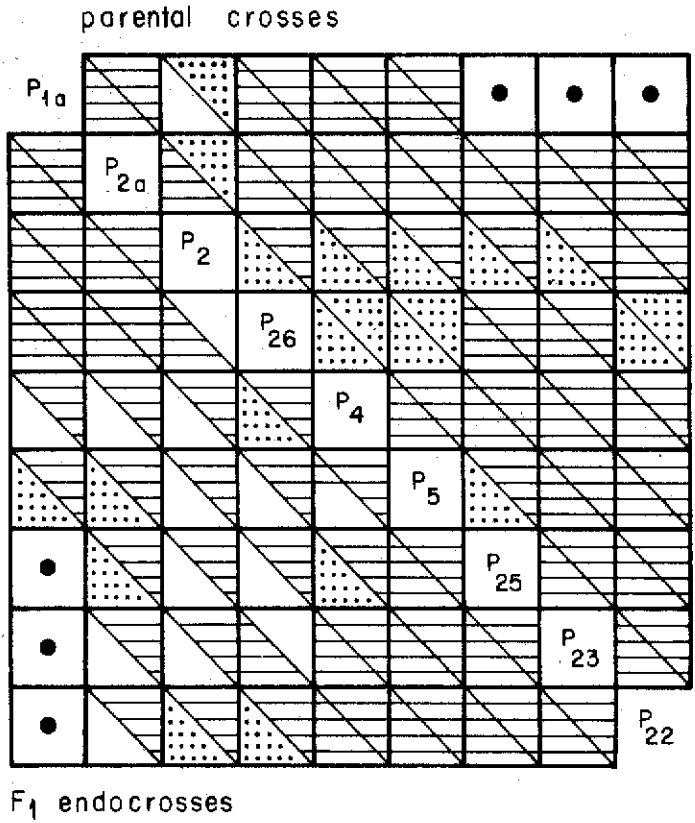

Figure 1. Fertility and Fecundity of the parental crosses and F_1 endocrosses of strains from Costa Rica (P_{1a}, P_{2a}, P_2), Panama (P_{26}), Trinidad (P_4), Colombia (P_5), Venezuela (P_{25}), and Guiana (P_{23}, P_{22}). Full lines = high fecundity; dotted lines = low fecundity; absence of lines = sterile crosses; a single dot = not prepared cross.

In both parental crosses and F_1 endocrosses fertility is indicated by the presence of lines (fertile crosses) or the absence of lines (sterile crosses); and fecundity is indicated by full lines (high productivity) or dotted lines (low productivity). Crosses which were not prepared because the strain P_{1a} was lost are indicated by a single dot in the center of the empty square.

Crosses in Figure 1 showed variable results. For example, the crosses of strains from Trinidad (P_4), Colombia (P_5), Venezuela (P_{25}), and Guiana (P_{23} and P_{22}) with each other were fertile and produced fertile progeny in both directions. In two cases, fecundity was low: in the parental crosses of P_5 females X P_{25} males and in F_1 endocrosses of P_4 females X P_{25} males. All the other combinations presented high fecundity.

The crosses of strains from Costa Rica (P_{1a}, P_{2a} and P_2) and Panama (P_{26}) with each other showed that, when P_{1a}, P_{2a} and P_{26} were involved, the fecundity of the parental crosses was high and their progeny was fully fertile However, in the tests involving any of these strains and P_2 (also from Costa Rica), a variable degree of isolation was detected. Productivity was low in all of the parental crosses in which P_2 were the females. In the crosses between P_{26} and P_2 the isolation affected also the fertility of F_1 progeny: their intercrosses were sterile in the direction P_{26} females X P_2 males.

Crosses of the strains from Costa Rica (P_{1a}, P_{2a} and P_2) and Panama (P_{26}) with the strains from Trinidad (P_4), Colombia (P_8), Venezuela (P_{25}) and Guiana (P_{23} and P_{22}) showed 3 situations concerning the parental crosses: (1) crosses in both directions were fully fertile; (2) crosses in one direction were fully fertile and the other exhibited a low productivity; and (3) crosses in both directions presented low productivities. The first situation was the most frequent. The second was detected in crosses in which the P_2 strain was used, and the third situation occurred in crosses which involved the P_{26} strain.

Most of the F_1 endocrosses in the 3 cases at hand exhibited one fully fertile direction while the reciprocal was sterile or yielded a low number of progeny, frequently half a dozen of pupae or adults at most.

The sterile or almost sterile F_1 endocrosses were produced in most cases by females from Central America and males from northern South America. Two exceptions were the combinations P_{2a} X P_{22} and P_{26} X P_{23}. In the first case, sterile F_1 endocrosses were produced in both directions of crosses, and, in the second, in the direction involving P_{23} females.

Figure 2 is arranged like Figure 1. Data included in it are concerned with the experiments involving the strain from Trinidad (P_4) and 9 strains from Brazil (P_{12}, P_6, P_{19}, P_{10}, P_{16}, P_{17}, P_{18}, P_{20} and P_{21}). Except for the crosses of P_{12} X P_6, P_{10}, P_{18} or P_{21}, and the crosses of P_{10} X P_{17}, all of the other combinations showed high productivity in both directions of the parental crosses and in both directions of the F_1 endocrosses.

The isolation degrees in crosses of P_{12} X P_6 and P_{12} X P_{18} were the strongest among Brazilian combinations: in both cases, a single direction was fertile and the small number of produced hybrids failed to yield F_2. In this direction females were P_{12}. In the intercrosses of P_{12} X P_{21} a single direction (also involving P_{12} females) was also fertile, but the progeny was numerous and fully fertile.

The isolation in the combinations P_{12} X P_{10} and P_{10} X P_{17} affected a single direction of the parental crosses, causing the production of a small number of progeny. This direction involved P_{10} females in the crosses with P_{17}, and P_{10}

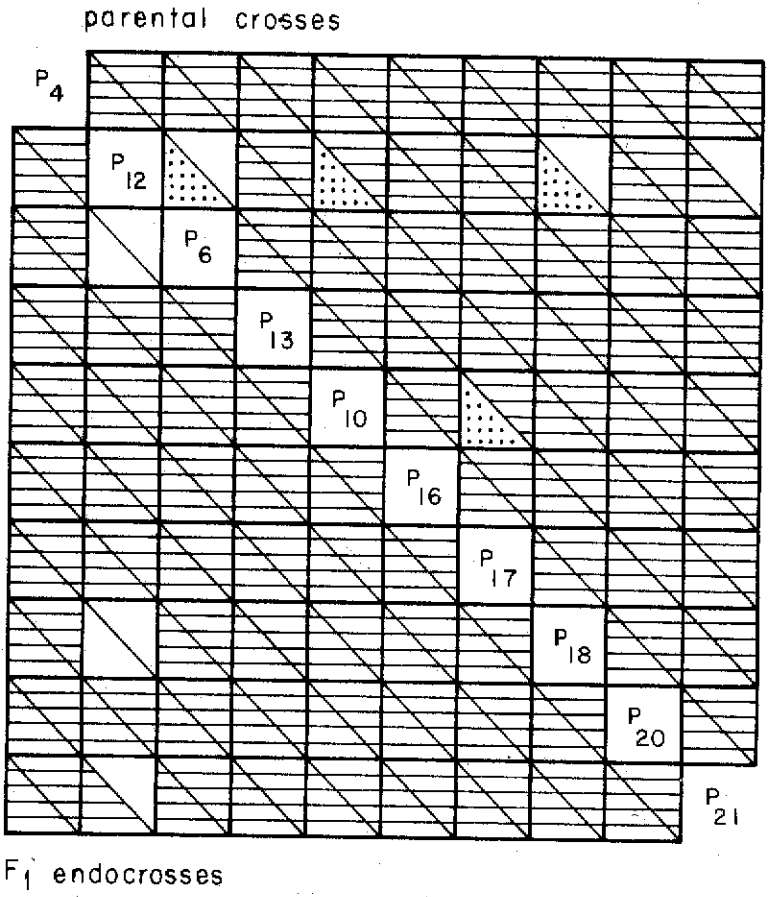

Figure 2. Fertility and fecundity of the parental crosses and F_1 endocrosses of strains from Trinidad (P_4) and Brazil (P_{12}, P_6, P_{13}, P_{10}, P_{16}, P_{17}, P_{18}, P_{20} and P_{21}). For symbols, cf. Figure 1.

males in the crosses with P_{12}. The F_1 endocrosses were fully fertile in both directions of these combinations.

The results of crosses between strains from Colombia (P_5), Venezuela (P_{25}), and Guiana (P_{23} and P_{22}) and 4 Brazilian strains (P_{12}, P_6, P_{10}, and P_{21}) are given in Figure 3. In this Figure, data on parental crosses and F_1 endocrosses are presented separately, (3a and 3b, respectively). The triangles inside the small squares refer to both directions of crosses of every combination: those under the diagonal are concerned with crosses of females from strains included in the vertical column with males from the horizontal column; those above the diagonal

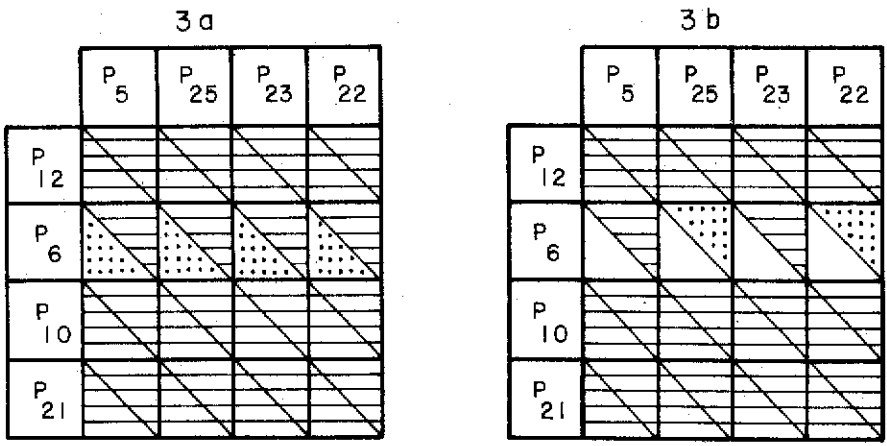

Figure 3. Fertility fecundity of the parental crosses (3a) and F_1 endocrosses (3b) of strains from Colombia (P_5), Venezuela (P_{25}) and Guiana (P_{23} and P_{22}) with Brazilian strains (P_{12}, P_6, P_{10} and P_{21}). For symbols, cf. Figure 1.

are concerned with the reciprocal crosses (females from horizontal column and males from vertical column).

The data in this Figure show that, except for the intercrosses which used the Brazilian strain P_6, all others succeded very easily at the parental and at the F_1 endocrosses level. The P_6 strain showed a decrease in its ability to hybridize and to produce fertile hybrids with any of the strains from northern South America. In all of the intercrosses involving that strain, the direction of parental crosses which used P_6 females showed low productivity and failed to produce fertile F_1 endocrosses.

Figure 4 (a and b) is arranged like Figure 3. It includes the results of the intercrosses between the strains from Costa Rica (P_{1a}, P_{2a}, P_2) and Panama (P_{26}) and the Brazilian strains P_{12}, P_6, P_{10} and P_{21}.

The combinations in this Figure exhibited strong degrees of isolation. Most of the parental crosses (Fig. 4a) were fertile in a single direction and this direction either failed to produce fertile F_1 endocrosses or produced only poorly fertile F_1 endocrosses (Fig. 4b). The origin of males and females in this fertile direction was variable except when parental crosses involved the strain P_2a. In this case every combination was fertile when females were P_{2a}. In general, the

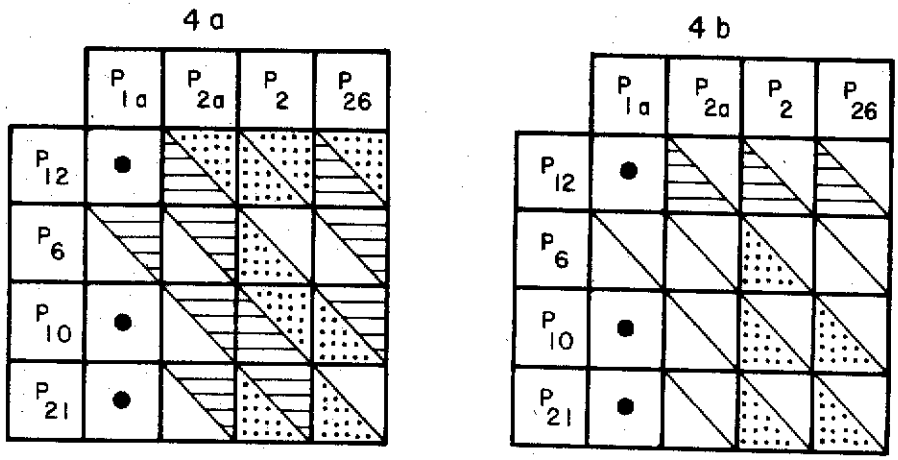

Figure 4. Fertility and fecundity of the parental crosses (4a) and F_1 endocrosses (4b) of strains from Costa Rica (P_{1a}, P_{2a}, P_2) and Panama (P_{26}) with Brazilian strains (P_{12}, P_6, P_{10} and P_{21}). For Symbols, cf. Figure 1.

sterility in the parental crosses was observed more often when females were from Brazil than when they were from Central America. The parental crosses of P_{12} were fertile in both directions, but one or both of these directions produced a small number of progeny. Their F_1 endocrosses did not yield any progeny when the males were P_{12}.

The other combinations which showed both fertile directions in the parental crosses (P_2 X P_{10}, P_2 X P_{21}, and P_{26} X P_{10}) produced fertile F_1 endocrosses only when Brazilian females were used, but even in these cases a small number of progeny was yielded.

Parental crosses involving P_6 showed a single fertile direction in every combination, but the F_1 endocrosses failed to produce progeny except in the crosses with P_2.

The number of females per sterile parental cross available for dissection at the time of analysis for any combination in this study varied from 6 to 20. No sperm was found in most of them. No inseminated female was detected in the sterile direction of the 3 combinations in Figure 2, and, among the 7 combinations which showed one sterile direction in Figure 4a, one inseminated female only was detected in the crosses of P_6 females X P_{26} males (1 inseminated: 6 dissected).

Females from several low productive parental crosses in Figures 1 to 4

were also dissected. From Figure 1: P_5 females X P_{28} males, P_2 females X P_{23} males, P_2 females X P_5 males, and P_{26} females X P_5 males. From Figure 2: P_{12} females X P_{18} males. From Figure 3: P_6 females X P_{22} males. From Figure 4: P_6 females X P_2 males. Most of the flies in every case had not been inseminated.

None of the dissected females from sterile or low productive crosses showed insemination reaction.

The backcrosses of females and males from sterile F_1 endocrosses showed that the males were sterile and the females were fertile in every case.

DISCUSSION

The genetic events leading to reproductive isolation and consequently to speciation are still unknown. Differences in gene regulation (King and Wilson, 1975, Wilson, 1975) or differences in structural genes not detected by current techniques (Ayala, 1976; Coyne, 1976) are hypotheses which still remain to be proved.

Species in "statu nascendi" (Dobzhansky and Spassky, 1959) constitute apparently the most promising material for the study of the genetic basis of speciation. Since evolution is a continuous process, many species are expected to present such borderline populations. However, examples of these species are relatively rare. The explanation advanced by Dobzhansky and his research group for this discrepancy is that the critical stages of the speciation process are passed rather rapidly (Dobzhansky, 1959, 1970; Richmond and Dobzhansky, 1976).

The most studied exemple of species in "statu nascendi" is *D. paulistorum*, considered as a superspecies evolving into 6 semispecies or incipient species (Dobzhansky and Spassky, 1959; Dobzhansky and Powell, 1975; Richmond and Dobzhansky, 1976; Ayala, 1976).

The present study indicated that *D. prosaltans* may constitute a species in the critical stages of the speciation process. Among the strains analyzed in this paper, those from Central America (Costa Rica and Panama) showed reproductive isolation in crosses with the strains from northern South America and from Brazil. The isolation degree was higher when Brazilian strains were used. In this case some combinations produced hybrids in a single direction of the parental crosses and the male hybrids were completely or almost completely sterile. Other combinations produced hybrids in both directions of crosses, but in many cases these hybrids were scarce and the males were completely sterile in one of the directions and almost completely sterile in the other.

The partial or complete absence of inseminated females among those

dissected from sterile parental crosses indicates that sexual isolation is an isolating mechanism which, with hybrid sterility, is acting to prevent laboratory crosses between strains from Central America and Brazil. In most cases, sexual isolation was stronger when the females were from Brazil than when they were from Central America, but when hybrids were produced in both directions of the crosses, male sterility was stronger in the direction involving females from Central America.

In crosses between strains from Central America and strains from northern South America, sexual isolation was less widespread than in crosses with Brazilian strains. This kind of isolating mechanism was only found in crosses involving strains P_{26} (Panama) and P_2 (Costa Rica). However, complete or almost complete hybrid sterility was detected in at least one direction of the crosses in every combination.

Despite the different behavior of strains from Brazil and northern South America in crosses with Central-American strains, most of the intercrosses of strains from northern South American and Brazilian strains were highly productive in both directions of the laboratory crosses and yielded fertile hybrids. However, one of the Brazilian strains, P_6, which intercrosses freely with the other Brazilian strains exhibited some sexual isolation and complete male sterility in the direction involving P_6 females and males from northern South America. The behavior of the Brazilian strain P_{12} should also be pointed out. This strain intercrossed easily with the strains from northern South America but showed a variable degree of isolation in crosses with other Brazilian strains. Thus, the behavior of P_{12} is closer to that of strains from northern South America than to that of other Brazilian strains. This fact was also observed in crosses with Central American strains: P_{12} showed a degree of isolation lower than that shown by other Brazilian strains and similar to that exhibited by strains from northern South America.

These results provided evidence for dividing the *D. prosaltans* strains into 3 isolation sets which obey the geographic origin of the strains on the basis of their ability to hybridize with each other. One of these sets is composed of strains from Central America, including Costa Rica and Panama; another is composed of strains from northern South America, including Trinidad, Colombia, Venezuela, Guiana and Brazil, north of the Amazon River; and the last is composed of the remaining Brazilian strains. In Figure 5 the 3 sets are respectively referred to as A, B, and C.

While the crosses of strains from different sets showed variable degrees of reproductive isolation, the results of tests within every set showed, with few exceptions, high productivity of parental crosses and fully fertile F_1 hybrids. The exceptions were strain P_2, in set A, and strain P_{26}, in set B. Both strains exhibited an incipient isolation in crosses with other strains from the same set.

Figure 5. Distribution area of the 3 isolation Sets. Set A = strains from Central America; Set B = strains from South America, north of the Amazon River; Set C = strains from Brazil, south of the Amazon River.

Strain P_4, from Trinidad, deserves a special mention. This strain barely intercrossed with a single strain from Set A (P_{26}), but intercrossed freely with the strains from Set B (except P_{25}) and with all of the strains from set C. The decision to include P_4 in set B and not in C was based mainly on geographical origin. In cases like this, it is possible that other methods may reveal lower degrees of isolation than those shown by mass mating crosses. On the other hand, we cannot discard the possibility that, if a greater number of mass crosses were prepared, few hybrids could be obtained in crosses which were completely sterile in these experiments. Aniway, the present data show very clearly the existence of a speciation process in progress in *D. prosaltans*.

In this study, laboratory strains were used. Many of them were kept as laboratory stocks for several years before these experiments were made. This situation must be mentioned because there is one well known case of origin of incipient isolation in the laboratory reported by Dobzhansky and Pavlowsky (1966, 1967, 1971) and Dobzhansky and Powell (1975). In *D. prosaltans*, however, the behavior of the strains consistent with their distribution area makes us believe that the isolation detected among the strains analyzed reflects the evolutionary stage of the natural populations from which they came, rather than an evolutionary response to laboratory conditions. Besides, the use of natural samples in studies of *D. prosaltans* is very difficult. This species, in spite of its large distribution area, is considered "rare". It has been collected, using different methods, in very low frequencies such as 1:10,000 (Cavalcanti, 1950), 1:2,700 (Pavan, 1959), and 1:200 to 1:2,000 (Mourão, 1966). A frequency of 1:50 was considered good (Bicudo *et al.*, 1978), but exceptionally good was a single collection of 28 *D. prosaltans* in 36 flies caught in the secretion of a *Campomanesia cagaiteira* Keaersk tree (Pavan, 1959). The impossibility of getting new samples using the same methods, and in the same places where the species was detected in previous collections, is another problem which has been mentioned by some authors (Cavalcanti, 1950; Pavan, 1959; this author's personal experience).

We still do not know if the "rarity" of flies in the collections really reflects the population size of *D. prosaltans* or is only the consequence of an almost complete absence of knowledge about feeding habits and their behavior in nature. Anyway, the "rarity" of the species makes the laboratory stocks very precious because they are the only available source of information on the species.

A discussion on the evolutionary status of *D. prosaltans* strains must involve concepts accepted by evolutionists. Ayala (1976) described 2 main stages in the geographic speciation. In the first, the allopatric populations (subspecies) exhibit incipient reproductive isolation in the form of partial hybrid sterility. In the second stage, the populations (semispecies) became sympatric and are

being submitted by natural selection to the development of more complete reproductive isolation mainly in the form of sexual isolation. Ayala (1976) mentioned, as examples of the first stage, 2 species from the willistoni group: *D. willistoni* and *D. equinoxialis* which are composed of 2 subspecies (*D. willistoni willistoni* and *D. w. quechua*, respectively, and *D. equinoxialis equinoxialis* and *D. e. caribbensis*). In both cases crosses of the subspecies in one direction produced sterile hybrid males, but no evidence of sexual isolation was obtained. The second stage of the speciation process was exemplified by *D. paulistorum* (also from the willistoni group), whose 6 semispecies show strong, although incomplete, ethological isolation and complete sterility of male F_1 hybrids. Some of the semispecies of *D. paulistorum* are sympatric, with apparently complete sexual isolation.

Although isofemale lines of *D. prosaltans* are not available at present for detection of sympatric reproductive isolation, the situation in *D. prosaltans* is to some extent comparable to the situation encountered in *D. paulistorum*. The presence of sexual isolation and male hybrid sterility operating between strains from Central America (set A) and Brazil (set C), and between Central America and northern South America (set B) is an argument to classify the 3 sets as 3 semispecies. The results of crosses between strains from sets B and C are similar to those of Transitional semispecies of *D. paulistorum* in crosses with Central American semispecies. Strains in set B are closely related to strains in set C, but, while some crosses of B x C succeeded easily and the hybrids were fertile, other crosses showed some sexual isolation and male progeny was sterile.

Accumulated data on chromosomal polymorphism of *D. prosaltans* revealed a total of 14 inversions distributed in the chromosome arms as follows: XL - 7 inversions; XR - 1 inversion; IIL - 1 inversion; IIR - 3 inversions; III - 2 inversions (Bicudo *et al.*, 1978). The gene arrangements of *D. prosaltans* strains used in the present study are shown in Table II. Among the 3 sets, set A is the most homogeneous as to chromosomal polymorphism: all of the strains in this set are homozygous for the inversion PXLa and for the standard arrangements in the other chromosomes (The P_{2a} strain has a homozygous and exclusive inversion overlapping PXLa: the PXLb inversion). However, the arrangements shared by the strains in set A are not exclusive of this set. The PXLa inversion was also found in the P_4 strain, included in set B and geographically close to set A; and the standard arrangements of chromosome arms XR, IIL, IIR and chromosome III were also found in some strains from sets B and C.

The distribution of the gene arrangements in the strains from sets B and C is as follows. Three inversions are shared by some strains from both sets (PIILa, PIIRa, PIIIb), while other inversions are exclusive of set B (PIIRb, PIIRc) or exclusive of set C (PXLd, PXRa). However, in both sets the exclusive inversions

Table II - Chromosomal arrangements of strains in every set. Data from Cavalcanti (1948); Bicudo (1967, 1973); Bicudo et al. (1978).

SET	STRAIN	Chromosomal arrangements				
		XL	XR	IIL	IIR	III
A	P_{1a}	a	+	+	+	+
	P_{2a}	ab	+	+	+	+
	P_{3}	a	+	+	+	+
	P_{3b}	a	+	+	+	+
B	P_{4}	+	+	+	+	+
	P_{5}	ac	+	+	+ a	a
	P_{25}	+	+	+ a	+ b	+
	P_{22}	+	+	+ a	+	+
	P_{23}	+	+	+	+ bc	+
	P_{12}	+	+	+ a	+ a	+ b
C	P_{6}	+	+	+ a	+	+
	P_{13}	+	+	a	+ a	+
	P_{16}	+ d	+	a	+	+
	P_{14}	d	+	+	+ a	+ b
	P_{17}	d	+	a	+	+
	P_{18}	+ d	+	+ a	+	+ b
	P_{20}	+	a	+	+	+
	P_{21}	+	+	+	+	+

are only present in some of the strains.

The chromosomal 1 polymorphism in *D. prosaltans* strains of sets B and C resembles that of *D. paulistorum,* in which some inversions are shared by 2 or more semispecies, while other inversions are found in some species and not in others (Kastritsis, 1967, Dobzhansky and Powell, 1975). The monomorphism of *D. prosaltans* strains in set A is very interesting because it may be indicative of the origin and dispersion of the species.

According to Carson (1959), new strains arise mostly from peripheral populations of parental species. The peripheral populations are usually monomorphic (da Cunha et al., 1959; Carson, 1959). In this way the polymorphic sets of *D. prosaltans* strains (B and C) may be considered ancestral to set A. This idea is reinforced by comparison of the present isolation data with Kaneshiro's (1976) data. This investigator, in a study of Hawaiian *Drosophila,* assumed that females of derived species mate randomly with males of ancestral species but females of ancestral species show strong sexual discrimination against males of the more derived species. The justification was that the courtship pattern of the derived species has elements in common with the ancestral population so that the females may recognize those elements in the courtship of males of ancestral species. The same does not happen between females of ancestral species and males of derived species since the courtship of these males contains only some elements of the pattern of ancestral species.

Crosses between *D. prosaltans* strains from Central America and strains from Brazil showed sexual isolation which in most combinations was stronger when females were from Brazil than when they were from Central America.

Thus, based on Kaneshiro's assumption, it is also possible to consider Central America strains of *D. prosaltans* to be derived from South American strains. These observations are also consistent with the ideas of Throckmorton (1974) on the origin and spreading of the saltans subgroup. On the basis of the geographic distribution of the species (Magalhães, 1962), this author suggested that the saltans subgroup originated in South America, and diffused northward into Central America, Mexico and the Caribbean.

The discovery of semispecies in *D. prosaltans* increases the opportunity of getting new information on the speciation process of the saltans group and may become useful in the approach to general problems such as the kind and the amount of genetic changes involved in speciation.

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