

REPRODUCTIVE ISOLATION
IN THE *SALTANS* GROUP OF *DROSOPHILA*.
I. THE *SALTANS* SUBGROUP

HERMIONE ELLY MELARA DE CAMPOS BICUDO

Departamento de Genética, Faculdade de Filosofia, Ciências e Letras de São José
do Rio Preto, São Paulo, Brasil

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In order to study isolation within the *saltans* subgroup, pair mating crosses involving its 7 species were performed. The results of testing different interspecific combinations varied from complete isolation at the insemination level to the production of fertile hybrids. Some isolation indices are proposed: Ii (isolation index at the insemination level), Fi (isolation index at the fertilization level), Ti (total isolation index), and Ai (average isolation index). A schema of the biological relationships on the basis of the isolation degree is also presented for the 7 species of the subgroup.

Introduction

This paper is the first in a series concerned with studies of isolation in the *saltans* group of *Drosophila* whose primary aim is to identify some of the isolating mechanisms that operate within the group.

Since an analysis of chromosomal variability of the same group is also being made, it can be assumed that the relationships between the cytological and the isolation data will contribute to a better knowledge of the evolution within the group.

This paper summarizes the results of a series of tests of intraspecific and interspecific mating, using the species in the *saltans* subgroup. Subsequent papers will deal with the other subgroups of the same group.

Materials and Methods

The taxonomy, morphology and geographical distribution of the *saltans* group were extensively analyzed by MAGALHÃES (1962). In summary, according to this author, the group contains 5 subgroups:

saltans, *sturtevanti*, *parasaltans*, *elliptica* and *cordata*, with 19 species in total. Two more species were later added by MOURÃO & BICUDO (1967).

The *saltans* subgroup, the subject of the present paper, includes 7 members: *Drosophila saltans*, *D. prosaltans*, *D. septentriosaltans*, *D. austrosaltans*, *D. lusaltans*, *D. pseudosaltans* and *D. nigrosaltans*. Our cytological studies confirm that these 7 species, originally established on the basis of a morphological analysis, are all full species.

The species of the *saltans* group are found in the Nearctic and Neotropical regions, being preponderant in the latter. Of the *saltans* subgroup, only *D. saltans* is present in both regions.

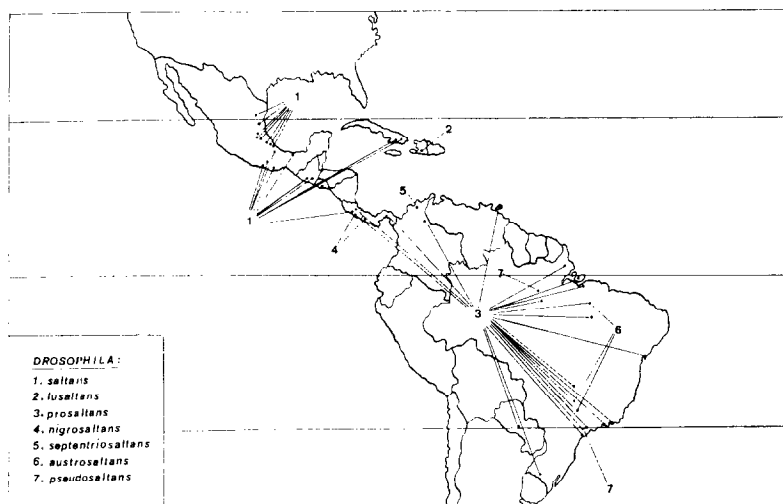


Fig. 1. Geographical distribution of the *saltans* group.

Figure 1 shows the geographical distribution of the *saltans* subgroup species according to MAGALHÃES (1962), with some new collection places added. It may be seen that *D. saltans* is present from Mexico to Costa Rica and *D. prosaltans* from Costa Rica to the south of Brazil and Paraguay. Before the publication of Magalhães' article, Mexico was believed to be the northern limit of distribution of *D. prosaltans*. This may be explained by the fact that the 7 species included in the *saltans* subgroup are sibling species and their mor-

phological distinction became possible only after the detailed study of the male genitalia and spermathecae performed by MAGALHÃES & BJORNBERG (1957) and MAGALHÃES (1962).

In this investigation the 7 standard strains (inversion free), originally prepared for detecting chromosomal variability, were used. Their geographical origins are as follow: *D. saltans* – San Salvador, El Salvador; *D. prosaltans* – Belém, Pará, Brazil; *D. septentriosaltans* – Sevilla, Colombia; *D. austrosaltans* – Mirassol, São Paulo, Brazil; *D. lusaltans* – Petionville, Haiti; *D. pseudosaltans* – Santarém, Pará, Brazil; *D. nigrosaltans* – Boquete, Panamá.

The following procedure was used:

Reciprocal pair mating crosses of virgin flies from 5 to 6 days old were made. The flies were transferred twice to new cultures at 3 day intervals. The analyses were made from 25 to 30 days after the mating. The females of the pairs which did not produce any progeny were dissected and their reproductive tracts examined for the presence of spermatozoa. Such dissections were made in Shen solution (NICOLETTI, 1959) that was also used in the preparations. Sex-ratio and number of progeny were recorded for the vials whose pairs produced progeny. The fertility of the F₁ flies was tested by means of F₂-crosses and back-crosses.

For a more detailed study on the isolation between *D. prosaltans* and *D. saltans*, pair mating tests were carried out using 7 strains of *D. prosaltans* and 5 strains of *D. saltans*. In this case only the number of fertile crosses was computed. The geographical origins and the symbols of these strains are as follow: *D. saltans* – San Jose, Costa Rica (S₁); San Salvador, El Salvador (S₂); Guatemala, Guatemala (S₃); Huychiuayan, Mexico (S₆); Chilpancingo, Mexico (S₇). *Drosophila prosaltans* – Turrialba, Costa Rica (P₁); San Isidro, Costa Rica (P₂); Balboa, Panamá (P₃); Sangre Grande, Trinidad (P₄); Bucaramanga, Colombia (P₅); Belém, Pará, Brazil (P₆); El Dorado, Rio Grande do Sul, Brazil (P₇).

Results

Table 1 presents the results of the interspecific pair mating tests. For the sake of comparison, intraspecific pair mating tests involving

TABLE 1

RESULTS OF INTERSPECIFIC PAIR MATING CROSSES IN THE *SALTANS* SUBGROUP¹

| Types of crosses | | Number of crosses | Fertile crosses | | Dissected females | Inseminated females | | Average number of progeny within 30 days | Fertility of F ₁ |
|------------------|-----|-------------------|-----------------|---------|-------------------|---------------------|---------|--|-----------------------------|
| ♀ | ♂ | | No. | (%) | | No. | (%) | | |
| P | S | 50 | 25 | (50.00) | 21 | 1 | (4.76) | 13.04 | Fertile ♀♂ |
| S | P | 50 | 14 | (28.00) | 33 | 32 | (96.96) | 5.31 | Fertile ♀ |
| P | Se | 144 | 2 | (1.38) | 95 | 0 | | 8.50 | Fertile ♀♂ |
| Se | P | 51 | 1 | (1.96) | 40 | 1 | (2.50) | 14.00 | Fertile ♀ |
| P | L | 102 | 48 | (47.00) | 39 | 0 | | 25.20 | Fertile ♀ |
| L | P | 54 | 17 | (31.40) | 16 | 0 | | 14.47 | Fertile ♀ |
| S | Se | 53 | 0 | | 46 | 44 | (95.65) | — | — |
| Se | S | 57 | 0 | | 51 | 2 | (3.92) | — | — |
| S | L | 105 | 17 | (16.19) | 63 | 12 | (19.04) | 5.17 | Fertile ♀♂ |
| L | S | 92 | 42 | (45.65) | 30 | 12 | (40.00) | 12.02 | Fertile ♀♂ |
| Se | L | 55 | 0 | | 44 | 1 | (2.27) | — | — |
| L | Se | 91 | 2 | (2.19) | 50 | 5 | (10.00) | 1.50 | — |
| A | N | 51 | 13 | (23.53) | 25 | 0 | | 22.38 | Fertile ♀ |
| N | A | 50 | 0 | | 22 | 0 | | — | — |
| Se | Pse | 50 | 0 | | 43 | 1 | (2.32) | — | — |
| Pse | Se | 50 | 0 | | 43 | 0 | | — | — |

¹) The following combinations showed complete isolation: P × A, P × N, S × Pse, Se × N, L × Pse, P × Pse, S × A, S × N, A × Pse, L × A, L × N, Pse × N, and Se × A.

P = *prosaltans*, S = *saltans*, A = *austrosaltans*, Se = *septentriosaltans* L = *lusaltans*, Pse = *pseudosaltans*, N = *nigrosaltans*.

the same 7 strains used in the interspecific tests were also made and their results are shown in Table 2.

An examination of Table 1 discloses that the 21 possible interspecific combinations of the 7 species studied can be divided into 3 major groups: 1. The combinations that do not yield hybrids or even insemination; 2. The combinations that yield insemination, but do not yield hybrids; 3. The combinations that yield hybrids.

The first group is the largest comprising 13 combinations that show complete reproductive incompatibility. As even insemination does not occur in this group (the number of crosses was at least 50 in each direction for each combination used), we may conclude that one or more "pre mating barriers" (as named by Mecham, 1961, in

TABLE 2

RESULTS OF INTRASPECIFIC PAIR MATING CROSSES IN THE *SALTANS* SUBGROUP¹

| Types of crosses | | Number of crosses | Fertile crosses | | Dissected females | Inseminated females | | Average number of progeny |
|------------------|-----|-------------------|-----------------|---------|-------------------|---------------------|---------|---------------------------|
| ♀ | ♂ | | No. | (%) | | No. | (%) | |
| P | P | 51 | 47 | (92.15) | 2 | 1 | (50.00) | 45.32 |
| S | S | 50 | 38 | (76.00) | 4 | 2 | (50.00) | 30.90 |
| A | A | 50 | 32 | (64.00) | 13 | 0 | | 48.37 |
| Se | Se | 58 | 26 | (44.82) | 29 | 4 | (13.79) | 47.92 |
| L | L | 50 | 40 | (80.00) | 8 | 0 | | 66.15 |
| Pse | Pse | 50 | 40 | (80.00) | 6 | 0 | | 55.45 |
| N | N | 52 | 31 | (59.61) | 3 | 1 | (33.33) | 22.77 |

¹) Cf. also note to Table 1.

MAYR, 1963) are acting in maintaining the complete genetic separation of these species. Sexual isolation is probably the responsible mechanism, since we never saw, in several observations of those crosses, any pair in copulation. As listed under Table 1, this group includes all the combinations of *pseudosaltans*, except its crosses with *septentriosaltans*; all the combinations of *austrosaltans*, except its crosses with *nigrosaltans*, and all the combinations of *nigrosaltans*, except its crosses with *austrosaltans*.

The second and third groups comprise interspecific combinations which share some compatibility at certain levels of the reproductive cycle.

The second group includes the combinations *septentriosaltans* × *pseudosaltans* and *saltans* × *septentriosaltans*. The first 2 of these species may be considered as completely isolated from each other, since only one female, in the crosses *septentriosaltans* ♀ × *pseudosaltans* ♂ was inseminated and exhibited a strong "insemination reaction" (PATTERSON, 1946). Gametic mortality was thus acting in the only case where the early premating barriers could be overcome.

A significant difference in relation to the percentage of inseminated females was found between the reciprocal crosses involving *saltans* × *septentriosaltans*: the crosses *saltans* ♂ × *septentriosaltans* ♀ showed a strong isolation at the insemination level (3.92% of ♀♀ were found to be inseminated), while in the reciprocal crosses there was a high percentage of insemination (95.65%, $\chi_1^2 = 81.77$, $P = 0.01$). However,

in both directions of the crosses there is a mechanism that is very effective in completely preventing the production of hybrids.

In the third group, besides pre mating barriers, "postmating barriers" (as also termed by Mecham, 1961, in MAYR, 1963) are present. It comprises 6 interspecific combinations: *prosaltans* × *saltans*, *prosaltans* × *septentriosaltans*, *prosaltans* × *lusaltans*, *saltans* × *lusaltans*, *septentriosaltans* × *lusaltans* and *austrosaltans* × *nigrosaltans*. Although having in common the capacity of producing hybrids, these combinations exhibit a number of variable characteristics involving: (1) the percentage of fertile crosses, (2) the percentage of inseminated females, (3) the fertility of F₁ flies, and (4) the average number of progeny.

The percentage of fertile crosses was found to be variable among the different combinations. The lowest percentages were obtained in *septentriosaltans* × *prosaltans* (1.38% and 1.96%) and *septentriosaltans* × *lusaltans* (2.19% and 0%). The highest percentages were obtained in the combinations *prosaltans* × *lusaltans* (47% and 31.48%) and *prosaltans* × *saltans* (50% and 28%). On the other hand, the percentage of fertile crosses was also found to be different between the reciprocal crosses of some combinations, being statistically significant for *prosaltans* × *saltans* ($\chi_1^2 = 5.08$, P = <0.05), and for *saltans* × *lusaltans* ($\chi_1^2 = 20.28$, P = <0.01).

From the biological point of view, the most extreme case is the combination *austrosaltans* × *nigrosaltans*: 23.53% of fertile crosses were obtained from *austrosaltans* ♀ × *nigrosaltans* ♂ while the reciprocal crosses did not yield hybrids, or even insemination ($\chi_1^2 = 14.62$, P = <0.01).

In the third group the percentage of inseminated females differs between the reciprocal crosses of some interspecific combinations: *prosaltans* × *saltans* ($\chi_1^2 = 45.91$, P = <0.01) and *saltans* × *lusaltans* ($\chi_1^2 = 4.54$, P = <0.05). In the combinations *prosaltans* × *saltans*, the highest percentage is in the direction of the crosses that yields less fertile crosses (*saltans* ♀ × *prosaltans* ♂); in contrast, *saltans* × *lusaltans* gives the highest percentage in the direction that yields more fertile crosses (*lusaltans* ♀ × *saltans* ♂). These facts suggest that the isolating mechanisms are probably more effective before insemination in the crosses *prosaltans* ♀ × *saltans* ♂, and after insemination in the crosses *saltans* ♀ × *prosaltans* ♂. On the other hand,

both the premating and the postmating mechanisms are probably more effective in the crosses *saltans* ♀ × *lusaltans* ♂ than in the reciprocal.

Several authors have shown that the heterogametic crosses of *Drosophila* the spermatozoa may be inactivated or killed when introduced into the reproductive tract of an alien female (e.g. PATTERSON, 1954). This phenomenon could explain the non-development of hybrids from the inseminated females, observed in the present investigation. Another possibility is that although fertilization has occurred the zygote is inviable. The first hypothesis receives support from the fact that immobile spermatozoa were predominantly found in the inseminated females. If this is the case, the fertile interspecific crosses could be explained by some spermatozoa that were resistant to the negative effect of the female reproductive tract.

In relation to the fertility of F₁, 3 situations were found: (1) Fertile males and females in both directions of the crosses, in the combination *lusaltans* × *saltans*; (2) Fertile females and sterile males in one direction of the crosses, and fertile females and males in the reciprocal, in *prosaltans* × *saltans* and *prosaltans* × *septentrionsaltans*; (3) Fertile females and sterile males in both directions of the crosses, in *prosaltans* × *lusaltans*, or in the only fertile direction of the crosses, in *austrosaltans* × *nigrosaltans*. The fertility of F₁ progeny from *septentrionsaltans* ♀ × *lusaltans* ♂ could not be analyzed because of the small number of F₁ flies.

Finally, the average number of progeny is variable for the different combinations of species in the third group. The highest averages were obtained in the crosses *prosaltans* ♀ × *lusaltans* ♂ (25.20) and *austrosaltans* ♀ × *nigrosaltans* ♂ (22.38); the smallest average was obtained in *lusaltans* ♀ × *septentrionsaltans* ♂ (1.50). The average number of progeny also varies between the reciprocal crosses, the difference being significant for *prosaltans* × *saltans* ($t_{37} = 2.742$, $P = < 0.01$), for *prosaltans* × *lusaltans* ($t_{51} = 2.492$, $P = < 0.01$), and for *saltans* × *lusaltans* ($t_{57} = 2.225$, $P = < 0.05$).

A comparison of the data in Tables 1 and 2 shows that the numbers of fertile crosses and the average numbers of the progeny are quite different in the interspecific and the intraspecific combinations. Both numbers are considerably smaller in the interspecific crosses. All the χ^2 values for the differences in numbers of fertile crosses are signifi-

cant at the 0.01 level. On the other hand, Student's "t" test gave significant values for the combinations *prosaltans* × *saltans*, *prosaltans* × *lusaltans*, and *saltans* × *lusaltans* versus the intraspecific crosses involving the same species, thus rejecting the hypothesis of equality of their average number of progeny. Table 3 shows that in a single case the "t" value is not significant: in *austrosaltans* ♀ × *nigrosaltans* ♂ versus *nigrosaltans* × *nigrosaltans*. The low fecundity of the intraspecific crosses of *nigrosaltans* (the lowest of the *saltans* subgroup, as shown in Table 2) is not unexpected. This species was always maintained in our culture stocks only with difficulty until it was completely lost. Student's "t" test was not applied for *septentriosaltans* × *prosaltans* and *lusaltans* × *septentriosaltans* because of their small *n* values.

TABLE 3

STUDENT'S *t* VALUES FOR THE AVERAGE NUMBER OF PROGENY OF THE INTERSPECIFIC CROSSES VERSUS THE AVERAGE NUMBER OF PROGENY OF THE INTRASPECIFIC CROSSES

| ♀ ♂ | P×P | S×S | L×L | A×A | N×N |
|-------|--------------|--------------|--------------|-------------|------------|
| P × S | 5.078 (70)** | 5.561 (61)** | — | — | — |
| S × P | 4.946 (59)** | 6.797 (50)** | — | — | — |
| P × L | 3.765 (93)** | — | 7.178 (86)** | — | — |
| L × P | 3.990 (62)** | — | 4.101 (55)** | — | — |
| S × L | — | 7.414 (53)** | 7.036 (55)** | — | — |
| L × S | — | 4.824 (78)** | 9.335 (80)** | — | — |
| A × N | — | — | — | 2.644 (43)* | 0.054 (42) |

1) In parentheses, degrees of freedom.

*) $P \leq 0.05$.

***) $P \leq 0.01$.

Table 2 also shows that some incipient isolating mechanisms may be seen even in the intraspecific crosses. For example, 13 females dissected in the intraspecific crosses of *austrosaltans* were found not to be inseminated; and 29 in the intraspecific crosses of *septentriosaltans* showed only 4 inseminated females. The percentage of fertile crosses also varies in the intraspecific combinations. The lowest percentage was found in *septentriosaltans* (44.82%) which, as mentioned above, also exhibits a high frequency of uninseminated females.

Tables 4 and 5 present percentages that reflect the isolation degrees between and within the species studied. They were calculated from

TABLE 4

MEASURES OF THE ISOLATION IN PERCENTAGE CALCULATED FROM TABLE 1¹

| Types of crosses | | Ti | Ii | Fi | Ai |
|------------------|-----|--------|--------|-------|--------|
| ♀ | ♂ | | | | |
| P | S | 50.00 | 47.62 | 2.38 | 61.00 |
| S | P | 72.00 | 2.26 | 69.84 | |
| P | Se | 98.62 | 98.62 | 0.00 | 98.33 |
| Se | P | 98.04 | 95.59 | 2.45 | |
| P | L | 53.00 | 53.00 | 0.00 | 65.76 |
| L | P | 78.52 | 78.52 | 0.00 | |
| S | L | 83.81 | 67.89 | 15.92 | 69.08 |
| L | S | 54.35 | 32.61 | 21.74 | |
| Se | L | 100.00 | 97.72 | 2.28 | 98.90 |
| L | Se | 97.81 | 88.03 | 9.78 | |
| A | N | 76.27 | 76.27 | 0.00 | 88.13 |
| N | A | 100.00 | 100.00 | 0.00 | |
| S | Se | 100.00 | 4.40 | 95.60 | 100.00 |
| Se | S | 100.00 | 96.10 | 3.90 | |
| Se | Pse | 100.00 | 97.68 | 2.32 | 100.00 |
| Pse | Se | 100.00 | 100.00 | 0.00 | |

¹) The interspecific combinations not included here (the ones mentioned in Table 1) showed 100% isolation at the Ii level. Ti = total isolation, Ii = isolation at the insemination level, Fi = isolation at the fertilization level, Ai = average isolation.

TABLE 5

MEASURES OF THE ISOLATION IN PERCENTAGE CALCULATED FROM TABLE 2¹

| Types of crosses | | Ti | Ii | Fi |
|------------------|-----|-------|-------|-------|
| ♀ | ♂ | | | |
| S | S | 24.00 | 12.00 | 12.00 |
| P | P | 7.80 | 3.90 | 3.90 |
| A | A | 36.00 | 36.00 | 0.00 |
| Se | Se | 46.34 | 36.42 | 9.92 |
| L | L | 20.00 | 20.00 | 0.00 |
| Pse | Pse | 20.00 | 20.00 | 0.00 |
| N | N | 40.39 | 26.94 | 13.45 |

¹) For explanation cf. note to Table 4.

the data of Tables 1 and 2 respectively, including the measure of the total isolation (Ti), represented by the percentages of crosses not yielding offspring, and, within the total isolation, the measure of the isolation acting at 2 different levels of the reproductive cycle: (1) at the insemination level (Ii), represented by the percentage of uninseminated females; (2) at the fertilization or early zygotic viability level which cannot be clearly distinguished here (Fi), represented by the percentage of inseminated females which did not yield hybrids. If *a* equals the number of uninseminated females; *b*, the number of inseminated females without progeny; and *c*, the number of females with progeny, then

$$Ti = \frac{a + b}{a + b + c}; Ii = \frac{a}{a + b}; \text{ and } Fi = \frac{b}{a + b}.$$

The measure of isolation for each interspecific combination (Ai) is the average of the total isolation in both directions of the crosses, hence

$$Ai = \frac{Ti(A \times B) + Ti(B \times A)}{2}.$$

Among the interspecific combinations the lowest values for Ti were found in *prosaltans* ♀ × *saltans* ♂ (50%), *prosaltans* ♀ × *lusaltans* ♂ (53%), and *lusaltans* ♀ × *saltans* ♂ (54.35%). Among the intraspecific crosses, the lowest value for Ti was found in *prosaltans* (7.80%) and the highest in *septentriosaltans* (46.34%). In general the Ii values are considerably greater than the Fi values, both in the inter- and in the intraspecific crosses. The reverse is true for *saltans* ♀ × *prosaltans* ♂ and for *saltans* ♀ × *septentriosaltans* ♂. Although in the crosses *lusaltans* ♀ × *saltans* ♂, the Ii value (32.61%) is greater than the Fi value (21.74%), the latter still can be regarded as great. In the intraspecific crosses of *saltans* and *prosaltans*, the Ii values equal the Fi values (12% for the first species; 3.90% for the second).

Among the 6 interspecific combinations which exhibit Ai values different from 100%, *prosaltans* × *saltans*, *prosaltans* × *lusaltans* and *lusaltans* × *saltans* showed the smallest values (61%, 65.76% and 69.08% respectively). In other words this means that these are the most intercrossable species in the *saltans* subgroup. Next in order of size is the Ai value showed by *austrosaltans* × *nigrosaltans* (88.13%).

The A_i values for *prosaltans* × *septentriosaltans* (98.33%) and *septentriosaltans* × *lusaltans* (98.90%) are very near the maximum value (100%).

TABLE 6
RESULTS OF INTERSPECIFIC CROSSES INVOLVING DIFFERENT STRAINS OF
PROSALTANS (MALES) AND *SALTANS* (FEMALES)¹

| <i>prosaltans</i> strains | <i>saltans</i> strains | | | | |
|------------------------------|------------------------|----------------|----------------|----------------|----------------|
| | S ₁ | S ₂ | S ₃ | S ₆ | S ₇ |
| P ₁ | 0 | 12 | 3 | 50 | 56 |
| P ₂ | 0 | 0 | 2 | 72 | 15 |
| P ₃ | 33 | 3 | 66 | 1 | 34 |
| P ₄ | 2 | 0 | 28 | 14 | 55 |
| P ₅ | 0 | 7 | 4 | 24 | 58 |
| P ₆ | 39 | 5 | 6 | 67 | 85 |
| P ₇ | 22 | 48 | 27 | 65 | 77 |

¹) The number of fertile crosses obtained in 100 crosses for each combination is recorded. Cf. text for further explanations.

TABLE 7
RESULTS OF INTERSPECIFIC CROSSES INVOLVING DIFFERENT STRAINS OF
PROSALTANS (FEMALES) AND *SALTANS* (MALES)¹

| <i>prosaltans</i> strains | <i>saltans</i> strains | | | | |
|------------------------------|------------------------|----------------|----------------|----------------|----------------|
| | S ₁ | S ₂ | S ₃ | S ₆ | S ₇ |
| P ₁ | 0 | 29 | 17 | 86 | 67 |
| P ₂ | 0 | 0 | 0 | 16 | 9 |
| P ₃ | 0 | 1 | 71 | 53 | 27 |
| P ₄ | 17 | 55 | 85 | 18 | 4 |
| P ₅ | 28 | 23 | 80 | 20 | 65 |
| P ₆ | 1 | 29 | 17 | 16 | 27 |
| P ₇ | 16 | 24 | 58 | 67 | 59 |

¹) Cf. note to Table 6.

Tables 6 and 7 record the results of a more detailed study on isolation between *prosaltans* and *saltans* using tests performed with 7 strains of *prosaltans* and 5 strains of *saltans*. The subfigures in the symbols, used for naming these strains, correspond to a ranking of the relative distance from their origin to Costa Rica, which is the geographical distribution limit common to both species: the highest figures correspond to the most distant strains (see Materials and

Methods). The results show that the strains from Costa Rica, where both species are geographically more proximate (the collecting sites were about 20 km apart, according to a personal information by Dr. L. E. Magalhães), are completely isolated. Besides the pair mating crosses, 15 mass crosses with 10 pairs per vial were tried for the combinations $P_1 \times S_1$ and $P_2 \times S_1$, and not even larvae were obtained. On the other hand, combinations involving other allopatric strains showed variable percentages of fertile crosses: from low, as in P_3 (Panama) \times S_2 (El Salvador) (1%; 3%) and P_2 (Costa Rica) \times S_3 (Guatemala) (2%; 0%), to high, as in P_7 (Brazil) \times S_7 (Mexico) (77%; 59%) and P_7 (Brazil) \times S_6 (Mexico) (65%; 67%). Different frequencies between the reciprocal crosses are striking for some allopatric combinations such as P_6 (Brazil) \times S_1 (Costa Rica) (39%; 1%) and P_4 (Trinidad) \times S_2 (El Salvador) (55%; 0%).

Discussion

Data concerned with reproductive isolation of the species of the *saltans* subgroup were presented for the first time by DOBZHANSKY & STREISINGER (1944). They used tests of sexual preference performed with 7 strains classified as *prosaltans* from Mexico (Huychiuayan, Chilpancingo and Zopilote), from Guatemala (Guatemala) and from Brazil (Belem, Bertioga and Iporanga). The present knowledge about the taxonomy, distribution (MAGALHÃES, 1962) and cytology (our data, unpublished) of the *saltans* subgroup, allow us to conclude that their study was not carried out with one species but with two: *saltans* (Mexico and Guatemala) and *prosaltans* (Brazil). From the origin of the strains it may be inferred that, among the 42 tests, 12 are intra-specific for *saltans*, 6 are intraspecific for *prosaltans*, and 24 are interspecific for both species. The 42 tests gave 27 positive and 15 negative isolation indices. Among these negative indices, one is intraspecific for *prosaltans*, 5 are intraspecific for *saltans* and 9 are interspecific. In the latter, the females preferentially inseminated were always *saltans*, as we also found for *prosaltans* \times *saltans*.

SPASSKY (1957) presents results of tests for reproductive isolation using strains from Brazil (Piraçununga) and Mexico (Chilpancingo), the latter also misclassified as *prosaltans*. Chilpancingo females mated

with Piraçununga males produced fertile females and sterile males while the reciprocal crosses produced fertile females and males. On the basis of his studies on the morphology of genitalia, the same author established a morphological differentiation between the strains of "*prosaltans*" from Mexico and Guatemala and 6 Brazilian strains of *prosaltans*. It is now known that the morphological variation recognized by Spassky is a character of interspecific distinction between *saltans* and *prosaltans*.

Other studies on the reproductive isolation in the *saltans* subgroup were performed using strains of *saltans* classified as *prosaltans*. Among them, MAYR & DOBZHANSKY (1945), who made tests for sexual preference using strains from Mexico (Chilpancingo and Zopilotte) and from Brazil (Belem, Iporanga and Bertioga) under several conditions, such as, presence or absence of light, males grown up in the absence of females, and different temperatures, and HOENIGSBERG & SANTIBAÑES (1950), who studied the courtship and mating discrimination using strains from Mexico, Trinidad and Brazil. In both studies the strains from Mexico were not *prosaltans* as they were classified, but rather *saltans*.

Our results, obtained by means of pair mating, provide evidence that there exist, in the *saltans* subgroup, different factors that influence, in varying degrees, the isolation among the 7 species.

The several interspecific combinations studied showed that isolation mechanisms ranging from those situations in which the pre-mating barriers are completely effective to situations in which the pre-mating and the postmating barriers are present without completely preventing the production of fertile hybrids, occur in the *saltans* subgroup. A predominant action of the isolating mechanisms at the insemination level is evident and may be understood if we recognize that the postponement of the isolation to the later levels of the reproductive cycle may represent a greater risk.

By and large, the studies of isolation and interspecific hybridization in animals have shown, in WHARTON'S (1944) words, that "in nearly every case where the production of fertile hybrids is possible in the laboratory, potent isolating mechanisms operate to prevent such gene exchange in nature." In the *saltans* subgroup also, none of the situations in which the reproductive isolating mechanisms are less effective endangers the genetical identity of the species concerned.

So far the data available show that in nature *saltans* and *lusaltans* are geographically isolated. The same is true of *prosaltans* and *lusaltans*, of *austrosaltans* and *nigrosaltans*, and even of *prosaltans* and *septentriosaltans* that, although in a low frequency, also produce fertile hybrids. On the other hand, *saltans* and *prosaltans*, which overlap in distribution in areas in Costa Rica, do not exchange genes either. Our results using different strains of these species showed that the strains from Costa Rica have a complete reproductive isolation.

It is accepted that the reproductive isolating mechanisms may originate in 2 possible ways: (1) accidentally, as a side-effect of genetic divergence (MULLER, 1942); (2) as a product of the natural selection acting on appropriate genetic variation when the allopatric populations that have an incipient isolation become sympatric (DOBZHANSKY, 1940). Evidence for both processes has been obtained by several authors.

The incipient isolation occurring between geographic strains of the same species that has been widely demonstrated in the genus *Drosophila* (e.g. DOBZHANSKY, 1944; MATHER, 1963), corroborates the hypothesis of the origin as a side-effect of the genetic divergence. Our results in the *saltans* subgroup show that some incipient isolating mechanisms are already acting between individuals of the same strain and suggest (at the level of our observations) that the isolating mechanisms which act in these intrastain crosses are apparently the same that act in the interspecific ones, although exhibiting obviously lower frequencies in the first case. Assuming that the reproductive isolation results from the concomitant occurrence of genetic variants which are incompatible (i.e., situations involving males and females with such antagonistic variants show a break of the reproductive process in some stage), we may conclude that incompatible variants are already present intrastain. Here, however, natural selection probably maintains such variants in a low frequency, or does not, at least, permit that in the case of 2 antagonistic variants both be highly frequent in the same strain. In geographical isolation, on the other hand, 2 populations, which at first belong to the same species, may undergo genetic divergence in a way that natural selection protects one of the antagonistic variants in one of the populations and the other antagonistic variant in the other population. Returning to sympatry both populations will show a higher or a lower isolation

degree dependent on the frequencies attained by those antagonistic genetic variants in them. This is obviously an oversimplification of the problem, since several incompatible variants may occur with different performance in the reproductive cycle.

The importance of natural selection in reinforcing the isolation is evidenced by the fact that among the allopatric species or allopatric strains of different species the isolation is less strong than among sympatric strains or species (e.g. DOBZHANSKY and al., 1964; GRANT, 1966; SMITH, 1959). Our results relative to crosses of different strains of *prosaltans* and *saltans* are an example of this situation: the strains from Costa Rica, where perhaps the 2 species coexist or have coexisted, show a complete isolation; high frequencies of fertile crosses are obtained only when the distance between the origins of the strains is great enough to prevent their crossing in nature. Thus it may be supposed that the genetic divergence between *prosaltans* and *saltans* took place in allopatric populations that became sympatric and, when this happened, natural selection favoured the reproductively isolated individuals, fixing, in this way, the isolation among the species in that region. Other examples involve *austrosaltans* and *prosaltans*. (sympatric in Piraçununga and Mirassol, Brazil), *nigrosaltans* and *prosaltans* (sympatric in Turrialba, Costa Rica), and *pseudosaltans*

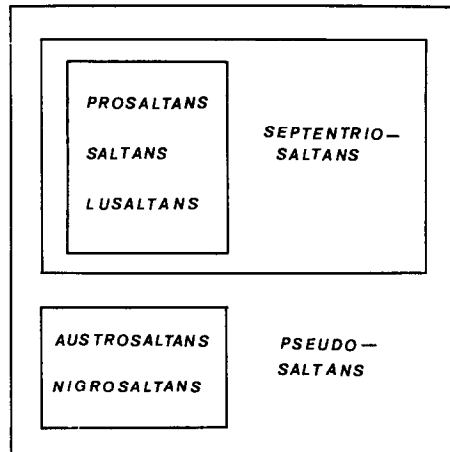


Fig. 2. Relationships among the *saltans* group species, established on the basis of the isolation data.

and *prosaltans* (sympatric in Cantareira, Brazil); all of them showed complete isolation at the insemination level. On the other hand, as indicated above, the intercressable species in the *saltans* subgroup are all allopatric in nature.

It is well known that incomplete reproductive isolation has been used by several authors as indicative of a close biological relationship between the intercressable species (e.g. FUTCH, 1962; PATTERSON, 1952; WHARTON, 1944). In other words, this means that the lower the isolation degree, the greater the biological proximity. Figure 2 shows schematically the evolutionary relationships among the *saltans* subgroup species determined on the basis of the interspecific average isolation degree (A_i) calculated for the 7 allopatric standard strains used in this paper. *Drosophila prosaltans*, *saltans* and *lusaltans* are reproductively closer to each other than to the remaining species. In its turn, *septentriosaltans* is closer to the first 3 than to the others. *Drosophila nigrosaltans* and *austrosaltans* are closely related to each other and apart from the remaining, and *pseudosaltans* is apart from all the others.

In order to evaluate to what extent these relationships may be accepted as real, a comparison of the analysis of the present isolation study and the cytological data reported in a forthcoming paper will need to be made.

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