

## REPRODUCTIVE ISOLATION IN THE SALTANS GROUP OF *DROSOPHILA*. IV. THE STURTEVANTI SUBGROUP

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### ABSTRACT

The reproductive isolation in the sturtevanti subgroup was studied by pair mating and mass mating crosses involving 3 species in the subgroup: *D. sturtevanti*, *D. milleri* and *D. magalhaesi*. The results showed high and variable degrees of reproductive isolation among combinations. The isolation indices showed stronger isolation at the insemination level (Ii) than at the fertilization level (Fi) in every case. Two different interpretations of the species derivation in the subgroup were presented and discussed on the basis of the available data.

### INTRODUCTION

This paper is part of a series concerned with isolation studies in the saltans group of *Drosophila*. In the preceding papers hybridization tests of species and strains in the saltans, parasaltans and elliptica subgroups were reported (Bicudo, 1973, 1978; Bicudo and Prioli, 1978a, b). Data on intraspecific and interspecific mating tests of species belonging to the sturtevanti subgroup are now presented.

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The *sturtevanti* subgroup was established on a morphological basis by Magalhães (1962) to include *D. rectangularis*, *D. sturtevanti* and *D. milleri*. Two other species — *D. magalhãesi* and *D. dacunhai* — were added by Mourão and Bicudo (1967), bringing to 5 the number of species accepted today as belonging to the subgroup.

The geographical distribution of *D. sturtevanti* is the largest among the species in the subgroup and even in the saltans group as a whole. It includes almost the entire distribution area of the group, from Mexico to the south of Brazil and the Caribbean Islands. In contrast, according to the available data, the other members of the subgroup exhibit a very restrict geographical distribution. Magalhães (1962) reported *D. milleri* exclusively in El Yunque, Puerto Rico (where *D. sturtevanti* was also recorded by the same author), and *D. rectangularis* in Orizaba and Tixtla, Mexico. Mourão and Bicudo (1967) reported *D. magalhãesi* in Eldorado, Brazil, and *D. dacunhai* in Kingston and Ocho Rios (Jamaica).

The 5 members in the *sturtevanti* subgroup are morphologically very similar, being distinguishable only by some minor details (Magalhães and Bjornberg, 1957; Magalhães, 1962; Mourão and Bicudo, 1967). They constitute close genetic systems which are, in general, considered important tools in speciation studies.

The data presented in this paper provide information on the degrees of isolation and on the isolating mechanisms operating among 3 species in the subgroup. An interpretation of the phylogenetic relationships of these species based on their isolation patterns is also presented and discussed.

## MATERIALS AND METHODS

*Drosophila mülleri*, *D. magalhãesi* and *D. sturtevanti* were available for the present study. The geographical origins and the symbols of the strains used are given in Table I. Except for St (from Mirassol), all of these strains have been maintained for a long time in the laboratory.

The methods followed were basically the same as described in detail in the first paper of this series (Bicudo, 1973). In summary, interspecific and intraspecific reciprocal pair mating crosses replicated twice were analyzed 30

days after preparation with respect to fertility (frequency of fertile crosses) and fecundity (average number of progeny). Females of sterile crosses were also examined for the presence of spermatozoa in their reproductive tracts.

The fertility of the  $F_1$  progeny was studied by means of mass mating endocrosses. In cases of no  $F_2$  production,  $F_1$  males and females were separately backcrossed to both parental species.

Fertility and fecundity were also studied in mass mating tests involving *D. sturtevanti* and *D. milleri*. Two mass crosses were prepared in every case using 10 pairs per vial with two changes of food. The crosses were classified with respect to their fecundity as: low productivity (+), medium productivity (++) , and high productivity (+++). These classes included crosses which yielded less than 10, less than 50 and more than 50 descendants respectively.

The experiments and the stocks were maintained on banana culture medium at a temperature of  $25^{\circ} \text{C} \pm 1^{\circ} \text{C}$ . Virgin flies aged 7 days were used in the experiments.

Table I - Species and strains used in this study

Species	Symbols	Origin
<i>D. sturtevanti</i>	St	Mirassol (SP), Brazil
	St <sub>1</sub>	El Yunque, Puerto Rico
<i>D. milleri</i>	Mi	El Yunque, Puerto Rico
<i>D. magalhães</i>	Mag	Eldorado (RS), Brazil

## RESULTS

The Results of interspecific pair mating crosses are shown in Table II. Both directions of the combination St x Mi produced fertile crosses. The combinations St x Mag and Mag x Mi produced fertile crosses in a single direction; in both cases the sterile direction involved Mag females.

The combination St x Mi showed 21.28 % fertile crosses when St females and Mi males were used, and 3.28 % in the reciprocal crosses. The difference between both directions of crosses was statistically significant

( $P < 0.001$ ). The combinations St x Mag and Mag x Mi produced low frequencies of fertile crosses in the single fertile direction, as follows: Mi x Mag – 1.44 % and St x Mag – 0.96 %.

The highest percentages of inseminated females found in the sterile crosses were obtained in the crosses Mi females x Mag males (21.62 %) and St females x Mi males (14.28 %). A significant difference with respect to the number of inseminated females was found between the reciprocal crosses in the combinations St x Mi ( $\chi^2_1 = 4.61, P < 0.05$ ) and Mi x Mag ( $\chi^2_1 = 6.89, P < 0.01$ ).

The highest average number of progeny was shown by St females x Mi males (15.60), followed in decreasing order by Mi females x Mag males (12.0), and Mi females x St males (5.0). The single fertile cross of St x Mag yielded only larvae.

With respect to the fertility of  $F_1$ , St x Mi showed fertile females and males in the direction of crosses involving St females and Mi males, and fertile females and sterile males in the reciprocal crosses. The single fertile direction of crosses in the combination Mag x Mi yielded fertile females and sterile males.

The results of intraspecific crosses are presented in Table III. Fertility was highest in St (67.06 %) followed in decreasing order by Mi (62.10 %) and Mag (46.67 %). The differences were significant for Mag versus St ( $\chi^2_1 = 7.77, P < 0.01$ ) and for Mag versus Mi ( $\chi^2_1 = 3.92, P < 0.05$ ). Fecundity was highest in St, closely followed in decreasing order by Mag and Mi. The Student's "t" test for comparison of the average numbers of progeny indicated that the differences are not significant. Inseminated females among those dissected from sterile crosses were detected in a single intraspecific cross: St x St (18.18 %).

A comparison of the data in Tables II and III shows that, as expected, the percentages of fertile crosses and their fecundity are clearly lower in the interspecific crosses than in the intraspecific ones.

Tables IV and V present isolation indices calculated from the data in Tables II and III, respectively, as proposed by Bicudo (1973). These indices are: Ti (total isolation), represented by the percentage of crosses not yielding offspring; Ii (isolation at the insemination level) represented by the percentage of uninseminated females; Fi (isolation at the fertilization level) represented by the percentage of inseminated females which did not yield hybrids; and Ai (average isolation), i.e. the average of the Ti values in both directions of

crosses in the interspecific combinations. The highest values for  $t_i$  in the interspecific combinations (Table IV) were found in the crosses involving Mag females and equals 100 %, while the lowest values (which are still high) were obtained in both directions of crosses between St and Mi (78.72% and 96.72%). The  $A_i$  value of the latter combination equals 87.72., while in the combinations St x Mag and Mi x Mag the same index equals 99.52 and 99.28, respectively. The  $I_i$  values obtained were in general considerably greater than the  $F_i$  values.

In the intrastrain crosses (Table V) the highest  $T_i$  value was obtained in Mag x Mag (53.33 %), followed in decreasing order by Mi x Mi (37.90) and St x St (32.93).

The isolation observed in Mi and Mag endocrosses occurred exclusively at the insemination level. Isolation at the fertilization level was only detected in St endocrosses. However, in this case the  $I_i$  value was also greater than the  $F_1$  value.

Table VI shows data on mass crosses prepared using *D. sturtevantii* from El Yunque ( $St_1$ ) or Mirassol (St) and *D. milleri* from El Yunque (Mi). It also shows data on the intraspecific crosses between *D. sturtevantii* from Mirassol and El Yunque, prepared for control. The results showed stronger isolation between sympatric strains than between allopatric ones. However, hybrids were produced in both cases.

Table II - Results of interpecific pair mating crosses.

Types of crosses		Number of crosses	Fertile crosses N♀ (%)	Dissected females	Inseminated females N♀ (%)	Average number of progeny within 30 days	Fertility of F <sub>1</sub>	
F	M							
St	Mi	94	20 (21.28)	63	9 (14.28)	15.60	Fert.	FM
Mi	St	61	2 ( 3.28)	44	3 ( 6.82)	5.0	Fert. F; Ster.	M
St	Mag	104	1 ( 0.96)	98	2 ( 2.04)	—	—	—
Mag	St	156	0	120	0	—	—	—
Mag	Mi	92	0	66	4 ( 6.06)	—	—	—
Mi	Mag	139	2 ( 1.44)	74	16 (21.62)	12.0	Fert. F; Ster.	M

St = *D. sturtevantii*; Mi = *D. milleri*; Mag = *D. magalhãesii*; F = females; M = males; Fert. = fertile; Ster. = sterile.

Table III - Results of intraspecific pair mating crosses.

Type of crosses		Number of crosses	Fertile crosses Nº (%)	Dissected females	Spermed females Nº (%)	Average number of progeny
F	M					
Mi	Mi	124	77 (62.10)	23	0	52.34
St	St	167	112 (67.06)	33	6 (18.18)	60.22
Mag	Mag	60	28 (46.67)	18	0	58.71

For symbols, cf. Table II.

Table IV - Measures of isolation (in percentage) calculated from Table II.

Type of crosses		Ti	li	Fi	Ai
F	M				
St	Mi	78.72	67.48	11.24	87.72
Mi	St	96.72	90.13	6.59	
St	Mag	99.04	97.02	2.02	99.52
Mag	St	100.00	100.00	0.00	
Mi	Mag	98.56	77.25	21.31	99.28
Mag	Mi	100.00	93.93	6.07	

Ti = total isolation; li = isolation at the insemination level; Fi = isolation at the fertilization level; Ai = average isolation. See text for explanation.

Table V - Measures of isolation (in percentage) calculated from Table III.

Type of crosses		Ti	li	Fi
F	M			
Mi	Mi	37.90	37.90	0.00
St	St	32.93	26.94	5.99
Mag	Mag	53.33	53.33	0.00

For symbols, cf. Table IV.

Table VI - Results of interspecific crosses using sympatric and allopatric strains of *D. milleri* and *D. sturtevantii*.

Type of crosses		Number of fertile crosses	Fecundity
F	M		
St <sub>1</sub>	Mi	1	+
Mi	St <sub>1</sub>	0	-
St	Mi	2	++
Mi	St	1	+
St <sub>1</sub>	St	2	+++
St	St <sub>1</sub>	2	+++

For control, intraspecific crosses of *D. sturtevantii* strains are also included. Two mass crosses were prepared in every case. F = females; M = males. For symbols of strains, cf. Table I.

## DISCUSSION

Reproductive isolation data on the sturtevantii subgroup were presented by Mourão and Bicudo (1967) in the same paper in which the species *D. magalhãesii* and *D. dacunhai* were described. The authors used reciprocal pair mating tests in two series of crosses involving *D. dacunhai*, *D. magalhãesii* and *D. sturtevantii* (*milleri* was not available at that time for study). In one of these series, *D. dacunhai* (from Kingston, Jamaica) and *D. magalhãesii* (from the State of Rio Grande do Sul, Brazil) were crossed to *D. sturtevantii* (from Brazil, Peru and Trinidad). In the other series, *D. dacunhai* (from Kingston, Ocho Rios and an unnamed place, all of them in Jamaica) was crossed to *D. sturtevantii* (from Costa Rica and Trinidad). In both series, the number of fertile crosses in a total of 100 performed in every type of cross was computed. The results showed a high degree of reproductive isolation between *D. dacunhai* or *D. magalhãesii* and *D. sturtevantii*. In the crosses between *D. sturtevantii* and *D. dacunhai* the isolation was stronger when *D. sturtevantii* females and *D. dacunhai* males were involved. In the crosses between *D. sturtevantii* and *D. magalhãesii*, the isolation was stronger when *D. sturtevantii* males and *D. magalhãesii* females were used. Mourão and Bicudo (1967) also intercrossed *D. magalhãesii* and *D. dacunhai*. The crosses involving *D. magalhãesii* females

and *D. dacunhai* males showed complete isolation while the reciprocal ones produced some fertile crosses.

In this paper, a more extensive study of reproductive isolation using *D. magalhãesi*, *D. sturtevantii* and *D. milleri* is presented. The availability of *D. milleri* was very useful; it made possible to check, by crossability, the evolutionary status of that species, previously established on a morphological basis in relation to the other members in the same subgroup.

The reproductive isolation was strong in every combination analyzed in this study. However it was stronger in *D. magalhãesi* x *D. sturtevantii* and *D. magalhãesi* x *D. milleri* than in *D. sturtevantii* x *D. milleri*. In the combination *D. sturtevantii* x *D. milleri* crosses in both directions were fertile, although with different degrees of fertility. In turn, the combinations *D. magalhãesi* x *D. sturtevantii* and *D. magalhãesi* x *D. milleri* exhibited a low fertility in a single direction of the crosses, in both cases involving *D. magalhãesi* males; the reciprocal crosses were completely sterile. The isolation indices showed  $I_i$  values greater than  $F_1$  values in every case, indicating that the isolating mechanisms which prevent insemination are stronger than those which prevent the production of hybrids by inseminated females. The nature of the pre-mating barrier was not analyzed but very probably sexual isolation is the mechanism acting to prevent insemination in these combinations. The action of mechanical isolation is less probable because the male genitalia are very similar in the 3 species studied.

Partial sterility of  $F_1$  hybrids was another isolating barrier found between the 3 species analyzed. In the combination *D. sturtevantii* x *D. milleri*, male and female hybrids were fertile in the direction *D. sturtevantii* females x *D. milleri* males while males were sterile and females were fertile in the reciprocal crosses. The same direction of parental crosses which yielded less progeny (*D. milleri* females x *D. sturtevantii* males) also yielded sterile males, showing that the incompatibility of both species is greater when *D. milleri* females are used. Hybrid sterility was also observed in the intercrosses involving *D. milleri* females x *D. magalhãesi* males: male  $F_1$  progeny was sterile while female progeny had low productivity.

The decreased productivity in the interspecific crosses when compared to intraspecific ones and also the detection of some inseminated females which did not produce descendants are indicative of other post-mating mechanisms operating among the species studied, involving gametic isolation or hybrid inviability in very early stages of development.



The hybridization of allopatric and sympatric strains of *D. sturtevantii* and *D. milleri* in the laboratory was also focused upon in this study. Extensive data on reproductive isolation of *Drosophila* have shown complete isolation between sympatric strains of different species while allopatric strains present a variable degree of crossability. This was observed, for example, in the saltans subgroup, in crosses between strains of *D. prosaltans* and *D. saltans* (Bicudo, 1973). Complete isolation of sympatric strains is a mechanism which prevents interspecific hybridization in nature.

In the interspecific crosses *D. milleri* x *D. sturtevantii*, crossability was lower between sympatric strains than between allopatric ones. However, progeny was yielded both when sympatric and allopatric strains were used.

Hybrids have also been mentioned to occur, in the laboratory, in interspecific crosses involving sympatric strains of other *Drosophila* species, such as *D. milleri* and *D. arizonensis* (Bicudo and Richardson, 1978). Since natural hybrids of these strains have never been found, additional isolating mechanisms are presumed to operate in nature perhaps involving habitat selection which has been considered an important factor in reproductive isolation (for an example, see Parsons, 1977). *Drosophila arizonensis* and *D. milleri* explore different genera of cacti as oviposition sites and larval substrates. But, in the case of species in the saltans group, ecological data are very limited. *Drosophila sturtevantii* was reported to be bred from citrus (Heed, 1957), and several kinds of fallen fruits such as jaca, genipapo, mango and others (Dobzhansky and Pavan, 1950; Knapp, 1953; Pavan, 1959). Pipkin (1965, in Throckmorton, 1974) mentioned this species as being bred from fallen fruits and blossoms. On the other hand, artificial baits prepared with tomato, papaya, orange, watermelon and banana showed that *D. sturtevantii* prefers banana, followed by citrus (Mourão, 1966). However, as far as we know, no information of this kind on the *D. milleri* species is available. In spite of this, the possibility of habitat selection cannot be discarded.

An attempt was made to interpret the phylogenetic relationships among the 4 species in the *D. sturtevantii* subgroup already submitted to isolation studies. It followed Kaneshiro's (1976) assumption that females of ancestral species show strong sexual discrimination against males of the more derived species since these males exhibit only a part of the total courtship pattern of conspecific males.

In every combination with *D. magalhãesi* the isolation was stronger when *D. magalhãesi* females were used than in the reciprocal crosses. These results were observed in *D. magalhãesi* females x *D. sturtevantii* males and *D. magalhãesi* females x *D. milleri* males. Data presented by Mourão and Bicudo (1967) also showed similar results in the combinations *D. magalhãesi* females x *D. sturtevantii* males and *D. magalhãesi* females x *D. dacunhai* males.

Thus, based on Kaneshiro's hypothesis, *D. magalhãesi* should be considered to be the ancestral among the 4 species. On the other hand, in the crosses between *D. sturtevantii* and *D. milleri* the isolation was stronger in the direction involving *D. milleri* females while data reported by Mourão and Bicudo (1967) on crosses between *D. dacunhai* and *D. sturtevantii* showed stronger isolation when *D. sturtevantii* females were used.

The phylogenetic sequence of derivation based on the isolation patterns would be:

*D. magalhãesi* → *D. milleri* → *D. sturtevantii* → *D. dacunhai*

However, the wide geographical distribution of *D. sturtevantii* (Magalhães, 1962) and its degree of inversion polymorphism (18 heterozygous inversions were detected by Knapp, 1953) could indicate that this species is the ancestral in the subgroup. *Drosophila magalhãesi* located at the extreme south in the geographical area of the subgroup could be the result of a differentiation process in the limits of *D. sturtevantii* distribution. Besides, *D. milleri* and *D. dacunhai*, reported respectively in Puerto Rico and Jamaica could result from local changes in genetic composition in response to environmental conditions, probably under the influence of a founder event. Another interpretation, following the indication based on Kaneshiro's hypothesis, would be that *D. sturtevantii*, although more recent than *D. milleri* and *D. magalhãesi*, attained such a wide range (and perhaps displaced the other species within its distribution area) because it proved to be a very successful evolutionary experience in the subgroup.

The information available on the subgroup is not sufficient to allow a decision to be made between these two possibilities. A comparison of chromosomal arrangements between the species might be useful to clarify this point and will be done in a next step.

Another aspect worth analyzing in the *sturtevantii* subgroup and probably helpful to get new information on its speciation process concerns *D. sturtevantii*. The large distribution area of this species, which includes many of the Caribbean Islands and extends from Mexico to southern Brazil

on the mainland, apparently provides many different environments. Their demands could lead to changes in genetic composition under control of natural selection. Thus the detection of populations in the evolutionary status of semispecies, or even new species, would not be unexpected in a study using strains of different origins. Some incipient sexual isolation between *D. sturtevantii* strains from different regions has already been shown by Dobzhansky (1944). The higher isolation indices computed on preference mating were obtained in tests involving strains from Tamazunchale (Mexico) and Bertioga (Brazil), and strains from Quiriguá (Guatemala) and Belém or Bertioga (Brazil). The large distribution area plus the great number of flies in the collections apparently make this species unique in the saltans group for studies of genetic changes involved in the speciation process.

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