

## REPRODUCTIVE ISOLATION IN THE SALTANS GROUP OF DROSOPHILA II. THE PARASALTANS SUBGROUP

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A comparison of the frequency of fertile crosses and average number of progeny of 3 *D. parasaltans* strains suggests that each of them has developed a different reproductive strategy in its evolutionary history. The strains Pa<sub>1</sub> and Pa<sub>2</sub> apparently give similarly good reproductive performances through different ways, while the strain Pa<sub>3</sub> has developed another strategy which allows it to survive with degrees of fertility and fecundity much lower than those seen in Pa<sub>1</sub> and Pa<sub>2</sub>.

Hybridization tests among these strains gave some information on the role played by males and females in the reproductive efficiency of each strain and on the interplay of their genetic systems when put together.

Tests of hybridization between *D. parasaltans* and *D. subsaltans*, the 2 species available from the *parasaltans* subgroup, were also performed. The results show complete reproductive isolation of both species.

### Introduction

Data concerned with isolation studies in the *saltans* group of *Drosophila* are being presented in a series of subsequent papers. In the first of them, the 7 species included in the *saltans* subgroup were analysed (Bicudo, 1973). This paper, the second in the series, is concerned with the *parasaltans* subgroup.

Magalhães (1962) included three species in this subgroup: *D. parasaltans*, *D. subsaltans* and *D. pulchella*. The geographical distributions of these species according to the same author are as follows: *D. para-*

*saltans* – Uaupés (AM), Brazil, and Villavicencio, Colombia; *D. subsaltans* – Belém (PA), Brazil; and *D. pulchella* – St. Vincent, B.W.I.

Later collections have shown *D. parasaltans* to occur in other Brazilian localities: Porto Velho (Rondônia), Várzea (Belém, Pará) and Tapuruquara (Amazonas). The known geographical distributions of *subsaltans* and *pulchella* remain as described by Magalhães.

*Drosophila pulchella* is a little known species. It was described by Sturtevant (1916). As far as we know, this species has not been taken in other collections. Wheeler (1957) included it in the *saltans* group and showed the similarity of this species to *D. subsaltans* and *D. parasaltans*. Only a single male of *D. pulchella*, 'in fair condition', from the AMNH collection, was available for both Wheeler's and Magalhães' studies.

Thus, only two species were available for the present study on the *parasaltans* subgroup: *D. parasaltans* and *D. subsaltans* in which their reproductive compatibility was analyzed. Further, 3 geographical strains of *D. parasaltans* were studied to detect genetic variation affecting some components of reproduction. The effect of this genetic variation in crosses between those strains was also examined.

### Material and methods

Information on the 4 strains used are in Table 1 and their geographical origin is given in Figure 1. All of them have been kept in the laboratory for a long

Table 1

Strains used in the present investigation

Species	Stock references	Symbols used in this paper	Origin
<i>D. parasaltans</i>	B <sub>14-7</sub>	Pa <sub>1</sub>	Porto Velho (RO), Brazil
	B <sub>17-5</sub>	Pa <sub>2</sub>	Belém (PA), Brazil
	2536.1	Pa <sub>3</sub>	Uaupés (AM), Brazil
<i>D. subsaltans</i>	2536.2	Su	Belém (PA), Brazil

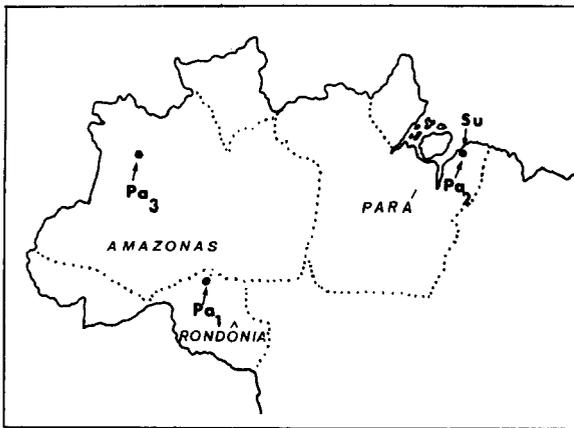


Fig. 1. Brazilian regions where the strains used come from.

period (probably since 1960) and were derived from stocks brought from The Genetics Foundations (University of Texas, Austin, U.S.A.) by Dr. Luiz Edmundo de Magalhães. The number of flies which originated the stocks is unknown.

As in the previous paper of this series, intraspecific and interspecific studies involved pair mating crosses in both directions, pairs being changed twice to new food, and progeny analyzed 25 days after preparation (for details, see Bicudo, 1973). Mass crosses using 10 pairs per vial were also prepared. Fertility (frequency of fertile crosses) and fecundity (average number of progeny) were studied. Females of sterile crosses were dissected and their reproductive tracts examined for the presence of spermatozoa. The fertility of F<sub>1</sub> flies was examined by mass matings individually prepared from the fertile crosses. The experiments were carried out at 25°C ± 1°C.

## Results

The results of intraspecific crosses in *Drosophila parasaltans* and *D. subsaltans* are presented in Table 2.

Intrastrain crosses in *D. parasaltans* showed higher frequencies of fertile crosses in Pa<sub>1</sub> than in Pa<sub>2</sub>, and both were more fertile than Pa<sub>3</sub>. The difference was statistically significant in every comparison of these results: Pa<sub>1</sub> versus Pa<sub>2</sub>,  $\chi^2 = 5.96$ ,  $P < 0.01$ ; Pa<sub>1</sub> versus Pa<sub>3</sub>,  $\chi^2 = 29.81$ ,  $P < 0.001$ ; and Pa<sub>2</sub> versus

Table 2

Results of intraspecific pair mating crosses in the *parasaltans* subgroup  
Symbols of strains cf. Table 1.

Types of crosses		Number of crosses	Fertile crosses		Dissected females	Inseminated females	Average number of progeny within 30 days	Number of mass crosses for testing fertility of F <sub>1</sub> (100% were fertile).
F	M		N	%				
Pa <sub>1</sub>	Pa <sub>1</sub>	72	71	(98.61)	—	—	137.08	69
Pa <sub>2</sub>	Pa <sub>2</sub>	72	61	(84.72)	6	0	156.21	60
Pa <sub>3</sub>	Pa <sub>3</sub>	74	37	(50.00)	11	0	24.30	28
Pa <sub>1</sub>	Pa <sub>2</sub>	73	71	(97.26)	1	0	78.19	71
Pa <sub>2</sub>	Pa <sub>1</sub>	73	71	(97.26)	0	—	74.13	68
Pa <sub>1</sub>	Pa <sub>3</sub>	72	37	(51.39)	26	16	66.35	35
Pa <sub>3</sub>	Pa <sub>1</sub>	75	25	(33.33)	8	2	45.48	23
Pa <sub>2</sub>	Pa <sub>3</sub>	73	52	(71.23)	7	0	93.30	50
Pa <sub>3</sub>	Pa <sub>2</sub>	73	27	(36.99)	13	2	49.93	26
Su	Su	67	55	(82.09)	2	0	62.00	53

$Pa_3$ ,  $\chi_1^2 = 13.66$ ,  $P < 0.001$ .

Interstrain crosses in *D. parasaltans* also showed different results in the 3 possible combinations of strains. The fertile crosses exhibited a decreasing frequency in the following order:  $Pa_1 \times Pa_2$ ,  $Pa_2 \times Pa_3$  and  $Pa_1 \times Pa_3$ . The fertility was the same in both directions of the crosses  $Pa_1 \times Pa_2$ . The level of fertility was very similar to that shown by intrastain crosses of  $Pa_1$  but showed a significant increase when compared with intrastain crosses of  $Pa_2$  ( $\chi_1^2 = 4.82$ ,  $P < 0.05$ ).

Reciprocal crosses in the combinations  $Pa_1 \times Pa_3$  and  $Pa_2 \times Pa_3$  gave different results. In both cases, when  $Pa_3$  females were used, the frequency of fertile crosses was smaller than in the reciprocal crosses: for  $Pa_1 \times Pa_3$  versus  $Pa_3 \times Pa_1$ ,  $\chi_1^2 = 3.34$ ,  $0.10 > P > 0.05$ ; for  $Pa_2 \times Pa_3$  versus  $Pa_3 \times Pa_2$ ,  $\chi_1^2 = 11.81$ ,  $P < 0.001$ . In the first comparison the P value is not significant but it is near the conventional limit of significance. The frequencies of fertile crosses involving  $Pa_3$  females in both combinations are not different ( $\chi_1^2 = 0.15$ ,  $P > 0.75$ ). However, the crosses involving  $Pa_3$  males in both combinations are statistically different ( $\chi_1^2 = 4.15$ ,  $P < 0.05$ ): the value obtained in crosses to  $Pa_2$  females was higher than that obtained in crosses to  $Pa_1$  females.

A comparison between intrastain results involving  $Pa_3$  males and females is summarised in Table 3. The combination  $Pa_1 \times Pa_3$  showed a statistically significant decrease in the frequency of fertile crosses in both directions when compared to  $Pa_1 \times Pa_1$ . This decrease brought down the level to about that obtained in the cross  $Pa_3 \times Pa_3$ . With the cross  $Pa_3$  females  $\times$   $Pa_1$  males the frequency of fertile crosses was even smaller than in the crosses  $Pa_3 \times Pa_3$ , with a P value near to the 0.05 level of significance.

In the combinations  $Pa_2 \times Pa_3$ , both directions of crosses also showed a decrease in the frequency of fertile crosses as compared to  $Pa_2 \times Pa_2$ . This difference was statistically significant in the direction  $Pa_3$  females  $\times$   $Pa_2$  males. The same interstrain combination, when compared with  $Pa_3 \times Pa_3$ , showed an increase in the frequency of fertile crosses in the direction  $Pa_2$  females  $\times$   $Pa_3$  males and a decrease in the reciprocal direction, although in the last case the P value is not significant.

Dissection of females from the sterile crosses showed exclusively uninseminated females in some combinations (intrastain crosses of  $Pa_2$  and  $Pa_3$ ; in-

terstrain crosses  $Pa_1$  females  $\times$   $Pa_2$  males, and  $Pa_2$  females  $\times$   $Pa_3$  males). Other combinations showed inseminated and uninseminated females (interstrain crosses  $Pa_1 \times Pa_3$ ,  $Pa_3 \times Pa_1$  and  $Pa_3 \times Pa_2$ ). The average numbers of progeny in intrastain crosses of  $Pa_1$  and  $Pa_2$  were greater than those of any other combination. The smallest average numbers were obtained in  $Pa_3 \times Pa_3$  and in the interstrain crosses involving  $Pa_3$  females. Student's 't' tests for comparisons of the average numbers of progeny were performed. The comparisons were made between both directions of crosses in the same combination, and between interstrain crosses and the parental intrastain crosses. The 't' values were not significant in 4 comparisons although in 2 of them P values were very near to the 0.05 level of significance:  $Pa_1 \times Pa_1$  versus  $Pa_2 \times Pa_2$  ( $t_{131} = 1.77$ ;  $0.10 > P > 0.05$ ),  $Pa_1 \times Pa_2$  versus  $Pa_2 \times Pa_1$  ( $t_{141} = 0.57$ ;  $P > 0.20$ ),  $Pa_3 \times Pa_1$  versus  $Pa_3 \times Pa_2$  ( $t_{51} = 0.42$ ;  $P > 0.20$ ), and  $Pa_3 \times Pa_1$  versus  $Pa_1 \times Pa_3$  ( $t_{61} = 1.68$ ;  $0.10 > P > 0.05$ ). All the other comparisons gave significant values ( $P < 0.05$ ).

All the combinations produced 100% of fertile mass crosses when  $F_1$  flies were used.

Taking the percentages of fertile crosses obtained in the 3 strain samples and the total numbers of progeny each of them yielded, the genetically effective population size (N) of the 3 *D. parasaltans* strains based in the original concept of Wright (1931) can be

Table 3

Statistical values obtained in the comparisons of fertility between intrastain and interstrain crosses involving  $Pa_3$  males and females.

In parentheses frequencies of fertile crosses; symbols of strains cf. Table 1.

♀	♂	$Pa_1 \times Pa_1$ (98.6%)	$Pa_2 \times Pa_2$ (84.7%)	$Pa_3 \times Pa_3$ (50.0%)
$Pa_2 \times Pa_3$ (71.2%)			$\chi_1^2 = 2.64$ $p < 0.20$	$\chi_1^2 = 4.72$ $p < 0.05$
$Pa_1 \times Pa_3$ (51.4%)		$\chi_1^2 = 29.73$ $p < 0.001$		$\chi_1^2 = 0.02$ $p < 0.90$
$Pa_3 \times Pa_2$ (37.0%)			$\chi_1^2 = 30.90$ $p < 0.001$	$\chi_1^2 = 1.72$ $p < 0.20$
$Pa_3 \times Pa_1$ (33.3%)		$\chi_1^2 = 47.00$ $p < 0.001$		$\chi_1^2 = 2.86$ $p < 0.10$

Table 4

Results of interspecific pair mating crosses in the parasaltans subgroup  
Symbols of strains cf. Table 1.

Method	Type of crosses		Number of crosses (they were all sterile)	Number of dissected females (none was inseminated)
	F	M		
Pair Mating	Pa <sub>3</sub>	Su	60	36
	Su	Pa <sub>3</sub>	67	48
Mass Mating	Pa <sub>1</sub>	Su	2	13
	Su	Pa <sub>1</sub>	2	15
	Pa <sub>2</sub>	Su	2	17
	Su	Pa <sub>2</sub>	2	18
	Pa <sub>3</sub>	Su	2	15
	Su	Pa <sub>3</sub>	2	14

estimated. The total number of progeny yielded by the analyzed sample of Pa<sub>1</sub> was 9,733; of Pa<sub>2</sub> was 9,529 and of Pa<sub>3</sub>, 899. Considering these values as the total number of individuals in each 'colony' and using the percentages of fertile crosses detected in each of them, the N values for Pa<sub>1</sub>, Pa<sub>2</sub> and Pa<sub>3</sub> were respectively 9,597.71, 8,072.96 and 449.50.

*Drosophila subsaltans*, the other species in the parasaltans subgroup, showed a great frequency of fertile intrastrain crosses (Tab. 2). However, it failed to produce hybrids in crosses with *D. parasaltans*. As shown in Table 4, pair mating crosses of *D. subsaltans* with *D. parasaltans* (Pa<sub>3</sub> strain) did not produce hybrids. No inseminated female was found among those dissected. The same results were obtained in mass mating crosses between *D. subsaltans* and each of the 3 available *D. parasaltans* strains.

## Discussion

Although Throckmorton & Magalhães (1962) mentioned obtaining a few hybrids between *D. parasaltans* and *D. subsaltans*, the attempt to intercross those species in the present study has failed. No hybrids or even inseminated females were obtained in mass mating crosses using each of the 3 strains of *D. parasaltans* and *D. subsaltans* or in pair mating crosses involving *D. subsaltans* and the strain Pa<sub>3</sub> also used

by the above mentioned authors. This difference of results possibly reflects differences between the methods in the two studies. However, since Throckmorton & Magalhães did not give information on the method they used to obtain the hybrids, such differences remain unknown.

In the present study, data on frequencies of fertile crosses and average numbers of progeny make it possible to detect geographical strain differences in the 3 strains of *D. parasaltans*: Pa<sub>1</sub>, Pa<sub>2</sub> and Pa<sub>3</sub>.

Many previous studies have shown that the changes determined by natural selection acting on populations of the same species, exposed to different conditions, may affect among others, their reproductive patterns. For example, studies on sexual preferences of several geographical strains performed by Dobzhansky & Streisinger (1944) in *D. prosaltans*, and Spieth (1966) in *D. ananassae* indicated that the courtship balance varied among populations of a single widespread species (see also Spieth, 1968). Also studies using several allopatric strains of *D. prosaltans* and *D. saltans* by Bicudo (1973) showed a large range of variation of fertility of crosses of each strain to several strains of the other species, denoting the geographical genotypic variation which led to a greater or a lesser interspecific reproductive compatibility.

The divergence of reproductive patterns of populations in the same species is one step in the speciation process from which the establishment of incipient isolating mechanisms may finally result. These mechanisms, in their turn, may be reinforced by natural selection when the geographical strains come into contact with each other.

In the present case, the performances of the strains Pa<sub>1</sub> and Pa<sub>2</sub> as to both characteristics studied (fertility and fecundity) were much better than those shown by Pa<sub>3</sub>. These results confirmed our observations on the laboratory stocks that, although all 3 strains are easy to maintain, density is always clearly greater in Pa<sub>1</sub> and Pa<sub>2</sub> cultures than in those of Pa<sub>3</sub>.

However, a comparison of Pa<sub>1</sub> and Pa<sub>2</sub> shows that they also differ from each other. The frequency of fertile crosses was greater in Pa<sub>1</sub> than in Pa<sub>2</sub> and the average number of progeny was greater in Pa<sub>2</sub> than in Pa<sub>1</sub>. Although the difference in the average number of progeny is not statistically significant, it is great enough to determine that, in every of these strains, a disadvantage in one of the characteristics is compen-

sated by an advantage in the other. The net result is that both strains yield very similar total numbers of progeny. In this respect, natural selection has apparently attained similar results in  $Pa_1$  and  $Pa_2$  by different ways. However, the genetically effective population size, in spite of a partial compensation produced by the higher average number of progeny, remains lower than in  $Pa_1$ .

In  $Pa_3$  another strategy which allowed the population to be maintained with lower levels of frequency of fertile crosses and average number of progeny was developed. Thus, apparently, in the course of the evolutionary process, the 3 strains have diverged in the genetic systems which control these steps of their reproductive patterns.

Another aspect of this divergence was studied through the way these diversified genetic systems interact in the interstrain crosses. The degrees of fertility and fecundity examined in intrastrain crosses increased, decreased or were maintained independently of each other, in different interstrain crosses, yielding several different combinations of these conditions. For example, the frequency of fertile crosses between  $Pa_1$  females and  $Pa_2$  males was as high as that detected intra- $Pa_1$ , but the average number of progeny decreased to about half the intrastrain values obtained in  $Pa_1$  and  $Pa_2$ . In their turn, crosses between  $Pa_1$  females and  $Pa_3$  males exhibited a frequency of fertile crosses similar to that detected intrastrain in  $Pa_3$ , but the productivity increased to more than twice the average obtained in the same strain. Another situation occurred in crosses of  $Pa_3$  females with  $Pa_1$  or  $Pa_2$  males: their fertility was lower than in  $Pa_3 \times Pa_3$  but the productivity increased about twice.

The indication is that the reproductive capability of each population reflects the balanced effect of the several genetic systems which control all the steps in the reproductive pattern of that population. In different populations each of these systems may present genetic variation detected not only by comparison of the intrastrain effects but also by the effects in hybridization tests.

Although the present study did not directly involve tests of sexual preference, the results of fertile crosses and the dissection of females from negative crosses allow us to infer to some extent the performances of the strains at that level. For example, crosses between  $Pa_1$  and  $Pa_2$  indicate that  $Pa_1$  fe-

males accept  $Pa_2$  males as readily as they accept their own males, but  $Pa_2$  females showed more acceptance of  $Pa_1$  males than of their own males. Sexual preference for 'foreign' individuals has been shown in several studies using various geographic strains of *Drosophila*, among them, Dobzhansky & Streisinger (1944) and Spieth (1968).

According to Spieth (1968) this preference may be attributed to the difference in the courtship threshold levels of the 2 sexes. In some strains the males may show a low threshold (they are aggressive) and the females may present a high threshold (they are lethargic in responding). In the present case this hypothesis needs verification.

Fecundity in intrastrain crosses of  $Pa_1$  and  $Pa_2$  was very high. However, interstrain crosses between  $Pa_1$  and  $Pa_2$  showed that hybridization is detrimental for this characteristic. Apparently the great fecundity exhibited by both strains is provided in each of them by genetic systems peculiar to the strain. The effect of hybridization was the same in both directions of the crosses. No insemination reaction and apparently no immotile spermatozoa in the spermathecae were observed, suggesting that probably the block to productivity occurs at early stages of zygotic development. In this case the difference between both systems might be in their structural genes and/or in their regulation. Anyway hybridization brings about a breakage of the coadapted system of each strain at this level of the reproductive pattern.

Fertility in interstrain crosses involving  $Pa_3$  males and  $Pa_2$  females shows an improvement when compared to crosses  $Pa_3 \times Pa_3$ . An improvement of crossability is also detected in crosses between  $Pa_3$  males and  $Pa_1$  females adding up the number of inseminated females among the dissected to the number of fertile crosses. But when  $Pa_3$  females were used in interstrain crosses, the fertility was even lower than that obtained with intra- $Pa_3$  strain crosses. This may indicate that some incipient reproductive isolation is taking place between  $Pa_3$  and the other strains analyzed. This idea is reinforced by the considerable frequency of inseminated females not yielding progeny in interstrain crosses involving  $Pa_3$ . On the other hand, the improvement of crossability of  $Pa_3$  males in interstrain crosses might also be indicative of sexual differences in courtship balance with the  $Pa_3$  strain. However, this suggestion also needs verification.

Fecundity in interstrain crosses involving  $Pa_3$  indi-

cates that in this case the control of this characteristic is female rather than male dependent. Fecundity was lower in crosses between Pa<sub>3</sub> females and Pa<sub>1</sub> and Pa<sub>2</sub> males than in the reciprocal crosses. But even in this case some improvement of fecundity in comparison with Pa<sub>3</sub> intrastain crosses was detected. In the interstrain crosses of Pa<sub>3</sub> males, fecundity was nearly as great as that obtained in crosses between Pa<sub>1</sub> and Pa<sub>2</sub>, or even higher. These data suggest that the low fecundity of the strain Pa<sub>3</sub> may be attributed to genes affecting mainly female reproductive capability; but other genes from males and females are also probably affecting survival of zygotes. The improvement of the fecundity of Pa<sub>3</sub> females detected in interstrain crosses might be attributed to the effect of heterozygosis originated by outbreeding, acting at the zygotic level.

Other studies on the reproductive patterns of these strains using some of the present observations as working hypothesis are desirable. The study of natural population samples would also be recommended to compare the behavior of the flies recently collected with that of the ones kept for a long period of culture in the laboratory. However, these species, like most of the species in the *saltans* group, are not easy to collect. A more extensive study using natural samples of these species is thus restricted by the poor knowledge of their behavior in nature.

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