

EXPERIMENTAL HYBRIDS BETWEEN *DROSOPHILA WILLISTONI* STURTEVANT AND *DROSOPHILA PAULISTORUM* DOBZHANSKY AND PAVAN FROM SOUTHERN MARGINAL POPULATIONS

HELGA WINGE and A. R. CORDEIRO
Departamento de Genética, Universidade do Rio Grande do Sul,
Pôrto Alegre, Brazil

Received 1.ii.62

1. INTRODUCTION

NOTWITHSTANDING the morphological similarity of "sibling species" (Mayr, 1942) they maintain complete reproductive isolation in nature showing that speciation might occur without morphological divergence.

The *willistoni* group of sibling species includes the two studied here and three others: *D. equinoxialis* Dobzhansky (1946), *D. tropicalis* Burla and da Cunha and *D. insularis* Dobzhansky. This is the most important group of *Drosophila* species in the Neotropical region. It is the best studied in several respects, for example, the mutational load, chromosomal polymorphism, comparative biochemistry, genetic effects of radiations on populations, ecology, incipient speciation, physiological characteristics. It has also one of the widest geographic distributions with highly dense wild populations in warmer seasons. Yet, *Drosophila insularis* Dobzhansky, the most recently described species in this group (Dobzhansky, Ehrman and Pavlovsky, 1957) was found in Lesser Antilles only.

Burla *et al.* (1949) found the usual taxonomic traits not reliable for identification of species in single individuals. Identification is achieved by the use of other morphological characteristics. Malogolowkin (1952) showed the usefulness of male genitalia in distinguishing these species. Nevertheless, the detailed study of disk patterns of polythenic salivary gland chromosomes remains one of the best methods of specific determination as Burla *et al.* (1949) suggested. However, this method and the intercrossing tests depend on the existence of fertile females and cannot be used in field work, ecology, etc. Spassky (1957) described slight but consistent difference between the external male genitalia, that allows a direct discrimination of the five sibling species. This is a helpful auxiliary method in spite of being limited to one sex.

Burla *et al.* (1949) obtained no hybrids between the first four known species. Dobzhansky, Ehrman and Pavlovsky (1957) discovered that *D. insularis* can produce hybrids with *D. tropicalis* and *D. willistoni*. This was the first instance of interspecific hybridisation in this group. Recently, Winge and Cordeiro (1960) have reported the first hybrids obtained between southern races of *D. willistoni* and *D. paulistorum*. It is well known that the study of interspecific hybrids

helps in understanding actual evolutionary processes underlying speciation. An extensive work on the affinities between all species and subspecies of the *willistoni* sibling group is in progress. This paper reports the experiments on sexual isolation, and the fertile hybrids obtained between a strain of *D. paulistorum* and another of *D. willistoni*.

2. ORIGIN OF THE STRAINS

The southernmost border of Neotropical rain forest reaches the north of the State of Rio Grande do Sul, at Torres County. Near the locality of Colônia São Pedro de Alcântara, we collected many samples of *Drosophila*, including those of 25th September 1959, from which the strains of *willistoni* and *paulistorum* here studied were obtained. In a total of 1180 flies, only 39 "*willistoni*" have been found. Each female collected was singly cultured in a vial. The 18 fertile cultures obtained have been test crossed with *white* virgin *willistoni* females from a laboratory stock, and *paulistorum* virgin females from Angra dos Reis (Rio de Janeiro) and Sapé (Minas Gerais). Two strains proved to be *paulistorum* but in further tests they produced larvæ or pupæ with three, and adults with one of the 16 *willistoni* strains.

3. SEXUAL ISOLATION

Multiple choice, crossing tests have been performed to determine the sexual isolation between the selected strains. In each culture vial (30 × 80 mm.) were placed ten males of one species and ten virgin females of each species. These individuals were all identically aged for three days before mating. The virgin females were marked by clipping a slight nick in either the right or the left wing, according to the species. All experiments were conducted at $25^{\circ} \pm 0.5^{\circ}$ C. under diffuse natural light together with artificial "daylight fluorescence tubes" lighting during the normal day period. After 10, 15 or 20 days the females were dissected and their spermathecae and ventral receptacles examined microscopically for spermatozoa. Table 1 summarises the results of these three differently lasting tests of sexual isolation.

The isolation coefficients in table 1 have been computed using the formulæ proposed by Levene (1949). These isolation coefficients become equal to zero when *no* isolation is present, equal to +1 if isolation is complete, and they take a negative value if there are more hetero- than homogamic inseminations.

Each isolation coefficient (table 1 : $K_{1,2}$ and $K_{2,1}$) yield a highly significant *t* value when tested against its standard error. These coefficients also show that there is stronger sexual isolation when *willistoni* males are used.

The application of the test for equality of two independent coefficients of isolation (Levene, 1949) disclosed significant disparity in the sexual isolation between the experiments of 10 and 20 days with

TABLE 1
 Number of females dissected (*n*) and per cent. inseminated (%) in experiments of male's multiple choice. Levene coefficients and its standard errors are used

Duration of crosses	Males	Females	Homogamic		Heterogamic		Coefficient of isolation $K_{1,2}$ and $K_{2,1}$	Coefficient of joint isolation K_1 and K_2	Coefficient of excess insemination $m_{1,2}$
			<i>n</i>	%	<i>n</i>	%			
10 days	<i>willistoni</i>	<i>willistoni</i> + <i>paulistorum</i>	106	96.23	104	7.69	0.95 ± 0.02	0.91 ± 0.02	0.04 ± 0.02
	<i>paulistorum</i>	<i>willistoni</i> + <i>paulistorum</i>	107	96.26	123	19.51			
15 days	<i>willistoni</i>	<i>willistoni</i> + <i>paulistorum</i>	141	99.29	110	12.73	0.95 ± 0.02	0.88 ± 0.02	0.07 ± 0.02
	<i>paulistorum</i>	<i>willistoni</i> + <i>paulistorum</i>	108	90.74	132	21.97			
20 days	<i>willistoni</i>	<i>willistoni</i> + <i>paulistorum</i>	127	97.64	106	7.55	0.96 ± 0.02	0.85 ± 0.03	0.11 ± 0.03
	<i>paulistorum</i>	<i>willistoni</i> + <i>paulistorum</i>	104	84.62	109	24.77			

paulistorum males ($t = 2.33$; $P < 0.02$) which clearly demonstrate a decrease of isolation with time. This is not observed when the males are *willistoni*. Hence either *paulistorum* males increase their activity in the periods studied or *willistoni* females lose their discrimination against these males. According to Spieth (1952) *D. paulistorum* males "seem identical to *willistoni* in their behaviour, but the *D. paulistorum* female differs from *willistoni* female". Furthermore, Spieth suggests a more aggressive behaviour on the part of *paulistorum* females compared with the relatively passive *willistoni* females. The fact that Levene coefficients (table 1) are less affected by changes in the length of time the flies are together than are other isolation indexes reinforces the significance of the results obtained.

The total sexual isolation between the two strains as measured by the coefficient of joint isolation (K_1 and K_2 , table 1), decreases to a significant extent ($P < 0.05$) with time. Nevertheless it is noticeable at table 1 that after 20 days even the homogamic insemination was not complete. Burla *et al.* (1949) observed that for a period of about 10 days after mating *willistoni* males \times (*willistoni* + *paulistorum*) there is 100 per cent. of homogamic insemination, but *paulistorum* males \times (*paulistorum* + *willistoni*) showed 89 per cent., which is comparable to our finding of 84.62 per cent. for 20 days. Dobzhansky and Koller (1938) found four to five days to be enough for almost all females to become impregnated in the homogamic crosses between *D. pseudoobscura* and *miranda* in multiple choice tests.

On the experimental conditions the gene flow goes chiefly from *paulistorum* to *willistoni* and increases significantly with time ($P < 0.05$) as expected by the results with the other coefficients.

Burla *et al.* (1949) found no heterogamic insemination in either the crosses of *willistoni* or *paulistorum* males in multiple choice tests. Yet, in tests without choice they found 5.7 per cent. of the 69 females inseminated by *paulistorum* which is not significantly different from our results for 10 days period ($\chi^2 = 0.23$; $P < 0.70$), with the same kind of crosses. On the other hand, Burla *et al.* (1949) found that *paulistorum* males inseminated 51.3 per cent. of the 230 *willistoni* females in the same period. This is significantly higher than we find in the same type of crosses ($\chi^2 = 33.7$; $P < 0.005$). Unfortunately, Burla *et al.* did not give the geographic origin of the strains used. But this lack of sexual isolation might well be explained if their *paulistorum* and *willistoni* samples were originally from allopatric populations. According to the results here reported and those of Dobzhansky, Ehrman and Pavlovsky (1957) there is more sexual isolation among sympatric populations of two species than among the allopatric ones, as expected on theoretical grounds. We may conclude also that this holds true for *paulistorum* males only.

In this connection it is interesting to mention that Dobzhansky and Spassky (1959) discovered a complex cluster of subspecies or species *in statu nascendi* formed by *D. paulistorum*. The Andean-South

Brazilian group appeared to be heterogeneous ; these facts emphasise the possibility of finding further sexual isolation differences among *paulistorum* populations when tested with *willistoni*. According to the excellent studies of Ehrman (1960a, 1960b, 1961) on *paulistorum* "matings between the subspecies succeed much less frequently than do those within a subspecies". The genic basis of sexual isolation is apparently "fostered by factors distributed over all the chromosomes", these polygenes having additive effect that add up to a nearly complete bar to crossing between the subspecies.

4. FERTILE HYBRIDS BETWEEN *DROSOPHILA WILLISTONI* AND *DROSOPHILA PAULISTORUM*

Ten males and ten virgin females were aged separately for three days before crossing in vials (30×80 mm.). A total of 100 females, *willistoni* or *paulistorum*, was crossed this way with equal numbers of males of the other species. Every third day culture transfers were made until the last female died. This occurred after about 45 days giving approximately 150 cultures for each type of cross, which were carefully observed for larvæ, pupæ and adults. The F₁ hybrids obtained were handled with the same technique to study their fertility. Backcrosses were made to both parental species strains. A specially rich culture media was used (with soybean and maize flours, wheat germ, sugar and yeast), which probably improves the survival of the hybrids.

The crosses of *D. willistoni* male × *D. paulistorum* female yielded few F₁ individuals, with a predominance of females. None gave an F₂. The F₁ hybrids were sterile when backcrossed to both parental species.

In contrast the reciprocal crosses, *D. paulistorum* male × *D. willistoni* female yielded a greater number of F₁ hybrids, which were mostly fertile and produced a fairly numerous F₂. Surprisingly the F₁-P₁ backcrosses gave descendants readily only when male or female F₁ hybrid were crossed with the *paulistorum* parental strain. Few offspring being obtained when F₁ males were backcrossed to *willistoni* females and none in backcrosses of F₁ females to *willistoni* males.

These results seem to indicate that the hybrids have a greater affinity with the *paulistorum* strain. A greater affinity between the F₁ hybrids and the male parental strain has also been observed in crosses of *D. equinoxialis* male (Tefé) × *D. paulistorum* female (Florianopolis—72) and *D. tropicalis* male (Trinidad—330) × *D. tropicalis* female (Palma) (Winge, 1961 and unpublished data).

5. SPERMATHECÆ AND HYPANDRIA OF THE TWO SPECIES AND OF THEIR HYBRIDS

The spermathecæ and hypandria of the two species and of their hybrids were dissected under a stereomicroscope and the chitinous parts of the genitalia mounted in slides with Euparal and protected

with coverslides. The spermathecae were dissected in *Drosophila* saline solution, and mounted on slides with coverslides as usual. The drawings of fig. 1 were made by camera lucida.

The spermathecae of the F_1 hybrids (*paulistorum* male \times *willistoni* female) is clearly intermediate between those of the parents. The

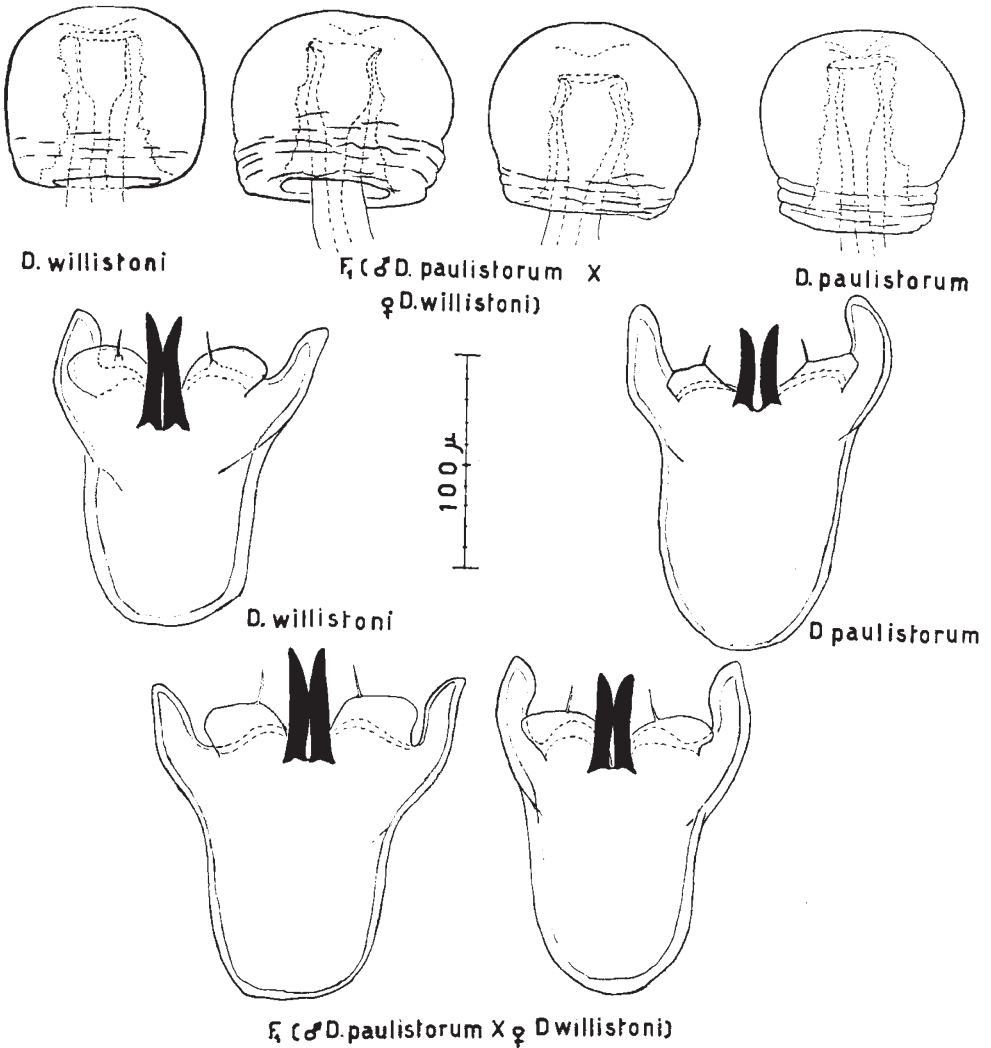


FIG. 1.—Spermathecae and hypandria of *D. willistoni*, *D. paulistorum* and of their hybrids.

general external shape varies more than the folds of the base which are always stronger than in *willistoni* and less conspicuous than in *paulistorum*. The canal uniting the spermatheca with the vagina is not cup-shaped as in *willistoni* and resembles more the vase-shaped type of *equinoxialis* rather than that of *paulistorum* itself (fig. 1).

The hypandria in the genitalia of F_1 male hybrids also varied. The edges of the expansions, or lobes, are rounded in *willistoni* and

angular in *paulistorum*. The hybrids vary greatly in this trait. In spite of this, in F_1 males, the small setæ are inserted in the edges of these lobes as in *paulistorum*. The two great teeth which have a lower insertion in *willistoni* than in *paulistorum*, are intermediate in the hybrids (fig. 1).

Whatever the extent of the variation among the F_1 hybrids for these anatomical traits it is always possible to distinguish them from the parental species. This possibility was recognised by Cordeiro (1952) from studies of the inheritance of hypandrium shape between two contrasting races of *paulistorum*, one from Osório, Rio Grande do Sul, and the other from S. Paulo. These racial differences in the hypandrium lobes proved to be controlled by a single mendelian factor. This need not be the case among the two species here studied. Spassky (1957) also noticed that the male external genitalia of F_1 hybrids of *D. willistoni* \times *D. insularis* are intermediate.

6. SUMMARY AND CONCLUSIONS

Two strains, one of *willistoni* and another of *paulistorum* derived from inseminated females collected in the seashore rain forest of the north of Rio Grande do Sul State, have been studied extensively because of their unexpected ability to yield fertile hybrids between two species which are usually completely isolated.

Insemination tests, analysed statistically with the aid of Levene's (1949) isolation coefficients, demonstrated an increase in the acceptance of *paulistorum* males by the *willistoni* females with time. The reciprocal crosses do not show this behaviour. Under the experimental conditions, the genic flux goes from *paulistorum* to *willistoni*, according to Levene's formulæ.

Only a few sterile F_1 hybrids, have been obtained when *willistoni* males were crossed to *paulistorum* females; yet the reciprocal crosses yielded fairly numerous fertile F_1 s and F_2 s. The backcrosses $F_1 \times P_1$ gave better results when mated to the male's parental species *paulistorum*.

These facts and the sex ratio deviation 3.3 males : 1 female observed in the F_1 , suggests a maternal effect which has not yet been elucidated.

The intermediate morphology of most traits of the spermathecæ and hypandria of the hybrids between these species, corroborates the findings of Spassky (1957) and Cordeiro (1952). Nevertheless, the genetical basis of the observed traits need not be a single mendelian factor as it is among the races of *paulistorum* studied by Cordeiro.

This is the first recorded instance of hybrids between these two species. Cytological and genetic studies of other strains show that the affinities and hybrid production, as well as chromosome pairing, vary greatly even among strains from a single locality which, according to data to be published elsewhere, has a restricted population.

Acknowledgments.—The authors wish to thank Miss Nena B. Morales for her interest and technical aid.

The collecting and laboratory expenses have been supported by The Rockefeller Foundation, The Conselho Nacional de Pesquisas and the University of Rio Grande do Sul.

7. REFERENCES

- BURLA, H., DA CUNHA, A. B., CORDEIRO, A. R., DOBZHANSKY, TH., MALOGOLOWKIN, C., AND PAVAN, C. 1949. The *willistoni* group of sibling species of *Drosophila*. *Evolution*, 3, 300-314.
- CORDEIRO, A. R. 1952. Inheritance of variations in the male genitalia in *Drosophila paulistorum*. *Amer. Nat.*, 84, 185-188.
- DOBZHANSKY, TH. 1946. Complete reproductive isolation between two morphologically similar species of *Drosophila*. *Ecology*, 27, 205-211.
- DOBZHANSKY, TH. 1951. *Genetics and the Origin of Species*. x+364 pp. Columbia University Press, New York.
- DOBZHANSKY, TH., EHRMAN, LEE, AND PAVLOVSKY, O. 1957. *Drosophila insularis*, a new sibling species of the *willistoni* group. Univ. Texas Publ., *Genetics of Drosophila*, 5721, 39-47.
- DOBZHANSKY, TH., AND KOLLER, P. CH. 1938. An experimental study of sexual isolation in *Drosophila*. *Biologischen Zentralblatt*, 58, 589-607.
- DOBZHANSKY, TH., AND SPASSKY, B. 1959. *Drosophila paulistorum*, a cluster of species *in statu nascendi*. *Proc. Nat. Acad. Sci.*, 45, 419-428.
- EHRMAN, LEE. 1960a. A preliminary study of mating behaviour in *Drosophila paulistorum*. *Amer. Nat.*, 94, 189-191.
- EHRMAN, LEE. 1960b. The genetics of hybrid sterility in *Drosophila paulistorum*. *Evolution*, 14, 212-223.
- EHRMAN, LEE. 1961. The genetics of sexual isolation in *Drosophila paulistorum*. *Genetics*, 46, 1025-1035.
- LEVENE, H. 1949. A new measure of sexual isolation. *Evolution*, 3, 315-321.
- MALOGOLOWKIN, CHANA. 1952. Sobre a genitália dos "Drosophilidae" (Diptera). III. Grupo *willistoni* do gênero *Drosophila*. *Rev. Bras. Biol.*, 12, 79-96.
- MAYR, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.
- SPASSKY, B. 1957. Morphological differences between sibling species of *Drosophila*. Univ. Texas Publ., *Genetics of Drosophila* 5721, 48-61.
- SPIETH, H. T. 1952. Mating behavior within the genus *Drosophila* (Diptera). *Bull. Am. Mus. Nat. Hist.*, 99, 395-474.
- WINGE, HELGA. 1961. Relações interespecíficas no grupo críptico *willistoni* (*Drosophila*). *Anais do II Congresso Brasileiro de Zoologia* (1961) (in preparation).
- WINGE, HELGA, AND CORDEIRO, A. R. 1960. Descoberta de uma ponte biológica entre *Drosophila willistoni* e *paulistorum* (Summ.) *Ciência e Cultura*, 12, 149.