EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. II. GEOGRAPHIC STRAINS OF DROSOPHILA PROSALTANS

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It has been shown[1] that when males of Brazilian strains of *Drosophila willistoni* are confined with females of their own and of other Brazilian strains, the females are inseminated at random. But when Brazilian or Guatemalan males are kept with mixtures of Brazilian and Guatemalan females, there is a tendency for a greater proportion of the Brazilian than of the Guatemalan females to be inseminated. A more complex system of sexual preferences, which exists within the species *D. prosaltans* Duda (= *D. sellata* Sturtevant), is described in the present article.

Strains of *D. prosaltans* coming from the following localities (Fig. 1) have been used: Huichihuayan, state of San Luis Potosi, Mexico; Chilpancingo and Zopilote Canyon, state of Guerrero, Mexico; Guatemala City, Guatemala; Belem, state of Pará, Brazil; Bertioga and Iporanga, state of São Paulo, Brazil. The strains from Mexico and Guatemala were obtained through the courtesy of Professors J. T. Patterson and A. H. Sturtevant. The Brazilian strains were collected by the senior author. All seven strains intercross readily, produce fertile hybrids, and doubtless belong to the same species. Yet the peculiar chromosome structure found in the South-Brazilian strains (Bertioga and Iporanga)[2] is absent in the strains from Belem, Guatemala and Mexico, which have V-shaped X's and Y's, one pair of V-shaped autosomes, and one pair of rod-like autosomes. Furthermore, in the Mexican strains the basal bands on the abdominal tergites are broader, lighter in color and sharper in outline than those of the Brazilian ones;[3] the Guatemala strain is intermediate in this respect and closer to those from Brazil than to those from Mexico. Finally, under the conditions of the experiments, sexual activity in the Mexican strains starts on the second day and in the Brazilian strains on the third day after hatching from the pupae.

A slight modification of the experimental techniques used for *D. willistoni*[1] was followed in the work with *D. prosaltans*. Ten freshly hatched females of each of two strains—i.e., twenty females in all—are placed in a vial with ten freshly hatched males of one of these strains. The right wings are clipped in one class of females and the left wings in the other, or else in one class a wing is cut and in the other left intact. The vials are kept at $24^1/_2$°C. for about 3 days, where cultures with Mexican males are concerned, or for 4 days if Brazilian males are used. The females are then

dissected, and their seminal receptacles are examined for sperm. The vials in which too few or too many (less than 20% or more than 80%) of the females are inseminated are discarded (such vials are infrequent). The tubular (ventral) receptacle in *D. prosaltans* is a very long tube, resembling a skein of wool that is folded, as a whole, into the shape of an M. Live spermatozoa lying parallel to the axis of the tube are easily seen. The spherical chitinous spermathecae contain a tangled mass of sperm in which only some moving threads can be seen.[4] If a female is killed while in coitu or shortly thereafter, the spermathecae and all but the proximal gyres of the tubular receptacle are free of sperm, but a viscous ejaculate is found in the vagina.

A summary of the data is presented in table 1. Numbers of the females dissected and percentages of them found inseminated are given for the homogamic (i.e., inseminated by males of the same strain) and the heterogamic (i.e., inseminated by males of the foreign strain) combinations. The χ^2's for the observed differences between the frequencies of the homogamic and the heterogamic matings, and the isolation indices[5] are also included. It is evident at a glance that preferential mating is the rule rather than an exception when different geographic strains of *D. prosaltans* are brought together, and that strong preferences for homogamic matings (positive isolation indices) as well as for heterogamic matings (negative isolation indices) are common. Taking the 0.05 or 0.01 probability levels (corresponding to χ^2's of 3.84 and 6.64) as the limits of significance, we find that 35 or 34 out of the 42 crosses made give results deviating from randomness of mating. Significant positive isolation indices are found in 23 or 24, and significant negative ones in 11 crosses.

The relationships observed can be described most simply as follows. The geographic strains examined form a hierarchic series: Chilpancingo $\geqslant$ Zopilote > Huichihuayan > Guatemala > Belem > Iporanga $\geqslant$ Bertioga. If males of a strain higher up in this series are confined with females of the same strain and of another strain lower down in the series, the frequency of homogamic matings exceeds that of the heterogamic ones, making the isolation index positive. But if males of a "low" strain are kept with a mixture of females of their own and of a "higher" strain, the frequency of heterogamic matings exceeds that of the homogamic ones, and the isolation index is negative. This regularity was found in 36 out of 42 crosses; the exceptions are discussed below.

Chilpancingo males always inseminate more of their own than of foreign females. This preference[6] is very strong in mixtures of Chilpancingo with Bertioga and Iporanga females, a little less strong in mixtures with Belem, Guatemala and Huichihuayan, and least strong in mixtures with Zopilote females. It may seem surprising, however, that, with the exception of the Zopilote males which show no preference, males of all other strains in-

TABLE 1

NUMBERS OF FEMALES DISSECTED (n) AND PER CENT CARRYING SPERM (%) IN VARIOUS CROSSES OF *Drosophila prosaltans*

FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	χ^2	ISOLATION INDEX
Chilpancingo, Zopilote	Chilpancingo	60	88.3	65	60.0	12.8	0.19
Chilpancingo, Zopilote	Zopilote	91	57.1	90	56.7	0.0	0.00
Chilpancingo, Huichihuayan	Chilpancingo	72	95.8	70	32.9	61.7	0.49
Chilpancingo, Huichihuayan	Huichihuayan	48	31.2	47	87.2	30.8	−0.47
Chilpancingo, Guatemala	Chilpancingo	60	96.7	63	28.6	60.7	0.54
Chilpancingo, Guatemala	Guatemala	93	40.9	106	71.7	19.3	−0.27
Chilpancingo, Belem	Chilpancingo	91	89.0	86	26.7	70.5	0.54
Chilpancingo, Belem	Belem	111	27.0	102	74.5	47.9	−0.47
Chilpancingo, Bertioga	Chilpancingo	48	91.7	48	6.2	70.0	0.87
Chilpancingo, Bertioga	Bertioga	103	17.5	111	65.8	51.0	−0.58
Chilpancingo, Iporanga	Chilpancingo	57	100.0	57	14.0	85.9	0.75
Chilpancingo, Iporanga	Iporanga	85	35.3	89	67.4	18.0	−0.31
Zopilote, Huichihuayan	Zopilote	71	71.8	72	34.7	19.7	0,35
Zopilote, Huichihuayan	Huichihuayan	105	26.7	114	69.3	39.7	−0.44
Zopilote, Guatemala	Zopilote	65	75.4	59	18.6	40.1	0.60
Zopilote, Guatemala	Guatemala	67	28.4	75	58.7	13.0	−0.35
Zopilote, Belem	Zopilote	46	95.6	42	26.2	45.5	0.57
Zopilote, Belem	Belem	112	49.1	113	61.1	3.2	−0.11
Zopilote, Bertioga	Zopilote	77	72.7	76	19.7	43.3	0.57
Zopilote, Bertioga	Bertioga	105	32.4	108	67.6	26.4	−0.35
Zopilote, Iporanga	Zopilote	50	92.0	53	32.1	38.8	0.48
Zopilote, Iporanga	Iporanga	82	26.8	80	70.0	30.1	−0.45
Huichihuayan, Guatemala	Huichihuayan	92	71.7	92	38.0	21.1	0.31
Huichihuayan, Guatemala	Guatemala	79	36.7	80	48.7	2.6	−0.14
Huichihuayan, Belem	Huichihuayan	86	77.9	82	13.4	70.1	0.71
Huichihuayan, Belem	Belem	106	57.5	100	30.0	15.8	−0.31
Huichihuayan, Bertioga	Huichihuayan	74	58.1	74	17.6	25.8	0.54
Huichihuayan, Bertioga	Bertioga	100	32.0	96	51.0	7.1	−0.23
Huichihuayan, Iporanga	Huichihuayan	76	81.6	75	48.0	18.7	0.26
Huichihuayan, Iporanga	Iporanga	100	33.0	95	45.3	3.1	−0.16
Guatemala, Belem	Guatemala	103	55.3	102	10.8	46.5	0.67
Guatemala, Belem	Belem	99	55.6	106	16.0	35.0	0.55
Guatemala, Bertioga	Guatemala	51	64.7	51	11.8	30.2	0.69
Guatemala, Bertioga	Bertioga	78	66.7	80	41.3	10.2	0.24
Guatemala, Iporanga	Guatemala	50	60.0	52	11.5	26.2	0.68
Guatemala, Iporanga	Iporanga	164	60.4	155	38.7	14.9	0.22
Belem, Bertioga	Belem	122	53.3	116	36.2	7.0	0.19
Belem, Bertioga	Bertioga	91	41.8	94	21.3	9.1	0.32
Belem, Iporanga	Belem	94	45.7	94	50.0	0.3	−0.04
Belem, Iporanga	Iporanga	85	43.5	80	41.2	0.1	0.03
Bertioga, Iporanga	Bertioga	70	42.8	67	38.8	0.2	0.05
Bertioga, Iporanga	Iporanga	99	61.6	108	48.1	3.8	0.12

seminate Chilpancingo females in preference to females of their own strains. The preference is exhibited most strongly by males from Bertioga, which give a negative isolation index as low as -0.58.

Zopilote flies are about equivalent to Chilpancingo, but the positive as well as the negative isolation indices tend to be lower where Zopilote flies are concerned. Huichihuayan males prefer their own females to those of Guatemala, Belem, Bertioga and probably Iporanga, but inseminate Chilpancingo and Zopilote females in preference to their own. Hiuchihuayan females placed with Chilpancingo and Zopilote are discriminated against by males of the latter strains, but are preferred by Guatemala, Belem, Iporanga and Bertioga males in the presence of their own females.

Guatemala and Belem strains behave somewhat differently from those discussed above. As might be expected from the position of the Guatemala strain in the hierarchic series, Guatemala males inseminate their own females preferentially in mixtures with Belem, Bertioga and Iporanga, but prefer foreign females in mixtures with Chilpancingo, Zopilote and probably Huichihuayan. But Guatemala females are not inseminated by males of any strain in preference to females of their own strain. Belem, Bertioga and Iporanga males, which stand lower in the hierarchic series than Guatemala, inseminate a greater proportion of their own than of Guatemala females; Chilpancingo, Zopilote and Huichihuayan males do likewise. There is, consequently, a true sexual isolation—that is, a reciprocal aversion—between the Guatemala and the Brazilian strains, and if flies from Guatemala and Brazil were mixed the matings would be mostly homogamic. As shown above, there is no reciprocal aversion between Guatemala and Mexican strains, or between Mexican and Brazilian strains, Mexican females being always preferred. The Belem strain seems to behave in a manner similar to the Guatemala. In accordance with the position of this strain in the hierarchy, Belem males give negative isolation indices in mixtures with Mexican and their own females, but positive isolation indices in mixtures with Bertioga females. However, Bertioga males placed with Bertioga and Belem females inseminate the former preferentially. Matings occur more or less at random in mixtures of Belem and Iporanga, or of Bertioga and Iporanga flies.

The hierarchy of sexual preferences of strains of *Drosophila prosaltans* is clearly correlated with the geographic origin of these strains (Fig. 1). The three highest strains all come from Mexico, which is the northern limit of the distribution area of the species. The two lowest strains are from southern Brazil, and the intermediate strains from Central America and from equatorial Brazil. Strains from localities geographically close to each other are more or less similar (Chilpancingo and Zopilote, Iporanga and Bertioga).

The discovery of negative isolation indices, which signify that matings

between representatives of different strains are more frequent than between those of the same strain, was unexpected. Since sexual activity starts sooner after hatching of the flies from the pupae in Mexican than in

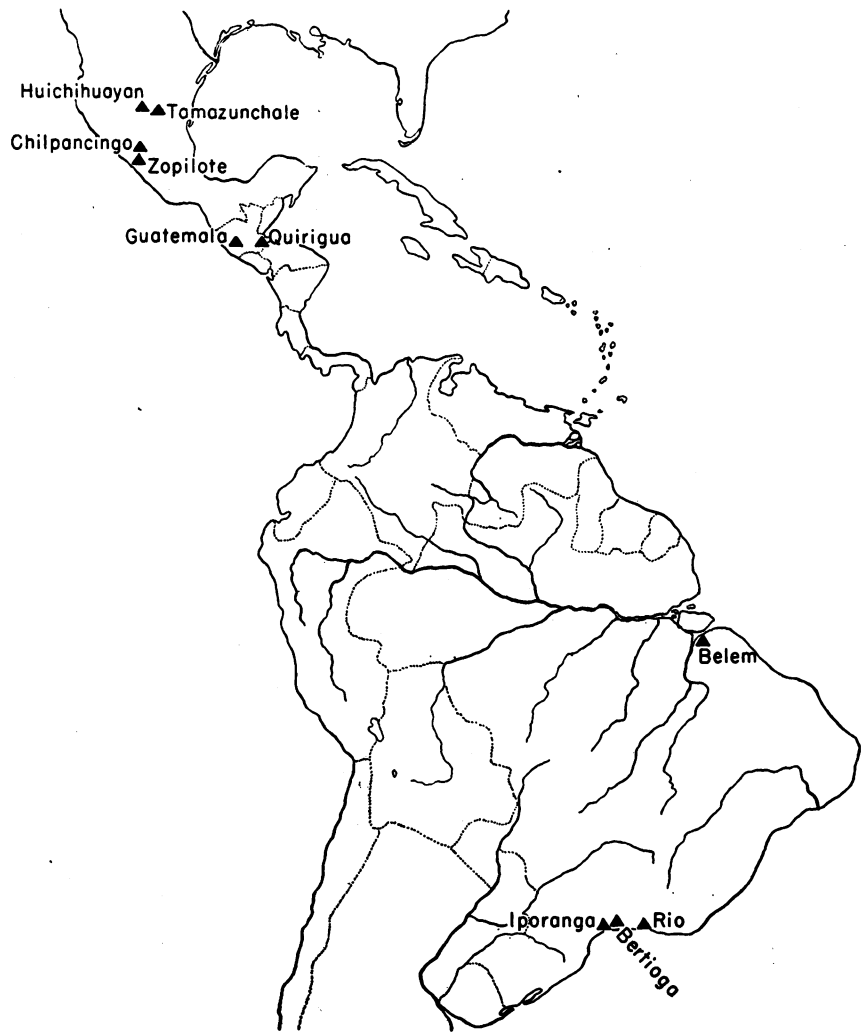

FIGURE 1

A map showing the localities in which strains of *D. prosaltans* and *D. sturtevanti* have been collected.

Brazilian strains, it seemed possible that the results obtained in our experiments, in which flies of the same chronological age are placed together, reflected merely the difference in biological age of the flies. Indeed, females

may become more sexually receptive as they grow older, and therefore older females may be inseminated in preference to younger ones. To test this possibility, Brazilian females were kept for two days on abundant food but without males, and then placed in vials with freshly hatched Mexican females and freshly hatched males of either kind. The females were dissected and examined as usual. The results are summarized in table 2. The rate of insemination clearly does not depend upon the age of the females alone, although the isolation indices in table 2 are lower than those for the corresponding crosses in table 1.

TABLE 2

NUMBERS OF FEMALES DISSECTED (n) AND PER CENT CARRYING SPERM (%) IN CROSSES IN WHICH BRAZILIAN FEMALES WERE OLDER THAN THE MEXICAN ONES

FEMALES	MALES	HOMOGAMIC n	HOMOGAMIC %	HETEROGAMIC n	HETEROGAMIC %	χ^2	ISOLATION INDEX
Chilpancingo, Bertioga	Chilpancingo	133	80.5	136	25.7	80.8	0.52
Chilpancingo, Bertioga	Bertioga	69	34.8	71	66.2	13.8	−0.31
Chilpancingo, Belem	Belem	41	53.7	40	80.0	5.3	−0.20

Summary.—The strains of *D. prosaltans* form a graded series with respect to their sexual preferences, and this seriation corresponds in a general way to the geographic origin of these strains. When northern (Mexican) males are kept with a mixture of northern and southern (Brazilian) females, more of the former than of the latter are inseminated. In similar experiments, the southern males also inseminate more northern than southern females. Females of the strains from Guatemala and from equatorial Brazil are discriminated against by South-Brazilian males in favor of females from the South-Brazilian strains.

[1] Dobzhansky, Th., and Mayr, E., these PROCEEDINGS, **30**, 238–244 (1944).

[2] Dobzhansky, Th., and Pavan, C., *Ibid.*, **29**, 368–375 (1943).

[3] This external difference is not sufficient to distinguish the Mexican and the South-Brazilian flies in all cases. Young Mexican flies have the same "faded-out" appearance as the older Brazilian flies, the "bright" coloration characteristic of the Mexican race developing only gradually with age. It does not seem advisable at this time to recognize the Mexican race taxonomically by attaching to it a Latin name.

[4] This condition, and the fact that the opening of the spermathecal duct is provided with a brush of chitinous needles protruding into the cavity of the spermatheca, suggests that the sperm stored in it is not used in fertilization of eggs. It seems possible that the function, in Drosophila, of the sperm in the chitinous spermathecae is to stimulate the development of the eggs in the ovary through a hormone-like mechanism.

[5] Stalker, H. D., *Genetics*, **27**, 238–257 (1942).

[6] It should be stressed that expressions like "preference" and "discrimination" are used merely to avoid circumlocutions. Our data show merely the frequencies of insemination of females of different strains, and it is not known whether these frequencies depend upon the behavior of males, of females or of both sexes.