THE GENETICS OF SEXUAL ISOLATION IN *DROSOPHILA PAULISTORUM*¹

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SEXUAL isolation is a "weakness or lack of mutual attraction between males and females of different species" (DOBZHANSKY 1951). Occurrence of rudiments of sexual isolation within a species, between races or subspecies, may indicate that a process of speciation, of splitting of a single species into two or several derived ones, is under way. It is, indeed, to be expected on theoretical grounds that genetic variability from which reproductive isolating mechanisms could be built should be found also intraspecifically.

The genetics of sexual isolation between two morphologically similar, sibling, species, *Drosophila pseudoobscura* and *Drosophila persimilis*, has been analyzed by TAN (1946). Factors distinguishing the mating behavior of *D. persimilis* from that of *D. pseudoobscura* were found in at least the X and the second chromosomes. The present article describes a series of experiments directed, for the first time, toward the study of the genetics of sexual isolation, i.e., of isolation in the process of formation, between subspecies.

Drosophila paulistorum Dobzhansky and Pavan is one of the five sibling species which constitute the *willistoni* group of sibling species. The morphological characteristics and the relationships of this species to *D. tropicalis*, *D. willistoni*, and *D. equinoxialis* were analyzed by BURLA *et al.* (1949). *D. tropicalis* males were found to inseminate some *D. paulistorum* females and *D. paulistorum* males occasionally copulated successfully with females of the other three species. However, these interspecific inseminations never resulted in the production of hybrid progenies. Later, DOBZHANSKY, EHRMAN and PAVLOVSKY (1957) analyzed the relationship between *D. paulistorum* and *D. insularis*, the last *willistoni* sibling species to be described. Here hybrid progenies, although wholly sterile, were produced in some instances by heterogamic insemination. Without doubt, the five sibling species are completely isolated reproductively.

Extensive collections of *D. paulistorum* in 1957 and 1958 from localities in Central and South America yielded a group of geographic strains which, when brought into the laboratory and intercrossed, gave varied and surprising results. Not all the strains could be outcrossed with equal ease; some matings produced no hybrids at all, while others yielded fertile female hybrids and F₁ males that were completely sterile (DOBZHANSKY and SPASSKY 1959). Eventually, six groups of geographic populations were distinguished:

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1. *Centro-American* group, from Guatemala to Costa Rica.
2. *Amazonian* group, from Panama through northern Brazil.
3. *Andean-South Brazilian* group, from southern Colombia, Ecuador, Peru, Bolivia to southern Brazil.
4. *Orinocan* group, from Panama and eastern Colombia to Venezuela and British Guiana.
5. *Guianan* group, from Georgetown, in British Guiana (a single strain).
6. *Transitional* group, from several localities in Colombia.

Crosses between the first three subspecies always produce fertile female and sterile male hybrids; this male sterility is genic in nature, and expresses itself via the genotype of hybrid mothers (EHRMAN 1960a). The male offspring of an intersubspecific hybrid female is sterile regardless of its own genotype and that of its male parent; the mother needs, however, be "hybrid" only with respect to any one chromosome in the diploid complement of three pairs to produce this remarkable phenomenon.

The purpose of the present work is to analyze three of the subspecies, the Centro-American, the Amazonian, and the Andean-South Brazilian, with regard to the genetic basis of another reproductive isolating mechanism, namely the sexual isolation between them.

MATERIALS AND METHODS

The plan followed in ascertaining the role of each of the two autosomes and of the X chromosome in the genetic architecture of the sexual isolation barrier via a mating preference technique was the same as the one employed to analyze the genetics of the sterility of the hybrid males (see EHRMAN 1960a for details). The object was to transfer one marked chromosome into the nuclear and cytoplasmic background of an alien subspecies. At no time could two marked chromosomes be transferred across the subspecific barrier simultaneously. *D. paulistorum* is a species delicate enough so that, at least in laboratory cultures, flies carrying several mutant genes are apt to have a viability too low to permit carrying out experiments on their mating preferences successfully.

Because of the difficulty of obtaining progeny from intersubspecific matings, the hybrid offspring necessarily come from a limited number of females. The initial intersubspecific crosses were therefore made in a direction which minimized the sexual isolation barrier. Thus, the F_1 hybrids shown in Table 1 were obtained by crossing Centro-American ♀♀ × Amazonian ♂♂; Tables 2, 3, 4 = Amazonian ♀♀ × Centro-American ♂♂; Tables 5, 6, 7, 8 = Centro-American ♀♀ × Andean-South Brazilian ♂♂; Table 9 = Andean-South Brazilian ♀♀ × Amazonian ♂♂.

Backcross progenies were obtained by crossing F_1 hybrid females carrying suitable marker genes in certain chromosomes to males of one or the other of the parental subspecies. The backcrosses were repeated in each of three successive generations, always selecting as female parents the carriers of subspecies-foreign

chromosomes. For each combination of parental subspecies, two series of recurrent backcrosses were made, to each of these subspecies. The F_1 hybrid females between subspecies A and B obviously contain one A and one B chromosome in each pair; the F_1 hybrid males have the X of their mother, and the Y of their father, and an A and a B autosome of each parent. In the backcrosses of the A/B hybrid females to A males, the B chromosomes, except the one with the gene marker, tend to be gradually replaced because only the one with the gene marker is selected for; in the backcrosses to B males, the A chromosomes tend to be eliminated. In the progeny of the third backcross, most of the flies will carry chromosomes of one subspecies only, except the "foreign" chromosome with the genetic marker (and sometimes also a foreign Y chromosome).

The "control" crosses involved the use of sisters of the same females which were used in the experimental series, but not containing the foreign chromosomes with the genetic markers. The progenies were, consequently, like the experimental ones, except that they did not contain the foreign marked chromosome.

In most instances the foreign chromosome contained only a single mutant gene which served as a marker. This was nevertheless deemed a satisfactory experimental technique, for two reasons: First, whenever more than one marker was present, crossing over was found to be suppressed in the intersubspecific crosses. Second, as shown by the unpublished data of DOBZHANSKY and PAVLOVSKY, the subspecies involved in these experiments differed in at least one inversion in each of their five chromosome "arms" (the X chromosome and second chromosome in *D. paulistorum* are metacentric). This does not, of course, exclude the possibility that some undetected crossing over occurs in the hybrids, but such crossing over will not be frequent. This makes using a single gene marker per chromosome a legitimate procedure; more than one could be used in some crosses, but this would have reduced the viability of the flies to a greater extent than would be desirable.

Flies to be tested for mating preferences were aged from four days to one week in isolation from flies of the opposite sex. They were then placed, without etherization, in glass observation chambers; small Petri dishes containing a small amount of food were used for this purpose.

Generally, the ratio of the sexes put into an observation chamber at one time was three hybrid females plus three males of one parental subspecies and three males of the other parental subspecies. Or, where hybrid males were to be tested, three hybrid males plus three females of one parental subspecies and three females of the other parental subspecies were used. These numbers were sometimes changed because of a shortage of virgin females. In all, 4871 virgin females were used in the course of these experiments, and *D. paulistorum* virgins must be collected every three to four hours. Observation chambers testing hybrid females were watched until approximately 20 copulae were recorded. Observation chambers testing hybrid males were watched until 20 copulae involving them were recorded.

A pictorial study of both the material and the method, and of the mating behavior in *D. paulistorum* has been published by EHRMAN and STRICKBERGER (1960).

Hybrids between the Centro-American and Amazonian races: Females of the Centro-American subspecies heterozygous for the second chromosome dominant gene Spot (a dark spot on the thorax) were crossed to the Amazonian subspecies males heterozygous for the second chromosome dominant marker Plum (an eye color). Among the F_1 hybrids, Spot/Plum females were selected to be the progenitors of the backcross progenies. Spot, Plum, and wild-type hybrid females and males were used for tests on their mating preferences. The females were placed together with equal numbers of wild-type males from the Honduras strain of the Centro-American subspecies, and from the Belem strain of the Amazonian subspecies. As shown in Table 1, the 22 copulae observed were all with Centro-American males. The males were given equal numbers of virgin Honduras females and Belem females. Of the 19 copulae observed, 17 were with Honduras and two with Belem males.

The Spot/Plum F_1 females were backcrossed to wild-type males of both parental subspecies. In two following generations, Spot or Plum females were backcrossed to wild-type males of the opposite subspecies. In every generation, females and males carrying the mutant markers were tested for their mating preferences by placing them together with equal numbers of wild-type individuals of the

TABLE 1

Copulae observed in crosses between Centro-American (CA) females and Amazonian (Am) males strains. The second chromosomes marked by Spot (CA) and Plum (Am) mutations

Number		Copulae with CA	Am	Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	22	22	0	72	20.04	<0.01
Backcrosses to Centro-American parent						
BC ₁	20	10	10	27		
BC ₂	20	19	1	25	14.45	<0.01
BC ₃	20	19	1	24	14.45	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	4	16	34	6.05	0.02-0.01
BC ₂	22	5	17	51	5.50	0.02-0.01
BC ₃	22	2	20	30	13.14	<0.01
Tests of hybrid males						
F ₁	19	17	2	48	10.32	<0.01
Backcrosses to Centro-American parents						
BC ₁	20	17	3	58	8.45	<0.01
BC ₂	20	19	1	36	14.45	<0.01
BC ₃	22	20	2	38	13.14	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	0	20	38	18.05	<0.01
BC ₂	19	0	19	51	17.04	<0.01
BC ₃	20	0	20	38	18.05	<0.01

opposite sex of the parental subspecies. All the results appear in Table 1. In the backcrosses to the Centro-American subspecies, the hybrids showed a decided preference for Centro-American partners (except for the BC₁ females which appeared to be neutral). In the backcrosses to the Amazonian subspecies, the preference for the Amazonian partners rapidly becomes emphatic.

To test the possibility that the source of the cytoplasm may be of importance, the same crosses as above were repeated starting with Plum Amazonian females and Spot Centro-American males. The results are reported in Table 2. Comparison with the data in Table 1 discloses an interesting situation. The F₁ hybrid females now prefer males of the Amazonian subspecies (16 out of the 20 copulations observed). This is also true of the F₁ hybrid males (17 of the 20 copulations with the Amazonian females). Effects on the sexual preference of the maternal cytoplasm or of the foreign X chromosomes are therefore indicated. These are, however, not borne out in other experiments (see below).

In any case, this maternal effect does not extend beyond the first generation of hybrids. Comparison of the data in Tables 1 and 2 show that the sexual preferences of all the backcross hybrids were similar or identical in the reciprocal crosses.

To test the effects on the sexual preferences of the foreign third chromosomes, Amazonian females heterozygous for the dominant marker Delta (wing venation) in the third chromosome were crossed to Centro-American males which

TABLE 2

Copulae observed in crosses between Amazonian (Am) females strains and Centro-American (CA) males strains. The second chromosome marked by Spot (CA) and Plum (Am) mutations
A Reciprocal Cross

Number		Copulae with CA	Am	Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	20	4	16	163	6.05	0.02-0.01
Backcrosses to Centro-American parent						
BC ₁	22	18	4	39	7.68	<0.01
BC ₂	21	20	1	26	17.04	<0.01
BC ₃	20	20	0	46	18.05	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	1	19	68	14.45	<0.01
BC ₂	20	0	20	41	18.05	<0.01
BC ₃	20	0	20	26	18.05	<0.01
Tests of hybrid males						
F ₁	20	3	17	61	8.45	<0.01
Backcrosses to Centro-American parent						
BC ₁	20	11	9	31	0.05	0.90-0.80
BC ₂	20	17	3	30	8.45	<0.01
BC ₃	20	19	1	31	14.45	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	2	18	33	11.25	<0.01
BC ₂	20	2	18	33	11.25	<0.01
BC ₃	20	1	19	27	14.45	<0.01

carried on one of their third chromosomes the dominant Minute (bristles) and the recessive ebony (body color). In the F_1 generation, the Delta/Minute-ebony females were used as progenitors of the backcross progenies, and their sibs were used for tests of the mating preferences of the F_1 hybrids (ebony is approximately 50 crossover units from Minute, and is used here as a check on the suppression of crossing over in the hybrids). The results are reported in Table 3. The F_1 hybrid females accepted Centro-American males, while the F_1 hybrid males appeared to be neutral (although the F_1 hybrid males shown in Table 2 were accepted preferentially by the Amazonian females, this difference in behavior is definitely not statistically significant).

The backcross progenies showed that the sexual preference of the hybrids were for the subspecies of the recurrent parent. It appears that the third chromosome, like the second chromosome, does not by itself determine the sexual preferences in these crosses.

To test the effects of the X chromosome, Amazonian females homozygous for the sex-linked gene for white eyes were crossed to wild-type Centro-American males from the Honduras strain (no gene markers are available in the X chromosome of the Centro-American subspecies). The gene white in *D. paulistorum* is incompletely recessive, heterozygous females being identifiable by their dilute red eye color. The tests of the sexual preferences of the F_1 hybrids are reported in Table 4. The hybrid females preferred Centro-American males despite their

TABLE 3

Copulae observed in crosses between Amazonian (Am) females and Centro-American (CA) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (Am) mutations

Number		Copulae with CA Am		Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	19	17	2	77	10.32	<0.01
Backcrosses to Centro-American parent						
BC ₁	20	18	2	37	11.25	<0.01
BC ₂	20	19	1	19	14.45	<0.01
BC ₃	20	19	1	44	14.45	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	2	18	52	11.25	<0.01
BC ₂	20	1	19	50	14.45	<0.01
BC ₃	20	1	19	49	14.45	<0.01
Tests of hybrid males						
F ₁	20	8	12	123	0.45	0.70-0.50
Backcrosses to Centro-American parent						
BC ₁	20	14	6	35	2.45	0.20-0.10
BC ₂	20	19	1	40	14.45	<0.01
BC ₃	20	19	1	35	14.45	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	2	18	45	11.25	<0.01
BC ₂	20	2	18	72	11.25	<0.01
BC ₃	20	2	18	60	11.25	<0.01

TABLE 4

Copulae observed in crosses between Amazonian (Am) females and Centro-American (CA) males strains. The first chromosome marked by a white (Am) mutation

Number		Copulae with CA	Am	Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	22	19	3	45	10.22	<0.01
Backcrosses to Amazonian parent						
BC ₁	20	0	20	44	18.05	<0.01
BC ₂	20	1	19	83	14.45	<0.01
BC ₃	20	0	20	37	18.05	<0.01
Tests of hybrid males						
F ₁	20	8	12	99	0.45	0.70-0.50
Backcrosses to Amazonian parent						
BC ₁	20	0	20	46	18.05	<0.01
BC ₂	20	0	20	28	18.05	<0.01
BC ₃	20	0	20	21	18.05	<0.01

Amazonian cytoplasm. The F₁ hybrid males (having white eyes) were not clearly preferred by females of either subspecies.

The F₁ hybrid females were backcrossed to white-eyed Amazonian males in three successive generations. The results are reported in Table 4. No backcrosses to the Centro-American parent could be made because of the lack of gene markers. Both females and males in the backcross progenies preferred and were preferred by Amazonian partners. Now, the backcross females carried one Amazonian and one Centro-American X chromosome. The backcross males had, however, the Centro-American X chromosome, or at least the part containing the wild-type allele of the gene white. They also had an Amazonian Y chromosome.

It appears then, that in the Amazonian × Centro-American hybrids, sexual preference is decided by which subspecies contributes more than half of the genome, no one chromosome being clearly more important than the others.

Hybrids between the Centro-American and Andean-South Brazilian races: The crosses made to study the sexual preferences of the hybrids carrying various combinations of the chromosomes of the Centro-American and the Andean-South Brazilian subspecies were quite analogous to those described above for the Centro-American × Amazonian hybrids. The X chromosome could not, however, be tested on account of the absence of suitable genetic markers.

Table 5 reports the results of the tests of the second chromosomes. The parents of the crosses were Centro-American females with the gene marker Spot, and Andean-South Brazilian males heterozygous for the dominant second chromosome gene Bubbled (inflated wings). As usual, the F₁ hybrid females carrying the two markers from the two parental subspecies were used as parents of the backcross progenies, and their siblings showing either or neither marker were tested for sexual preferences. As shown in Table 5, the F₁ hybrids of both sexes were neutral between the parental subspecies.

The behavior of the backcrosses is most interesting, and clearly different from

TABLE 5

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The second chromosome marked by Spot (CA) and Bubbled (ASB) mutations

Number		Copulae with CA	ASB	Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	20	9	11	24	0.05	0.90-0.80
Backcrosses to Centro-American parent						
BC ₁	21	11	10	24		
BC ₂	25	18	7	30	4.00	0.05-0.02
BC ₃	20	15	5	29	4.05	0.05-0.02
Backcrosses to Andean-South Brazilian parent						
BC ₁	20	9	11	29	0.05	0.90-0.80
BC ₂	20	9	11	28	0.05	0.90-0.80
BC ₃	20	10	10	26		
Tests of hybrid males						
F ₁	20	8	12	26	0.45	0.70-0.50
Backcrosses to Centro-American parent						
BC ₁	23	9	14	39	0.69	0.50-0.30
BC ₂	20	7	13	46	1.25	0.30-0.20
BC ₃	20	6	14	52	2.45	0.20-0.10
Backcrosses to Andean-South Brazilian parent						
BC ₁	22	11	11	36		
BC ₂	19	10	9	48		
BC ₃	20	9	11	28	0.05	0.90-0.80

what we observed in the Centro-American $\times$ Amazonian hybrids described above. In the Centro-American $\times$ Andean-South Brazilian hybrids (Table 5), the sexual neutrality is retained as long as the fly carries a subspecies-foreign chromosome. Even in the progeny of the third backcross, when the genotype must have been mostly that of the recurrent parent, no clear sexual preference is apparent.

The above conclusion was tested by a control experiment shown in Table 6. The pure Centro-American females with the gene Spot preferred Centro-American males; the pure South Brazilian males with the gene Bubbled were as clearly preferred by South Brazilian females (Angra strain). In the progenies of the third backcrosses, females and males were selected which did not carry the mutant markers, and therefore presumably were free of subspecies-foreign chromosomes. The test showed they preferred, and were preferred by, individuals of the subspecies to which the recurrent parent belonged.

The tests of the effects of third chromosomes are reported in Table 7. The original cross was of Minute-ebony Centro-American females to Delta Andean-South Brazilian males. The F₁ hybrid females and males were neutral in sexual preference between the parental subspecies. This agrees with what was observed in the tests of the second chromosomes (see above). The behavior of the backcross progenies was different: the females perhaps showed a slight preference for males

TABLE 6

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The second chromosome marked by Spot (CA) and Bubbled (ASB) mutations
Control

	Number	Copulae with		Number of	Chi-square	P
		CA	ASB	hybrids tested		
Tests of Spot (Centro-American) females						
P	20	16	4	20	6.05	0.02-0.01
Backcrosses of Centro-American parent—tests of hybrid females						
BC ₃	20	16	4	23	6.05	0.02-0.01
Backcrosses to Andean-South Brazilian parent						
BC ₃	20	4	16	25	6.05	0.02-0.01
Tests of Bubbled (South Brazilian) males						
P	20	4	16	20	6.05	0.02-0.01
Backcrosses to Centro-American parent—tests of hybrid males						
BC ₃	20	16	4	20	6.05	0.02-0.01
Backcrosses to Andean-South Brazilian parent						
BC ₃	20	3	17	24	8.45	<0.01

TABLE 7

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (ASB) mutations

	Number	Copulae with		Number of	Chi-square	P
		CA	ASB	hybrids tested		
Tests of hybrid females						
F ₁	20	9	11	25	0.05	0.90-0.80
Backcrosses to Centro-American parent						
BC ₁	22	13	9	37	0.41	0.70-0.50
BC ₂	20	12	8	25	0.45	0.70-0.50
BC ₃	20	12	8	33	0.45	0.70-0.50
Backcrosses to Andean-South Brazilian parent						
BC ₁	20	9	11	29	0.05	0.90-0.80
BC ₂	22	9	13	37	0.41	0.70-0.50
BC ₃	20	8	12	38	0.45	0.70-0.50
Tests of hybrid males						
F ₁	20	15	5	39	4.05	0.05-0.02
Backcrosses to Centro-American parent						
BC ₁	20	18	2	42	11.25	<0.01
BC ₂	20	18	2	41	11.25	<0.01
BC ₃	20	18	2	44	11.25	<0.01
Backcrosses to Andean-South Brazilian parent						
BC ₁	20	11	9	38	0.05	0.90-0.80
BC ₂	20	3	17	38	8.45	<0.01
BC ₃	20	3	17	54	8.45	<0.01

of the subspecies of the recurrent parent, but in no case did their preference reach the level of statistical significance. On the other hand, the backcross males

have become gradually shifted towards being preferred by the females of the subspecies of the recurrent parent. The F_1 hybrid males, and the males from the first backcross to the South Brazilian parent, are neutral or slightly preferred by the Centro-American females. In the second and third backcrosses the preference by the females for the recurrent parental subspecies becomes obviously significant.

To make sure that the interpretation of the data reported in Table 7 was valid, a control experiment was run, and reported in Table 8. The Centro-American

TABLE 8

Copulae observed in crosses between Centro-American (CA) females and Andean-South Brazilian (ASB) males strains. The third chromosome marked by Minute (CA), ebony (CA), and Delta (ASB) mutations
Control

Number		Copulae with CA ASB		Number of hybrids tested	Chi-square	P
Tests of Minute-ebony (Centro-American) females						
P	20	16	4	20	6.05	0.02-0.01
Backcrosses to Centro-American parent—tests of hybrid females						
BC ₃	20	16	4	23	6.05	0.02-0.01
Backcrosses to Andean-South Brazilian parent						
BC ₃	20	2	18	24	11.25	<0.01
Tests of Delta (South Brazilian) males						
P	20	2	18	20	11.25	<0.01

Minute-ebony females prefer, as expected, Centro-American (Honduras) males, and the Delta South Brazilian males are preferred by South Brazilian (Angra) females. The "control" females from the progenies of the third backcross behaved like the females of pure subspecies.

Hybrids between the Amazonian and Andean-South Brazilian races: Only the effects of the X chromosomes in the progenies of the hybrids between these subspecies have been studied. Wild-type females of South Brazilian (Angra) subspecies were crossed to white-eyed males of the Amazonian (Belem) subspecies. As indicated above, the white gene behaves as a semidominant in this species. The F_1 hybrid females from this cross accept males of no subspecies (EHRMAN 1960b). (Backcross progenies were obtained by etherizing the females and immediately placing them with mature, unetherized males. The males will then approach and will often mount the females while they are still partly anesthetized. Subsequently, the females deposited fertilized eggs.) The F_1 hybrid males seem to be accepted significantly more frequently by females of the maternal subspecies (South Brazilian).

The behavior of the backcross progenies is interesting in that the preference for the subspecies of the recurrent parent grows only slowly or not at all. The females coming from the third backcross to the Amazonian parent still accepted Amazonian and South Brazilian males about equally. The same is true of the

backcross males. It appears that the South Brazilian X chromosome contains genetic modifiers that are strong enough to neutralize the Amazonian genes in the remainder of the genome.

DISCUSSION

The genetic architecture of reproductive isolating mechanisms is known only in very few instances. DOBZHANSKY (1936) studied the hybrid sterility and TAN (1946) studied the sexual isolation between the sibling species, *D. pseudoobscura* and *D. persimilis*. The sterility and the sexual isolation proved to be similarly constructed: they are due to summation of the effects of polygenes borne in all the chromosomes which those species have (except for the tiny fifth chromosome which was not studied). The subspecies, or incipient species, of *D. paulistorum* are also sexually isolated, and they also produce sterile male hybrids. But here the genetic mechanisms underlying the sterility and the sexual isolation are quite different. The evidence, as regards the cause of this sterility, is consistent with the view that any female which carries any mixture of the chromosomes of different subspecies deposits eggs giving rise to sterile male zygotes and to fertile female ones. The sterility of a male depends, in the backcrosses, upon the genetic constitution of his mother, and not on his own genetic constitution. In other words, the properties of an egg are determined, as far as the sterility of the backcross males is concerned, by the genotype present in it before meiosis, and not by that formed following fertilization. It should be noted that the presence in the mother of just one subspecies-foreign chromosome is already sufficient to make it produce sterile sons (EHRMAN 1960a).

The sexual isolation, which makes matings between females and males of the three subspecies of *D. paulistorum* much less likely to succeed than matings within a subspecies, is due to polygenes scattered in every one of the three pairs of chromosomes which these flies have. The subspecies of *D. paulistorum* resemble, in this respect, the pair of species, *D. pseudoobscura* and *D. persimilis*. As shown above, there exist, however, certain interesting differences between the subspecies.

Genes controlling the sexual preferences of both the male and female hybrids of the Centro-American and the Andean-South Brazilian subspecies, are concentrated particularly in the second chromosome (see Tables 5 and 6). As long as the Centro-American second chromosome is retained in the hybrids (its loss is prevented by selection for it each generation), repeated backcrosses to the Andean-South Brazilian subspecies do not neutralize the tendency to copulate with Centro-American individuals. On the other hand, as long as the Andean-South Brazilian second chromosome is retained in the hybrids (its loss by replacement is prevented by selection for it each generation), repeated backcrosses to the Centro-American subspecies do not dilute the tendency to copulate with Andean-South Brazilian flies. Note that none of the P values in Table 5 are significant.

The control experiments (Table 6) clearly validate the above conclusion in

that they indicate just the opposite situation. Both sexes show a pronounced preference for mating with individuals of the recurrent parental subspecies. The "BC₃s" to the Centro-American parent prefer Centro-American mates, and they lack the Andean-South Brazilian second chromosome. The "BC₃s" to the Andean-South Brazilian parent prefer Andean-South Brazilian mates, and they similarly lack the Centro-American second chromosome.

To express what is happening in the backcrosses in terms of percentage dilution of the alien chromosome, one might say that in repeated backcrosses between subspecies "A" and subspecies "B", where subspecies "B" is the recurrent parent, by the time the third backcross generation is reached, the percentage of "A" in the entire genome should be about 20.8 percent, while that of "B" is approximately 79.2 percent. In the controls, the theoretical percentage of "A" is 4.2 percent and that of "B" is 95.8 percent (see EHRMAN 1960a; Table 1 for details).

Some of the genes controlling the sexual preferences of the Centro-American and the Andean-South Brazilian subspecies and of their hybrids, are carried also in the third chromosome and in the X chromosome, as is shown by the data in Tables 7-9. The sexual preferences of the Centro-American vis-a-vis the Amazonian subspecies show, in a way, a more complex genetic structure (Tables 1-4). No one chromosome is here clearly more influential than the others. In the backcrosses, both sexes of the hybrids prefer and are preferred by the opposite sex of the subspecies which serves as the recurrent parent in the backcross. For the combination Amazonian $\times$ Andean-South Brazilian subspecies, only the effects of the X chromosome were studied, and even these not in a satisfactory manner, on account of the paucity of genetic markers (Table 9). The X chromosome clearly contains some genes determining the sexual preference, but most likely the autosomes would show such genes also.

A more detailed analysis of the genetics of the sexual isolation in *D. paulis-*

TABLE 9

Copulae observed in crosses between Andean-South Brazilian (ASB) females and Amazonian (AM) males strains. The first chromosome marked by a white (Am) mutation

Number		Copulae with ASB		Number of hybrids tested	Chi-square	P
Tests of hybrid females						
F ₁	These females do not accept <i>any</i> males (EHRMAN 1960b)*					
Backcrosses to Amazonian parent						
BC ₁	20	5	15	62	4.05	0.05-0.02
BC ₂	21	6	15	44	3.06	0.10-0.05
BC ₃	20	13	7	55	1.25	0.30-0.20
Tests of hybrid males						
F ₁	20	4	16	66	6.05	0.02-0.01
Backcrosses to Amazonian parent						
BC ₁	20	12	8	62	0.45	0.70-0.50
BC ₂	20	5	15	48	4.05	0.05-0.02
BC ₃	20	8	12	43	0.45	0.70-0.50

* With the reciprocal cross, Amazonian ♀♀ $\times$ Andean-South Brazilian ♂♂, 18 out of 20 copulae observed were with Andean-South Brazilian ♂♂, and two were with Amazonian ♂♂ ($P < 0.01$).

torum will have to await the availability of more genetic markers than are at our disposal at present. Although, as stated in the discussion of the experimental procedures, crossing over is probably largely suppressed in the subspecies hybrids, a better control over this point would clearly be desirable. The different genetic architecture of the hybrid sterility and of the sexual isolation is, in any case, beyond doubt. Which of these isolating mechanisms is more important is an interesting problem but only speculation is now possible. Sexual isolation is, adaptively, a more economical method than hybrid sterility, since it avoids the production of useless, sterile progeny. It is nevertheless likely that the peculiar chromosomal-cytoplasmic mechanism of sterility arose first, in the allopatric subspecies becoming adapted to different environments in their respective geographic areas. Sexual isolation would then be built by natural selection as a reinforcement of the bar to gene exchange between the subspecies. When this isolation became strong or complete, the subspecies, or incipient species, became able to coexist sympatrically, as they have been found to do in at least three different localities (DOBZHANSKY and SPASSKY 1959).

SUMMARY

The subspecies (or races, or incipient species) of *Drosophila paulistorum* show a pronounced sexual isolation. Matings between the subspecies succeed much less frequently than do those within a subspecies. The genetic basis of the sexual isolation has been studied in the hybrids between the Centro-American, Amazonian, and Andean-South Brazilian subspecies. Crosses have been made in which the distribution of a certain pair of chromosomes was followed with the aid of mutant genes which served as genetic markers. The sexual preferences were studied in the F_1 hybrids between the subspecies, and also in a series of back-crosses to each of the parental subspecies. A technique of direct observation was employed. The evidence thus obtained shows that the sexual isolation is fostered by factors distributed over all the chromosomes. The polygenes controlling the sexual preferences seem to produce additive effects, the sum of which makes the bar to crossing nearly complete between the subspecies. The sterility of the male hybrids between these subspecies has been previously shown to be due to a quite different genetic mechanism (EHRMAN 1960a). It is conjectured that the hybrid sterility is here the older of the two isolating mechanisms, but the sexual isolation is more effective in preventing gene exchange between the natural populations of the subspecies.

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