MATERNALLY INHERITED "SEX-RATIO" CONDITIONS IN *DROSOPHILA WILLISTONI* AND *DROSOPHILA PAULISTORUM*

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MAGNI (1952, 1954) and CAVALCANTI and FALCÃO (1954) have independently discovered strains of *Drosophila bifasciata* and of *Drosophila prosaltans*, respectively, which produce progenies consisting mostly or only of females. These abnormalities are transmitted exclusively from mothers to their daughters. Females from the "sex-ratio" strains produce unisexual, or almost unisexual, progenies when crossed to males from a variety of strains giving normal proportions of the two sexes; at the same time the occasional sons of "sex-ratio" mothers do not differ in their breeding behavior from normal males. The abnormal "sex-ratio" condition is thus transmitted through the female line only, leading to the conclusion that the factor causing this condition is inherited in the cytoplasm. The "sex-ratio" conditions in *D. bifasciata* and *D. prosaltans* are, therefore, completely different from those described and studied much earlier by GERSHENSON (1928), STURTEVANT and DOBZHANSKY (1936), DOBZHANSKY (1939) and others in *D. pseudoobscura*, *D. persimilis*, *D. azteca* and other species. In these latter, the abnormality is transmitted through the X chromosome, in such a way that a "sex-ratio" male produces mostly or only daughters regardless of the genotype of its mates.

The purpose of the present article is to report the existence of maternally inherited "sex-ratio" conditions in two new species, namely in *D. willistoni* and *D. paulistorum*. Interestingly enough, these new cases differ in not unimportant details of their behavior, both from those in *D. bifasciata* and *D. prosaltans*, and from one another. The present paper is concerned with the inheritance of these "sex-ratio" cases in various crosses; the embryological aspects of the situation will be described in a paper jointly with PROFESSOR D. F. POULSON of Yale University. The transmission of the causative agent by injection of the oöplasm of the infected eggs into normal females has been reported in a preliminary communication by MALOGOLOWKIN and POULSON (1957), and it is hoped that a more detailed report will follow.

Origin of the material

Owing to the courtesy of PROFESSORS WILSON STONE and MARSHALL WHEELER of the University of Texas, samples of the population of *willistoni*-like species of *Drosophila* collected by Drs. W. B. HEED and M. WASSERMAN in the West Indies and in Colombia were sent for study to the laboratory of PROFESSOR TH. DOB-

ZHANSKY in New York. The females collected in their natural habitats were placed, after they had been received at Columbia University, in individual culture bottles, and allowed to produce offspring (females collected in nature are usually inseminated). Among numerous females of *D. willistoni* collected at Bath, Jamaica, on February 21–25, 1956, there was one which, when examined by Mr. B. SPASSKY, proved to have produced several dozen daughters and no sons (no exact count has been made). Mr. SPASSKY crossed some of these daughters to males from the laboratory strain containing the sex-linked mutants eosin (w^e), singed (sn), yellow (γ), Incomplete (Inc), and roughoid (ru). The next generation consisted of numerous females and some few males. Mr. SPASSKY continued the backcrossing of the females from the “sex-ratio” stock to $w^e sn \gamma Inc ru$ males for four more generations until November, 1956, when he most generously passed it to the present writer for study. The strain consisted exclusively of females, and from then on it has been maintained by crossing its females to $w^e sn \gamma Inc ru$ males from the mutant stock (which, of course, showed a normal sex ratio). The “sex-ratio” strain soon became, and continued to be, homozygous for the mutants indicated above.

A sample of *willistoni*-like flies was collected on September 6, 1956, on the southern exposure of Sierra Nevada de Santa Marta, in Colombia. On October 12, 1956, Mr. B. SPASSKY found that one of the females, which belonged to the species of *D. paulistorum* (a close relative of *D. willistoni*), had produced a progeny of 26 females and no males. Mr. SPASSKY very generously handed this culture to the present writer for study. A part of the females were crossed to males from another strain of *D. paulistorum* derived from the same locality. A strain of the “sex-ratio” was established from these crosses.

The “sex-ratio” strains of both species are maintained in the laboratory at room temperatures by outcrossing in every generation some of the “sex-ratio” females to $w^e sn \gamma Inc ru$ males (in the case of *D. willistoni*), or to males from the strain Santa Marta No. 6 (in the case of *D. paulistorum*).

RESULTS

Inheritance of “sex-ratio” in D. willistoni in crosses to different strains of this species

Females from the “sex-ratio” strain have been crossed and backcrossed to males of several other strains from the collection maintained at Columbia University. Usually groups of 4–5 females from the “sex-ratio” strains were mated to equal numbers of males of the kinds chosen for this purpose. Complete counts of the progenies were made. Most of them are summarized in Tables 1 and 2.

In the first place, the “sex-ratio” strain was outcrossed to mutant strains which contained genetic markers known to be located in each of the three pairs of the chromosomes which the species possesses (SPASSKY and DOBZHANSKY 1950). One of the purposes of these crosses was to replace the chromosomes of the original Jamaican “sex-ratio” strain by chromosomes of other origins. The results are shown in Table 1. Later on, similar crosses and backcrosses were made to some

TABLE 1

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males from certain mutant strains

Genera- tion	Flies counted	Percent males	Genera- tion	Flies counted	Percent males	Genera- tion	Flies counted	Percent males
<i>w^e sn γ Inc ru</i> males			<i>w^e sn γ Inc ru</i> males			<i>abb bw</i> males		
5	500	0	19	346	0	2B	82	0
6	793	0	20	286	0	3B	281	30.6
7	617	0	21	146	0	pink males		
8	606	0	22	367	0	1A	2667	0
9	348	2.8	23	169	0	2A	2438	0
10	285	0	24	166	0	3A	439	0
11	692	0	<i>abb bw</i> males			..		..
12	742	0	1A	1699	0	1B	832	0
13	206	0	2A	1243	1.8	2B	428	0
14	338	0	3A	397	0.1	3B	140	0.7
15	90	0	4A	474	15.4	4B	265	0.3
16	285	0	5A	1249	42.6	ebony males		
17	102	0	..		..	1A	216	0
18	56	0	1B	415	0	2A	1341	0
						3A	479	0
						4A	217	0

TABLE 2

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males from certain wild strains

Genera- tion	Flies counted	Percent males	Genera- tion	Flies counted	Percent males	Genera- tion	Flies counted	Percent males
Barbados-5 males			Jamaica-9 males			Recife-3 males		
1A	381	0	1B	421	0	1A	1333	0.2
2A	43	0	2B	1713	0	2A	1067	3.3
3A	28	0	3B	930	0	3A	563	20.0
1B	207	0	4B	416	13.5	4A	108	44.4
2B	4	0	St. Kitts males			5A	490	51.4
3B	223	0	1A	477	0	1B	499	0
Costa Rica-2 males			2A	594	0	2B	1385	11.7
1A	781	0	3A	489	2.2	3B	838	20.0
2A	403	0	4A	974	1.0	Recife-6 males		
3A	201	0.5	1B	332	0	1A	196	0
4A	967	0	2B	1751	0.05	2A	992	3.3
1B	577	0	3B	1293	0	3A	90	22.2
2B	1036	0	4B	276	0	1B	318	0
3B	1045	0	St. Lucia males			2B	1206	17.5
4B	342	0	1A	520	0	3B	758	36.5
Jamaica-9 males			2A	387	0	Recife-8 males		
1A	164	0	3A	267	10.5	1A	662	0
2A	690	0	4A	1002	1.9	2A	927	0
3A	517	0.2	1B	227	0.4	3A	432	9.7
4A	1573	0.06	2B	740	6.8	4A	1017	0.1
			3B	105	4.7			

strains of diverse geographic origins which contained no obvious mutant genes. These data are summarized in Table 2.

Backcrosses to *w^e sn γ Inc ru* males were made and counted for 24 generations (in the first four of them they were made but not counted by Mr. B. SPASSKY). As shown in Table 1, the entire progenies consisted exclusively of females, except in the ninth generation in which one of the two cultures made produced 148 females and 10 males. In the tenth generation, when all the flies in the cultures showed all the sex-linked mutant genes indicated above, some females were crossed to males containing the second chromosome mutants Star (*S*), Hooked (*Hk*), abbreviated (*abb*), and brown (*bw*). This cross, denoted in Table 1 as generation 1A, produced, in three cultures, 1,699 females and no males. Star Hooked females were next selected and crossed to *abb bw* males; the progeny, generation 2A in Table 1, consisted of 1,243 flies among which 23 were males. Three further backcrosses, 3A to 5A in Table 1, resulted in a breakdown of the "sex-ratio" condition. In the generation 5A, three cultures produced each only slight excesses of females over males. To insure that this breakdown of the "sex-ratio" condition was not accidental, the experiment was repeated by making new crosses of females from the *w^e sn γ Inc ru* "sex-ratio" stock to *S Hk abb bw* males, and backcrosses to *abb bw* males. The results are summarized in Table 1 under the designation of generation 1B to 3B. The second backcross again resulted in production of 30.6 percent of males in the cultures.

In the 11th generation of the *w^e sn γ Inc ru* "sex-ratio" lines, some females were crossed to males from the stock containing the mutants Delta and pink (third chromosome). The *F*₁ females showing Delta were crossed to pink males for two generations (1A to 3A in Table 1). A repetition of the backcrosses for four generations (1B to 4B) was made later. Except for single males in the 3B and 4B generations, the progenies consisted exclusively of females. The "sex-ratio" strain was also crossed and backcrossed for four generations to the mutant ebony (third chromosome). The results were unisexual female progenies (Table 1).

Table 2 summarizes the results of crossing and backcrossing females from the *w^e sn γ Inc ru* "sex-ratio" stock to males from 8 wild stocks the progenitors of which were collected on the islands of Barbados, St. Kitts, St. Lucia, Jamaica, and also in Costa Rica and at Recife, Brazil. Each set of experiments was repeated twice, except for that with the strain Recife-8. The two series are reported in Table 2 with letters A and B respectively after the number of the generation of the cross.

The results fall into three fairly noticeably distinct classes. To the first of these classes belong the crosses to Barbados and Costa Rica. In these crosses the unisexual progenies are maintained intact with rare exceptions (three males in the generation 3A from the backcross to Costa Rica). The second class are the crosses to the strains from Jamaica, St. Kitts, St. Lucia, and Recife-8. Here the progenies of the first cross and backcross to the respective geographic lines usually consist of females only, while further backcrosses produce some males, but still nothing approaching the normal 1:1 sex ratio. Finally, the third class consists of the

strains Recife-3 and Recife-6. Crossing "sex-ratio" females to males from these strains results usually in unisexual progenies in the F_1 generation, the first backcross gives some males in every culture, while from the second backcross on, the normal sex ratio is approached in most cultures.

Nontransmission of the "sex-ratio" condition through the males

Whenever the cultures of the "sex-ratio" lines first produced a few males among numerous female sibs, attempts were made to cross these males to their sisters or to unrelated strains giving normal sex ratios, to see whether such males may transmit to their offspring a tendency to produce higher proportions of males. Outcrosses to unrelated females gave completely negative results; in seven series of such outcrosses a total of 1,329 females and 1,331 males were produced. This is obviously a normal proportion of the sexes.

The outcrosses of the males sporadically occurring in "sex-ratio" cultures to their sisters gave results summarized in Table 3. Examination of these results shows no consistent influence of the male on the sex ratio in its progeny. Thus, the ninth generation of the *w^e sn y Inc ru* "sex-ratio" line was the only one which happened to give some males (see above). These males crossed to their sisters produced unisexual progenies (a total of 285 females and no males, Table 3). On the contrary, males from the outcrosses to *abb bw*, St. Lucia, and Recife strains gave, when outcrossed to their sisters, progenies which did include males. However, comparison of the data in Table 3 with those in Table 2 shows that these results do not differ from those obtained when other sisters of the same males were mated to unrelated males from certain strains.

Lacks of temperature effect

MAGNI (1954) discovered that the "sex-ratio" condition in *D. bifasciata*, which is inherited in a manner similar to that described here for *D. willistoni*, can be "cured" by exposure of the flies to temperatures higher than optimal for the

TABLE 3

Progenies of females from "sex-ratio" strains which have produced some males, outcrossed to their brothers

Strain males	Generation	Flies counted	Males percent
<i>w^e sy y Inc ru</i>	9th (see Table 1)	285	0
<i>abb bw</i>	2A (see Table 2)	230	43.5
<i>abb bw</i>	3A (see Table 2)	289	2.8
<i>abb bw</i>	4A (see Table 2)	635	47.2
pink	F_1 (see Table 1)	60	0
St. Kitts	3A (see Table 2)	59	0
St. Lucia	3A (see Table 2)	284	37.1
Recife-3	2A (see Table 2)	195	31.3
Recife-3	2B (see Table 2)	425	0
Recife-6	2A (see Table 2)	375	44.8

species. In the case of *D. bifasciata*, MAGNI obtained only a few males from eggs deposited at 21° C, but normal sex ratios were approached when ovipositing females were exposed for several days to 26° C. Such thermic "cures" have also been observed for another cytoplasmically inherited character, sensitivity to carbon dioxide, in *D. melanogaster* (L'HERITIER 1951). Experiments have therefore been devised to test the effects of high temperatures on the "sex-ratio" in *D. willistoni*.

Although being a species native in the tropical zone of the Western Hemisphere, *D. willistoni* does not easily tolerate under laboratory conditions a temperature of 28° C for several generations. The experiments were therefore arranged as follows. "Sex-ratio" females from the *w^e sn γ Inc ru* "sex-ratio" strain developed at room temperatures (about 22° C) were crossed to males from other strains, and the cultures were placed for five days in an incubator at 25° C. The eggs deposited in these cultures were allowed to develop at the same temperature, and the distribution of the sexes among the adult flies which hatched is reported in the upper line "Generation P, 25° C" in Table 4. The parents were next transferred to fresh culture bottles which were placed for a 5-day period in an incubator at 28° C. The progeny hatching from the eggs deposited at that temperature developed also at 28°; the results are presented in the line "Generation P, 28° C" in Table 4. Finally, the surviving parents were transferred once more to fresh cultures, returned to 25° for another 5-day period, and the progeny allowed to develop at the same temperature at which the oviposition occurred. The results are found in the lower line "Generation P, 25° C" in Table 4.

The flies developed at a given temperature were then allowed to mate and to oviposit at that same temperature for three generations. The results are reported

TABLE 4

Numbers of flies counted and percent of males in crosses and backcrosses of "sex-ratio" females to males of three groups of strains carried out at different temperatures.
(Further explanation in text)

Generation	t° C	First group		Second group		Third group	
		Flies counted	Percent males	Flies counted	Percent males	Flies counted	Percent males
P	25°	1447	0.2	3598	0.3	1380	0.6
	28°	771	1.2	845	0.7	329	0
	25°	1186	0	1151	0	463	9.5
F ₁	25°	3750	0	5692	1.1	3962	10.5
	28°	1402	2.8	1702	5.3	3916	8.4
F ₂	25°	1631	0	2353	0.04	2789	23.4
	28°	1132	0	860	0.1	4204	10.5
	25°		..		..	2583	6.1
F ₃	25°	970	0	957	5.9		..
	28°	400	0	440	0	608	5.0

in Table 4 in the lines marked F_1 , F_2 , and F_3 , 25° and 28°. However, in the F_2 and the F_3 , 28° lines became so weak that their loss was threatened; for this reason, the progeny from the eggs deposited at 28° was allowed to develop to maturity at 25°.

In Table 4, the crosses are divided in three groups, corresponding to the behavior of the respective strains in the experiments carried out at room temperatures and reported in Tables 1 and 2 (see above). The data in Table 4 should, hence, be compared with those in Tables 1 and 2. The first group (crosses to *w^e sn y Inc ru*, to pink, ebony, Barbados, and to Costa Rica) has produced almost pure female progenies, except for scattered males in several cultures of crosses to pink, Barbados, and Costa Rica at 28°. The F_1 generation also produced a few males at 28°, but none appeared in F_2 or F_3 .

To the second group, belong the crosses to strains from Jamaica, St. Kitts, St. Lucia, and Recife-8. In these crosses (Table 4) the progenies consisted predominantly of females except for about five percent males in the F_1 generation at 28°, and about six percent males in the F_3 generation at 25°. Finally, the third group contains the crosses to *abb bw*, Recife-3, and Recife-6. The progeny of the first outcross (P) which was produced at 25° and at 28° consisted, as usual, of females and only a scattering of males, but the outcross to *abb bw* gave 26 females and 29 males when returned to 25° from 28°. The outcross to Recife-3 gave 195 females and 15 males, while the outcross to Recife-6 produced only females. In the backcrosses males appeared in most cultures both at 25° and at 28°, and no consistent relationship with temperatures at which the flies developed was noticeable.

Reference to Tables 1 and 2 will show that the three groups of strains behaved differently also in the crosses to the "sex-ratio" stock carried out at room temperatures. The third group of strains yielded numerous males, or even normal proportions of the two sexes beginning in the second backcross. There is, hence, no consistent difference between the crosses at room temperature, at 25° and at 28°. No thermal "cure" of the "sex-ratio" condition is observed in *D. willistoni*, although the possibility is not excluded that the scattered males which occur in the "sex-ratio" stock are slightly more frequent at 28°.

Permanent loss of "sex-ratio"

At whatever temperature a strain of "sex-ratio" females crossed to males from strains of the third group (see above) gives normal proportions of sexes, the condition becomes permanent from then on. Females from such strains were outcrossed to a variety of males and their progenies showed no restoration of the capacity to produce unisexual progenies.

"Sex-ratio" in Drosophila paulistorum

The "sex-ratio" condition in *D. paulistorum*, although inherited in a manner generally similar to that in *D. willistoni*, *D. prosaltans*, and *D. bifasciata*, has certain characteristics which are not found in the other species. As shown above,

the "sex-ratio" in *D. willistoni* has a very nearly absolute character, producing almost completely unisexual progenies, unless outcrossed to strains of a certain group which evidently contain genes which disrupt the transmission of the "sex-ratio" causative agent. This is not the case in *D. paulistorum*.

Fourteen females were taken from the culture of the "sex-ratio" of *D. paulistorum* obtained from MR. B. SPASSKY and outcrossed in individual cultures to single males from the strains Santa Marta-144 and Santa Marta-185. These strains came, then, from wild progenitors collected in the same natural locality as the "sex-ratio" strain itself. Table 5 shows the results. Nine of the females produced progenies consisting of females only, while five females gave approximately equal numbers of females and males.

TABLE 5

Progenies of single females from the original "sex-ratio" cultures of Drosophila paulistorum crossed to males from a normal strain

Culture	Total number flies	Percent males	Culture	Total number flies	Percent males
I	67	0	VIII	134	0
II	65	0	IX	98	0
III	48	0	X	152	0
IV	104	0	XI	272	48.5
V	12	41.6	XII	132	50.0
VI	58	50.0	XIII	110	0
VII	61	49.1	XIV	9	0

The cultures "sex-ratio" I, II, III, and VI were chosen for further study (cf. Table 5). In each generation, single females were taken at random from the lines I, II, III, and VI, and were mated with single males from the strains VI and XI. As can be seen in Table 5, the strains I, II, and III originally produced strictly unisexual progenies, while VI and XI gave approximately equal numbers of females and males. The results are summarized in Table 6. The lines I and II have continued for at least 12 generations (and are still continuing at the date of this writing) to produce predominantly unisexual progenies but with a scattering of males. These lines are used to perpetuate the "sex-ratio" stock. It is worth noting that the males do not appear in the progeny of just a few pair matings, but occur rather uniformly in all matings (compare the columns "Pairs mated" and "Matings producing males" in Table 6). Females from line III were outcrossed to males from the original culture, and produced 96 females and no males in 4 pair matings (generation 2, Table 6). From this generation on, females of line III were crossed to males from a strain of a recessive brown-like eye color mutant. This mutant was sent to the laboratory at Columbia University by DR. A. B. DA CUNHA of the University of São Paulo, but the geographic origin of the strain in which it has arisen has been lost. This outcrossing resulted in a rapid increase of the proportion of males in the cultures (generations 3-6, Table 6), and eventual

TABLE 6

Outcrosses of different "sex-ratio" lines of Drosophila paulistorum to males from the strains VI and XI

Generation	Flies counted	Percent males	Pairs mated	Matings producing males
"Sex-ratio" I				
2	709	0	9	0
3	779	3.8	15	3
4	419	0	10	0
5	98	3.1	4	1
6	724	0.9	15	3
7	405	0	10	0
8	1371	0.9	15	4
9	764	0.9	8	3
10	878	1.4	16	2
11	256	0	7	0
12	502	0.7	7	3
"Sex-ratio" II				
2	74	0	3	0
3	767	3.1	9	4
4	68	0	3	0
5	812	0.1	16	1
6	119	0	6	0
7	1302	9.4	20	14
8	686	0.1	8	1
9	486	0.6	16	3
10	102	0	5	0
11	323	0	5	0
"Sex-ratio" III				
2	96	0	4	0
3	314	36.3	3	3
4	300	10.7	4	4
5	599	24.0	9	9
6	724	43.5	12	12
7	745	38.1	14	14
8	338	45.4	7	7
9	1089	40.0	14	14
10	338	45.2	7	7
11	1166	37.6	10	10
"Sex-ratio" VI				
2	1121	46.5	14	14
3	600	44.6	8	8
4	1431	45.2	18	18
5	587	44.4	12	12

disappearance of the "sex-ratio" condition. Finally, females from line VI, crossed to their brothers, produced a strain with normal proportions of the two sexes.

Table 7 gives the records of the results of outcrossing females from the strain I to males from a strain from Bucaramanga, Colombia, from Costa Rica, from Içana, Brazil, and from the same strain of the brown-like mutant which has been mentioned above. In all these crosses the "sex-ratio" condition has been lost within several generations, except that the cross to Içana males has produced a sterile F_1 progeny.

The "sex-ratio" condition in *D. paulistorum* seems, thus, much less stable than in *D. willistoni*. At any rate, most of the strains of *D. paulistorum* to which the "sex-ratio" has been outcrossed contained genetic factors which acted unfavorably with respect to the regular transmission of the causative agent of "sex-ratio."

DISCUSSION

In recent years geneticists have become acquainted with several instances of inheritance through the cytoplasm. Such inheritance occurs among animals as well as among plants, among lower as well as among higher organisms. It is instructive to compare the known properties of the "sex-ratio" in *Drosophila willistoni* and *Drosophila paulistorum* with these other instances of cytoplasmic inheritance.

Perhaps the closest analogy is found in the "killer" character described and analyzed in *Paramecium aurelia* by SONNEBORN (1951, 1954) and PREER (1948). This character depends upon the presence of self-reproducing particles ("kappa") in the cytoplasm of the infusoria. However, the "kappa" particles can multiply

TABLE 7
Outcrosses of "sex-ratio" I females to males from unrelated strains

Generation	Flies counted	Percent males	Pairs mated	Matings producing males
Bucaramanga ♂ ♂				
1	721	16.5	9	7
2	1339	31.5	22	21
3	661	45.5	9	9
Mutant ♂ ♂				
1	84	2.4	3	1
2	70	49.9	1	1
3	2028	50.7	10	10
Costa Rica ♂ ♂				
1	14	0	1	0
2	682	1.7	8	2
3	816	0.9	9	3
4	909	35.5	6	6
Içana ♂ ♂				
1	149	0	3	0

successfully only in animals which carry alleles of a certain gene, *K*, in their nuclei. Animals which lack this gene lose kappa within a certain number of cell divisions, but the presence of *K* is not itself sufficient to initiate the production of kappa in the cells which have not inherited these particles. In our "sex-ratio" strains the original progenitors evidently carried nuclear genes favorable for the reproduction of the cytoplasmic factor responsible for the death of the male zygotes, analogous to the gene *K* in *Paramecium*. On the other hand, the strains *abb bw*, Recife-3, and Recife-6 in *D. willistoni*, as well as the eye-color mutant strain and the Bucaramanga and Costa Rica strains in *D. paulistorum*, carry genes analogous to *k* in *Paramecium*, which suppress the reproduction of the cytoplasmic factors responsible for the "sex-ratio."

The admirable studies of L 'HÉRITIER and SIGOT (1946), L 'HÉRITIER and SCOEUX (1947), and L 'HÉRITIER (1951, 1955) have clarified the details of a most interesting case of cytoplasmic inheritance in *Drosophila melanogaster*, that of the sensitivity of the flies to poisoning by carbon dioxide. This character depends upon the presence in the cytoplasm of an "integrated virus" (virus intégré), which, however, can be transmitted not only through the egg cytoplasm but also, though less efficiently, through male sex cells. This last capacity is apparently not shared by the "sex-ratio" factors. On the other hand, the virus which induces the sensitivity to carbon dioxide can be transmitted from flies to flies not only through the sex cells but also, in experiments, by injection of hemolymph and by implantation of organs of the sensitive strain. It has been shown (MALOGOLOWKIN and POULSON 1957, and unpublished data), the causative agent of the "sex-ratio" can also be transmitted to previously uninfected strains by injection of the oöplasm of dying male eggs into normal females. The other property of the virus studied by L 'HÉRITIER and his collaborators, namely its low resistance to treatments with high temperatures, is not shared by our "sex-ratio" factors.

Other cases resembling the ones described in the present article are, as already stated in the introduction, those of the "sex-ratio" in *Drosophila bifasciata* (MAGNI 1952, 1954), in *D. prosaltans* (CAVALCANTI and FALCÃO 1954, and CAVALCANTI, FALCÃO and CASTRO 1957), and in *D. borealis* (CARSON 1956). However, MAGNI was able to effect a "cure" of the "sex-ratio" by high temperatures, while CAVALCANTI and FALCÃO found strains containing nuclear genes which disrupt the transmission of the "sex-ratio" particles. A further analogue is the character "extra setae on the forelegs" of the mite *Tetranychus cinnabarrinus* (Boudreaux, a report read before the Entomological Society of America in December 1956, which its author kindly permits us to mention here). MITCHELL and MITCHELL (1956) described a most suggestive case in the fungus, *Neurospora crassa*. Here a trait, "poky", is inherited through the cytoplasm, but its phenotypic manifestation is suppressed by a nuclear gene, *F*, which does not, however, destroy the cytoplasmic particle. The "sex-ratio" strains in *D. willistoni* and *D. paulistorum* show, therefore, an interesting combination of properties which are encountered separately in several other known cases of cytoplasmic inheritance.

SUMMARY

1. A female of *Drosophila willistoni* which produced a unisexual progeny consisting exclusively of daughters was found by Mr. SPASSKY among 137 females of the same species collected on the island of Jamaica by Dr. W. B. HEED. Similarly, Mr. SPASSKY noticed that one of the 85 females of *D. paulistorum*, collected by Dr. HEED on the Sierra Nevada de Santa Marta, Colombia, produced a progeny consisting mostly of daughters and only few sons. These two females have been the progenitors of the "sex-ratio" strains of the two species, which are characterized by the production of unisexual, or nearly unisexual, female progenies.

2. The "sex-ratio" condition is inherited strictly through the female line. Females from the "sex-ratio" strains produce only or mostly daughters, regardless (with the exception noted below) of the genetic nature of the males to which they are crossed. The occasional sons which appear in the progenies of "sex-ratio" females do not differ from sons of normal females in their influence on the proportions of the sexes in their offspring.

3. The inheritance of the "sex-ratio" is due to the transmission of a causative agent through the cytoplasm of the egg. The "sex-ratio" condition is retained even when all the chromosomes of the original "sex-ratio" strain are replaced by their homologues from lines giving normal progenies. This causative agent acts by making the eggs fertilized by the Y-bearing sperms lethal.

4. The cytoplasmic factor causing the "sex-ratio" condition is, nevertheless, not independent from chromosomal genes. This is evidenced by the fact that some wild and mutant strains induce reversions to normal proportions of the sexes in the crosses to "sex-ratio" strains. However, a loss of the "sex-ratio" is irreversible; females from the progenies of flies containing about equal numbers of daughters and sons breed like normal females.

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