# ON THE ETIOLOGY OF THE STERILITY OF HYBRIDS BETWEEN CERTAIN STRAINS OF *DROSOPHILA PAULISTORUM*

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DOBZHANSKY and SPASSKY (1959) and DOBZHANSKY and PAVLOVSKY (1966, 1967) discovered that *Drosophila paulistorum* DOBZHANSKY and PAVAN is a superspecies of five incipient species (or semispecies, to use the term proposed by AMADON, 1966). These are the Centroamerican, Amazonian, Orinocan, Andean-South Brazilian, and Transitional semispecies. Recently a sixth semispecies, the Interior, has been encountered in southern Colombia (DOBZHANSKY *et al.*, unpublished). A very closely related form, now considered a separate species, is *Drosophila pavlovskiana* KASTRITSIS and DOBZHANSKY (1966). Excepting the Transitional, crosses of representatives of different semispecies produce either no progeny because of a strong ethological isolation, or produce progenies consisting of fertile female and completely sterile male hybrids. The Transitional semispecies usually gives fertile hybrids of both sexes with at least one other semispecies, particularly with Centroamerican. The hybrid sterility in crosses of the Transitional with other semispecies appears often to be incomplete, so that the same two strains may give some sterile and some fertile hybrids.

Still other complications are known to occur in certain crosses: the Santa Marta and Mesitas (Transitional) strains give male hybrids the sterility of which appears to be due to an interaction of Santa Marta cytoplasm with Mesitas *Y* chromosome; the reciprocal cross gives sometimes fertile and sometimes sterile hybrid males. EHRMAN and WILLIAMSON (1965) and WILLIAMSON and EHRMAN (1967) showed that the hybrid male sterility is transferable to nonhybrid sons of Mesitas females injected with homogenates of sterile hybrid males and crossed to pure Mesitas males. This induced sterility is, however, not transmitted by the daughters of the injected females to males of the backcross generations, as the sterility of the hybrids between semispecies normally is. DOBZHANSKY and PAVLOVSKY (1966, 1967) observed an interesting change in the properties of a strain from Llanos of Colombia. When collected and brought into the laboratory in 1958, this strain behaved as a member of the Orinocan semispecies, and produced fertile male hybrids with other Orinocan strains. By 1963 the Llanos strain was found to be changed, so that it produced no fertile male hybrids with any other strains then available in the laboratory. The new Llanos did, however, produce fertile hy-

DEDICATION—Dedicated to our favorite septuagenarian, Professor THEODOSIUS DOBZHANSKY, on the occasion of his seventieth birthday.

brids with a strain from Marco, Brazil, sent to our laboratory by Professor H. CARSON in 1964. We now know that Marco is a representative of the recently discovered Interior semispecies. This does not mean, however, that the Llanos strain underwent a transformation in the laboratory from the Orinocan to the Interior semispecies. Indeed, while the new Llanos strain showed no sexual isolation from Orinocan strains even after its change, Marco is isolated from the Orinocan (DOBZHANSKY and PAVLOVSKY 1967, and unpublished data).

The relationships between the New Llanos, Marco, and Orinocan strains present many challenging problems (*loc. cit.*). For instance, some experiments with cross injections of supernatants of ground flies of these strains have been described in a preliminary communication of WILLIAMSON and EHRMAN (1968). We wish now to report the results of more extensive experiments, which were performed with the flies to be tested coded (by D.L.W), so that the person making the fertility-sterility tests (L. E.) did not know what was injected into their female parents.

## MATERIALS AND METHODS

Three strains of *Drosophila paulistorum* were used: (1) New Llanos (No. 13A). Formerly behaving as a member of the Orinocan semispecies, but now giving sterile male hybrids with Orinocan but fertile with Interior strains. This strain carries a sex-linked recessive mutant, *rough eye.* (2) Marco, Amazonas, Brazil (No. 107), a member of the Interior semispecies. Marco produces sterile male hybrids with (3). (3) Georgetown, Guiana (No. 45F), a member of the Orinocan semispecies. This strain carries a sex-linked recessive mutation, *yellow eye* (possibly a member of the *white* series).

The names of these strains will be abbreviated below as Ll, Ma, and Or, respectively.

## RESULTS

*The tests and the control experiments:* The gist of the experiments is injections into females of the Ll, Ma and Or strains of supernatants of ground male hybrid flies between these strains, followed by tests of fertility of the sons of the injected mothers. Control experiments consisted of fertility tests of sons of the females of the respective strains injected with supernatants of ground males of the same strains.

The preparations for, the injection techniques, and the post-injection procedures have been described by EHRMAN and WILLIAMSON 1965, and WILLIAMSON and EHRMAN 1967, 1968. In order to allow for coding, the injections were performed at Woman's Medical College in Philadelphia, and all post-injection experiments were done in New York City at The Rockefeller University. There were 25 coded lines in all, as indicated in Table 1. Recipients of injected materials were always females, since our previous experiments have shown that no sterility is induced by injecting males. An average of a dozen virgin females was injected to initiate each of the 25 tests reported in Table 1. Lots of 100 $F_1$ males from each of the six crosses, plus equal numbers of males from each of the three strains used for making the crosses, were homogenized separately at 4°C in 0.5 ml 0.25 M sucrose, Tris buffered at pH 7.2. The crude homogenates were centrifuged at 3,000 rpm for 15 min at 4°C and the supernatants from each transferred to sterile tubes and kept at 4°C. All females to be injected were collected as virgins and were one to six days old at the time the injections were performed. Each group of females was lightly etherized and injected with the appropriate supernatant, using proboscis extension as the criterion of volume (0.2 — 0.3 $\mu$l).

TABLE 1

*Male sterility in five single matings (1 ♂ × 3 ♀ ♀) per brood, in the male progeny of injected* Drosophila paulistorum *females Ll = Llanos; Ma = Marco; Or = Orinocan.* The total number of males individually tested for fertility = 947.

| | Recipient | Donor | Total broods | Percent male sterility in broods: 1–3 | 4–6 | 7–9 | 10–12 | 12 | Overall percentage |
|---|---|---|---|---|---|---|---|---|---|
| 1. | Ll | Ll | 14 | 6.7 | 6.7 | 6.7 | 13.0 | 0.0 | 7.4 |
| 2. | Ma | Ma | 7 | 6.7 | 6.7 | 0.0 | .. | .. | 6.5 |
| 3. | Or | Or | 6 | 6.7 | 0.0 | .. | .. | .. | 3.3 |
| 4. | Ll | (Ll × Ma) | 11 | 40.0 | 50.0 | 26.7 | 30.0 | .. | 37.0 |
| 5. | Ll | (Ma × Ll) | 7 | 61.5 | 33.3 | 60.0 | .. | .. | 48.5 |
| 6. | Ll | (Ll × Or) | 11 | 46.7 | 69.2 | 46.7 | 80.0 | .. | 58.5 |
| 7. | Ll | (Or × Ll) | 8 | 15.4 | 66.7 | 11.1 | .. | .. | 35.0 |
| 8. | Ll | (Ma × Or) | 11 | 13.3 | 60.0 | 46.7 | 20.0 | .. | 36.4 |
| 9. | Ll | (Or × Ma) | 13 | 53.3 | 60.0 | 53.3 | 33.3 | 0.0 | 49.2 |
| 10. | Ma | (Ll × Ma) | 6 | 27.3 | 60.0 | .. | .. | .. | 46.2 |
| 11. | Ma | (Ma × Ll) | 3 | 46.7 | .. | .. | .. | .. | 46.7 |
| 12. | Ma | (Ll × Or) | 1 | 0.0 | .. | .. | .. | .. | 0.0 |
| 12a. | Ma | (Ll × Or) | 3 | 40.0 | .. | .. | .. | .. | 33.3 |
| 12b. | Ma | (Ll × Or) | 13 | 75.0 | 53.3 | 60.0 | 40.0 | 60.0 | 58.5 |
| 13. | Ma | (Or × Ll) | 13 | 69.2 | 66.7 | 50.0 | 26.7 | 0.0 | 50.0 |
| 14. | Ma | (Ma × Or) | 10 | 80.0 | 60.0 | 60.0 | 50.0 | .. | 66.0 |
| 15. | Ma | (Or × Ma) | 4 | 73.3 | 100.0 | .. | .. | .. | 80.0 |
| 15a. | Ma | (Or × Ma) | 11 | 53.3 | 60.0 | 53.3 | 71.4 | .. | 57.7 |
| 16. | Or | (Ll × Ma) | 10 | 13.3 | 50.0 | 42.9 | 66.7 | .. | 34.1 |
| 17. | Or | (Ma × Ll) | 9 | 0.1 | 36.4 | 54.5 | .. | .. | 29.7 |
| 18. | Or | (Ll × Or) | 9 | 0.0 | 53.3 | 58.3 | .. | .. | 35.7 |
| 19. | Or | (Or × Ll) | 9 | 0.0 | 0.0 | 44.4 | .. | .. | 10.3 |
| 19a. | Or | (Or × Ll) | 5 | 66.7 | 30.0 | .. | .. | .. | 52.0 |
| 20. | Or | (Ma × Or) | 8 | 23.1 | 46.7 | 0.0 | .. | .. | 28.6 |
| 21. | Or | (Or × Ma) | 11 | 20.0 | 18.2 | 26.7 | 14.3 | .. | 20.8 |

As soon as the injected females had revived, they were placed in food vials and mailed to New York for further handling.

When the injected females arrived at The Rockefeller University they were checked for survival, and then each line was mated *en masse* to appropriately-marked males. These males were taken from the same strains as the recipient females, to insure the genetic nonhybridity of subsequent offspring. The *rough eye* and *yellow eye* mutant markers served as checks of female virginity. Though the Marco strain has no such marker, its wild type sufficed here since pure Marco offspring were the only wild types in these experiments. At the time of crossing to males, the injected females were only about three days old. This is why several broods were obtainable from these mothers; see the third column in Table 1. Since this crossing was done at The Rockefeller University, the strains to which the recipient females belonged, but not the kind of material injected into them, were known.

A brood spanned 3½ to 4 days. At the end of such a period, a new brood was begun until the aged females ceased to deposit eggs or deposited eggs which did not hatch at all. Five males per brood were tested for fertility; each male was confined with three virgin sisters the day they eclosed or one day after eclosion. These flies were first placed in vials, and two days afterward transferred to half pint culture bottles. For four days after such crosses were made, any dead females were replaced. The additional virgin females were obtained from stock bottles. At the very most, only one "stock female" was assigned to any given male, the other two were then always his sisters. Sterile *D. paulistorum* males court and mate as do fertile ones (EHRMAN 1961).

The half pint culture bottles containing one male and three females were kept for two weeks and checked daily for larvae. The presence of larvae was the criterion of male fertility. Professor DOBZHANSKY and one of us (L. E.) have found in extensive tests, that dissection for motile spermatozoa as the criterion of fertility or sterility is more prone to error than single male tests. Simply, a Drosophila male may produce some apparently normal motile spermatozoa and still be sterile.

*Frequencies of sterile and fertile males in the progenies of injected mothers.* Table 1 summarizes the results of the experiments. The uppermost three lines (rows 1–3) giving the lowest percentage of male sterility were the three control lines. These three sets of females, one from each *D. paulistorum* strain employed, received only homogenates prepared from fertile males of their own strains. All of the other sets of females received homogenates of hybrid male material, and in the second column of Table 1 the crosses are listed. The female parent in the cross providing the $F_1$ males which were homogenized is always listed first. All possible reciprocal crosses and tests were performed. Rows 12a and b, 15a, and 19a represent repeats done later when it became apparent that the first set of injected females was producing either few broods (row 12), or extremely low (row 19) or high (row 15) proportions of sterile sons.

In all of the lines except those indicated in rows 1 to 3 (the controls) and rows 4,5,10,11,16, and 17 (the Llanos-Marco hybrids), sterile hybrid individuals furnished the material injected. Other than the controls, females in the rows 4,6,11,14,19,19a, and 21 received material derived from individuals with the same source of cytoplasm. All others received alien cytoplasm. The derivation of the cytoplasm is important in *Drosophila paulistorum* since this superspecies exhibits maternally-transmitted, though chromosomally controlled, hybrid male sterility (EHRMAN 1960, 1962, 1963).

We may then consider (while excluding the controls, of course) the average induced sterility in the sons of mothers receiving their own cytoplasmic material in homogenates derived from fertile hybrid males, their own cytoplasm in homogenates derived from sterile hybrid males, alien cytoplasm in homogenates derived from fertile males, or alien cytoplasm in homogenates derived from sterile males:

| | | |
|---|---|---|
| A. Own + Fertile | Rows 4 and 11 | 41.9% |
| B. Own + Sterile | Rows 6, 14, 19, 19a, and 21 | 41.5% |
| C. Alien + Fertile | Rows 5, 10, 16, and 17 | 39.6% |
| D. Alien + Sterile | Rows, 7, 8, 9, 12a, 12b, 13, 15, 15a, 18, and 20 | 46.4% |

The four comparisons above exhibit no significant differences from one another, but are significantly different from the controls (row 1, 2, and 3) which average only 5.7% sterility. So, neither the fertility, the sterility, nor the ultimate cytoplasmic source seems to determine the properties of the "infectious" material. Rather, these semispecies are able to tolerate whatever resides in their own cytoplasm; they appear to recognize their own symbionts (EHRMAN 1969). They do not "recognize" infectious material extracted from hybrids, however, and its establishment and maturation in the germinal tissues of recipient females eventually destroys the functional capacities of the reproductive tissues of their sons.

Table 1 shows a tendency toward increasing percentages of sterile sons being produced by older mothers. The percentages recorded in the fourth column for broods 1 through 3 (excluding the controls), are infrequently the highest. In fifteen lines it is not the highest percentage for that line, in four lines it is the highest, and in the three lines (11, 12, and 12a) no decision is possible. Furthermore, in the three lines in which the first recorded percentage of male sterility was the highest, two of these three (rows 5 and 13) have values nearly the same as that achieved in broods hatched later on. Any evaluation of the *final* entry for each line however, must take into account the fact that varying numbers of broods and, therefore, of individually tested males are involved. For instance, lines 4, 6, 8, 15a, and 21 have 11 broods each (see the third column). Therefore, entries for broods 1–3, 4–6, 7–9 each tally the same numbers of tested males and the same number of broods (5 × 3 for each), but the terminal entry, e.g. 20.0% in line 8, is for only the two remaining broods produced. This inequality in the final percentages recorded is also true of lines 1, 2, 5, 7, 9, 12b, 13, 14, 15, 16, 19a, and 20. But there is no way, even given the identical age of all the mothers at the start, to secure the production of precisely the same number of broods by all these females.

## DISCUSSION

Intercrosses between the three strains of *Drosophila paulistorum* utilized in our experiments produce sterile male hybrids in two cases, and fertile hybrids in one case, namely:

Orinocan × Marco = sterile male progeny
Orinocan × Llanos = sterile male progeny
Llanos × Marco = fertile male progeny

Two semispecies are represented here—the Orinocan and the Interior (Marco). The Llanos strain is a special case, since at one time it produced fertile hybrids with Orinocan, while more recently it produces sterile male hybrids with its erstwhile mating partners. Since the Interior semispecies has been discovered only recently, it is known only that the new Llanos strain gives fertile hybrids with it, but not how its ancestors would have behaved if crossed to Interior.

What is remarkable about the experiments reported in the present article is that injection of hybrid material into females of the three strains induces sterility in a fraction of their sons, regardless of whether the hybrids themselves were sterile. The males with induced sterility are, of course, not themselves hybrid in a genetic sense—they are "pure" representatives of their strains. The sterility inducing agent is, however, capable of reaching the eggs of the injected mothers and affecting their male progeny.

The nature of this agent, transmissible by injection, remains conjectural. KERNAGHAN and EHRMAN (1968) and TANDLER, WILLIAMSON, and EHRMAN (1968) have depicted cytoplasmic symbionts in the paragonia of *Drosophila paulistorum* males. These are probably RNA viruses. KING (1968) reports the widespread occurrence of a Rickettsia-like symbiont in *Drosophila melanogaster*. He locates these in, among other places, female germinal tissue. Whether there is a connection between these intracellular inclusions, symbionts or commensals, and the sterility of hybrid males is unknown.

Another approach to the problem of the causation of sterility is through a study of the effects of heat (EHRMAN 1967) and of certain antibiotics (EHRMAN 1969). Neither heat nor antibiotics effect a "cure", in the sense that neither will permanently stop the formation of sterile male hybrids. Yet either treatment permits some fertile hybrids to appear in crosses in which the males are normally sterile.

Taken as a whole, the results reported above strongly suggest that, at least in some instances of sterility in *Drosophila paulistorum*, transmissible symbionts or commensals are somehow implicated. The same suggestion was derived from previous experiments, in which genetically pure Mesitas males were made sterile by injecting their mothers with material derived from Santa Marta × Mesitas hybrids (EHRMAN and WILLIAMSON 1965), and from experiments in which injection of material from Llanos flies into females of various strains resulted in sterility of male progeny or even in death of the recipients (WILLIAMSON and EHRMAN 1967).

The transformation which the Llanos strain underwent in the laboratory between 1958 and 1963 may also have been due to a loss of old symbionts, acquisition of new ones, or to both (DOBZHANSKY and PAVLOVSKY 1967). We have already mentioned above that this transformation involved the unexpected appearance of hybrid sterility in the male progeny of Llanos × Orinocan crosses. The new Llanos strain produces, however, fertile male hybrids with Marco and, as has been learned from as yet unpublished results of experiments of DOBZHANSKY and PAVLOVSKY, with other strains belonging to the Interior semispecies. It is, however, not to be concluded that what happened with the Llanos strain was a transformation of the Orinocan into the Interior semispecies. Although the fertility-sterility relationships might suggest this, the no less significant characteristic of the semispecies, the sexual behavior which leads to a premating ethological isolation between them, did not undergo change at the same time. The new Llanos strain still mates freely with strains of the Orinocan semispecies; its behavioral relationship with the new Interior semispecies is now being investigated.

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## SUMMARY

Three strains of *Drosophila paulistorum*, Llanos, Marco, and Orinocan, have been used in the experiments described in this article. The crosses Llanos × Orinocan and Marco × Orinocan produce sterile male hybrids, while Llanos × Marco give fertile hybrids. Male hybrids between these strains were ground, centrifuged, and the supernatants were injected into Llanos, Marco, and Orinocan nonhybrid females. The injected females were then crossed to males of their own strains, so that the progenies obtained were nonhybrids as far as their genes were

concerned. Nevertheless, a considerable proportion of the sons of the injected females was sterile. A control experiment, injecting material from males into females of the same strains, produced no sterility. These results support the hypothesis, suggested also by other data, that the different semispecies of the *D. paulistorum* complex may be carriers of different commensal or symbiont associates, and that these associates may under some circumstances make these carriers sterile.

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