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Induction of Sterility in *Drosophila paulistorum*: Effect of Cytoplasmic Factors

(electron microscopy)

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ABSTRACT **Male hybrids from Mesitas and Santa Marta (Colombia) strains of *Drosophila paulistorum* are sterile. Homogenates of males belonging to these strains and from the hybrids were injected into Mesitas or Santa Marta females, which were crossed to males of their own strains, so that their progenies were genetically nonhybrid. Nevertheless, some of the males from these progenies were sterile. Male progenies of females that were injected with homogenates from males that disagreed in the source of cytoplasm with the recipients were sterile. No sterility was induced if the donors and the recipients had similar cytoplasm.**

At least three kinds of male sterility occur in hybrids between strains of *Drosophila paulistorum* (1). F_1 hybrid males are invariably sterile because of their own genetic constitution; however, some or all of the backcross males are sterile because of their mothers' genotypes (2, 3). In a series of publications, we (4–8) have reported a third type of sterility, probably caused by the mother's genotype. Suppose that two strains of *D. paulistorum* denoted as A and B produce only sterile hybrid sons when crossed. Sterile A/B hybrid males are ground, suspensions are centrifuged, and the supernatant is injected into B females, which are subsequently crossed to uninjected B males. The resulting progeny belongs to the nonhybrid B strain; however, the male progeny of the injected females are often sterile. In the present report, we examine the influence of cytoplasmic sources and reproductive condition of donor males on the induction of this "infectious" sterility.

MATERIALS

Crosses between strains of Santa Marta (Transitional semispecies) and Mesitas (Andean semispecies), both from Colombia, South America, have resulted in sterile male progeny since the superspecies *D. paulistorum* was first investigated (9). The reciprocal cross originally produced fertile males, but as of summer 1966, it produced sterile hybrids. The origin and history of each of these two strains have been described (4, 10, 11).

METHODS

The method of injecting *Drosophila* females and testing their male offspring for fertility has been described by Ehrman and Williamson (5, 7). In the present experiments, Mesitas or Santa Marta females were injected with material derived from males of the same strains, or from their hybrids, as indicated in Table 1. About 17 females were injected with male homogenates for each of the eight experiments recorded in Table 1. 100 males were used to prepare each homogenate. However, unlike the previously used procedures, these males were at least 1-month-old before homogenization. More mycoplasma-like intracellular inclusions were seen by electron microscopy in the reproductive tracts of 1-month-old *D. paulistorum* males than in younger ones (12). Each male was tested for fertility by placing it with five virgin females. These males were taken from the fourth, fifth, and sixth broods (3–4 days per brood) of egg-laying females, which were previously crossed to males of their own strains. These were the middle broods that had the highest proportion of males displaying induced sterility (7). Sources of injected materials in the eight homogenates were coded. 15–30 males were individually tested in each of the eight experiments reported in Table 1.

RESULTS

Rows 1 and 5 of Table 1 are the controls, which produced little or no male sterility above the background level that is characteristic of *D. paulistorum* under laboratory conditions. The incidence of sterility in the controls is about the same as in rows 3 and 7. These rows report experiments in which the females were injected with homogenates from hybrid males that came from crosses in which the females belonged to the same strains as the recipient mothers. Thus, the source of the cytoplasm in the hybrid-male donors was the same as in the recipient females. In contrast to this, the results reported in rows 2, 4, 6, and 8 of Table 1 show a much higher incidence of sterility. In all these experiments, the source of the cyto-

TABLE 1. *Percent sterility in male progeny of Mesitas (M) or Santa Marta (S) females injected with homogenates from males of the same strains or their hybrids*

	Mother	Donor	Father	Sterility (%)
1.	M	Fertile M	M	3.3
2.	M	Fertile S	M	80.0
3.	M	Sterile F_1 (M♀ × S♂)	M	6.7
4.	M	Sterile F_1(S♀ × M♂)	M	60.0
5.	S	Fertile S	S	6.7
6.	S	Fertile M	S	53.3
7.	S	Sterile F_1(S♀ × M♂)	S	6.7
8.	S	Sterile F_1(M♀ × S♂)	S	60.0

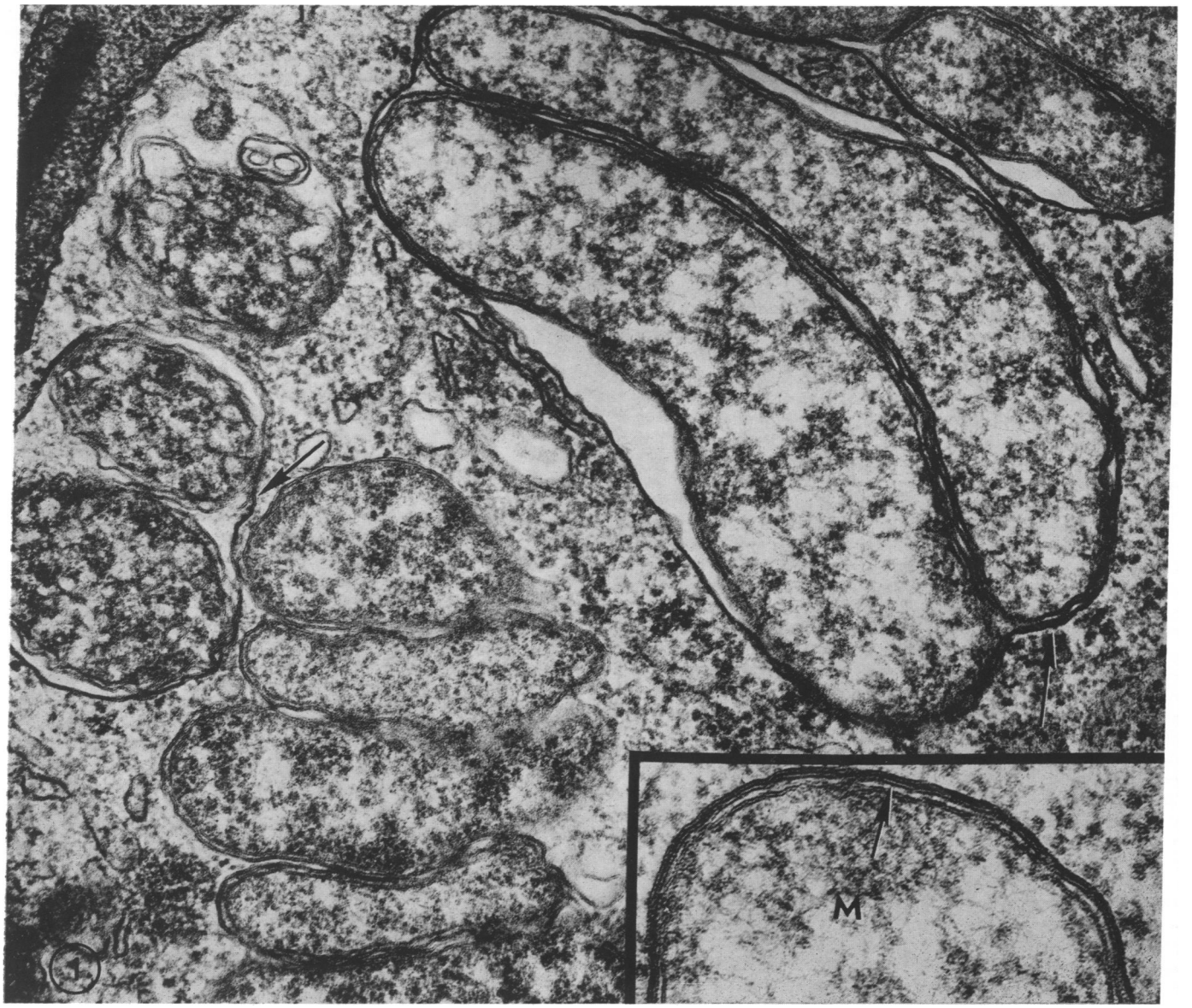

FIG. 1 An electron micrograph of a section from the testis of a sterile F_1 hybrid male from a cross of Mesitas ♀ × Santa Marta ♂ showing pleomorphic mycoplasma-like microorganisms. Each microorganism has two or more membranes. A cytoplasmic membrane encloses one or more organisms in a vacuole. Arrows distinguish the vacuolar membrane from the membraneous structure of the microorganism. ×90,000. The insert shows the outermost membrane of the microorganism (M) in juxtaposition to the vacuolar membrane of the host cell (arrow). ×115,000.

plasm in the donor was different from that in the recipient. Injection with homogenates from sterile hybrid males (rows 4 and 8) is about as effective in inducing sterility in the male progeny of recipient mothers as is injection with homogenates from fertile nonhybrid males (rows 2 and 6). Hence, the source of the cytoplasm, and not the fertility or sterility of the donor males, determined the outcome of these experiments.

DISCUSSION

The nature of the factor causing sterility in the male offspring of injected female *Drosophila* may tentatively be inferred. One of us (R.P.K.) has found in preparations examined by electron microscopy a mycoplasma-like symbiont (13), which has been seen in the cells of the testes (spermatids), and apparently outside the cells, between developing spermatids and sperm bundles (Fig. 1). It has also been seen intracellularly in the females, in oogonia, nurse cells, follicular cells, and the egg cytoplasm. Thus far the identity of the symbiont has not been clearly established, nor has it been grown *in vitro*. More studies aimed at such identification are now underway. As mentioned above, until about 1964 or 1965, the cross M ♀ × S ♂ produced fertile male hybrids. Experiments were then made by injecting S females with homogenates of fertile (M ♀ × S ♂) F_1 males, and no sterility was observed (4). At present, the hybrid males produced by this cross are sterile, and homogenates of these males injected into S females induce sterility in their male offspring (row 8, Table 1). The same is true of the homogenates of fertile nonhybrid M males injected in S females (row 6, Table 1). This situation parallels the findings of Dobzhansky and Pavlovsky (14) in the New Llanos strain of *D. paulistorum*.

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