

Microorganismal Basis of Infectious Hybrid Male Sterility in *Drosophila paulistorum*

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THE five semispecies constituting the *Drosophila paulistorum* complex exhibit strong reproductive isolation from one another. The most effective isolating mechanism is ethological, but when hybrids are produced (apparently only with difficulty in the laboratory), only the females are fertile^{2, 20}. Morphologically, these semispecies (Andean, Amazonian, Orinocan, Central American and Interior—plus a complex of populations, the Transitional) are almost indistinguishable¹⁹, but they do differ profoundly in the gene arrangements in some of their chromosomes (Kastritis⁹ and references therein).

Our concern here is with hybrid male sterility. In some crosses, i.e., between Santa Marta females and Mesitas males (both from Colombia, South America), the factor responsible for this sterility can be transmitted by injection⁵ and partially suppressed by heat shocks⁴.

This sterility can also be partially alleviated by treatment of the Santa Marta mothers with the antimycoplasmal antibiotics tylosin tartrate and tetracycline hydrochloride¹². The proportions of hybrid males in which there are mature motile spermatozoa are higher in the progenies of drug-treated mothers than in the controls. Electron microscopy reveals mycoplasma-like bodies in the untreated controls; these have not been seen in the sons of treated females^{11, 12}. These mycoplasma-like symbionts may be implicated in the production of the infectious hybrid male sterility, transmitted via the egg cytoplasm.

We present here electron microscopic evidence of the presence of intracellular mycoplasma-like entities in the cytoplasm of cells in the ovaries. This is also an extension of our work with antibiotic treatments to unique strains that exhibit an apparently spontaneous origin of hybrid male sterility¹. The particular strain involved is the "new Llanos," descended from a progenitor collected in the Llanos of Colombia in 1958. This strain, which formerly

produced fertile hybrids with the Orinocan semispecies of *Drosophila paulistorum*, is now producing sterile male hybrids. In evolving the sterility, however, new Llanos has not become sexually isolated from its progenitor; new Llanos males continue to be accepted by Orinocan females and vice versa. Professor Th. Dobzhansky (*Nature*, April, 1971) is seeking to modify this relationship by selecting for sexual isolation between the strains.

Materials

The strains of *Drosophila paulistorum* used in this study have been described previously (Kernaghan¹, and earlier papers referred to therein).

Crosses between any two of the strains mentioned in this article produce sterile F₁ male hybrids; this is so regardless of the direction of the cross.

Drug Treatments

In attempting to test the possible causal relations between the presence of mycoplasma-like bodies and the sterility of hybrid males, we examined the effects of two antimycoplasmal antibiotics: tylosin tartrate (Lilly:Tylen) and Achromycin (Lederle: tetracycline hydrochloride). Tylosin has been found effective against mammalian and avian mycoplasma in broth cultures¹⁷, against a mycoplasma contaminant in a murine leukemia tissue culture⁶, and against the arthropod-borne mycoplasma-like causative agent of the plant disease, aster yellows²⁷. Tetracycline, too, is effective against the aster yellows disease²⁷, and against the mycoplasma-like structures in plants infected with mulberry dwarf disease⁸. (Stewart, *et al.*²⁶ and Kawakita, *et al.*¹⁰ provide further information; Shikata and Maramorosch²⁴, and Whitcomb and Davis²⁷ provide excellent summaries.)

Both drugs were administered in a 100 mg/ml distilled water solution; 0.3 ml of the drug solution was added to each 250 ml culture bottle of *drosophila* food, which was Ohba's medium (8 percent), consisting of distilled water, agar, dry dead yeast and Tegosept, an antimold agent¹⁸. Only the "new Llanos," Colombia (no. 13A, Interior semispecies)¹, strain was placed on drug-impregnated medium. Crosses to the Georgetown, British Guiana (no. 42, Orinocan semispecies)¹ males were made on

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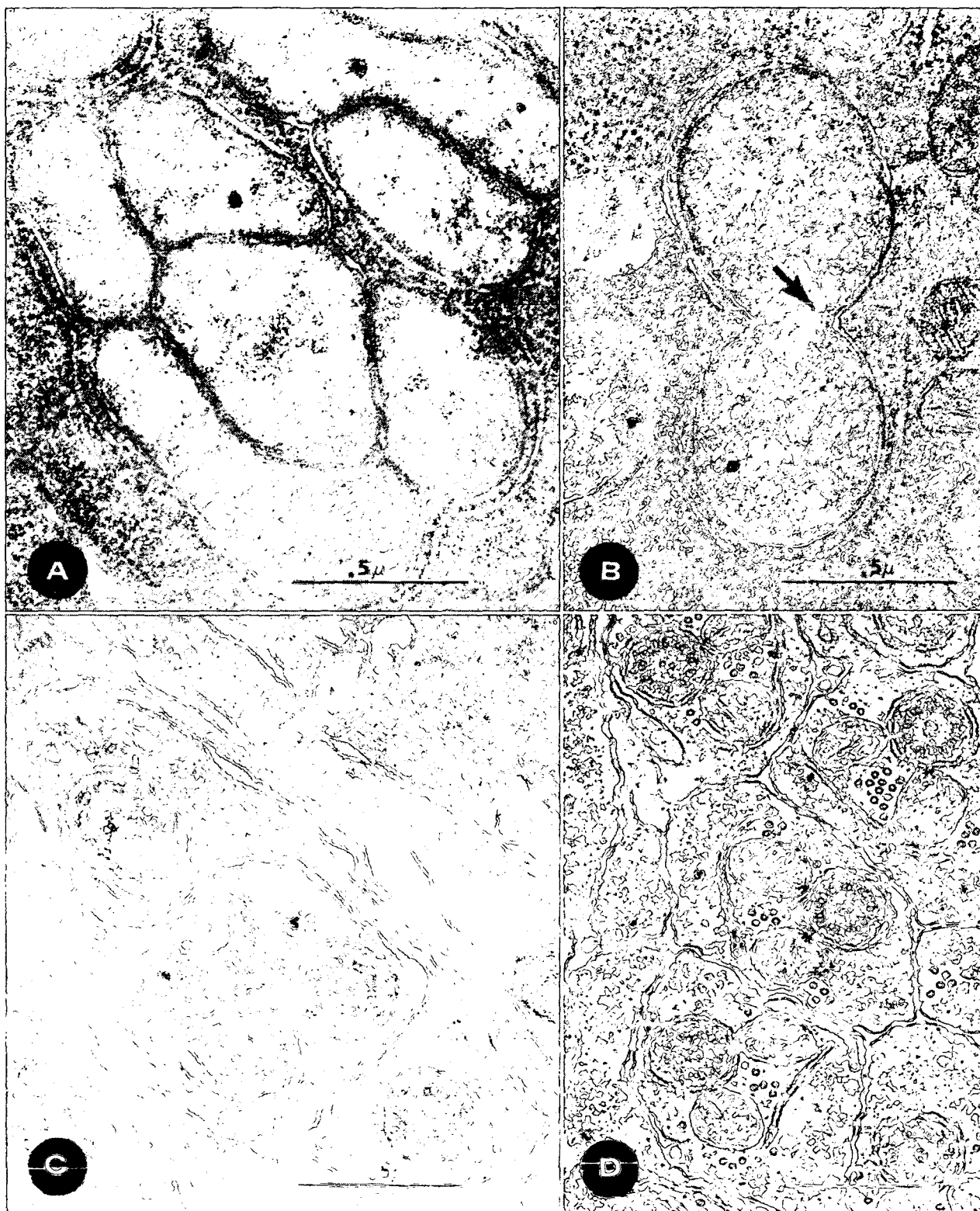


FIGURE 2—*A* shows a cluster of mycoplasma-like inclusions in a developing egg within the ovariole of an F₁ hybrid from a cross between Llanos ♀ × Georgetown ♂. A central fibrillar network and peripheral granulation is evident in each inclusion. Identical inclusions are observed to accompany spermatid degeneration in the sterile F₁ testes in males produced by the same cross (× 35,400). *B*—enlarged section of an unfertilized egg within the ovary of an F₁ female from a Llanos ♀ × Georgetown ♂ cross. It contains a mycoplasma-like inclusion. Note encapsulation within the cytoplasmic matrix by adhesion of ribosomal material to the exterior of the inclusion. In the area of the con-

striction a single limiting membrane (arrow) may be observed (× 36,500). *C*—section through a developing spermatid bundle in the sterile testis of an F₁ hybrid from a Llanos ♀ × Georgetown ♂ cross showing asymmetry in the organization of the axial filament complex. Similar disorders in the axial filament complex are common in such sterile males (× 32,000). *D*—symmetrical arrangement of the axial filament complex in a developing spermatid bundle in a drug-treated, fertile F₁ hybrid male from a Llanos ♀ × Georgetown ♂ cross. Extensive areas of degeneration customary in untreated, sterile F₁ males are not observed (× 29,000).

ordinary drug-free drosophila food devised by Spassky²⁵. Progeny resulting from these crosses were also raised and tested on drug-free Spassky medium, as were all the control generations.

To test the fertility of the F_1 hybrid males, 30 or more cultures were initiated with 8 females and 8 males from each experiment reported in Table I. No fertile cultures were obtained in the control F_1 generations (row 9 of Table I—actually repeated three times), or in the F_1 hybrid cultures derived from mothers treated with tylosin (row 1). In all the other experiments, where many males had motile sperm, some of those males were also fertile. (No experiments testing the progeny of Georgetown females \times Llanos males are reported in Table I because they all gave results indistinguishable from the controls.)

Electron Microscopy

The *Drosophila paulistorum* tissues were fixed with 3 percent glutaraldehyde in 0.15 M phosphate buffer at pH 7.4 for 30 minutes at room temperature²², washed in buffer, postfixed with 1 percent Millonig's osmium tetroxide¹⁶ for one hour, dehydrated, and embedded in Epon 812¹⁴. Silver gray sections were cut with an LKB microtome, mounted on Formvar-coated grids, stained with 50 percent alcoholic uranyl acetate⁷ and lead citrate²¹ and observed with a JEM 6C electron microscope.

An ultrastructural analysis of the ovaries of hybrid females produced from intersemispecific matings shows mycoplasma-like inclusions in the follicle cells (Figure 1A) and cystocytes (Figure 1B). In the cytoplasm of developing egg cells in the ovary of an F_1 hybrid female from the cross between Llanos $\text{♀} \times$ Georgetown ♂ , numerous single or clustered inclusions are observed that are ultra-

structurally identical to those described earlier as accompanying spermatid degeneration in sterile hybrid males¹¹. Figure 2A illustrates a cluster of such inclusions showing the characteristic peripheral granulation and central fibrillar network. At high magnification, Figure 2B exhibits a mycoplasma-like inclusion surrounded by a band of cytoplasmic matrix separated from the host cell. In regions where encapsulation is incomplete the single limiting membrane of the mycoplasma-like inclusion body is evident. Transovarial passage through the egg is essential to the involvement of this inclusion in the hybrid male-sterility phenomenon in *Drosophila paulistorum*. Further support for this hypothesis is added by the demonstration of mycoplasma-like bodies within the unfertilized egg.

Figure 2C demonstrates dissymmetry in the axial filament complex in developing spermatids in an untreated hybrid male from a Llanos $\text{♀} \times$ Georgetown ♂ cross. Unusually numerous asymmetric complexes as well as mycoplasma-like inclusions are observed in conjunction with extensive spermatid degeneration in these sterile males. The fertile males reported upon in row 6 of Table I are depicted in Figure 2D. They are the result of twelve generations of treatment with tetracycline. Ultrastructurally, spermatogenesis in these males appears to proceed normally, without any trace of extensive degeneration or observation of any inclusions resembling the customary mycoplasma-like bodies characteristic of sterile *D. paulistorum* hybrids.

Discussion

Treatment with tetracycline, but not with tylosin, results in the appearance of motile sperm and fertility in a fraction of the F_1 hybrid males. Both tetracycline and tylosin increase the frequency of motile sperm in backcross hybrid males. The longer the exposure to tetracycline, i.e., 3, 9 or 12 generations, the greater the increase over the control levels. However, after 12 generations of drug treatment, a plateau may have been reached; note the increase of only one percentage point to 42.0 percent in the sixth row of Table I. We are continuing to maintain the "new Llanos" strain on tetracycline; tylosin proved too toxic to the flies for such lengthy exposure. After three generations on tylosin-impregnated medium, no individuals were eclosing from the puparia although there were thousands of pupae.

The twelfth generation of tetracycline-treated flies was also subjected to single male tests for fertility, i.e., one drug-treated male isolated with five virgin females to see if larvae were subsequently produced. This procedure also serves to check on the relationship between dissections in physiological saline for the presence of motile spermatozoa and the actual ability of a male to father offspring. This relationship need not be 1:1. Actually, the difference reported in Table I is six percentage points (42.0 motility:36.0 fertility). With the F_1 controls consistently displaying neither sperm mo-

Table I. The effects of tylosin tartrate and tetracycline on hybrids between the Llanos (L) and Georgetown (G), Orinocan, strains of *Drosophila paulistorum*

	Percent- age of ♂ with motile sperm	Number of ♂ dis- sected	Larvae pro- duced
<i>After 3 generations on tylosin</i>			
1. F_1 ($L \text{♀} \times G \text{♂}$)	0	100	—
2. BC_1 ($L \times G$) $F_1 \text{♀} \times G \text{♂}$	37.7	53	+
3. BC_1 ($L \times G$) $F_1 \text{♀} \times L \text{♂}$	22.6	84	+
<i>After 3 generations on tetracycline</i>			
4. F_1 ($L \text{♀} \times G \text{♂}$)	13.0	100	+
<i>After 9 generations on tetracycline</i>			
5. F_1 ($L \text{♀} \times G \text{♂}$)	41.0	100	+
<i>After 12 generations on tetracycline</i>			
6. F_1 ($L \text{♀} \times G \text{♂}$)	42.0*	100	+
7. BC_1 ($L \times G$) $F_1 \text{♀} \times G \text{♂}$	55.0	100	+
8. BC_1 ($L \times G$) $F_1 \text{♀} \times L \text{♂}$	24.0	100	+
<i>Untreated controls</i>			
9. F_1 ($L \text{♀} \times G \text{♂}$)	0	100	—
10. BC_1 ($L \times G$) $F_1 \text{♀} \times G \text{♂}$	7.0	100	+
11. BC_1 ($L \times G$) $F_1 \text{♀} \times L \text{♂}$	8.0	100	+

* Single ♂ tests ($1 \text{♂} + 5 \text{♀}$) for fertility, i.e., the production of larvae, in this twelfth generation gave 36.0% fertile

tility nor fertility, we may conclude that the small difference is due to sampling error.

The injection of gold sodium thiomalate into females, undertaken because of reports by Sabin and Warren²³ and Marmion and Goodburn¹⁵, produced entirely negative results. There was no diminution of the hybrid male sterility of their sons. Dosages ranging from 0.1 mg/ml through 1.0 mg/ml were tested, including some double injections. These gold salt injection experiments were performed with the collaboration of Professor D. L. Williamson, Medical College of Pennsylvania, who is now testing the effects of the ingestion of this compound.

Ultrastructurally, we have previously demonstrated the presence of numerous mycoplasma-like bodies within and between spermatid bundles, and within spermatids themselves in the testes of *Drosophila paulistorum* males^{11, 12}; the number and size of these inclusions increases as the bundles degenerate in sterile males. King has described similar mycoplasma-like inclusions, termed "A bodies," in the ovaries of *D. melanogaster* and *D. willistoni*¹³. In *D. paulistorum* "A bodies" were observed as inclusions in ovarian nurse cells of the Mesitas strain but not in the Santa Marta strain¹³, p. 158. If these inclusions are involved in the etiology of the maternally transmitted hybrid male sterility in *D. paulistorum*³, mycoplasma-like bodies or their progenitors should be present in the ovaries of hybrid females whose sons and brothers are sterile. That this is so is shown in Figures 1A and B and 2A and B. Figure 2C and D plus the data in Table I present evidence that successful antimycoplasmal antibiotic treatment of the maternal line enhances the fertility of hybrid sons. This evidence is consistent with the supposition that there is a causal relation between the presence of numerous mycoplasma-like bodies in the testes and the maternally transmitted sterility of hybrid males. Apparently, the presence and the rate of multiplication of the mycoplasma-like entities is genetically controlled. Discord between the genotype of the host and the symbiont or parasite in a hybrid may allow the microorganism to multiply excessively. The result is the sterility of inter-semispecific hybrid males.

Summary

Male hybrids between the Llanos and Georgetown strains of *Drosophila paulistorum* are sterile. This sterility can be partially alleviated by treating the Llanos mothers with the antimycoplasmal antibiotics tylosin tartrate and tetracycline hydrochloride. Of the two drugs, treatment with tetracycline is more successful in elevating the levels of fertility in hybrid males. Electron microscopy reveals the presence of mycoplasma-like bodies in untreated controls but not in the sons of treated females. The involvement of these mycoplasma-like symbionts with the maternally transmitted sterility observed in hybrid males is further attested to by the electron-microscopic demonstration of their

presence in the ovaries and eggs of hybrid mothers of sterile sons and sisters of sterile brothers.

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Key Lecture and AGA-cosponsored Symposium Planned for AIBS Annual Meeting

THE eighth Wilhelmine Key lecture, sponsored annually by the American Genetic Association, will be presented by Dr. Harold H. Smith, Brookhaven National Laboratory geneticist. Dr. Smith's address, "Broadening the base of genetic variability in plants," will be delivered on Tuesday evening, August 31, during the 1971 meeting of the American Institute of Biological Sciences at Colorado State University, Ft. Collins. As part of the same program, the Meyer Memorial Medal, sponsored jointly by the American Genetic Association and the Office of Plant Introduction of the U. S. Department of Agriculture, will be awarded for distinguished achievement in the field of plant introduction. The scientist chosen to receive the medal will be announced at a later date.

On September 1 and 2, the AGA will cosponsor (with the American Society of Animal Science, Poultry Science Association, American Society of Zoologists, and the American Association for Laboratory Animal Science) a symposium entitled "Approaches toward development of isogenic stocks and their use." During the two-day session 12 speakers will discuss the following topics: E. G. Buss, Consequences of inbreeding turkeys during a 20-

year period; Hans Abplanalp, Experimental techniques for breeding highly inbred lines of poultry; Howard A. Stone, The usefulness and application of highly inbred chickens to research programs; Robert C. Carter, Development and testing of inbred and single trait selection lines in beef cattle; James S. Brinks, Inbreeding, livecrossing, and topcrossing results from inbred lines of beef cattle; S. K. Ercanbrack, Results from developing and testing 57 inbred lines of sheep; M. W. Olsen, Diploid parthenogenesis—An effective tool in establishment of isogenic lines of turkeys; James E. Wright, Inbreeding and heterosis in trout; James H. Asher, Optimal methods of obtaining isogenic and congenic strains using combinations of bisexual and parthenogenetic reproduction; Carl T. Hansen, Comparisons of reproductive performance among inbred strains of laboratory animals; Hans Meier, Genetic determination of neoplasia and expression of C-type RNA viral genomes in inbred mice; Benjamin Taylor, Recombinant inbred lines—A new approach to genetic analysis. In conclusion, there will be a summary panel discussion by the four session chairmen—L. B. Crittenden, K. P. Bovard, M. N. Runner, and T. M. Sutherland—led by Robert C. Carter.