
In an attempt to ascertain the cause of sterility, the mating behavior, reproductive tract morphology, sperm motility, and ultrastructure of spermiogenesis of certain male-sterile mutants have been studied. Previous work in this general area has been carried out by Kiefer (1969), Romrell, et al., (1972) and Tokuyasu et al., (1972).

Analysis of courtship patterns was carried out on males homozygous for tapered (ta), shaven-depilate (svde) and scute\(^{260-15}\) (sc\(^{260-15}\))(See Lindsley and Grell, 1968). From one to five sterile males (2-7 days old) were paired with one to five wild-type, virgin females (2-3 days old). In addition to normal females, wild-type virgin females whose antennae were removed and wingless, fertile apterous virgins were also employed. The flies were placed in small, 1" x 1" x 3/8" clear plastic breeding boxes and were observed from 15 minutes to two hours. Some were left up to 24 hours in which cases the females were isolated and the eggs were checked for fertilization for as long as five days. Approximately 100 tests were made for each mutant and the mating behavior was compared to the wild type in accordance with Spieth (1952). Normal courtship pattern begins with the 360° circling of the female by the male; next the male vibrates his wings holding one at a 90° angle to his body; then the male intermittently touches the female with fore legs and/or proboscis; next the male expresses acceptance by spreading her wings and/or vaginal plates; the male then mounts female and completes intromissions; following insemination the female begins rejection of the male by kicking; and finally the male rotates 180°, dismounts and withdraws. When a female is non-receptive she exhibits the kicking phenomenon and other avoidance techniques. Presumably the non-receptivity is caused by a previous mating (Merle, 1968) or the courtship by the male is unsatisfactory.

It was found that ta and svde cannot mate. Vague interest in the form of circling and approaching the female was displayed by the ta and svde males, up to 15 min. in many cases. Both mutants displayed wing vibrations periodically at 90° and proboscis movements. No intermittent touching or any other type of behavioral steps were observed. Using the kicking motions, the females always prevented the males from coming close to them. Presumably the courtship steps employed by the mutants were insufficient to induce acceptance by the female. It is interesting to note that non-receptivity was also exhibited by the antennaless and wingless females. Furthermore it was shown that females made receptive by wild-type males would not accept the mutants. In this experiment 7-day Oregon-R wild-type males were placed in with one or more females, and just prior to intromission the normal males were replaced with mutant ones. With regard to the sterile mutant sc\(^{260-15}\), the normal courtship pattern was displayed, mating was completed, but fertilization did not take place.

In order to examine the reproductive tract morphology and sperm motility the complete tract was dissected out in Drosophila Ringer's solution placed on a slide with fresh Ringer's solution, and examined under the light microscope. Spermatozoan motility was then checked in flies whose seminal vesicles had been dissected open.

In ta there were a frequent number of abnormalities in the reproductive tract, the most common was atrophy of one of the accessory glands, or both in some cases. Accompanying these abnormalities club-like structures usually were present on the apical ends of either of the testes. Frequently either the right or left testis was atrophied, and occasionally both. Out of 300 flies examined the ones with two or more of the malformations had non-motile spermatozoa. This number amounted to about 20% of the total number of flies studied. Preliminary electron microscopic studies revealed a large number of degenerating spermatids and in numerous instances double and triple axonemes per cell. There were also cases where several axonemes share one major mitochondrial derivative.

In svde abnormalities in the reproductive tract morphology were more common. In many cases either one of the accessory glands were shrivelled and in a few instances, both. Either one or both of the testes in several flies were completely atrophied. Similar to ta the percentage of flies with non-motile spermatozoa increases greatly with the number of abnormalities present. Out of 300 flies examined approximately 50% had non-motile spermatozoa when containing more than two of the above abnormalities. Preliminary fine-structural analysis showed normal development within the testes. Both pre and post individualization stages appear consistent with wild type.

At the light microscope level the testes of sc\(^{260-15}\) appeared normal in most cases, but in a few flies the accessory glands were constricted toward the apical end. Spermatozoa were motile but somewhat less than normal. About 15 minutes following copulation the vaginal tract (Continued at bottom of next page)
We reported previously the presence of a pair of anal organs at the posterior end of Drosophila larvae (Gloor and Chen 1950). These can easily be demonstrated by immersing the larvae briefly in a 0.5% AgNO₃ solution. Subsequent to a short exposure to light, two symmetrical dark-brown plates located on each side of the anus are visible. From their analysis of the ionic concentration of larval hemolymph in solutions of different salinities it was concluded that these organs absorb chloride and sodium ions from the environmental medium. In connection with our studies on the lethal mutant 1(3)tr, which accumulates an enormous amount of hemolymph of very low osmotic concentration (Hadorn 1949 and unpublished data), we undertook an electron microscope analysis of the anal organs of both the wild type and the ltr-homozygous larvae of D. melanogaster.

By using a scanning electron microscope (Cambridge Stereoscan S4) it was observed that the cuticular surface in the anal organ region has a porous structure, due to infoldings of the epicuticle. Studies of thin sections with a Hitachi transmission electron microscope revealed that underneath the cuticle the plasma membrane of the giant hypodermal cells show numerous folds which are oriented perpendicular to the cuticular surface. On the other hand, the plasma membrane on the basal surface appears quite smooth. The hypodermal cells are rich in mitochondria and other organelles such as free ribosomes, endoplasmic reticulum, microtubules, lysosomes, vacuoles of heterogeneous sizes and electron dense bodies have also been noticed.

Of particular interest are the profound changes of the fine structure of the hypodermal cells in larvae treated with solutions of different salt concentrations. When the larvae were immersed for one hour in a hypotonic medium (distilled water), the folds of the plasma membrane become greatly increased and penetrate more deeply into the cell. Mitochondria are also increased in number and many of them move into the interspace between the folds. Conversely, in a hypertonic medium (1.5-5.85% NaCl) there is a distinct reduction of both the folds and mitochondria. At still higher salinity (10% NaCl) the folds of the plasma membrane nearly disappear. Similar to that already reported for the anal papillae of the mosquito larvae (Copeland 1964, Sohal and Copeland 1966) the present results indicate that in Drosophila larvae the uptake of inorganic ions through the anal organs can be regulated by variations of the surface area of the plasma membrane of the hypodermal cells on the cuticular surface. Furthermore, alterations in the number and distribution of mitochondria at different salinities suggest that the ionic absorption must be an energy-consuming process.

We have so far detected no morphological difference in the ultrastructure of the anal organs between the wild type and mutant larvae. If osmoregulation is involved in the ltr lethal mutation, the effect must occur at some other level.


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of the female was dissected out and the spermatozoa in the tract did not appear as active as wild-type. Most spermatozoa were confined to the region just distal to the vaginal plug and none were observed in the spermathecae. Electron microscopy of the testis showed a large percentage of degenerating sperm bundles. In many individualized spermatids the axial filament was attached to the major mitochondrial derivative lateral to the normal position. This abnormality may be a symptom of the underlying cause of sterility.

With regard to sc6-15 the cause of sterility must be associated with the spermatozoa or the seminal fluid. With regard to ta and svde the interesting problem lies in the causal relationship of the two reproductive phenomena studied: courtship behavior and reproductive tract morphology. Are the two abnormalities related or do they occur independently? Does one give rise to the other or are they both induced by a third cause? (Supported by N.S.F. Research grant GB12969 and N.I.H. Biomedical Institutional Research Funds).