Wood, D.D. and F.M. Butterworth. Oakland
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behavior and reproductive tract morphology
of male-sterile mutants.
In an attempt to ascertain the cause of steril-
ity, 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 (sv^{de}) 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 re-
moved 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 fertization 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 inter-
mittently touches the female with fore legs and/or proboscis; next the female expresses
acceptance by spreading her wings and/or vaginal plates; the male then mounts female and com-
pletes intromission; following insemenation 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 sv^{de} cannot mate. Vague interest in the form of circling and
approaching the female was displayed by the ta and sv^{de} males, up to 15 min. in many cases.
Both mutants displayed wing vibrations periodically at 90° and proboscis movements. No inter-
mittent 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 wing-
less 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 sperma-
tozoa. 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 sv^{de} 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 per-
centage of flies with non-motile spermatozoa increases greatly with the number of abnormal-
ities 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 consistant 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

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land. The ultrastructure of the anal
organs of Drosophila larvae.

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 concen-
tration 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 l(3)tr, which accumulates an enormous amount of hemolymph of very
low osmotic concentration (Hadorn 1949 and unpublished data), we undertook an electron micro-
scope 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 re-
vealed 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, micro-
tubules, 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 mem-
brane 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 salin-
ities 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.

Literature cited: Copeland, E. 1964, J. Cell Biol. 23:253-264; Gloor, H. and P.S. Chen
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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 microcroscopy of the testis showed a large
percentage of degenerating sperm bundles. In many individualized spermatids the axial fila-
ment 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 sc[260-15] the cause of sterility must be associated with the spermatozoa
or the seminal fluid. With regard to ta and sv[de] 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.
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