Lipids of Drosophila: A Newly Detected Lipid in the Male

Abstract. *A thin-layer chromatographic analysis of* Drosophila melanogaster *revealed a lipid to be found almost exclusively in the adult male. The compound or class of compounds having an* R_F *close to that of methyl oleate is present in substantial amounts and is located predominantly in the ejaculatory bulb. It appears from genetic studies that the formulation of the lipid is not mediated by the Y chromosome.*

The general classes of lipids such as triglycerides, steroids, fatty acids, phospholipids, hydrocarbons, and waxes have been identified in *Drosophila* previously (*1, 2*). Using thin-layer chromatography (TLC) I was able to separate at least ten lipid components. One of these components is of immediate interest, since it is found almost exclusively in the male (see Fig. 1). This compound or class of compounds has a mobility close to that of methyl oleate. The R_{ST} (relative R_F) of the male lipid with the methyl oleate standard is about 0.9.

Generally the following procedures were employed. The flies which were reared according to methods described earlier (*3*) were anesthetized with ether, teased open in *Drosophila* Ringer solution, blotted dry on filter paper, and extracted in a mixture of chloroform and methanol (2:1) for 24 hours at room temperature. After removal of the tissue residue the supernatant extract was concentrated at 45° to 50°C under nitrogen. The extracts were applied to commercially prepared TLC plates (Analtech, Inc., silica gel G, E. Merck; 250 μ) that were activated at 120°C for 30 minutes. The plates were developed in hexane, diethyl ether, glacial acetic acid (85:15:1), and the lipids were detected by charring at 190°C after spraying with 10 percent sulfuric acid. No attempt was made to extract the tissues completely or remove the nonlipid residue from the extract. Generally 10 to 20 flies were used for each experiment; however, the lipids extracted from as few as one or two flies can be detected by the above method. Each experiment was repeated at least once.

Although chloroform-methanol is the usual solvent system for lipid extraction (*4*), esters of fatty acids have been shown to arise as artifacts when lipids from both mammalian (*5*) and insect (*6*) tissues were extracted with solvents containing methanol or other alcohols of low molecular weight. The workers in question (*5, 6*) were able to distinguish between native and artifactual esters if the extracting solvents were free of these alcohols. However, there are cases (*7, 8*) where lipids extracted with chloroform-methanol contained methyl esters that were not artifacts. Since the male lipid has a TLC mobility close to that of methyl oleate, steps were taken to rule out the possibility that the male lipid may be an artifact caused by postmortem changes or alcohols present in the anesthetic and extracting solvent. To this end unanesthetized flies were frozen with dry ice, split open, and extracted in either chloroform-methanol, chloroform (Chromatoquality; Matheson, Coleman, and Bell), or hexane (Criterioquality; Matheson, Coleman, and Bell) at −15°C for either 30 minutes or 24 hours. Extractions with the same solvents were also done at room temperature for either 30 minutes or 4 hours. In order to prevent the artifactual production of methyl esters during the concentrating procedure (*7*), the extracts were evaporated rapidly at 40°C under reduced pressure. In every case the extracts of male flies contained the male lipid and in the same proportion to the other classes of lipids in each extract. We conclude that the male lipid can be demonstrated with or without the presence of methanol in the extracting solvent. Although the latter two extracting solvents were free of alcohols of low molecular weight, it is possible that such alcohols may exist in the living tissues (*5*). Since the compound is extracted in abundant amounts (approximately equivalent to triglyceride) from male flies at −15°C, it is unlikely that the male lipid is either a product of autolysis or due to an enzymatic reaction occurring during the period of extraction.

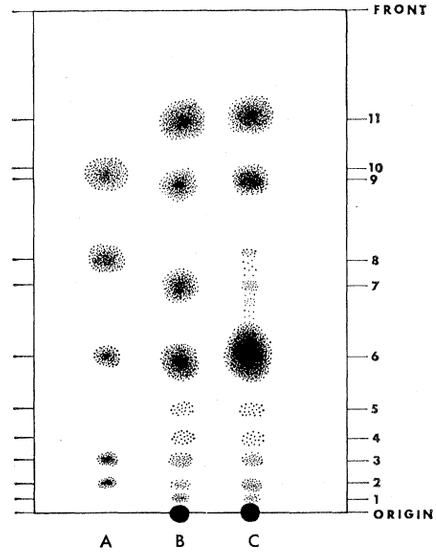

Fig. 1. Drawing of a typical thin-layer chromatogram. Column A, standard TLC mixture; B, lipid mixture extracted from adult males; C, lipid mixture extracted from adult females. The spots at the origin are presumably polar lipids. In column A, positions are 2, cholesterol; 3, oleic acid; 6, triolein; 8, methyl oleate; and 10, cholesteryl oleate. Position 7 is the male lipid. Between 6 and 8 on column C there is a faint streak which appears to have spots within it. The spot at 7 is absent in virgin females. The other "spots" are extremely faint and difficult to distinguish from the background.

The male lipid was found in the highest concentration in males that were at least 7 days old. Similar amounts were present in both mated and unmated males that were 1, 2, and 3 weeks old. The lipid was found in only trace amounts in recently emerged male adults and could not be detected at all in male larvae just ready to pupate. The lipid was not present in similarly aged female larvae either. The male lipid was found in trace amounts in mated female flies that were 1, 2, and 3 weeks old. A definite spot with an R_F similar to the male lipid could not be detected in virgin females, although there was a very faint streak of material between positions 6 and 8 of Fig. 1.

The fact that a definite spot could not be detected in virgin females suggested that the lipid might be located in the male reproductive system and transferred to the female during mating. To answer this question extracts were made of various regions of the male genital tract. Although the lipid was not detected in the testes, seminal vesicles, or accessory glands, it was present in extremely high concentration in the ejaculatory bulbs. The ejaculatory bulbs in this experiment contained a portion of the anterior and posterior ejaculatory ducts. Additional studies showed that the lipid was not present in the head, thorax, or hemolymph. However, trace amounts were detected in the fat body, gut, and Malpighian tubules. The male lipid in the gut and Malpighian tubule extract may have come from adhering strands of adipose tissue.

A similar study was made of mated and virgin females. Extracts were made of the spermathecal region which also contained the seminal receptacle, accessory gland, uterus, rectum, associated adipose tissue, and external genitalia. It was found that the male lipid was definitely present in the spermathecal region of mated females but absent from the same region of virgin females. Extracts of the remaining parts of both mated and virgin females did show a faint streak between positions 6 and 8 of Fig. 1.

There is a mutation in *Drosophila* which causes karyotypic females to develop into phenotypic males. For example, karyotypic females homozygous for *transformer* (*tra*) (*9*) develop into normal-looking, sterile, adult males. The gonads of such an individual are abnormal, but accessory glands and an ejaculatory bulb are present. A TLC analysis of *tra* "females" indicated that they are typically masculine with respect to the male lipid. Since the karyotype of the *tra* "female" used was XXY, it was thought that perhaps the Y chromosome mediated the synthesis of the male lipid. However, a TLC analysis of normal males lacking a Y chromosome (XO) showed the male lipid to be present. Alternatively, normal females with an XXY karyotype did not have the male lipid. It appears, then, that the formation of the male lipid is not regulated by genes on the Y chromosome.

The function of the lipid is unknown. The fact that it is present in the male genital tract and appears to be transmitted to the female suggests that the compound may serve a reproductive function.

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New Antheridiogen from the Fern Onoclea sensibilis

Abstract. *An antheridium-inducing hormone (antheridiogen) derived from the fern species* Onoclea sensibilis *(Polypodiaceae) is chromatographically distinct from the native antheridiogen of* Pteridium aquilinum *(Polypodiaceae). It also differs from the native antheridiogens of* Lygodium japonicum *and* Anemia phyllitidis *(Schizaeaceae).*

Prothalli of *Pteridium aquilinum* elaborate a hormone [antheridogen (*1*)] which induces antheridia in many if not all species of the fern family Polypodiaceae (*2, 4, 9*). The isolated hormone is active at a concentration of less than one part in ten billion, and it contains a carboxylic group that is necessary for activity (*1*). The antheridiogen of *P. aquilinum* failed to induce male sex organs in *Anemia phyllitidis* and *Lygodium japonicum* (Schizaeaceae) (*3*).

The prothalli of *Anemia phyllitidis* also elaborate an antheridiogen that is highly active toward the prothalli of this species but fails to induce male sex organs in *Onoclea sensibilis*, the species used to assay for the hormone elaborated by *P. aquilinum*. This, and other results, led to the conclusion that the antheridiogens of *A. phyllitidis* and *P. aquilinum* are different molecular entities (*3*). In turn, the prothalli of *Lygodium japonicum* elaborate an antheridiogen which differs both from the antheridiogens of *A. phyllitidis* (*4*), and of *P. aquilinum* (*4, 5*). More recently, Schraudolf (*6, 7*) demonstrated that known gibberellins are capable of inducing antheridia in tested species of the fern family Schizaeaceae. Like the native *Anemia* antheridiogen (*3, 5*), these phytohormones fail to hasten antheridium formation in other fern families (*6, 8, 9*). The native antheridiogen of *A. phyllitidis* does not seem to be identical with any of the several tested gibberellins (*9*).

In undisturbed cultures of *O. sensibilis* an antheridiogen could be detected at most in traces. However, clear-cut activity was detected after the prothallial extract or the medium from 16-day-old cultures was heated. Low *p*H increased the yield. It could not yet be decided conclusively whether heating converts an inactive precursor to an antheridiogen or whether it destroys a heat-labile inhibitor of antheridiogen action (*10*). An attempt was made to learn whether this antheridiogen differs from those demonstrated earlier.

The methods used in spore inoculation and in the culture of the prothalli have been described (*11*). Descending chromatography on filter paper (Whatman No. 1) with an isopropanol, water, and ammonia system (8:1:1) was performed. Chromatography was discontinued when the solvent front was 20 cm from the origin. After drying, the chromatogram was cut into ten sections, each section being eluted with water. Antheridium-inducing activity of the eluates was detected by bioassay (*11*). To obtain active antheridiogen, agar-solidified medium from 16-day-old cultures of *O. sensibilis* was harvested and frozen. After thawing, the liquid was filtered off and its *p*H adjusted to 2.0. The liquid was then boiled for 10 minutes; upon cooling the *p*H was readjusted to that of the fresh culture medium (5:8).

This activated preparation induced antheridia in *O. sensibilis* to a dilution of 1/100 as observed 12 days after spore inoculation. The active preparation was extracted twice with half the volume of ethanolacetate for chromatography. The combined extracts were evaporated in a vacuum, and the residue was dissolved in an amount of water 1/200 that in the original preparation. Fifty microliters of this concentrate was spotted. Of the *Pteridium* antheridiogen 25 μl of an ethanol extract was spotted which induced antheridia in *O. sensibilis* to a dilution of about 1/60,000. The antheridiogens of *O. sensibilis* and *P. aquilinum*

Table 1. Antheridium-inducing activity of *Onoclea* antheridiogen in *Onoclea sensibilis*, *Anemia phyllitidis*, and *Lygodium japonicum*. Values are averages of antheridium-bearing individuals in two samples of 30 prothalli 12 days (*O. sensibilis*) or 20 days (*A. phyllitidis*, *L. japonicum*) after spore inoculation.

Dilution	Activity toward		
	O. sensibilis	*A. phyllitidis*	*L. japonicum*
1/10	25.5	0.0	0.0
1/30	19.0	0.0	0.0
1/100	4.5	0.0	0.0
1/300	0.0	0.0	0.0
Co*	0.0	0.0	0.0

* Control, with no *Onoclea* antheridiogen.