Studies
on
Hymenopteran Parasitism
of
Drosophila

Thesis

by

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Studies on Hymenopteran Parasitism of Drosophila

Flies of the genus Drosophila are subject to attack by a number of parasitic forms. Sturtevant (1921) has listed records of parasitism by protozoa (Leptomonas), fungi (Muiaria and Stigmatomyces), nematodes, mites and various hymenoptera. According to Sturtevant, Perkins (1913) has bred at least five species of hymenoptera, belonging to the proctotrupoid, cynipoid and chalcidoid groups, upon Drosophiline flies. H.S. Smith has bred an unidentified proctotrupoid and a chalcidoid, Pachycrepoideus dubius Ashmead* from both Drosophila melanogaster and D. hydei. Kieffer (1913) has described three species of hymenoptera from Africa collected by Silvestri and stated by him to be parasitic on Drosophila, species not given. They are Trichopria (Planopria) rhopalica (Diapriidae), Ashmeadopria drosophilae (Diapriidae), and the insect which forms the subject matter of the present investigation, Eucoila drosophilae (Figitidae).

There are in addition a number of predacious enemies among wasps, spiders, flies and beetles.

The present account is concerned with parasitism of various species of Drosophila by Eucoila drosophilae Kieff. The wasps were found by Dr. W. P. Spencer who exposed traps in an effort to collect Drosophila at Long Lake, Ohio, in Sept. 1934. Drosophila larvae from the trap gave a large number of pupae from which wasps emerged in considerable proportions. Since that time stocks have been maintained in culture on Drosophila melanogaster.

* Psyche 17: 112: 1910

Systematic Position

Class: Insecta

Order: Hymenoptera

Superfamily: Cynipoidea

Family: Figitidae

Subfamily: Eucoilinae

Genus: Eucoila

Species: drosophilae

I am indebted to Dr. A.C. Kinsey for information to the effect that the present object of investigation was Eucoila drosophilae Kieff. The type material has not been seen but the published description fits very well. The present insect is readily distinguishable from all the type material I have seen which comprise 14 species from North, South and Central America, all described by Kieffer (1907).

The following description is given by Kieffer (1913).

Eucoila drosophilae n.sp.

Female: Noir, brillant et lisse; antennes, hanches et pattes jaunes, abdomen brun noir, extremite posterieure brun roux. Antennes de 13 articles, avec une massue peu distincte de 7 articles; 3^{e} article plus long que le 2^{e}, 3--6 subcylindriques, au moins de moitie plus long que gros, sans aretes, 7--12 un peu allonges, a 4 aretes sensorielles partant de la base de chagne article qu'elles depassent a peine, 13^{e} article, ellipsoidal, un peu plus gros que les precedents, carene obliquement dans sa moitie distale. Cupule presque plane ellipsoidale, sans enfoncement distinct.

Ailes hyalines, cellule radiale fermee, 2 fois aussi longue que large, 1^{e} partie du radiusarque, 3 fois aussi longue que la 3^{e} partie de la sous-costale, a peine plus courte que la 2^{e} partie du radius, qui est arquee en sens inverse, cubitus nul dans sa moitie proximale, bien marque dans sa moitie distale. Abdomen comprime, ceinture peu distincte. Long. 1.5 mm - Guinea francais: Konakry, parasite de Drosophila (Silvestri).

Some details are shown in figures 1--4.

Geographical Distribution

Members of the genus Eucoila apparently have a rather wide distribution. I have examined specimens from Brazil, Nicaragua, Cuba, Mexico, California, Wisconsin, and Pennsylvania. Cresson (1865) has described two species from Cuba. Ashmead (1900) records ten different species from the West Indies and Viereck (1906) describes species fromTexas (Galveston) and Arizona (Oak Creek Canyon, 6,000 ft.) and three species from Connecticut (1910).

von Ibering has described a new species from Brazil (1914) and one from Kostroma, U.S.S.R., (1927). Hedicke has described new forms from New Pomerania, (2 spp.n.), (1922); and two from Stavropol, U.S.S.R. (1928); one from Argentina (1924); one from France (1928); and one each from Syracuse,(Sicily), Tunis, and Germany (1928). No host records are given for this material.

P.H. Timberlake informs me that members of the genus Eucoila are extremely abundant in the islands of the Hawaiian group. This list makes it clear that the genus is cosmopolitan.

Fig. I Adult male
of Eucoila drosophilae
(X17)

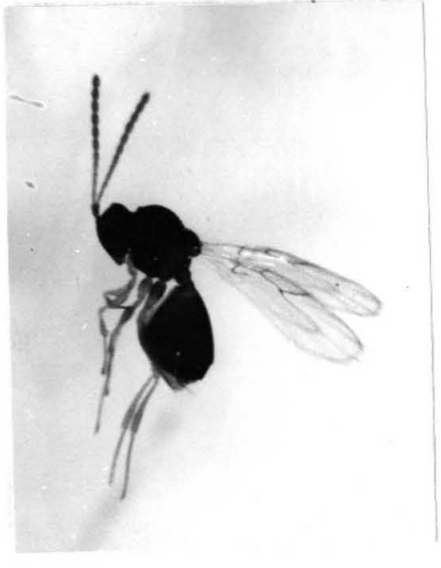

Fig. II Adult female
of E. drosophilae
(X 17)
Note distinct sexual
dimorphism

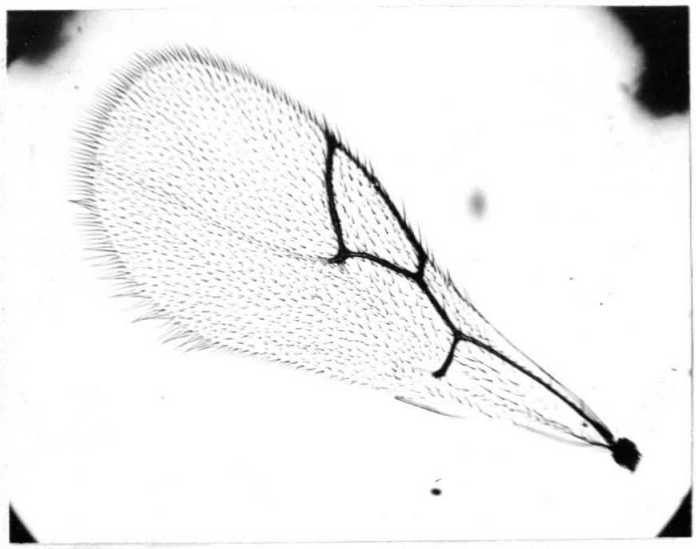

Fig. III Photograph showing venation of fore wing

(X 35)

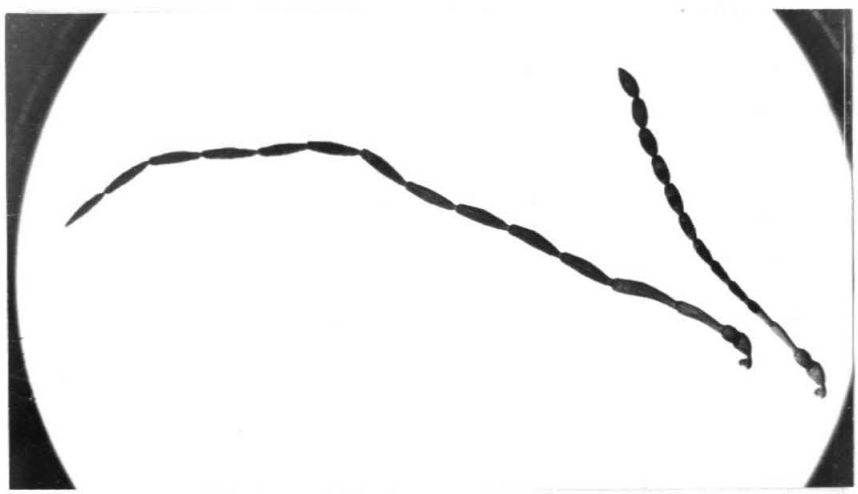

Fig. IV Male (left) and female antennae

(X 35)

Records for Eucoila drosophilae are so far limited to French Guinea and Ohio.

Life History

The reproductive system of the female Eucoila drosophilae is shown in Fig. 5. Fertilization probably takes place in the common oviduct near the site of the receptacle (Fig. 5.A). The function of the gland (5B) and resevoir (5C) are unknown; they are usually referred to as acid glands. The resevoir (5C) is probably homologous to the resevoir figured by James (1928; fig. 4. p. 298). James refers to this structure as an "acid gland resevoir" in Figites anthomyiarum but its contents are neutral or alkaline in Eucoila drosophilae. This may be shown by dissecting the resevoir out in citrate phosphate buffer, pH 6.0 and placing it with needles on a piece of Nitrazine test paper (Squibb). The dry paper is olive green in color. A minute drop of buffer pH 6.0 makes a distinct yellow spot. A drop of buffer pH 7.0 causes a distinct blue spot. The paper is sensitive to 1/10,000 N. acid or base. The macerated gland produces a distinct blue spot on the olive green paper indicating an approximate pH of 7 or higher. The resevoir produces the same reaction indicating that whatever their contents may be there is little free acid.

The ovarian eggs all mature at about the same time; there is very little nurse cell tissue evident in the adult ovary. Detailed studies are at hand on the reproductive systems and morphology of true gall wasps which resemble Eucoila drosophilae rather closely. Fruhauf (1924) has figured the reproductive systems and ovipositing apparatus of Biorhiza aptera and Rhodites rosae. James (1928) has published descriptions of the reproductive

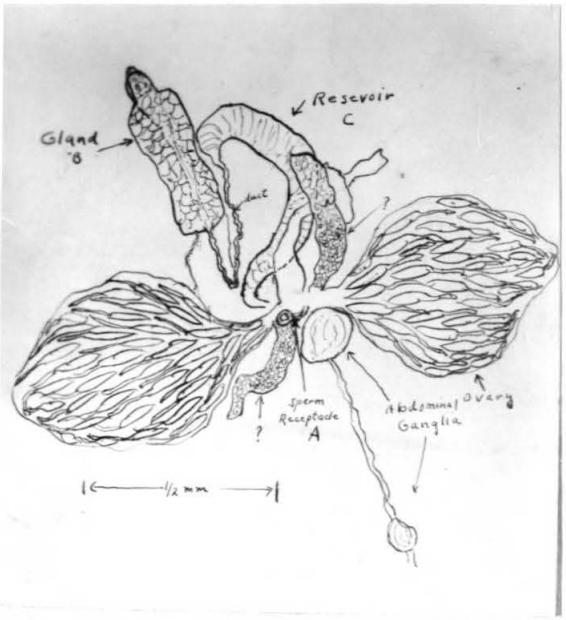

Fig. V The female reproductive system of E. drosophilae.

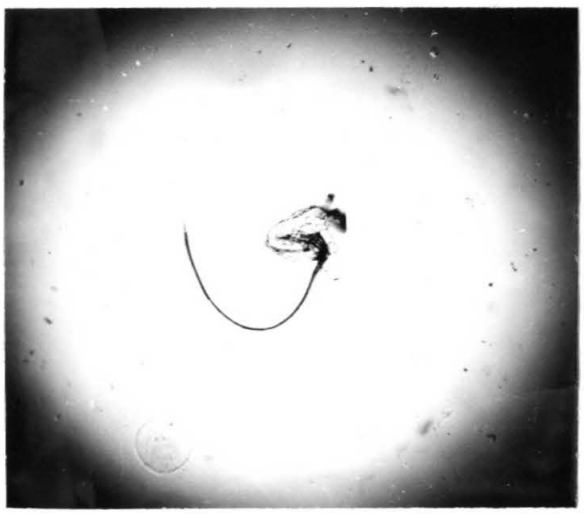

Fig. VI The ovipositor.

system,eggs, and larvae of the closely related Figites anthomyiarum and Kleidotoma marshalli, parasitic on saprophagous dipteran larvae. They were reared from various species of Calliphora, Lucilia, Musca and Sarcophaga.

Eucoila females, unlike some of their near figitid relatives, which are strongly negatively phototropic while ovipositing, (James, l.c.) may be watched under a binocular during this operation.

Oviposition takes place only into larvae. Tests with eggs, larvae, and pupae in mixed dishes show that only larvae are recognized as proper hosts. Larvae just hatched from the egg are suitable, and parasitism at this early stage does not result inan appreciable mortality among the host larvae. Larvae inthe second instar may be readily attacked and the eggs so deposited give rise to adults. However parasitism in the third instar is not common. Such large larvae are apparently not recognized as suitable hosts. Parasites may, however, attack them on occasion. The large Drosophila larva frequently draws away from the parasite before the ovipositor can be properly inserted. Such eggs as may be laid usually undergo degeneration, though not until considerable development of the embryo has occurred.

Eucoila females once induced to commence oviposition by exposure to a fermenting medium, find their hosts by patient and rapid exploration with the ovipositor. Cracks and holes in the medium are favorite sites for exploration; they often push the ovipositor several millimeters down into the food in search of hosts. For the most part, the ovipositor is the only

part of the parasite that comes in contact with the host larva. If the larva is on the surface and is fairly large the feet may aid in holding the larva within range. These observations assume the existence in the ovipositor of a suitable sensory system capable of detecting proper hosts. Fig. 6.

When the ovipositor comes in contact with a Drosophila larva the female raises the abdomen and extends the ovipositor to its full length. Actual drilling is quickly accomplished. This process occupies two or three seconds at most. No particular site on the larva seems to be preferred. Any site which brings the ovipositor into the haemocele appears suitable. The total time occupied by the process from insertion to withdrawal of the ovipositor is shown for a series of cases timed with a stop watch.

Table 1.

Times (in secs.) required to oviposit in larvae of D. melanogaster.

17.4	19.4
22.0	14.8
20.0	15.4
19.0	17.0
22.4	20.2
25.4	12.2
20.0	

18.7 secs. average.

The whole process then occupies approximately 19 seconds. Upon withdrawal of the ovipositor the parasite remains quiescent for a period of four to seven seconds and then continues the search for hosts. The overall efficiency of this process for a density of fifty hosts per square inch is about 25 ovipositions per

hour. No very conspicuous reaction is manifest on the part of the host just parasitized. Large larvae, difficult to parasitize, may at times be observed to become less active after the ovipositor has been inserted a few seconds. The host, if handled, appears somewhat flaccid, though is still capable of some movement. No very profound paralysis results.

Development: The ovarian egg is shown in Fig 7. It varies somewhat in size and in the relative proportions of ooplasm and filament. The egg is approximately 0.036 mm. in thickness, which is almost twice the outside diameter of the ovipositor. It must, then, undergo considerable compression during passage down the ovipositor. It is very elastic, for it can be stretched with blunt needles more than three times its normal length without tearing. The egg on entering the host's haemolymph begins to throw off a membrane which covers the entire egg with the exception of the filament. See Fig. 8. This process takes place very quickly and is usually complete within 1 1/2 minutes after withdrawal of the ovipositor. I have been able to witness this only when the process was about two thirds complete as some 50 seconds are required to get the larva dissected and on the stage of the microscope. Membrane formation is usually complete at the anterior end of the egg first.

The factors causing the egg to form this membrane under natural conditions are not clear. The early work by Tichomiroff (1902), who found that sulphuric acid could activate the silk worm egg suggested the study of pH. Analogy with the fertilization membrane of marine eggs suggested experiments on the effects of osmotic pressure. The following observations and

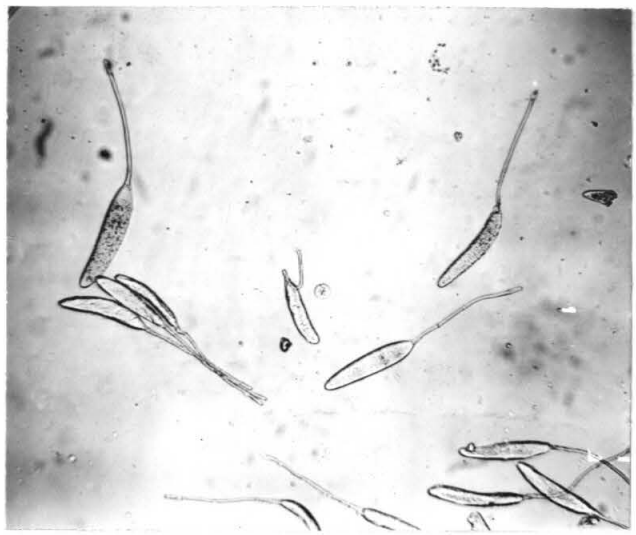

Fig. VII Ovarian egg dissected in Ringers.

(X 35)

Fig. VIII Ovarian eggs after treatment with distilled water. (X 35)

experiments were undertaken to elucidate this question.

The ovarian egg dissected out in approximately isotonic saline (0.127 molal) does not form a membrane but soon loses its clear opalescence, turns brown. The egg dissected out in the blood of Drosophila does not form a membrane. If, however these eggs are treated with distilled water or acid a considerable proportion throw off typical membranes. This structure appears first as a bubble-like protrusion at the periphery. Growing larger it extends to include the whole egg except the pedicel. Fig. 8 shows such an activated egg. Thus two factors appear able to initiate membrane formations, pH and osmotic pressure. These two factors were investigated one at a time while the other was controlled. The pH of the blood of the mature larvae of Drosophila melanogaster was measured (in collaboration with Dr. John B. Buck) by means of the Beckman micro glass electrode. Four determinations gave 6.79, 6.80, 6.84, and 6.80, average value 6.81.

A citrate phosphate buffer system was made up from 0.127 molal citric acid and 0.127 molal K_2HPO_4. Portions of these were mixed and adjusted to pH 6.8 with the glass electrode. This solution was diluted 1:1, 1:3, and 1:10 with freshly distilled water and the pH readjusted to 6.8. Groups of young female Eucoila were dissected in these solutions and after 10 minutes the eggs were counted and classified for membrane formation. Below are the results.

Table 2.

Effect of lowering osmotic pressure on membrane formation. pH 6.8

	No. eggs without membranes	No. eggs partial membranes	No. eggs complete membranes
0.127 m. buffer	41	9	0
dil 1:1 dist. water	25	4	0
dil 1:3 dist. water	38	16	14
dil 1:10 dist. water	56	17	37
distilled water	19	64	39

Thus lowering the osmotic pressure has a decided influence on membrane formation though some eggs resist activation completely.

The effect of changing the pH while keeping the osmotic pressure approximately constant was next investigated. 0.127 molal citric acid has a pH of 2.2; that of 0.127 molal K_2HPO_4 is about 8.7. These were mixed in various proportions to give intermediate pH values and adjusted with the aid of the glass electrode. Ovarian eggs were dissected out in these media and after 10 minutes counts made on membranes formed.

Table 3.

Effect of pH on membrane formation at constant osmotic pressure,

pH	No. eggs without membranes	No. eggs partial membranes	No. eggs complete membranes
2.2	2	3	36
3.0	2	17	21
4.0	8	27	1
5.0	29	20	0
6.8	41	11	0
8.5	52	6	0

If the ovarian eggs are treated for five minutes with distilled water and then transferred to three cc of buffer pH 8.5 the membranes persist. If a few milligrams of pure crystalline trypsin (Northrop) are added the membranes collapse within two minutes and are apparently digested in the course of some hours. In control experiments without trypsin the eggs die but the membranes persist. This indicates that protein is an important constituent of the membrane.

Most of the eggs which respond to osmotic pressure and pH changes by membrane formation show signs of degeneration after fifteen minutes indicating that the treatment is rather drastic.

Attempts to make the activated eggs develop by injecting them into the body cavities of second instar larvae have so far not succeeded. They may be found dead in the haemocele after twelve hours. No development was visible in twenty two trials although they may have been damaged by over exposure to distilled water.

Mention has been made of the compression to which eggs are subjected in passing down the ovipositor. In view of the results of massaging the silk worm egg (Tichomiroff et al.), it is possible that the stimulus to membrane formation under natural conditions arrises from passage through the ovipositor.

This membrane formation is probably not of general occurrence among Cynipidae since it has apparently not been seen before by others working on parasitic cynipids.

We shall next sketch in outline, the general features in the ensuing life history for reference in abnormal situations.

Approximately 100 young Drosophila larvae, 6 hours old, were subjected to parasitism by a single female parasite. She was watched until she had made fifty ovipositions. Each larva was removed immediately after oviposition had occurred and placed in a vial with food. One vial was used for all the larvae parasitized within atwenty minute interval; a fresh vial for those parasitized in the next twenty minute interval etc. The time limits were then marked, and the whole left at 22 C. At intervals 5 or 6 larvae were dissected, the living embryos therein examined and photographs taken of typical specimens.

A series of larvae dissected between 1 and 4 hours after oviposition showed no marked changes. Despite the fact that the egg contains a very small amount of yolk, no polar body formation has been seen. Cleavage first becomes clearly evident at about 6 hours after oviposition. Fig. #9 shows an egg in the cleavage stage 8 hours after oviposition. Cleavage continues rapidly and at sixteen hours the blastoderm is clearly formed. The pole cells which form at the posterior end of the embryo, and whichby analogy with other insects probably give rise to the germ cells, may be seen in fig. 10.

Between 16 and 20 hours the embryo does not change greatly in appearance. The pole cells may still be seen at the posterior end. The blastoderm which was seen as a single layer of cells at 16 hours, at 20 hours appears to have undergone considerable thickening.

At 28 hours the proctadaeal invagination has been completed, the pole cells have disappeared. The proctadaeum may be faintly seen.

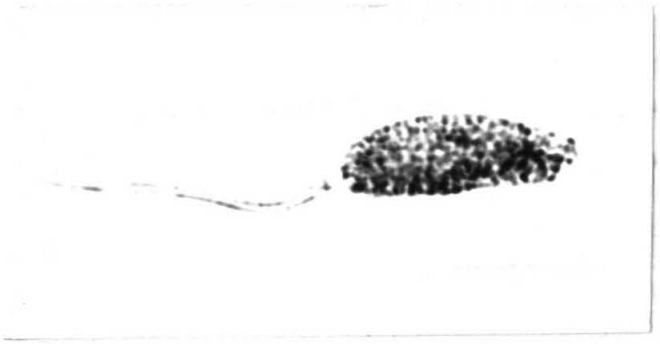

Fig. IX Egg of Eucoila undergoing cleavage 8 hours after oviposition. (X 90)

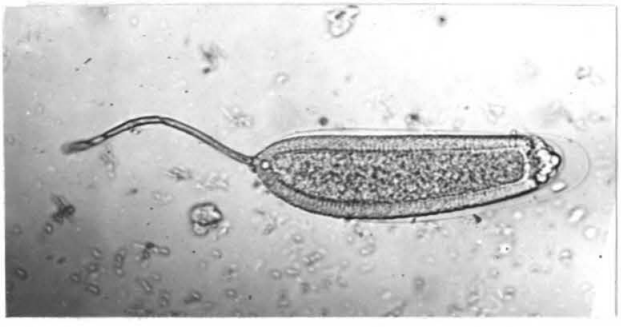

Fig. X Photograph of living embryo 16 hours after oviposition with blastoderm, pole cells, and egg membrane clearly visible. (X 107)

The first signs of segmentation are visible between 32 and 34 hours. The anterior end of the embryo shows a characteristic head fold. Fig. 11 shows the appearance at 34 hours. Segmentation continues, the larval head becomes defined, and at 40 hours the caudal appendage begins to form as an outpouching from the posterior end, ventral to the proctadaeum. At 48 hours the larva is completely formed inside of the membrane which has been described previously. At this time the larva is moving feebly. Fig. 12 shows a fully formed motile larva enclosed in the egg membrane. The cauda is folded under the ventral surface. The thoracic appendages may be seen in part. Eclosion occurs at this time and begins by puncture of the egg membrane by the mouth parts of the larva. The whole process has not been observed in this form though larvae with only a portionof the head extruded have been seen. James (l.c.) is of the opinion that the cauda is of material assistance in eclosion from the egg (Cothonaspis rapae).

The primary larva of Eucoila drosophilae is typically eucoiliform and very similar in appearance to those figured by Keilen and Pluvinel (1913) for Eucoila Keilini (parasite of Pegomyia winthemi) and James for Kleidotoma marshalli. The larva has ten segments exclusive of head and cauda. The first three (thoracic) segments are equipped with pairs of appendages.

The surface, of which mention will be made later, is waxy or lipophilic. The larva lies free in the host's haemolymph and may be found in very different situations. Its food at this stage is haemolymph. Fig. 13 shows a photograph of a living parasite taken through the body wall of a

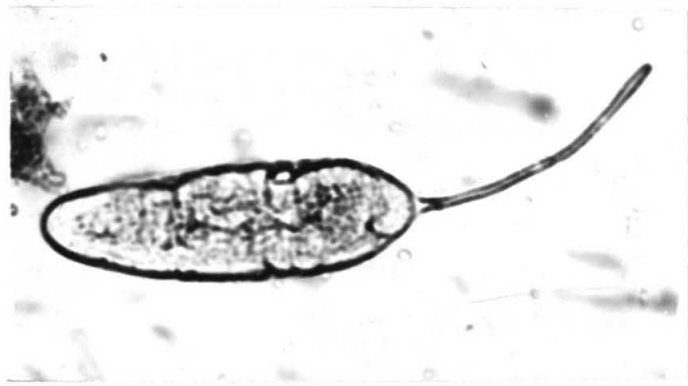

Fig. XI 34 hour old living embryo showing the beginnings of segmentation and a characteristic head fold. (X 140)

Fig. XII Fully developed motile larvae ready to hatch from the egg membrane, 48 hours old. The cauda is folded along the ventral surface. Two thoracic processes are visible.

mature Drosophila larva.

At the time of eclosion the parasite larva is about 0.35 mm long exclusive of cauda. In the course of two days considerable growth occurs and by the time the Drosophila larva pupates the parasite has doubled its size. The gut contents become yellow, the thoracic processes disappear and the cauda becomes progressively shorter. Fig. 14 shows the parasite larvae 4 1/2 days after oviposition. Parasitized and unparasitized Drosophila larvae of the same age pupate at the same time.

At 6 1/2 days of age (1 1/2 days after Drosophila pupation) parasite larva has become a typical hymenopterous grub with mandibles but with no appendages of any kind. Fig. 15 shows this form.

The parasitized Drosophila larva has up to this time proceeded in its usual pattern of development. It has passed through histolysis,the imaginal discs are forming the surface of the body including the eyes, leg and wing rudiments. At about this stage the grub emerges through the body wall and feeds upon the newly formed hypoderm. The internal structures for the most part have been entirely consumed. The remains of a few trachael shreds, some fat body and fragments of the malpighian tubules may occasionally be seen. The eyes do not form pigment. The grub consumes the remainder of the drosophila pupa inthe ensuing four days and by the eleventh day pupates within the drosophila pupa case and commences its own metamorphosis. If removed at this time by careful dissection it will continue to develop. The eyes are well formed and have pigment by the 15th day. The black eye color develops

Fig. XIII Photograph taken through the body wall of a large Drosophila larvae. The head and cauda and a number of segments can be clearly seen. The larvae is not attached.

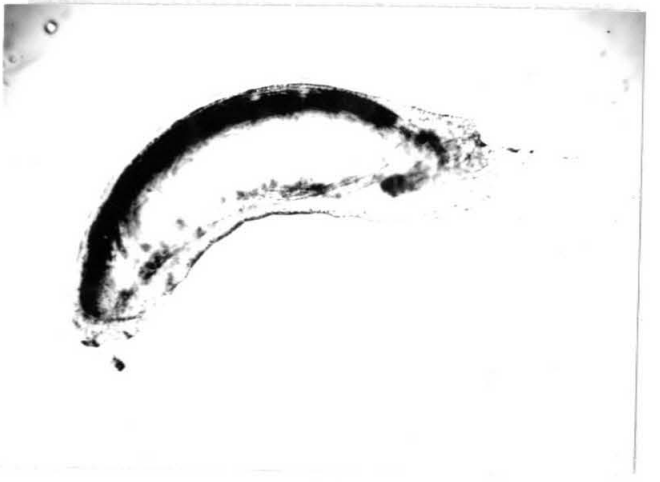

Fig. XIV Four and one half day old parasite larvae, 0.7 mm long.

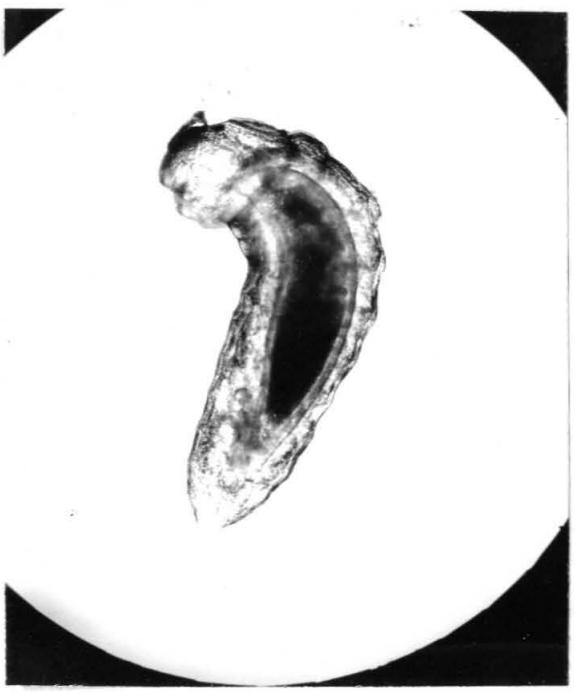

Fig. XV Large Eucoila grub.
Note food in gut.

first as a light red pigment which gradually darkens. The body becomes black by the 17th day.

Within the pupa case it becomes oriented with its head in the anterior end of the pupa case and turned slightly on its longitudinal axis. (Pachycrepoidius dubius (p l.) does not orient itself within the pupa case. I have observed that approximately 50 per cent. of the insects of this form come to lie with their heads in the posterior end of the pupa case.)

The Eucoila drosophilae males hatch from the pupa case on the 19th day and females on the 20th. Mating occurs almost immediately and oviposition may proceed without appreciable ageing of the females. Females isolated before hatching produce only males. If females are produced thelytokously they are rare. The adults live less than three weeks at ordinary temperatures. Thus generations do not overlap one another.

The above description of the life history is based on observations in the Florida wild type strain of Drosophila melanogaster. A study was next made of the reaction of various mutant races to these parasites.

Development in Mutant Races

Mutant genes which alter the morphology and physiology such as dwarf, giant, and minutes were investigated together with many others in the hope that these differences might affect the susceptibility to parasitism. This expectation has not been realized and the results thereof though occupying considerable detail will be reported briefly.

In general for the preliminary survey eight female and five male adult parasites were placed with the flies being tested

in the regular culture bottles. When larvae began to pupate, a series of fifteen to twenty of various sizes were dissected to ascertain whether oviposition and development were proceeding in the usual way. When flies had hatched they were classified and counted; adult parasites were recorded at a later date.

In this way the parasites were successfully grown on the following stocks:

M/c_3el	gt w^a/gt bb
M_w/c_3el	fes bt/Cy, al lt L^4 sp^2
bb	l 36el6/Cy hom 3
lh B car bb/y^2w^aec f $\overline{xx}$	X'ple
Inv. st^v, st^vdw	all
chubby	rucuca

The two minutes, bobbed, and late hatching are characterized by having life cycles slightly longer than the normal. They are nevertheless completely susceptible hosts. The late hatching attached - X stocks produced 316 parasites of both sexes, 13 B males, 11 y^2w^aec f females, no superfemales. 3 females with yellow malpighian tubules, hence superfemales, were isolated as larvae; gave, 2 male and one female adult parasites. The tests of dwarf, chubby, and giant, which have rather extreme morphological differences from wild type showed that these stocks nevertheless behave in the same way. This was true of the female sterile stock, the females of which have rudimentary ovaries. The stock 136el6/Cy is a balanced stock; both homozygous second chromosomes are lethal in the pupal stage. In two experiments, a total of 166 parasites of both sexes, 20 Cy males and 27 Cy females were produced.

25 pupae isolated gave 3 Cy females, 2 Cy males, and 19 parasites. This indicates that pupae which are unable to become adults nevertheless are satisfactory hosts for complete wasp development.

It appears that the mere piling up of mutant genes in a fly does not prevent the wasp's development. This has been demonstrated with three stocks each of which contain a number of mutant characters. The wasps develop to maturity on the Xple stock containing: scute, bifid, eosin, echinus, ruby, and tan in the X chromosome; likewise on the second chromosome stock "all" containing the mutant genes aristaless,dumpy, black, pupple, curved, plexus, and speck; and on the third chromosome mutant stock "rucuca" containing the genes roughoid, hairy, thread, scarlet, curled, striped, sooty, and claret.

There is no a priori reason why genes which would serve to protect the fly should be expected to occur in a mutant stock rather than in a wild one.Accordingly the following melanogaster wild stocks were tested but were found to behave like Florida wild type: Swedish-B, Swedish-C, Oregon-R, Calif.-C, Idaho Falls, and Painted Canyon.

Parasitism of Other Species of Drosophila.

The course of events in parasitism of other species of Drosophila was investigated in some detail. Many were found to be completely susceptible, others regularly immune,while some were found to be partially susceptible. There appears to be no distinct correlation between susceptibility and taxonomic affinity. There are numbers of each of the two common drosophila

subgroups among the immune, susceptible, and partially immune species. This may be seen from the following list in which the letters F-1 and F-2 are placed after the name to indicate whether the species is more closely related to Drosophila melanogaster (the yellowish or reddish species) or to D. affinis (the blackish or greyish species). One of the immune forms, D. busckii, is not closely related to either, belonging to quite another group (E).

Completely Susceptible

D. pseudoobscura F-2 Race A (Texas)

D. pseudoobscura F-2 Race B (Santa Lucia)

D. hydei (true hydei) F-2

D. duncani F-1

D. funebris F-1

D. robusta (st) F-2

D. affinis F-2

D. virilis F-2

D. repleta F-2

D. immigrans F-1

Partially Susceptible

D. montium F-1 (Formosa)

D. mulleri F-2

D. ananassae F-1

Mutants: scute

forked cardinal

reduced

Completely Immune

D. takahashii F-1

D. bipectinata F-1

D. ananassae F-1

Wild strains: Texas

Formosa

Yucatan

Tuscaloosa

Mutants: white Beadex

D. busckii E

D. melanica F-2

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To the list of susceptible species may be added D, simulans F-1. I have not studied the species, but Dr. W. P. Spencer informs me that the parasites will develop on this as a host. Tests on D. immigrans have not been very satisfactory because of the tendency of this species to over-populate the cultures. This results in a considerable mortality in the pupal stage. Many isolated pupae produced neither flies nor wasps. However the parasite has been grown in small numbers on this species. The heavy mortality is thought to be due to the overcrowding and not to the parasitism, although this is not certain.

Drosophila mulleri and D. montium have been listed as partially susceptible because it has not been possible to grow the wasps on these stocks with any marked success, although the incidence of parasitism, has been shown by dissections, to have been reasonably high.

Of twelve cultures of D.mulleri subjected to parasites

(8 females and 5 males) only three produced any wasps and these in small numbers, although numerous adult flies appeared. Thus culture 2510 was made up with 8 female and 8 male flies. 8 female and 5 male parasites were introduced. Dissection showed 7 out of 10 larvae to contain living parasites. Yet 67 females and 55 male flies hatched and only 4 females and 1 male wasps. A selection effect was tested for by exposing some of these flies to the five wasps. Fifteen larvae gave nine parasites on dissection, but 50 flies hatched (sexes approximately equal) and only 1 female parasite. In the next generation only flies were produced. The cause of this anomaly is not known.

In D. montium, oviposition is normal. Living parasite larvae may be recovered from 2nd instar drosophila larvae. They cannot be found in pupae after histolysis in any large proportion. Of seven cultures only one produced wasps. It gave, flies: 68 females and 74 males, wasps: 11 females and 23 males. These wasps were used in an attempt to raise a second generation on this host. Ten montium females and some males were placed in each of two bottles together with the wasps. No adult parasites were produced in the next generation.

As in the preceding observation onD.mulleri, selection does not seem to avail the parasite of much. If selection were at work it seems to have been most effective in favoring the flies (selection for immune individuals rather than selection of parasite genotypes adapted to these species). It is difficult to visualize how selection can be of much avail since both the flies and parasites used were rather highly inbred.

All the wild strains of D. ananassae are immune to parasitism. Tests were made in the usual way of several mutant stocks available. These were the white Beadex stock from which no parasites were produced in two cultures, and scute, forked cardinal,and reduced which gave a few adults. The reduced stock gave 1 female parasite from two cultures and was not studied further.

Culture 2520 scute, ananassae, produced 182 flies of both sexes, 16 female and 8 male parasites.

Culture 2521 scute produced 5 female and 2 male parasites.

Culture 2522 forked cardinal ananassae produced 213 flies of both sexes, 9 female and 8 male parasites.

Culture 2523 forked cardinal, produced 137 flies, 5 female and 3 male wasps.

Pair matings were made in attempt to secure a completely susceptible ananassae stock. Six single pair cultures of scute gave 2 cultures containing wasps, 13 females, 11 males, and 9 females, 12 males. Second generations raised from these produced no wasps. Reciprocal hybrids between wild type (Yucatan) and forked cardinal ~~cardinal~~ were grown. There were no wasps among the F_1 generation from either cross (15 cultures each; single pair matings). Single pairs were isolated from each of these thirty cultures and exposed to parasitism. No wasps were produced. The parents of the hybrids were takenfrom cultures never exposed to parasitism, hence were not "selected" stocks.

Development in Immune Species

It has not so far been possible to raise the parasites on D. melanica, D. busckii, D. takahashii, D. bipectinata, or the wild type strains of D. ananassae. Dissection of approximately 75 larvae per species showed that oviposition is normal but that development usually proceeded to a certain stage and then ceased. Eggs which fail to develop in these hosts turn brown and then black. Although melanization of the parasite embryo makes it difficult to ascertain to what stage development has proceeded before death occurs, these details can be made out if the embryo is seen while turning brown. In many cases these black, melanized, eggs survive and histolysis may be found in the body cavity or implanted in the hypoderm of adult flies.

In D. melanica death usually occurs within 24 hours of oviposition. Dissection at this time shows partially developed embryos beginning to turn brown. The blastoderm can be made out in most such cases; however a fair proportion (25%) have apparently not gone further than cleavage. 4 cases out of 92 dissections at the time of larval pupation gave black objects not certainly identified as larvae but which may have been.

D. busckii presents the most striking case of active immunity which I have seen. Eggs deposited in this host undergo degeneration while cleavage is in process. The eggs turn first brown then coal black and may be seen through the body wall of the larva. They retain their characteristic shape with pedicel intact and lie free in the haemolymph.

In D. takahashii development proceeds through blastoderm

and pole cell formation, then ceases. The rapid degeneration makes the determination of the exact stage of death a matter of some difficulty. No living wasp larvae (hatched) have been seen in 84 dissections, but in three cases black objects having the form and dimensions of a larva have been seen. However no body segments or thoracic appendages could be made out.

Development in D. bipectinata is somewhat variable. Approximately 15% of the embryos undergo degeneration in the egg stage. (65 dissections). The rest may hatch from the egg but die without further growth.

Parasite eggs deposited in the wild type strains of D. ananassae (four were tested) produce larvae which hatch from the egg. In D. melanogaster the following two days witness a rapid increase in the size of this larva and the accumulation in the gut offood which gives it a bright yellow appearance. In D. ananassae the parasite continues to live but fails to increase in size, or to take food into the gut. By pupation time approximately 50% have died but are not absorbed immediately. They do not pass through a melanized stage as do the preceding in which death occurs in the egg. In general their bodies do not survive histolysis and no trace of parasitism can be found in the adult fly.

Immunity

There is at present no general agreement concerning the importance of phagocytes in the defense reactions of insects to their parasites. Metalnikov (1907) studied the resistance of Galleria melonella to the tubercle bacillus and came to the

conclusion that this immunity was due principally to ingestive powers of the phagocytes. He was unable to find any evidence of antibodies as they are known in the vertebrates and concludes (1932) that they are of little importance in bacterial infections of Galleria.

This conclusion has been reached by numerous observers working with hymenopteran parasites. Pemberton and Willard (1918), Hollande (1920), Strickland (1923), Meyer (1926) have observed and described the encapsulation and destruction of parasite eggs by syncytia of host blood cells. This has led to the ~~trait~~ tacit assumption onthe part of numerous observers that phagocytes are the immediate cause of parasite destruction.

Thompson (1930) on the basis of observations of parasites in unfamiliar hosts concludes that the phagocytes are of little importance so long as the parasite remains alive. Phagocytes were observed to gather around dead larvae but never while they were still living.

Ekstein (1931) has called attention to the formation of melanin deposits around foreign bodies, including parasites, that have been introduced into the bodies of insects. He is of the opinion that this is an additional (chemical) defense mechanism. He found that the blood of Tipula caterpillars placed on tyrosine test paper caused a black spot. The blood of a Tipula caterpillar which was parasitized by a tachina possessed the same property. If, however, the tachina blood was mixed with its host(s blood, the reaction was inhibited. On the basis of this obscure phenomenon Eckstein has formulated a theory of immunity in which the phagocytes have an indirect role.

Recently, Flanders (1937,B) has suggested that the alleged "immunity" of certain insects to their parasites is a matter of unsuitability of the host blood as food. He found (1937,A) that the two sexes of certain chalcidoids require different hosts for their development. The males normally live in the body fluids of other hymenopteran parasites (i.e. as secondary parasites). The females live in the body fluids of lecanine scale insects. If both came to lie in an immune host only the female hatches as long as both are surrounded by body fluids. The female dies and is encapsulated. The male lives for long periods without molestation by the phagocytes of the host. This suggests that the female dies because the food is unsuitable. The male persists because he is adapted to live for long periods without food.

Such information as I have concerning immunity of Drosophila points to the conclusion that the phagocytes (true amoeboid cells) play little or no role in the destruction of living parasites. In those forms which resist the parasite in the egg stage, such as D. busckii or D. melanica, phagocytes cannot be found in the vicinity of the parasite embryo until the egg has begun to turn brown. In D. melanica eggs undergoing degeneration have been seen which were not yet in contact with phagocytes. If D. annanassae is used as a host the parasite egg produces a larva which fails to grow, but nevertheless lives for two or three days. It appears that in this case the parasite destruction is brought about by improper nourishment.

One of the most striking properties of the Eucoila

larva is their waxy surface. Such a surface is not wet by the larval blood or by acqueous media. A parasite larva placed in contact with a drop of parafin oil is immediately engulfed by the drop and cannot be separated from it. This surface property is of such a nature as to make it impossible for phagocytes to adhere to the larva since they must wet particles which they are to engulf or surround. Tests for this waxy surface made on dead parasite larvae in the immune D. ananassae showed that this property had not been lost. Thus, although the parasite was already effectively disposed of, the waxy surface remained. The inference is that the phagocytes were not directly responsible for the parasite's death.

It was thought desirable to attempt to confer the immunity of D. tackahashii, D. busckii, and D. ananassae, on D. melanogaster by injecting immune blood. Approximately 0.1 cmm was injected into parasitized second instar melanogaster larvae. Nevertheless from 28 injected larvae 16 parasites emerged (4 from blood of ananassae, 5 from busckii blood, 4 from tackahashii blood, 3 controls: melanogaster blood). The remaining 12 died as larvae.

The reciprocal experiment was tried using D. ananassae (Yucatan) as host and D. melanogaster as a source of blood. 19 D. ananassae larvae known to have been parasitized were injected in the second instar with approximately 0.1 cmm of D. melanogaster larval blood. 12 survived the operation and produced adult flies. It appears that the immunity or susceptibility could not be transferred by blood transfusions made under these conditions.

Superparasitism

In view of the very interesting experiments of Salt (1934) on superparasitism by Trichogramma evanescens, it was thought desirable to get evidence as to whether a faculty of distinguishing parasitized from unparasitized larvae existed in Eucoila drosophilae.

Fiske (1910) has defined superparasitism as the condition that results "when any individual host is attacked by two or more species of primary parasites or by one species more than once." Smith (1916) has restricted Fiske's definition of superparasitism to "that form of symbiosis occurring when there is a superabundance of parasites of a single species attacking an individual host insect." The definition accepted here is that of Smith. The emphasis is on the idea of superabundance.

Thompson (1924) has applied the mathematics of random distribution to the parasite problem and has developed an equation for the expected number of hosts parasitized in a population onthe assumption ofrandom distribution of parasite eggs.

It is $y = N(1 - e^{-\frac{x}{N}})$

where y = number of hosts parasitized

N = number of hosts

x = number of parasite eggs distributed.

Stoy in Salt (1932) has derived a more general expression for the expected number of hosts carrying 0, 1, 2, 3 --- parasite eggs in a population where parasite eggs are distributed at random. His expression is,

$$z = N\,{}_xC_p \left(\frac{1}{N}\right)^p \left(1 - \frac{1}{N}\right)^{x-p}$$

inwhich z = the number of hosts carrying p eggs

x = number of eggs distributed

N = number of hosts

$${}_xC_p = \frac{x!}{p!(x-p)!}$$

It is clear that for $p = 0$

$$z = N \frac{x!}{x!}\left(\frac{1}{N}\right)^0\left(1 - \frac{1}{N}\right)^x$$

$$= N\left(1 - \frac{1}{N}\right)^x = N\left\{\left(1 - \frac{1}{N}\right)^N\right\}^{\frac{x}{N}}$$

when N is large, $z = Ne^{-\frac{x}{N}}$

The number of hosts parasitized is then,

$$N - z_{p_o} = N - Ne^{-\frac{x}{N}} = N(1 - e^{-\frac{x}{N}})$$

which is Thompson's formula.

On the basis of Stoy's general equation Salt (1934) has analysed the existing adequate field data and shown that a number of parasites in the field do not distribute their eggs among the hosts in a random manner. He has in addition shown that Trichogramma exercises a sense of perception during oviposition, the effect of which, is to insure an "efficient" distribution of eggs and a low frequency of superparasitism. In a later work (1937) he has shown that the perceptive faculty depends upon a substance left by parasites on the surfaces

upon which they walk and inside the host eggs in which they oviposit.

The effects of enforced superparasitism in artificial populations have been studied and Salt has shown (1936) that it results in a net diminution of the parasite progeny and in alteration of the sex ratio in the direction of an increased proportion of males. He has shown that the amount of superparasitism is increased by increasing the ratio of parasites to hosts.

A limited number of observations and experiments suggest that in Eucoila the faculty of discriminating between already parasitized and unparasitized larvae is likewise present. Direct observation is of limited use in this material since the hosts unlike those of Trichogramma are moving. However the following observations are pertinent.

Single females have beenseen to make:

a. Two attacks on a single larva in 10 seconds. At dissection two eggs were found.

b. Two attacks on a si ngle larva within 25 seconds. The second insertion was brief. Dissection gave only one egg.

c. Four attacks on a single larva in thirty seconds. The first two attacks resulted in insertion of the ovipositor. The second insertion was brief (8 seconds).The third and fourth attacks were momentary explorations confined to the surface. Dissection gave only one egg.

These observations are independent and seen at different times. They suggested a more elaborate experiment to ascertain whether the distribution of eggs among hosts was a random one.

Two hundred young larvae (6 hours old) of D. melanogaster

were placed in a dish with food and exposed to 6 female parasites for 24 hours. At the end of this time, the parasites were removed, the larvae dissected and the number of larvae containing 0, 1, 2, etc. parasite eggs was recorded. The errors due to not finding parasite eggs are thought to have been very low since the eggs lie free in the haemolymph and emerge as soon as the larva is opened. 205 eggs were found. The expected number of larvae containing 0, 1, 2, etc. parasite eggs was calculated using the formula of Stoy. Below are the observed and calculated results.

	Number of larvae with 0, 1, 2, etc. parasite eggs.					
	0 eggs	1 egg	2 eggs	3 eggs	4 eggs	5 eggs
Expected 199.56	71.4	73.6	37.8	12.8	3.3	0.66
Observed 200	1	193	6	0	0	0

It is immediately apparent that far too many larvae received only one egg, too few received no egg, too few received more than one egg, than is demanded by the assumption of random distribution.

An experiment to ascertain whether increasing the ratio of parasites to hosts could affect the amount of superparasitism was undertaken.

Two dishes of the same dimensions were made up as follows: One contained 200 larvae (0 - 6 hours old) the other 100 larvae of the same age. In the first dish 6 female parasites were placed and left for 24 hours. Into the second dish 12 female parasites were introduced and left for 24 hours. Thus the ratio of parasites to hosts was four times as high in dish

one. The parasites were removed and 25 larvae from eachdish were dissected. Results:

Dish #1. 200 larvae: 6 parasites	Dish #2. 100 larvae: 12 parasites
2 larvae contained 0 eggs.	0 larvae contained 0 eggs.
21 larvae contained 1 egg.	2 larvae contained 1 egg.
2 larvae contained 2 eggs.	22 larvae contained 2 eggs.
0 larvae contained 3 eggs.	1 larva contained 3 eggs.

Thus superparasitism can be made to occur by increasing the density of parasites.

Salt (1934) has shown that in enforced superparasitism that the parasite Trichogramma elects to place its supernumary eggs in the larger hosts.This effect was tested for inthe following way.

A dish containing D. melanogaster larvae of various sizes was placed under a binocular microscope equipped with a micrometer eyepiece. Larvae were measured and those of two suitable sizes were selected for the experiment.In this way 35 larvae measuring 2.5 divisions and 35 larvae measuring 1.25 divisions were selected. They were placed together in a third dish and exposed to 8 parasites for 24 hours. At the end of this time the larvae were removed, dissected, and the number containing 0, 1, 2, etc. eggs were recorded. The results were:

Number of larvae containing:

	0 eggs	1 egg	2 eggs	3 eggs	4 eggs	5 eggs
Large larvae	1	4	20	9	1	0
Small larvae	0	28	7	0	0	0

It appears then from this table that although the amount of enforced superparasitism was not great the supernumary eggs were most often placed in the larger larvae. Since almost twice as many eggs were placed in large larvae as in small ones it is not excluded that the larger sized, and consequently,more active larvae were not simply encountered more frequently.

The Fate of Supernumary Eggs.

Drosophila larvae inwhich more than one egg is laid give rise to one and only one adult.The supernumary eggs do not develop. This phenomenon is of very common occurrence among the parasitic hymenoptera but the mechanisms concerned are not known. The modification of the sex ratio in Trichogramma has already been referred to and seems to be of such general occurrence in cases of superparasitism that Smith (1929) has been led to attribute this to "intrinsic" superiority of males in competition. I have no observations on the sex ratio of adult parasites emerging from superparasitized hosts as compared to hosts not superparasitized.

If more than one egg is deposited in a larva the second egg generally undergoes degeneration at about the time of blastoderm formation (12 - 16 hours). They may,however, continue to develop and hatch out of the egg as larvae. Their further growth is retarded, no increase in size can be seen, and by drosophila pupation time they invariably die. Experiments designed to study the relationbetween the time interval between the two ovipositions and the amount of subsequent development of the younger embryo should be

illuminating.

Thompson and Parker (1930), on the basis of their observationin Eulimneria crassifemur, a parasite of the European cornborer, in which supernumary parasites die, have suggested that this is probably due to production by the oldest larva of an enzyme which has a lethal effect on the younger larvae. This idea had been advanced years ago by Timberlake (1910) in connection withthe aphid parasites.

In Eucoila drosophilae I have observed cases in which two eggs were present but only one was developing. If this is due to inhibition of one egg by another, it implies either absorption or secretion of materials through the membrane of the developing egg.

Host Selection

Thompson and Parker (1927) in the last general review of the problem of host selection have taken a rather too pessimistic view. They say, "the laws underlying the problem of host selection are not capable of expression in scientific terms or discoverable by scientific methods."

Picard (1922) has recorded an observation of extreme interest in his paper on the parasites of Pieris brassicae. If a small paper cone is soaked with Pieris blood and placed within range of a female Pimpla instigator, the parasite pierces the cone and continues to do so until the paper is dried up, though she lays no egg. This observation clearly suggests a reflex mechanism.

It has been shown by Ullyett (1936) for Microplectron fuscipennis and by Thorpe and Jones (1937) for Nemeritis canescens that host finding is governed by an olfactory sense situated in the antennae, but that actual oviposition depends on some other factor such as texture, and to some extent, size.

It has been demonstrated by Salt(1935) that Trichogramma selects its hosts on the basis of size alone, olfactory stimuli being of little importance in this insect.

In Eucoila drosophilae the stimulus to unsheath the ovipositor does not come from the larvae since females will explore food chips which contain no larvae. The stimulus is probably provided by some factor produced by yeast. The larvae of Sciara are not recognized as possible hosts so long as they remain on their customary substrate. If they are transferred to a drosophila medium containing yeast , parasites will attack them. The ovipositor is inserted for three or four seconds and then withdrawn. However, no eggs are deposited. It is evident then that some specific property of drosophila blood which Sciara does not possess is required for actual oviposition.

No evidence has been found of a distinct preference for hosts in which Eucoila drosophilae is able to develop as opposed to host larvae in which it cannot. They apparently oviposit almost as readily in D. ananassae or D. montium as in D. melanogaster.

James (1928) found that the related Figites anthomyiarum which is normally parasitic on several different carrion feeding dipterous maggots, could be induced to oviposit in the cabbage root maggot, Hylemyia brassicae, only if these larvae

were carefully washed and then placed for a time in very putrid meat. Subsequent dissection proved that eggs were actually deposited. The eggs do not develop. The normal parasite of the cabbage root maggot (Cothonaspis rapae) could not, however, be induced to oviposit in the carrion feeding larvae.

Effects on Mixed Cultures

D, montium has been described as a species partially immune to parasitism by E. drosophilae. This faculty enables the species to successfully compete with D. melanogaster in mixed populations providing the parasite is present. This is shown in the following way.

A culture made up using 5 females and 3 males each of D. montium and D. melanogaster (white forked) and 10 female parasites produced 46 females and 61 males D. montium; 159 male and 62 female parasites; 25 females and 26 males of D. melanogaster. The net advantage is slightly in favor of D. montium despite the higher fertility of melanogaster. To offset this, cultures were made up with 100 young larvae (6hours old) of each species and 10 female parasites. Four such cultures and two controls (no parasites) gave:

	Culture	montium		melanogaster (w f)		parasites	
		female	male	female	male	female	male
	2531	4	0	0	0	4	40
	2532	6	7	0	0	19	75
	2533	10	8	0	0	26	62
	2534	7	1	0	0	21	73
control	2535	22	18	49	43	--	--
control	2536	24	31	49	47	--	--

Parasitism under such conditions may enable one species to thrive where it would otherwise be impossible. The above results might mean that the female parasite prefers to place its eggs in larvae of D. melanogaster, its customary host. This was found not to be true in the following experiment.

50 young larvae of each kind were placed in a dish together with 15 female parasites. After 24 hours the parasites were removed and 40 larvae of each kind dissected. (The 2 kinds of larvae are distinguishable by the light colored malpighian tubules of white forked larvae)

The results were:

	Number of larvae containing:			
	0 eggs	1 egg	2 eggs	3 eggs
montium	0	20	19	1
melanogaster (wf)	0	8	28	4

There appears to be a slight preference for the larvae of D. melanogaster. This effect is small, however, for , of the total eggs deposited (137), 61, or 44.5%, were in D. montium. The difference in supernumary eggs is thought to have been due to a slight though systematic difference in host size. The important fact is that no larvae of either kind escaped parasitism.

Conclusion

The experiments and observations on Eucoila drosophilae Kieff. which are recorded in the preceding pages represent an attempt to prepare the ground-work for extensive studies on parasitism of Drosophila. Because of its introductory nature the investigations have been comprehensive rather than exhaustive. From this point of view it has had the character of a survey rather than that of an analysis.

Much of the work has been descriptive -- far more than suited the author's taste -- but this is necessarily the case with a subject in its beginning.

It is a pleasure to acknowledge the assistance and advice of many of my friends both in this department and at the Citrus Experiment Station.

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Summary

1. The life history and development of Eucoila drosophilae from oviposition to hatching of the adult is outlined.

2. A peculiar egg membrane formation is described, and its natural formation simulated in vitro by treatment of ovarian eggs with acid and distilled.water.

3. It is shown that not all species of Drosophila are suitable hosts; the stages at which development ceases in immune forms are described.

4. Factors contributing to the immunity of these forms are discussed in the light of our present knowledge of immunity in insects.

5. The presence of a faculty in the parasite which enables them to discriminate between parasitized and unparasitized larvae is demonstrated.

6. The problem of host selection is discussed and it is demonstrated that the parasites are unable to discriminate between immune and susceptible species.

7. It is shown that the stimulus to oviposition is not received from the larvae but from something in the food on which they live.

8. It is suggested that the choice of a proper host depends on a suitable composition of the host's body fluid.

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