A BIOLOGICAL AND DEVELOPMENTAL STUDY OF *COELOIDES BRUNNERI* VIER., A PARASITE OF THE DOUGLAS-FIR BEETLE, *DENDROCTONUS PSEUDOTSUGAE* HOPK.

by

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INTRODUCTION

The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopkins, has been responsible for large losses over the last few decades because of the trees it has killed. Entomolgists have touched on various phases of the biology of this insect and of its natural enemies. Still, the study of the complex interrelationships between it and the many factors contributing to its abundance is still in its infancy. The life histories of all the parasites and predators which prey on the beetle are incomplete, or in some cases virtually unknown.

The present investigation is an attempt to close these gaps in our knowledge by supplying some basic data on the life history of a primary insect parasite of the Douglas-fir beetle, *Coeloides brunneri* Viereck (Hymenoptera:Braconidae). This parasite is incompletely effective in controlling the abundance of the Douglas-fir beetle because of its inability to occupy fully the distribution of its host within a tree due to bark thickness. It appears to be present, however, in any geographic locality where its host is present, and shows promise of becoming more effective in the years to come as old growth stands are converted to second growth.

The problem was broken down into two major phases. One portion consisted of field and laboratory experiments and observations to determine the biology of the parasite and the host-parasite relationships, with the ultimate aim of developing silvicultural practices which will aid the parasite in reducing the frequency of beetle outbreaks and their severity when they occur. The other portion consisted of a developmental study of the parasite commencing with cleavage within the egg and tracing the embryonic development and the external morphology of the immature stadia.

The writer wishes to express his appreciation to Dr. J. A. Rudinsky of the Department of Entomology, Oregon State University, under whom this work was conducted. Financial support has been provided by the Forest Research Division, Oregon Agricultural Experiment Station. The writer also wishes to express his thanks to Dr. F. H. Butt, Professor Emeritus, Cornell University, for certain interpretations regarding embryological development; to Karl Drlica for aiding in the collection of certain data; and to Miss Margaret Hsieh for making the drawings from which Figures 61 and 62 were reproduced.

Since the completion of this work, some specimens sent to the United States National Museum were identified as Coeloides scolyti Cushman by C. F. W. Muesebeck. He is under the opinion that C. scolyti will need to be suppressed as a synonym of C. brunneri.

MATERIALS AND METHODS

Techniques in Laboratory Rearing

Source of breeding stock- The original stock for laboratory rearing was collected in late spring as larvae overwintering in cocoons. These original collections were from several sources: the McDonald Forest of Oregon State College, approximately seven miles north of Corvallis, Oregon; the eastern slope of Mary's peak, approximately 11 miles west of Corvallis; Pedee Creek, approximately 17 miles northwest of Corvallis; and Weyerhaeuser's McDonald tree farm near Centralia, Washington. The bark infested with beetles and parasites was stored at 45°F. until needed. By transferring it to 75°F. adult parasites would emerge starting after approximately 10 days.

Mating and handling adults- Once a supply of newly emerged adults was on hand the first proceedure was to mate the females. Either individual males and females were placed together in small vials or the females were collectively placed in a small container (Figure 2) with the males.

The insects were kept and fed individually in vials for some experiments and collectively in ice cream containers for others. In the former case double ended vials were used. On a waxed paper strip protruding into each vial

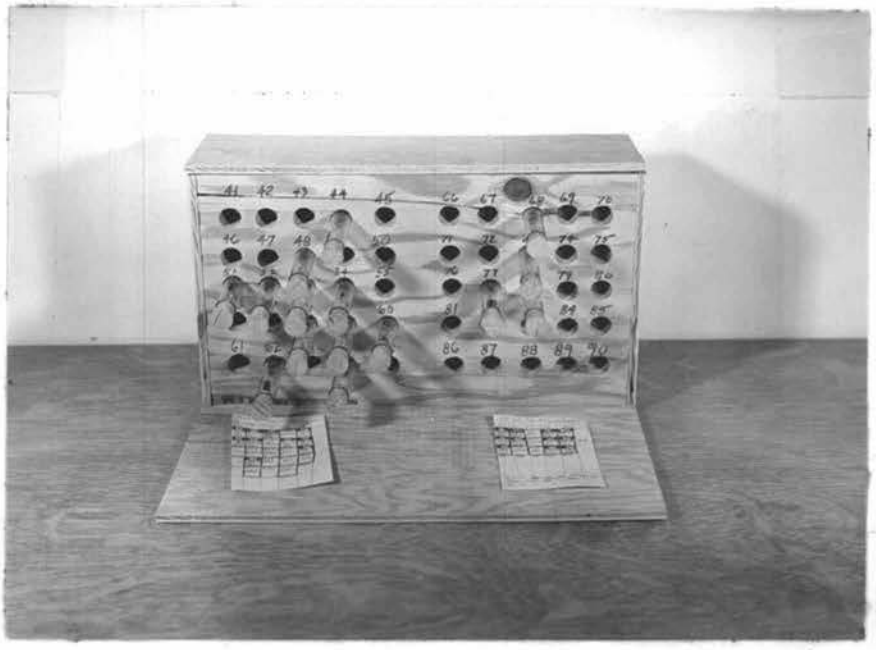

Figure 1. Type of vial holder used for holding adults individually.

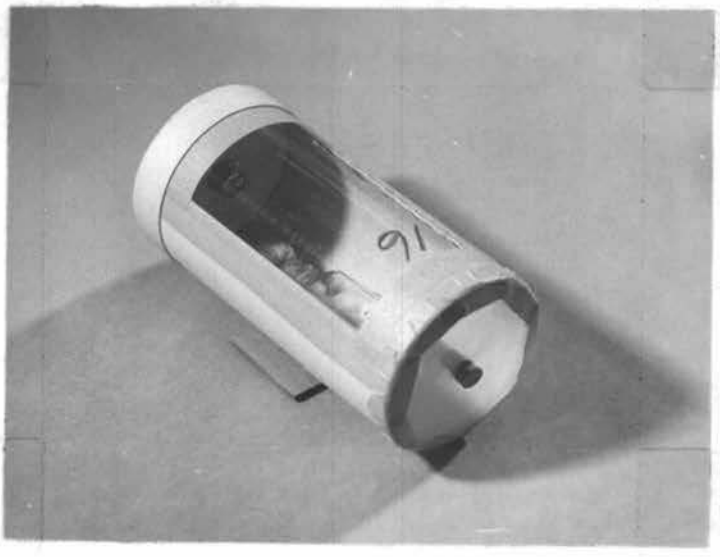

Figure 2. Ice cream carton transformed into a holding cage for adult parasites. The plastic window allows entrance of light for photoperiodic treatments.

and held in place with a cork, a drop of liquid food was placed. In some cases a dried raisin was also placed in the vial as food. The other end of the vial was placed through a hole in a plywood board and abutted against plastic screening stapled to the other side of the board (Figure 1). A wooden chamber was constructed and used to maintain a high humidity inside the vials by means of moist paper towels and a pan of water. Ice cream cartons used to hold parasites (Figure 2) were fitted with screen on one end, a plastic window on one side and a cork on each end to allow access for an aspirator. Water was supplied from a small bottle with a cotton ball stopper placed inside the container. Liquid food on waxed paper strips and raisins were placed on a paper towel lining the bottom of the unit.

Rearing in logs- Oviposition was secured by placing females in cages under suitable environmental conditions with a log section infested with susceptible host larvae. Ovipositing females were collected every two days and held overnight in ice cream cartons for purposes of feeding.

When oviposition cages were placed under greenhouse conditions the light intensity was reduced by covering portions of the glass with tarpaper in a checkerboard pattern. In rearing rooms where there was no natural light, artificial light was supplied by banks of fluorescent

lamps suspended approximately two feet above the tops of the cages giving an intensity of approximately 40 foot-candles at the top of the cages. Photo period was controlled by time switches[1] and by the use of black cloth placed over the ice cream containers and cages. Attempts were made to hold ambient temperatures constant, usually at 75°F., but for some experiments 85°F. was maintained.

Figure 3. Log section being shaved so that the bark would be thin enough for parasites to reach through with their ovipositors at any place.

1. Time switches were obtained from the International Register Company, 2624 W. Washington Blvd., Chicago 12, Illinois.

Cages for the most part were similar to those used in previous studies (86, p. 12-13) with the exception that clear plastic was used instead of screening for most of the cage and a sliding sleeve was added to each. Humidity was maintained at a high level by frequently wetting the floor. The logs and cages were set on white cloth with two objectives in mind: first, to aid in locating dead insects which fell to the floor; and second, to aid in holding moisture on the floor of the cages to increase the humidity.

After oviposition was complete or when sufficient time had elapsed so that the insects from the first eggs laid were almost ready to emerge, any ovipositing females still remaining were removed. The same cage was then used to collect the emerging progeny.

Rearing individual insects- Exact oviposition sites were marked on the logs by means of two insect pins placed one above and one to the side of the ovipositing female. The time the female completely withdrew her ovipositor from the bark was considered the time of oviposition and was marked on a card and placed on a third pin directly over the oviposition site. The bark was carefully removed and the egg and host larva were transferred to a small Syracuse watch glass lined with moist paper towel. The unit (Figure 4) was covered with a 22 mm. square cover slip and incubated under constant temperature conditions for observation. Moisture was replenished in the dishes

at the time of observation.

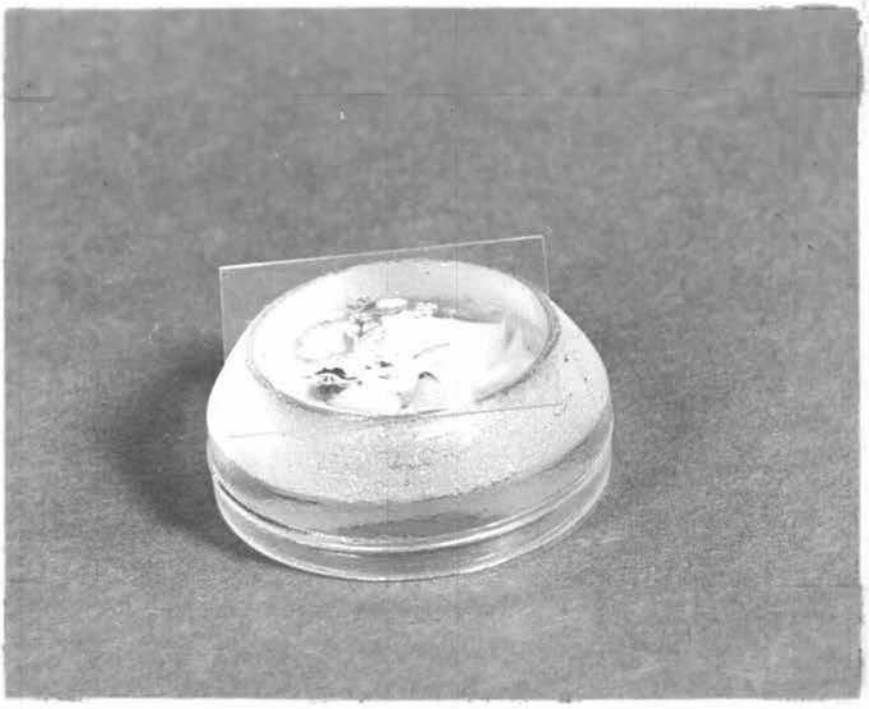

Figure 4. Watch glass unit used for observing the development of individual parasites.

The first instar sometimes commenced feeding on the host within a few hours, but often it was necessary to place it on the host with a camel hair brush. Head capsule measurements were taken with a calibrated eyepiece micrometer in a dissecting microscope at a 90X magnification.

Temperature control- Rearing rooms were heated for the most part with steam heat. Controlled temperature was achieved with supplemental heat produced by an electric space heater regulated by a separate control placed some distance from the heat source. The control unit

consisted of a bimetallic thermoregulator[1] and a single pole, single throw, magnetic relay[1]. Cooling of the rearing rooms during summer was provided by thermostatically controlled Frigidaire room air conditioners.

A walk-in cooler was maintained at 45°F. For temperatures between 45°F. and room temperature an incubator[2] and two plywood temperature cabinets with small heating elements and control units similar to those described above were placed in the walk-in cooler. These same cabinets and incubator were placed in one of the rearing rooms maintained at 75°F. for temperatures above the latter. Temperature controls were adjusted and periodically checked by consulting recordings taken on a portable relative humidity and temperature recorder[3] and were set so that the desired temperature was midway between the maximum and minimum reading of each cycle. A glass mercury thermometer[4] marked at intervals of 1°C. was left permanently in each temperature unit as a check on temperature conditions.

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1. The thermoregulator (catalog no. 99012) and the relay (catalog no. 99725) were obtained from the Central Scientific Company, 1040 Martin Avenue, Santa Clara, California.

2. The incubator was from the National Appliance Company, 7634 S. W. Capitol Highway, Portland 19, Oregon.

3. Foxboro Company, Foxboro, Massachusetts.

4. Owens-Illinois, Toledo 1, Ohio.

Measuring Log Area Available to Parasites

In trying to establish a relationship between bark thickness and the effectiveness of Coeloides brunneri in parasitizing its host, it was realized that factors such as maximum bark thickness, minimum bark thickness, number and extent of crevices and parasite ovipositor length should all be considered. These factors were incorporated into a single measurement as follows.

The average length of the parasite ovipositor was determined by measuring one hundred individuals with a calibrated eyepiece micrometer of a dissecting microscope. A piece of metal was filed to this measurement and was used as a gauge to compare with bark thickness to determine if the thickness of bark was greater than, equal to, or less than the average length of the ovipositor. This gauge was fitted onto a device constructed to determine the percentage of the bark at a given height in a tree which was less in thickness than the measurement of the gauge, or the mean ovipositor length (Figure 5).

The device consisted basically of two units. There was a circular base with a raised lip around its outer edge and a central assembly pivoted at the center of the base which was movable through 360 degrees. The central assembly itself consisted of two main parts, a base and a movable block running within it. A long hollow tube was

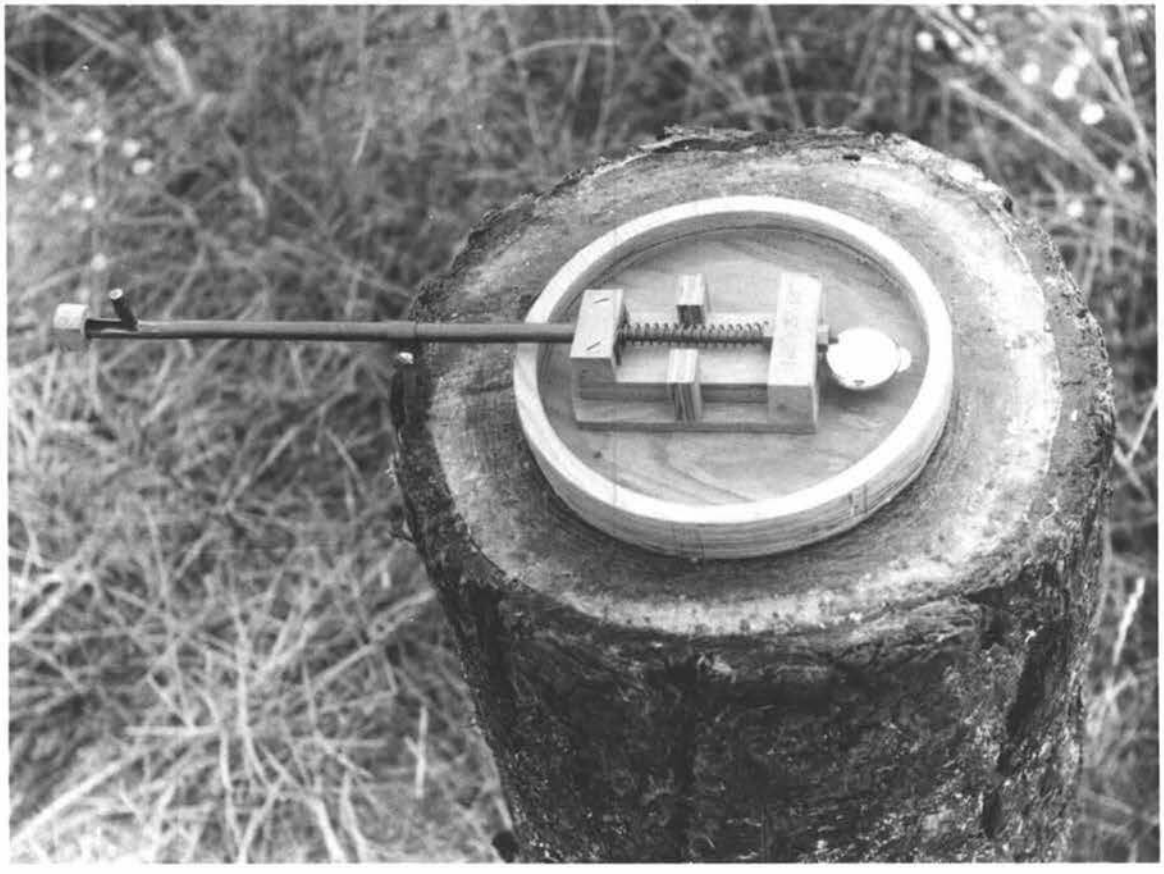


Figure 5. Device used for determining the percentage of the log circumference which had a bark thickness less than the mean parasite ovipositor length.

mounted on the movable block of the central assembly. A rod ran through the tube and was rigidly fastened to the base of the central assembly. The distal end of the rod was turned up and ran in a slot in the end of the tube. Together the tube and rod formed an arm extending out over the circular main base. A map measurer was affixed within one end of the movable block of the central assembly and ran along the inside of the lip of the circular main base as the central assembly was turned. The tube and movable block of the central assembly were spring loaded so that the map measurer only recorded when the spring was compressed by squeezing together the end of the tube and the turned up end of the rod. Thus, the map measurer was made to record intermittently by alternately squeezing and releasing at the end of the arm as the central assembly was rotated. The gauge filed to the mean ovipositor length was mounted on the arm and was moved in or out depending on the diameter of the log being measured.

In use the device was applied to the center of a cross section of a log. The gauge was slid along the arm until it was above the bark and the arm and central assembly were rotated through 360 degrees while the circular base was held stationary. Whenever the outer bark thickness was less than the gauge, the end of the arm was squeezed, allowing the map measurer to record. When the outer bark thickness was greater than the gauge the map measurer was disengaged by releasing the spring pressure. To determine the

percentage of the log cross section which had an outer bark thickness less than the mean ovipositor length, the reading on the map measurer, after going once around the log in this manner, was compared with a reading when the measurer was engaged for a complete revolution.

Morphological Preparations

Embryos- Eggs were fixed in Kahle's consisting of:

95% ethanol	- 15cc.
40% formalin	- 6cc.
glacial acetic acid	- 1cc.
distilled H_2O	- 30cc.

at a temperature of 80°C. They were left in the fixative for approximately one minute. At the end of this time they were transferred to a small Syracuse watch glass along with some of the hot fixative where a portion or all of the chorion, depending on the age of the embryo, was removed. For very young embryos, before the formation of the blastoderm, that portion of the chorion on each end of the elongate egg was removed to allow better penetration of the fixative and other fluids during subsequent treatment. After the formation of the blastoderm the whole chorion was removed in the following manner. First, the anterior end of the egg was cut off with a curved scalpel immediately anterior to the embryo itself. This was at a place far enough back so that the hole would be large

enough for the embryo to be pushed through. Next, the posterior end of the egg behind the embryo was held in place with pressure against the bottom of the dish with a curved pair of jeweler's forceps, and the embryo was gradually worked forward by alternately squeezing and relaxing the chorion immediately posterior to the embryo. In some cases gentle pressure on the embryo itself was necessary. Utmost care was needed to prevent crushing the cells during this operation, but it was generally accomplished without injury.

After removal of the chorion the serosa could be removed in much the same manner, the whole proceedure involving the removal of it and the chorion usually taking no longer than a minute. The embryo was then replaced in hot fixative, allowed to cool, and was left for 2 to 24 hours. It was washed in four changes of 70 percent alcohol during the next 24 hours and were stored in the last change until needed. For eggs stained with the Feulgen reaction, however, an additional 12 hours of washing in running water was necessary to remove all traces of the formaldehyde.

To trace the nuclear history eggs stained with the Feulgen reaction proved best (see Appendix for procedure). For studying the external morphology of embryos past the blastular stage they were stained with Harris' hematoxylin for only a few seconds, so that the surface relief was

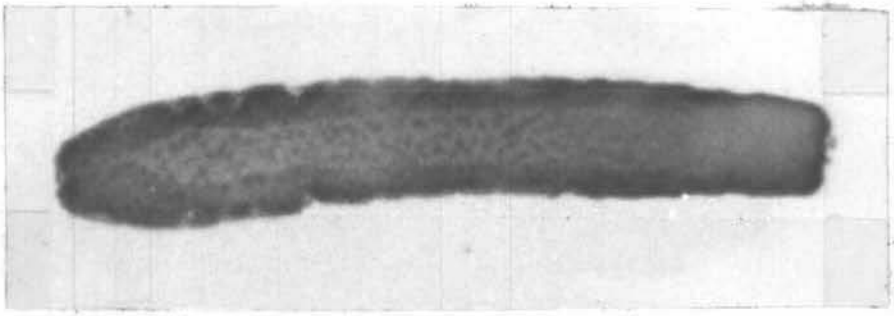


Figure 6. Embryo incubated for 13 hours at 75°F. and stained whole in Harris' hematoxylin. Dorsal view. Kahle's fixative. Chorion removed. X130.

accentuated, the most exposed areas staining the heaviest while the deep invaginations and the dorsal part of the embryo before dorsal closure remained virtually unstained (Figure 6) (see Appendix for procedure). This is a modification of Wheeler's second method (115, p. 141).

Examination of whole embryos stained in hematoxylin was made while in an uncleared condition under a 90X magnification of a dissecting microscope. The embryos were studied in alcohol with reflected light for two reasons. If they were cleared and mounted on slides they could be viewed from only two sides unless the balsam was softened and they were rolled over. But even more of an objection to clearing and mounting and using transmitted light was that the picture of the surface contours tended to be confused by the internal details. The tendency was to look through rather than at the surface.

Internal morphological studies of the embryos were

made from sectioned material after staining whole in alum carmine or dilute Harris' hematoxylin (see Appendix for procedure).

Larvae- Observations on the external morphology of the larvae were made for the most part on whole specimens, either live or preserved in alcohol. Slide preparations of the cuticle after treatment with KOH and stained in acid fuschin proved valuable for discerning the detailed structure, particularly of the larval head. Gross dissections of fresh material were used for study of the body musculature and tracheal system.

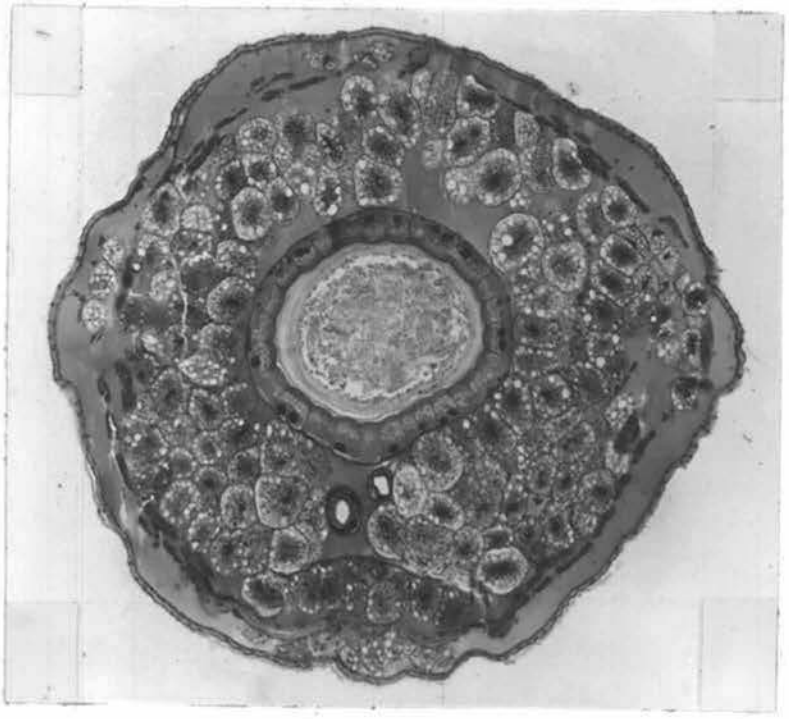


Figure 7. Photomicrograph of a section through the abdomen of a fifth instar. Relaxed in hot water. Bouin's fixative. Harris' hematoxylin and eosin. 10μ. X40.

Specimens were pinned open in physiological saline, fixed in Bouin's fluid and stained in alum carmine, although eosin worked almost as well and was easier. Proper tissue orientation was ascertained from sections (Figure 7) stained with Harris' hematoxylin and eosin (see Appendix for procedure).

LIFE CYCLE OF *DENDROCTONUS PSEUDOTSUGAE* UNDER NATURAL CONDITIONS

An intimate knowledge of the life history of the Douglas-fir beetle, *Dendroctonus pseudotsugae*, is an important part of a biological study of *Coeloides brunneri* because this beetle is the primary host of this parasite. The life history and habits of the Douglas-fir beetle have been investigated in whole or in part by many entomologists including the writer (4, 31, 86, 111, 112).

Briefly, the life cycle with special emphasis on aspects influencing the success of *Coeloides brunneri* may be summarized as follows. There is one generation per year. Adults emerge from infested logs in April and May and establish broods between the inner bark and wood of standing or recently down trees. A portion of these adults reemerge during late spring or early summer after construction of this first gallery is completed and establish a second brood in the same or other trees. These adults then die. Adults invading during this second period during late spring or early summer are joined by adults emerging from logs infested late in the previous year's flight period. The broods from these late invasions have not completed development by the time winter sets in, consequently they are not ready to emerge the following spring with beetles overwintering in the adult stage, but additional time is needed to transform to the adult stage.

There may be some overlapping of the first and second flight periods, but generally two distinct peaks are evident.

Some of the larvae of the broods extend their mines completely into the inner bark as they grow, where they are slightly closer to the surface of the log and more easily reached by the parasites. A cursory examination of the inner surface of the inner bark will not reveal these larvae or parasites feeding on them. Therefore, in any detailed analysis of parasitism the inner bark must be completely and laboriously chipped away. The first beetle larvae to reach sufficient size to be susceptible to parasitism by C. brunnëri occur during May as a result of April attacks. Due to the extended egg laying and the long attack periods of the beetle, at least some larvae susceptible to parasitism by C. brunneri are present from May throughout the summer and into the fall. The flight periods and the time when susceptible larvae are present are shown diagramatically along the bottom of Figure 8.

LIFE CYCLE OF COELOIDES BRUNNERI UNDER NATURAL CONDITIONS

This insect has three complete generations a year with a portion of each generation overwintering in the fifth instar. The portion which overwinters is different for each generation, the approximate percentages being about 5 percent in the first, 50 percent in the second and 95 percent in the third generation. The seasonal history is shown diagramatically in Figure 8, the details of which are as follows.

The insect overwinters as mature larvae in cocoons spun in the larval galleries of its host. During late spring the larvae pupate and by late June the first adults may be found in the cocoons. Within one day after becoming adults the parasites chew through the cocoons and bark to the outside, filling the cocoons with bark fragments as they emerge. During the first half of the emergence period more males than females emerge, while the opposite is true during the latter half. Emergence of these overwintered insects continues for about three weeks, although by the end of the first week of July most have emerged.

Females live only a few weeks, but before dying, which happens usually before August first, they lay eggs on susceptible larvae through the bark of trees attacked by Douglas-fir beetles in April. Approximately 95 percent of

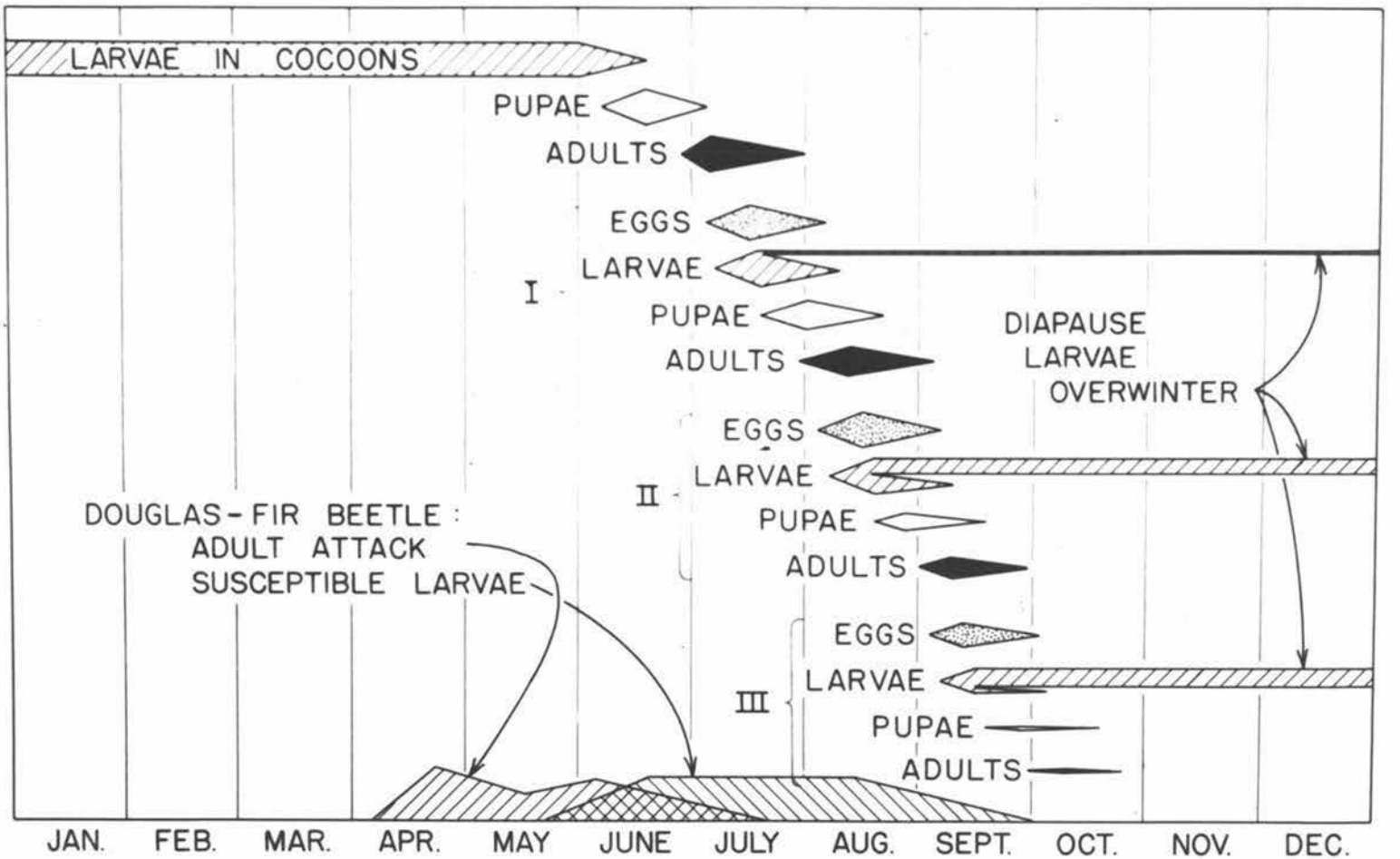


Figure 8. Diagramatic life cycle of C. brunneri. Also shown are the attack periods of its main host, Dendroctonus pseudotsugae, and the period when host larvae of susceptible size are present.

the eggs laid during July by the overwintered population develop to the adult stage and emerge during August to parasitize susceptible larvae from later or second (summer) beetle invasions. The remaining 5 percent, however, enter diapause in the fifth instar after cocoon formation, pass the remainder of the season and finally overwinter in this stage. Those parasites emerging in August lay eggs on larvae resulting from May and June beetle invasions. Approximately 50 percent of the progeny of this, the second generation, develop to the adult stage and emerge in September, while the other 50 percent enter diapause and overwinter with the 5 percent of the first generation which entered diapause. The third generation occurs in September and October and results from eggs laid in September by the 50 percent of the second generation which emerged during that month. Only about 5 percent or less of the third generation develop to the adult stage before winter. The remaining 95 percent enter diapause, and like the portions of the first two generations which entered diapause, overwinter. While the percentage of insects of the third generation which overwinter is greater than the percentage of the second, the absolute number is probably about the same since the third generation is smaller than the second. Thus, the overwintering individuals are of three different generations. All overwintering individuals emerge at approximately the same time, late June or early July of

the following year.

PARASITE BEHAVIOR

Emergence and Mating

After emergence from the pupal skin the adult remains inside the cocoon for several hours while the wings are expanded to full size and the exoskeleton hardens. Within the day the adult commences its exit from the cocoon and through the layer of bark which has served as its protection from the moment of egg deposition. A circular hole just wide enough to wriggle through is cut with the mandibles in the side of the cocoon toward one end. The bark fragments are pushed behind by the insect advancing toward the exterior, filling the cocoon behind. From the overwintered population the males emerge on the average slightly ahead of the females. During the summer generations, however, the timing of the male and female emergence in relation to one another depends largely on the time each egg was laid and upon the size of the host, which influences the rate of development. During the summer generations egg laying proceeds over many days and generally the first individuals emerging are those from eggs laid first; while from eggs laid on one day the first individuals to emerge are those developing on the smallest beetle larvae.

Sexual excitation is not acute in the males immediately upon emergence. They frequently rest motionless on logs or

nearby foliage for long periods for one or two days after emergence. By the time the first females emerge these males are seen on the surface of the log from which they emerged, now flitting and running from place to place, now stopping to listen for emerging females. Females are frequently located, apparently by the noise or vibrations produced by their chewing, before they have broken through the surface of the log. Commonly several males are found waiting for an emerging female. Such females are fertilized immediately. The appearance of the female sends the males into a frenzy of sexual excitement. All males try to copulate with the female at once, some even with each other. The female at this time is usually quite receptive to the attentions of the males and drops the seventh sternum, opening the genital chamber to allow insertion of the male genitalia. When one male succeeds in penetration the other males usually drop off the female. The successful male has grasped the female with his legs and has his abdomen bent around to one side of the female's and the extruded genitalia are within the female's genital chamber immediately above the seventh sternite. The male's wings and antennae are pumped rhythmically while the head is moved from side to side. Coition lasts from 15 seconds to two minutes.

The males awaiting the emergence of the females are apparently unable to distinguish between males and females,

for they attempt sexual intercourse with newly emerged males as well as with females.

Should a female emerge at a time when no males are in the immediate vicinity she may be mated within the next day by any male she encounters. However, she loses her attractiveness to males if more time elapses than about a day. Then, females are extremely unreceptive to the advances of the males, and it is only a very strong and persistent one which will succeed if the female is not willing. The genital chamber is no longer held open, and if a male does mount the female she promptly brushes him off with her hack legs. If the male happens to be successful in penetration, the female continues to struggle, often dragging the male around behind her (Figure 9) or even flying away with the male still inserted.

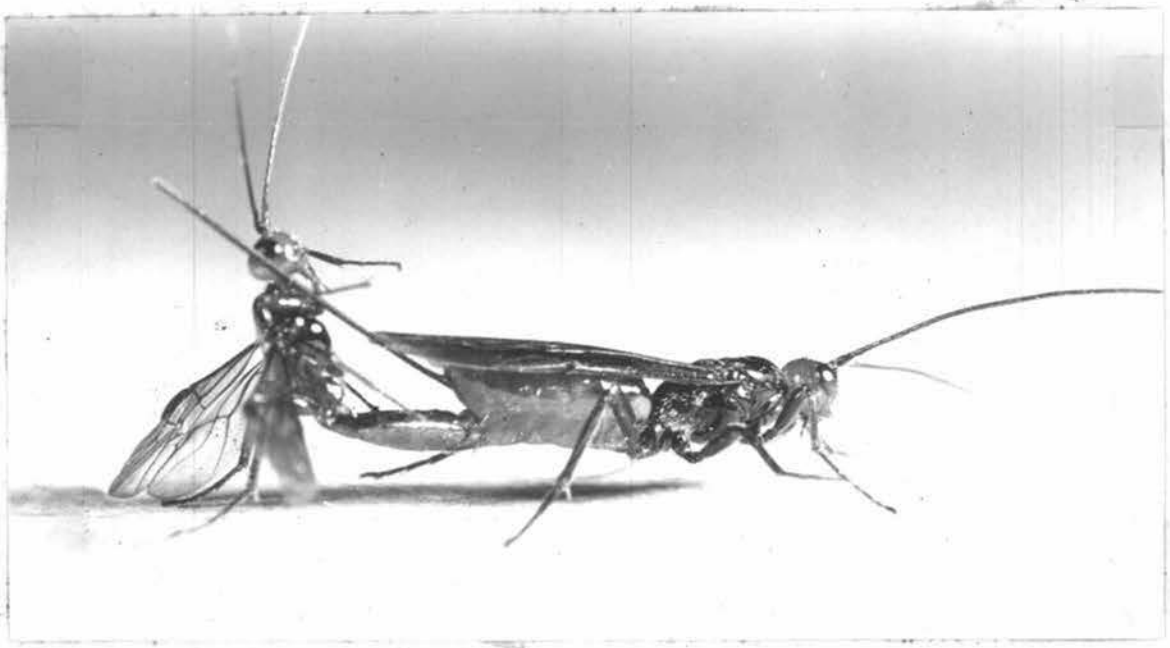


Figure 9. Male and female C. brunneri mating.

Once mated, even if newly emerged, the females usually resist further advances of the males.. The latter, however, will mate many times with different females. In the laboratory a single male was mated with nine different females over a period of two weeks. It is suspected through experience in laboratory mating and rearing that the potency of the males is reduced with both age and number of matings.

Female Searching and Oviposition

The females of this species are rather slow moving, but thorough searchers, that tehd to follow the crevices of beetle-infested logs. They pause frequently to listen for noises or vibrations from beneath the bark surface. Most time is spent on the undersurface and sides of logs where the light intensity is less than on top. These places are also where the greatest numbers of its principle host, the Douglas-fir beetle, are found. Parasites are sometimes found on the tops of logs exposed to the direct rays of the sun, but oviposition was never observed in such locations. It is felt that the similarity in light intensity preferences of Coeloides brunneri and Dendroctonus pseudotsugae is a major factor contributing to parasitism of the latter by the former in preference to other species found in Douglas-fir and sometimes attacked, such as Melanophila drummondi. Adults of M. drummondi are attracted to sunny

locations where they lay their eggs. The incidence of parasitism by C. brunneri is low in this species. Probably it is parasitized only on cloudy days or at other times when the light intensity is at a low level.

Hosts are apparently located by the noise produced by their chewing activity rather than by odor, for only larvae and adults elicit ovipositional responses in searching females, never pupae (see "Stage of Host Attacked"). In the laboratory females have been induced to lay eggs through a piece of bark, which was removed from a log, simply by scratching the undersurface of the bark gently with a pin.

Once the host has been heard the female pauses and shifts her position frequently until the exact source of the sound has been located. She places herself so that the exact spot is centered approximately midway between her meso- and metathoracic legs (Figure 10 a). Once confident of her position, she raises her abdomen as high as possible and swings her ovipositor ventrally (Figure 10 b) until it is exactly vertical (Figure 10 c). This procedure takes only a few seconds and may be repeated several times before actual drilling starts. Once drilling has started the female retains the position of the pro- and mesothoracic legs, although the metathoracic legs may be shifted frequently. Actual drilling time varies considerably. It may be as short as ten minutes or more

Figure 10. Picture series of a female in the process of oviposition. X4. The complete process usually takes between 15 and 45 minutes.

a. Searching female. Time zero.

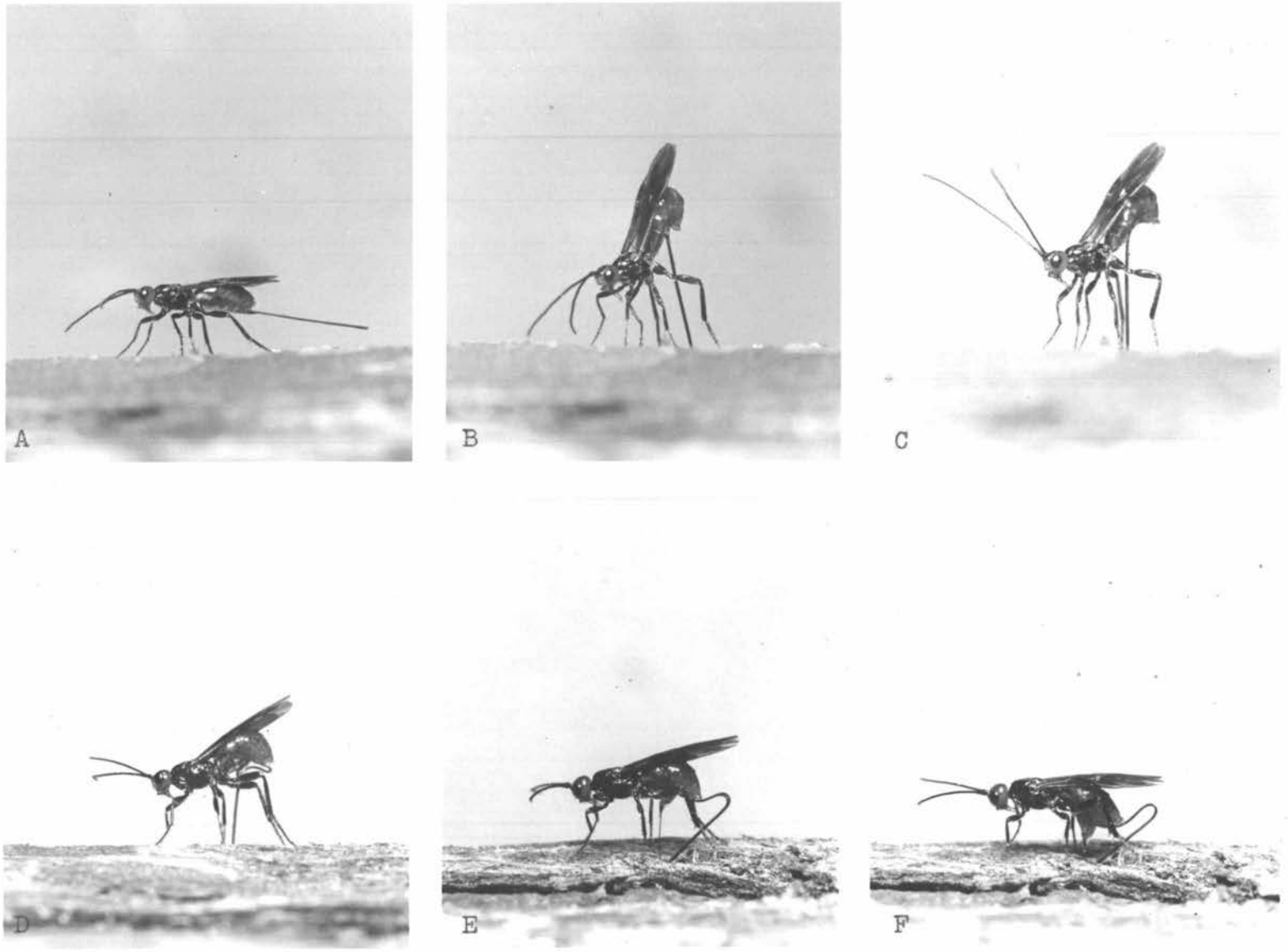
b. Abdomen raised and ovipositor being swung vertically. Time zero plus three minutes.

c. Ovipositor vertical. Penetration of first and second valvulae begun. Time zero plus five minutes.

d. Partial penetration. Third valvulae being pushed into a tight loop behind. Time zero plus 10 minutes.

e. Third valvulae slip away from first and second as penetration is half completed. Time zero plus 20 minutes.

f. Penetration almost complete. Time zero plus 35 minutes.


A
B
C
D
E
F

than three hours, but in most cases is between 15 and 45 minutes. Only the first and second valvulae are inserted into the bark. As the abdomen is brought closer and closer to the log the proximal parts of the third valvulae at first are pushed into a tight loop directed posteriorly beneath the abdomen, while the distal portions still surround the united first and second valvulae (Figure 10d). After a certain point is reached the third valvulae slip away from the first and second and form a broad loop behind (Figures 10 e and 10 f). Insertion continues usually up to the limit, where the insect remains for several minutes, motionless, except for slight vibrations in the abdomen while the egg is laid. She may partially withdraw the ovipositor for perhaps one-fourth its length once or twice and then thrust it to the limit. This probably is when the host larva is injected with the paralyzant. The egg is laid within the host gallery but external to the host itself (Figure 11) and frequently adheres to the head of the host or to its body immediately posterior to the head.

Extrusion of the egg from the ovipositor was observed in a few cases. These females were stimulated to oviposit through the mesh of a plastic screen and were observed under a binocular microscope. The united first and second valvulae were waved violently back and forth while the egg was passed to the exterior in a semi-fluid state.

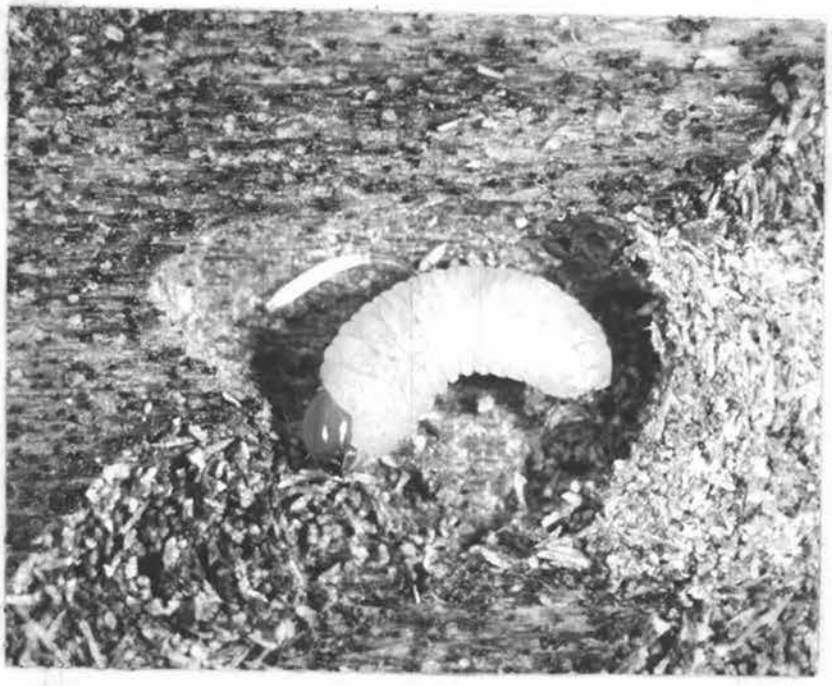


Figure 11. Douglas-fir beetle larva and an egg of C. brunneri which has been laid within the larval gallery. X15.

It appeared first as a small drop near the tip of the ovipositor and gradually took form as more and more was squeezed through until finally it dropped free. Only one or two seconds were required for full penetration in these cases since there was no bark to offer resistance, and from the time of complete insertion until the egg dropped free no more than about ten seconds elapsed. It seems safe to say, then, that most of the time spent in ovipositing on beetles in logs is spent penetrating the bark, since actual egg deposition and withdrawl of the ovipositor from bark have been observed to take approximately ten seconds each.

General noise level in rearing rooms such as produced by noisy fans, etc. appear to result in fewer eggs being deposited. Perhaps the parasites can not as readily locate their hosts under these conditions. However, females are not easily distracted once a host has been located. Even when pushed aside bodily they return to the spot again and again to oviposit. They are most easily disturbed after the ovipositor is in position but before penetration has started, but even then they are persistent and return. Once penetration has started they are almost immovable. The approach of another female brings a flurry of wing fluttering from an ovipositing female, but she keeps right on. The second one may even try to oviposit on the same host. Several cases have been observed where two females were ovipositing side by side, apparently oblivious to one another, and in an extreme case two females were observed

ovipositing, a small one directly beneath a large one.

Preferred Oviposition Sites

Many hours were spent observing adult habits in the field. Particular attention was given to one ideally situated tree. This particular tree was felled by the writer in the spring of 1959 so that it dropped in an east-west direction within a small opening in a stand of second growth Douglas-fir at McDonald forest. The opening in the canopy allowed the sun's rays to fall on a portion of this log for part of the day. Adult parasites were observed to frequent most the less exposed portions of the log, toward the bottom and on the shady side where the light intensity was less than on the exposed portions. Oviposition was most frequently observed in these places, while never in direct sun. Toward the end of the summer a partial sampling in this tree was conducted to obtain data pertinent to these observations. Unfortunately, the tree was salvaged without the writer's knowledge before sampling was completed, but a partial sample was obtained.

The sampling procedure was to remove the bark completely for a length of the log at regular intervals up the hole. At each sampling point the bark was divided into fourths by scoring with an axe along the lines of the horizontal and vertical tangents to the log, thereby dividing it into top right, top left, bottom right and

bottom left portions. Each quadrant was then removed and analyzed separately. The data from the quadrants were grouped into top and bottom, and right and left halves for analysis. The numbers of Douglas-fir beetle larvae which were parasitized were compared with the numbers not parasitized by the chi square test of independence (66, p. 410-412). The parasitism in the top of the log proved to be significantly different at the 0.5 percent level from that underneath (Appendix, Table 10), while the parasitism on the sunny and shady sides did not differ at the 5 percent level (Appendix, Table 11).

Larval Feeding

The yolk present within the newly hatched larva is sufficient to sustain it for at least a day without feeding on its host. It wanders about within the confines of the host gallery until it encounters its host, which has been completely immobile since about 12 hours after the injection of the paralyzant by the adult female at the time of oviposition. The young parasite crawls over its host with slow movements frequently retaining attachment with only the last few segments of its abdomen while the head and anterior portion of its body are waved slowly back and forth. The parasite tests the host frequently by appressing its mouthparts close to the host. Finally the young

parasite settles down to feed. The place may be anywhere on the body of the host except the heavily sclerotized head capsule. The cup shaped mouthparts are held against the host apparently by suction while the mandibles are used first to break through the integument and then as paddles to keep the wound unobstructed during feeding. During feeding the mandibles wave back and forth within the preoral cavity while the host fluids are sucked by the strong pharyngeal pump. The larva frequently discontinues feeding to shift its position on the host, and of course, during molting the insect does not feed. At first only the body fluids of the host are consumed, and it is not until the last instar that appreciable quantities of the vital organs are ingested. Although paralyzed the host remains quite alive until the parasite in its final instar consumes it completely except for the head capsule, integument and portions of the gut and its contents. The only recognizable portions of the host visible in sections of the parasite midgut at this time are fragments of tracheae.

If a paralyzed host is kept under suitable conditions and is kept from parasite feeding it will remain alive for approximately two weeks at 75°F. During this time the heart continues to beat, although gradually growing slower and weaker until at the end it is almost imperceptible. But this is more than enough time for the parasite to reach

the final instar.

Cocoon Formation

Pupation without cocoon formation has been observed in a few cases, and these were all in insects developing without a diapause. Most larvae spin cocoons (Figure 12). At 75°F. spinning commences about a day after the host is completely consumed, and in the case of nondiapause larvae the cocoon is not added to after about a day

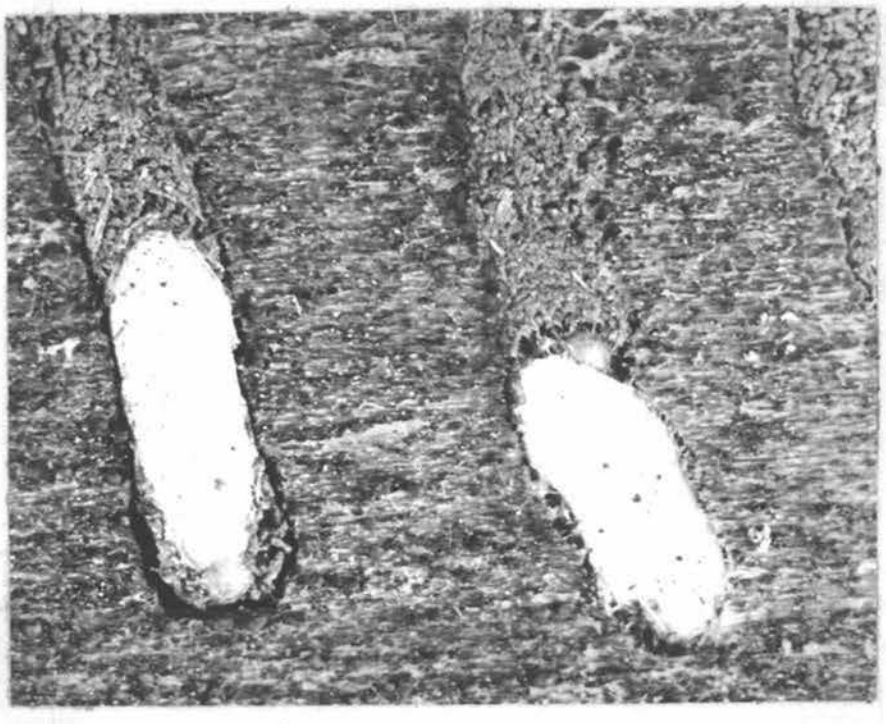


Figure 12. Cocoons of C. brunneri. X6.

later. Cocoons of larvae developing without diapause are rather thin and consist of only a single layer of silk, whereas frequently two thicker layers are evident in cocoons of diapausing larvae. The shape and size of the cocoons depend upon the size of the host and its gallery. If the host and its gallery are small the cocoons are roughly oval in shape and are flattened on the top and bottom, and therefore, rectangular in cross section due to the restrictions imposed by the size of the host gallery. When larger hosts are parasitized the cocoons do not completely fill the host gallery and are more cylindrical in overall shape and roughly circular in cross section. Any irregularities of the host gallery are frequently reflected in a similar configuration in the cocoons.

FECUNDITY

Ovigenesis and Oösorption

In order to trace egg maturation in this insect some females were dissected after intervals of from zero to 20 days after adult emergence from the cocoon. Each was kept at 75°F. in an individual vial and was continuously supplied with a water-honey-yeast-raisin diet (see p. 3 for procedure). After anesthetization with CO_2 dissections were performed in Bouin's fluid under a 45X magnification of a dissecting microscope. Counts were made of the number of mature oöcytes within the ovaries, and the average for each day has been plotted in Figure 13. An oöcyte was considered mature if the trophocytes were not visible and if the oöcyte was full size and covered with a chorion. Each point on the curve represents the average of three individuals, except for the tenth day when 11 females were dissected.

It is evident that at the time of emergence there are no mature oöcytes, although each female at this time had a series in various stages of development. Some were mature after one day at 75°F. Oöcyte maturation continued apparently unchecked until on the third day an average of nine mature oöcytes per individual was present. But starting on the fourth day the average declined steadily

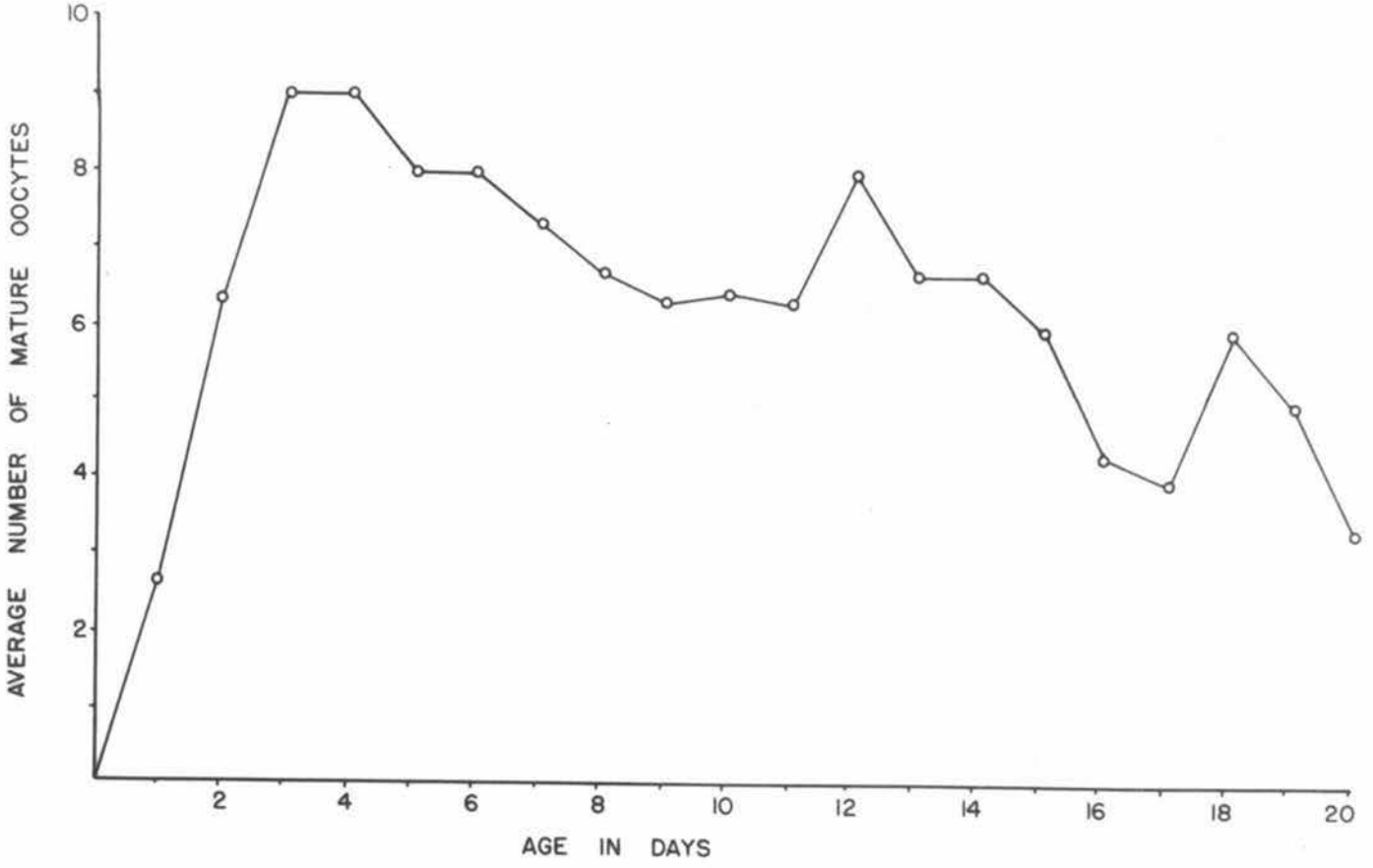


Figure 13. The mean number of oöcytes present in C. brunneri females of various ages after being held at 75°F. and fed a water-honey-yeast-raisin diet.

until the twentieth day, the end of the experiment, when there was an average of slightly better than three. Subsequent to the fourth day it was common to find some of the full-sized oocytes appearing shrivelled.

This check in the increase of the average number of mature oöcytes and appearance of shrivelled ones was expected since Flanders (37, p. 253) has shown that in some species at least, when they are denied access to their host, oösorption sets in. Thus, the nutritive material in unovulated eggs is conserved to be used in later vitellogenesis. The observed steady decline in number indicates that oösorption proceeds at a faster rate than oögenesis, although Flanders states that, "when hosts are lacking, oösorption occurs at the same rate as oögenesis." He states elsewhere (p. 252) that, "the germarium as a rule is not depleted of oögonia before the death of the female..." In these experiments it was not possible to discern individual degenerate eggs in the ovaries after a certain point in oösorption was passed.

The effects of different foods on the number of mature oöcytes present at the end of 10 days were studied. Newly emerged females were completely randomized to receive one of four diets: honey; raisin; honey and active dry yeast; and a multi diet consisting of honey, inactive powdered yeast and a commercial dietary supplement sold

under the trade name of "Metrical". Ten insects were used in each treatment. Each was held in an individual vial, continuously supplied with the food and dissected after a period of ten days at 75°F. to count the number of mature oöcytes present.

An analysis of variance (66, p. 167-169) performed on the data led to the conclusion at the 5 percent level that the means of the four treatments were the same (Appendix, Table 12).

Number of Eggs Laid

In proövigenic species where the entire complement of eggs is ripened at approximately the same time, it is possible to determine the absolute number of eggs produced by simply dissecting the female and counting. In Coeloides brunneri, however, where oögenesis proceeds throughout adult life it is impossible to determine the capacity by observing the static picture by any single or series of dissections.

An effort was made to establish the number of eggs laid per day and the total number laid in the female's lifetime. Approaching the problem directly was almost impossible. Females generally would oviposit only into logs containing their hosts. Debarking the logs could not be done carefully enough to count the small eggs themselves. Watching ovipositing females was not only entirely too time

consuming, but frequently females apparently oviposited but laid no eggs. The approach which was finally adopted, although with several drawbacks, was to count the resulting cocoons. This method entirely disregarded egg and larval mortality if there were any and individuals which pupated without spinning cocoons, but had the advantage that examination could be done at any time.

Newly emerged females were mated and kept overnight in ice cream containers where they were fed a diet of a water-honey-yeast mixture plus dried raisins. The following day the females were placed in a cage with a log section infested with Douglas-fir beetle larvae and allowed to oviposit. A day length of 16 hours and a temperature of 75°F. were maintained throughout the experiment. Every second day the insects were removed from the cages and held overnight in ice cream containers for feeding.

It was apparent after several trials that oviposition was restricted due to lack of hosts. To alleviate the situation another trial was made using a fresh beetle-infested log every two days with the exception that the log presented on the thirteenth day was used for five days since all females except two had died by the fifteenth day. These two were dead by the end of the seventeenth day.

Each female laid eggs resulting in an average of 21.2 cocoons. In Table 1 is listed the number found in each log. These figures represent the total production of nine

females, disregarding, of course, mortality occurring before cocoon formation, if there were any. The daily emergence of the progeny from the logs is shown in Figure 14 in which zero on the horizontal scale represents the day on which the parent females emerged from their cocoons.

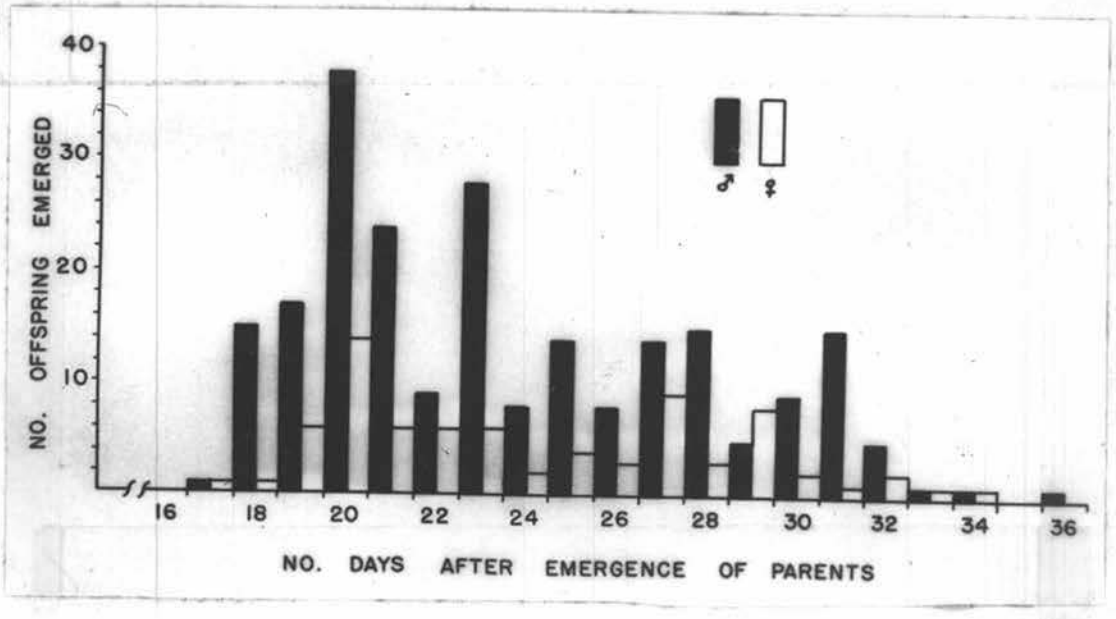


Figure 14. Daily emergence of progeny of nine females ovipositing until their death. Experiment conducted at 75°F.

Table 1. Total progeny as determined by cocoon counts of nine females offered a fresh supply of hosts every two days.

No. days after emergence of females	No. females on day of introduction	No. cocoons
1-2	9	22
3-4	9	50
5-6	9	60
7-8	9	0
9-10	9	36
11-12	8	12
13-17	7	11
Total		191
Average		21.2

LONGEVITY

Longevity in the Field

Field observations on the emergence dates and the presence or absence of parasites, on or in the vicinity of beetle-infested logs, were used to estimate the longevity of parasites in the field. Observations in 1959 on the adults from overwintered individuals may be taken as representative.

The first and last emergence of adult males from caged infested bark were recorded on June 27 and July 17, respectively. Males were abundant on the logs from which they had emerged during the first two weeks of July, but none were seen after July 25 until emergence of the first generation from newly infested logs started on August 5. The mean longevity was probably between 15 and 20 days.

The emergence of the females from the same caged material lasted from June 30 to July 16. They were abundant on beetle-infested logs during July where some individuals were still present when the first generation started emerging. The new and old females could not be distinguished. The mean female longevity was probably between 20 and 30 days.

Longevity in the Laboratory

Effects of temperature on longevity- The length of

life of males and females was studied separately at constant temperatures at intervals of five degrees, between and including 60° and 90°F. when fed the same food, and at 75°F. when fed different foods. Each insect was held in a separate vial, continuously supplied with food (see p. 3 for procedure) and observed daily until death. The diet consisted of a dilute honey and yeast mixture. Approximately fifteen individuals of each sex were observed at each temperature with the exception that at 70 and 80 degrees only males were observed. In Figure 15 are plotted separately the mean longevity of males and females at each temperature. Due to the restricted number of temperature cabinets available observations could not be conducted concurrently. Therefore, individuals could not be randomized among treatments, and the differences between means plotted in the figure may in part be due to the variation in condition of the insects used at each temperature.

The females lived longer than the males in each case, and the differences are regarded as real. Differences were compared at five temperatures by the t test (66, p. 131-133), and three were found significant at the 1 percent level. There were not enough observations to prove significance at the 5 percent level for the other two cases, however. A summary of the results is presented in Table 2 and in Figure 15.

Table 2. Summary of male and female longevity at various constant temperatures.

Temp. °F.	No. of deservations		Mean longevity in days		t	95% confidence interval limits of the difference between means
	males	females	males	females		
60	19	18	32.9	74.0	4.06[1]	20.5 61.6
65	15	12	11.4	20.2	1.96[2]	-0.5 18.0
70	15		18.8			
75	15	15	16.3	33.8	3.01[1]	5.6 29.5
80	15		12.4			
85	15	15	7.8	10.5	1.63[2]	-0.7 6.0
90	15	15	14.9	23.0	3.29[1]	3.1 13.2

1. Significant at the 1 percent level.
2. Not significant at the 5 percent level.

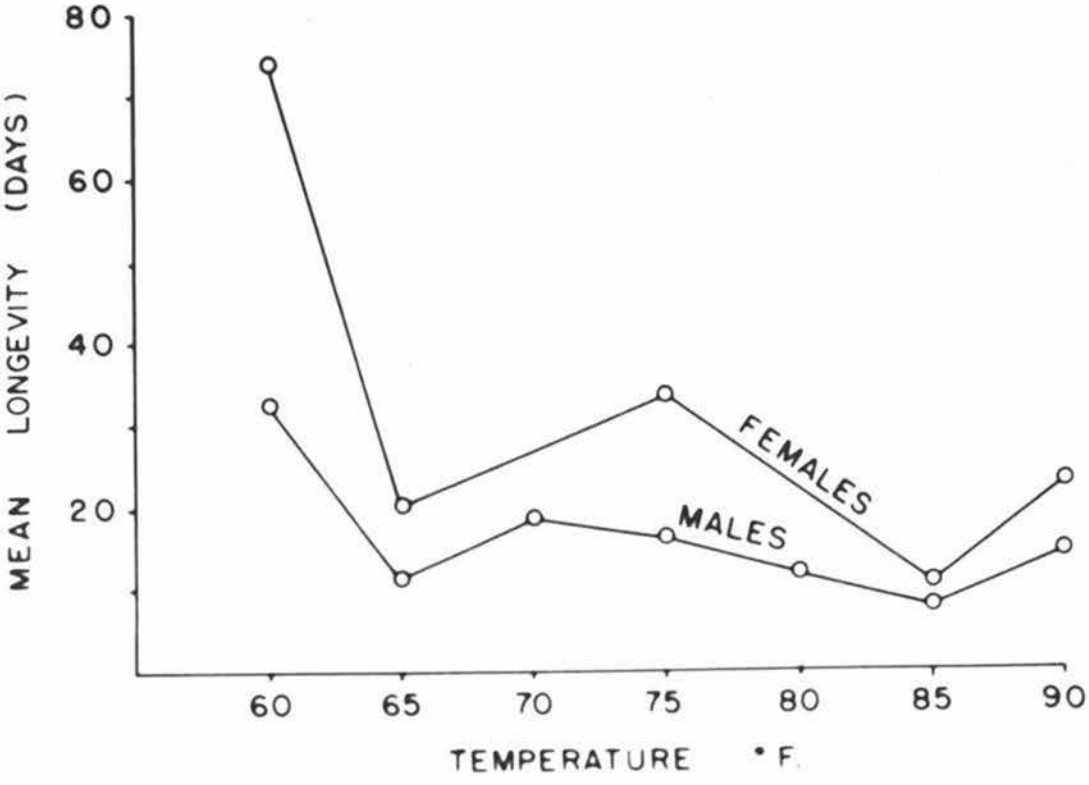


Figure 15. Male and female longevity at various constant temperatures when fed a water-honey-yeast diet.

Effects of food on longevity- The effects of food on the longevity of males and females were compared separately at 75°F. A completely randomized design was used in these experiments using 10 insects per treatment. The natural diet of the adults is unknown. Five artificial diets were tested: no food; raisin; honey; honey and yeast; and honey, yeast and raisin. The honey used in the treatments was diluted about half with water. Insects were assigned to the treatments with a random number table and isolated in separate vials as described previously.

The means of the 10 observations in each treatment are listed in Table 3. Analyses of variance (66, p. 167-169)

Table 3. Mean longevity of males and females at 75°F. when fed different diets[1]. (Means based on ten observations).

Sex	Diet				
	None	Raisin	Honey	Honey & Yeast	Honey & Yeast & Raisin
Male	5.1	15.3	22.2	17.0	9.9
Female	5.0	35.6	32.9	26.9	33.7

1. The least significant difference (5 percent level) between treatment means is 8.5 for males and 15.9 for females.

were performed on the data (Appendix, Tables 13 and 14) and led in each case to the conclusion that the treatment means differed significantly at the 5 percent level. The least significant difference (5 percent level) between treatment means (66, p. 233-238) was computed to be 8.5 for males and 15.9 for females.

It is clear that the four diets tested lengthened longevity compared to the controls which were not fed, although in the case of the males fed a honey, yeast and raisin diet the number of observations was not sufficient to prove significant the difference observed. When any food at all was supplied the treatment means for insects of the same sex were not significantly different, with one exception. Longevity of males fed a honey diet was significantly greater than that of males fed a honey, yeast and raisin diet.

STAGE OF HOST ATTACKED

This insect successfully parasitizes only the larval stage of its hosts. This conclusion is based on three lines of evidence: first, observing the stage of host actually being fed on by the parasites; second, examination of host remnants at the cocoon site; and third, observing the stage of host present at the oviposition site at the time of oviposition.

Parasites have been observed feeding only on host larvae, never eggs, pupae or adults. Examination of the host gallery in the immediate vicinity of the parasite cocoon invariably revealed remnants of the host larval head capsule, although this would have been present also had the parasite attacked a pupa or an adult before it left its pupal cell. Adult remains have never been found at the cocoon site. By removing the bark at several hundred oviposition sites immediately after the females withdrew their ovipositors it was found that eggs were laid only on larvae. Sometimes females would go through all the motions of egg laying at a place over an adult, but in these cases eggs were never found and were probably never laid. Never was a female stimulated to oviposition movements by an egg or a pupa.

Measurements of larval head capsule width of the Douglas-fir beetle measured by Vité and Rudinsky (11, p. 159)

were compared with measurements of head capsule remains at cocoon sites to estimate the instar of the host at the time of parasitism. These measurements were very helpful when the host head capsule was intact. However, frequently it was broken along the coronal and frontal sutures, and head capsule width was impossible to determine accurately. Bedard (3, p. 1132) also recognized this fact. The triangular fronto-clypeal region, however, was always intact. Bedard also measured this region, but since he apparently was mistaken as to the number of instars (11, p. 160) it seemed advisable to establish the width of this region for each instar. Of course, the highly sclerotized mandibles were also always intact.

Three measurements were taken on each of approximately two hundred Douglas-fir beetle larvae: head capsule width, maximum mandible length and fronto-clypeal width. These data have been plotted in Figure 16 as two scatter diagrams, one representing head capsule width and mandible length and the other head capsule width and fronto-clypeal width (marked clypeus width in the figure). The area of each circle is proportional to the number of individuals represented by it. The frequency of the individuals in each unit of measure are represented by histograms along the horizontal and right vertical axes.

Head capsule widths are essentially the same as obtained by Vité and Rudinsky, with the exception that those

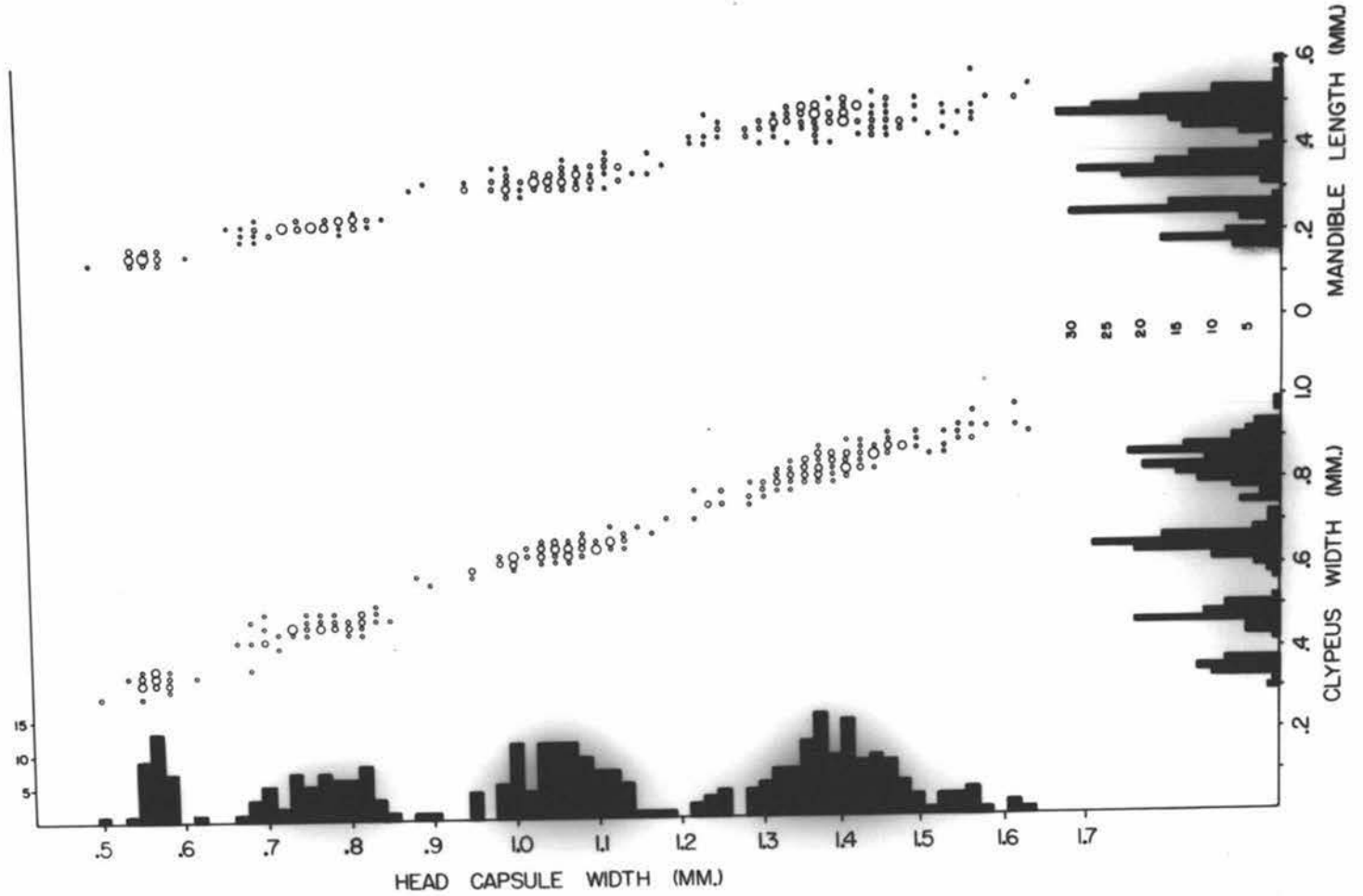


Figure 16. Scatter diagrams and frequency distributions of Douglas-fir beetle larval head capsule widths, mandible lengths and clypeus widths.

in the fourth instar were found by the writer to have a slightly larger variance. The measurements thus obtained were used to determine the instar of the host at the time of parasitism. Another approach was followed to arrive at essentially the same information. Vité and Rudinsky (111, p. 160) measured the distance from the egg gallery to where the head capsules of the individual instars were found. These data were used in conjunction with measurements of the writer on the lengths of 200 larval galleries containing parasite cocoons to determine the instar at the time of parasitism. Some of these measurements are plotted in Figure 17. In this consideration the vertical scale and lines of regression may be disregarded. Consider only the position of the points with reference to the horizontal scale. The broken vertical lines are the average locations of the exuviae as determined by the authors mentioned with the exception that the location of the pupal chamber, or the upper limit of the fourth instar in the figure, was moved to the right to include measurements taken by the writer.

As indicated in Figure 17 the parasites commonly attack the third and fourth instars of the Douglas-fir beetle but can also attack the second. This conclusion based on gallery lengths of parasitized larvae was confirmed by measuring head capsule remnants at cocoon sites.

In the laboratory eggs were laid on all instars except the first. The parasites developed to the adult stage with

equal facility on all three instars, although on smaller hosts they were smaller as adults (see p. 65) and the development appeared slightly more rapid than when reared on larger hosts.

In the laboratory larvae in the first instar were also observed to elicit normal ovipositional responses from the searching females, although no eggs were found in the few cases observed. It is possible that eggs are laid also on individuals of the first instar but the amount of food afforded by such a small larva is so small that the parasite is not able to complete development. This would explain the lack of first instar head capsules at cocoon sites. In this case the death of any parasitized first instar would probably be attributed to other causes.

DIAPAUSE

The Incidence of Diapause in Trees Felled on Different Dates

Infested material examined in September, 1959, from each of three trees felled at Mayy's peak that same year, had a different percentage of empty (frass filled) cocoons. The exact date of felling was known for two of these trees, and for the third the approximate date was known. All insects had emerged from the tree felled in April. Aside from those insects which had died for one reason or another all cocoons were filled with the characteristic frass pushed back into the cocoon by the adult emerging through the bark. The other two trees had different percentages of cocoons with living larvae, the overwintering stage, present (Table 4).

Table 4. The influence of felling date of the tree on the diapause percent in C. brunneri.

Date tree felled	No. of cocoons examined [1]	No. of cocoons with living larvae present	% Diapause	95% confidence interval of mean
4/20	68	0	0	0
5/28	214	81	37.9	31.0-45.0
7/1 (approx.)	1152	681	59.1	56.3-62.0

1. Not included in these totals are insects which died in the larval stage. If dead insects were found in the pupal or adult stage they were considered as having emerged since they did not go into diapause.

A chi square test of independence (66, p. 415-416) led to the conclusion that the three means differed significantly (Appendix, Table 15).

The trees felled later in the season naturally were attacked by Douglas-fir beetles later than those felled earlier, therefore the beetle larvae resulting from late attacks reached the stage susceptible to parasitism later. It was felt that the different environmental conditions existing at the time of parasitism or during the immature stages of the parasite were the cause of the difference in the incidence of diapause observed.

The Influence of Photoperiod and Temperature on Diapause Initiation

Laboratory investigations were conducted in order to ascertain the influence of temperature and photoperiod on the diapause in this species, since both factors are known to play an important role in insect diapause (64).

Cocoons were removed from storage at 45°F. Half were placed at a temperature of 75°F. and the other half at 85°F. As the adults emerged at each temperature the females were mated to the emerging males. The females at each temperature were assigned to one of four containers by means of a random number table. Each of the four containers with its clear plastic window to admit light was then subjected to one of four photoperiod treatments, 10, 12, 14 or 16 hours, and remained so for one week. During this time these

females were fed daily a diet of a water-honey-yeast mixture plus dried raisins. When they were one week old the four containers of females at each temperature were assigned at random to one of four cages containing a log infested with Douglas-fir beetles in the larval stage. The females were left in the cages for oviposition for one week, being removed during that time every two days for an overnight feeding period, after which they were reintroduced into the cages. During this week the cages and the ovipositing females continued to receive the photoperiodic treatment initiated at emergence, however, after the one week oviposition period, when all females were removed, all cages received a 16-hour day length. Sufficient time was allowed for any eggs laid on the last day to complete development and emerge before the logs were debarked. Cocoons were examined to ascertain the incidence of diapause induced by the treatments. The whole procedure was repeated twice more for a total of three replications. The results are presented in Table 5.

At both 75° and 85°F. a higher percentage of the progeny entered diapause in the fifth instar at the shorter day lengths than at the 16-hour day length. These differences proved real when separate chi square tests of independence were performed on the counts at each temperature (Appendix, Tables 16 and 17). The effects of temperature at each photoperiod were similarly tested by chi square

Table 5. The effect of photoperiod and temperature treatments of adult females on the percent of their progeny entering diapause.[1]

temperature °F.	photoperiod-hours of light per 24			
	10	12	14	16
75	65(49-78)	70(58-78)	73(62-82)	8(3-16)
85	66(58-69)	76(66-86)	60(48-71)	0(0-4)

1. Figures in parenthesis are the 95 percent confidence intervals of the means.

tests of independence. At the 16-hour day length the means were different at the 5 percent level, however, they were not significantly different at the 10-, 12- or 14-hour day lengths. Thus, it appears that within the ranges of conditions tested both temperature and photoperiod play a role in the diapause in C. brunneri, with the latter being more influential than the former.

The Effect of Maternal Age on the Incidence of Diapause

To determine if the age of C. brunneri females had any effect on the percentage of diapause in their progeny, a group of nine females emerging on the same day were allowed to oviposit under conditions previously shown to be effective in averting diapause, a day length of 16 hours and a temperature of 75°F. The eggs laid during each two day period for the entire lives of the females were isolated

to observe the subsequent emergence of the resulting adults. All cocoons were examined at the end of the emergence period to ascertain if there were any larvae still remaining in the cocoons, which would be those in diapause. All individuals developed without arrest, indicating that maternal age does not influence the percent of diapause.

The Critical Stage

There are two cases cited by Lees (64, p. 36) where there apparently is transovarial transmission of a diapause determining factor (19; 97). Simmonds found that in Spalangia drosophilae Ashmead "the percentage of progeny entering diapause increases with the age of the parent female at the time of oviposition" (97, p. 388), indicating that some factor in the physiology of the parent parasite changes with increasing age. This effect is transmitted to the egg to determine whether or not the progeny will enter diapause as larvae. Earlier Simmonds (95, p. 95) indicated that a species of Cryptus showed the same effect, but this was influenced by diet (97, p. 402-404).

Lucilia sericata Meigen is another insect where apparently a diapause factor is transferred from parent to offspring. Eggs laid in the laboratory by flies collected from the field at different times of the year, showed differing percentages of diapause when reared under conditions shown

to avert diapause in eggs collected from laboratory-reared flies. The later in the season the flies were collected in the field, the higher was the incidence of diapause in the progeny (19, p. 601). The factors involved in this cases may be different than in S. drosophilae since in the latter insect "approximately the same percentage of larvae entered diapause in both the early and the later parts of the seasonal period of parasitism..." (97, p. 387). Cragg and Cole tested the diapause percent in the offspring of various aged laboratory-reared flies and found no difference (19, p. 602-603), which is another difference between L. sericata and S. drosophilae. In S. drosophilae the temperature at which the parent females are reared and oviposit influences the percentage of diapause in the offspring (97, p. 390), whereas in L. sericata it is probably the photoperiod which exerts the controlling influence. I take this to be the case even though Dickson (25, p. 534) states "although there is a considerable amount of true diapause in this species, the photoperiod has no effect." Although he does not state that the photoperiodic treatments were imposed upon the larvae, I take this to be the case in view of his work on Grapholitha molesta (Busck) and Carpocapsa pomonella (Linnaeus).

These three examples are extremely interesting from the physiological standpoint since Lees (64, p. 32-36) states that "the physical factors which control the onset

of diapause often operate on the insect long before growth is finally arrested", even as much as almost a complete generation earlier. However, "the final events leading up to the onset of diapause take place within the life of the individual", and earlier, that (with the possible exceptions noted above), "the sensitivity to external factors is only acquired when the central nervous and endocrine systems are already in existence". It is probable as Lees indicates that these systems are involved in the transmission of a stimulus which later determines whether or not diapause will intervene later.

C. brunneri appears to be another exception to these statements. In the experiments described above the adult females were subjected to the different photoperiods from the time of emergence from the cocoons until the eggs had been deposited. Since the eggs were deposited within the larval cells of the host, they were shielded from the alternate periods of light and darkness by a thickness of bark, and it seems unlikely that they could be affected by photoperiodic influence. The different day lengths, therefore, must have acted upon the maternal physiology, the effects of which were then transferred to the subsequent generation through the eggs. The only other alternative to this conclusion is that there was a small amount of light penetrating the bark which was effective in acting upon the developing embryos or larvae. Experiments were

started to determine if this was the case, but unfortunately, due to unforseen technical difficulties no results were obtained.

EFFECTS OF THE HOST ON THE PARASITE

Effects of Host on Size of Parasite

During the course of these investigations it was observed that in general the smaller cocoons producing males, were at the ends of the shorter Douglas-fir beetle larval galleries, while the larger cocoons producing females, were at the ends of longer galleries. To ascertain if these observations had any basis in fact, a sample of Douglas-fir beetle larval galleries in which the larvae had been parasitized were measured, and the cocoon from each was isolated in a separate vial. Galleries which could be traced for their entire length were chosen to avoid the possibility of getting a cocoon from another gallery. A length of string laid from the parent gallery along the larval gallery to its end was compared with a millimeter rule as a measure of larval gallery length. The restriction of being able to trace each gallery for its entire length reduced somewhat the number of galleries available for measurement, since in much of the material on hand the beetles were crowded and individual mines could not be traced with certainty. No randomization was done during the course of sampling since the 200 galleries measured represented virtually a 100 percent sample.

From the 200 cocoons 59 male and 43 female parasites emerged. They were anesthesized with carbon dioxide and

measured with a calibrated eyepiece micrometer in a dissecting microscope at 15X magnification.

The data were analyzed to determine the relation between the length of the host gallery and the size of the adult parasite. A test of linearity of regression (66, p. 295-298) performed separately on males and females led in each case to the conclusion that the regression of the size of the adult on the length of host larval gallery was linear (Appendix, Tables 18 and 19). Next, the hypotheses that the population regression coefficients were equal to zero were tested using the F test (66, p. 263-264). F values of 93.79 with 1 and 57 degrees of freedom and 27.06 with 1 and 41 degrees of freedom were obtained for males and females, respectively, and led to the conclusion in each case that the population regression coefficient was not equal to zero. A test of homogeneity of regression coefficients (66, p. 344-349) led to the conclusion that the population regression coefficients were not the same for the males and females (Appendix, Table 20).

Measurements are plotted in Figure 17. Individual lines of regression have been plotted for males and females with the regression equations and correlation coefficients listed in the figure.

Thus, there is an obvious relationship between parasite size and host larval gallery length. We may

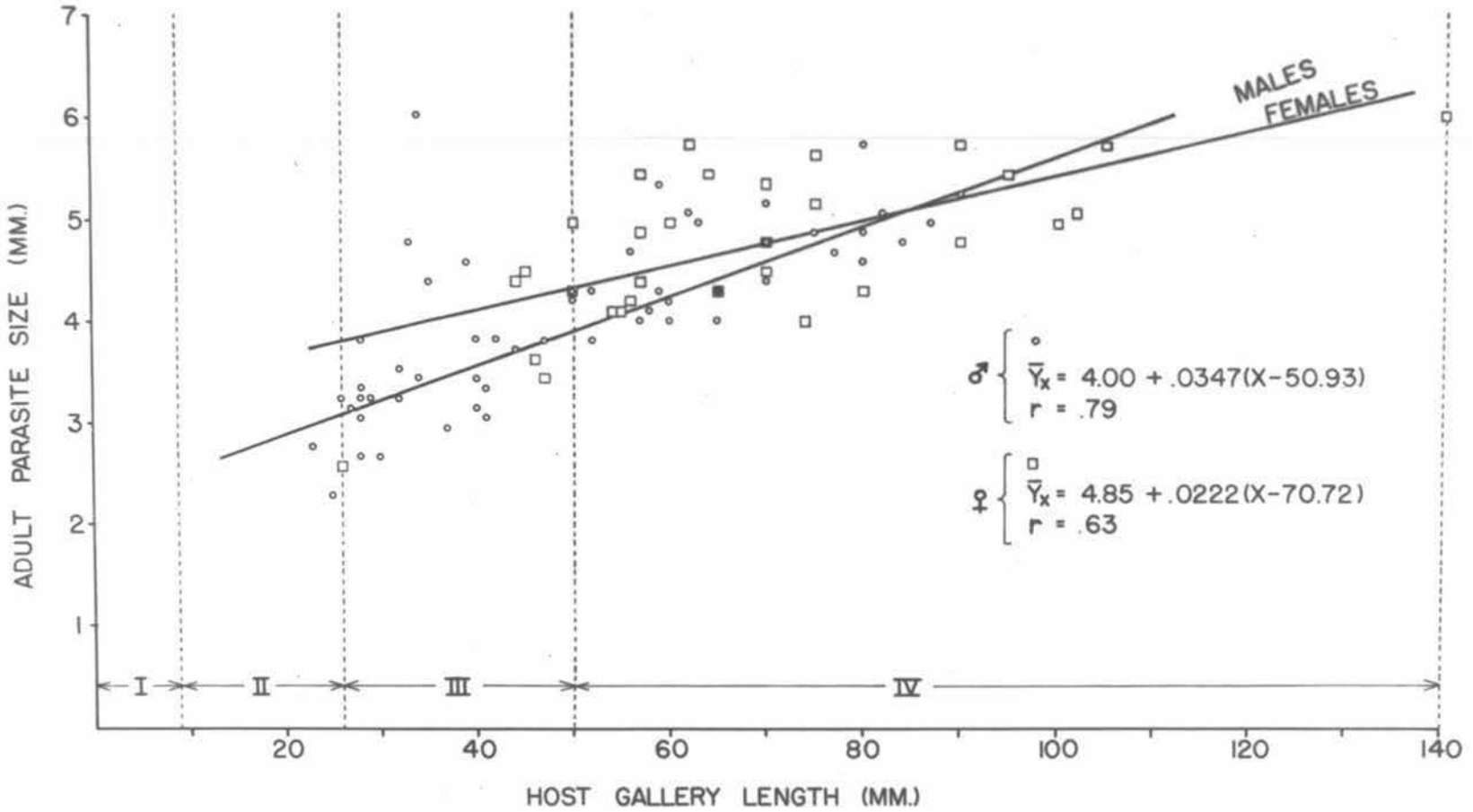


Figure 17. Regressions of male and female parasite size on length of host larval gallery length. Vertical lines indicate the larval gallery lengths of the four host instars.

reasonably assume that the longer galleries had larger beetle larvae than the shorter ones, and that the parasite larvae, having more food available in the larger hosts, were bigger as a result. Further evidence as to the validity of this conclusion is lent by observations on the size of this parasite after feeding on different host species. When reared on a smaller species, Scolytus ventralis LeConte, the adults of Coeloides brunneri are smaller than when reared on their main host, the Douglas-fir beetle. Similarly, they are larger when reared on the larger species, Melanophila drummondi, although no actual measurements were taken in these two cases.

Effects of Host on Sex of Parasite

It has been observed many times before that the sex ratio of parasites issuing from large hosts is frequently heavily in favor of females, while the reverse is true for parasites from small hosts (16, p. 1-9; 35, p. 20-21; 43, p. 325-334; 54, p. 247-259; 92, p. 67-70). In the case of C. brunneri the observation was made that males usually came from short host galleries while females usually came from long host galleries. Since the shorter galleries have smaller larvae the same relationship was apparently true. As a test, the data presented in the previous section were analyzed. Lengths of galleries having cocoons producing males were compared with those

having cocoons producing females by a t test (66, p. 131-133). A calculated value of 4.99 with 100 degrees of freedom, significant at the 1 percent level, led to the conclusion that the observation was a correct one. It seems likely to the writer that in the case of C. brunneri the pitch or volume of the vibrations produced by the tunnelling of the larger Douglas-fir beetle larvae has more of a stimulating effect on the female to activate the sperm and produce a female than does that produced by the smaller larvae. Flanders (35) has given an excellent discussion of the phenomenon.

DURATION AND RATE OF DEVELOPMENT OF THE IMMATURE STADIA AT VARIOUS CONSTANT TEMPERATURES

Parasite eggs and host larvae were removed from within the bark of logs immediately after females finished ovipositing (see p. 7 for procedure). Each egg with its accompanying larva was transferred to a small Syracuse watch glass and quickly placed in one of several constant temperature cabinets for incubation, with the lapse of time between completion of oviposition and start of incubation usually not exceeding 10 minutes. Time of oviposition and all pertinent observations regarding temperature and development were recorded. Examinations were made at intervals varying from 30 minutes to 12 hours depending on the temperature, stadium and time since the last molt. Larval head capsule measurements were taken at each observation, thereby indicating when a molt had occured. In addition, setation of the first and fifth instars were distinctive enough to separate them at a glance from the second and fourth instars, respectively (see Figure 56).

Following the development of a single insect from egg to adult in this manner gave observations on the duration of all seven immature stadia as well as a measurement of the head capsule width of the five instars with absolute certainty of the instar being measured. A total of 87 insects was observed for all or a part of their development. The duration of each stadium in hours and the velocity of

development expressed as the reciprocal of time are presented in Table 6 for each stadium at each temperature studied. These figures have been plotted in Figure 18.

It was difficult to obtain observations on the duration of the instars at the higher temperatures. At the lower and middle range of temperatures the host larvae, although paralyzed, lived long enough for the parasites to consume them completely. However, at the higher temperatures the host larvae died quickly and were soon invaded by saprophytic fungi. The parasites would not feed on larvae in this condition. That is the reason for the lack of observations at 95 and 100 degrees in Table 6. There was a tendency, most marked at the lower temperatures, for the fifth instar to go into quiescence or diapause, thereby reducing the number of observations and consequently the reliability of the lower part of the curve for this instar. Whether this arrested development was a true diapause or merely quiescence due to the low temperature as observed by Dickson (25, p. 529-531) in Grapholitha molesta was not determined. This same factor created a shortage of observations on the duration of the pupal stage. Therefore, overwintering fifth instars being held at 45°F. were transferred to the various constant temperatures to obtain additional observations on the duration of the pupal stage. This perhaps has caused a slightly lower average duration for the pupal stage at

Table 6. Average duration and velocity of each stadium at various constant temperatures.

Stage	Time and velocity	Temperature °F.									
		55	60	65	70	75	80	85	90	95	100
Egg	no. hours	151	70	54	40	33	29	24	22	21	27
	1/time	.0066	.0143	.0185	.0250	.0303	.0345	.0417	.0455	.0476	.0370
First instar	no. hours	105	56	51	28	25	25	26	18	24	
	1/time	.0095	.0179	.0196	.0357	.0400	.0400	.0385	.0556	.0417	
Second instar	no. hours	70	39	33	21	18	17	15	18	32	
	1/time	.0143	.0256	.0303	.0476	.0556	.0588	.0667	.0556	.0313	
Third instar	no. hours	73	42.5	29	23	17	13	12	15		
	1/time	.0137	.0235	.0345	.0435	.0588	.0769	.0833	.0667		
Fourth instar	no. hours	118	52	41	30	23	20	17	18		
	1/time	.0085	.0192	.0244	.0333	.0435	.0500	.0588	.0556		
Fifth instar	no. hours		310	320	163	130	125	132	150		
	1/time		.0032	.0031	.0061	.0077	.0080	.0076	.0067		
Pupa	no. hours	945		313	178	131	125	116	135		
	1/time	.0011		.0032	.0056	.0076	.0080	.0086	.0074		

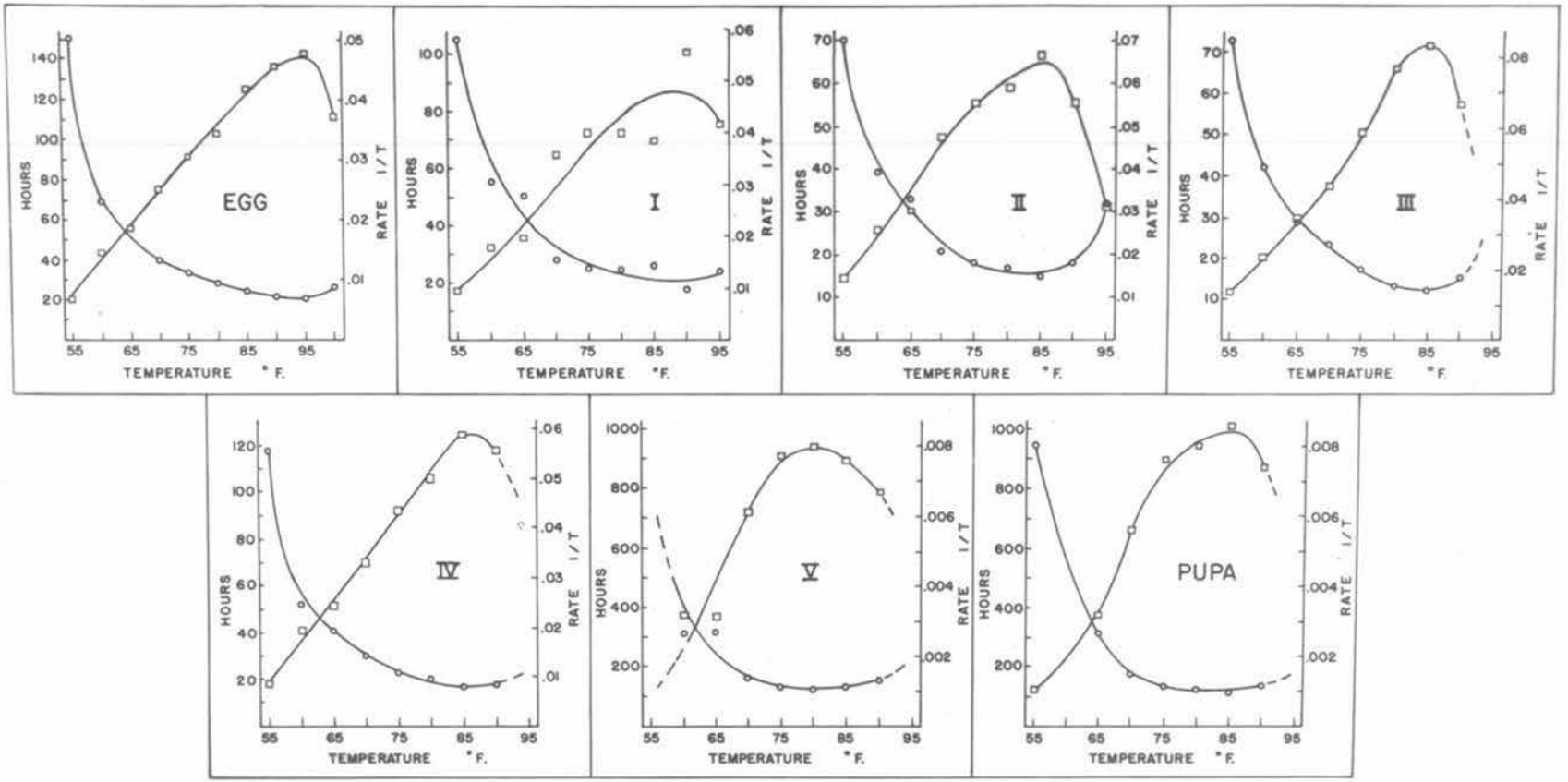


Figure 18. Time and velocity curves for the durations of the seven immature stadia of C. brunneri at various constant temperatures.

the higher temperatures than would have been observed had the insects been held at these temperatures for their entire development (55, p. 13-16).

As shown in Figure 18 the means of the observations at each temperature on each curve, except those for the first instar, lie quite close to the rather smooth sigmoid curve so often observed in biological data. In general form this is the reciprocal of the catenary curve of Janisch (58, p. 140). After hatching from the egg some individuals of the first instar wander, often for as long as 24 hours or longer, before settling down to feed, while others start feeding almost immediately. It is felt that this fact has contributed greatly to the lack of correspondence between points and curve for this instar.

Different vertical and horizontal scales have been used for some curves in Figure 18 for ease of plotting, but the similarity of their general shape is readily apparent. Based on these empirical data the optimum temperature for the development of each stadium along with the duration of each and its velocity at the optimum temperature are shown in Table 7.

Table 7. Temperature optima for development of each immature stadium with duration and velocity of each.

Stadium	Temperature optimum °F.	Duration in hours	Velocity at optimum (1/time)
egg	95	21	.0476
1st instar	88	21	.0476
2nd instar	85	15.5	.0645
3rd instar	85	12	.0833
4th instar	85	17	.0588
5th instar	80	125	.0080
pupa	82	117	.0085

The total time of development from egg deposition to emergence of the adult from the pupa has been summarized for temperatures between 55° and 90°F., inclusive, in Table 8. The times are in days and each represents the converted sum of the seven individual values in hours for each stadium read from the curves in Figure 18.

Table 8. Total time of development at various constant temperatures as determined by summing curved durations of individual stadia.

Temperature °F.	Time (days)
55	98.4
60	51.5
65	31.4
70	20.5
75	15.8
80	14.8
85	14.1
90	15.8

Average field temperatures were derived from hygrothermograph recordings and were used in conjunction with field observations of developmental times. These corresponded very closely to values obtained in the laboratory.

The variation which may occur in the total developmental time is indicated in Figure 19. It represents the emergence of parasites reared in the laboratory at 75°F. Females were allowed to oviposit into beetle-infested logs for 33 hours. Cages were examined at the end of each day and emerging progeny were collected. From the time when adults first could have laid eggs until the collection of the first of the offspring from the cages no more than 14½ days had elapsed. The longest developmental time was at least 23 days. It is felt this variation is due largely to the size of the host attacked. Observations indicate that parasites developing on small larvae develop faster than those on large larvae.

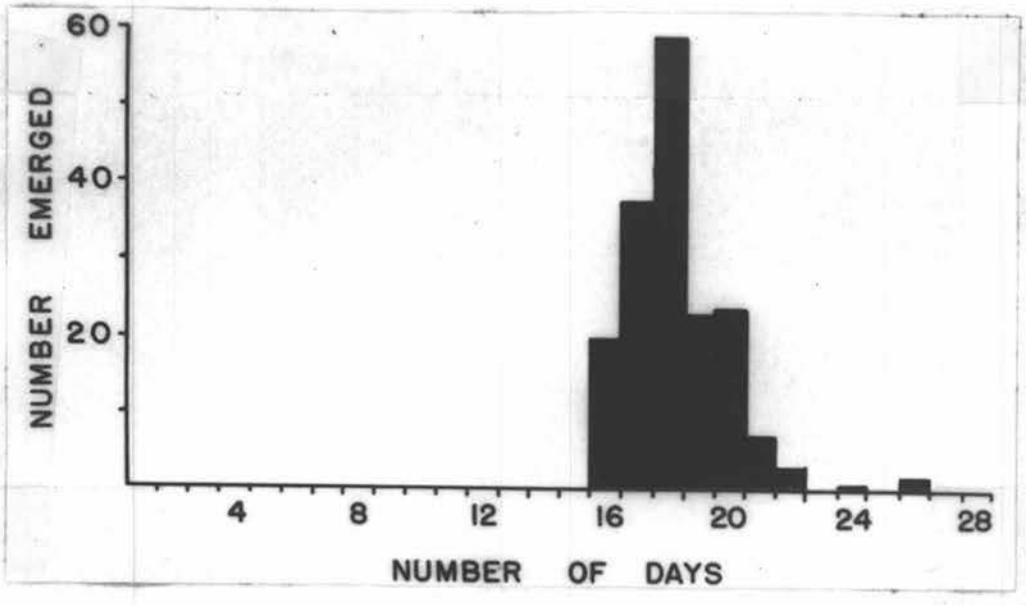


Figure 19. The daily emergence of adults resulting from eggs laid during a 33-hour period, showing the variation in developmental time at 75°F.

EFFECTIVENESS IN PARASITIZING *DENDROCTONUS PSEUDOTSUGAE*

The percentage of the individuals in any particular Douglas-fir beetle brood which will be parasitized by *C. brunneri* depends on many factors. Some of these are; the location of the tree i.e., whether it is under the closed canopy of a dense stand or in the open; characteristics of the bark over-lying the brood; whether the brood is in a standing or a down tree, and if in a down tree whether on the top, bottom or sides of the bole; the stadium of the individuals in the brood when parasites are present; the percentage of the individuals which are destroyed by other factors and the time of their destruction; the number of beetle broods in the immediate vicinity; the number of parasites present (which itself depends on the time of the year, the number of host individuals which were present the previous generations, the distance to the logs from which they emerged and other factors); the parasite sex ratio; the size of the females; and the weather. Some of these factors have been discussed previously. A few of those remaining are discussed below.

Effects of Bark Thickness on Parasitism

C. brunneri females cannot parasitize larvae that are under bark thicker than the length of their ovipositors. But even though the outer bark of Douglas-fir may reach a

maximum thickness of many times the average parasite ovipositor length, the thickness in the crevices separating these thick places is frequently thin enough for C. brunneri females to parasitize larvae in the inner bark. Thus, although two potential host larvae may be separated by only an inch or two of circumferential distance, one may be inaccessible and the other accessible to the parasite due to differences in bark thickness. Also, the same larva may alternate one or more times between being accessible and inaccessible as it tunnels tangentially under different thicknesses of bark. Of course, when the maximum bark thickness at any given height in a tree is less than the ovipositor length of a female parasite, then any larva at this height may be reached at any time by the female. This occurs in small trees and in the tops of larger trees. Yet, another female searching at the same height in the same tree may not be able to reach all host larvae because of a shorter ovipositor.

To obtain data on the effectiveness of C. brunneri in parasitizing the Douglas-fir beetle in areas of different bark thickness, samples were taken at different heights in a number of infested trees. Eight trees were selected in a stand of second growth Douglas-fir in Weyerhaeuser Company's McDonald tree farm near Centralia, Washington and sampled approximately every 16 feet in April, 1959. The trees had blown down during the winter of 1957-58 and had

been attacked by Douglas-fir beetles in the spring of 1958. The trees were bucked into log lengths and the bark was removed from the basal two feet in each log. Percent parasitism was determined for each sample on the basis of cocoon counts and counts of the number of Douglas-fir beetle larval galleries longer than one inch. The figure of one inch was used since it has been shown (Figure 17) that unless the larvae have tunnelled at least this far they are too small to be parasitized. In addition, for each sample the following data were recorded: maximum bark thickness, circumference of the log at the middle of the sample area and the percentage of the circumference which had outer bark thinner than the mean ovipositor length of the parasites (see p. 10 for description of a device and its use in obtaining this percentage).

The trees sampled were of different heights. In all eight trees at least three samples containing at least some beetle galleries were obtained. In five of the trees four samples were obtained and in three trees there were five usable samples. The samples were grouped starting with the topmost sample in each tree, thereby reducing much of the variation between corresponding sample points which would have resulted if the samples had been grouped starting with the bottom sample in each tree. The mean percent parasitism at each of the sampling points has been plotted in Figure 20. An analysis of variance with a tree

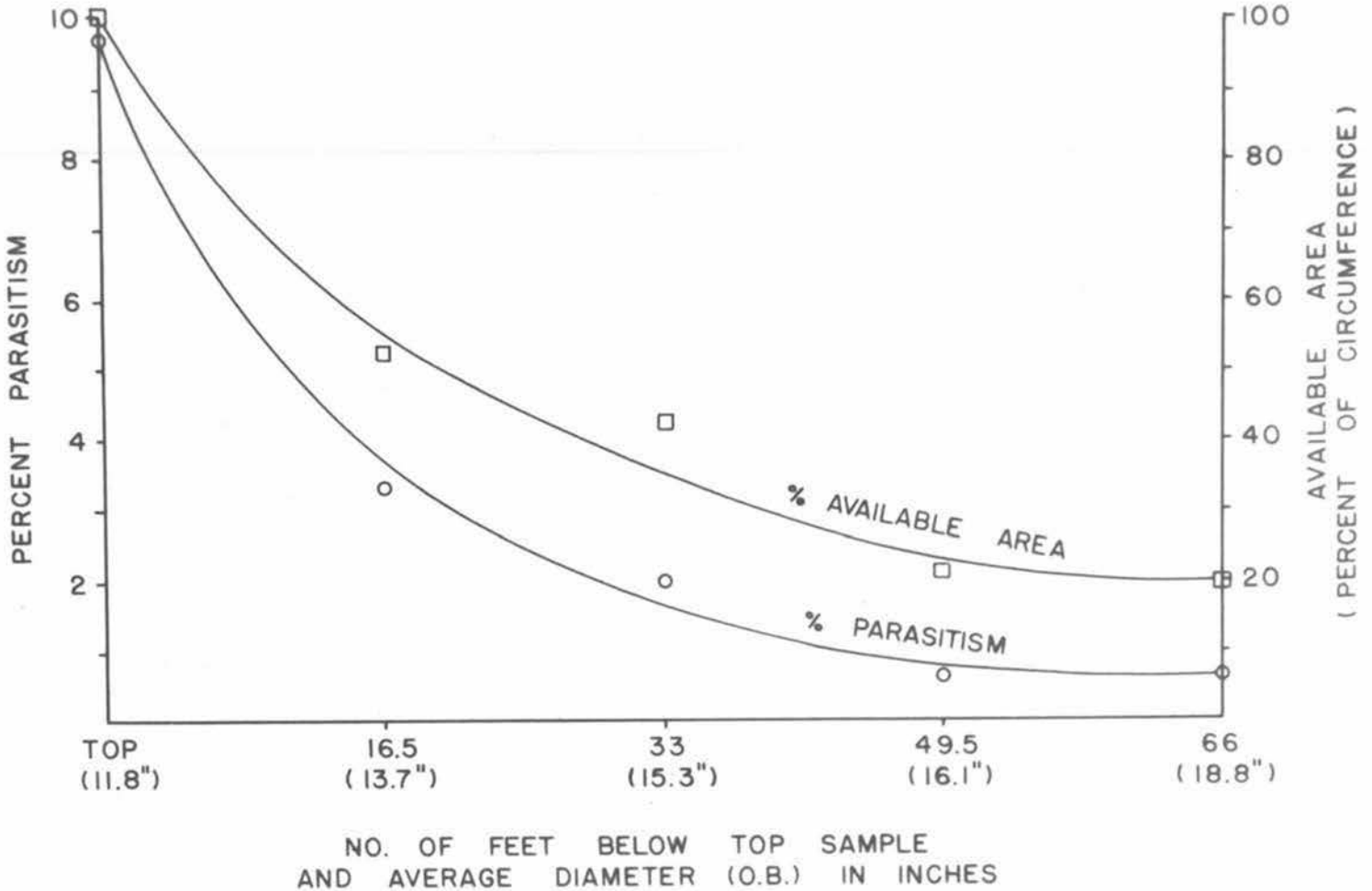


Figure 20. The relationship between the percent parasitism and the percent available area at different heights in trees. For discussion see text.

being a replication was conducted on the percent parasitism. Due to the incompleteness of observations at the fourth and fifth sampling points because of tree height variation it was possible to analyze only the top three samples in each tree according to the randomized block design. The replication effect proved to be insignificant at the 5 percent level, so an analysis of variance was conducted as it it were a completely randomized experiment (66, p. 167-169). It was possible to utilize the data at all five sampling points in this analysis (Appendix, Table 21). The conclusion reached was that the percent parasitism is not the same at different heights in a tree. It is higher at the top of the tree than at the bottom.

The reason for the difference in parasitism seems obvious when the percentage of the log circumference at each sampling point which had outer bark thinner than the mean ovipositor length of the parasites is considered. The mean percentages at each sampling point have been plotted in Figure 20. These percentages actually represent the area of the sample available to the parasites for oviposition. The curves of percent parasitism and percent available area closely parallel each other, with the slight deviations of the means of percent available area from their curved values being reflected by similar deviations in the means of the percent parasitism.

However, the curves are not exactly parallel. There is convergence toward the top of the tree. This seems reasonable when the gallery pattern of the Douglas-fir beetle is considered in relation to the pattern of crevices in the bark. The adult beetles obtain entry to the inner bark at a crevice and construct longitudinal egg galleries, from which the larval galleries extend at right angles. Toward the top of the tree a parasite may reach a beetle larva at any time it happens to be searching in that area, since all of the bark area is thin enough. If the parasite proceeded down the tree bole it would be less likely to encounter a host larva beneath thin bark, because not only does the percent of the circumference available for oviposition decrease (Figure 20), but also the distance between available areas increases (Figure 21). A particular beetle larva may actually complete its development toward the base of the tree without ever passing beneath a thin area, even though thin areas were present elsewhere on the circumference at that height in the tree.

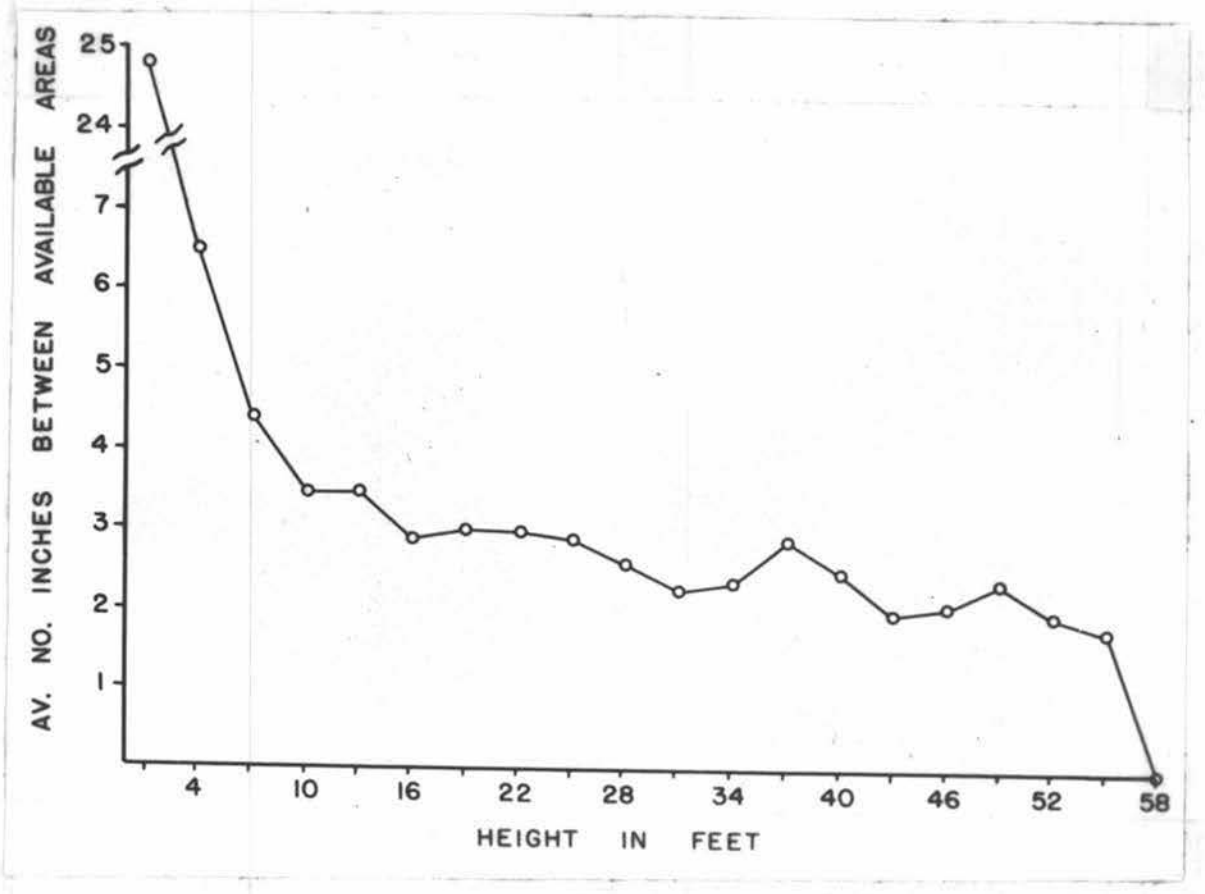


Figure 21. Graph of the average number of inches between available areas at different heights in a tree. For discussion see text.

IMPORTANCE OF TIMING OF HOST INVASIONS ON PARASITE SUCCESS

The majority of the Douglas-fir beetle invasions from overwintering adults occurs during April and May. Gallery construction and egg laying proceed over a several week period, or longer if weather conditions are particularly unfavorable. Generally, the individuals from the first eggs laid pass out of the susceptible stage (become pupae) about mid-July, while those from the last eggs laid in these galleries pupate about mid-August. Adults of C. brunneri which overwintered as larvae are present during July and use these early beetle broods as hosts for their progeny, the first generation. Parasite numbers increase greatly during this generation. Since the photoperiod was relatively long during the egg-laying period, most of the individuals of the first parasite generation emerge the same season (see p. 58) for a discussion of the effects of photoperiod on diapause).

As hosts for the second parasite generation, the adults of the first generation may use the late developing individuals from the early beetle invasions. However, some of these have already pupated by the time adults of the first parasite generation emerge. Were not additional hosts made available as a result of second beetle invasions, the full reproductive capacity of C. brunneri would not be realized. This is even more true for the third generation

which finds hosts exclusively in broods from late beetle invasions.

The second and third parasite generations contribute the majority of the individuals to the overwintering population (see p. 20). Therefore, the importance of late or second beetle invasions in regulating the abundance of C. brunneri the following year is apparent. If there were no late beetle invasions the overwintering parasite population would be much smaller than it normally is. It would consist of only those larvae of the first generation which went into diapause (5 percent or less) plus diapause larvae (approximately 50 percent in the second generation) from adults of the first generation which were able to find late developing beetle larvae from early invasions. The parasite is able to utilize other species as hosts, but it is felt that these are not too important in building up large populations. The writer was unable to find any parasite larvae which overwintered more than once, so the factor of holdovers from previous years apparently does not enter the picture. The knowledge of the dependence of C. brunneri on late beetle invasions is made use of in a later section in which control practices for the Douglas-fir beetle are recommended.

Alternate Hosts

Only Dendroctonus pseudotsugae and Scolytus ventralis

are listed as hosts in catalogs by Muesebeck, Krombein and Townes (72, p. 160) and by Thompson (107, p. 106). Both of these species have been confirmed as hosts by the writer.

One other species should be added with certainty to this list. Melanophila drummondi is successfully parasitized. It is only because of a partial ecological separation between it and C. brunneri (see p. 27) that the incidence of parasitism is not greater.

Adults of C. brunneri have been reared from cocoons found in galleries of other species found in Douglas-fir, but identification in these cases is based solely on gallery patterns. The probable hosts are Pseudohylesinus granulatus (LeConte) and at least one species of Cerambycidae.

That C. brunneri lays eggs on several species beneath the bark of Douglas-fir is not surprising since females have been stimulated to oviposit through bark merely by scratching the inner surface (see p. 27). With the exception of S. ventralis all species of known hosts are found in Douglas-fir. The existence of an alternate host in another tree species could be especially valuable to the parasite during times when few Douglas-fir trees suitable for breeding for its main host are available.

DOUGLAS-FIR BEETLE CONTROL RECOMMENDATIONS INVOLVING COELOIDES BRUNNERI

The current Douglas-fir beetle control methods consist of a rapid salvage of beetle-infested trees so that the beetles may be destroyed during the milling operations before emerging. This method does little to shift the population balance in favor of natural control agents because the latter are often destroyed along with the beetles. Because of this fact serious beetle infestations may continue or reappear. Methods need to be devised which will take advantage of the biological control agents and aid them in holding beetle numbers at low levels.

C. brunneri is a parasite, which under certain conditions can effect a high degree of control. Evidence as to its ability is presented in Figure 22 where virtually every beetle larva in the one gallery shown has been parasitized under natural conditions. This parasite has been seriously hampered in its efforts by being unable to occupy fully the distribution of its host within a tree because the thickness of Douglas-fir bark is often greater than the parasite ovipositor length. This drawback will become less serious as old growth stands are converted to younger second growth and management becomes more intensive. The trees will be smaller and the bark thinner.

It is felt by the writer that populations of C. brunneri should be maintained at a level sufficiently

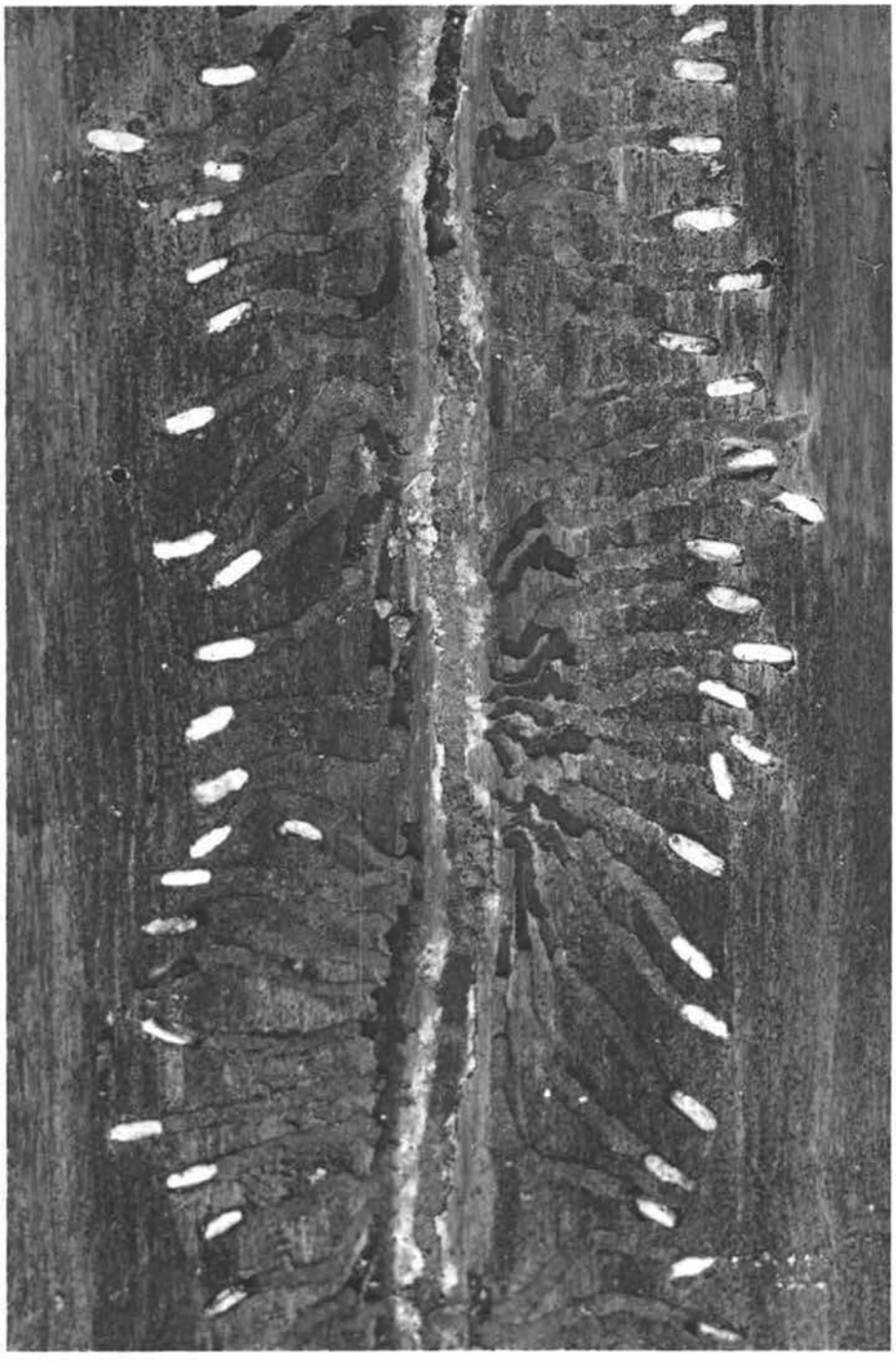


Figure 22. A Douglas-fir beetle gallery in which the brood has been heavily parasitized by C. brunneri under natural conditions.

high to act as a damper on any potential large scale increase in beetle numbers, for actually it is only when beetle populations become excessive that there is large scale tree killing. This will necessitate fostering beetle populations in small diameter trees where parasite increase will be favored, and postponing removal of these trees until parasites have emerged. The present practice of rapid milling to destroy beetle broods before they emerge should continue, but discretion should be used in selecting the trees and the time of the year for their removal. The specific trees which should be removed and the times of the year during which this should be done will be discussed below along with other recommended practices.

All trees invaded by beetles in early spring should be removed from the woods some time between August 15 and April 1 of the following year. By August 15 the first generation of C. brunneri will have emerged, and soon after April 1 of the following year the beetle broods will emerge.

From a study of the diapause in C. brunneri (p. 57) it is known that the percentage of parasites which overwinter depends on the date of parasitism, which in turn depends somewhat on the date of initial beetle invasion. The earlier in the season that beetle invasion and parasitism occur in any given log, the smaller will be the

percentage of parasites overwintering in that log, and vice versa. Many of the trees that beetles use as breeding material are windthrows occuring during the winter and are heavily invaded during spring. The percentage of parasites overwintering as a result of these early invasions is small. The majority of the parasites emerge the same season and parasitize broods from later beetle invasions or larvae resulting from eggs laid late in galleries established early. Some of the parent beetles reemerge after the first gallery is complete to establish a second brood. They often re-enter the same log. It is recommended that trees be felled just prior to this time so that only late beetle invasions will be made in these freshly felled trees. This practice could be integrated with thinning operations, for trees which should be thinned would probably possess the qualifications of being excellent parasite brood trees. That is, they are likely to be suppressed and therefore small, and after felling they would be located under a closed canopy, which has been observed to be preferred by parasites for oviposition. Research is needed to determine the number of trees which should be felled on any given area.

Probably during June is the best time to fell these trees, but this may vary according to the locality and from year to year. They should be left in the woods until the middle of July of the following year after which time

they may be salvaged if economically feasible. If the thinned trees are large the basal portions should be removed before April 1 of the following year, and only the tops should be left. It appears now that a diameter of approximately 12 to 14 inches will be marginal for the decision as to whether to remove a log early or to leave it until after parasite emergence. However, further research is needed to determine more exactly this limit. The trees and portions of trees left will act as reservoirs of overwintering parasites which will be ready the following spring to check any rapid increase in beetle numbers due to suddenly favorable conditions, such as an abundant food supply due to a severe winter windstorm.

Bedard (3, p. 1134) speculated on a method which would reduce beetle numbers without reducing parasite numbers. He pointed out that there is a delay between invasion by the Douglas-fir beetle in the spring and parasitsm of its brood by C. brunneri in the early summer. If these newly invaded trees were debarked and the bark burned during this time, he said, the parasite would not be destroyed. He did not categorically advocate this practice, however, due to the fire hazard at that time of the year. Any attempts to use direct control methods against the beetle before the broods are parasitized, such as he mentioned, necessarily involves early detection which is often difficult on a large scale. Too, the labor

force and money for control are often not available to do the job in the necessary time. A further objection to this method of control is that parasites are thereby deprived of hosts. By waiting until after August 15 to do the control (or removal) not only are the overwintering parasites assured of hosts on which to lay their eggs, but also subsequent parasite generations are assured of hosts, because of the offspring from reemerged parent beetles.

It must be realized that the recommendations set forth here do not take into consideration their possible effects on other natural control agents such as Medetera aldrichii. Therefore, they may need modification when more complete life histories of the other biotic agents are known.

EMBRYOLOGY AND MORPHOLOGY OF THE IMMATURE STADIA

Description of the Egg

The egg is an elongate cylindrical shape with a long tapering tail (Figure 23) and corresponds to the "stalked type" of Clausen (17, p. 14). Its widest portion is approximately one-fifth of the distance from the anterior end. Anterior to the widest region it tapers concavely to the broad, rounded anterior end. The gradually tapering tail is usually slightly curved and ends in a small acute point. Average overall length is approximately 1.3 millimeters, while at the widest point the eggs average approximately 0.15 millimeters.

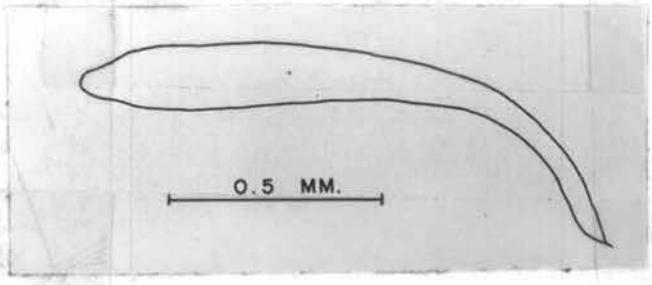


Figure 23. Outline of an egg of C. brunneri.

The newly laid egg is an homogeneous, translucent white color, although the ends soon clear and become transparent. The chorion is thin, elastic and devoid of any conspicuous sculpturing. Internal to the chorion is the thin vitelline membrane enclosing the egg contents. Inside the vitelline membrane lies a layer of peripheral protoplasm, the cortical layer, or periplasm, which is continuous with the internal protoplasmic reticulum

extending throughout the yolk or deutoplasm. In the center toward the posterior pole is a mass of germinal cytoplasm, the oösome, which stains heavily with alum carmine or Harris' hematoxylin.

Embryological Development[1]

Cleavage and blastoderm formation- During the first two hours of incubation the yolk withdraws slightly from the anterior end of the egg and almost completely from the tail. After this time the egg is in the 32-nucleus stage. The cleavage center appears to be located in the center of the yolk mass approximately one-fifth to one-fourth of the way from the anterior pole, for this is the area from which the cleavage nuclei spread. Two hours after egg deposition the nuclei are arranged in an elongate group in the center of the yolk extending from approximately one-eighth to one-third of the distance from the anterior pole. Each is surrounded with a rather well defined but irregularly outlined mass of protoplasm. The discreteness of these protoplasmic masses, each with a nucleus, has led to their being called cleavage "cells" (78, p. 16; 100, p.21), but they are not, strictly speaking, cells,

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1. The following observations were made on eggs after incubation for various periods at 75°F. At this temperature the total development from egg deposition to rupture of the chorion by the emerging first instar is approximately 33 hours (see p. 72).

since they are not bounded by cell membranes at this time. By three hours the nuclei have multiplied to number 256, have spread out further caudad and exhibit a cigar-shaped configuration. Figure 24 is a cross section through the anterior region of a three-hour egg. The nuclei are arranged in a circle concentric with the center of the egg. It can be seen that the multiplying nuclei in the center of the egg have gathered around themselves the protoplasm formerly held in the reticulum of the central region, leaving the yolk in this area devoid of protoplasm. The reticulum peripheral to the cleavage nuclei as well as the periplasm remain as before.

By the fourth hour, and with a further increase in number, most of the nuclei in the anterior half of the egg have migrated to the egg periphery and have entered the periplasm. Those in the posterior half may still be seen in the process of their peripheral migration, with the nuclei in advance and the accompanying protoplasm strung out behind for a length of several times the nuclear diameter. Some nuclei never migrate to the periphery but remain within the yolk as primary yolk cells or vitellophags. Since both the cleavage and differentiation centers are located in the anterior portion of the egg the posterior portion lags behind in this peripheral migration of nuclei as well as all stages of later development. By the fifth hour all nuclei which are to reach the

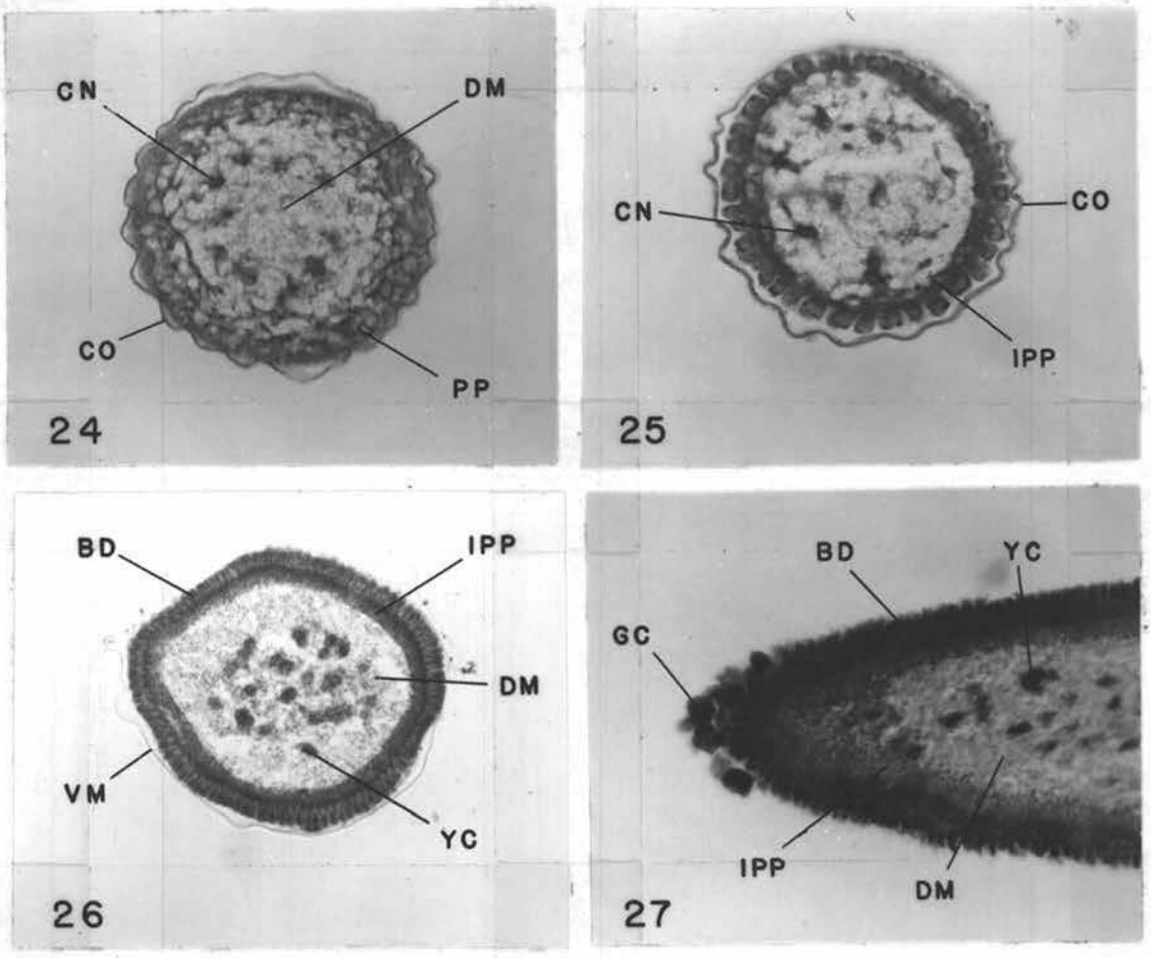

Figure 24. Cross section through the anterior region of a three-hour embryo showing the arrangement of the cleavage nuclei. Kahle's fixative. Alum carmine. 10μ. Photomicrograph.

Figure 25. Cross section through the anterior region of a five-hour embryo showing the beginning of cell wall formation in the blastoderm. Kahle's fixative. Alum carmine. 10μ. Photomicrograph.

Figure 26. Cross section through the anterior region of a seven-hour embryo. Kahle's fixative. Alum carmine. 10μ. Photomicrograph.

Figure 27. Sagittal section through the posterior pole of an eight-hour embryo showing the germ cells. Kahle's fixative. Harris' hematoxylin. 5μ. Photomicrograph.

periphery have done so. Bronskill (9, p. 659) termed the egg at this time the blastema, contrasting it to the blastoderm which is after the cell walls have formed.

Up to the time the nuclei reach the periplasm the divisions appear to be synchronous. However, once at the periphery this synchronization throughout the egg gives way in favor of posteriorly passing waves of activity where only the adjacent nuclei are at approximately the same stage of division. In a five-hour egg a complete cycle of mitosis from interphase to interphase can be observed in contiguous cells in a region occupying approximately one-fifth the length of the blastema. It is obvious, therefore, that the posterior region of the egg may lag the anterior region by several divisions. The plane of division is normal to the egg surface, resulting in a single layer of cells.

Between the fourth and fifth hour in the anterior region and between the fifth and sixth hour in the posterior portion of the egg there appear depressions between the nuclei. The depressions rapidly deepen, cutting off each nucleus laterally from its neighbor (Figure 25). This is the beginning of cell wall formation. The partially completed cells are still connected centrally by the periplasm at this time. Soon cell walls are extended centrally from these infoldings until between the sixth and seventh hour in the anterior portion of the egg, the

blastoderm cells are completed by the union of the walls centrally. Internal to the blastoderm cells a layer of protoplasm is thereby cut off, the inner periplasm (Figure 26) which disappears between the eighth and ninth hour. Meanwhile, the blastoderm cells have increased in number, by posteriorly passing waves of mitotic activity and have become columnar.

The completed blastoderm at eight hours is of approximately uniform thickness on the ventral and lateral sides. In the dorsal region the cells soon to be differentiated into the serosa are slightly less columnar.

During the process of cell wall formation the vitellophags are scattered quite uniformly throughout the yolk except for the region immediately adjacent to the blastoderm cells where there are none (Figure 26).

Formation of the inner layer and mesenteron rudiments- Between the eighth and ninth hour anteriorly along a lateroventral line on either side, the lateral folds begin to form. They are visible externally as shallow grooves and divide the blastoderm into a middle (ventral) and two lateral plates. By the ninth hour these lateroventral grooves have progressed to the posterior end of the embryo, diverging from the midventral line somewhat more than at their anterior ends. Cephalad the middle plate occupies approximately one-fifth or less of the circumference of the egg, while caudally it occupies approximately

one-fourth.

While the lateral folds are differentiating in a posterior direction, anteriorly the lateral plates are growing beneath the middle plate, which at the same time is spreading out laterally inside the lateral plates. Concurrently, there is an infolding of the blastoderm anterior to the middle plate. This is the site of the anterior midgut rudiment[1]. Because the posterior regions lag the anterior regions much of this process can be observed in successive sections from the same embryo. Successive stages of this process are shown in Figures 28-30, three sections through a single embryo at nine hours through the regions of the anterior midgut rudiment, immediately posterior to the anterior midgut rudiment and toward the posterior end.

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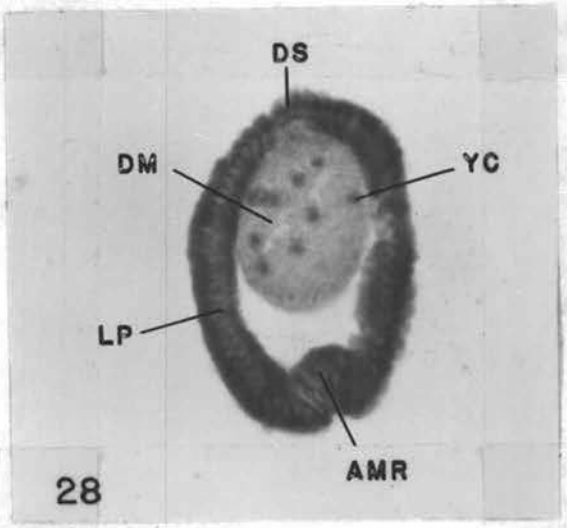
1. Nelson concluded that the anterior midgut rudiment in Apis arose as a result of immigration of the surrounding blastoderm cells (78, p. 62) and it was only later that its further growth was due to proliferation. This is in contrast to the conclusion reached by Carrière and Bürger for Chalcidoma who stated, "Es findet keine Einsenkung, Einfaltung oder Ueberwachsung statt, sondern auf gewissen Gebieten (Inseln) des Vorder- und Hinterfeldes vermehren sich die Blastodermzellen durch mitotische tangentiale Theilung" (15, p. 293). Figure 28 strongly suggests that C. brunneri is similar to Apis in this respect.

Figures 28-30. Three cross sections through a nine-hour embryo showing the initial differentiation of the anterior midgut rudiment and the middle and lateral plates. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrographs. 28. Through the anterior midgut rudiment. 29. Through the anterior portion of the embryo posterior to the anterior midgut rudiment. 30. Toward the caudal end of the embryo.

Figure 31. Cross section through the anterior midgut rudiment of a 10-hour embryo. Kahle's fixative. Alum carmine. 10μ. Photomicrograph.

Figures 32-33. Two cross sections through an 11-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrographs. 32. Through the mid-abdominal region. 33. Through the posterior midgut rudiment.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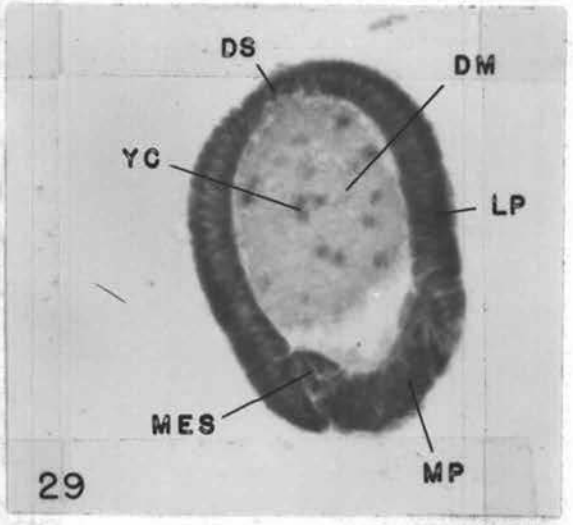
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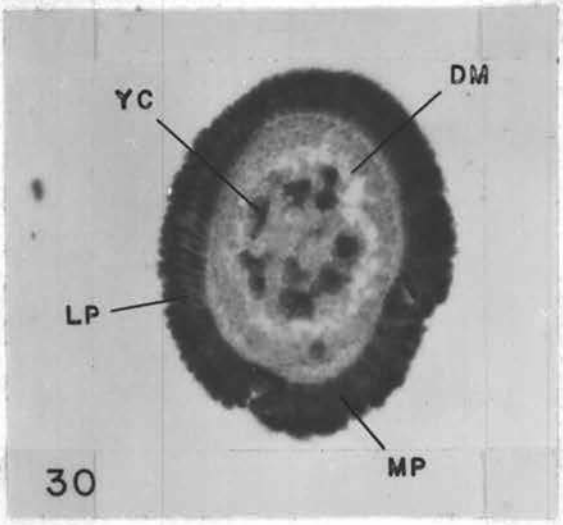
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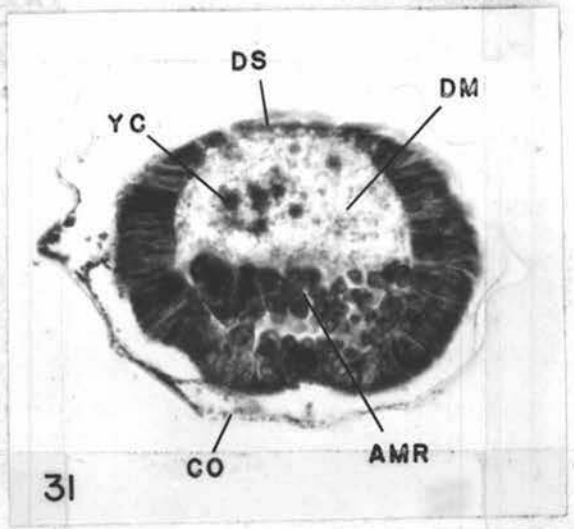
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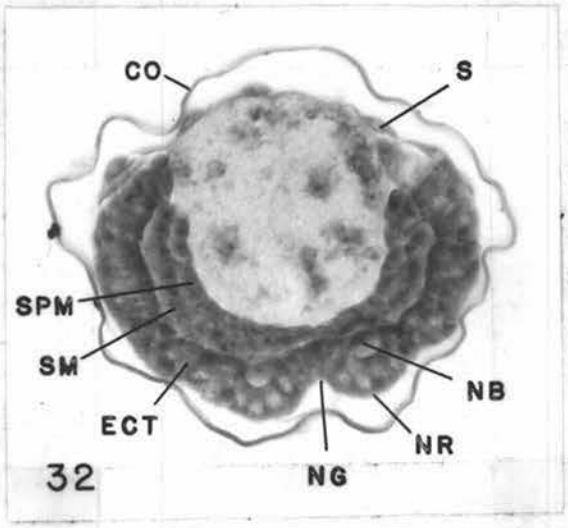
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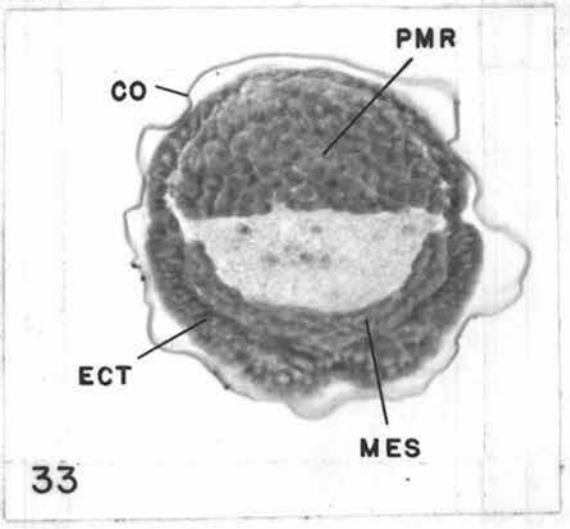
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By the tenth hour anteriorly the lateral plates (ectoderm) have approximated each other along the midventral line and are closing over the anterior midgut rudiment which is now composed of loosely aggregated cells projecting dorsally into the yolk (Figure 31). Immediately caudad of the anterior midgut rudiment the middle plate (mesoderm) has spread laterally to occupy between one-third and one-half of the inner circumference. There is now more than one layer of cells in the mesoderm. Caudoventrally the cells of the middle plate have lengthened forming a definite thickened area which by the eleventh hour has migrated dorsally as the posterior midgut rudiment and has been partially overgrown by the lateral plates. The lateral plates by the eleventh hour are approximating each other caudodorsally as well as having fused ventrally for almost their entire length (Figure 34). The mesoderm has spread out further within the ectoderm and now occupies greater than one-half of the internal circumference (Figure 32). The cells have become arranged into an outer somatic layer and an inner splanchnic layer.

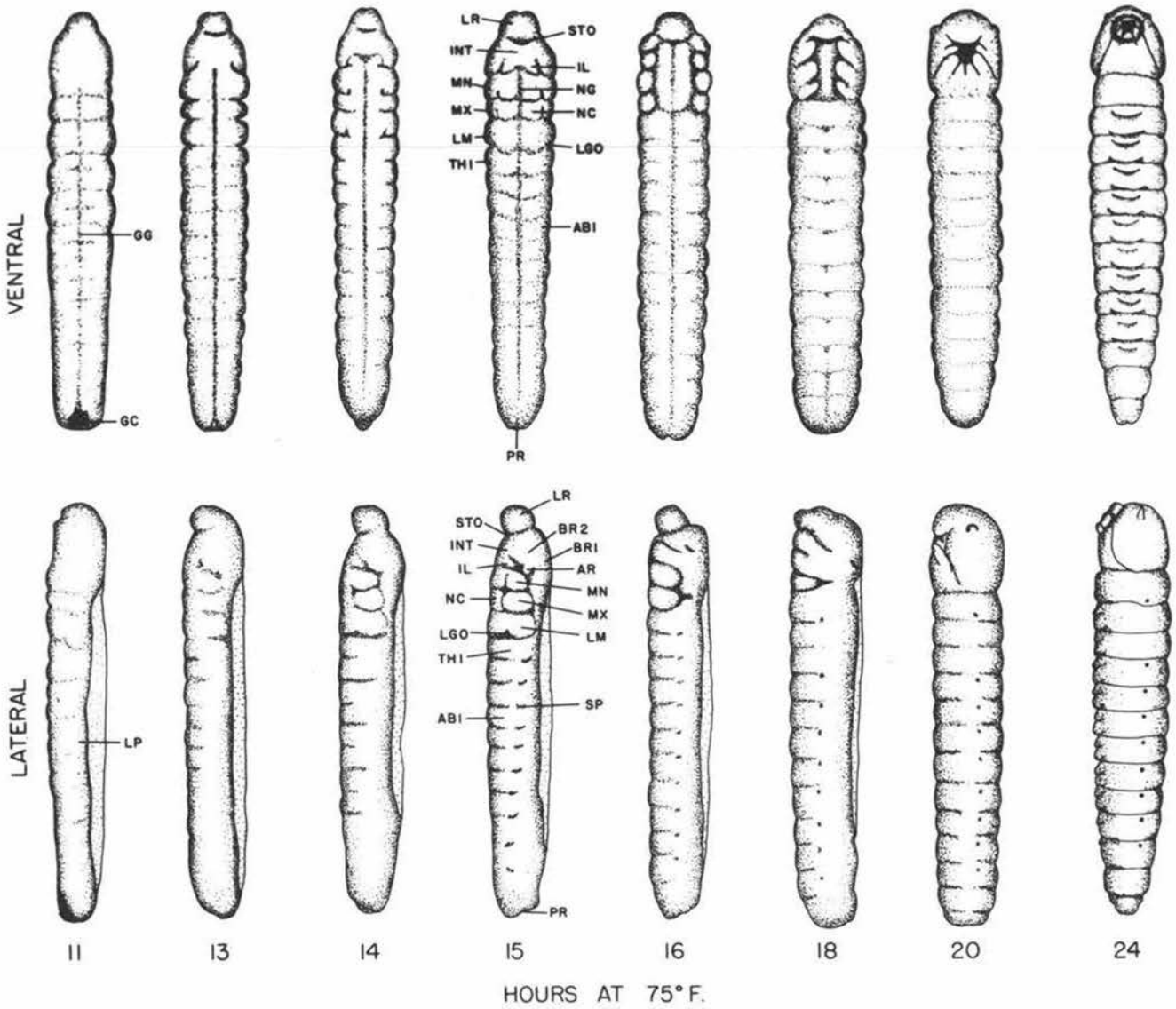
External segmentation and definitive head formation- The first external evidences of segmentation occur between the ninth and the tenth hour when the second maxillary segment is delimited by transverse grooves. The cells of the ectoderm cephalad of the anterior midgut rudiment

lengthen while cephalodorsally the cells of the serosa are rounding up. By 10 hours the serosal cells are severing their attachment with the lateral ectoderm and faint transverse segmental grooves may be seen marking off the first maxillary segment and the first two thoracic segments. At the eleventh hour the labrum is distinct as a single ventral lobe cephalad of the anterior midgut rudiment. The lateral plates in this region have undergone a dorsomesial extension and now approximate each other anteriorly. The mandibular, thoracic and several abdominal segments are all now evident.

There has been a tendency for alternate segments to become more distinctly delimited than the others, and this is best illustrated in the accompanying figures by the ventral view of the 11-hour embryo and the lateral view of the 14-hour embryo in Figure 34.

The labral lobe assumes a median cleft by 13 hours. By this time segmentation has proceeded caudally in the abdomen until all but the last few segments are at least faintly visible. The constrictions between the gnathal segments deepen sharply laterad, particularly those on either side of the second maxillary segment. The labral lobes are larger and their ventrolateral margins are marked by distinct grooves extending cephalodorsally form just anterior to the anterior midgut rudiment. Cephaloventrally to what are to become the mandibular lobes faint

Figure 34. Drawings of lateral and ventral views of whole embryos after incubation for various periods at 75°F. Chorion and serosa removed. X85.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HOURS AT 75° F.

evidences of the intercalary segment may be seen. The stomodaeum commences its invagination about this time.

By 14 hours the antennal rudiments appear at the laterocaudal angles of the deutocerebrum. The lateral appendage lobes are becoming distinct from the ventral neural crests in the gnathal segments, while the labrum is further constricted and appears as a large, bilobed, bulbous appendage. The later development of the head as seen in external view involves: completion of the differentiation of the gnathal appendages and their cephalomesial migration; the approximation of the second maxillary lobes mesially by the twentieth hour and finally their fusion into the labium by 24 hours; the fusion of the two labral lobes by the sixteenth hour; the cephalodorsal migration of the antennal rudiments; the disappearance of the intercalary lobes; a sinking in of the neural crests beneath the migrating gnathal lobes; the overgrowth of the brain lobes by the ectoderm and the dorsal fusion of the latter.

The labial glands arise as a pair of paired invaginations of the ectoderm at the caudoventral margins of the labial lobes between 14 and 15 hours. As the four invaginations extend caudad the tubes of each side fuse anteriorly. The common duct on either side lengthens as the distal ends extend caudad, the latter reaching the fifth abdominal segment by 18 hours and the seventh by 24 hours. The fork

of the labial gland is in the second abdominal segment in embryos past 24 hours (Figure 52). As the labial lobes approach each other the single narrow ducts of the glands of each side fuse into a short median duct at about 20 hours (Figure 50).

The embryonic tentorium appears to form as in the honeybee (78, p. 175-179), however the anterior arms are much more slender and no lateral spurs were observed on them.

The three thoracic and the ten abdominal segments, the difinitive number, are not all visible until 16 hours. Dorsal closure is complete at 20 hours.

Between 22 and 24 hours the cells in the anterior and posterior portions of the midventral hypodermis of the meso-and metathoracic and the first seven abdominal segments thicken to form an anterior and posterior lobe on each segment (Figures 34 and 51). On the eighth abdominal segment there is only an anterior lobe. These largely disappear before eclosion. The cuticle is secreted between 28 and 30 hours.

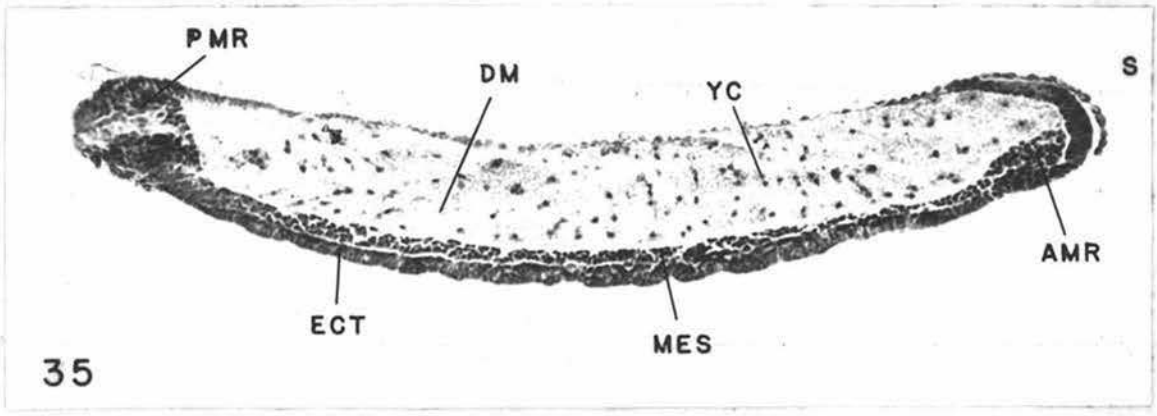
The processes of cephalization and dorsal closure can be seen in Figure 34, lateral and ventral views of whole embryos of various ages.

Figure 35. Sagittal section through an embryo at about 10½ hours. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 36. Sagittal section through the head of a 15-hour embryo. Kahle's fixative. Harris' hematoxylin. 5 μ. Photomicrograph.

Figure 37. Parasagittal section near the mid-line through the head of an 18-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 38. Sagittal section through the head of a 20-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.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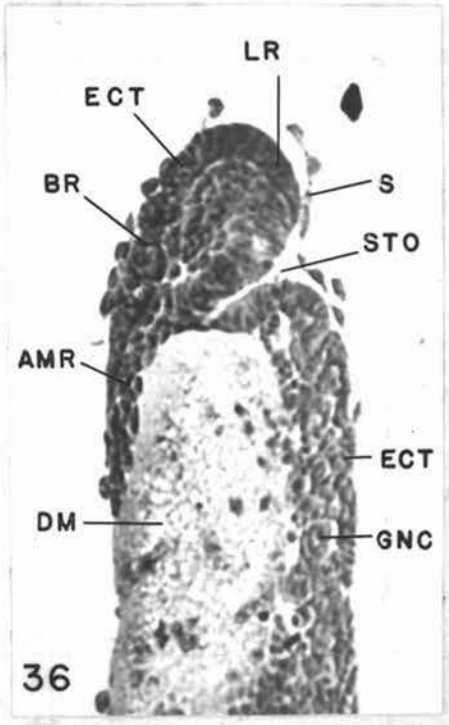
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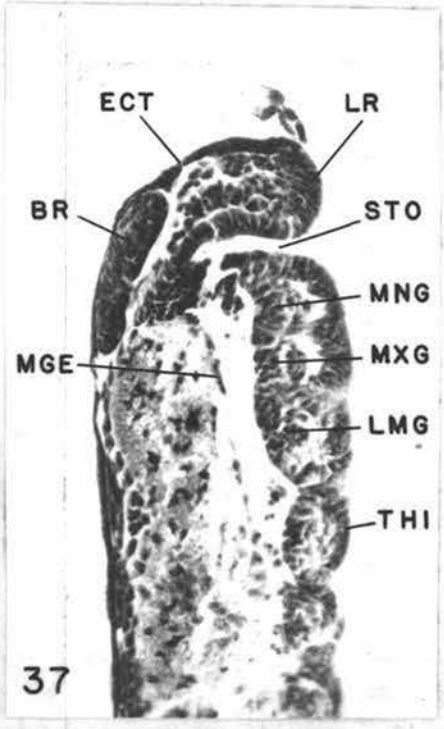
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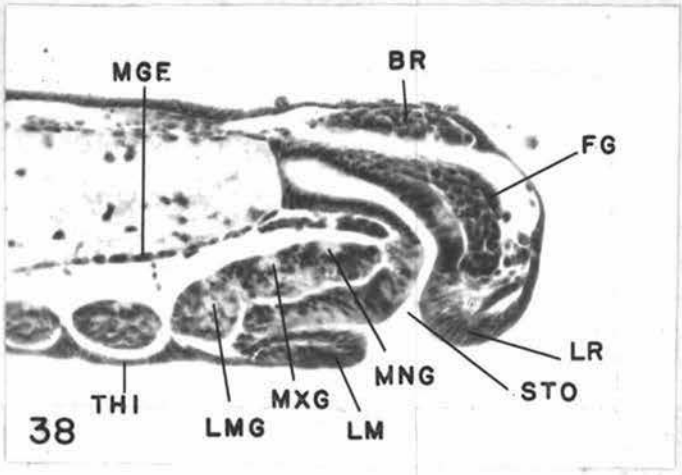
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Development of the serosa- The serosa forms from a narrow band of cells of the dorsal blastoderm, the dorsal strip. Originally columnar, the cells of the dorsal strip round up at about the time the lateral plates begin to close beneath the middle plate (Figure 29). In the anterior region of the egg this takes place at about nine hours. The cells sever their connection with the lateral plates at about 10 hours, begin to spread cephalad and laterad and cover the dorsal end of the embryo (Figures 35 and 39). The serosa then spreads ventrally and finally fuses along the midventral line. Its differentiation from the lateral

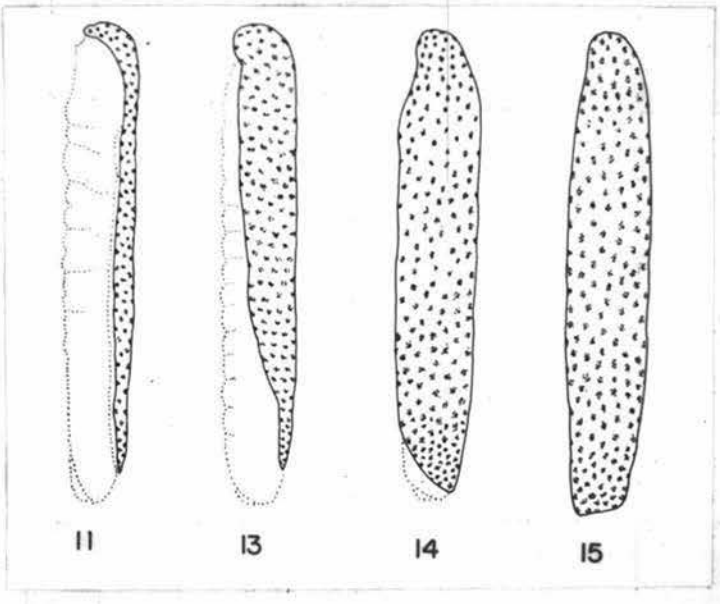


Figure 39. Development of the serosa. The time of incubation at 75°F. in hours is given by the numbers in the figure.

plates and its ventral growth over the embryo is slower caudad than cephalad (Figure 39). The envelope is complete in 15-hour embryos. The serosa was not observed to secrete a cuticular layer as in Pteronidea ribesii (93, p. 103-104). Although an amnion is formed in some Hymenoptera (2, p. 66; 9, p. 666), it is absent in others (57, p. 185; 78, p. 82-88). None was observed in C. brunneri.

Organogenesis- The alimentary canal: The initial formation of the anterior and posterior mesenteron rudiments has been discussed previously (p. 99). By the time of the initial invagination of the stomodaeum at about 13 hours the cells of the anterior midgut rudiment have proliferated dorsally and laterally around the anterior end of the yolk. From this position it grows posteriorly as a single dorsal ribbon of cells (Figures 36 and 44) and meets with a similar but broader ribbon from the posterior midgut rudiment. The two unite slightly cephalad of the middorsal position between 16 and 18 hours. The ribbons meanwhile are spreading out laterally and then ventrally around the yolk to complete the midgut epithelium by 20 hours. In Ammophila campestris there are two ribbons anteriorly and two posteriorly which unite one-fifth of the way back in the embryo (2, p. 65). In Angitia vestigialis there are no anterior and posterior ribbons, but "individual yolk cells are deposited at the surface of the yolk syncytium and they coalesce to form the continuous wall of the midgut " (57, p. 187). This is similar to Eurytoma aciculata (56, p. 15). The process of sinking in of the posterior midgut rudiment at about 11 hours appears to correspond with the initial stages of the proctodaeal formation. The ectoderm seems to follow the posterior midgut rudiment inward and finally closes in behind it in a manner still uncertain by about 14 hours. The proctodaeum

initially is more ventral than terminal but assumes its terminal position by 14 hours. The two Malpighian tubules first appear at the tip of the proctodaeal invagination at 16 hours. They extend cephalad beneath the yolk, their ends reaching the third abdominal segment by 24 hours and the first abdominal segment by 26 hours.

Several folds appear in the enlarged posterior portion of the stomodaeum between 22 and 24 hours (Figure 50). The cells comprising the floor of the stomodaeum are at first rather large and restrict communication between the fore- and midintestine (Figure 38). By 24 hours only a thin membrane separates the two (Figure 50). The center of this membrane is broken down in 30-hour embryos.

The gonads: The formation of the germ cells in insects has been divided by Nelsen (76, p. 554) into several groups according to the time of their differentiation. C. brunneri is in the first category where they are differentiated before blastoderm formation. The pole (germ) cells are recognizable as early as five hours as a group of round cells lying outside the blastema at the posterior pole. They retain this position while cell walls are formed in the blastoderm (Figure 27). Between the eighth and ninth hour they migrate cephaloventrally to lie beneath the site of the posterior midgut rudiment (Figure 40). As the latter sinks beneath the lateral plates the germ cells migrate inwards and come to rest

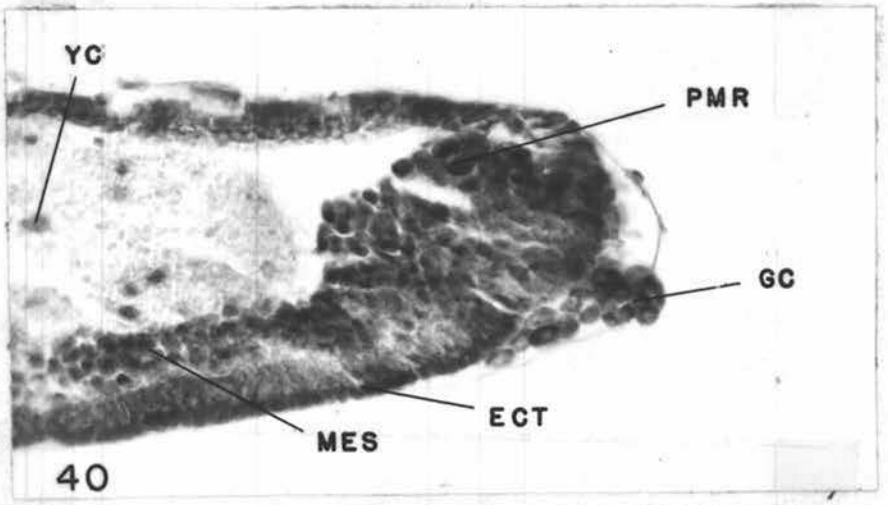
beneath the proliferating midgut rudiment. It appears that not all of the pole cells thus enter, but some remain outside indefinitely and form a compact group exterior to the proctodaeum. In 15-hour embryos the germ cells have separated into two groups, one ventrolateral on either side of the midgut, and are closely appressed by mesodermal cells dorsally, ventrally and laterally. By 16 hours some mesodermal cells have begun to ensheath the germ cells and are distinguishable from the latter by their smaller size and darker staining qualities (Figure 42). This mesodermal covering becomes quite thin by 18 hours. By 20 hours the gonads occupy their definitive dorsolateral position in the eighth abdominal segment and appear fully formed with their ducts leading ventrally and attaching to the ventral body wall. No gross differences were noted between the structure of male and female gonads. However, the ducts in some cases were attached to the posterior portion of the seventh abdominal segment and in others to the posterior portion of the ninth, allowing identification of females and males, respectively.

Figure 40. Parasagittal section near the mid-line at the caudal end of an embryo at about 10½ hours. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 41. Sagittal section through the caudal end of a 14-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 42. Cross section through the germ cells of a 16-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 43. Cross section through the gonads of a 20-hour embryo. Kahle's fixative. Alum carmine. 10 μ. Photomicrograph.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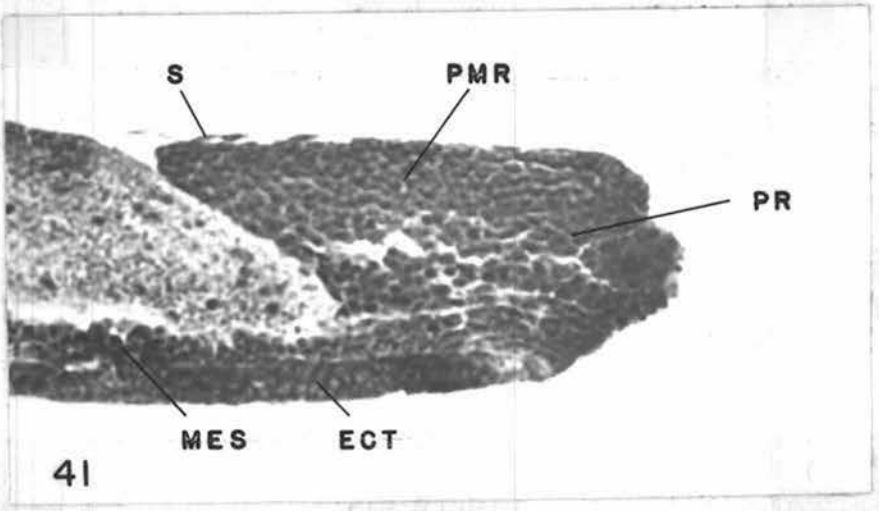
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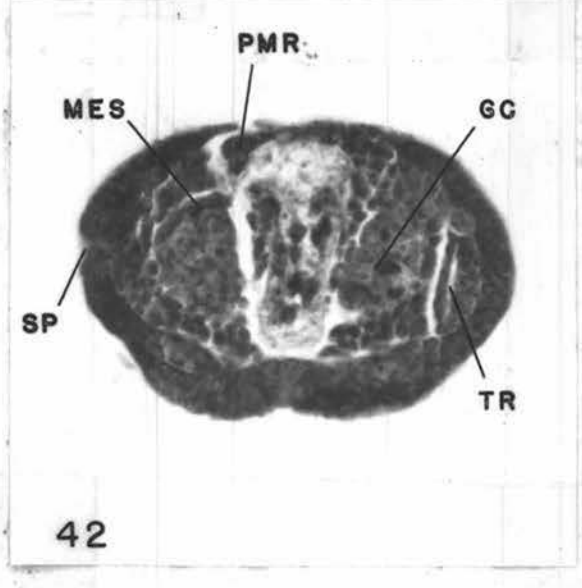
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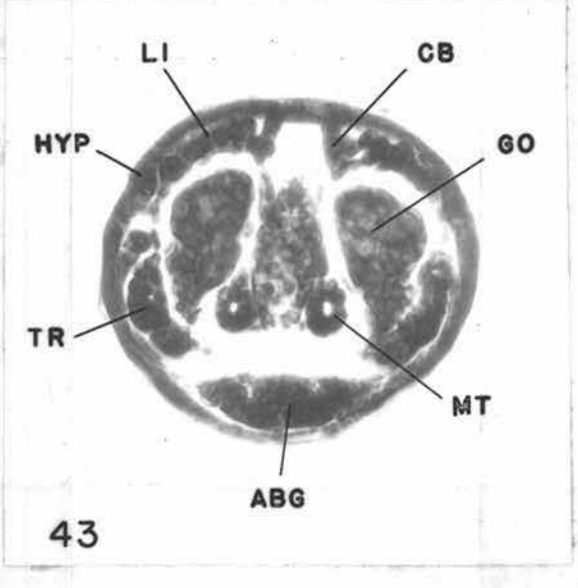
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The ventral nerve cord: Soon after the ventral closure of the lateral plates toward the anterior portion of the embryo between the tenth and eleventh hour, a neural crest develops on either side of the median neural groove. Neuroblasts have become differentiated within the neural crests by 11 hours (Figure 32). The multiplication of the neurogenic cells and their differentiation from the ventral hypodermis follow during the next several hours so that by the sixteenth hour the ganglion cells have spread out laterally on either side of the now shallow neural groove (Figure 46). The ventral hypodermis at this time is distinct from the nervous tissue, but the two are still closely appressed. By 18 hours the ganglion cells have become compacted together and have completely separated from the hypodermis in the intrasegmental regions of each of the three gnathal and first few trunk segments (Figure 37). The intersegmental regions still retain their connections with the ectoderm, however, and the ganglia in the posterior abdominal region are still differentiating at this time. In 20-hour embryos all ganglia have completely severed their attachment with the hypodermis except in the eighth, ninth and tenth abdominal segments, and fusion of the ganglia of the mandibular, maxillary and labial segments into the suboesophageal ganglion has begun. The fusion is complete by 24 hours (Figure 50). Fusion of the tardily developing eighth,

ninth and tenth abdominal ganglia commences about this time but is not complete until about 28 hours.

Thus, the definitive ventral nerve cord consists of the suboesophageal ganglion representing the fused mandibular, maxillary and labial ganglia, followed by three thoracic, seven individual abdominal ganglia and one compound terminal ganglion representing the fused ganglia of the eighth, ninth and tenth abdominal segments.

Figure 44. Cross section through the intercalary segment of a 15-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

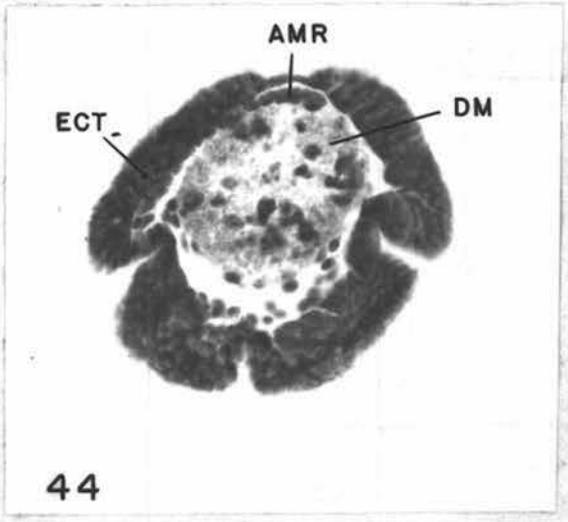
Figure 45. Cross section through the mandibular segment of a 16-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 46. Cross section through the second thoracic segment of the same embryo as in Figure 45.

Figure 47. Cross section through the second thoracic segment of an 18-hour embryo. Kahle's fixative. Harris' hematoxylin. 7 μ. Photomicrograph.

Figure 48. Cross section through the brain, circumoesophageal connectives and suboesophageal ganglion of a 20-hour embryo. Kahle's fixative. Alum carmine. 10μ. Photomicrograph.

Figure 49. Cross section through the head of the same embryo as in figure 48 slightly more caudad, intersecting the optic lobe of the brain, the tentorium and the suboesophageal ganglion.



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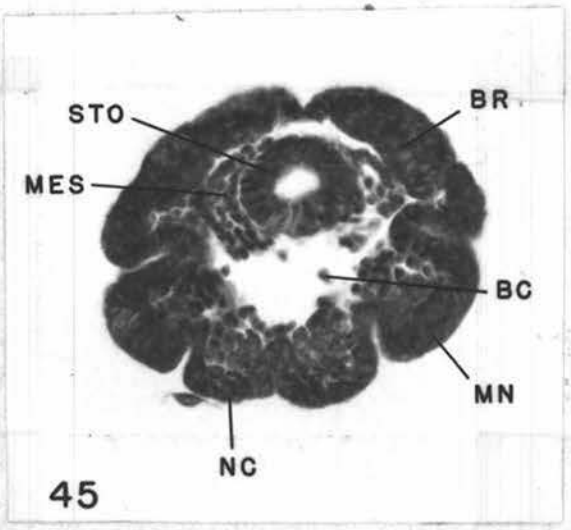

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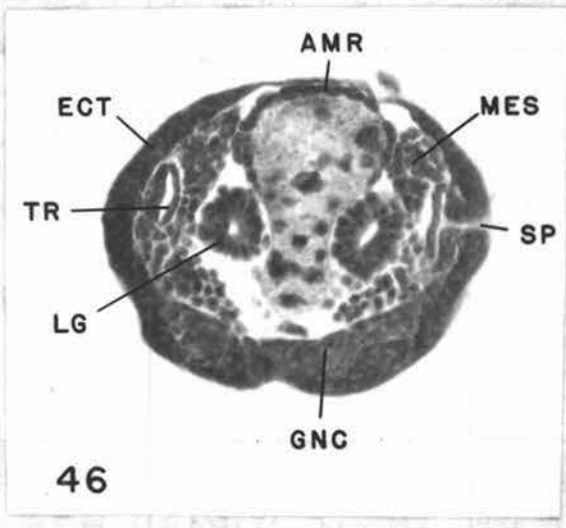

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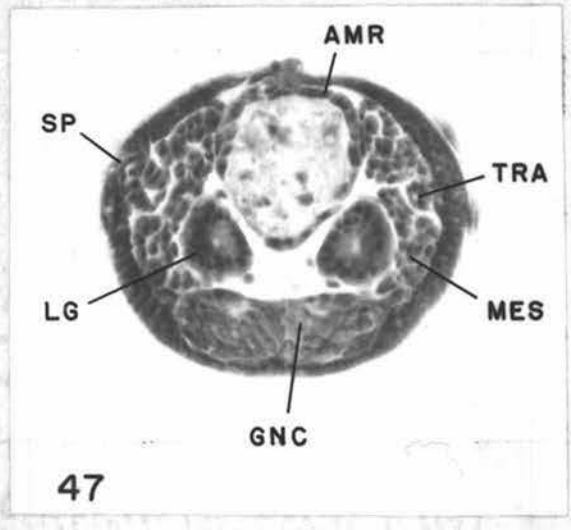

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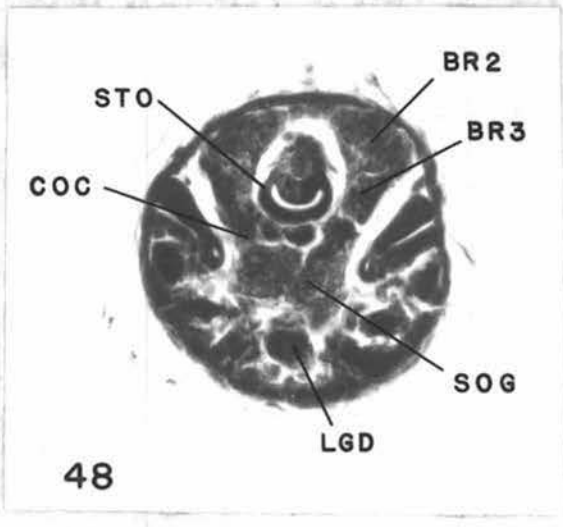

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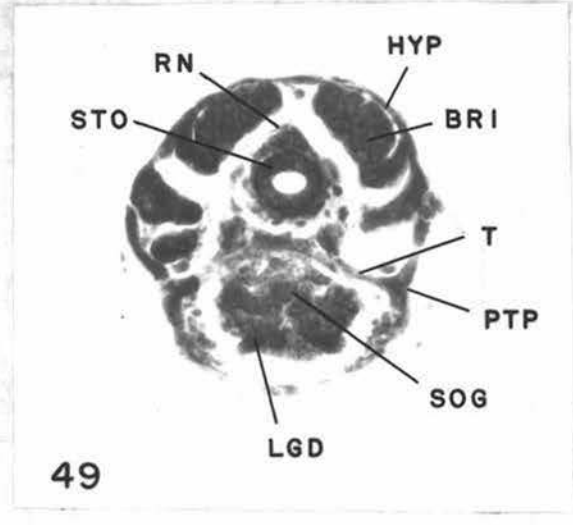

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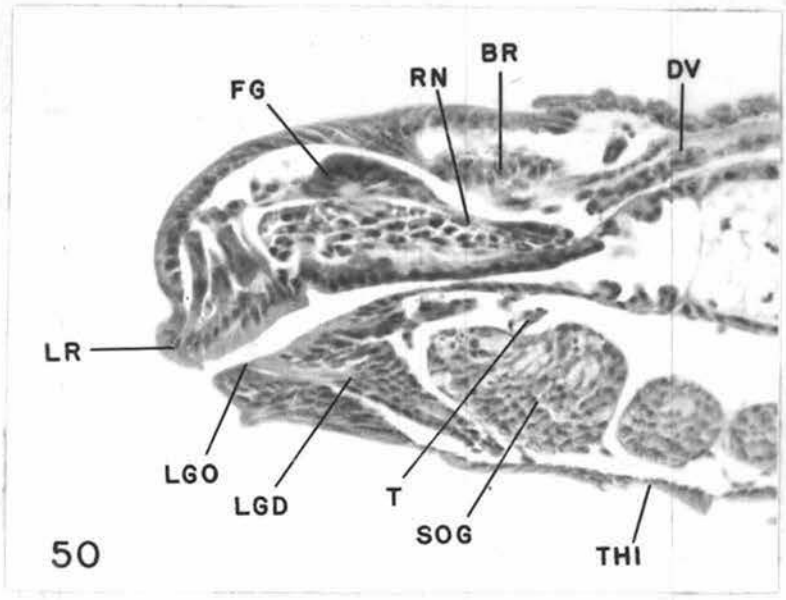
The brain and stomodaeal nervous system: As far as could be determined the development of the brain is similar in most respects to that in Apis (78, p. 142-156). However, the proto-and deutocerebrum are not as evident as distinct lobes separate from the rest of the ectoderm on the external surface of whole embryos as in the honeybee. The neurogenic area of the intercalary segment (tritocerebrum) becomes evident in surface view in 14- and 15-hour embryos as small lateral swellings at the anterior ends of the neural crests cephalomesiad of the mandibular lobes (Figure 34). The deutocerebrum is represented by the area cephalodorsal to the tritocerebral swellings between the lateroventral mandibular lobes and the labrum. The antennal rudiments mark the caudolateral angles of the deutocerebrum. The protocerebrum is dorsal and somewhat posterior to the deutocerebrum but is not distinctly separated from the latter.

During the course of cephalization the intercalary segment becomes reduced, and much of the area appears to sink in with the stomodaeal invagination. The intercalary lobes, very evident in 15-hour embryos, are no longer visible on the surface of 16-hour embryos. The invagination of the caudolateral portion of the protocerebral optic lobes (Figure 49) are evident on the surface of 18-hour embryos (Figure 34).

The frontal ganglion originates at 15 hours as a

group of cells of the dorsal stomodaeal wall. By 24 hours the fusiform ganglion (Figure 50) occupies its definitive position medially above the stomodaeum cephaloventral to the deutocerebral lobes of the brain. Extending caudad from the frontal ganglion, the recurrent nerve enlarges slightly into a rudimentary occupital ganglion above the posterior enlargement of stomodaeum (Figure 50).

Figures 50-52. Three sections through a 24-hour embryo. Kahle's fixative. Harris' hematoxylin. 5 μ. Photomicrographs. 50. Sagittal section through the head. 51. Parasagittal section through the head intersecting the circumoesophageal connectives. 52. Parasagittal section intersecting the labial gland.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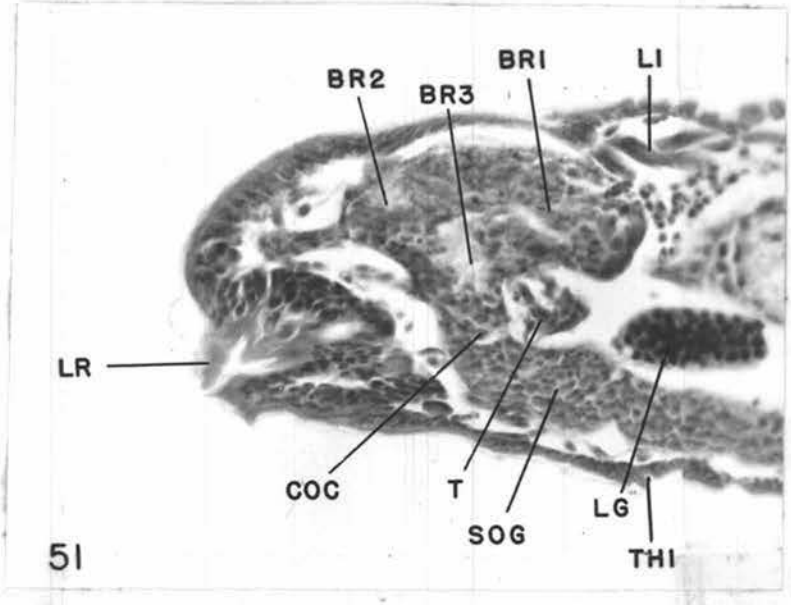
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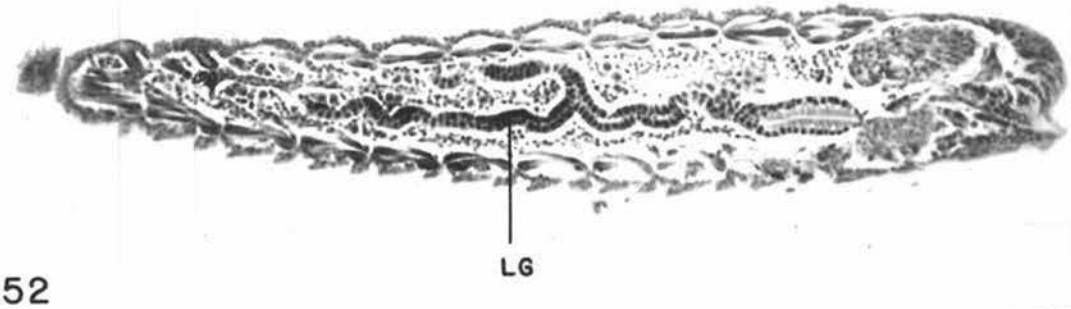
51


LG

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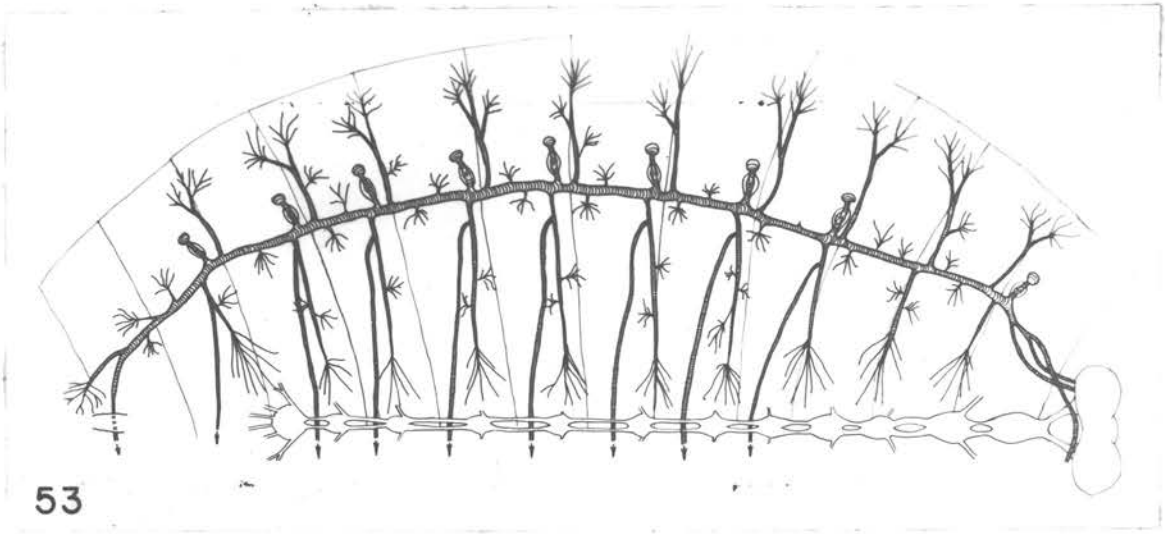
The tracheal system: Slight indentations are present in 12-hour embryos, however it is not until 15 hours that the tracheal invaginations are plainly visible on the external surface. Ten pairs of arcuate pits are present, one pair on the cephalic margins of the meso- and metathoracic and the first eight abdominal segments. They are aligned in a straight row along the side of the embryo (Figure 34). The line of invaginations is slightly more dorsal cephalad than caudad. By 16 hours the line of invaginations has become oriented with the long axis of the embryo and their arcuate shape has changed to circular. Internally each invagination sends branches dorsad, ventrad, cephalad and caudad. By 18 hours the cephalic and caudal branches have united into the lateral longitudinal trunks, the dorsal and ventral branches have lengthened and the ventral branches in the abdomen have divided into two. Also by this hour the invagination originally on the cephalic margin of the mesothoracic segment has migrated to a position on the posterior portion of the prothoracic segment, and that on the metathoracic segment has closed over leaving no visible trace on the external surface. By 24 hours one of the ventral branches of each abdominal segment has united beneath the nerve cord with its mate from the opposite side. The posterior branches from the eighth abdominal segment have extended caudad and ventrad

to unite beneath the rectum and a cephalic branch from the first pair of spiracles have united behind the brain. The tracheal system in the embryo as far as could be determined is similar to that in all the instars. Since the fifth instar is larger and more easily studied than the embryo, the complete tracheal system of a mature larva is illustrated instead of an embryonic system (Figure 53). The structure of the spiracle is shown in Figure 54.

Figure 53. Tracheal system of the left side of a fifth instar flattened into one plane. Drawn from dissections and corrected with the aid of sections.

Figure 54. Spiracle and valve of a fifth instar. Bouin's fixative. Harris' hematoxylin and eosin. 10μ. Photomicrograph.

Figure 55. Somatic musculature of the left side of a fifth instar flattened into one plane. Drawn from dissections and corrected with the aid of sections.



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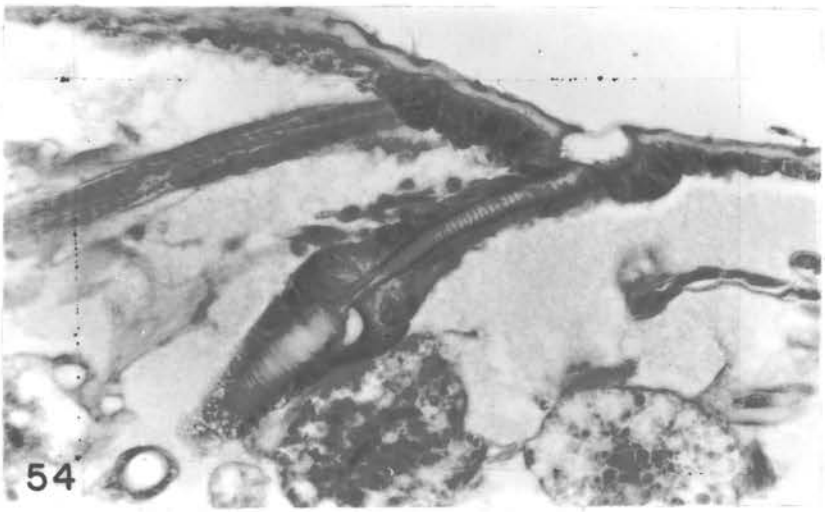

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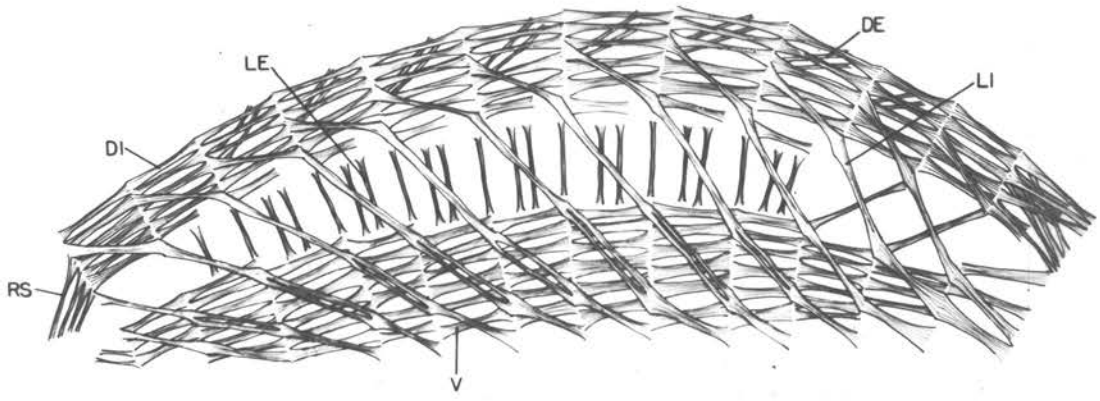

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The musculature and circulatory systems: The development of these systems appears to correspond closely to that in Apis (78, p. 189-212), so no extended discussion will be presented. In Figure 50 anterior termination of the dorsal vessel is seen immediately dorsad of the stomodaeum and beneath the brain.

The mesodermal cells forming muscles elongate and become aligned in their respective positions starting at 18 to 20 hours. Attachment to the hypodermis occurs by 24 hours. Instead of illustrating the somatic musculature of an embryo which is difficult because it must be studied from sections, that of a fifth instar is shown (Figure 55). The system in both stages appears similar, and the fifth instar because of its size allows gross dissections to be used for study.

External Morphology of the Larvae

First instar- The first instar is clear white in color and consists of a head and 13 body segments, subequal in length but diminishing gradually in diameter toward the caudal end. Overall length at eclosion averages approximately 1.1 millimeters. The sides of the head in dorsal view are nearly straight for approximately two-thirds of the length of the head, converging slightly anteriorly and merging with the broadly rounded cephalic margin. The conical antennae are in a cephalolateral

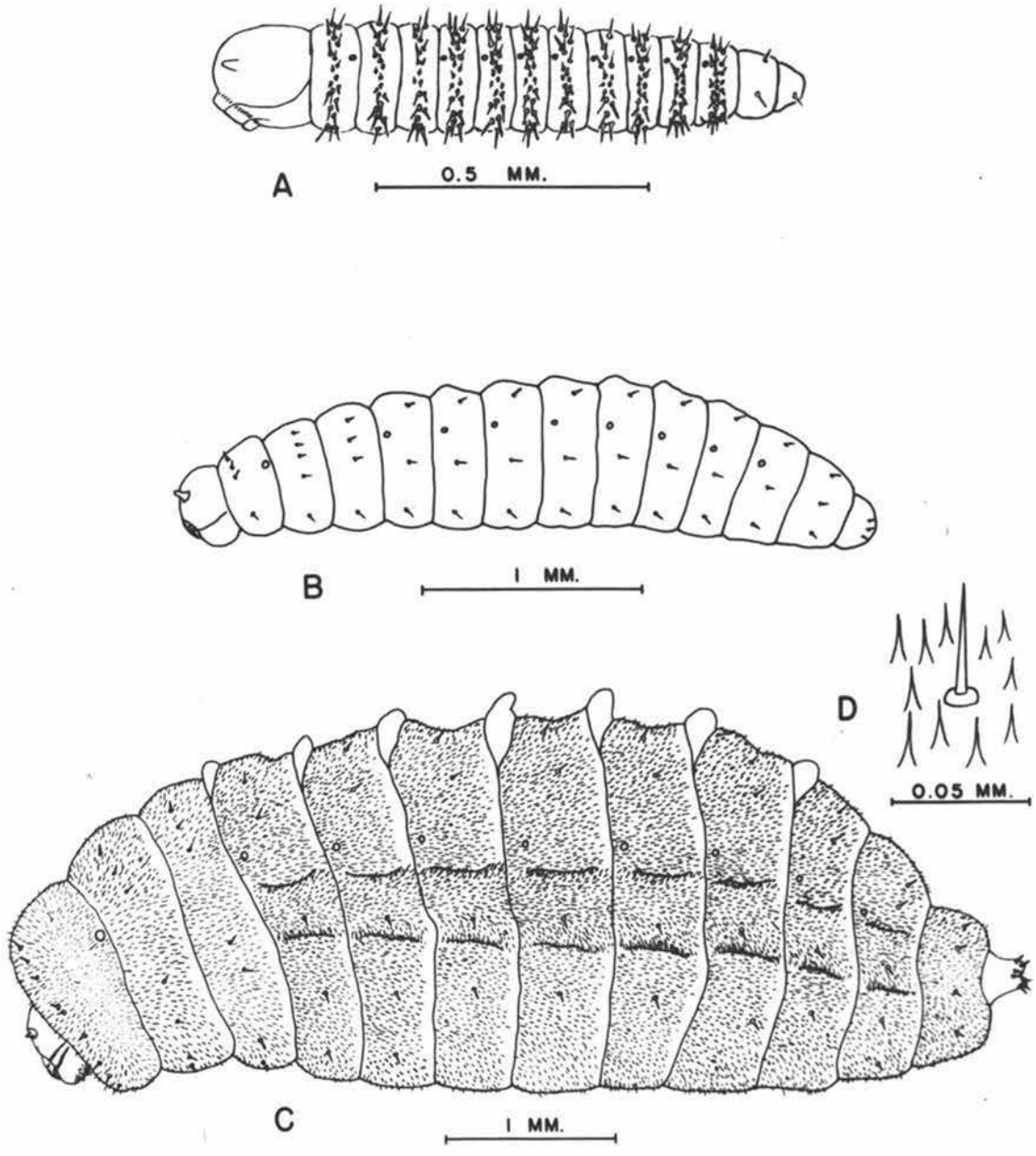


Figure 56. Larvae of C. brunneri A. First instar. B. Fourth instar. (The second and third instars are similar except for size.) C. Fifth instar. D. Sensory setae and cuticular spines of a fifth instar.

position where the straight sides of the head give way to the round anterior margin. Head capsule width measurements for all the instars are given in a later section. In lateral view the antennae are seen to lie slightly above the midline (Figure 56A). The labrum, maxillae and labium are grouped about the mouth opening as a cup-shaped prominent cephaloventral projection from the head capsule proper.

Each of the first 11 body segments bears a prominent band of sensory setae around the middle in addition to several smaller scattered setae. The twelfth and thirteenth segments have only a few scattered setae. Those in the bands around the segments are approximately 0.012 millimeters long. Nine pairs of spiracles measuring approximately 0.0014 millimeters in diameter are present, one on each of the first and fourth to the eleventh body segments. All are located slightly dorsad of the mid-lateral line, that on the first segment just cephalad of the caudal margin of that segment, while those on the fourth to the eleventh segments lie just caudad of the cephalic margins of their respective segments.

The first instar is distinct from the other four instars in having the prominent bands of setae around the first 11 body segments and in having a head capsule with nearly straight lateral margins when viewed from above.

Second, third and fourth instars- The external morphology of the second, third and fourth instars is similar, so they will be discussed together. Variation in size is often greater between individuals in the same instar than it is between individuals in different instars, so measurements mean little except when considering averages. The average overall length of individuals in the second, third and fourth instars are approximately 1.4, 2.3 and 3.3 millimeters, respectively. Head capsule measurements are given in a following section.

The head capsules of these instars are more spherical than the first instar. The outline of the head capsule in dorsal view is no longer straight sided but curved, with the widest part being that in the center. The mouthparts do not project as in the first instar (Figure 56B). Thirteen body segments are present as in the first instar, but the body is now widest in the center and tapers toward both ends. No prominent bands of setae are present, although from four to eight individual setae are aligned around the center of each thoracic segment. In the abdominal segments a seta is present laterad of the mid-dorsal and mid-ventral lines and at the mid-lateral position on each side. Figure 56B is an individual in the fourth instar, but except for the size it could also represent the second and third.

Fifth instar- The fifth instar (figure 56C) is distinct at once from the preceding instars by the presence of numerous cuticular spines over most of the body and by having seven dorsal protrusile areas in the successive intersegmental areas, the most anterior being that between the metathoracic and first abdominal segments.

Sensory setae (Figure 56D) are slightly more numerous than in the preceding instars and now measure approximately 0.045 millimeters in length. The cuticular spines (Figure 56D) are approximately 0.017 millimeters long. The latter are not present on the head or terminal body segment but are found everywhere on the first 12 body segments except along the intersegmental lines and on the dorsal protrusile areas. In living larvae the first eight abdominal segments are regularly seen to have swellings in the mid-lateral areas. These are produced by the tension on the cuticle by the external lateral muscles (Figure 55) in these segments. The arrangement of the spiracles is similar to the preceding instars (Figures 53, 54 and 56). The heads of full grown braconid larvae have been particularly useful in classification (94), therefore a detailed description of the head of a fifth instar is desirable. The terminology used here is after Vance and Smith (108)[1].

1. Some of the interpretations of Vance and Smith (108) have been questioned by Short (94, p. 34-45), but it is beyond the scope of this work to determine who is right and who is wrong on these points.

The head in frontal view (Figure 57A) is roughly obovate and is constricted slightly below the midline by the hypostoma. Dorsally on the epicranium there is a slight depression on either side of the midline, formed by the tension of the pharyngeal pump muscles. The metoptic (coronal) and the frontal sutures, the postocciput and the temporal fossae are not evident. The bases of the short, conical antennae are surrounded by the broadly elliptic antennal foramina. Six pairs of setae are placed as in Figure 57A dorsad of the mouth armature.

The mouth armature is well developed. The hypostoma, pleurostoma, epistoma, stipital sclerome, maxillary sclerome, labiostipital sclerome and the tips of the mandibles are all rigid, brown pigmented areas which stand out in contrast to the rest of the head which is white. The ligular sclerome is lacking. The labrum is not distinct from the clypeus. It bears three pairs of small papillae and two pairs of setae. The mandibles (Figure 57C) bear a row of six small, comb-like teeth at the base of the sickle-shaped tip. The stipes of the maxillae each bear a pair of setae, a rudimentary palpus and a small papilla, while the cardo bears a single seta. The horizontal slit-like orifice of the silk gland is located at the dorsal margin of the labium between the dorsal arms of the labiostipital sclerome. A pair of rudimentary palpi and two pairs of setae are present on the labiostipes. The labiobase bears

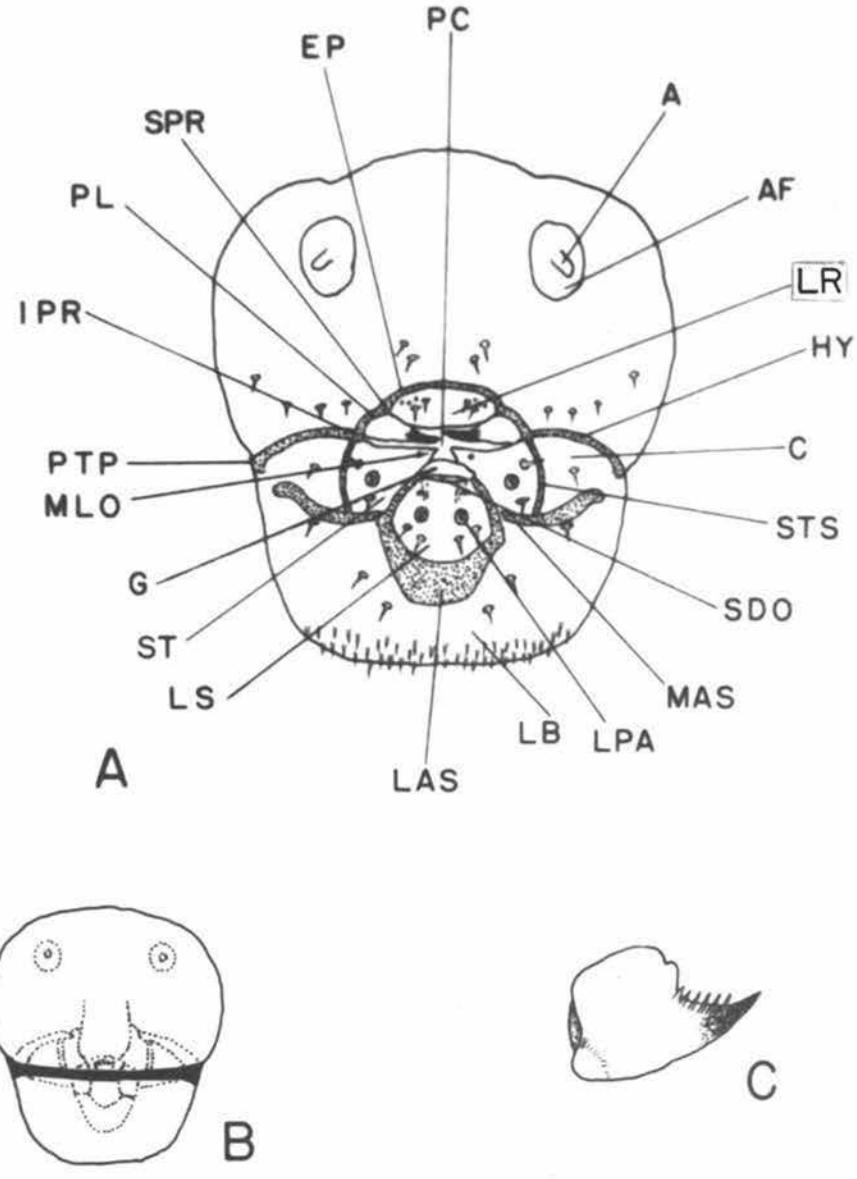


Figure 57. Morphology of the head of a fifth instar. A. Front view. B. Internal view from behind. C. Right mandible. Ventral view.

three pairs of setae dorsally and many cuticular spines ventrally.

The transverse tentorial bar (Figure 57B) is well developed and originates at the tentorial pit at the posterior end of the hypostoma of each side. No anterior or dorsal tentorial arms were observed, although slender anterior arms are present in the embryo (see p. 107).

Head capsule width distribution of the larval instars- Head capsule measurements were taken periodically on larvae being reared individually to determine the length of each stadium at various constant temperatures (see p. 70). By this method the instar of the individual at the time of each measurement was positively known. Measurements were taken at a 90X magnification of a dissecting microscope with a calibrated eyepiece micrometer divided into 100 units. Each unit at this magnification was equivalent to 0.0168 millimeters.

The individual head capsule widths are shown as histograms in Figure 58. The widths used for ease of plotting were the number of units subtended by each on the eyepiece micrometer. A statistical summary of these data converted to millimeters is given in Table 9.

Table 9. Summary of larval head capsule measurements in millimeters.

Instar	Sample mean	Standard deviation	Standard error	Sample mean ± 3 standard deviations
first	.1707	.0118	.0014	.1352 .2063
second	.2199	.0159	.0020	.1722 .2676
third	.2825	.0242	.0031	.2101 .3550
fourth	.3660	.0407	.0056	.2438 .4881
fifth	.4612	.0777	.0111	.2280 .6944

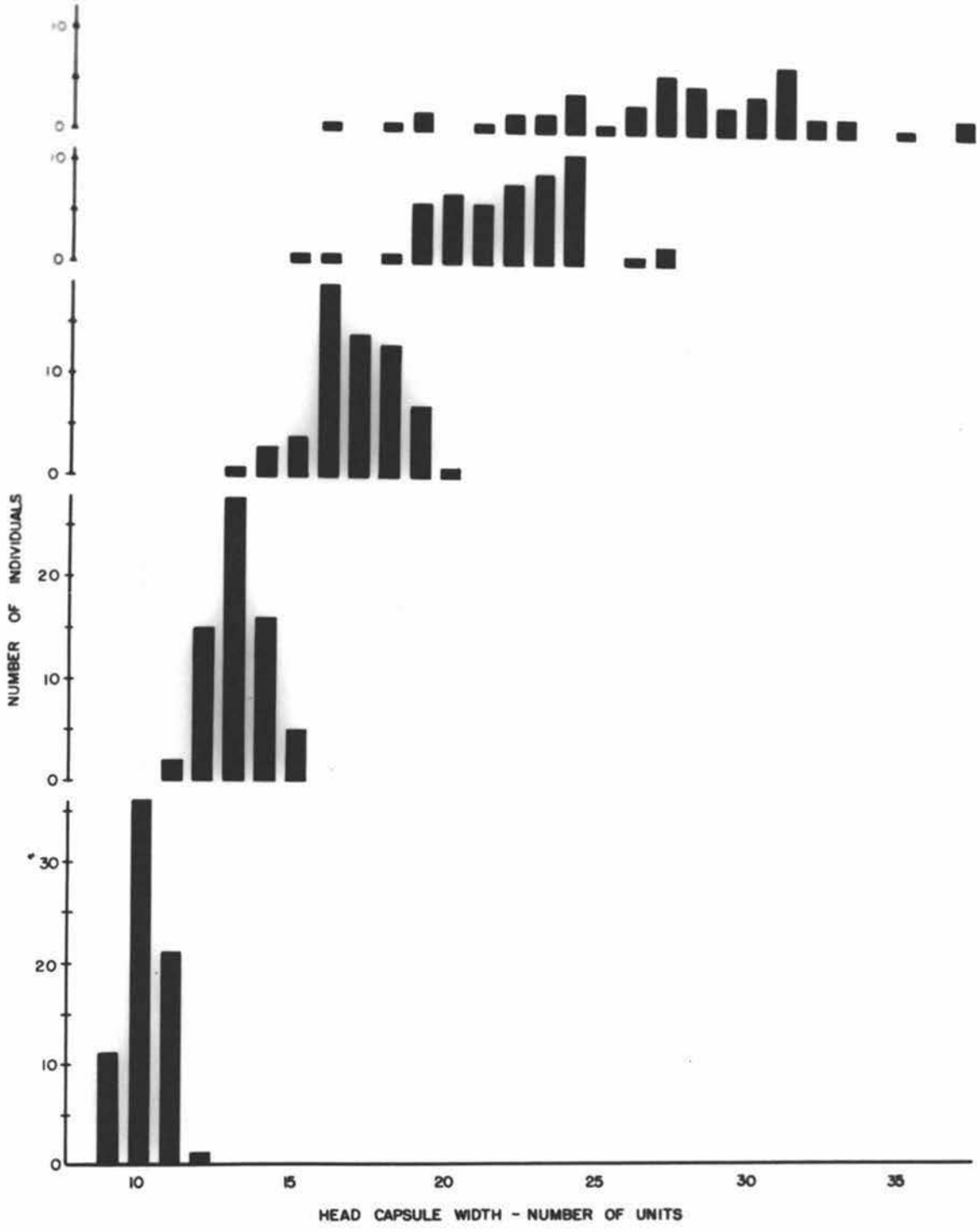


Figure 58. Histograms of the head capsule width of the five instars of C. brunneri.

With only a knowledge of the head capsule width distribution for each instar it is difficult to determine in which instar individuals are at the time of any one examination, since there is considerable overlapping between successive instars. For example, even if individuals outside of a three standard deviation range on either side of the mean for each instar are disregarded, the range of the fifth instar includes all that of the fourth, most of the third and a part of the second. An individual with a head capsule width of 0.24 millimeters could actually be in any instar except the first.

Pupal Morphology and Progress of Coloration

The pupae are of the typical exarate type. Dorsal and ventral views of a female pupa are shown in Figure 59. The last larval cuticle typically remains covering the ovipositor as shown in the figure and is shed with the pupal cuticle at eclosion. Figure 60 shows several stages in the life of a male as it changes from a mature larva to an adult.

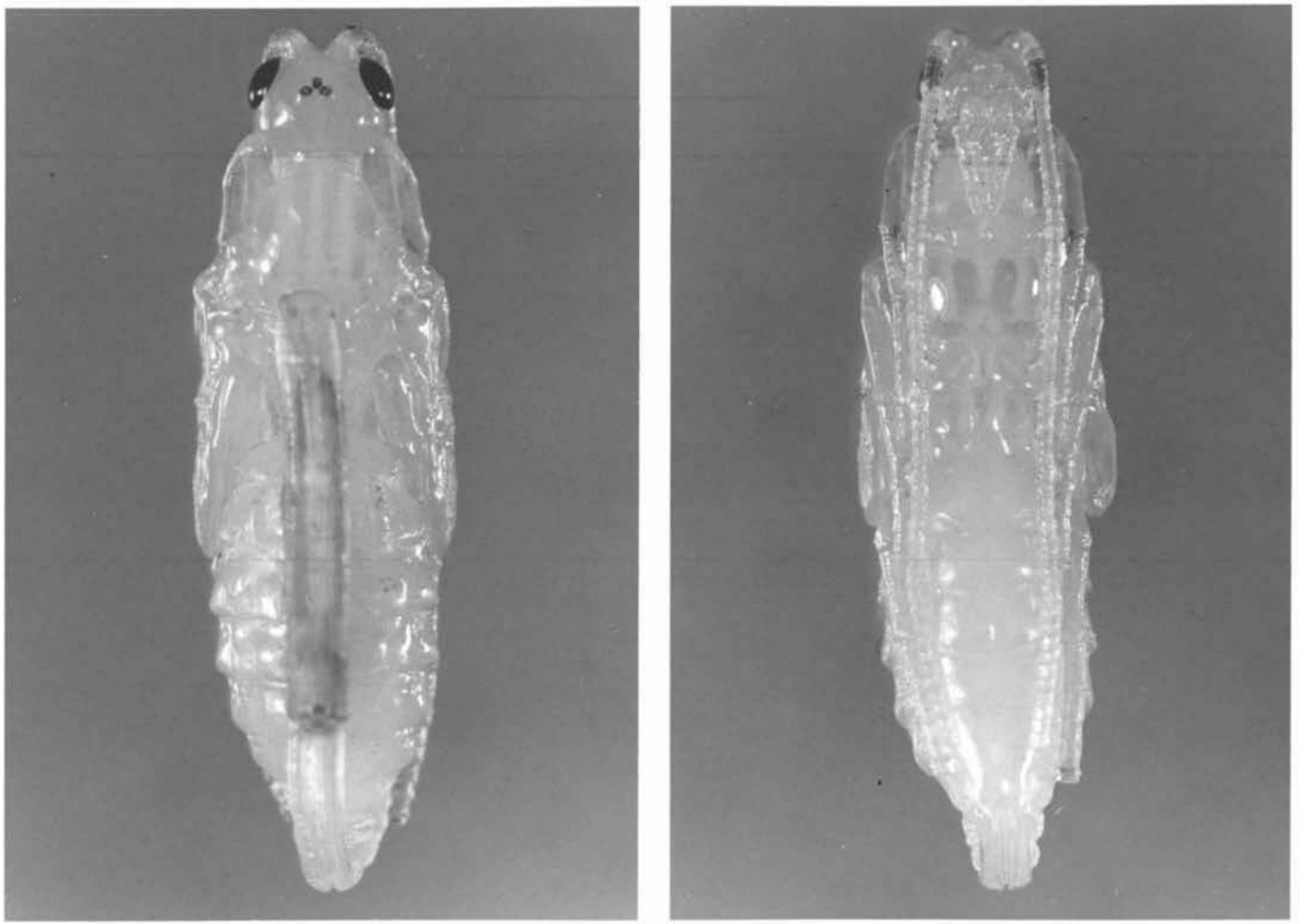
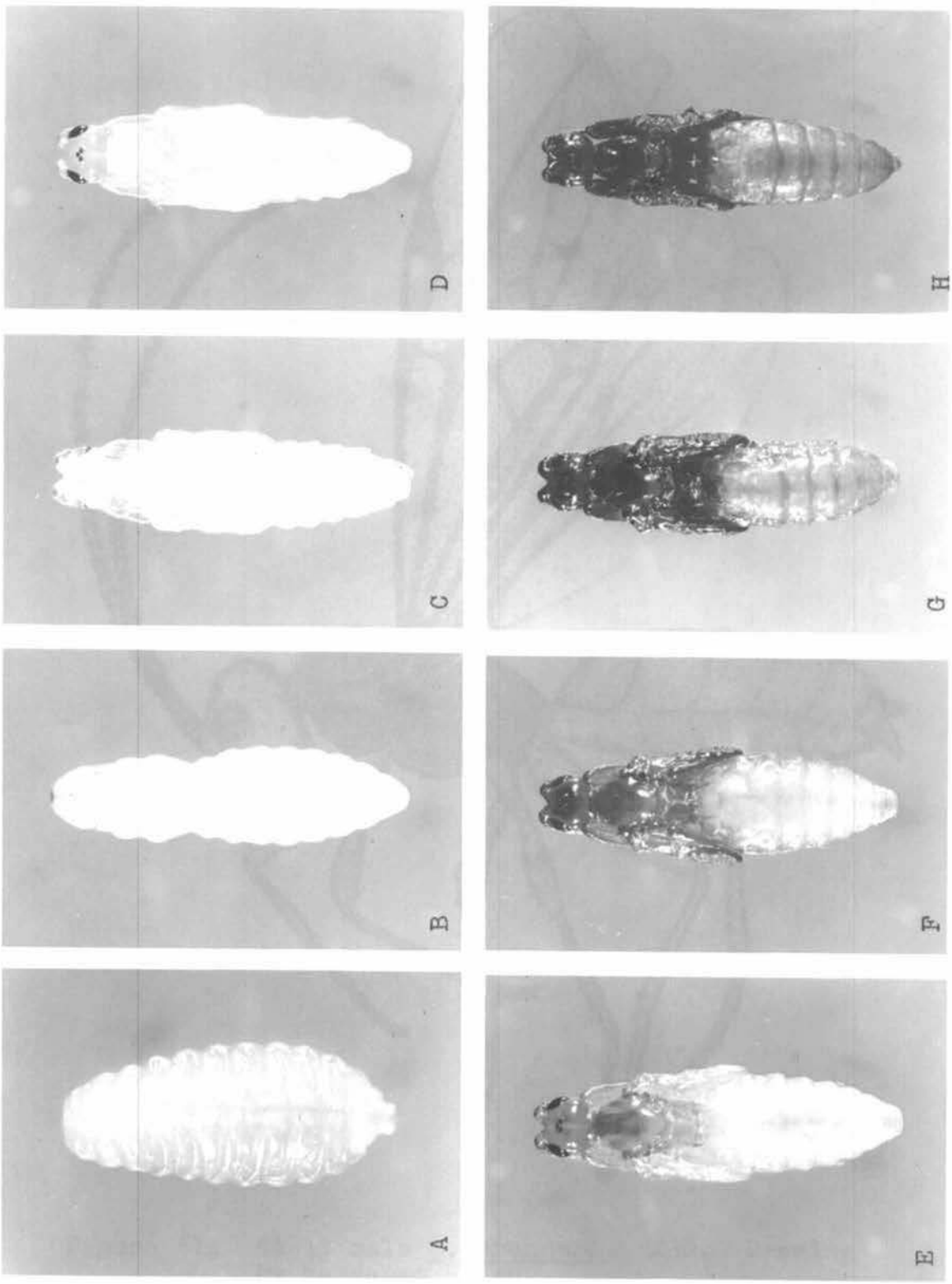


Figure 59. Female pupa of C. brunneri. X25. Left: dorsal view. Right: ventral view.

Figure 60. Several stages in the life of a male as it changes from a mature larva to an adult. X 15. Insects were held at 75°F. and photographs taken periodically. At this temperature the average durations of the last larval and pupal stages are approximately 130 hours each. A. Mature larva. B. Prepupa, a few hours before pupation. C. Pupa, immediately after pupation. D. Pupa, 65 hours after pupation. E. 85 hours after pupation. F. 95 hours after pupation. G. 110 hours after pupation. H. Just before eclosion.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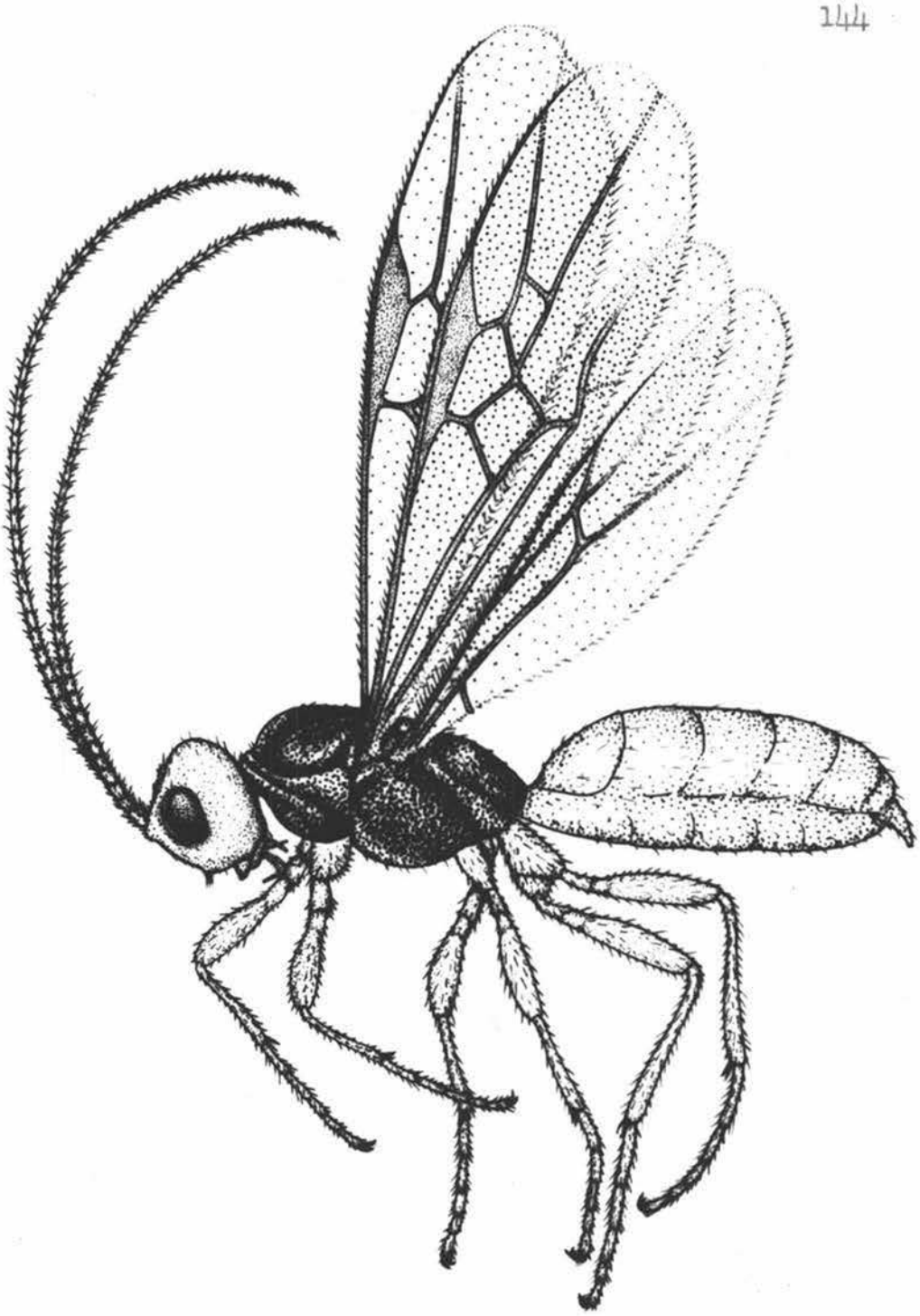

Figure 61. Adult male C. brunneri. X30. Drawing by Margaret Hsieh.

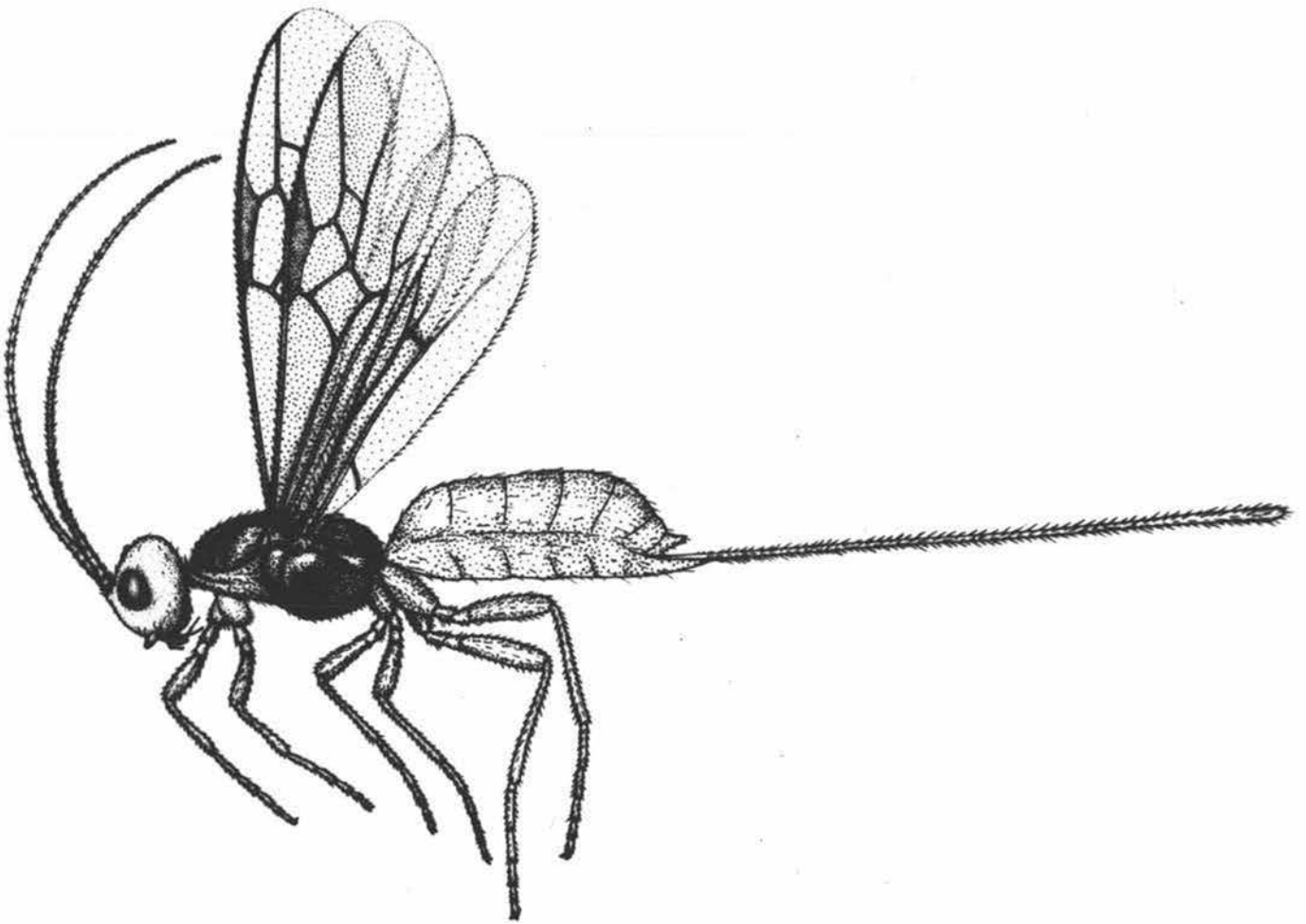

Figure 62. Adult female C. brunneri. X16. Drawing by Margaret Hsieh.

LIST OF ABBREVIATIONS USED IN THE FIGURES

A - antenna
ABG - abdominal ganglion
AB1 - first abdominal segment
AF - antennal foramen
AMR - anterior midgut rudiment
AR - antennal rudiment
BC - blood cell
BD - blastoderm
BR - brain
BR1 - protocerebrum
BR2 - deutocerebrum
BR3 - tritocerebrum
C - cardo
CB - cardioblast
CN - cleavage nucleus
CO - chorion
COC - circumoesophageal connective
DE - external dorsal muscle
DI - internal dorsal muscle
DM - deutoplasm
DS - dorsal strip
DV - dorsal vessel
ECT - ectoderm
EP - epistoma
ES - epineural sinus
FG - frontal ganglion
G - glossa
GC - germ cells
GG - gastrular groove
GNC - ganglion cells
GO - gonad
HY - hypostoma
HYP - hypodermis
IL - intercalary lobe
INT - intercalary segment
IPP - inner periplasm
IPR - inferior pleural ramus
LAS - labiostipital sclerome
LB - labiobase
LE - external lateral muscle
LG - labial gland
LGD - labial gland duct
LGO - labial gland orifice
LI - internal lateral muscle
LM - labium
LP - lateral plate
LPA - labial palpus
LR - labrum
LS - labiostipes
MAS - maxillary sclerome
MES - mesoderm
MGE - midgut epithelium
MN - mandible
MNG - mandibular ganglion
MP - middle plate
MPA - maxillary palpus
MT - Malpighian tubule
MX - maxilla
MXG - maxillary ganglion
NB - neuroblast
NC - neural crest
NG - neural groove
OES - oesophagus
PC - preoral cavity
PL - pleurostoma
PMR - posterior midgut rudiment
PP - periplasm
PR - proctodaeum
PTP - posterior tentorial pit
RET - reticulum
RN - recurrent nerve
RS - rectal suspensor muscle
S - serosa
SDO - silk duct orifice
SOG - suboesophageal ganglion
SM - somatic mesoderm
SP - spiracle
SPM - splanchnic mesoderm
SPR - superior pleural ramus
ST - stipes
STO - stomodaeum
STS - stipital sclerome
T - tentorium
TH1 - first thoracic segment
TR - trachea
V - ventral muscles
VM - vitelline membrane
YC - yolk cell

SUMMARY

There are three generations of C. brunneri per year, one each during July, August and September. Approximately 5 percent of the first generation, 50 percent of the second generation and 95 percent of the third generation enter diapause and overwinter in the fifth instar. The primary host is the Douglas-fir beetle.

Parasite behavior during emergence and mating, searching and oviposition, larval feeding and cocoon formation is described.

Laboratory and field studies have led to the following conclusions: females prefer to oviposit under reduced light conditions; hosts are probably located by the vibrations produced rather than by odor; females kept at 75°F. are ready to deposit at least some eggs on the second day after emergence; peak egg production occurs about the fourth day although ovulation may continue until death; females must feed if maximum egg production is to be realized; females exposed to a 16-hour day at 75°F. and fed a honey and yeast diet laid eggs resulting in an average of 21 cocoons per female; female longevity is approximately twice male longevity, and is greater for both at 60°F. than at any other temperature up to and including 90°F.; only the larval stage of its hosts is parasitized; the insect has a facultative diapause controlled primarily by photo-

period; although diapause intervenes in the fifth instar there is evidence that the critical stage is the adult female before egg deposition; the size of the parasite is correlated with that of its host; fertilized eggs are laid more frequently on large hosts than unfertilized eggs, and vice versa; the temperature optimum for development and the average duration for each stadium at this temperature is: egg, 95°F., 21 hours; first instar, 88°F., 21 hours; second instar, 85°F., 15.5 hours; third instar, 85°F., 12 hours; fourth instar, 85°F., 17 hours; fifth instar (non-diapause), 80°F., 125 hours; pupa, 82°F., 117 hours; no important hyperparasites were found, although predation by Medetera sp. is significant; parasitism of the Douglas-fir beetle ranges up to 100 percent for individual invasions under certain described conditions.

It is recommended that parasite populations be fostered in small diameter trees by leaving selected trees from thinning operations in the woods until after parasite emergence.

A description of the external morphology of all immature stages is given. The embryological development is traced in detail from early cleavage and blastoderm formation, including gastrulation, segmentation and formation of the organ systems.

BIBLIOGRAPHY

1. Allen, Donald G., Robert R. Michael and Solon A. Stone. Sounds of Douglas fir beetle activity... recorded and interpreted...equipment techniques. Corvallis, 1958. 18p. (Oregon Forest Lands Reserch Center. Research Note no. 36)

2. Baerends, G.P. and J.M. Baerends-van Roon. Embryological and ecological investigations on the development of the egg of Ammophila campestris Jur. Tijdschrift voor Entomologie 92:53-112. 1949.

3. Bedard, W.D. The number of larval instars and the approximate length of the larval stadia of Dendroctonus pseudotsugae Hopk., with a method for their determination in relation to other bark beetles. Journal of Economic Entomology 26:1128-1134. 1933.

4. _____ The Douglas-fir beetle. Washington, 1950. 10p. (U.S. Dept. of Agriculture. Circular no. 817)

5. Bijjani, George Yousuf. Studies on the embryological development of the mealworm, Tenebrio molitor Linné (Tenebrionidae, Coleoptera) Ph.D. thesis. University of Illinois, 1954. 114 numb. leaves. (Abstracted in Dissertation Abstracts 14:1852-1853. 1954)

6. Bischoff, H. Biologie der Hymenopteren. Berlin, Springer, 1927. 598p.

7. Bodenstein, D. Embyryonic development. In: Kenneth D. Roeder's, Insect physiology. New York, Wiley, 1953. p. 780-821.

8. Bonhag, Philip F. Ovarian structure and vitellogenesis in insects. Annual Review of Entomology 3:137-160. 1958.

9. Bronskill, Joan F. Embryology of Pimpla turnionella (L.) (Hymenoptera:Ichneumonidae) Canadian Journal of Zoology 37:655-688. 1959.

10. Brunson, M.H. The influence of the instars of host larvae on the sex of the progeny of Tiphia popilliavora Roh. Science, new ser. 86:197. 1937.

11. Butt, F.H. Embryology of Sciara (Sciaridae:Diptera) Annals of the Entomological Society of America 27:565-579. 1934.

12. _____ The origin of the peritrophic membrane in Sciara and the honey bee. Psyche 41:51-56. 1934.

13. _____ The role of the premandibular or intercalary segment in head segmentation of insects and other arthropods. Transactions of the American Entomological Society 83:1-30. 1957.

14. _____ Head development in the arthropods. Biological Reviews of the Cambridge Philosophical Society 35:43-91. 1960.

15. Carrière, Justus and Otto Bürger. Die Entwicklungsgeschichte der Mauerbiene (Chalcidoma muraria, Fabr.) im Ei. Nova Acta Academiae Caesareae Leopolino-Carolinae Germanicae Naturae Curiosorum 69:253-420. 1897.

16. Clausen, C.P. The effect of host size upon sex ratio of hymenopterous parasites and its relation to methods of rearing and colonization. Journal of the New York Entomological Society 47:1-9. 1939.

17. Clausen, Curtis P. Entomophagous insects. New York, McGraw-Hill, 1940. 688p.

18. Counce, S.J. The analysis of insect embryogenesis. Annual Review of Entomology 6:295-312. 1961.

19. Cragg, J.B. and Paticia Cole. Diapause in Lucilia sericata (Mg.) Diptera. The Journal of Experimental Biology 29:600-604. 1952.

20. Cushman, R. A. Some types of parasitism among the Ichneumonidae. Proceedings of the Entomological Society of Washington 28:29-51. 1926.

21. DeBach, Paul. The importance of host-feeding by adult parasites in the reduction of host populations. Journal of Economic Entomology 36:647-658. 1943.

22. DeLeon, Donald. The morphology of Coeloides dendroctoni Cushman (Hymenoptera:Braconidae) Journal of the New York Entomological Society 42:297-317. 1934.

23. _____ The biology of Coeloides dendroctoni Cushman (Hymenoptera-Braconidae) and important parasite of the mountain pine beetle (Dendroctonus monticolae Hopk.) Annals of the Entomological Society of America 28:411-424. 1935.

24. Dickel, Otto. Entwicklungsgeschichtliche Studien am Bienenei. Zeitschrift für Wissenschaftliche Zoologie 77:481-527. 1904.

25. Dickson, R.C. Factors governing the induction of diapause in the oriental fruit moth. Annals of the Entomological Society of America 42:511-537. 1949.

26. Dillon, Lawrence S. Some notes on preparing whole insects for sectioning. Entomological News 65:67-70. 1954.

27. Doten, Samuel. Concerning the relation of food to reproductive activity and longevity in certain hymenopterous parasites. Reno, 1911. 30p. (Nevada. Agricultural Experiment Station. Technical Bulletin no. 78)

28. Doutt, Richard L. The biology of parasitic Hymenoptera. Annual Review of Entomology 4:161-182. 1959.

29. Eastham, L.E.S. The formation of germ layers in insects. Biological Reviews of the Cambridge Philosophical Society 5:1-29. 1930.

30. Edwards, Roy L. The effect of diet on egg maturation and resorption in Mormoniella vitripennis (Hymenoptera, Pteromalidae) The Quarterly Journal of Microscopical Science 95:459-468. 1954.

31. Evenden, James C. and Kenneth H. Wright. Douglas-fir beetle. Washington, 1955. 4p. (U.S. Dept. of Agriculture. Forest Service. Forest Pest Leaflet 5)

32. Finlayson, L.R. and Thelma Finlayson. Influence of adult food on viability of early stages of Aptesis basizonia (Grav.) (Hymenoptera:Ichneumonidae), a parasite of pine sawflies (Diprionidae) The Canadian Entomologist 89:507-509. 1957.

33. Finlayson, L.R. and Thelma Green. A note on the effect of certain foods upon fecundity and longevity in Microcryptus basizonius Grav. (Hymenoptera) The Canadian Entomologist 72:236-238. 1940.

34. Flanders, S. E. Host influence on the prolificacy and size of Trichogramma. The Pan-Pacific Entomologist 11:175-177. 1935.

35. Flanders, Stanley E. Environmental control of sex in hymenopterous insects. Annals of the Entomological Society of America 32:11-26. 1939.

36. _____ Metaphycus helvolus, an encyrtid parasite of the black scale. Journal of Economic Entomology 35:690-698. 1942.

37. _____ Oösorption and ovulation in relation to oviposition in the parasitic Hymenoptera. Annals of the Entomological Society of America 35:251-266. 1942.

38. _____ The rôle of mating in the reproduction of parasitic Hymenoptera. Journal of Economic Entomology 36:802-803. 1943.

39. _____ Diapause in the parasitic Hymenoptera. Journal of Economic Entomology 37:408-411. 1944.

40. _____ Elements of host discovery exemplified by parasitic Hymenoptera. Ecology 28:299-309. 1947.

41. _____ Culture of entomophagous insects. The Canadian Entomologist 81:257-274. 1949.

42. _____ Regulation of ovulation and egg disposal in the parasitic Hymenoptera. The Canadian Entomologist 82:134-140. 1950.

43. _____ The mechanisms of sex-ratio regulation in the (parasitic) Hymenoptera. Insectes Sociaux 3:325-334. 1956.

44. Fulton, B.B. Notes on Habrocytus cerealellae, parasite of the angonmois grain moth. Annals of the Entomological Society of America 26:536-553. 1933.

45. Furniss, Malcolm M. Reducing Douglas-fir beetle damage--how it can be done. 1959. 6p. (U.S. Dept. of Agriculture. Forest Service. Intermountain Forest and Range Experiment Station, Ogden. Research Note no. 70)

46. Gatenby, J. Bronté. The embryonic development of Trichogramma evanescens, Westw., monembryonic egg parasite of Donacia simplex, Fab. Quarterly Journal of Microscopical Science, new ser. 62:149-187. 1917.

47. Genieys, Paul. Habrobracon brevicornis Wesm. tr. by L.O. Howard. Annals of the Entomological Society of America 18:143-202. 1925.

48. Glover, P.M. The developmental stages of Bracon tachardiae, Cam. (Hym.) Bulletin of Entomological Research 25:521-539. 1934.

49. Gray, Peter. The microtomist's formulary and guide. New York, Blakiston, 1954. 794p.

50. Grosch, Daniel S. Starvation studies with the parasitic wasp Habrobracon. The Biological Bulletin 99:65-73. 1950.

51. Guyer, Michael F. Animal micrology. 5th ed. Chicago, University Press, 1953. 327p.

52. Harries, F. H. Some effects of temperature on the development and oviposition of Microbracon hebetor (Say) The Ohio Journal of Science 37:165-171. 1937.

53. Hegner, Robert W. Studies on germ cells. IV. Protoplasmic differentiation in the oocytes of certain Hymenoptera. Journal of Morphology 26:495-561. 1915.

54. Holdaway, F.G. and H. Fairfield Smith. A relation between size of host puparia and sex ratio of Alysia manducator Pantzer. The Australian Journal of Experimental Biology and Medical Science 10:247-259. 1932.

55. Huffaker, Carl B. The temperature relations of the immature stages of the malarial mosquito, Anopheles quadrimaculatus Say, with a comparison of the developmental powers of constant and variable temperatures in insect metabolism. Annals of the Entomological Society of America 37:1-27. 1944.

56. Ivanova-Kazas, O.M. Biology and embryonic development of Eurytoma aciculata Ratz. (Hymenoptera, Eurytomidae) Entomologicheskoe Obozrenie 37:5-23. 1958. (Tr. in Entomological Revue 37:1-18. 1958)

57. _____ Embryonic development of Angitia vestigialis Ratz. (Hymenoptera, Ichneumonidae) - an internal parasite of the sawfly Pontania capeae L. (Hymenoptera, Tenthredinidae). Entomological Review 39:181-189. 1960)

58. Janisch, Ernst. The influence of temperature on the life-history of insects. The Transactions of the Entomological Society of London 80:137-168. 1932.

59. Johannsen, O.A. and F.H. Butt. Embryology of insects and myriapods. New York, McGraw-Hill, 1941. 462p.

60. Johnson, Floyd A. Estimating past diameters of Douglas-fir trees. 1955. 3p. (U.S.Dept. of Agriculture. Forest Service. Pacific Northwest Forest and Range Experiment Station, Portland. Research Note no. 112)

61. Keen, F.P. Progress in bark-beetle control though silviculture in the United States. Proceedings of the Tenth International Congress of Entomology 4:171-180. 1956.

62. Kennedy, Clarence Hamilton. Methods for the study of the internal anatomy of insects. Columbus, H.L. Hedrick, 1932. 103p. (Mimeographed)

63. Korschelt, E. and K. Heider. Text-book of the embryology of invertebrates. Tr. by Matilda Bernard. New York, MacMillan, 1899. 441p.

64. Lees, A.D. The physiology of diapause in arthropods. London, Cambridge University Press, 1955. 150p.

65. _____ The physiology and biochemistry of diapause. Annual Review of Entomology 1:1-16. 1956.

66. Li, Jerome C.R. Introduction to statistical inference. Ann Arbor, Edwards, 1957. 553p.

67. Maezawa, K. Bark thickness at several heights in young Douglas-fir trees. British Columbia University, Forest Club, Research Note no. 13. 1956. 3p.

68. Mashhood, Alam S. A contribution to the biology of Stenobracon deesae Cameron (Braconidae, Hymenoptera), and the anatomy of its pre-imaginal stages. Zeitschrift für Parasitenkunde 15:159-182. 1952.

69. Maslennikova, V.A. The conditions determining diapause in the parasitic hymenopteron Apanteles glomeratus L. (Hymenoptera;Braconidae) and Pteromalus puparum L. (Hymenoptera,Chalcididae) Entomologicheskoe Obozrenie 37:538-545. 1958. (Tr. in Entomological Review 37:466-472. 1958)

70. McCowan, Vaughn F. and Julius A. Rudinsky. Biological studies on the Douglas fir bark beetle. Tacoma, Weyerhaeuser Timber Company, 1954. 14p. (Forestry Research Notes. Millicoma Forest. Coos Bay, Oregon. Progress Report)

71. Morris, R.F. The interpretation of mortality data in studies on population dynamics. The Canadian Entomologist 89:49-69. 1957.

72. Muesebeck, C.F.W., Karl V. Krombein and Henry K. Townes. Hymenoptera north of Mexico. Synoptic catalog. Washington, 1951. 1420p. (U.S.Dept. of Agriculture. Agriculture Monograph no. 2)

73. Myser, Willard C. The larval and pupal development of the honey bee Apis mellifera Linnaeus. Annals of the Entomological Society of America 47:683-711. 1954.

74. Narayanan, E.S. and P.B. Mookherjee. Effect of nutrition on the longevity and rate of reproduction in Trichogramma evanescens minutum Riley (Chalcidoidea:Hymenoptera) The Indian Journal of Entomology 17:376-382. 1955.

75. Narayanan, E.S. and T.V. Venkatraman. Technique of mass multiplication of Stenobracon deesae (Cam.) Hymenoptera: Braconidae for use against sugarcane and maize borers. Proceedings of the Indian Academy of Sciences B 36:12-18. 1952.

76. Nelsen, Olin E. The segregation of the germ cells in the grasshopper, Melanoplus differentialis (Acrididae;Orthoptera) Journal of Morphology 55:545-575. 1934.

77. Nelson, James A. Morphology of the honey bee larva. Journal of Agricultural Research 28:1167-1214. 1924.

78. Nelson, James Allen. The embryology of the honey bee. Princeton, Princeton University Press, 1915. 282p.

79. Parker, H.L. Recherches sur les formes post-embryonnaires des Chalcidiens. Annales de la Société Entomologique de France 93:261-379. 1924.

80. _____ Macrocentrus gifuensis Ashmead, a polyembryonic braconid parasite in the European corn borer. Washington, 1931. 62p. (U.S.Dept. of Agriculture. Technical Bulletin no. 230)

81. Pschorn-Walcher, H. Einige Beispiele für Korrelationen zwischen Körper- und Organgrösse bei den Proctotrupoidea (Hymenopt. parasit.) Mitteilungen der Schweizerischen Entomologischen Gesellschaft 28:214-219. 1955.

82. Reid, R.W. The bark beetle complex associated with lodgepole pine slash in Alberta. II. Notes on the biologies of several hymenopterous parasites. The Canadian Entomologist 89:5-8. 1957.

83. Richards, A. Glenn. Comments on the origin of the midgut in insects. Journal of Morphology 53:433-441. 1932.

84. Rudinsky, J.A. and J.P. Vité. Effects of temperature upon the activity and the behavior of the Douglas fir beetle. Forest Science 2:258-267. 1956.

85. Russo, Giuseppe. VI. Contributo alla conoscenza dei Coleotteri Scolitidi. Fleotribo: Phloeotribus scarabaeoides (Bern.) Fauv. Bollettino del R. Laboratorio di Entomologia Agraria di Portici 2:3-420. 1938-39.

86. Ryan, Roger Baker. Some environmental factors affecting development and emergence of the Douglas-fir beetle, Dendroctonus pseudotsugae Hopkins (Coleoptera:Scolytidae), in the laboratory. Master's thesis. Corvallis, Oregon State College, 1959. 62 numb. leaves.

87. Salt, George. Parasites of the wheat-stem sawfly, Cephus pygmeus, Linnaeus, in England. Bulletin of Entomological Research 22:479-545. 1931.

88. Schlüter, Josefa. Die Entwicklung der Flügel bei der Schlupfwespe Habrobracon juglandis Ash. Zeitschrift für Morphologie und Ökologie der Tiere 27:488-517. 1933.

89. Schmieder, Rudolf G. The significance of the two types of larvae in Sphecophaga burra (Cresson) and the factors conditioning them (Hymenoptera:Ichneumonidae). Entomological News 50:125-131. 1939.

90. Schnetter, Martin. Morphologische Untersuchungen über das Differenzierungszentrum in der Embryonalentwicklung der Honigbiene. Zeitschrift für Morphologie und Ökologie der Tiere 29:114-195. 1935.

91. Seurat, L.G. Contributions a l'étude des Hyménoptères entomophages. Annales des Sciences Naturelles, ser.8, Zoologie, 10:1-159. 1899.

92. Seyrig, André. Relations entre le sexe de certains ichneumonides (Hym.) et l'hôte aux dépens duquel ils ont vécu. Bulletin de la Société Entomologique de France 40:67-70. 1935.

93. Shafiq, Saiyid Ahmad. A study of the embryonic development of the gooseberry sawfly, Pteronidea ribesii. Quarterly Journal of Microscopical Science, ser. 3, 95:93-114. 1954.

94. Short, J.R.T. The morphology of the head of larval Hymenoptera with special reference to the head of the Ichneumonoidea, including a classification of the final instar larvae of the Braconidae. The Transactions of the Royal Entomological Society of London 103:27-84. 1952.

95. Simmonds, F.J. A factor affecting diapause in hymenopterous parasites. Bulletin of Entomological Research 37:95-97. 1946.

96. _____ Some factors influencing diapause. The Canadian Entomologist 79:226-232. 1947.

97. _____ The influence of maternal physiology on the incidence of diapause. Philosophical Transactions of the Royal Society of London, ser. B 233:385-414. 1948.

98. Smith, Owen J. Biology and behavior of Microctonus vittatae Muesebeck (Braconidae) with descriptions of its immature stages. University of California Publications in Entomology 9:315-344. 1952.

99. Snodgrass, R.E. Morphology and evolution of the insect head and its appendages. Washington, Smithsonian Institution, 1928. 158p. (Smithsonian Miscellaneous Collections vol.81, no. 3)

100. _____ Principles of insect morphology. New York, McGraw-Hill, 1935. 667p.

101. Speicher, Benjamin Robert. The embryology of a parasitic wasp, Habrobracon juglandis. Mater's thesis. Pittsburgh, University of Pittsburgh, 1931. 37 numb. leaves.

102. _____ Oögenesis, fertilization and early cleavage in Habrobracon. Journal of Morphology 59:401-421. 1936.

103. Strindberg, Henrik. Zur Kenntnis der Hymenopteren-Entwicklung. Vespa vulgaris nebst einigen Bemerkungen über die Entwicklung von Trachusa serratulae. Eine embryologische Untersuchung. Zeitschrift für Wissenschaftliche Zoologie 112:1-47. 1915.

104. Tanquary, M.C. Biological and embryological studies on the Formicidae. Bulletin of the Illinois State Laboratory of Natural History 9:417-479. 1913.

105. Taylor, Raymond L. The biology of the white pine weevil, Pissodes strobi (Peck), and a study of its insect parasites from an economic viewpoint. Entomologica Americana, n.s. 9:167-246. 1929. 10:1-86. 1930.

106. _____ On "Dyar's rule" and its application to sawfly larvae. Annals of the Entomological Society of America 24:451-466. 1931.

107. Thompson, W.R. A catalogue of the parasites and predators of insect pests. sec. 2.Host parasite catalog. 2. Hosts of the Hymenoptera (Agaonidae to Braconidae). Ottawa Commonwealth Institute of Biological Control, 1953. 190p. (Multigraph)

108. Vance, A.M. and H.D. Smith. The larval head of parasitic Hymenoptera and nomenclature of its parts. Annals of the Entomological Society of America 26:86-94. 1933.

109. Venkatraman, T.V. and B.R. Subba Rao. The mechanism of oviposition in Stenobracon deesae (Cam.) (Hymenoptera:Braconidae). The proceedings of the Royal Entomological Society of London, ser. A 29:1-8. 1954.

110. Viereck, H.L. Descriptions of one new genus and three new species of ichneumon flies. Proceedings of the United States National Museum 41:293-295. 1912.

111. Vité, J.P. and J.A. Rudinsky. Contribution toward a study of Douglas fir beetle development. Forest Science 3:156-167. 1957.

112. Walters, J. Biology and Control of the Douglas-fir beetle in the interior of British Columbia. Ottawa, 1956. 11p. (Canada. Dept. of Agriculture. Science Service. Division of Forest Biology. Publication 975)

113. Way, M.J. and B.A. Hopkins. The influence of photoperiod and temperature on the induction of diapause in Diataraxia oleracea L. (Lepidoptera) The Journal of Experimental Biology 27:365-376. 1950.

114. Weaver, Nevin and Russell C. Thomas, Jr. A fixative for use in dissecting insects. Stain Technology 31:25-26. 1956.

115. Wheeler, William Morton. A contribution to insect embryology. Journal of Morphology 8:1-160. 1893.

116. Wilde, J.de, C.S. Duintjer and L. Mook.Physiology of diapause in the adult Colorado beetle (Leptinotarsa decemlineata Say) - I. The photoperiod as a controlling factor. Journal of Insect Physiology 3:75-85. 1959.

117. Willard, H.F. Parasites of the pink bollworm in Hawaii. Washington, 1927. 15p. (U.S.Dept. of Agriculture. Technical Bulletin no. 19)

118. Worthington, Norman P. and George R. Staebler. Commercial thinning of Douglas-fir in the Pacific Northwest. Washington, 1961. 124p. (U.S.Dept. of Agriculture. Forest Service. Technical Bulletin no. 1230)

APPENDIX

SCHEDULES FOR MORPHOLOGICAL PREPARATIONS

Feulgen Reaction for Staining Nuclei

1- fix, remove the ends of the egg, and wash in alcohol and running water according to the directions on p. 13.

2- 1N HCl; 10 minutes

3- 1N HCl at 60°C.; 20 minutes

4- 1N HCl at room temperature; rinse

5- Schiff's reagent (add 1 gm. of basic fuschin and 1.9 gm. sodium metabisulfite to 100 ml. 0.15 N HCl; shake at intervals for two hours; add 0.5 gm. activated charcoal and shake vigorously for one minute; filter); 1½ hours.

6- SO_2 water (0.25 gm. sodium metabisulfite, 2.5 ml. N HCl plus 52.5 ml. H_2O made up immediately before use); 3 changes, 1½ minutes each

7- tap water; 15 minutes

8- distilled water; rinse

9- dehydrate by dropwise addition of 95% alcohol

10- absolute alcohol; 3 changes, 2-3 minutes each

11- 1/3 cedarwood oil: 2/3 absolute alcohol; until embryo sinks

12- 2/3 cedarwood: 1/3 absolute alcohol; until embryo sinks

13- cedarwood oil; until embryo sinks

14- xylol; rinse

15- mount in balsam

Harris' Hematoxylin Staining of Whole Embryos

1- fix, remove chorion and wash according to the directions on p. 13.

2- hydrate by dropwise addition of distilled water

3- Harris' hematoxylin; 2-3 seconds (The embryo was contained in a lens paper sac during this step.)

4- tap water; until stain turns blue

5- acid alcohol;
5-30 seconds or until depressions are clearly visible

6- tap water; until stain turns blue

7- distilled water; 2-3 minutes

8- bring up to 70% alcohol gradually

Harris' Hematoxylin Sections of Embryos

1- fix, remove chorion and wash according to the directions on p. 13.

2- hydrate by dropwise addition of distilled water

3- dilute Harris' hematoxylin (1 part in 30 parts distilled water); 24 hours

4- tap water; 1-2 hours

5- dioxan; 5 changes, 30 minutes each

6- xylol; rinse

7- melted paraffin (56-58°C.); 5 changes, 30 minutes each

8- embed, section and mount

9- (the sections may be counterstained with eosin if desired. If overstained with hematoxylin a short period in acid alcohol may be necessary to remove the excess stain.)

Acid Fuschin Staining for Skeletal Structures

1- KOH (10% aqueous); 6-48 hours or until non-chitinous tissue is dissolved

2- distilled water acidulated with dilute acetic acid; 4-5 changes, 20-30 minutes each

3- acid fuschin (0.5% in 10% HCl); 10-60 minutes or until stain has penetrated all areas

4- 95% alcohol; 2-60 minutes or until desired stain is achieved

5- absolute alcohol; 2 changes, 3-4 minutes each

6- xylol; 2-3 minutes

7- balsam

Larval Whole Mount; Tracheal and Muscular Systems; Eosin Proceedure

1- pin open in physiological saline (NaCl, 10.9 gm.;

KCl, 1.57 gm.; $CaCl_2$, 0.85 gm.; $MgCl_2$, 0.17 gm.; H_2O, 1000 cc.)

2- drain saline and flood with Bouin's fixative (picric acid, saturated aqueous, 75 parts; 40% formalin, 25 parts; glacial acetic acid, 5 parts); remove all extraneous tissues; 1 hour

3- 50% alcohol saturated with $LiCO_3$; 4 changes, 20 minutes each

4- 50% alcohol; 2 changes, 5 minutes each

5- eosin (0.5% in 70% alcohol, acidulated with a few drops of dilute acetic acid); 1 hour

6- 70% alcohol; change until desired stain is reached (2-48 hours)

7- 95% alcohol; 5 minutes

8- absolute alcohol; 3 changes, 3-4 minutes each

9- xylol; 2-3 minutes

10- balsam

Hematoxylin and Eosin Sections of Larvae

1- place larva in water and raise to 70°C.

2- cool in cold water

3- cut larva into sections or open widely with a razor blade

4- Bouin's fixative; 2-6 hours

5- tap water; rinse

6- 50% alcohol saturated with $LiCO_3$; 4 changes, 30

minutes each

7- dioxan; 5 changes, 30 minutes each; hold in last change overnight

8- xylol; rinse

9- melted paraffin (56°-58°C.) 5 changes, 30 minutes each

10- embed, section, mount and stain with Harris' hematoxylin and eosin according to standard procedures

Table 10. Chi square test of independence for parasite success on the top and bottom of a down tree. (For discussion see p. 35)

Position of sample	Observations	Freq. (f)	Hypo. freq. (h)	(f-h)	$(f-h)^2$	$(f-h)^2/h$
Top	Success (parasitized)	62	82.9	-20.9	436.81	5.2691
	failure (not parasitized)	118	97.1	20.9	436.81	4.4986
Bottom	Success	83	62.1	20.9	436.81	7.0340
	Failure	52	72.9	-20.9	436.81	5.9919
Total		315	315	0		22.7936[1]

1. Significant at 0.5 per cent level.

Table 11. Chi square test of independence for parasite success on the sun and shade sides of a down tree. (For discussion see p. 35)

Position of sample	Observations	Freq. (f)	Hypo. freq. (h)	(f-h)	$(f-h)^2$	$(f-h)^2/h$
Sun side	Success (parasitized)	14	18.9	-4.9	24.01	1.2704
	Failure (not parasitized)	27	22.1	4.9	24.01	1.0864
Shade side	Success	131	126.1	4.9	24.01	0.1904
	Failure	143	147.9	-4.9	24.01	0.1623
Total		315	315	0		2.7095[1]

1. Not significant at the five per cent level.

Table 12. Analysis of variance of the number of mature oöcytes present in ten day old females fed different diets. (For discussion see p. 42)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	65.8750	3	21.9583	1.87[1]
Error	423.1000	36	11.7528	
Total	488.9750	39		

1. Not significant at the five per cent level.

Table 13. Analysis of variance of male longevity at 75°F. when fed different diets. (For discussion see p. 51)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	1739.0000	4	434.7500	4.94[1]
Error	3963.5000	45	88.0778	
Total	5702.5000	49		

1. Significant at the 0.5 per cent level.

Table 14. Analysis of variance of female longevity at 75°F. when fed different diets. (For discussion see p. 51)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	6375.0800	4	1593.7700	5.11[1]
Error	14048.3000	45	312.1844	
Total	20423.3800	49		

1. Significant at the 0.5 per cent level.

Table 15. Chi square test of independence for diapause percentages in trees felled on different dates. (For discussion see p. 58)

Date tree felled	Obser.	Freq. (f)	Hypo. freq. (h)	(f-h)	$(f-h)^2$	$(f-h)^2/h$
4/20	Success (emerged)	68	31.9	36.1	1303.21	40.852978
	Failure (diapause)	0	36.1	-36.1	1303.21	36.1
5/28	Success	133	100.3	32.7	1069.29	10.66091724
	Failure	81	113.7	-32.7	1069.29	9.40448548
7/1 (approx.)	Success	471	539.8	-68.8	4733.44	8.76887736
	Failure	681	612.2	68.8	4733.44	7.73185233
Total		1434	1434	0		113.51911041[1]

1. Significant at the 0.5 per cent level.

Table 16. Chi square test of independence for the number of progeny entering diapause following treatment of the parent females with different day lengths at 75°F. (Combination of three replicates) (For discussion see p. 59)

Hrs. light per 24	Obser.	Freq. (f)	Hypo. freq. (h)	(f-h)	$(f-h)^2$	$(f-h)^2/h$
10	Success (emerged)	15	20.64	-5.64	31.8096	1.541162790
	Failure (diapause)	28	22.36	5.64	31.8096	1.422611806
12	Success	26	41.28	-15.28	233.4784	5.65596899
	Failure	60	44.72	15.28	233.4784	5.22089445
14	Success	23	40.32	-17.32	299.9824	7.44003968
	Failure	61	43.68	17.32	299.9824	6.86772893
16	Success	80	41.76	38.24	1462.2976	35.0167049
	Failure	7	45.24	-38.24	1462.2976	32.3231122
Total		300	300	0		97.48822375[1]

1. Significant at the five per cent level.

Table 17. Chi square test of independence for the number of progeny entering diapause following treatment of the parent females with different day lengths at 85°F. (Combination of three replicates). (For discussion see p. 59)

Hrs. light per 24	Obser.	Freq. (f)	Hypo. freq. (h)	(f-h)	$(f-h)^2$	$(f-h)^2/h$
10	Success (emerged)	26	33.44	-7.44	55.3536	1.655311004
	Failure (diapause)	50	42.56	7.44	55.3536	1.300601503
12	Success	19	34.76	-15.76	248.3776	7.14550057
	Failure	60	44.24	15.76	248.3776	5.61432188
14	Success	28	30.80	-2.80	7.8400	0.25454545
	Failure	42	39.20	2.80	7.8400	0.20000000
16	Success	47	20.68	26.32	692.7424	33.49818181
	Failure	0	26.32	-26.32	692.7424	26.32000000
Total		272	272	0		75.98846221[1]

1. Significant at the five per cent level.

Table 18. Test of linearity of regression of size of male parasite on length of host larval gallery. (For discussion see p. 66)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	3763.0424	37	101.7038	3.18[1]
lin. reg.	2758.2133	1	2758.2133	86.26[1]
dev. from lin.	1004.8291	36	27.9119	0.87[2]
Error	671.5000	21	31.9762	
Total	4434.5424	58		

1. Significant at the 0.5 per cent level.
2. Not significant at the 5 per cent level.

Table 19. Test of linearity of regression of size of female parasite on length of host larval gallery. (For discussion see p. 66)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	2092.6847	29	72.1615	3.0075[3]
lin. reg.	956.0586	1	956.0586	39.8460[4]
dev. from lin.	1136.6261	28	40.5938	1.6918[5]
Error	311.9200	13	23.9938	
Total	2404.6047	42		

3. Significant at the 2.5 per cent level.
4. Significant at the 0.5 per cent level.
5. Not significant at the 5 per cent level.

Table 20. Test of homogeneity of regression coefficients; size of male and female parasite on host larval gallery length. (For discussion see p. 66)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Regression due to $\bar{b}$	35.4820	1	35.4820	1.11[1]
Variation among b's	3678.7899	1	3678.7899	115.37[2]
Pooled residual	3124.8752	98	31.8865	
Within sample	6839.1471	100		

1. Not significant at the 5 per cent level.
2. Significant at the 0.5 per cent level.

Table 21. Analysis of variance of the per cent parasitism at different heights in trees. (For discussion see p. 82)

Source of variation	Sum of squares	Degrees of freedom	Mean square	F
Treatment	304.2076	4	76.0519	5.37[1]
Error	382.6074	27	14.1706	
Total	686.8150	31		

1. Significant at the 0.5 per cent level.