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INTRA-UTERINE NUTRITION OF THE "BEETLE-ROACH" *DIPLOPTERA DYTISCOIDES* (SERV.) DURING EMBRYOGENESIS, WITH NOTES ON ITS BIOLOGY IN THE LABORATORY (BLATTARIA: DIPLOPTERIDAE)

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INTRA-UTERINE NUTRITION OF THE "BEETLE-ROACH" *DIPLOPTERA DYTISCOIDES* (SERV.)[1] DURING EMBRYOGENESIS, WITH NOTES ON ITS BIOLOGY IN THE LABORATORY (BLATTARIA: DIPLOPTERIDAE)

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Diploptera dytiscoides is a common viviparous cockroach that injures the bark of several kinds of trees in Hawaii and other Pacific Islands (Fullaway and Krauss, 1945). Its embryology has been extensively studied by Hagan (1951) who indicated that the embryo increases over five times in size during development. This growth was not accompanied by a decrease in yolk, and Hagan suggested that the developing embryo acquired nutriment from another source. The embryos have greatly elongated pleuropodia to which Hagan (1939, 1951) tentatively ascribed nutritional or respiratory functions, or both. Because of the embryo's increase in size and its modified pleuropodia, Hagan (1951) cited *D. dytiscoides* as the one example of pseudoplacental viviparity among cockroaches. Although Hagan's suggestion is a logical hypothesis, the acquisition of nutriment by the embryo has never been examined experimentally.

The eggs of the false ovoviviparous cockroach, *Nauphoeta cinerea* (Oliv.), also increase in size during embryogenesis; this increase is closely correlated with absorption of water, whereas solids are slowly lost until hatching (Roth and Willis, 1955). In order to determine whether

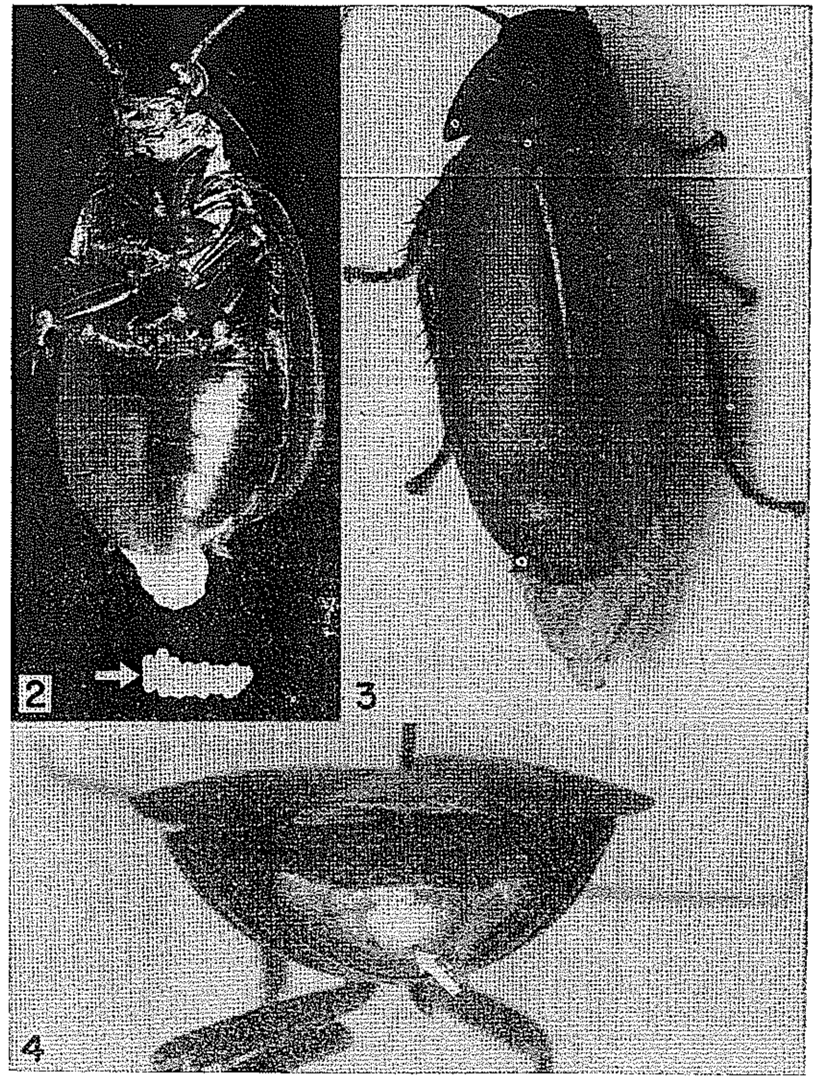
[1]We are greatly indebted to the United States Department of Agriculture for permission to import this species, and to Mr. Fred A. Bianchi of the Hawaiian Sugar Planters Association, who kindly sent us several hundred living specimens of *Diploptera*. The insects were cultured on Purina dog chow checkers.

absorption of water alone could account for the increase in the size of *Diploptera* eggs, we have studied the changes, with age, in wet weight and in dry matter and water content of the developing eggs.

Female *Diploptera* were removed from the cultures, anesthetized with CO_2, and their oöthecae were expelled by finger pressure against the base of the abdomen. Because oötheca formation was rarely observed, the exact age of the eggs was unknown. Therefore, the length of an end egg in each oötheca was measured with an ocular micrometer and its size was used in lieu of age. All weights were determined on torsion balances sensitive to 0.01 and 0.05 mg. Oöthecae were air-oven dried at about 100°C. to constant weight. All determinations were made on eggs enclosed in oöthecae (rather than on individual eggs), but calculations are presented on a per egg basis. The oötheca itself (fig. 5, arrow) is so reduced in this species that its inclusion in the calculations is a negligible factor, particularly with older eggs which have greatly increased in size. Sometimes an oötheca contained one or two eggs which failed to develop; these were easily removed without damage to the remaining eggs and were not included in the weight determinations. The usual number of eggs in an oötheca is 12 (Hagan, 1941); of 51 oöthecae examined by us, the number of eggs ranged from 9 to 13 (including undeveloped eggs), with a mean of 11.4 per oötheca. Hagan (1954) thinks that this average may be due to altered environment, food, and captivity. In Hawaii he seldom found less than 12 eggs per oötheca and usually more than that number, although he did not record the data.

EXPLANATION OF PLATE 4

Figures 2-4. *Diploptera dytiscoides.* Fig. 2. Female with an everted uterus (as a result of once having had her abdomen squeezed to remove an oötheca) and a recently formed oötheca (arrow) which she extruded because it could not be retracted into the brood sac (×3.8). Fig. 3. Female, after accidentally being exposed to high temperatures, prematurely aborting an oötheca containing well-developed embryos (×3.8). Fig. 4. End view of abdomen of female; genital segments separated to show 4 embryos visible in the brood sac. Note eye (arrow) of embryo (×7.5).



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Evidence for Intra-uterine Nutrition

The weight, water, and dry matter changes which accompanied the increase in egg size are shown in figure 1; a series of oöthecae containing eggs of different sizes are shown in figures 5-9. The smallest eggs (0.93 mm.) undoubtedly were from a very recently formed oötheca. Hagan (1951) stated that the freshly deposited egg is approximately 1.20 mm. long. The largest eggs (6.9 mm.) represent fully matured embryos, inasmuch as some of the eggs in this oötheca began to hatch shortly after they were manually extruded from the female (figs. 9-13). The difference in size between the smallest and largest eggs represents more than a seven-fold increase during embryogenesis. The size and weight of the eggs increase with acquisition of both water and solids. However, most of the weight increase arises from absorption of water. From the smallest to the largest eggs there was an increase of about 50 times in the amount of dry matter and about 85 times in water content. Visible differentiation was accompanied by the first observed increase in dry matter (fig. 1, arrow). Prior to differentiation the eggs absorbed water only, and the dry matter content dropped from about 34% to about 26%. After differentiation became visible, the dry matter content remained at approximately 26% throughout development. The increase in the size of the egg, as Hagan (1951) pointed out, cannot arise from the small amount of yolk available, nor could the increase in dry weight be interpreted on this basis. Obviously, additional nutriment is supplied by the mother to the eggs in the brood sac. The exact source of this nutriment is not known; Hagan (1941) suggested that nutriment may be secreted by the maternal accessory (colleterial) glands.

Diploptera is unique among cockroaches in gaining solid matter from the mother during embryonic development. In oviparous cockroaches (*Blatta orientalis* L., *Blattella vaga* Heb., *Blattella germanica* (L.)) and false ovoviviparous species (*Nauphoeta cinerea*, *Pycnoscelus surinamensis* (L.), and *Leucophaea maderae* (F.)) solid matter is lost during embryogenesis, although water is absorbed from the oötheca or directly from the mother (Roth and

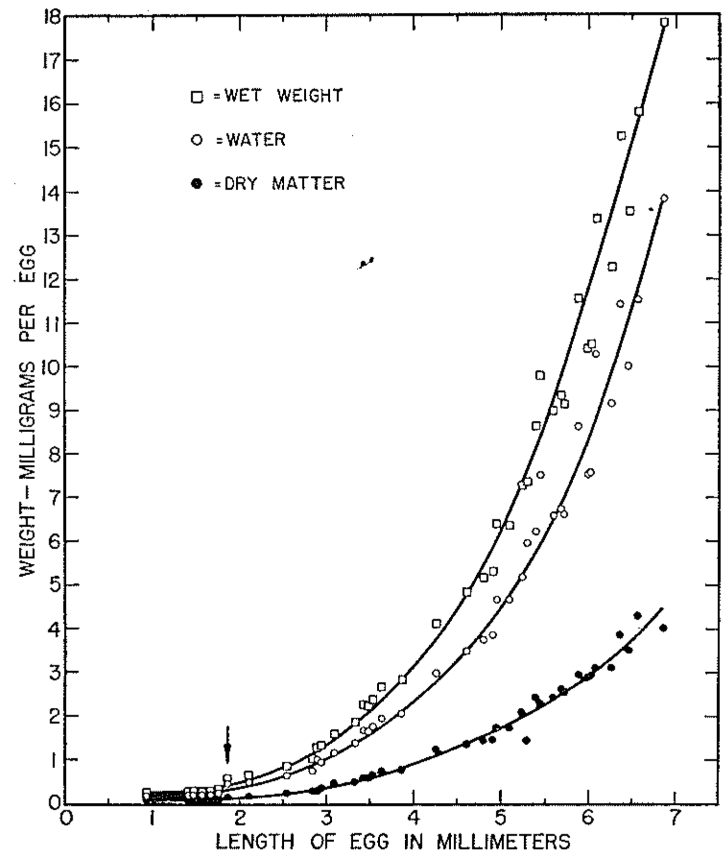


Figure 1. Wet weight, water, and dry matter changes of the eggs of *Diploptera dytiscoides* with increasing age (as indicated by increase in size). All determinations were made on oöthecae containing 9 to 13 eggs, but the data are plotted on a per-eggs basis and include the weights of the oöthecae. Arrow indicates first sign of visible differentiation of the embryo.

Willis, 1955). The gain or loss in water or solids by the eggs of various cockroaches during embryogenesis is compared in table 1. Only the egg of *Diploptera* changes greatly with a truly tremendous gain in water and solids.

And yet, morphologically, the reproductive system of *Diploptera* is comparable to that of *Blatta orientalis*, although some of the structures in *Diploptera* have become modified with its altered physiology (Hagan, 1941).

TABLE 1

Changes in wet weight, water, and solids of the eggs of several species of cockroaches during embryogenesis[1]

Species	Factors by which initial weights changed, per egg[2]		
	Wet weight	*Water*	*Solids*
Blatta orientalis[3]	1.21	1.35	0.96
Blattella vaga	1.12	1.32	0.81
Blattella germanica	1.21	1.49	0.74
Nauphoeta cinerea	2.11	4.62	0.81
Diploptera dytiscoides	73.47	85.80	49.28

Of all the viviparous cockroaches, *Diploptera* is the most highly evolved: the oöthecal covering is reduced, and the small size and number of eggs and the comparatively large size of the vestibule apparently make it possible for the female to transfer the oötheca into her brood sac without exposing the majority of eggs outside her body. Strictly speaking, *Diploptera* may be the only known case of true viviparity among cockroaches, in that the eggs are apparently rarely extruded beyond the vestibule and the embryos derive nourishment (other than yolk or water) from the female.

Biological Observations

We have frequently observed courtship and copulation.

[1]The data for species other than *Diploptera* were computed from Roth and Willis (1955).

[2]Except for *B. orientalis* (see footnote 3), the weights of the oöthecae were included in the computations, and therefore the actual changes would be somewhat larger; the youngest eggs had been recently deposited and the oldest were about ready to hatch. Factors less than unity indicate a decrease in weight.

[3]Based on eggs removed from the oötheca; the youngest eggs were 11 days old and the oldest 32 days old (about 1 week prior to hatching), so that the changes might have been somewhat higher if recently laid and fully developed eggs had been used.

The active male follows the female and palpates her body or genital region. He then partly raises and flutters his wings, turns his terminal abdominal segments toward the female, pushes backward under her and grasps her genitalia. Once they are hooked together, the male swings around into the typical opposed position with his head 180° from the female's. Newly emerged, teneral females are very attractive to, and are courted by, older males; 2 males were attracted to a female that was only partly out of its last nymphal skin and went through weak courting movements. Surprisingly, these young females mate normally (fig. 14) and in laboratory cultures the mating of teneral females is a common occurrence. According to Hagan (1951, p. 320), sexual maturity in *Diploptera* ". . . follows physical maturity rather promptly, if the presence of well developed follicles in the last nymphal instar is any criterion." Teneral females have been seen in copula before their wings had become fully extended; examination of one such female, after she had been in copula for more than an hour and had separated from the male, showed that she had received a spermatophore (fig. 15). Another teneral female, isolated while still in copula, separated from the male about 30 minutes after isolation. Sixty-seven days later, she give birth to 13 nymphs; temperature and humidity were uncontrolled during the gestation period.

We have observed four females forming oöthecae. When the first female was seen, her terminal abdominal segments were slightly separated. We anesthetized the insect and, upon microscopic examination, found about 6 eggs aligned vertically in a double row in her vestibule. After the female had recovered, she began pulsating movements, expanding and telescoping the end segments of her abdomen. While viewing the posterior end of the female during these movements, we could see the first egg through a hand lens. None of the eggs protruded completely beyond her body. About three hours after the female ceased her ovipositing motions, she was dissected. The oötheca, containing 10 eggs, had rotated (micropyles to the left) and it lay partly in the vestibule and partly in the brood sac.

The other three females were discovered in the stock colony by Dr. Barbara Stay of our laboratory. Two of these were observed expanding the end of the abdomen. Although the oöthecae did not protrude beyond the ends of the abdomens, the distal ends of the oöthecae could easily be seen in profile as the females separated their genital segments. The axes of the eggs were vertical at this time. These oöthecae were rotated a short time later, cephalic ends of the eggs to the females' left, and retracted into the brood sac. The females were then dissected; the oöthecae contained 11 and 10 eggs, respectively.

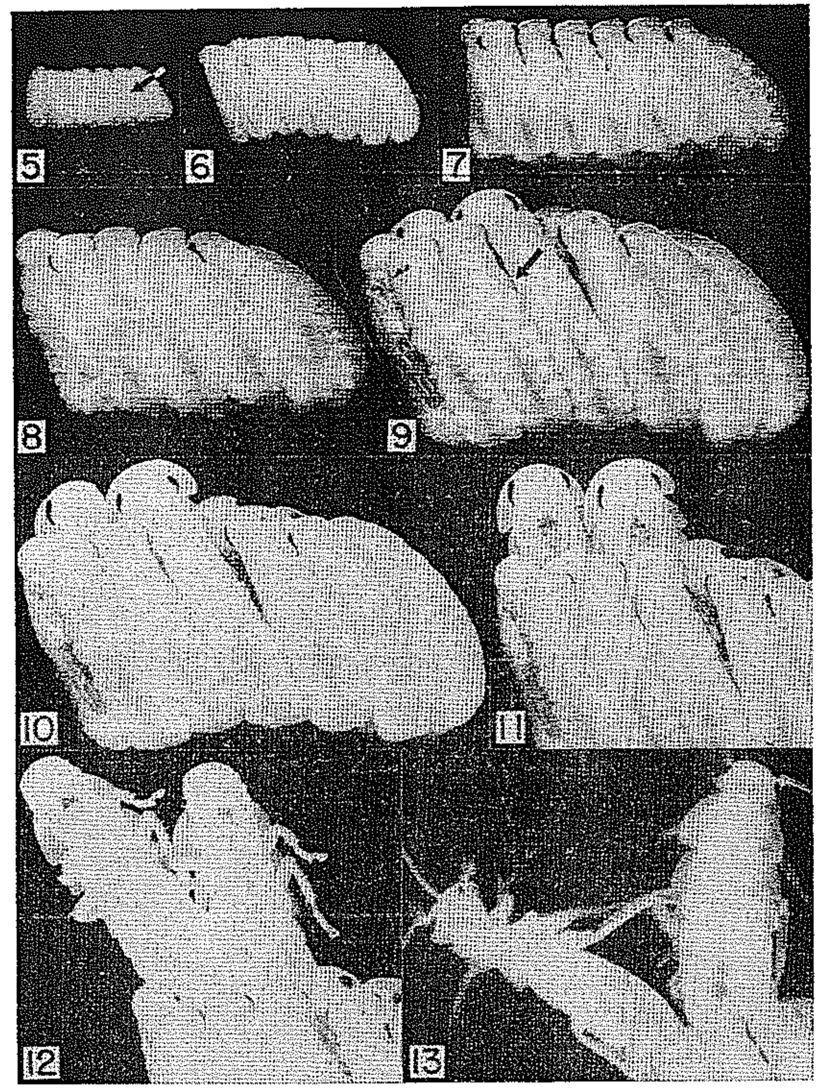
The fourth female was seen with the first egg and parts of the succeeding two eggs protruding beyond the end of her abdomen. The axes of the eggs were vertical, but within a few minutes the female rotated the oötheca, as above, and retracted it. This oötheca was later found to contain 13 eggs and the female's right ovary had seven and the left six ovarioles. These observations show that our a priori hypothesis of oöthecal formation in *Diploptera* (Roth & Willis, 1954) is correct, being similar to that found in other cockroaches.

Protrusion of eggs beyond the end of the abdomen during oöthecal formation apparently depends on such factors as size and number of eggs and size of the female. From our observations, it seems likely that few eggs protrude beyond the female's body and that protrusion occurs rather infrequently.

Since writing the above we have seen oviposition several more times; ovipositing females were recognized when they repeatedly expanded and contracted their genital segments. The first laid egg was visible for only a short time from behind or from the side. Rotation and retraction of the oötheca occurred rapidly, in a matter of minutes.

Explanation of Plate 5

Figures 5-13. Eggs of *Diploptera dytiscoides* (×5). Figs. 5-9. Eggs of various ages showing increase in size. Actual lengths of the eggs as follows: fig. 5. 1.7 mm. (note lack of visible differentiation; upper limit of thin oöthecal membrane indicated by arrow); fig. 6. 3.1 mm.; fig. 7. 4.3 mm.; fig. 8. 5.7 mm.; fig. 9. 6.9 mm. (arrow indicates a strand of greatly stretched, perforated oöthecal membrane). Figs. 10-13. Sequence showing hatching of 2 eggs from oötheca shown in figure 9; hatching occurred within 3-minute period.



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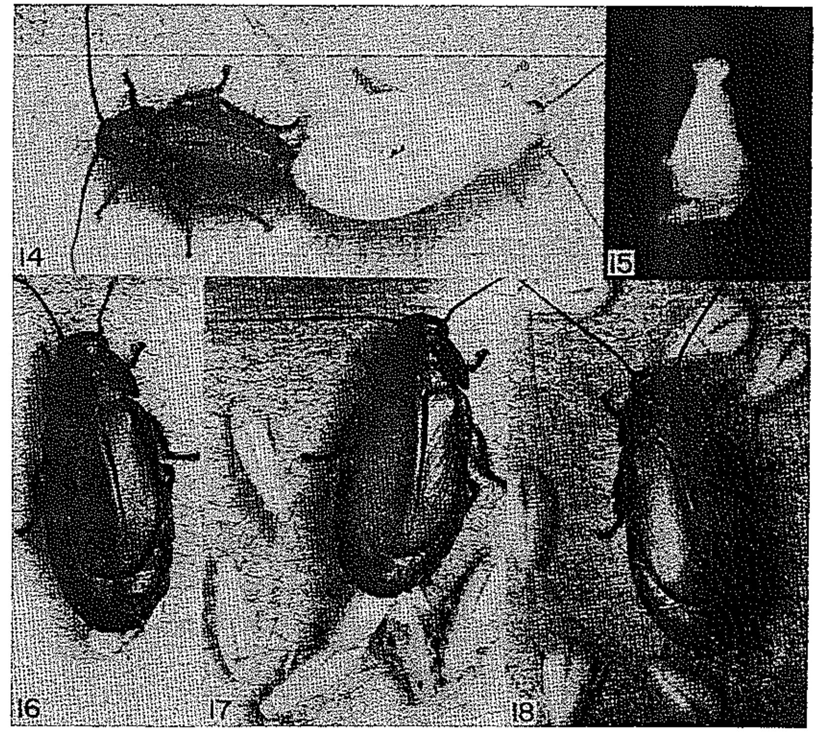
The rapidity of the ovipositional sequence explains why oviposition had not been seen previously in *Diploptera.*

In only one instance did we see a newly formed oötheca completely extruded by a female. We had previously removed an oötheca from this female by finger pressure against her abdomen; apparently this manipulation had damaged her reproductive organs and she was unable to direct the subsequently formed oötheca into her brood sac (fig. 2). A similar abnormality has been observed in *Nauphoeta cinerea* (Roth and Willis, 1955). Females of *Diploptera* that were accidentally exposed to temperatures above 100°F. were seen to abort oöthecae containing apparently dead eggs in various stages of development (fig. 3); this effect of high temperature has been observed in *Pycnoscelus surinamensis* by Roeser (1940).

Kotinsky (1909) found a female of *Diploptera dytiscoides* which, upon being captured, dropped a "batch of embryos" which had been projecting from her abdomen; he concluded from this that the species is viviparous. In *Diploptera,* the fully developed eggs lie diagonally in the brood sac with their cephalic ends pointed posterio-laterally. The head of the full-grown embryo nearest the genital opening of the female may protrude slightly from the brood sac; if the female's genital segments are spread apart, the embryo's head may be readily seen (fig. 4). We have observed hatching of *Diploptera* several times. The abdomens of females carrying fully developed embryos become so distended that the intersegmental membranes between the abdominal sternites are visible. The mature embryos are extruded head first (figs. 16-18). They appear by pairs, swallowing air, the heads of successive pairs

EXPLANATION OF PLATE 6

Figures 14-18. *Diploptera dytiscoides.* Fig. 14. Older, dark-colored male in copula with a recently emerged, teneral female. Fig. 15. Spermatophore (narrow region is the anterior part) removed from a mated teneral female. Figs. 16-18. Female giving birth; time interval between figures 16 and 17 was 9 minutes; that between 17 and 18 was 22 minutes. This female gave birth to only 6 individuals; the others of the brood failed to emerge from the uterus. (Figure 15 about ×13; all other figures about twice natural size.)



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appearing before the preceding pair has completely freed themselves from their embryonic membranes. The embryonic membranes slip back toward the nymphs' caudal segments while they are still held by the genital segments of the female. When the nymphs drop from the mother and move off, the membranes are left behind attached to the vestigial oötheca in the posterior part of the female's genital cavity. The oötheca and embryonic membranes adhere to the female until all the eggs hatch, after which the female expels them, if necessary, with the aid of her hind legs. Thus, the birth product of *Diploptera* is an embryo devoid of a chorion, oötheca, and even the embryonic membrane. This type of birth is characteristic of truly viviparous insects (Hagan, 1951). In *Nauphoeta* and a number of other so-called ovoviviparous species the final birth product is frequently, if not always, the oötheca containing fully matured embryos which begin to hatch after the oötheca has passed beyond the caudal extremity of the female (Roth and Willis, 1954). We have found the pleuropodia of the embryos left behind with the oötheca and embryonic membranes; apparently the pleuropodia are severed from the body at or before hatching time.

We (1954) have questioned whether the so-called ovoviviparous cockroaches are truly viviparous because their oöthecae are first extruded externally during formation and then retracted into the brood sac. Hagan (1954) suggests that the extrusion and retraction of the oötheca into a brood sac represents ". . . a special case of maternal care of the oötheca and its ova." Some oviparous Homoptera place their eggs in a fold in the hypodermis, a location foreign to the reproductive system, until they hatch (Hagan, 1953). The extrusion and retraction of the eggs of the so-called ovoviviparous cockroaches is comparable to the homopteran behavior, with the exception that the oviposition site is a brood sac within the reproductive system.

When the new-born nymphs of *Diploptera* eliminate the air which distends their bodies (fig. 18), they may eat the embryonic membranes and sometimes the oötheca as well. These first instar nymphs are unduly large in comparison with the nymphs of other species of cockroaches, even those with adults twice the size of the adult *Diploptera*.

Mr. George Riser, formerly of our laboratory, working on the life history of *Diploptera*, has found that at 85°F. eggs hatch about 58 days after the female has mated; allowing about a week after copulation for oöthecal formation, embryogenesis takes about 50 days. He also found that nymphal development took about 38 days for males (9 individuals) which molted only 3 times; females (13 individuals) took about 50 days to mature and underwent 4 molts. Molted skins are not eaten by nymphs or adults.

We thank Dr. Harold Hagan for his interest and for critically reading the manuscript.

Summary

The eggs of the viviparous cockroach *Diploptera dytiscoides* absorb both water and solid matter from the female. During embryogenesis, dry matter in the eggs may increase about 50 times and the water content may increase about 85 times. In its embryonic development, *Diploptera* is unique among cockroaches in that the embryo gains solids from the mother in addition to the yolk initially present in the egg.

In laboratory colonies, teneral females mate normally with older males. The oötheca is formed as in other species of cockroaches: the eggs are extruded from the oviduct and arranged vertically in an oötheca within the vestibule. Sometimes one egg (the first laid) may protrude beyond the end of the abdomen, but most of the eggs remain within the vestibule while the female rotates the oötheca and starts retracting it into her brood sac.

The number of eggs per oötheca ranged from 9 to 13, with a mean of 11.4. Embryogenesis takes 50 or more days. The embryos hatch in pairs, being extruded head first from the female. Male nymphs took about 38 days to mature, molting 3 times; females became adults after about 50 days, molting 4 times.

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