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A COMPARATIVE STUDY OF OÖCYTE DEVELOPMENT IN FALSE OVOVIVIPAROUS COCKROACHES*

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Recently Engelmann (1960) compared various internal and external factors which affect the activity of the corpora allata in *Leucophaea maderae* (Fabricius) and *Diplotera punctata* (Eschscholtz). In these two species the stimuli resulting from mating, food intake, gestation, and parturition differed in the degree to which they influenced production of gonadotropic hormone.

In this paper we report our experiments on control of oöcyte development in several species of cockroaches that incubate their eggs internally in a brood sac or uterus. We classify these species as false ovoviviparous forms because the uterine eggs increase in water content only (Roth and Willis, 1955) as opposed to false viviparous species, like *Diploptera,* in which the embryos take up both water and solids from the mother (Roth and Willis, 1955a). In both groups the oviposition behavior is similar. The eggs do not pass directly from the ovaries into the uterus but are first extruded to the outside of the body and then retracted into the brood sac (Roth and Willis, 1954, 1958).

Cockroaches that incubate their eggs internally have two birth products, the egg and nymph (Roth and Willis, 1958). Ovulation and oviposition refer to the eggs being released from the ovaries, oriented by the ovipositor, and covered by the oötheca. After the eggs are in the uterus the females are pregnant (gestation) for a certain period of time and give birth (parturition) to nymphs.

MATERIALS AND METHODS

Except for one series of experiments on *Nauphoeta* (see page 174), all insects were reared on dog chow checkers and maintained at 24°

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to 25° C. and 50 to 70% relative humidity. Engelmann (1957, 1959) showed that yolk deposition and growth of the oöcytes are correlated with and dependent upon activity of the corpora allata in *Leucophaea* and *Diploptera* and we have used oöcyte development as an indicator of endocrine activity. Measurements were made, with an ocular micrometer, of oöcytes that were dissected from ovaries in Ringer's solution. Our measurements of the oöcytes of *Leucophaea* are larger than those reported by Engelmann (1960). This discrepancy is probably due to the fact that he measured the oöcytes after fixation (Engelmann, 1957). We measured one large oöcyte per female; in establishing the normal ovarian cycle or the sizes of the oöcytes at a specific period a number of females were usually dissected to give some indication of the extent of variation. Various operations (allatectomy, nerve cord severance, etc.) were performed on insects kept under carbon dioxide anesthesia.

The species reported on in this paper are *Pycnoscelus surinamensis* (Linnaeus), *Byrsotria fumigata* (Guérin), *Blaberus craniifer* Burmeister, *Blaberus giganteus* (Linnaeus), *Nauphoeta cinerea* (Olivier), and *Leucophaea maderae*. There are two strains of *Pycnoscelus surinamensis* which differ physiologically. The bisexual strain cannot reproduce parthenogenetically and the parthenogenetic strain females when mated to males of the bisexual form show a reduction in fertility and the resulting offspring are all females which reproduce parthenogenetically (Roth and Willis, 1961). Practically all of the experiments on *Pycnoscelus* were done on the parthenogenetic strain but a few were performed on the bisexual form. A similar study on control of oöcyte development in *Diploptera* and two species of *Blattella* has been reported elsewhere (Roth and Stay, 1961, 1962).

RESULTS AND DISCUSSION

Oöcyte development in virgin and mated females

Pycnoscelus surinamensis: Biological data for the two strains are given in table 1. The basal oöcytes of the ovarioles of females less than a day old are large and may already contain yolk. In fact yolk may be present in the oöcytes of some newly-emerged adults indicating that perhaps gonadotropic hormone had already been released in the nymphal stage. The ovarian cycle from emergence to the formation of the second oötheca in the parthenogenetic strain is shown in figure 1. During gestation the oöcytes remain small and increase only slightly in length during the development of the eggs in the uterus. Yolk deposition occurs after parturition and the oöcytes increase rapidly in size.

Table 1 — Biological Data For Two Strains of *Pycnoscelus surinamensis*

BIOLOGICAL OBSERVATION	PARTHENOGENETIC STRAIN				BISEXUAL STRAIN			
	Min.	Max.	Mean ± S.E.[1]	N[2]	Min.	Max.	Mean ± S.E.	N
Length (mm.) of oöcytes less than 1 day after emergence of adult	0.86	0.99	0.91 ± 0.03	5	0.73	0.94	0.85 ± 0.02	10
Length (mm.) of mature oöcytes at oviposition[3]	2.97	3.36	3.21 ± 0.04	10	2.69	3.36	2.99 ± 0.04	15
Length (mm.) of new basal oöcyte at time of oviposition	0.50	0.57	0.53 ± 0.01	6	0.50	0.74	0.60 ± 0.02	16
Length (mm.) of basal oöcytes less than 1 day after parturition	0.67	0.79	0.74 ± 0.01	10	0.69	0.79	0.74 ± 0.01	5
Age (days) at first ovulation								
Virgins	10	20	12.8 ± 0.1	244	8	25	13.6 ± 0.2	138
Mated	—	—	— —	—	9	22	11.9 ± 0.1	59
Gestation (days)[3]								
Virgins	53	58	55.4 ± 0.3	20	—	—	— —	—
Mated	—	—	— —	—	50	56	52.8 ± 0.2	37
Number days to ovulation following parturition[3]	14	17	15.5 ± 0.3	11	10	16	14.2 ± 0.7	8

[1]S.E. = standard error, here and in all following tables.
[2]N = number of insects, here and in all following tables.
[3]Data from Roth and Willis (1961).

In the parthenogenetic strain the first ovulation occurs when the female is about 13 days old whereas the second ovulation takes place about 16 days after birth of young. This 3 day difference is explained by the difference in size of the oöcytes in the newly-emerged female and in the female at parturition; the oöcytes are smaller after the female gives birth and it takes about 3 days to attain the same degree of development as they are at adult emergence. In *Diploptera* the reverse is true and the second preovulation period is 3 days shorter than the first although, as in *Pycnoscelus* the growth rate of the oöcytes is about the same during the first and second preovulation periods. In *Diploptera* the oöcytes at parturition are about the size of those of a 3-day-old mated female which explains the shorter period required for ovulation after parturition (Engelmann, 1959).

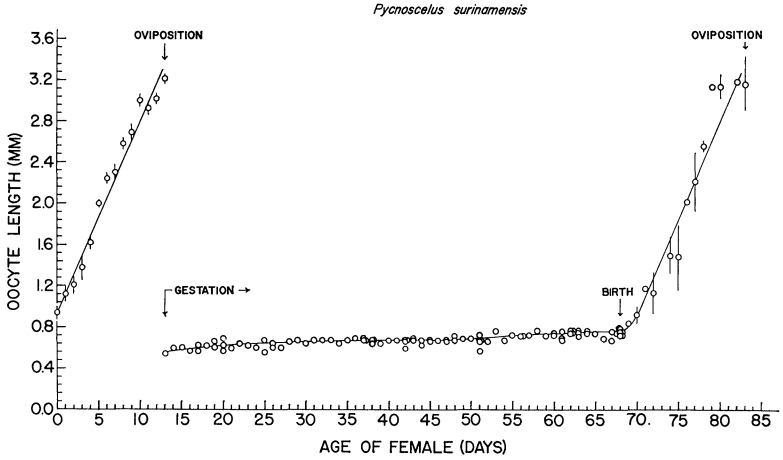


Fig. 1. Ovarian cycle of *Pycnoscelus surinamensis* (parthenogenetic strain). Each point on the curve for oöcyte development from 0 to 13 days is the mean of 6 to 13 measurements (N=134). Each point for the gestation period from 13 to 68 days represents individual measurements (N=99; when 2 or more points were similar for a particular age only one is indicated). The part of the curve representing the growth of the oöcytes after parturition (birth) is based on 1 to 3 individuals (N=24) for each point. Vertical bars =standard errors of mean values.

In the parthenogenetic strain of *Pycnoscelus* it is obvious that mating is unnecessary for development of the oöcytes. The initial development of the oöcytes in the bisexual strain is similar to that found in the parthenogenetic form but differs in that mating slightly stimulates the growth rate and also is necessary for normal retraction of the oötheca into the uterus. Mating a parthenogenetic strain female with a male

of the bisexual strain has no stimulating effect on growth of the oöcytes as indicated by age of the female at ovulation (Roth and Willis, 1961).

Six parthenogenetic strain females, allatectomized when 1 - 2 days old, did not oviposit within a month after the operations. Five of these females had 2 pairs of corpora allata implanted at 29 to 30 days after allatectomy. Four produced oöthecae in less than 35 days and one died after 44 days. At 111 days after allatectomy one female that still had not ovulated received corpora allata implants and oviposited in less than 21 days. This strain normally oviposits about 13 days after emergence (table 1). The delay in oviposition after implanting corpora allata may have been due to the presence of degenerating oöcytes in the ovaries since the oöcytes already have yolk one to two days after emergence (the age at which allatectomy was performed). In *Leucophaea,* oöcytes in resorption inhibit the corpora allata (Engelmann, 1957).

Table 2 — Effect of mating on oöcyte development and oviposition in *Byrsotria fumigata*

OBSERVATION	MATED	VIRGIN
Total number observed	63	213
Number oviposited	53 (84%)	102 (48%)
Oötheca retracted normally	46	92
Oötheca incompletely retracted	7	5
Oötheca dropped	0	5
Number failed to oviposit	10 (16%)	111 (52%)
Oöcytes large, well developed or matured but degenerating and being resorbed	4[1]	68[2]
Oöcytes small, abnormal in shape, being resorbed	6[3]	35[4]
Oöcytes small, normal in appearance but only slightly or not at all developed	0	8

[1]Three of the 4 females had sperm in their spermathecae; one lacked sperm.

[2]These females were 35 to 60 days old when dissected.

[3]All had sperm in their spermathecae.

[4]Twenty-one of these females were 32 to 60 days old. The other 14 were 11 to 24 days of age but since their oöcytes were small and abnormal they would not have oviposited.

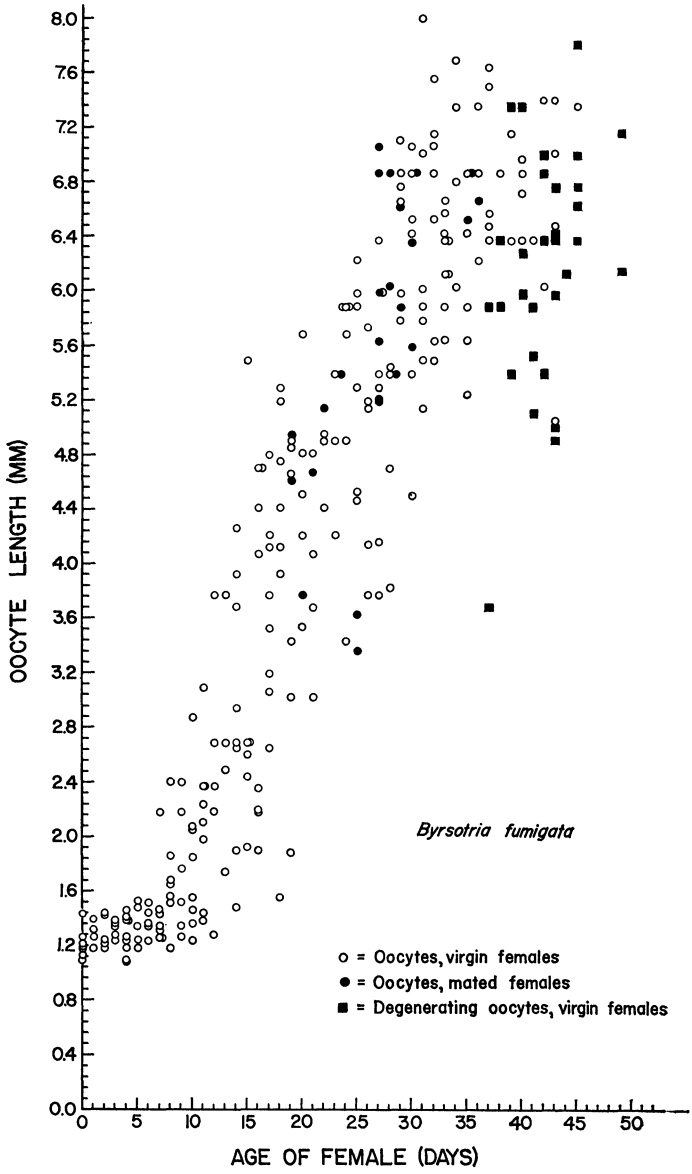


Fig. 2. Growth of oöcytes in virgin and mated females of *Byrsotria fumigata*. Each point represents one female. Females were mated when 1 to 17 days old.

Byrsotria fumigata: The effects of mating on oöcyte development and oviposition in *Byrsotria* are shown in table 2 and figure 2. About 50 percent of the virgin females failed to oviposit but of these 111 females 61% had large well-developed oöcytes that were degenerating or were being resorbed (fig. 14E). It is evident from figure 2 that after the thirty-fifth day of age the oöcytes of many virgins degenerate although most of them may reach a length of 5 mm. or more. About 16% of the virgins and about 10% of the mated females had small abnormally-shaped oöcytes that were being resorbed. It is unlikely that lack of hormone is responsible for this type of abnormality since Barth (personal communication) has dissected pheromone-producing *Byrsotria* females which had small degenerating oöcytes but accessory glands filled with secretion.

In those females that mate, copulation has little, if any, effect on the growth rate of the oöcytes (fig. 2). Mated females oviposited at 26 to 41 days of age ($\bar{x}=32.4\pm0.4$ days; $N=53$); virgin females oviposited 26 to 44 days after emergence ($\bar{x}=34.3\pm0.4$ days; $N=121$). That there is little effect on the rate of growth resulting from mating is further borne out by the fact that the females oviposit at about the same age regardless of their age when mated. In our series the females were with males continuously until they mated; copulation occurred from 4 to 25 days after female emergence. The oöcytes may vary considerably in size in females between these age limits (fig. 2). A female with large oöcytes mated when 25 days old may ovulate 10 days later whereas one with small oöcytes mated at 4 days of age may take 30 days to ovulate (fig. 3). This is quite different from the effect of mating in *Leucophaea* (Engelmann, 1960) where the average interval between mating and oviposition is about the same regardless of the age of the female when mated (fig. 3) because the females tend to mate more readily when their oöcytes reach a certain size (see below). Barth (1961) found that *Byrsotria* females begin to produce sex pheromone 10 to 30 days after the imaginal molt; however, recently (1962) he has found that some females may mate as early as 4 days after adult emergence.

It seems that in *Byrsotria* mating (perhaps the presence of sperm in the spermathecae) serves as a stimulus to oviposition. This is indicated by the fact that the oöcytes in many virgin females apparently mature yet ovulation does not occur. The oöcytes in virgin females at ovulation vary in length from 5.90 to 7.60 mm. ($\bar{x}=6.79\pm0.06$; $N=7$). Although the mean ages at ovulation of mated and virgin females are very similar a breakdown of the data (fig. 4) shows that

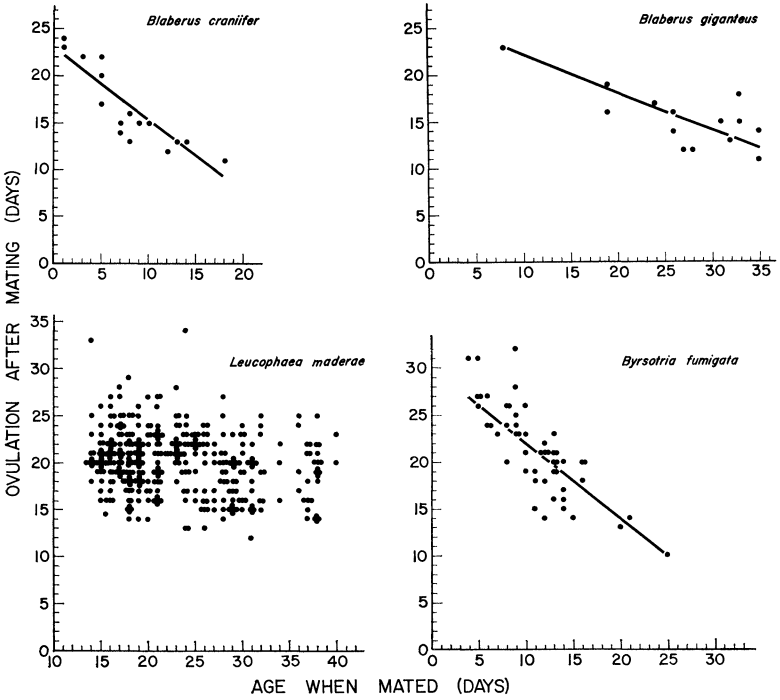


Fig. 3. Relationship between age at mating and ovulation (as indicated by oviposition) in 4 species of cockroaches. Each point represents one female. (N=356 for *Leucophaea*).

38% of the virgins oviposited after the thirty-fifth day as compared to 13% of mated females.

Fifteen virgin females allatectomized when 1 to 2 days old did not produce oöthecae within more than 50 days. At 52 to 210 days after allatectomy, corpora allata were implanted; 9 females ovulated in less than 82 days and one oviposited in 128 days; 3 died without ovipositing and two dissected after 150 days had small undeveloped oöcytes. One allatectomized female that oviposited after receiving corpora allata implants had well developed oöcytes although the oötheca was in the uterus (fig. 14D). Of 25 sham operated females 16 oviposited in less than 56 days. We don't know how to account for the delay in ovulation after implantation of corpora allata into allatectomized females. Four pregnant females (i.e. with an oötheca in the brood sac) had corpora allata implanted and were dissected at 35 to 41 days of pregnancy. Their oöcytes measured 4.90 to 6.81 mm. in length

indicating renewed growth of the ovarian eggs as a result of the implants.

Nauphoeta cinerea: The oöcytes of virgins develop but unless mating occurs the oöcytes in many females may degenerate before they reach ovulation size (fig. 5). Virgin females that ovulate do so in 31 to 47 days ($x=35.8\pm1.2$ days; $N=17$). Mating results in stimu-

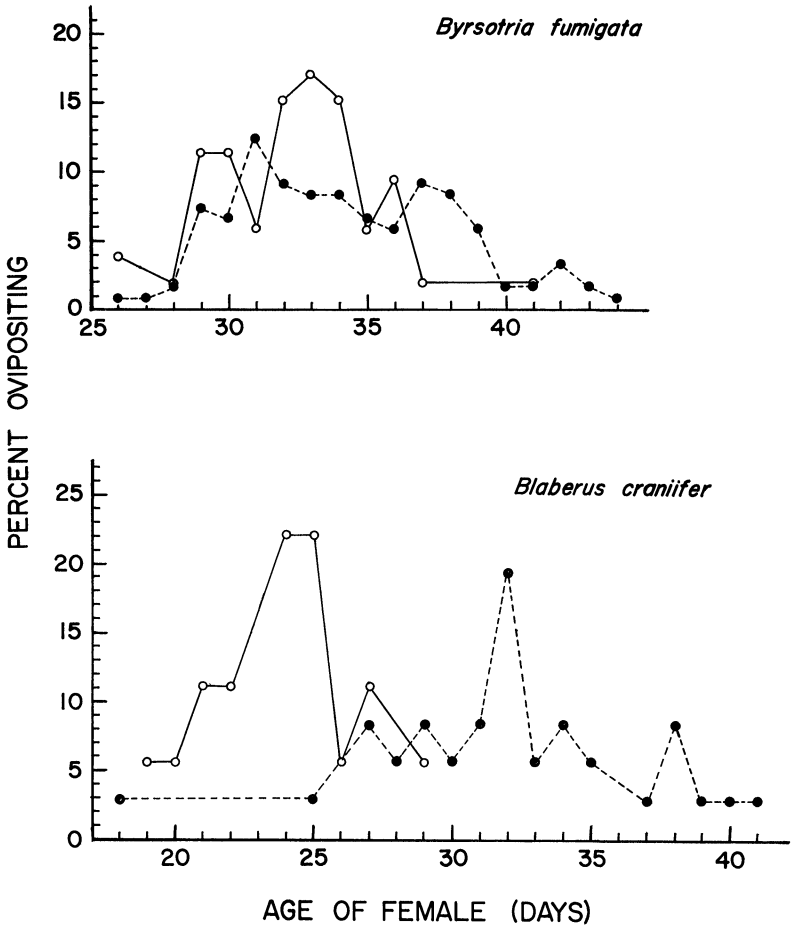


Fig. 4. Effect of mating on oviposition. Solid circles=virgin females. Open circles=mated females. The curves for *Byrsotria* are based on 53 mated and 121 virgin females. The curves for *Blaberus* are based on 18 mated and 36 virgin females.

lation of the corpora allata so that the oöcytes develop rapidly (fig. 5) and oviposition occurs in 18 to 21 days ($\bar{x}=18.9\pm0.40$; $N=8$). Copulation is completed in 17 to 30 minutes ($\bar{x}=20.4\pm0.81$ min.; $N=17$). Of 22 females kept with males continuously, 19 mated 5 days after emergence; the other 3 mated after 6, 8, and 10 days respectively.

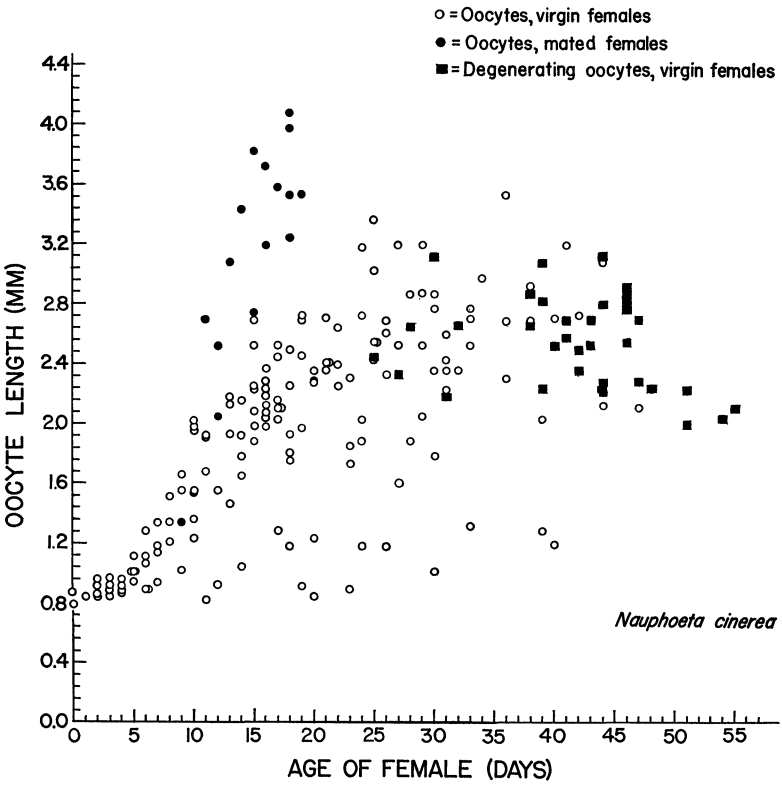


Fig. 5. Growth of oöcytes in mated and virgin females of *Nauphoeta cinerea*. Each point represents one female. Except for 2 individuals mated at 8 and 10 days of age, all others were mated when 5 days old.

In a series of experiments performed at Harvard University, temperature was uncontrolled but usually higher than 24° to 25° C.; the insects were maintained on Purina Laboratory Chow. Both virgin and mated females oviposited earlier than in the above experiment but virgin females still oviposited later (24 to 35 days) than mated indi-

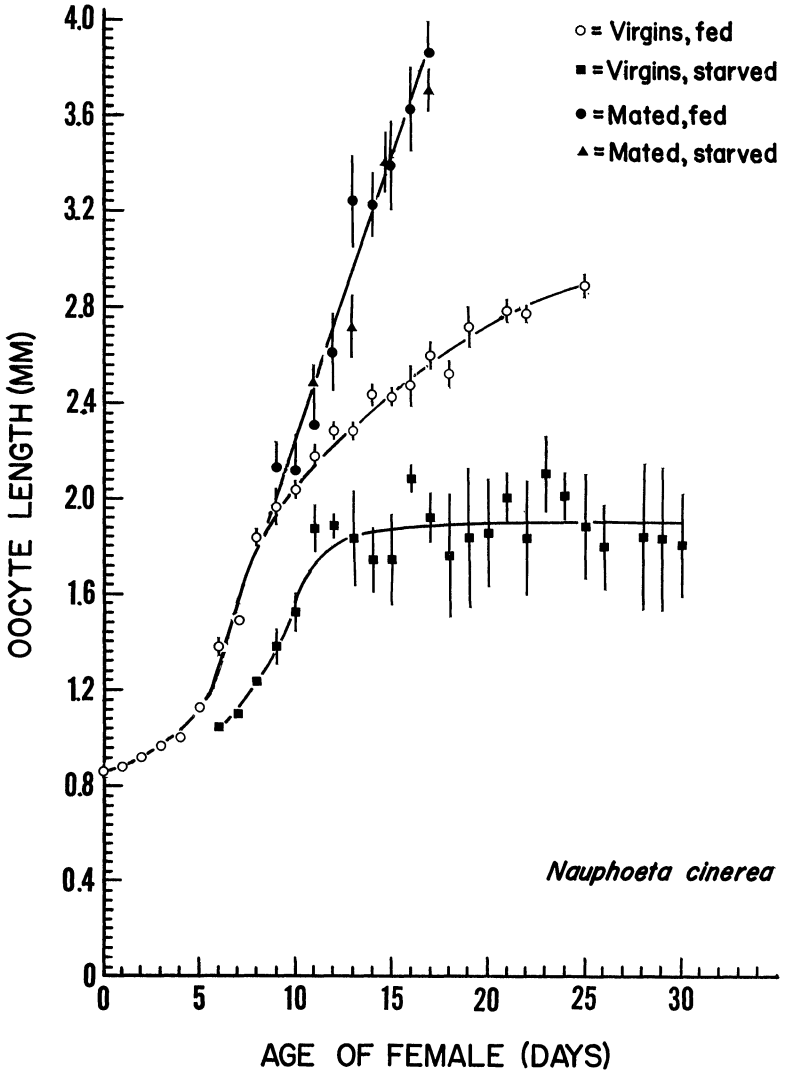


Fig. 6. Effect of mating, starvation, and combined starvation and mating on oöcyte development in *Nauphoeta cinerea*. The points are mean values; fed virgins, N=707; starved virgins, N=133; fed mated, N=58; starved mated, N=21. Females were mated when 4 to 6 days old. Vertical bars are one standard error (only positive halves of standard errors are indicated wherever errors overlapped); no vertical bars indicate standard errors of ±0.02 mm. or less.

viduals (15 to 18 days). The difference in rate of oöcyte development in virgin and mated females is shown in figure 6. The oöcytes of starved females that have mated develop at the same rate as fed mated females (fig. 6).

Of 17 virgin females that oviposited, 10 retracted the oötheca completely into the uterus; several of these females aborted the egg cases several days after oviposition. Four females partly retracted the egg cases so that some of the eggs remained protruding from the abdomen; three dropped the egg cases while or after they were formed without retracting them. In most virgin females (including those that retract the oötheca normally) some mature oöcytes remain in the ovaries.

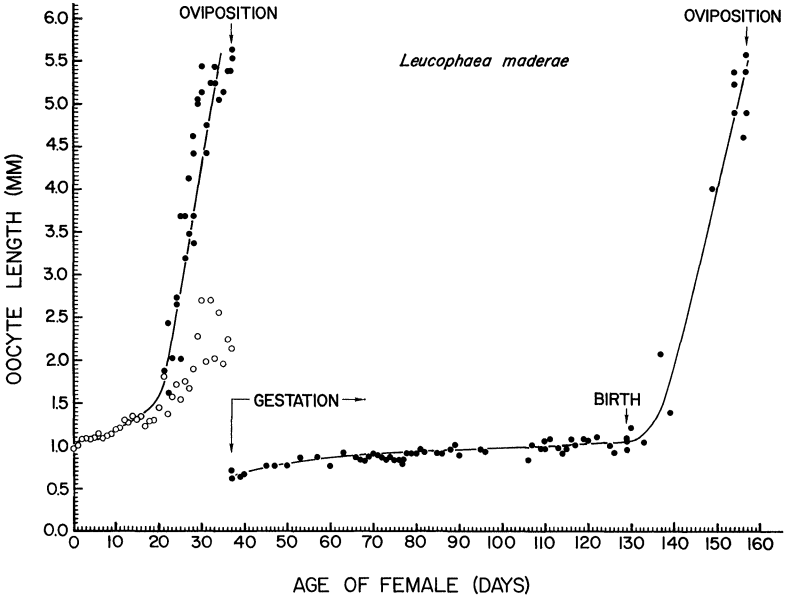


Fig. 7. Ovarian cycle (first and second ovipositions) in *Leucophaea maderae*. The points for the oöcytes of unmated females (open circles) are means of 5 to 21 individuals (N=349). For the first preoviposition period, females were mated (solid circles) when 16 days old and each point represents 1 individual. Each point for the gestation and post parturition periods represents one individual.

Leucophaea maderae: At emergence the oöcytes of *Leucophaea* are 0.97±0.01 mm. (N=10). Mature oöcytes at ovulation are 5.56± 0.12 mm. (N=20: 10 mated and 10 virgin females). The new basal oöcyte at oviposition is 0.66±0.01 mm. (N=11) and at parturition 1.05±0.01 mm. (N=4). Under our conditions gestation lasted 91.8

±0.7 days (N=35) and the second oviposition occurred 27.8±0.3 days (N=10) after parturition.

The ovarian cycle in this species is shown in figure 7. Mating shortens the egg maturation period so that the female ovulates at a more or less definite time (fig. 3) after copulation (Engelmann, 1960). It is almost impossible to predict what the extent of oöcyte development would be in virgins of known age (fig. 8). Only 25 of 381 mated females failed to oviposit. These were 16 to 40 days old when mated and were dissected 30 to 62 days later. Eighteen had large degenerating oöcytes; 5 had small (1.01 to 1.73 mm.) and 2 had large but normal appearing oöcytes. As Engelmann found mating results in the rapid growth of the oöcytes (fig. 7). Of the large number of virgin females dissected (fig. 8) only 2 had oöcytes that were degenerating.

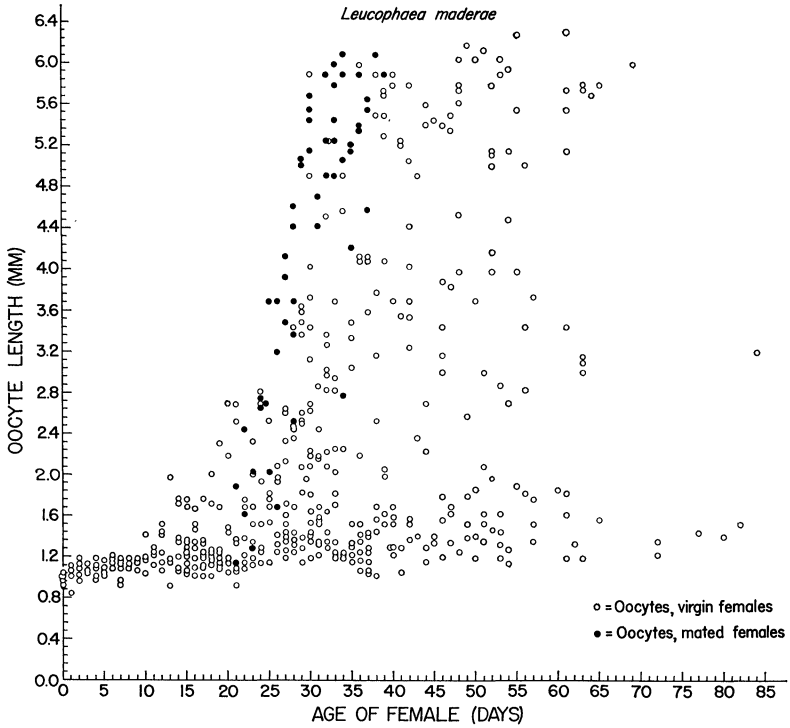


Fig. 8. Growth of oöcytes in mated and virgin females of *Leucophaea maderae*. Each point represents one female. Females were mated when 17 to 23 days old.

Of 47 virgin females that oviposited, only 11 retracted the oötheca into the uterus; the others dropped the egg cases while they were being formed. Virgin females frequently retain mature oöcytes in their ovaries and the egg cases are incomplete (Engelmann, 1957a).

Engelmann (1960) found that when females of *Leucophaea* had constant access to males, mating occurred when their oöcytes averaged 1.08±0.01 mm. and none mated that had oöcytes exceeding a size of 1.46 mm. He concluded (1960, 1960a) that the corpus allatum hormone must be present in low titer for mating to occur, and as soon as a certain titer is surpassed, the females did not mate even with ready access to males. We exposed females of various ages to males for relatively brief periods (the longest time females were with males was 2 days), and measured the oöcytes of those that did and did not mate. One hundred and fifteen females between 14 and 52 days of age were mated and their oöcytes varied in size as follows:

1.08 mm. to 1.95 mm. ($\bar{x}$=1.43±0.02, N=83)
2.00 mm. to 2.97 mm. ($\bar{x}$=2.34±0.06, N=21)
3.11 mm. to 3.72 mm. ($\bar{x}$=3.30±0.08, N= 9)
4.90 mm. to 5.88 mm. ($\bar{x}$=5.39±0.49, N= 2)

A breakdown of the data into two age groups when mated was as follows:

Size of oöcytes (mm.) when mated	Age (days) when mated and number mated	
	14-25	26-52
1.08 - 1.95	50	33
2.00 - 2.97	8	13
3.11 - 3.72	0	9
4.90 - 5.88	0	2

As pointed out earlier our measurements are larger than Engelmann's because in our experiments the oöcytes were dissected and measured in Ringer's solution whereas he measured fixed oöcytes. The majority of the females mated when their oöcytes averaged 1.43±0.02 mm. This value probably corresponds to Engelmann's mean of 1.08 ±0.01 mm. However, 28% of the females mated when their oöcytes were more developed. Thirty-six females that failed to mate when exposed to males along with the above females that copulated had oöcytes that ranged from 1.01 to 1.68 mm. ($\bar{x}$=1.19±0.02 mm.; N=25) and 2.05 to 5.88 mm. ($\bar{x}$=4.54±0.38 mm.; N=11).

There was a slight but not very significant shortening of the inter-

val between age at mating and age at ovulation when older females mated (Engelmann, 1960). Engelmann suggested that this shortening of the period needed for egg maturation could be explained by the presence of larger amounts of reserve substances that would allow for more rapid growth of the eggs and might not be due to the presence of larger oöcytes at the later mating. Our findings confirm Engelmann's in that *Leucophaea* tend to mate more readily when their oöcytes reach a certain size. However, some females mate even though their oöcytes have grown beyond this critical size and the shortening of the period between mating and ovulation is undoubtedly due to the presence of large oöcytes in these older females; some females mate even when there is a high titer of corpus allatum hormone (as indicated by large oöcytes).

Engelmann (1960) found that when the nerve cord of *Leucophaea* was severed 0 to 2 days after mating, oöcyte maturation occurred about a week later than normal mated females. When the cord was severed 3 to 19 days after mating, the females oviposited at the same age as normal mated females indicating that an intact nerve cord is necessary for at least 2 days after mating for the mating stimulus to be effective. When the nerve cords of virgin females were severed and they were not mated, ovulation occurred at the same time as females that had their nerve cords severed 0 to 2 days after mating. Engelmann concluded that severance of the ventral nerve cord in virgins either stimulates the corpora allata or cuts off an inhibitory center for the corpora allata but he favored the latter hypothesis.

We severed the nerve cords of females *prior* to mating them and found that in most cases the spermatophore was not inserted properly. Of 27 females that mated after their nerve cords were severed, only 8 had spermatophores that were apparently transferred by the male normally. Four females had spermatophores that were visible in the genital region but they had not been inserted properly in the bursa. In one mating the spermatophore was dropped by the male without being transferred to the female. Fourteen females had no spermatophores after mating and originally it was believed that none had been transferred by the male. However, it was discovered that in some females the male pierced the wall of the uterus and inserted the spermatophore in the body cavity near the right ovary (fig. 13C). This was found in 7 females but may have occurred in 6 others that apparently had no spermatophore inserted but were not dissected because we did not realize that the spermatophore could be inserted into the body cavity. One female had no spermatophore after mating, based on dissection. It seems that the female takes an active role in the

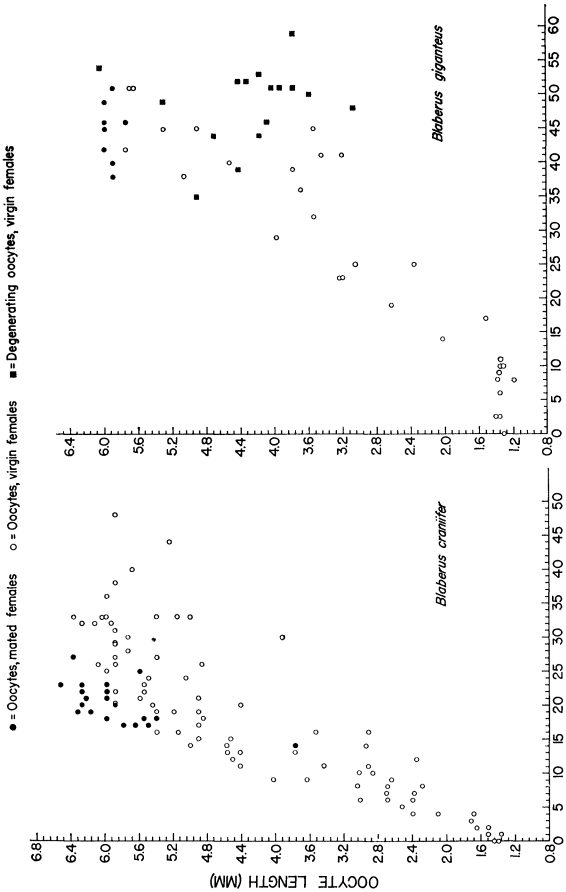


Fig. 9. Oöcyte development in mated and virgin females of *Blaberus*. Each point represents one individual. Females of *B. craniifer* were mated when 1 to 16 days old. Females of *B. giganteus* were mated at 19 to 35 days of age and all were ovipositing when their oöcytes were measured.

proper positioning of the spermatophore in the bursa copulatrix, and an intact nerve cord is needed for proper muscular movements of the female genitalia. Of 11 nerve-cord-severed virgin females that oviposited 7 dropped their egg cases when they were formed and 4 retracted the oötheca into the uterus but aborted some time later.

Blaberus craniifer: The growth of the oöcytes of virgins is rapid but mating affords sufficient additional stimulation (fig. 9) so that ovulation occurs about a week earlier than in unmated individuals (fig. 4). Eighteen mated females oviposited in 19 to 29 days ($\bar{x}$= 23.9±0.6 days); sixteen oviposited normally, 1 dropped its oötheca and 1 failed to retract the egg case completely. Virgin females oviposited in 18 to 41 days ($\bar{x}$=32.0±0.8; N=36). Stimulation from mating results in either an additional production of gonadotropic hormone or it may possibly serve as an oviposition stimulus. The relationship of age at mating and age at oviposition of the female is similar to that found in *Byrsotria* (fig. 3). The older the female when mated the shorter the interval to ovulation indicating that the oöcytes of these older females are large when mating occurs.

Of 40 virgin females that oviposited 23 (58%) failed to retract the egg case completely and some of the eggs protruded beyond the abdomen. This may be due to the fact that in some females the eggs are not aligned properly in the oötheca and may even be arranged in 3 rows (rather than 2) which may make it difficult to retract the egg case completely into the uterus. Generally, in most virgin females that ovulate, the proper amount of colleterial gland secretion does not flow out over the eggs since the accessory glands are usually quite full even after the egg case is formed. Sometimes not all of the eggs are laid and mature oöcytes remain in the ovaries and are eventually resorbed. Thirteen females retracted the oötheca normally into the uterus. Four females dropped the oötheca although some eggs remained in the uterus. Perhaps this is related to the lack of proper amount of colleterial gland secretion being poured out around the eggs; the result may be the formation of a weak oötheca which cannot support the weight of the eggs as they are extruded some distance beyond the end of the abdomen prior to their being retracted. In addition to the above 40 females, 5 unmated females that did not oviposit in 46 to 51 days had oöcytes that had obviously matured (based on size) but were degenerating.

Six allatectomized virgin females that had not ovulated had corpora allata implanted at 62 to 82 days of age; all 6 oviposited within 31 days after implantation. Nine allatectomized virgin females kept for 66 to 238 days failed to ovulate.

All the nerves to the corpora allata were severed in 11 virgin females and the glands were left in the animals; ten oviposited in 21.8±0.49 days which is similar to ovulation in mated females. This would indicate that the brain tends to inhibit the corpora allata in virgin *B. craniifer* and mating overcomes this inhibition.

Blaberus giganteus: The oöcytes of virgins grow and yolk is deposited but after about a month they may degenerate unless mating occurs (fig. 9). In general mating appears to be necessary for completion of oöcyte development, at least more so than in *B. craniifer*. Fourteen females kept with males until mating occurred, mated at 8 to 35 days of age and oviposited when 35 to 51 days old ($\bar{x}$=42.6 ±1.3 days). Of 8 virgin females, not shown in figure 8, kept for 51 to 68 days, only 2 oviposited when 51 days old, and in both individuals the oöthecae were dropped and not retracted; the 6 females that did not oviposit had small abnormally shaped oöcytes that failed to develop.

The relationship between age when mated and age at ovulation (fig. 3) appears to be similar to *Byrsotria* and *B. craniifer* rather than *Leucophaea.* The females of *B. giganteus* which have continuous access to males, mate over a rather wide age range, and their oöcytes may vary considerably in size at the time of mating.

Thirteen of 14 virgin females that had all the nerves to the corpora allata severed at 0 to 19 days of age ovulated in 35.1±1.2 days after the operations; one oviposited 153 days after the operation at 163 days of age. Severing the connectives to the corpora allata apparently removed the inhibition from the brain.

The effects of mating vary in degree among the species of cockroaches that incubate their eggs internally or carry them externally during the incubation period. In the summary given below, data from Engelmann, (1957, 1959, 1960), Roth and Willis (1961) Roth and Stay (1961, 1962), and the present study have been used.

I. Effect of mating on oöcyte development.
   1. Oöcytes of virgins may degenerate:
      a. before reaching ovulation size *(Nauphoeta cinerea* and *Blaberus giganteus)*
      b. before or after reaching ovulation size *(Byrsotria fumigata)*

   Mating prevents degeneration of the oöcytes in the above 3 species. The oöcytes of virgins generally do not degenerate in *Blaberus craniifer* (rarely), *Diploptera punctata, Leucophaea maderae, Pycnoscelus surinamensis* (parthenogenetic and bisexual strains), *Blattella germanica,* and *Blattella vaga.*

2. Mating increases rate of oöcyte development so that the first preoviposition period is less than in virgin females. Preoviposition period shortened on an average of about:
   a. 1 day *(Pycnoscelus surinamensis,* bisexual strain; *Blattella).*
   b. 9 or more days *(Blaberus craniifer, Blaberus giganteus).*
   c. 17 days *(Nauphoeta cinerea).*
   d. 30 or more days *(Leucophaea maderae).*
   e. Majority of virgins do not oviposit for months or not at all. Oviposition occurs about 10 days after mating *(Diploptera punctata).*
3. Mating apparently has little effect on the rate of oöcyte development but may stimulate oviposition *(Byrsotria fumigata).*
4. Mating has no effect on rate of oöcyte development or on the length of the preoviposition period *(Pycnoscelus surinamensis* — parthenogenetic strain mated to males of the bisexual form).

II. Effect of mating on ovulation and oviposition.

1. Ovulating virgins frequently retain mature oöcytes in some part of the reproductive tract so that not all of the eggs are laid *(Blaberus craniifer, Blattella vaga, Byrstotria fumigata, Leucophaea maderae, Nauphoeta cinerea, Pynoscelus surinamensis* bisexual strain).

Mated females usually oviposit all of the mature oöcytes.

2. Oötheca is incompletely formed and oviposition is abnormal in a large percentage of virgins.
   a. Oötheca usually dropped when formed *(Leucophaea maderae, Pycnoscelus surinamensis* [bisexual strain], *Blattella vaga).*
   b. Oötheca dropped or partly retracted into the uterus *(Nauphoeta cinerea).*
   c. Oötheca usually only partly retracted into the uterus so that some of the eggs protrude from the end of the abdomen *(Blaberus craniifer).*

Mating in a large percentage of females results in normal formation of the oötheca and complete retraction of the egg case into the uterus in the above species.

3. Oötheca may be retracted normally into the uterus in a high percentage of virgins *(Byrsotria fumigata, Diploptera punctata,* and *Nauphoeta cinerea).*
4. Mating has no effect on normal oviposition (*Pycnoscelus*

surinamensis — parthenogenetic strain mated to males of the bisexual form).

From the preceding summary one finds two extremes of dependence upon mating for stimulation of the corpora allata. In *Diploptera,* the majority of females require mating for maturation of the oöcytes and its effect is the most striking since ovulation occurs about 10 days after mating, whereas virgin females may go for months without ovipositing or they may never do so (Engelmann, 1959, 1960; Roth and Stay, 1961). At the other extreme is the parthenogenetic strain of *Pycnoscelus surinamensis* where mating is unnecessary and the oöcytes mature in virgins about 13 days after emergence. In this species some newly-emerged females already may have yolk in their oöcytes. Between these two extremes are species which show varying degrees of dependence on external mating stimuli for overcoming inhibition of the corpora allata. The oöcytes in virgins grow but unless mating occurs the ovarian eggs do not mature and may degenerate before reaching ovulation size. This is particularly true in *Nauphoeta, Byrsotria,* and *Blaberus giganteus* and apparently in these species the corpora allata in many virgin females secrete an insufficient amount of hormone for the oöcytes to mature; and in many of these females the partly developed oöcytes are not maintained but degenerate unless the corpora allata are stimulated by mating.

Various species show different degrees of dependence on mating for normal formation and retraction of the oötheca into the uterus. This is of particular interest, for the ability of virgin females to place the oötheca in the brood sac is a prerequisite to the evolution of parthenogenesis in false ovoviviparous cockroaches (Roth and Willis, 1961). Not all females of a species behave similarly which explains why some forms are included in more than one category in the above summary. It is this variation in behavior which may make possible the evolution of parthenogenesis in bisexual species of cockroaches. From the few species studied one can arrange the forms in a series to show the gradual evolution of retraction of the oötheca into the uterus in virgin females, although we do not imply that one gave rise to the other. Almost invariably in the bisexual strain of *Pycnoscelus surinamensis* the oötheca is dropped at formation in virgins. In *Nauphoeta* the oötheca is dropped at formation, partly retracted, or completely retracted. In *Blaberus craniifer* the oötheca is usually only partly retracted into the uterus. In *Byrsotria* the oötheca of virgins that ovulate is usually normally retracted into the brood sac. Although parthenogenesis is uncommon in false ovoviviparous cockroaches (other than the parthenogenetic strain of *Pycnoscelus*) it does occur

rarely. Nine females of *Nauphoeta cinerea* had eggs that developed parthenogenetically and in 8 individuals the eggs hatched; two unmated females that developed from unfertilized eggs gave birth to 3 nymphs (Roth and Willis, 1956). We have encountered only one case of parthenogenesis in *Leucophaea* (20 undeveloped eggs and 5 well developed embryos with pigmented eyes in an oötheca 89 days after ovulation) and one in *Byrsotria* (2 well developed embryos, 55 days after oviposition); Barth (personal communication) has reared a single adult female of *Byrsotria* that was produced parthenogenetically. Only one unmated female of the bisexual strain of *Pycnoscelus* was found that had a developed embryo in one of the eggs of the oötheca (Roth and Willis, 1961). Parthenogenesis in false ovoviviparous cockroaches depends upon (1) the ability of virgin females to mature their oöcytes, ovulate, and form and retract the oötheca into the uterus, and (2) the capacity for unfertilized eggs to develop. Although parthenogenesis cannot occur unless the above requirements are met, the insects must first be capable of retracting the oötheca into the uterus for unless this occurs the eggs desiccate since the oötheca does not prevent water loss in cockroaches that incubate their eggs internally (Roth and Willis, 1955).

No experiments were performed on the species, in this study, to determine the mechanism of stimulation during mating. However, in *Leucophaea* (Engelmann, 1960) and *Diploptera* (Engelmann, 1959, 1960; Roth and Stay, 1961) it is a mechanical stimulus that activates the corpora allata and it is probably similar in *Pycnoscelus* (bisexual strain), *Nauphoeta,* and *Blaberus.*

Food intake and maturation of the oöcytes

Food intake stimulates maturation of the oöcytes in *Leucophaea* (Scharrer, 1946; Johansson, 1955; Engelmann, 1960) and *Blattella germanica* and *B. vaga* (Roth and Stay, 1962) but is unnecessary for oöcyte development in *Diploptera* (Engelmann, 1960; Roth and Stay, 1961). The effect of starvation on oöcyte development in several species used in this study was determined; all females were isolated from food at emergence.

Pycnoscelus surinamensis: Fifteen females of the parthenogenetic strain were starved without water. All oviposited in 14.1 ± 0.4 days, which was about 1 day more than in unstarved females (table 1). Nine virgin females of the bisexual strain starved without water oviposited in 14.3 ± 0.5 days, which was similar to unstarved individuals (cf. table 1). Food is unnecessary in both strains to activate the corpora allata or for maturation of the eggs for the first ovulation.

Nauphoeta cinerea: The effect of starvation on oöcyte development in *Nauphoeta* is shown in figure 6. The oöcytes of starved virgin females develop more slowly and to a lesser degree than those of fed virgin females. When starved females were mated the oöcytes matured in the same period as fed mated females. In *Leucophaea,* females that were starved but mated failed to deposit yolk in the oöcytes and Engelmann (1960) concluded that the brain properly integrated the different afferent stimuli (inhibitory during starvation and activating from mating) into messages to the corpora allata and the endocrines were not activated. *Nauphoeta* differs from *Leucophaea* in that the oöcytes of starved females become well developed and mating adds sufficient stimulation to the corpora allata for the oöcytes to mature normally in spite of the absence of food.

Blaberus craniifer: Seven virgin females of *Blaberus craniifer* were starved (with water) for 22 to 39 days. In 6 females the oöcytes measured 4.82±0.19 mm.; one female had oöcytes that did not develop (1.38 mm.). The oöcytes of fed females 22-38 days of age were 5.61±0.11. Although there may be a slightly slower rate of growth of the oöcytes in starved females, food is unnecessary for initiating activity of the corpora allata.

Byrsotria fumigata: Twenty-four virgin *Byrsotria* were starved (with water) for 20 to 45 days. Six females starved 20 to 24 days had oöcytes 4.22±0.35 mm. long. Eight starved 29 to 40 days had oöcytes 4.86±0.36 mm. in length (several had oöcytes that had begun to degenerate). Two females had small undeveloped oöcytes (0.88 ±0.01 mm.) and 8 had small, round, abnormally shaped oöcytes. Thirty-three virgin females were starved without water for 26 to 50 days. Nine (starved 26 to 43 days) had oöcytes 5.01±0.30 mm. long. Twelve had large oöcytes that were degenerating. Five females oviposited in 34 to 38 days; four had undeveloped oöcytes (1.14±0.08 mm.) and 3 had small abnormally shaped oöcytes. The oöcytes of virgin females fed for 20 to 24 days were 4.85 ±0.32 (N=17) and for 29 to 40 days, 6.36±0.22 (N=39). Although the oöcytes of starved females may not grow quite as rapidly as unstarved individuals, neither food nor water are necessary for growth of the oöcytes in *Byrsotria.*

The degree to which cockroaches depend upon food intake for stimulation of the corpora allata varies among the species. The forms may be arranged in a series showing complete dependence to complete independence upon food for oöcyte development. The effects of starvation may be summarized as follows:

1. Oöcytes do not develop *(Leucophaea, Blattella germanica,* and *Blattella vaga).*

2. Oöcytes develop but at a slower rate and to a lesser degree than in fed females *(Nauphoeta)*.
3. Oöcytes develop at a normal or slightly slower rate than fed females *(Blaberus craniifer, Byrsotria)*.
4. Oöcytes mature about as rapidly as fed females (*Diploptera, Pycnoscelus surinamensis* — bisexual and parthenogenetic strains).

Inhibition of the corpora allata during pregnancy

During the first gestation the basal oöcytes, in all of the species investigated in this study, usually remain undeveloped except for a small increase in length; some *Nauphoeta* females may have oöcytes containing yolk at parturition. Yolk deposition occurs in these basal oöcytes only after parturition (except in *Diploptera* and some *Nauphoeta*). This has already been pointed out in *Pycnoscelus* (fig. 1). Very similar cycles occur in *Blattella* (Roth and Stay, 1962), *Leucophaea* (Engelmann, 1957) and *Diploptera* (Engelmann, 1959; Roth and Stay, 1961). However in *Diploptera* the oöcytes begin to show deposition of yolk about 3 days before parturition (Engelmann, 1959; Roth and Stay, 1961). Although complete ovulation cycles are not given for *Blaberus, Byrsotria,* and *Nauphoeta,* measurements of the new oöcytes at ovulation, and at parturition show that inhibition of the corpora allata during gestation also occurs in these forms. In *Leucophaea* (Engelmann, 1957, 1960), *Diploptera* (Engelmann, 1959; Roth and Stay, 1961), and *Pycnoscelus* (Roth and Stay, 1959) removal of the oötheca results in resumption of growth of the oöcytes prematurely, indicating that the oötheca in the uterus, in some manner, inhibits the activity of the corpora allata. The following experiments were performed to investigate the nature of inhibition of the corpora allata during gestation.

Pycnoscelus surinamensis: The oöthecae were removed from 84 females of the parthenogenetic strain, 62 (74%) of which subsequently ovulated. Of the 22 females that failed to oviposit 25 to 37 days after the operation, 15 had oöcytes that showed essentially no development (0.64 to 0.79 mm.) and 7 had oöcytes with definite yolk deposits (0.84 to 2.39 mm.); one female had oöcytes that had apparently matured but had not been laid and were being resorbed. There is an inverse relationship between the age of the oötheca at the time it is removed from the uterus and the time required to ovulate again. Less time is required to ovulate again, the older the uterine eggs are when removed (fig. 10). This relationship also has been found in *Blattella* and *Diploptera* (Roth and Stay, 1961, 1962). One

of the factors which might account for this may be that the oöcytes increase in size during gestation so that at the time an older oötheca is removed the oöcytes are larger when again subjected to gonadotropic hormone. The larger oöcytes may contain greater amounts of reserve substances allowing for a more rapid maturation of the eggs.

Since the period between the first and second ovulations is about 70 days (table 1) it is evident from figure 10, showing the relatively

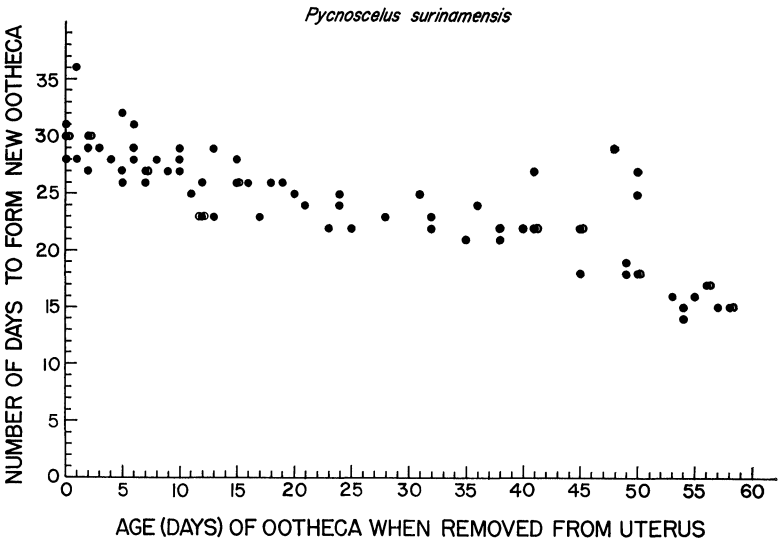


Fig. 10. Relationship between the age of the oötheca at the time it was removed from the uterus of *Pycnoscelus surinamensis* (parthenogenetic strain) and the time required to form a new oötheca. Each point represents one individual. The points at 53 to 58 days on the x axis, are for females that gave birth normally; all of the other points are based on females that had their oöthecae removed manually.

rapid development of the oöcytes (as indicated by oviposition) after removal of the oötheca, that the oöcytes are inhibited by the presence of the eggs in the uterus.

Virgin females of the bisexual strain of *Pycnoscelus* almost invariably fail to retract their oöthecae into the uterus (Roth and Willis, 1961). Fourteen virgin females that had dropped their oöthecae when they were formed, oviposited again in 28 to 39 days ($\bar{x}=32.9\pm1.1$ days). The normal interval between the first and second ovulations in this strain is about 67 days (53 days of gestation plus 14 days postparturition, table 1) and the absence of uterine eggs in the brood sac resulting from aberrant oviposition hastened the development of

the oöcytes. Three mated bisexual form females that failed to retract their oöthecae also formed the second egg case in 30.3±2.7 days. Three mated females that had their oöthecae removed 2, 5, and 7 days after oviposition ovulated again in 32 to 33 days. Both strains of *Pycnoscelus* are similar in that the presence of an oötheca in the uterus inhibits the development of the oöcytes.

Leucophaea maderae: The oöthecae were removed from 102 pregnant females at different stages in pregnancy to determine the time required for the next ovulation. Forty-three females oviposited during the period of the experiment and these results are plotted in figure 11. As in *Pycnoscelus* the time required to ovulate after removal of the oötheca varied with the age of the oötheca when it was removed; the younger the oötheca the longer it took to mature the oöcytes. Of the remaining females, 45 showed little or no growth of the oöcytes; those whose oöthecae were removed 0 to 18 days after ovulation had oöcytes 1.06±0.03 mm. (N=22), 62 to 82 days later and 23 females whose

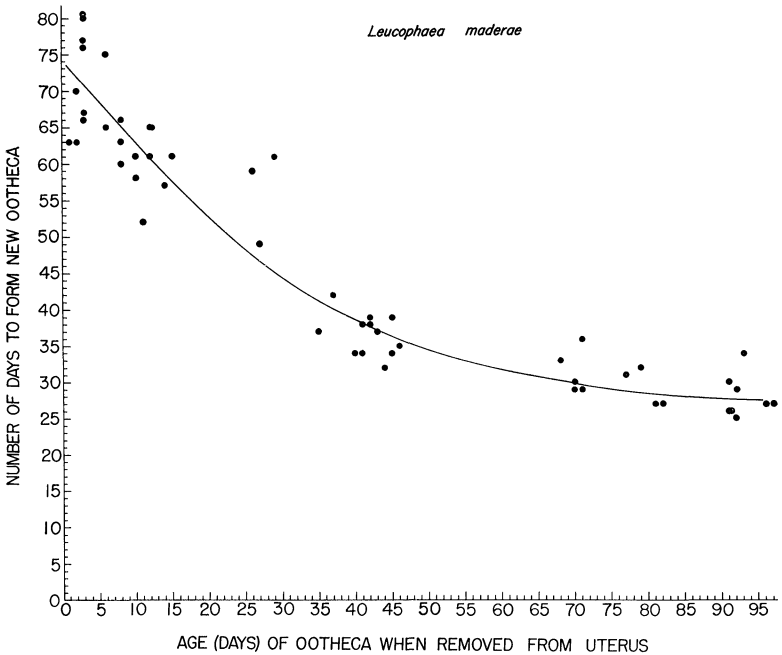


Fig. 11. Relationship between the age of the oötheca at the time it was removed from the uterus of *Leucophaea maderae* and the time required to form a new egg case. The points plotted at 82 to 97 days on the x axis are for females that gave birth normally; all of the other females had their oöthecae removed manually. Each point represents one female.

oöthecae were removed 23 to 76 days after oviposition had oöcytes 1.19 ±0.04 mm., 34 to 63 days later. Fourteen females that had their oöthecae removed 0 to 77 days after ovulation had developed oöcytes 3.72±0.30 mm. long, 34 to 71 days later. It is unknown why about 45% of the females failed to show oöcyte development after removal of the oötheca; the presence of degenerating oöcytes that were not laid in the first ovulation may account for some of these cases.

Byrsotria fumigata: In *Byrsotria* gestation lasts from 71 to 82 days ($\bar{x}$=76.2±1.4; N=6). The basal oöcytes at parturition vary in length from 1.43 mm. to 1.71 mm. ($\bar{x}$=1.53±0.04 mm.; N=10). The second ovulation occurs 21 to 30 days ($\bar{x}$=24.8±1.6; N=5) after parturition. Oöcyte development during pregnancy in mated females is inhibited and no yolk is deposited until after the young are born.

Five mated females had their oöthecae removed at various periods during pregnancy. One whose oötheca was removed 27 days after ovulation oviposited 45 days later. Two females whose oöthecae were removed 28 and 40 days after oviposition had practically mature oöcytes, 5.98 mm. and 6.22 mm. (fig. 14B) respectively, 32 days later. The oöcytes (1.23 and 1.29 mm. long) of two females whose oöthecae were removed on the first and thirty-first day of pregnancy failed to develop when examined after 75 and 32 days. The oöthecae of 10 virgin females were also removed with the following results. Three females whose oöthecae were removed 29 to 34 days after ovulation had mature oöcytes that were being resorbed 53 days later. One female whose oötheca was removed 38 days after oviposition ovulated again 39 days later. Six females whose oöthecae were removed from 1 to 24 days after oviposition failed to develop their oöcytes ($\bar{x}$=1.53 ±0.05 mm.) when examined 35 to 59 days after the operations. In the mated and virgin females that failed to develop oöcytes after removal of the oöthecae, several unlaid degenerating oöcytes were present from the previous ovulation which may account for the results.

Virgin females of *Byrsotria* that deposit their unfertilized eggs normally in the brood sac frequently carry these oöthecae for a longer period of time than mated females. When the undeveloped eggs are finally extruded the ovarian oöcytes may be large and contain considerable yolk in spite of the fact that an oötheca was present in the uterus during the entire "pregnancy" period. Thirteen females that carried their unfertilized eggs for 71 to 90 days had oöcytes 1.51± 0.04 mm. which is normal for the size of the oöcytes at parturition of mated females. However, the oöcytes of 14 virgins that had carried their oöthecae for 87 to 97 days had oöcytes that varied in length from

2.86 to 6.12 mm. ($\bar{x}$=4.60±0.23 mm.). One mated female that aborted an oötheca with undeveloped eggs after carrying for 79 days had oöcytes 3.72 mm. long. It is apparent that toward the end of the "gestation" period in virgin females or once the time at which parturition should normally take place is passed, the inhibition of the corpora allata (due to the presence of the oötheca in the uterus) breaks down and these endocrines again secrete the gonadotropic hormone. Eleven virgin females that aborted their oöthecae 91 to 104 days after depositing, were kept until they ovulated a second time. Five of the females oviposited in 21 to 30 days ($\bar{x}$=25.6±1.6) which is the same as mated females indicating that their oöcytes at the time of aborting were relatively undeveloped. The other 6 virgin females ovulated in 11 to 18 days ($\bar{x}$=14.8±1.4) undoubtedly because their oöcytes were already well developed when the egg cases containing undeveloped eggs were extruded from the uterus.

Blaberus craniifer: The oöcytes of this species at emergence are about 1.39 to 1.44 mm. (N=2) in length. The mature oöcytes are about 6.12 to 6.37 mm. (N=3) and at oviposition the new basal oöcytes vary from 1.02 to 1.16 mm. ($\bar{x}$=1.09±0.03; N=7). At parturition the oöcytes are 1.34 to 1.85 mm. ($\bar{x}$=1.56±0.07; N=6). Gestation lasts 73 to 87 days ($\bar{x}$=79.2±2.4; N=5). After birth, a second ovulation occurs in 16 to 27 days ($\bar{x}$=22.0±1.9; N=5).

Six virgin females had their oöthecae removed on the day of oviposition. One oviposited again 47 days later. The others were dissected 44 to 60 days later and all had well-developed oöcytes (3.96± 0.43 mm.). One female whose oötheca was removed 8 days after ovulation had oöcytes 5.88 mm. long, 54 days later. Two females whose oöthecae were removed 73 days after oviposition (i.e. close to parturition in mated females) had oöcytes 3.23 mm. and 3.82 mm. long, only 10 days later. Removal of the oötheca in *B. craniifer* results in renewed development of the oöcytes.

The principal evidence for Engelmann's (1957) hypothesis that a hormonal factor from uterine eggs inhibits the corpora allata via the brain was his claim that implantation of uterine eggs into the abdomen of females of *Leucophaea* inhibited oöcyte development, and nerve cord severance of pregnant females only had a slight but temporary effect on growth of the oöcytes. However, more recently, Engelmann (1960) found that severance of the nerve cord in pregnant females results in growth of the oöcytes indicating that nervous stimuli may also be responsible for inhibition of the corpora allata during pregnancy.

We have repeated these and performed additional experiments on the following species of cockroaches:

Pycnoscelus surinamensis (parthenogenetic strain): Some of the experiments on this species were briefly described elsewhere (Roth and Stay, 1959). The oötheca was removed from the uterus of each of 10 females 1 to 16 days after oviposition and one-half of each oötheca was implanted into the body cavity of the donor female. Twenty-three days after the operation the oöcytes ranged in length from 2.12 mm. to 3.19 mm. ($\bar{x}=2.70\pm0.10$ mm.) clearly larger than the oöcytes of females that have been pregnant for 24 to 39 days which vary from 0.59 to 0.66 mm. Implantation of uterine eggs into the abdomens of females that had their oöthecae removed did not prevent subsequent growth of the oöcytes. Two of the 10 females had oöcytes that had practically matured and the oöcytes of the remaining 8 females were approaching maturity (2.97 to 3.36 mm., cf. table 1) and undoubtedly would have matured in about the time one would expect ovulation following removal of the oötheca (cf. fig. 10). One-half of young oöthecae were implanted into the body cavities of 6 females one day old or less; after 11 days the oöcytes were 2.65 to 3.14 mm. ($\bar{x}=2.91\pm0.10$) in length. The oöcytes of untreated 11-day old females averaged 2.93 ± 0.06 mm. ($N=10$). These results show that uterine eggs when implanted into the abdomen of a recently emerged female have no effect on the initial development of the oöcytes. Nor does implantation of uterine eggs into the abdomen of a female that had her oötheca removed inhibit subsequent development of the oöcytes.

The oöthecae of 20 pregnant females were removed 13 to 25 days after ovulation and a wax "oötheca" about the size and shape of a normal oötheca was inserted into the uterus. Examined 20 to 37 days later all had small oöcytes (fig. 13A) similar in size to those found in females that were pregnant for 36 to 52 days (table 3). However

EXPLANATION OF FIGURE 12

Fig. 12A. *Pycnoscelus surinamensis* (parthenogenetic strain). Oötheca (upper) and ovaries (lower) of a female that had been pregnant 60 days and whose nerve cord was severed on the thirty-second day of pregnancy. When dissected 28 days after the operation, the embryos in the oötheca began to hatch. The oöcytes in the ovary had matured but were being resorbed. (Note the abundant colleterial gland secretion [arrow]). Vertical line=3 mm.

Fig. 12B, 12C. *Blaberus craniifer*. B. Mated female whose nerve cord was severed 26 days after oviposition. The oötheca (0) containing well developed embryos (note pigmented eyes) was being aborted 34 days after the operation. The oöcytes (arrow) were 5.88 mm. long. C. Virgin female that had carried an oötheca with undeveloped eggs for 93 days (well beyond the normal gestation period). The oöcytes were 3.92 mm long. Vertical line=5 mm.

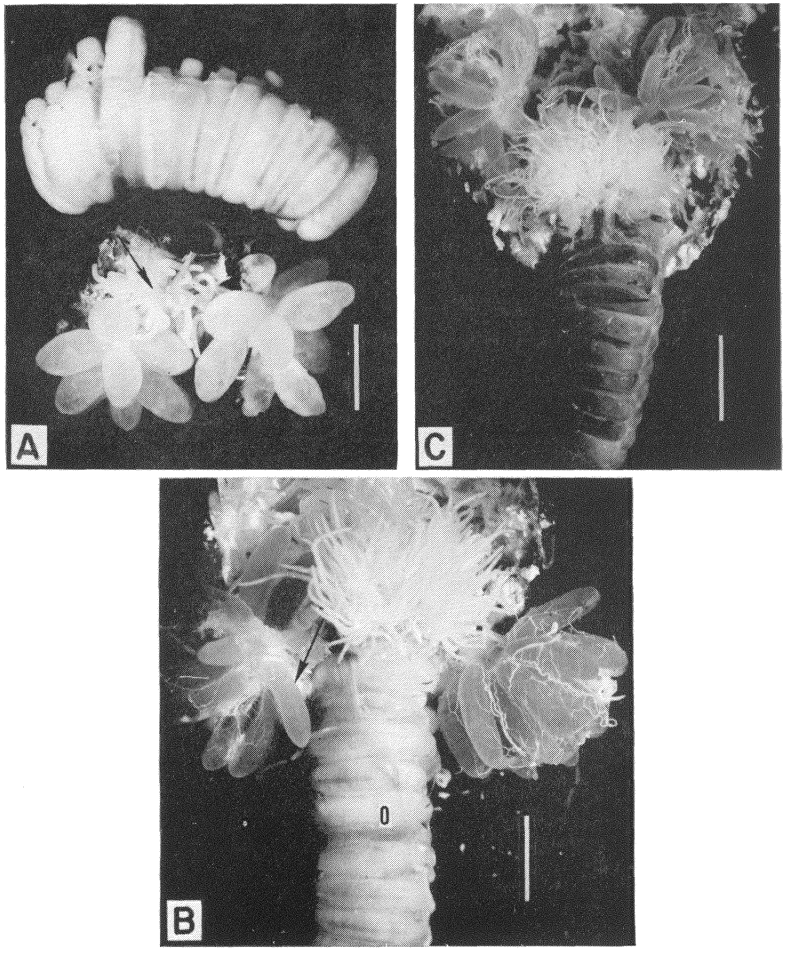

A
C
B
0

Table 3 — Effect of inserting a wax "oötheca" into the uterus and subsequent nerve cord severance on oöcyte development in the parthenogenetic strain of *Pycnoscelus surinamensis*

AGE (DAYS) OF OÖTHECA WHEN REMOVED AND WAX WAS INSERTED INTO UTERUS	DAYS AFTER INSERTION OF WAX, OÖCYTES WERE MEASURED	DAYS ♀ HAD OÖTHECA OR OÖTHECA THEN WAX IN UTERUS	OÖCYTES (MM.) MEAN ± S.E.	N
13-25	20-37	36-52	0.70±0.01	20
Control (oötheca in uterus)	—	36-52	0.71±0.01	17
Nerve cord severed after insertion of wax				
15	32(8)[1]	47	1.09	1
15	33(9)	48	1.18	1
13	37(13)	50	2.40±0.03	3
Control (oötheca in uterus; nerve cord intact)	—	47-50	0.72±0.01	8

[1]Numeral in ( ) = number of days, prior to measuring oöcytes, nerve cord was severed.

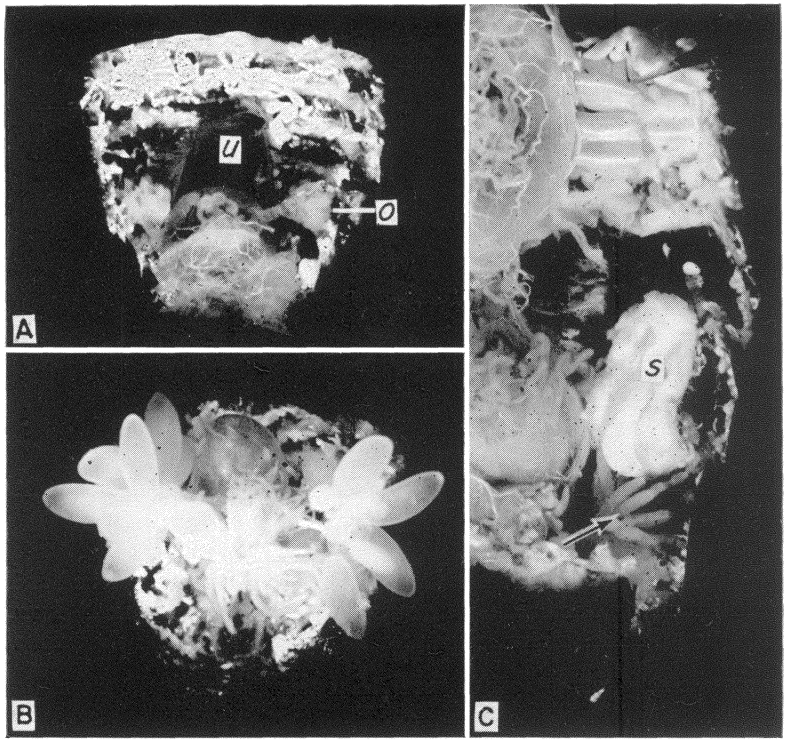


Fig. 13A-B. *Pycnoscelus surinamensis* (parthenogenetic strain). A. Ovaries (0) undeveloped as the result of the presence of a wax "oötheca" in the uterus (u). The oötheca was removed on the thirteenth day of pregnancy, and replaced with wax. The female was dissected 37 days later. B. Oöcytes which developed in a female that had a wax "oötheca" in its uterus for 37 days. The oötheca was removed 13 days after ovulation and replaced by wax. Twenty-four days later the nerve cord was severed and the female was dissected 13 days later.

Fig. 13C. *Leucophaca maderae.* Dissection of a female that mated after her nerve cord had been transected. The spermatophore (s) was inserted by the male into the body cavity near the right ovary (arrow).

Table 4 — Effect of nerve cord severance on oöcyte development in the parthenogenetic strain of *Pycnoscelus surinamensis*

DAYS AFTER OVIPOSITION NERVE CORD WAS SEVERED	DAYS AFTER OPERATION OÖCYTES WERE MEASURED	OÖCYTES (MM.) MEAN ± S.E.	N
<1	24	2.08±0.07	3
4	25	2.94	1
<1	26	2.57±0.30	2
0[1]	29	3.06[2]	1
<1	31	3.52±0.16[2]	2
<1	32	2.94	1
27-32	23-33	Oöcytes matured and degenerating[3]	5
Controls *(sham operated)*			
1-4	25	0.67±0.01	4
<1	29	0.65±0.02	4
1	38	0.66±0.02	5

[1]Operated on just after the female retracted the oötheca.

[2]Oöcytes matured.

[3]The uterine eggs of these females were completely developed and parturition was imminent. The eggs began to hatch (fig. 12A) from 3 of the 5 females after their oöthecae were removed from the uterus.

when the nerve cords were severed in five females that had been carrying a wax "oötheca" in the uterus for 24 days, the oöcytes were well developed (fig. 13B) 8 to 13 days later (table 3).

The effect of nerve cord severance in pregnant females on development of the oöcytes is shown in table 4. The oöcytes could mature (2.97 to 3.36 mm., table 1), in females carrying oöthecae once the nerve cord was severed. The time required for the oöcytes to mature in pregnant nerve-cord-severed females was essentially the same as that taken by females after their oöthecae were manually removed. When removed at 0 days a new oötheca was formed in about 28 to 31 days. When removed after 27 to 32 days of pregnancy ovulation occurred about 22 to 25 days later (fig. 10). The five females that had their nerve cords severed 27 to 32 days after oviposition all had mature oöcytes that were degenerating or being resorbed 23 to 33 days later at the time the uterine eggs were ready to hatch (fig. 12A). Apparently oviposition could or did not occur while an oötheca was

in the brood sac, and the mature oöcytes degenerated. In addition to the fifteen females shown in table 4, two females had their nerve cords severed prior to ovulation and oviposited normally; 24 and 25 days later their oöcytes had grown considerably and were 2.18 mm. and 2.72 mm. respectively. The nerve cord may be severed at any site between the second and sixth segments to eliminate the inhibition of the corpora allata during pregnancy. Two females had their nerve cords severed between the second and third abdominal segments 4 days after oviposition; 29 days later their oöcytes were 2.75±0.01 mm. Six females had their cords severed between the third and fourth, fourth and fifth or fifth and sixth abdominal segments, 4 days after oviposition; 22 days later their oöcytes were 1.89±0.24 mm. long. Six pregnant females taken from cultures (histories unknown) had their nerve cords severed between the fourth and fifth, or fifth and sixth segments; 20 days later their oöcytes measured 2.28±0.33 mm.

Unmated females of the parthenogenetic strain oviposited normally in 98 percent of 248 individuals examined (Roth and Willis, 1961). Twenty-two females had their nerve cords severed prior to oviposition. Of these, 15 (68%) ovulated in the normal period of time and deposited eggs in the uterus; 8 oviposited all their eggs and had normal oöthecae but the other 7 had small abnormally shaped oöthecae and from 1 to 12 mature oöcytes remained in their ovaries. The remaining 7 of the 22 females operated upon failed to retract the oötheca into the brood sac; in 4 of these one or more mature oöcytes remained in the ovaries but in the others all the eggs were laid. Of 9 females that were sham-operated when 1 to 4 days old, all oviposited normally and no mature oöcytes remained in their ovaries. Apparently an intact nerve cord is necessary for normal deposition of mature oöcytes and for normal formation and retraction of the egg case in some females of *P. surinamensis* (parthenogenetic strain). Some center, possibly in the brain, may be involved in this behavior.

Virgin females of the bisexual strain almost invariably fail to retract their oöthecae into the uterus (99% of 138 females, Roth and Willis, 1961) and drop the incompletely formed oötheca. Thirteen virgin females had their nerve cords severed when 1 to 9 days old. All oviposited abnormally, which is the typical behavior of virgins of the bisexual strain; 10 dropped their oöthecae and all had mature oöcytes left in their ovaries. The other 3 carried their oöthecae extruding from the abdomen but failed to retract them; 2 had some mature oöcytes left in the ovaries but the third had none. Virgin females of the bisexual strain with severed nerve cords behaved like unoperated virgin females in oviposition and deposition of mature oöcytes.

In *Blattella* pressure on the oöthecal chamber by the oötheca appears to be responsible for the inhibition of the oöcytes, the stimulus being transmitted via the nerve cord (Roth and Stay, 1959, 1962). When the oötheca is in the uterus of *Pycnoscelus* the ovipositor is bent forward and is held in that position by the egg case. This suggested the possibility that the gonapophyses might be involved in transmitting nervous stimuli to the brain which then inhibits the corpora allata. Two experiments on *Pycnoscelus* were performed to test this hypothesis. Glass beads (3-3.5 mm. in diameter) were inserted into the vestibule of 7 females 1 to 2 days of age. A small drop of ferrule cement on the anal segments prevented the beads from being extruded; the beads exerted pressure on the ovipositor. The oöcytes were measured at 3 different periods. At 5 days of age they were 1.77 ±0.13 mm. (N=4), at 7 days, 2.20 mm. (N=1), and at 13 days, 2.85±0.01 mm. (N=2). Normal females at 5, 7, and 13 days of age had oöcytes 2.03±0.08 mm. (N=9), 2.28±0.08 mm. (N=15), and 2.94±0.04 mm. (N=6) respectively. The presence of a bead and the resulting pressure on the ovipositor of recently-emerged females had essentially no effect on the development of the oöcytes.

To determine if release of pressure by the oötheca on the ovipositor during gestation would result in resumption of oöcyte development, the oöthecae of pregnant females were partly extruded, a portion of the egg case was cut off and the remainder was pushed back into the uterus. This was done to 8 females 11 to 12 days after oviposition and their oöcytes were measured on the fifty-fourth to fifty-sixth days of pregnancy when the females gave birth or parturition was imminent. Five operations were successful in that the ovipositors were

EXPLANATION OF FIGURE 14

Fig. 14. Reproductive organs of *Byrsotria fumigata.*

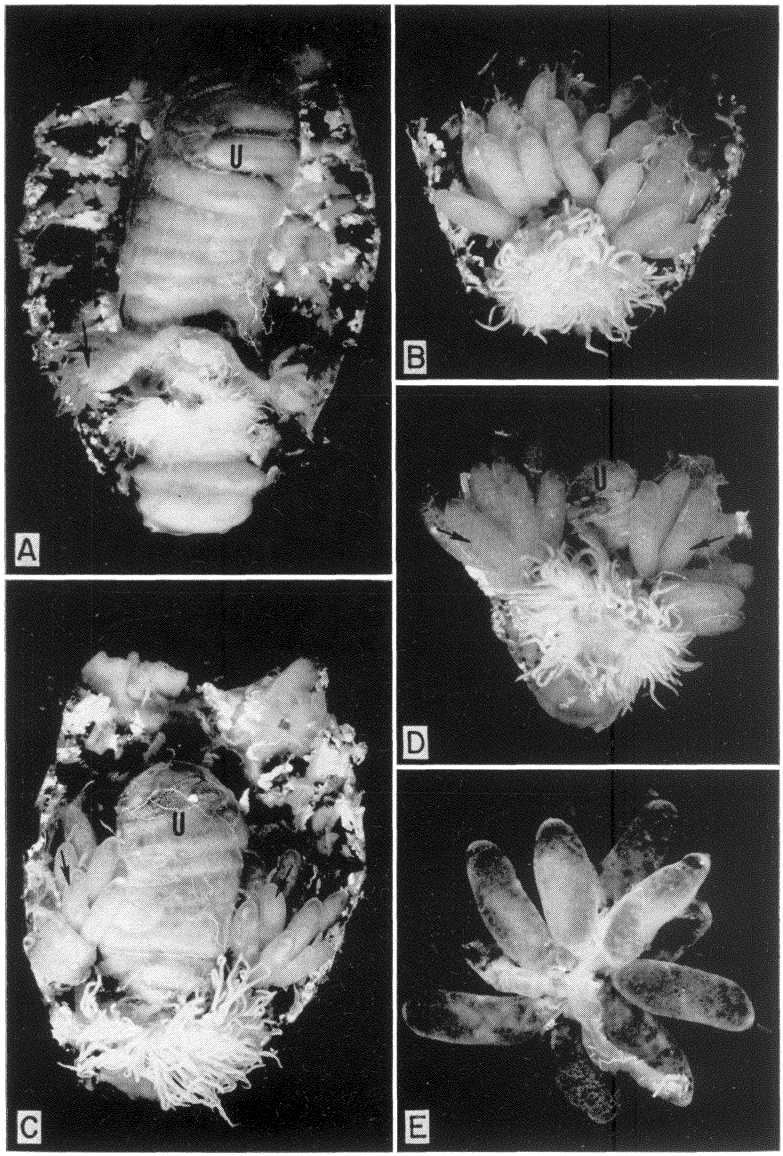
A. Normal mated female sham operated (nerve cord) when pregnant 38 days and dissected after 70 days of pregnancy. The eggs (arrows) in the ovaries are undeveloped; U=uterus containing oötheca.

B. Mated female whose oötheca was removed 40 days after oviposition and dissected 32 days later. The eggs in the ovaries have almost matured (6.22 mm. long).

C. Mated female whose nerve cord was severed at 39 days of pregnancy and dissected 32 days after the operation (71 days pregnant). The eggs (arrows) in the ovaries have nearly matured (5.88 mm. long); U=uterus containing oötheca.

D. Virgin female allatectomized at one day of age. After 52 days, corpora allata from two females 9 to 10 days of age were implanted. Oviposition occurred 28 to 35 days after implantation of corpora allata. The eggs (arrows) in the ovary are almost full grown (5.88 mm.) although an oötheca remains in the uterus (U).

E. Ovary of a virgin female 43 days old. The oöcytes are large and degenerating (A-D=×2; E=×4).


U
A
B
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D
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C
E

Table 5 — Effect on oöcyte development of various implants into the abdomens of virgins of *Byrsotria fumigata*

TYPE OF IMPLANT INSERTED INTO ABDOMEN	AGE (DAYS) OF FEMALE AT OPERATION	AGE (DAYS) WHEN OÖCYTES WERE MEASURED	OÖCYTES (MM.) MEAN ± S.E.	N
Portion of an oötheca of *Byrsotria*	<1	17	3.21±0.28	5[1]
	1	19	3.77	1
	<1-1	20	4.38±0.17	4[1]
	1	23	4.53±0.12	2
Entire oötheca of *Pycnoscelus*	<1	17	3.56±0.02	2
	2	20	4.50±0.20	4
Portion of an oötheca of *Leucophaea*	<1,2	17	3.56±0.02	2
Controls				
Wax "oötheca"	1	17	3.69±0.14	3[1]
	1	20	4.70±0.29	4
Untreated	-	17	3.81±0.23	7
	-	19	3.79±0.50	6
	-	20	4.55±0.35	5
	-	23	4.83±0.34	3

[1]One female had oöcytes that were abnormally shaped and was not included in the measurements.

Table 6 — Effect of severing the nerve cord on development of the oöcytes, in *Byrsotria* females that were carrying oöthecae

DAYS AFTER OVULATION NERVE CORD WAS SEVERED	DAYS AFTER OPERATION OÖCYTES WERE MEASURED	OÖCYTES (MM.) MEAN ± S.E.	N
Virgin Females			
0[1]	56-59	3.69±0.39	4
18	27, 33	4.03±1.11	2
28	33	5.39	1
0[1]	43-62	1.34±0.01	13
21-27	33	1.39±0.07	4
Controls (sham operated)			
0[1]	43-57	1.37±0.02	7
Mated Females			
12, 19	41, 43	6.01±0.02	2
27-28	29-32	3.68±0.48	4
30-36	31-32	5.02±0.40	6
42-44	32	5.50±0.71	3
21-39	30-37	1.39±0.02	5
Controls (sham operated)			
12, 30, 38	32, 43	1.43±0.02	4

[1]The nerve cords of these females were severed prior to oviposition and therefore they may be considered to have had the cords cut when the female was ovipositing.

freed for the period of the experiment. The oöcytes of these females measured 0.78±0.01 mm. In 3 females the remaining portion of the oötheca in the uterus continued to apply pressure on the ovipisitor and their oöcytes measured 0.77±0.05 mm. As controls 6 females were sham operated, i.e. their oöthecae were partly extruded and pushed back, without being cut off, into the uterus 11 to 13 days after ovulation. They all gave birth at 54 to 56 days of age and their oöcytes measured 0.74±0.01 mm. These experiments indicate that relieving the pressure of the oötheca on the gonapophyses during pregnancy had no effect on oöcyte development.

One mated female of the bisexual strain that had oviposited normally failed to give birth in the usual period of time (53 days). It was

dissected after 62 days of pregnancy and the oöcytes were 1.56 mm. long and contained yolk. The uterine eggs were degenerating and were undeveloped but the oöcytes had developed although the egg case had been in the uterus. This failure of endocrine inhibition during "pregnancy" was also found in *Blaberus* and *Byrsotria* (see below).

Byrsotria fumigata: The effect of various implants into the abdominal cavities of virgins is shown in table 5. Portions of egg cases of *Byrsotria* and *Leucophaea* and entire oöthecae of *Pycnoscelus* failed to inhibit the development of the oöcytes in *Byrsotria.*

Severance of the nerve cord in pregnant virgin and mated females resulted in resumption of oöcyte development in some females (cf. figs. 14A and C) although an oötheca was in the uterus (table 6). However, the oöcytes developed only in 7 of 24 virgins as compared to 15 of 20 mated individuals. All of the virgins that failed to develop oöcytes had many degenerating oöcytes that had not been laid during the initial ovulation which may account for the negative results in many of these females. Of the 7 virgin females that developed their oöcytes after nerve cord severance, 5 had no old degenerating oöcytes, one had one old oöcyte and the last had several oöcytes that had remained from the previous oviposition. In addition to the 24 virgin females that had been operated upon after ovulating (table 6), 11 females had their nerve cords severed 7 to 24 days after emergence and 8 others were sham operated when 5 to 20 days old. These females failed to oviposit and were dissected 31 to 38 days after the operations. Of the nerve-cord-severed females 7 had matured degenerating oöcytes and 4 had small oöcytes with some yolk but these had degenerated. Of the sham operated females 5 had mature degenerated oöcytes, 2 had small degenerated oöcytes and 1 had oöcytes that failed to develop. The oöcytes in females that had been operated on prior to oviposition were essentially similar to those found in unoperated virgin females.

Experiments were performed on several females to determine the effect of removing the ovipositors or relieving the pressure of the oötheca on the gonapophyses. The ovipositors were cut off of 9 virgin females 6 to 26 days after oviposition. The oöcytes were measured after the females had carried their oöthecae for 75 to 84 days. In 8 females the oöcytes measured 1.62 ± 0.06 mm. indicating no growth other than might be expected in unoperated females (1.53 ± 0.04 mm. at parturition). One female whose ovipositor was cut off 13 days after ovulation had oöcytes 6.37 mm. after 82 days of pregnancy. The oöthecae of 13 virgin females were partly extruded

manually, part of the egg cases were cut off and the remainder pushed back into the uterus in an attempt to free the pressure normally exerted on the ovipositor. In 6 successful operations the ovipositors were freed 9 to 12 days after ovulation, and at 73 to 75 days of "pregnancy" their oöcytes were 1.47±0.03 mm. Seven females in which the operations (7 to 18 days after ovulation) did not free the ovipositors, had oöcytes 1.49±0.05 mm. after carrying their oöthecae for 71 to 76 days; one female that was unsuccessfully operated upon 8 days after ovulation had oöcytes 3.77 mm. 64 days later. These experiments indicate that removing the ovipositor or releasing the pressure of the oötheca on the ovipositor during the gestation period does not influence the development of the oöcytes. The two individuals in which the oöcytes grew may be explained by the fact that inhibition of the corpora allata in some virgins of *Byrsotria* may break down during gestation.

Blaberus craniifer: Parts of oöthecae (about 5 mm. x 10 mm.) of *B. craniifer* were implanted into the abdomens of 9 virgin females less than 1 to 3 days old (one female had an entire oötheca implanted). Eight females dissected 15 to 30 days later had well developed oöcytes 4.84±0.28 mm.; one female dissected 28 days after the implant showed no growth of oöcytes (1.48 mm. long). The oöcytes of unoperated virgins 15 to 30 days old were 5.10±0.05 mm. The length of mature oöcytes are about 6.25±0.07 mm. (N=3); the new basal oöcyte at ovulation is 1.10±0.03 mm. (N=5). Uterine eggs implanted into the abdomens of virgin females did not inhibit oöcyte development in *B. craniifer.*

Six mated females had their nerve cords severed on the twenty-second to twenty-sixth days of pregnancy and were dissected 34 to 39 days later. Four of these females (operated on the twenty-fifth to twenty-sixth day of pregnancy) had oöcytes 4.09±0.84 mm., 34 to 38 days later (fig. 12 B); two females operated on the twenty-second and twenty-third days of pregnancy showed very little oöcyte development (1.74±0.12 mm.), 34 and 39 days later (the oöcytes at parturition are 1.56±0.07 mm. long; N=6).

Two virgin females of *B. craniifer* carried their oöthecae for 93 and 107 days, which is longer than the normal gestation period (about 79 days) of mated females. When the undeveloped uterine eggs were extruded the oöcytes measured 3.92 mm. and 3.68 mm. respectively (fig. 12C). Inhibition of the corpora allata in *B. craniifer* apparently can break down in the late stage of "pregnancy" in virgin females, as it does in *Byrsotria* and in *Pycnoscelus.*

Blaberus giganteus: Eight pregnant females were taken from cul-

tures (histories unknown), their nerve cords were severed and their oöcytes were measured on the day they gave birth or aborted their oöthecae. Five females gave birth and 3 extruded oöthecae containing well developed embryos in 19 to 33 days after the operations. In every female the oöcytes grew, as a result of nerve severance, and measured 4.24±0.33 mm. At ovulation the mature oöcyte is 5.86±0.04 mm. (N=11) and the new basal oöcyte is 0.97±0.01 mm. (N=6). Normally at parturition the oöcytes are 1.67±0.03 mm. long (N=10). Gestation in this species lasts about 95 to 103 days. Nerve cord severance at least 33 days before parturition eliminated the inhibition of the corpora allata resulting from the presence of the egg case in the uterus.

Leucophaea maderae: The oöthecae of 7 females were removed and part of the egg cases were implanted into the abdomens of the female donors. Five females which had their egg cases removed and implanted 10 to 15 days after oviposition, ovulated 61 to 65 days later. This is about the time one would expect ovulation after removal of the oötheca (fig. 11). One female had oöcytes 2.18 mm. long 65 days after an implant (made 10 days after ovulation). One female whose oötheca was removed and implanted 22 days after ovulation had oöcytes 5.14 mm. long 41 days later. Four females whose oöthecae were removed 14 to 40 days after ovulation and had a wax "oötheca" inserted into the uterus showed no yolk deposition in the oöcytes (1.05±0.05 mm.) 58 to 65 days later.

The implantation of uterine eggs into the abdomens of females did not prevent the oöcytes from maturing. The results with wax "oöthecae" insertions indicate that the corpora allata may be inhibited by pressure of the oötheca in the uterus.

To determine whether there was a hormonal influence on oöcyte development in *Leucophaea,* Engelmann (1957) removed the eggs from the uterus and implanted about one half of the oötheca into the abdominal cavity. He found that the eggs (in the oötheca) still affected the corpora allata when they were implanted into the abdomen (as they did when in the uterus). To rule out any possible effect of a mechanical pressure on the abdomen, or the effect of other substances resulting from decay of tissues (i.e. decaying implanted uterine eggs) he implanted paraffin blocks, muscle tissue, or agar blocks of about the size of half an oötheca after removal of the egg case. These implants did not inhibit the corpora allata and Engelmann concluded that the arrest of the corpora allata was not caused by mechanical pressure. However, it should be pointed out that pressure exerted by an implant in the abdominal cavity may be quite different from pres-

sure exerted in the uterus by the growing eggs (or by an implant into the uterus). In his more recent work (1960) Engelmann found that nerve cord severance did in fact result in renewed growth of the oöcytes in pregnant females and that nervous stimuli are primarily responsible for inhibition of the corpora allata during pregnancy. However, he found a statistically significant delay of egg maturation after severance of the nerve cord, compared with animals from which egg cases were removed (35.2 ± 0.7 versus 39.1 ± 1.4 days in animals operated on 29 to 37 days after ovulation; 64.7 ± 1.9 vs. 73.4 ± 1.5 days in animals operated on 0 to 1 day after ovulation). He concluded that other factors play an important role in inhibiting the corpora allata during pregnancy. By injecting 0.1 ml. of clear supernatant fluid from homogenized uterine eggs every fifth day for 30 days, he inhibited the corpora allata of *Leucophaea.* However, the injection of muscle homogenate resulted in a similar inhibition and Engelmann suggested that a non-specific substance inhibited the corpora allata during pregnancy.

Although Engelmann has shown a delay in ovulation in females that had nerve cords cut compared to females from which oöthecae were removed and has demonstrated that extracts of uterine eggs and muscle tissue have an inhibitory effect on the corpora allata, he has not demonstrated that there is a substance normally produced by the uterine eggs which acts to inhibit the corpora allata. Our experiments do not corroborate Engelmann's finding that a substance from uterine eggs inhibits the corpora allata. We find that removing eggs from the uterus and implanting them into the abdomen (in *Pycnoscelus, Byrsotria, Blaberus craniifer* and *Leucophaea*) removed inhibition of oöcyte development, i.e. oöcytes developed in the ovaries. We also find that cutting the nerve cord of pregnant females allows the oöcytes to develop in the ovaries of *Pycnoscelus, Byrsotria, Blaberus craniifer,* and *B. giganteus;* we therefore conclude that the inhibition of the corpora allata during gestation, in these species at least, is dependent upon nervous stimuli resulting from the presence of the egg case in the uterus.

Engelmann (1960) concluded that in *Leucophaea* the inhibitory influence of the oötheca may act on the last abdominal ganglion either by nervous or chemical factors and that there was "no reason to believe that the presence of an egg case in the brood sac is mechanically recorded in the brain (Roth and Stay, 1959). The question is still undecided." Our conclusions in the 1959 paper were based on studies of *Pycnoscelus surinamensis* and *Blattella germanica.* In the parthenogenetic strain of *Pycnoscelus* there is no inhibition of corpora allata

in virgin females prior to the first pregnancy, and severance of the nerve cord may affect the ability of the female to oviposit but has no influence on the rate of maturation of the oöcytes. There is no inhibitory center in the last abdominal ganglion in this species before the first oviposition. The insertion of wax into the uterus, after removal of the oötheca, results in inhibition of the corpora allata, and indicates that a chemical substance from uterine eggs is not necessary for inhibition of corpora allata in *Pycnoscelus*. We interpret these results to mean that pressure from the stretched uterus regulates the secretion of the corpora allata. As suggested by Engelmann (1962) the inhibitory center may be caudal to the site of the operation and "the brain may act only as a way station for the transmission of nervous impulses."

In *Rhodnius prolixus* the release of brain hormone was triggered by the distension of the insect's abdomen following a blood meal. Since cutting the nerve cord eliminated this effect, Wigglesworth (1934) inferred that the neurosecretory cells were influenced by nerve impulses arising in abdominal proprioceptors. The two stretch receptors found in each abdominal segment of *Rhodnius* adapt scarcely at all and will continue to discharge as long as the abdomen is stretched (Van der Kloot, 1961). In all of the false ovoviviparous cockroaches the uterus becomes greatly distended as the eggs increase in size as a result of water uptake and growth (Roth and Willis, 1955). It is possible that inhibition of the oöcytes during pregnancy may be due to pressure on abdominal stretch receptors as in *Rhodnius*. However, it is also conceivable that there are mechanoreceptors in the uterus itself. The present evidence indicates that the ovipositor is not involved in transmitting the pressure stimulus from the oötheca in the uterus or genital chamber of *Pycnoscelus* and *Byrsotria;* similarly, the ovipositor in *Blattella germanica* is not involved in corpora allata inhibition while the female carries its egg case (Roth and Stay, 1962).

In *Blattella,* which carries its oötheca externally, and in all cockroaches that incubate their eggs internally, the oötheca swells during embryogenesis, particularly in the latter species (Roth and Willis, 1955, 1955a, 1958). We (Roth and Stay, 1959, 1961, 1962) have suggested that during pregnancy inhibition of the corpora allata is due to nervous stimuli resulting from pressure of the oötheca. The changing pressure stimulus resulting from the increase in size of the oötheca would tend to prevent or retard adaptation of the receptors involved so that the corpora allata are inhibited during the entire (except in *Diploptera* and some *Nauphoeta*) gestation period. However, in virgins of *Blattella germanica* (Roth and Stay, 1962) *Blaberus craniifer, Byrsotria fumigata,* and *Pycnoscelus surinamensis*

(mated bisexual strain females whose uterine eggs do not develop) where the oötheca does not increase markedly in size because the eggs remain undeveloped, inhibition of the corpora allata, resulting from the presence of the oötheca, ceases, and consequently the oöcytes develop in spite of the presence of the egg case; it seems that because of the constant, more or less unchanging pressure stimulus resulting from an oötheca that is not increasing in size, pressure receptors (or the central nervous system) become adapted and nervous inhibition of the corpora allata ceases.

ABSTRACT

The effect of mating on oöcyte development and oviposition in *Pycnoscelus surinamensis, Byrsotria fumigata, Blaberus craniifer, Blaberus giganteus, Nauphoeta cinerea,* and *Leucophaea maderae,* all cockroaches that incubate their eggs internally, was investigated. In *Diploptera punctata,* the majority of females require mating for maturation of the oöcytes. In *Pycnoscelus* mating is unnecessary for egg maturation. Between these two extremes are species which show varying degrees of dependence on external mating stimuli for overcoming inhibition or for stimulating corpora allata. Various species also show different degrees of dependence on mating for normal formation and retraction of the oötheca into the uterus.

The extent to which cockroaches depend upon food intake for stimulation of the corpora allata also varies. The species may be arranged in a series showing complete dependence to complete independence upon food for oöcyte development.

Experiments to determine the nature of inhibition of the corpora allata during pregnancy indicate that inhibition is due to nervous stimuli resulting from pressure of the growing eggs in the uterus.

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