J. ins. Physiol., 1957, *Vol.* 1, *pp.* 229 *to* 239. *Pergamon Press Ltd., London*

THE PRODUCTION OF SEX ATTRACTANT SUBSTANCE AND OF OÖTHECAE BY THE NORMAL AND IRRADIATED AMERICAN COCKROACH, *PERIPLANETA AMERICANA* L.

MARTHA L. WHARTON and D. R. A. WHARTON

Pioneering Research Division, U.S. Army Quartermaster Research and Development Center, Natick, Massachusetts

(*Received* 20 *May* 1957)

Abstract—A correspondence has been shown to exist between the production of an attractant substance and the mating period of *Periplaneta americana*. The attractant is produced principally by virgin females and, sporadically, by mated females, and is conducive to mating; however, mating depresses the production of attractant. Manipulation of the female can depress the production of attractant. Production of attractant normally decreases with age and as oöthecal production increases. Mating induces an increase in oöthecal production, especially among younger cockroaches. Cathode-ray irradiation damages oöthecal and attractant production. With oöthecal production totally inhibited by irradiation, the females recovered their capacity to produce attractant and exceeded the normal yield. A relationship is indicated between attractant production and the processes which regulate ovulation.

INTRODUCTION

THE sex attractant produced by the female American cockroach has been studied from three aspects: (1) its production under different conditions; (2) an assay for the attractant as measured by the response of male cockroaches (WHARTON *et al.*, 1954a, b); and (3) its extraction, concentration, and purification. This paper deals with the first of these.

ROTH and WILLIS (1952) found that paper taken from jars containing virgin females of *Periplaneta americana* stimulated males to overt sexual activity, whereas paper taken from jars containing old, non-virgin females failed to do so, indicating that the sex attractant is produced chiefly by virgin females. These authors noted that the approach of the American cockroach to the mating act is direct, lacking in courtship, but accompanied by characteristic motions. We have confirmed these observations, and find further that the effect of the receptive female or of paper impregnated with the attractant is to arouse the interest of the male, as manifested first by its sudden alertness, then movement of the antennae, then active search for the source of odour, then more or less vigorous fluttering of the wings, usually accompanied by protrusion of the abdomen. Repeated tests with our extracts have consistently shown that this sequence of activity corresponds with the influence of increasing concentrations of attractant (WHARTON *et al.*, 1954a). Except for copulation, wing flutter (when it occurs in the presence of a sex stimulus) is the surest visible sexual response and is a certain indication of the

recognition of the specific odour by the male. It is on this response that we have based our assay of the attractant (WHARTON *et al.*, 1954b).

EXPERIMENTAL

Production of attractant

The attractant is obtainable by extraction from the virgin female cockroach or from objects with which it has been in contact during the period of emission. It occurs in minute quantities; nevertheless, the attractant from a highly productive virgin female kept on filter paper in a 250 ml beaker may so permeate the paper in a period of 2 hr that this paper, when placed in a jar with a dozen males, may stimulate several of them sexually.

To test for the production of attractant, we keep isolated females in 250 ml beakers lined at the bottom with a disk of filter paper which is removed every week for testing and replaced with a new paper. We maintain several groups of male cockroaches arranged in separate jars containing 11 insects each, and the paper from each female is assigned to its own group of 66 males throughout the experiment. The number of males responding to the weekly accumulation of attractant on the paper is the basis of comparison in this simple type of test. Over a period of weeks a reliable set of responses may be obtained and compared with controls that are run simultaneously.

Both male and female nymphs of *Blattella germanica* can stimulate adult males sexually (ROTH and WILLIS, 1952). We have found with *P. americana*, however, that nymphs ordinarily do not produce attractant, at least in detectable quantity. Nevertheless, one of the most productive of our cockroaches was a nymph which stayed in her last instar for 5 months. During the last 2 months prior to emergence she produced attractant consistently. Attractance could not be demonstrated on the second or third day after she became an adult, but had definitely returned at the end of one week. This adult cockroach was presented to males on several occasions but failed to mate.

In general, the newly emerged female produces very little attractant. After a week, however, the paper, and the cockroach itself, may be quite active in stimulating males; maximum production of attractant is usually attained by the second week. The level of attractant production subsides gradually over a period of several months, as we have observed with many females (Figs. 1 and 4). Fig. 1 covers the first 8 weeks and contrasts the slow decline in attractant production in unmated cockroaches with the abrupt decline that occurs when the insects are bred. The solid lines are lines of regression which represent the trend of response by males to normal (i.e. not bred) and bred groups of females, respectively, as defined by the method of least squares. The line of regression for the bred group was calculated from the first week after breeding. The broken line in the figure represents the actual response of the males to attractant from females which have been bred. It shows the drastic drop that occurs in production of attractant after breeding. The drop occurs within a period of 18 hr after mating, as actually demonstrated both by presenting the papers and the mated females to males and

noting the relative lack of interest on the part of the males. To determine the effect of mating we exposed each of twenty-five females to eleven males in combined day and electric light and observed the insects in copula. After copulation the

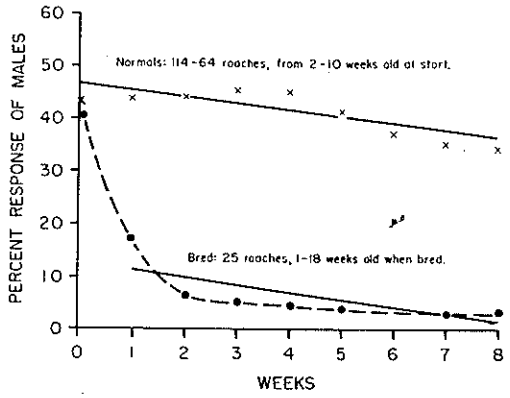

FIG. 1. Effect of breeding (observed) on weekly production of attractant by female *P. americana*. Solid lines show trend of response; the line for the bred group was calculated from the first week after breeding. Broken line shows actual curve of data representing responses of males to bred females, except that response at week zero was obtained before breeding. Note sharp drop in response to attractant from females 1 week after breeding. Test begun with 114 females, reduced to 64 by withdrawal for breeding.

female was returned to its freshly lined container and tested weekly as before. It was found, as shown in Fig. 1, that the production of attractant was permanently reduced by mating. (The terms mating and breeding are used interchangeably to mean that the female has been in copula with the male and has been demonstrated or assumed to have received the spermatophore from the male.) Nevertheless, even though a female cockroach may have passed several weeks in an apparently non-productive state, it is capable of sporadic outbursts of attractant, as shown in Fig. 2. During the non-productive phase the female does not attract the male and mating does not occur. On the other hand, during a shower of attractiveness mating may occur, even after a long period of inactivity (Fig. 2). We have known five females to mate for a second time. The effect of such an event is again to reduce or stop the production of attractant (Fig. 2). The probability (P), obtained from *Chi square* calculations, that the reduction in attractiveness is due to chance is as small after the second mating as it is after the first. Outcroppings of attractant productivity may occur after the second mating (Fig. 2), and, as this may take place quite early in a cockroach's maturity, we may infer that female *P. americana* may breed more than twice in nature. We have known one cockroach to mate for the second time when 33 weeks of age; and one to mate for the first time at 49 weeks. Multiple matings have been observed in other cockroaches, in *Blattella germanica* by KHALIFA (1950) and in *Diploptera punctata* (Eschtz) by STAY and ROTH (in press), who find it a frequent occurrence in *Diploptera*.

We have actually observed twenty-five female cockroaches mating, in all cases in a well-lit room. Time of copula was not less than 1 hr, nor more than 4 hr, the

average being 1 hr 44 min. To determine whether or not copulation has occurred, it is preferable to observe mating, instead of looking in the genital chamber for a spermatophore, this being less disturbing to the animal, and the effect of breeding alone being shown to have an adverse effect on production of the attractant, as seen in Fig. 1, and as indicated by $P = < 0{\cdot}0000001$. On the other hand, at first we were thwarted by the fact that *P. americana* tends to mate at night rather than in the day-time under our laboratory conditions of 8 hr of artificial light.

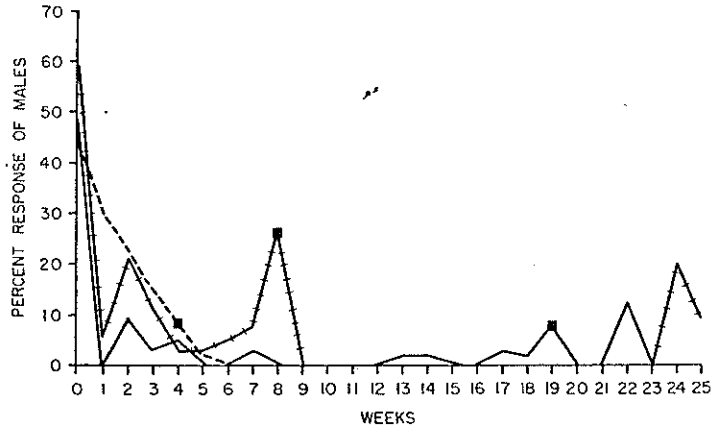

FIG. 2. Effect of breeding on weekly production of attractant by three different female *P. americana*, as determined by response of males to weekly accumulation of attractant deposited on paper by each female after being bred for the first and second time. ■ Bred for the second time.

This tendency prevails especially in the female, which often will not accept the male but rather retreats from its pursuit, only to mate later at night. Out of a total of 94 females aged 1–33 weeks which were exposed to males during a period of 16 hr at night, 56 (60 per cent) were bred. On the other hand, out of a total of 206 exposed to males during 8 hr of daylight only 26 (13 per cent) were bred. We found no seasonal variation in the percentages bred. The males, on the other hand, court actively during the day with females presented to them. There thus appears to be a difference between the sexes in periodism of sexual activity. A sex difference was not reported by MELLANBY (1940), GUNN (1940), or CLOUDSLEY-THOMPSON (1953) in the photoperiodism of the cockroach with respect to locomotor activity. Night matings, which occurred more frequently, were certified by finding the spermatophore in the genital atrium of the female the following morning. To make this observation we anaesthetized the females with CO_2 for 45 sec and examined them under the microscope while gently separating the genital segments with forceps. The effect of mating combined with these procedures affected production of attractant greatly. To determine how much of this effect was due to mating and how much to the other manipulations we compared two groups of females of similar age, one of which was mated, anaesthetized, and examined, and the second of which was exposed (but not mated), anaesthetized, and examined.

Thus, 24 females from 2 to 24 weeks of age were tested for their activity and found to arouse 39 per cent of the males on an average. They were then mated, and 1 week after evoked a response of only 7 per cent, from which point there was a continuous decline. A similar though less drastic decline occurred with the unmated group of 15 females. The difference was significant as indicated by P being $< 0{\cdot}002$. It could be deduced, therefore, that exposure, CO_2, and examination together had a depressing effect, though not as profound as when mating occurred.

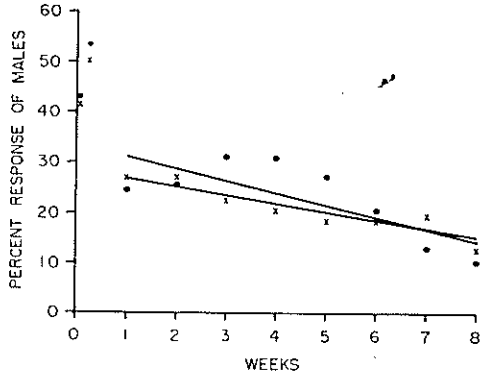

FIG. 3. Effect on attractant production induced by exposing females to males without breeding, CO_2, or examination. The probability of the deviations from the normal being due to chance $=> 0{\cdot}81$, indicating a lack of significant effect. ● Upper line = exposed when 2–6 weeks old. 15 roaches. × Lower line = normals, 5 roaches 2–6 weeks old at start.

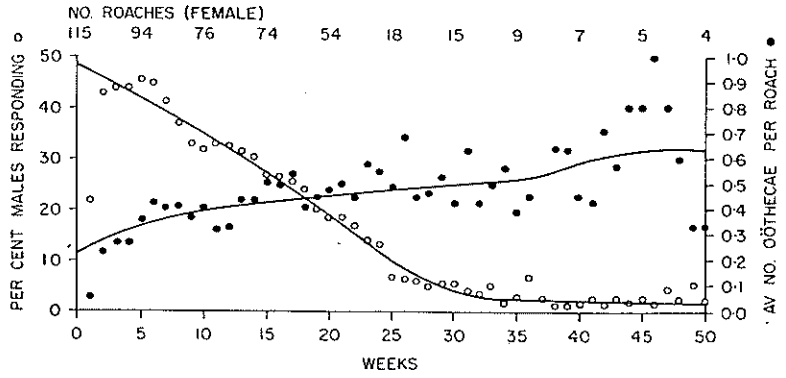

FIG. 4. Weekly production of oöthecae and attractant in unmated *P. americana*. Note that as oöthecal production increases, production of attractant decreases. Both of these changes are accelerated by mating.

Further analysis of the three factors, exposure, CO_2, and examination, showed that when the cockroaches were exposed to males but did not mate, and were not anaesthetized or examined under the microscope, no significant depressing effect was found to have been caused by exposure to males alone (Fig. 3), as indicated by the fact that the probability, P, of the deviations from the normal being due to

chance was greater than 0·81. From the weekly examination of three groups of females we find no significant difference ($P = 0{\cdot}91$) caused by treatment with CO_2 for 45 sec.

We have not detected a periodism such as occurs with mammalian oestrus. Until interrupted by breeding, the attractiveness of the female is maintained fairly evenly and tapers off gradually (Fig. 4). Nor do we know the mechanism of depression of the attraction. Perhaps the seminal material exerts an inhibitory effect on the olfactant or it may absorb it. Or production of the attractant may be under hormonal control, and the seminal fluid, on entering the spermatheca, may induce a stoppage of endocrine function such as the apparent suppression of the stimulus of the corpus allatum by the increasing secretion of the prothoracic gland at the last instar (BODENSTEIN, 1953; WIGGLESWORTH, 1951; WILLIAMS, 1948); or such as oestrogens exert in inhibiting the liberation of the lactogens in mammals (BROWN, 1950); or the cessation of oestrus by conception.

There is a question whether the attractant is being constantly produced or whether it is stored, as neurosecretory material produced in the brain may be stored in the corpus cardiacum (SCHARRER, 1952), and hence, whether its apparent loss represents a decrease in productiveness, or is due to changes in a release mechanism such as has been suggested. The present data cannot answer this question.

Production of oöthecae

Periplaneta americana can reproduce parthenogenetically, although unmated females produce fewer oöthecae during their lifetime than do mated ones, as GRIFFITHS and TAUBER (1942) and ROTH and WILLIS (1956) have demonstrated. Hence we have a means of determining the possible relationship between oöthecal production and the attractant. Fig. 4 shows that attractant continues to be emitted during the course of normal oöthecal production. However, a gradual decline in attractant takes place while oöthecal production increases. Also, in Fig. 5, mating is shown to enhance the rate of oöthecal production sharply, whereas, as we have seen (Fig. 1), it depresses the attractant.

Copulation, in fact, increases the rate of oöthecal production, as indicated by the findings of GRIFFITHS and TAUBER (1942) and ROTH and WILLIS (1956), who found that females placed with males (and presumably mated) produced more oöthecae than unmated ones. The latter authors also found that fertilized female *P. americana* produced their oöthecae sooner than unmated females, and that this was also true of *Blatta orientalis*. We have examined the effect of mating at different periods of adulthood on the production of oöthecae by *P. americana* (Table 1 and Fig. 5).

Table 1 shows the average number of oöthecae produced per female during 5 weekly intervals and compares the performance of normal (unmated) females with that of females mated during successive 5 weekly periods of adulthood. The data show that all groups of mated females increased oöthecal production soon after mating, regardless of whether they were bred at 1–5, 5–10, 10–15, or

15–20 weeks after emergence. The increase is independent of any seasonal variation that may occur in oöthecal production such as noted by RICCI (1950) in *Blatta orientalis*. Fig. 5, in which the cumulative increases are plotted against the normal production, represented by the base line, shows that the increase is

TABLE 1—EFFECT OF MATING ON OÖTHECAL PRODUCTION BY *P. americana*

	Number of oöthecae per female during 5 weekly periods								
	1–5	5–10	10–15	15–20	20–25	25–30	30–35	35–40	40–45
54 unmated	1·3	2·3	2·2	3·1	3·3	2·7	3·0	2·8	2·6
36 mated during weeks 1–5	2·2	4·5	4·1	4·5	3·3	4·8	3·2	3·2	2·3
8 mated during weeks 5–10	1·0	2·9	7·4	6·8	5·8	4·4	3·7	3·0	
9 mated during weeks 10–15	1·8	2·0	2·5	3·1	4·8	3·8	2·8	2·6	2·0
9 mated during weeks 15–20	1·7	2·6	1·6	3·9	5·1	4·8	2·2	2·6	

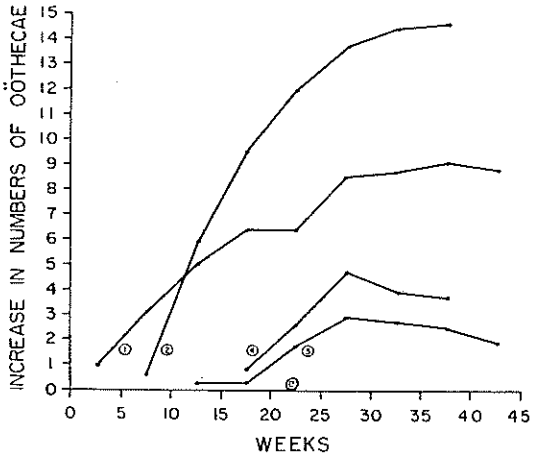

FIG. 5. Increase in oöthecal production per roach induced by breeding. (1) bred at 0–5 weeks; (2) bred at 5–10 weeks; (3) bred at 10–15 weeks; (4) bred at 15–20 weeks; (C) base line = unmated controls. Number of roaches at start: (1) 36; (2) 8; (3) 9; (4) 9; (C) 54.

not haphazard but on the contrary continuous, and it is manifested most strongly by the groups mated at an early age. The oviposition stimulus lasts throughout the life of the cockroach and attains its maximal effect several weeks after impregnation.

The sharpness and extent of the rise among those insects mated at 5–10 weeks after emergence indicates that the optimal time for the stimulus to be effective may be during this period.

The stimulus of breeding is not always sufficient to accelerate the production of oöthecae, however. For example, two of our cockroaches, neither of which had produced an oötheca, were mated at 2 and 4 weeks of age, respectively. They had been strongly attractive and were demonstrated to have received the spermatophore from the male, yet neither cockroach produced an oötheca during its lifetime; at the same time, attractant yield suffered the usual reduction. The connexion between attractance and oöthecal production is obscure; its study may well unfold an important endocrine relationship.

There may be significance in the lag observed in Fig. 5 (3) among the cockroaches mated during the 10–15 week period. This is suggested also by the decline in the rate of production of oöthecae that takes place at about this time in all groups of insects observed (including several groups of unmated cockroaches which provided the data for the composite base line of Fig. 5), except those mated during the 5–10 week period.

Egg maturation is under the control of hormones originating in the corpora allata and in the neurosecretory cells of the brain (SCHARRER, 1952; THOMSEN, 1952). We may suppose, therefore, that fertilization stimulates one or both of these centres to produce greater amounts of hormone. On the other hand, since the corpus cardiacum acts as a reservoir for secretions of the neurosecretory cells (SCHARRER, 1952), the effect of fertilization may rather be to cause a greater rate of release of the hormones stored in this body. It is entirely possible, however, that the contents of the spermatophore supply a gonadotropic or even a gonadal hormone which acts directly or indirectly on the ovaries. The stimulus, on the other hand, may be partly nervous. Furthermore, the contents of the spermatophore may supply nutrients which stimulate egg maturation, either by being absorbed or by fertilization. The view that the state of fertility of the ovum may be a factor in regulating ovulation is supported by the findings of ROTH and WILLIS (1956) that among the progeny of mated and unmated female *Blatta orientalis*, failure to ovulate occurred in the following order: in 2 out of 30 mated females; in 10 out of 20 unmated females; and in 25 out of 34 unmated females from parthenogenetic parents. On the other hand, when parthenogenetic females were mated there was a very great increase in oöthecal production which exceeded even the normal.

The effect of radiation

The effect of radiation on the production of attractant was studied to determine (a) how low a dose of non-lethal radiation could affect the production of attractant, and (b) whether, in view of the possibly significant relationship between production of attractant and of oöthecae, a connexion between the two might be further indicated by the action of the radiation on the ovaries. For this purpose sets of 12 two-week-old females were put into 5 × 12 in. polyethylene bags, the necks of which were then tied about polyethylene ventilating tubes, and irradiated with 1000, 2500, 5000, and 50,000 r by means of a 2 MeV Van de Graaff electron accelerator and then returned to their battery jars. At weekly periods the papers

lining these jars were replaced with fresh papers and tested by bioassay against a standard solution of attractant. The assay was done by using a test of two rounds in the first of which the entire paper was folded and presented to the male cockroaches. For the second round, half of the paper was used after it had been cut so as to halve the apparent soilage; halves made in this way were found by assay to be approximately equal in potency. Table 2 summarizes our findings.

TABLE 2—THE EFFECT OF CATHODE RAYS ON SURVIVAL AND ON OÖTHECAL AND ATTRACTANT PRODUCTION BY FEMALE *P. americana*

	Days,								
	3	5	6	7	14	21	28	35	42
	No. dead								
1000 r	0	0	0	0	0	0	0	0	0
2500 r	0	0	0	0	0	1	2	3	3
5000 r	0	0	1	2	3	6	10	10	11
50,000 r	7	10	10	10	12				
Controls	0	0	0	0	0	0	0	0	0
	No. of oöthecae								
1000 r				0	0	0	0	0	0
2500 r				0	0	0	0	0	0
5000 r				0	0	0	0	0	0
Controls				5	11	17	24	30	36
	Rel. potency*								
1000 r				0·29	0·9	0·86	2·3	1·6	1·8
2500 r				0·38	0·83	0·68	0·2	1·3	2·9

* Attractant yield (determined by bioassay of papers from jars against a standard solution) given in terms of paper from control jar as having a value of 1.

Doses of 50,000 and even 5000 r were too lethal to permit continuous tests for attractant and oöthecal production. However, a dose of 1000 r was not lethal to animals observed over a period of several weeks, and 2500 r proved to be about the lethal threshold. A most striking fact is that the irradiated cockroaches did not produce any oöthecae during the 6 week period of observation, whereas the unirradiated controls produced 36 oöthecae. Attractant production also was adversely affected. No great difference was shown in the effects of 1000 and 2500 r; at the end of the first week after irradiation the cockroaches of both groups had emitted about one-third as much attractant as the controls and continued to show the damaging effect of radiation for another 2 or 3 weeks. A remarkable reversal then occurred in production of attractant, so that at 4 weeks the relative activity of the females irradiated with 1000 r was 2·3, while at 6 weeks the relative activity of those irradiated with 2500 r was 2·9. Presumably recovery was retarded in the latter group because of the greater damage done by the larger dose. It is

interesting that the recovery of attractant production among these cockroaches should overshoot the normal mark. In view of the fact that oöthecal production was destroyed, or at least totally inhibited by irradiation, while in normal females there is an inverse relationship between oöthecal production and attractant production, these findings suggest that the over-recovery of attractance in the irradiated insects may be due to the destruction of a controlling mechanism associated with oöthecal production.

SUMMARY

A correspondence has been shown to exist between the production of attractant and the mating period of *Periplaneta americana*. The attractant is produced principally by virgin females and, sporadically, by mated females, and is conducive to mating; however, mating depresses the production of attractant.

Manipulation of the female can depress the production of attractant.

Production of attractant normally decreases with age and as oöthecal production increases.

Mating induces an increase in oöthecal production, especially among younger cockroaches.

Cathode-ray irradiation damages oöthecal and attractant production. With oöthecal production totally inhibited by irradiation, the females recovered their capacity to produce attractant and exceeded the normal yield.

A relationship is indicated between attractant production and the processes which regulate ovulation.

Acknowledgements—Irradiation of the cockroaches was supervised by Dr. E. F. DEGERING. We have appreciated having helpful discussions on the physiology of the cockroach with Dr. L. M. ROTH and Dr. E. R. WILLIS.

REFERENCES

BODENSTEIN D. (1953) Endocrine control of metamorphosis with special reference to Holometabola. *Trans. 9th int. Congr. Ent. Amsterdam* **2**, 58–62.

BROWN JR., F. A. (1950) Endocrine mechanisms. *In Comparative Animal Physiology* p. 748. PROSSER C. L., BISHOP D. W., BROWN JR., F. A., JAHN T. L. and WULFF V. J. (Editors). Saunders, Philadelphia.

CLOUDSLEY-THOMPSON J. L. (1953) Studies in diurnal rhythms. III. Photo-periodism in the cockroach *Periplaneta americana* (L.). *Ann. Mag. nat. Hist.* (12) **6**, 705–712.

GRIFFITHS JR., J. T. and TAUBER O. E. (1942) Fecundity, longevity, and parthenogenesis of the American Roach, *Periplaneta americana* L. *Physiol. Zool.* **15**, 196–209.

GUNN D. L. (1940) The daily rhythm of activity of the cockroach *Blatta orientalis* L. Aktograph experiments especially in relation to light. *J. exp. Biol.* **17**, 267–277.

KHALIFA A. (1950) Spermatophore production in *Blattella germanica* L. (Orthoptera: Blattidae). *Proc. R. ent. Soc. Lond.* **25**, 53–61.

MELLANBY K. (1940) The daily rhythm of activity of the cockroach, *Blatta orientalis* L. II. Observations and experiments on a natural infestation. *J. exp. Biol.* **17**, 278–285.

RICCI M. (1950) Note sulla biologia di *Blatta orientalis* L. *Riv. Parassit.* **11**, 219–231.

ROTH L. M. and WILLIS E. R. (1952) A study of cockroach behavior. *Amer. Midl. Nat.* **47**, 66–129.

ROTH L. M. and WILLIS E. R. (1956) Parthenogenesis in cockroaches. *Ann. ent. Soc. Amer.* **49**, 195–204.

SCHARRER B. (1952) Neurosecretion XI. The effects of nerve section on the intercerebralis–cardiacum–allatum system of the insect *Leucophaea maderae*. *Biol. Bull., Woods Hole* **102**, 261–272.

STAY B. and ROTH L. M. The reproductive behavior of *Diploptera punctata* (Eschtz) (Blattaria: Diplopteridae). In press.

THOMSEN E. (1952) Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blow-fly, *Calliphora erythrocephala* Meig. *J. exp. Biol.* **29**, 137–172.

WHARTON D. R. A., MILLER G. L. and WHARTON M. L. (1954a) The odorous attractant of the American cockroach, *Periplaneta americana* (L.). I. Quantitative aspects of the response to the attractant. *J. gen. Physiol.* **37**, 461–469.

WHARTON D. R. A., MILLER G. L. and WHARTON M. L. (1954b) The odorous attractant of the American cockroach, *Periplaneta americana* (L.). II. A bioassay for the attractant. *Ibid.* **37**, 471–481.

WIGGLESWORTH V. B. (1951) Source of moulting hormone in *Rhodnius*. *Nature, Lond.* **168**, 558.

WILLIAMS C. M. (1948) Prothoracic glands and insect development. *Biol. Bull., Woods Hole* **94**, 60.

PRINTED IN ENGLAND BY JOHN WRIGHT & SONS LTD., AT THE STONEBRIDGE PRESS, BATH ROAD, BRISTOL 4