1985

The Biology of Egg Production in the House Cricket, Acheta Domesticus L.

Craig William Clifford
Louisiana State University and Agricultural & Mechanical College

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THE BIOLOGY OF EGG PRODUCTION IN THE HOUSE CRICKET,
Acheta domesticus L.

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
in
The Department of Zoology and Physiology

by
Craig W. Clifford
B.S., Louisiana State University, 1972
M.S., Louisiana State University, 1974
May 1985
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ABSTRACT

Oogenesis commenced on day 3 (day 0 = adult ecdysis) in Acheta domesticus L., which, based on degeneration of the flight muscles, was approximately when juvenile hormone was released. Starvation did not prevent the onset of oogenesis, although egg production was greatly reduced. Virgin females did not oviposit and the oviducts filled with eggs by day 15. Compaction appeared to be a factor limiting oogenesis. The digestive tract was compressed and feeding reduced, which might be the primary factors terminating oogenesis. The concentration of juvenile hormone esterase (JHE) did not change as virgins filled with eggs, which indicated that JHE was not involved with termination of oogenesis. Prostaglandin topically applied to the genital opening was 3X more effective in initiating oviposition than when injected. Testectomized males stimulated oviposition of unfertilized eggs, indicating that the prostaglandin synthetase was not produced in the testes. The male cemented the spermatophore in the female genital chamber; this seal probably reduced chances for degradation of the enzyme and increased the efficiency of semen transfer.
The metabolic rate of virgin females was greatly reduced because the oxygen consumption by unfertilized eggs is very low. Although more than 90% of the eggs were derived directly from food, fat body reserves were used up during egg production. Mated females produced more eggs and lost more fat body than virgins or ovariectomized females. As females lost fat body, their survival time when starved decreased. Ovulated eggs stored in the oviducts could not be resorbed.

The vitellogenin was partially purified and isolated by low-ionic-strength precipitation and DEAE-affinity chromatography. The molecular weight was estimated at 400,000 daltons by calibrated gel filtration, and the pI estimated at 5.7-6.4 by isoelectric focusing. It was a glycolipoprotein containing 9.6% lipid and 6.6% carbohydrate. At least two subunits having molecular weights of 112,000 and 40,000 daltons, respectively, were identified by calibrated SDS-polyacrylamide gels. Using an integrating densitometer on stained SDS-PAGE preparations, it was determined that starvation, age, and egg production reduced the vitellogenin titre and that ovariectomy elevated vitellogenin titre.
PART 1.

Control of Oogenesis and Oviposition in the House Cricket, *Acheta domesticus* L.

KEYWORDS: *Acheta domesticus*, house cricket, oogenesis, oviposition, egg production, JH esterase
ABSTRACT

Egg production in the house cricket, *Acheta domesticus* L., began about three days following the last ecdysis even when conditions were inappropriate for successful reproduction. Ten days of starvation from the middle of the last instar into the early adult stage caused depletion of the fat body (FB) reserves but did not prevent the initiation of oogenesis. The amount of FB determines how long a cricket can be starved because adult crickets cannot resorb sequestered nutrients from the eggs already produced. The presence of juvenile hormone (JH) in adult crickets causes the degeneration of the flight muscles and the initiation of oogenesis. The flight muscles of starved crickets degenerated on schedule (day 3), and oogenesis commenced but could not be sustained. Starvation did not interfere with JH release but limited the extent of oogenesis.

The effects of hemiovariectomy, testectomy, and virginity on oogenesis and oviposition indicated that mating did not directly stimulate egg production. Prostaglandin E₂ (PGE₂) synthetase transferred to the females during mating via the spermatophore produced PGE₂, which stimulated oviposition. PGE₂ applied at the opening of the female reproductive tract stimulated significantly more oviposition than when it was injected into the hemocoel. Oogenesis started on day 2 (day of
adult ecdysis = day 0) and seemed to be a continuous process. Oviposition began by day 9, peaked on day 11, and continued for the 60-day life of the female, gradually declining with age. The percent of eggs that hatched increased with age because with the declining rate of oviposition, the efficiency of fertilization was greater. There was no difference in JH esterase (JHE) titre in the blood whether a female was virgin or mated, or whether a female was or was not full of eggs. Therefore, there was no evidence for JHE regulation of oogenesis.
INTRODUCTION

Oogenesis begins in early adult life and, along with oviposition, is the female's principal activity throughout her life. Although various cricket species are basically similar in anatomy (Snodgrass, 1933; Spann, 1934), there seems to be variation in the oogenic control mechanisms present. Egg production in house crickets (*Acheta domesticus* L.) is well studied but only from a life history approach (Nowosielski and Patton, 1965; Bate, 1971; and Patton, 1978). Few studies have been conducted relating oogenesis with the mechanisms involved in its control.

Whereas the initiation of oogenesis in *Acheta domesticus* is caused by the release of juvenile hormone (JH) from the corpora allata (Bocharova-Messner et al., 1970), the control of oviposition might be hormonal as well. Leahy (1973a) reported increased oogenesis and oviposition following implantation of male accessory gland in female *Schistocerca gregaria*. The male accessory gland of *Melanoplus sanguinipes* was directly or indirectly capable of stimulating egg laying (Pickford et al., 1969; Friedel and Gillott, 1976). The substances responsible for ovipositional stimulation are prostaglandins (PG's) produced by the PG synthetase transferred from the male during mating in *Teleogryllus commodus* (Loher et al., 1981; Tobe and Loher, 1983) and
in *Acheta domesticus* (Destaphano and Brady, 1977).

The initiation of oogenesis by JH, and of oviposition by PG, seems to be established for *A. domesticus*, but the means, if any, to terminate either process have not yet been clearly identified. JH esterase (JHE) was suggested for the control of cyclic reproduction in *Diploptera punctata* (Rotin et al., 1982). Renucci et al. (1984) made a similar case for JHE in the control of oogenesis in *A. domesticus*. A well-adapted control mechanism should be capable of terminating oogenesis in response to inappropriate conditions such as starvation. Inadequate protein for the developing oocytes increased oocyte resorption in *S. gregaria* (Highnam et al., 1963) and starvation induced egg resorption in *Periplaneta americana* (Bell, 1971).

I have observed mating and used experimental manipulation of mating to study the control of oogenesis and oviposition. Hemiovariectomy, testectomy, enforced virginity, starvation, and PG treatment were techniques used to determine the controls for the maturing and ovipositing of the eggs.
METHODS

Rearing

The crickets used in this study were maintained on a LD 12:12 photoperiodic regimen at 30° C and otherwise aged and reared according to Clifford et al. (1977). Water was provided by means of plastic vials, with slots (10 x 5 mm) cut for water flow, inverted in plastic petri dishes. Special drownproof slit-type water containers (slit 2 mm wide x 10 mm tall) had to be used to prevent the unexplained drowning behavior of the older, and starved, crickets.

Eggs

Eggs were collected by providing mated females with a 5 cm diameter x 3 cm deep plastic container partially filled with moist sand. The eggs were washed from the sand by alternately adding water, swirling, and pouring the wash water with eggs onto a fine mesh plastic screen until no more eggs appeared. Although sand was a more convenient oviposition medium for separating and counting eggs because eggs and sand separate easily, moist peat moss is the medium of choice for routine incubation and hatching because it retains moisture longer than sand (Clifford and Woodring, 1985a).
The viability of the oviposited eggs was determined by incubating groups of 50 eggs in separate petri dishes with moist sand at 30°C and observing emergence over the 2- to 3-day hatching period.

The number of eggs stored in a cricket was determined by making a middorsal cut from the last abdominal segment to the anterior prothorax. When the cricket was pinned open, the ovaries and oviducts were removed, opened, and the eggs counted. For some determinations of the number of eggs present in female crickets, the total weight of ovaries and oviducts was divided by the average weight of an egg at the appropriate age.

Prostaglandins

Prostaglandin $E_2$ (PGE$_2$) was dissolved in 100% ethanol and then added to Freund's Incomplete Adjuvant. Most of the ethanol was evaporated with a stream of $N_2$. The adjuvant served as a carrier for the tiny droplets of ethanol with the PGE$_2$ and the mixture was vortexed prior to use. The prostaglandin could not be injected in the ethanol alone because even 1 ul of ethanol caused visible injury to the cricket. The prostaglandins do not have to be dissolved in the carrier to be effective (Destaphano and Brady, 1977). The dose used (50 ug/cricket) was based on the dose-response curve of Destaphano and Brady (1977). The drug was either
injected into the body cavity or placed topically at the opening of the genital chamber in the experimental protocol. Adjuvant alone was used for the sham injection or sham topical application. A second control to investigate the effects of tactile stimulation on oviposition activity was also included.

Testectomy

Testes were removed from males on day 2 of the adult stage. Following light CO₂ anesthesia, a 2-3 mm vertical cut in the dorsolateral area between abdominal segments 3-5 on each side and slight pressure anterior and posterior to the cuts resulted in the protrusion of one testis and then the other testis from their respective slit. The exposed testes were removed and inspected to be certain that the entire testis had been excised. The survival rate exceeded 90% and the castrated males stridulated soon after the surgery, produced spermatophores, mated, and lived as long as intact animals. The principal reason for testectomizing males was to investigate the response of the female reproductive system to mating without fertilization. Approximately 10 female crickets were mated with these testectomized males. Ten more females were mated with normal males as the mated control, and some crickets without males served as the virgin control. Egg totals for a 25-day test period were measured.
**Spermatophores**

Spermatophores were obtained by squeezing a male to cause extrusion and then the spermatophore ampulla (Figure 2) was grasped and the whole structure removed with forceps. Seminal fluid containing the sperm flowed from the ampulla into physiological saline or water.

**Starvation**

Food was withheld from groups of crickets to investigate the effects of starvation on oogenesis and on egg resorption. If the period of starvation was short (2-3 days), then the food dish was simply removed from the cardboard rearing cartons containing the crickets. To starve the crickets for longer periods, individual crickets were isolated in glass jars with a screen lid and a drownproof (slit-type) water container to prevent cannibalism, drowning, and their eating the cartons.

**Fat body**

The amount of fat body (FB) can be difficult to measure, because it is a diffuse organ spread throughout the animal. A rinse-and-pick method gave reproducible results in *A. domesticus* and permitted comparisons between treatment groups to be made. The FB was washed out of the pinned-open carcass (gut removed) with a jet
of water from a plastic wash bottle. Two or more washings, and picking with forceps at trapped FB masses, were usually necessary. The runoff was collected and placed in a preweighed disposable plastic beaker and the oven-dried weight (70°C) gave a measure of the amount of FB.

Hemiovariectomy

Hemiovariectomy (removal of one ovary) on 1-day-old adult females under light CO2 anesthesia required a 2-3 mm slit as described for testectomy for the entire ovary to be protruded out of the slit. The ovaries were removed and checked for completeness. Any other structures that protruded could be pushed back in with a blunt forceps. The wound did not bleed, but closed quickly. Bradley and Edwards (1978) used sterile procedure and dusted the wound with an antiseptic powder when ovariectomizing larval *A. domesticus*. Sterile surgical techniques, antibiotics, or even cleaning the site of the incision was unnecessary. Out of the hundreds of operated crickets, none died due to postoperative complications.

Experimental categories

The three experimental categories of adult female crickets were distinguished in these experiments. Virgin females (virgins) had never been exposed to males
as adults and they rarely laid eggs. There were two types of mated crickets based on their laying activity. Mated female crickets lay eggs if provided suitable oviposition media and do not lay eggs if suitable oviposition sites are lacking. Therefore, it was possible to obtain mated crickets that would or would not lay eggs.

Juvenile hormone esterase

The titre of juvenile hormone esterase (JHE) in the blood and tissues was determined for virgin females starting on day 0 (emergence) and every other day through day 20. The blood of mated females was assayed on day 12. The substrate concentration was $10^{-6}$ JH III/ul, with sufficient $^3$H-JH added to produce an average of 6800 cpm/ul. The blood was diluted (50 or 100 x) with chilled 0.15 M phosphate buffer (pH 7.3) so that hydrolysis was linear over the course of the incubation (15 min). Separation and quantitation of the hydrolysis product, JH acid, from the unhydrolized substrate was performed by a rapid solvent-partition assay using water and iso-octane as described by Hammock and Sparks (1977). In order to assay for JHE titre in the body tissues, the head was removed and the gut dissected out. The adhering blood was rinsed from the body cavity and tissue surfaces with cold phosphate buffer. The carcass was homogenized with 5 ml cold
buffer in a Tekmar® Homogenizer, centrifuged 15 min at 10,000 xg, and a sample of the supernate was diluted 40 x with buffer and assayed as for the blood. Protein concentration was determined with the Biorad® assay.

Mating effect

In order to determine the duration of the mating effect on the stimulation of egg laying, male-female pairs were isolated in a series of cartons. The male member of a pair was removed from one carton every three days over a 21-day period (7 cartons). Eggs were collected daily and counted to determine how many were laid during the next 40 days.

Both living (saline) and dead (70% ethanol) specimens of both sexes and of younger and older adults were dissected to make the outline drawings of the female reproductive system.
RESULTS

Anatomy of the reproductive system

The internal reproductive system of the female house cricket is very similar to that of the field cricket, *Gryllus assimilis*, as described by Snodgrass (1933). The ovaries are supported anteriorly by suspensory ligaments and consist of 120 ovarioles per ovary. The lateral oviducts join to form a common oviduct as it forms a pouch fused to the ventral wall at the anterior end of the genital chamber (Figures 1A & 1B). The long coiled spermathecal duct enters into, and traverses through, a cone-shaped structure that is heavily sclerotized ventrally. The cone-shaped structure is labelled the fertilization spout. It is tightly encased in a dorsal pouch of the genital chamber that is fused to the dorsal wall. The tip of the fertilization spout projects into the flattened posterior area of the genital chamber where the handle (Figure 2) of the spermatophore fits. During dissection the fertilization spout was seen to move from side to side but for what purpose was not determined. The spermathecal duct continues to the pointed tip of the fertilization spout. Eggs are inseminated as they pass from the common oviduct into the base of the ovipositor (Figure 1A). The genital chamber is used for egg
fertilization as well as for holding the spermatophore during mating.

Spermatophore

Spann (1934), for Gryllus assimilis, and Loher and Renee (1978), for Teleogryllus commodus, described the entry of the spermatophore tube (Figure 2) into the spermathecal duct of the female. The identical process occurs in A. domesticus. An aspect of mating not previously described in any cricket species involves the mechanism whereby the spermatophore fuses with the female's reproductive system. The fusion of the cup of the spermatophore handle (Figure 2) to the fertilization spout in the genital chamber and the cup itself is described for the first time. The curved spermatophore handle wraps up and around the base of the ovipositor and is held there by the epigenital plate (S8). The fit of the spermatophore cup over the fertilization spout of the female during emptying of the semen confirmed the relationship of these structures. Khalifa (1949) described a "gelatinous white material" in the cup region of the spermatophore in A. domesticus. In the present study, it was determined that the gelatinous material forms a glue between the cup and the spout that apparently prevents the spermatophore from detaching and falling out of the female. The guarding behavior of the male prevents the female from pulling the spermatophore
...out before semen transfer is complete (Khalifa, 1950; Loher and Renee, 1978). The female must eventually break the cup from the spout and remove the spermatophore in order to fertilize and lay the eggs.

The intact spermatophore contained a fluid necessary to produce pressure osmotically and to cause semen flow (Khalifa, 1949). Khalifa determined, and it was verified here, that only salt concentrations of 1.5-2.0 M or more prevented the normal emptying of the spermatophore. Although all the sperm and seminal fluid was evacuated, some of the granular substance in the posterior half of the ampulla remained.

**Structure of the ovary**

The ovaries of adult female house crickets began a period of rapid growth at about day 2 of the adult stage (day of ecdysis = day 0). Control levels for average cricket weight, average ovary weight, and number of eggs are given in Figure 3. Prior to this growth period, the adult ovaries were indistinguishable from those in the last instar. Ovary wet weight (all weights include both ovaries) in a day-1 adult virgin female cricket was 2.8±0.4 mg (n=10) and reached a peak followed by a plateau at day 15 (210±18 mg (10)), which is in agreement with the findings of Bradley and Edwards (1978). Throughout early female growth, larger virgins had larger ovaries but after 15 days all virgins had...
ovaries that were 35±1.7% of the total body weight. The very first eggs ovulated into the oviduct by day 5 had a very high average weight, 0.89±0.07 mg (10), but the weight declined to 0.46±0.02 mg (10) by day 10 (when oviposition started) and remained at that level through day 30.

The best time to observe the detailed structure of the ovary in *A. domesticus* is in the 1- to 2-day adult just prior to the beginning of oogenesis. The individual ovarioles that make up the ovary are surrounded and bound together by a peritoneal sheath (Snodgrass, 1935). The ovariole can be divided into three regions: the terminal filament, the egg tube, and the pedicel. The individual terminal filaments, a continuation of the noncellular outer tunica propria, from the ovarioles join to form the suspensory ligament of each ovary. The egg tube is divided into a short anterior germarium which contains the mixed germ and follicle cells, and a posterior vitellarium, lined with follicle cells, where the oocyte growth occurs primarily by the addition of yolk (vitellogenin). The short pedicel is simply the connection of the individual ovariole to the lateral oviduct. The house cricket ovary contains an average of 120±4 ovarioles (4). The egg tube is a linear arrangement of at least 18-27 easily discernible, developing oocytes. These oocytes are in various stages ranging from very small spherical
oocytes at the anterior end of the gerarium which measure 0.025 mm in diameter to one full-size egg 2.64 mm long and ready to be ovulated. House crickets have panoistic ovarioles (Snodgrass, 1935). These ovarioles are characterized by a single layer of follicle cells surrounding the oocyte and it is the oocytes themselves that absorb the materials for growth directly from the fluid in the egg tube. The ovarioles in a 3-day adult female average 4.8±0.18 mm (11) long with the most proximal oocyte (the largest) averaging 1.13±0.10 mm (6). The ovarioles in a 5-day female average 4.00±0.20 mm (11) long and the most proximal oocyte averages 1.24±0.13 mm (10). By day five, however, a mature egg (2.48±0.04 mm long (10)) has already had the chorion (egg shell) deposited by the follicle cells and been ovulated from most of the ovarioles into the oviduct (Figure 3). Apparently, as the mature egg is released from the ovariole, it recoils to a shorter length until it is stretched again by the growth of another mature egg. Precise physiological aging, based on terminal oocyte length as is done in many grasshoppers, is not practical in crickets. Prior to oviposition, the mature eggs are held in the highly distensible oviduct that serves as an egg reservoir. The ovaries of a 15-day virgin cricket became distorted and difficult to find as the eggs filled the oviducts which then occupied the body cavity from the sides of the crop in the prothorax.
to the posterior-most end of the animal. If each ovary developed all 27 oocytes per ovariole, a female could lay 6480 eggs (27 oocytes/ovariole X 120 ovarioles/ovary X 2 ovaries). This is roughly twice the number of eggs that an average female lays in her lifetime.

**Egg production**

The number of eggs laid throughout the reproductive life of a female is 2994±245 (7) (Figure 4). Once egg laying begins at 9.4±0.6 days (13), it is continuous for about 60 days or until death. The average rate of egg laying, when plotted against time (Figure 4), shows peaks at an interval of 3-4 days (3.2±0.39 days (12)). The plots of average food consumption (Figure 5) during the first 40 days of the adult also shows peaks at 3-4 day intervals (3.6±0.26 days (8)). Though continuous, the rate of egg laying decreases with age (Figure 4).

**Juvenile hormone esterase**

The titre of juvenile hormone esterase (JHE) in the blood of virgin female crickets was very low (3-5 nmoles/min/ml, 0.1 nmoles/min/mg protein) with no significant differences from day 0 to day 20 when assayed every other day (3 separate assays). The blood titre of JHE in 12-day mated, laying females was 3.0 nmoles/min/ml (4). With the gut and head removed and the carcass rinsed free of blood, the remaining tissues
of virgins contained only 0.1-0.3 nmoles/min/mg tissue protein.

Effect of mating on egg production

There was no difference in the number of eggs produced and stored by virgin females (516±56 (6)) and by mated females denied access to oviposition sites (538±34 (9)) after 15 days (Table 1). The egg content of the mated, laying female, however, dramatically decreases to 65 eggs (Clifford and Woodring, 1985b) The primary effect on the female caused by mating involves a stimulation of egg laying.

Prostaglandins

Application of 50 ug PGE₂ at the opening of the genital chamber of 15-day virgin A. domesticus caused a significant increase in ovipositional activity (p<0.05) over control levels (Table 2). The lack of a difference between control and experimental values on the second day after treatment indicated that the effects of PGE₂ were shortlived. The effect on ovipositional activity was significantly greater when the prostaglandin was applied at the genital opening than when it was injected into the body cavity (p<0.05) (Table 2).

Females mated with testectomized males

Groups of 10 females that had mated with
Testectomized males laid approximately the same total number of eggs in the 25-day test period (4570 eggs vs. 5673 eggs) as normal females mated with normal males. Testectomized crickets produced spermatophores lacking sperm but that were otherwise normal and capable of stimulating egg laying. Control virgin-female oviposition levels were much lower (175 eggs in the 25-day test period) than either normals or females mated with testectomized males.

Ovipositional stimulation by mating

Adult crickets did not mate before day 3 (6) (Table 3). Observations indicated lack of interest in mating on the part of the female prior to the release of JH (as determined by flight muscle degeneration). When females did mate, the effect of the mating(s) on the ability to stimulate oviposition lasted an average of 17 days. Virgin crickets did not lay many eggs, but they started ovipositing soon after mating (Table 3, Carton A).

Hemiovariectomy

When only one ovary was removed, the other ovary partly compensated for the missing one. At the end of the experiment, the two ovaries of the normal female weighed 215±15 mg (10) but the single ovary of the hemiovariectomized animal weighed 156±12 mg (10) (Table 4). There was no significant difference between the fat
Resorption of eggs

In order to test for the possible resorption of the ovulated oocytes, matched total weight sets of 15 day virgin crickets were starved. There were no significant differences between the body weights, ovary weights, number of eggs, or average egg weights between the starved and fed groups after 5-days starvation (Table 5). The FB weights of the starved crickets, however, were significantly less than in the fed controls (p<0.01). Fifteen-day old virgin crickets were used because at this age a maximum and stable weight of eggs had been produced (Figure 3) and the crickets would not gain weight when fed. Fifteen-day virgins were only able to tolerate 4-5 days of starvation because they had used most of the FB stores for egg production.

Starvation and the initiation of oogenesis

Starvation of the virgins from day 1 to day 7 reduced egg production by one-fifth. When starved from the middle of the last instar, A. domesticus can endure 10 or more days without food. Starvation starting from the middle of the last instar through day 7 of the adult resulted in ovarian weights that were one-tenth of normal (Table 6). The flight muscles degenerated at the same time and to the same extent in the starved animals.
as they did in normally fed animals, which indicated the release of JH at the normal time in starved crickets.

**Egg viability**

Although the total number of eggs laid/day declined with age (Figure 4), the percent of eggs that hatched improved with age. The percent of eggs laid by 25-day-old females that hatched was 54.7±4.8% (n=150). A significantly higher percent of eggs laid that hatched was found for 45-day-old females (68.5±2.6% (200))(p<0.05). Retained or oviposited eggs that are not opaque and creamy in color with uniform-sized lipoprotein droplets in the cytoplasm were likely to be damaged or dead and would not hatch. Brown eggs, which became more numerous with age, were observed inside the ovaries of older crickets but were rarely laid. The egg shells varied from light to dark brown. There was no brown color in the cytoplasm which was partially or completely transparent and not creamy as in normal eggs. The brown eggs, the few eggs laid by virgins, or the normal colored eggs laid by females mated with testectomized males failed to hatch. These eggs were not dead because they remained intact long past the typical incubation period. Dead eggs, by contrast, decomposed and were rapidly covered with mold growth.
Food consumption

Food consumption by unmated males, virgin females, and mated males and females was high for the first 4-5 days, but then decreased to a stable lower rate for both the unmated males and females (Figure 6). Food consumption by mated males and females maintained itself at a higher level as the females continued to produce and lay eggs. When the rates of food consumption of both unmated males and females were added together, then subtracted from the rate for the mated pairs, an estimate of the quantity of food consumed to produce and lay the eggs could be made (375 mg food through day 15). This quantity compares well with the biomass (340 mg) of eggs laid through day 15 in A. domesticus (Clifford and Woodring, 1985b).
DISCUSSION

Initiation of oogenesis and the degeneration of the flight muscles

One of the basic tenets of insect physiology is that the initiation of egg production and associated changes are caused by the release of juvenile hormone (JH) from the corpora allata (Engelmann, 1970). Bocharova-Messner et al. (1970) presented evidence that this trigger, JH release, initiated flight muscle degeneration as well as oogenesis in *A. domesticus*. The flight apparatus first matured after adult ecdysis and then was useful for flight until oogenesis began. This maturation period for the muscles was temperature dependent and at 29°C occurred within the 1st day postecdysis (Chudakova and Bocharova-Messner, 1968). The time of flight muscles degeneration was used as an easy means to determine the time of JH release. In the stock of crickets used here, examination of the flight muscles revealed well-developed muscles from day 0 until about day 2, when the flight muscles degenerated coincident with the beginning of oogenesis. Before they degenerated, the flight muscles were bright yellow to orange in color, full bodied and rounded. Degenerated flight muscles were thin, straplike, and pale yellow in color.
One would expect there to be differences in the condition of the flight muscles in males and females because of the different functions served by the wings of the two sexes. During the first day or two of adult life, the wings provide a means of dispersal for both sexes (Chudakova et al., 1975). This may be a hypothetical function in this population because only rarely was there any flight activity among these house crickets maintained at 30° C. In the female, both pairs of wings could be easily broken off at an autotomy plane near the base of the wing with little or no blood loss. When the wings were cut with scissors at any other point, there were always drops of blood at the cut veins. There was no autotomy plane in the forewings of the male, but the hindwings had an autotomy plane and the wings came off as easily as in the female. The males use the forewings to stridulate to attract the females and mate (Spann, 1934; Loher and Renee, 1978; and unpublished observations). They also sing to alert to danger, establish territory, and defend against potential competitors (Alexander, 1957). Because there are differences in the functions of the mesothoracic wings, there should be differences in the condition of the wing muscles between males and females. Inspection revealed that in male house crickets (age ≥ 10 days), the hindwing muscles are degenerated to the same extent
as both pairs of the female's flight muscles. The male's mesothoracic wing muscles, however, were not degenerated, but were still yellow to orange in color and the same size as before JH release. The degeneration of the metathoracic wing muscles began in young males (age < 3 days). The flight muscle degeneration demonstrated an example of fine control by means of different receptors to the same hormone. In the female, it is only the flight muscles that are sensitive to JH and the dorsoventral flight muscles respond to JH 5 days before the longitudinal flight muscles (Chudakova and Gutmann, 1978). The male house cricket flight muscle degeneration was even more specific because the mesothoracic set was maintained while the metathoracic set degenerated. This may be the result of disuse of the hindwings while the forewings continue to be used for song production. Chudakova and Bocharova-Messner (1968) prevented the movement of the wings and found an acceleration of both degeneration and oogenesis in A. domesticus.

Starvation

Starvation prevented normal ovarial growth but did not block the initiation of oogenesis. JH release occurred on schedule (as indicated by flight muscle degeneration), but the ovarial weights of females starved from day 0 to day 7 was one-fifth of the control
ovarian weight. Ovarial growth was greatly reduced due to lack of sufficient nutrients for egg development even with the degeneration of the flight muscles and the depletion of most of the FB reserves. Crickets starved from the middle of the last stadium had less FB reserves when oogenesis began than the crickets starved from adult emergence, and they therefore had ovaries that were one-tenth the size of those in the control. Crickets in the middle of the last instar had more FB reserves than at any other time in development (Woodring et al., 1979), but at no time, even in the adult, did these reserves contain enough stores to produce many eggs compared to total egg production. Thus, FB reserves seem to be insignificant for egg production, but it may determine the duration of survival during starvation. Adults, starved from the day of emergence, survived at least 8 days whereas 15-day adults only lived 5 days when deprived of food. FB reserves supplied a ready but limited store for short-term survival, but feeding served as the principal source of nutrients for egg production.

Egg production

Weaver and Pratt (1977) stated that the number of eggs laid by insects is influenced by food quality and quantity, temperature, humidity, photoperiod, and group interactions. Food and temperature are probably the
major factors involved. Bowling (1955) found that at room temperature (21-27° C) house crickets deposited an average of 1195±269 eggs (6) and at 35° C they laid an average 1763±396 eggs (6). In our study, egg production/female was determined to be 2994±287 eggs (7) at the rearing room temperature of 30° C. This then implies that temperature may be very important in determining egg production. However, if 35° C is the optimum rearing temperature for the house cricket (Roe et al., 1980; Bowling, 1955), it seems anomalous for more eggs to be laid at 30° than at 35° C. Chudakova et al. (1975) stated that the optimum temperature for *A. domesticus* is 29° C. Different criteria upon which the optimum is evaluated will give different findings. The differences between our results and those of Bowling may be due entirely to rearing procedures or nutrition. Purina Cricket Chow was used in the present study and Bowling used a broiler mash.

Pickford and Gillott (1976), working with *Melanoplus sanguinipes*, found that females allowed to mate at two-week intervals laid twice the number of eggs as females mated only in the first few days of adulthood. The periodic mating in *M. sanguinipes* is comparable to the continuous male presence used in this study with *A. domesticus* and may be responsible for the large number of eggs laid compared to the other observations of egg production in the house cricket.
Mating in house crickets, besides supplying the sperm, provided an ovipositional stimulant (PGE$_2$ synthetase, Destaphano and Brady, 1977). Additional matings were required because the stimulatory effect from the spermatheca lasted only about 17 days (Table 3).

The rate of egg production (Figure 4) and the rate of feeding (Figure 5) peaked at an interval of 3-4 days. The peaks and valleys are probably the result of the time necessary to feed and the time required to convert these foodstuffs into eggs. Most processes accelerate with increased temperature (Roe et al., 1980) and, therefore, the duration of the interval between peaks would be affected by temperature. The time required to oviposit a large quantity of eggs in the cricket *Teleogryllus commodus* demanded a cessation in one activity while the other was occurring (Loher, 1979). Because adult female house crickets are most active in the scotophase (Cymborowski, 1973), the actual time available to allot among feeding, mating, and ovipositing may be considerably less than 24 hr/day. This may result in alternating periods where feeding or ovipositing activities predominate, which could produce a 3-4 day alternating cycle of feeding and oviposition. There seems to be no good evidence for any type of clock-driven infradian gonotrophic cycles in *A. domesticus* as are found in certain viviparous cockroaches (Engelmann, 1960).
Structure of the ovary

The number of oocytes and ovarioles and their development have not been described previously in *A. domesticus*, and our findings (see Results) indicate a basic similarity to those of related species (*Gryllus assimilis* by Spann, 1934). A factor, implied (Bradley and Simpson, 1981; Renucci and Strambi, 1983) but not previously mentioned in *A. domesticus*, was the important functioning of the oviducts as an egg storage area. This was most noticeable when the crickets were forced to hold eggs under conditions of enforced virginity. It was difficult to find the ovarioles, or even much structure to the ovaries, in virgin females more than 10 days old because of the distention and distortion caused by the large mass of eggs in the oviducts. A 30-day virgin female would have an average of 519 mature eggs (Figure 3). In an extreme case, a 25-day adult virgin cricket (weighing 756mg) containing 809 eggs whose ovary weight represented 42% of her total wet weight was obtained.

Crowding with eggs causing restrictions on egg production

One of the best ways to understand the factors involved in the regulation of normal mating, egg production, and oviposition is to interfere with these
processes. Some of the methods that have been used include studies of the virgin condition, testectomy, and ovariectomy. Loher and Edson (1973) found that mating in *Teleogryllus commodus* caused an increase not only in the number of eggs laid but also in the number of eggs produced by mated versus virgin crickets. Their statement about the increase in the number of eggs produced was based on 30-day totals of eggs laid plus eggs retained in the female. Mated females produced 541 eggs whereas the virgins produced 300 eggs (no statistics given). In my opinion, internal crowding with eggs may be what prevented the virgin *T. commodus* from producing as many eggs as the mated ones. Virgins retained 220 eggs in comparison to the 70 retained by the mated ones. Although no nervous or hormonal feedback mechanism has been found for *T. commodus*, the release of the eggs from the body might remove a direct or indirect inhibition on oogenesis and thus allow more eggs to be produced.

I suggest that the major difference in total eggs produced by mated and virgin *T. commodus* may not lie in the lack of oogenic stimulation as claimed by Loher and Edson (1973), but in the lack of ovipositional stimulation that would allow the virgins to lay some eggs and produce more. In the house cricket, there is no difference between the number of eggs produced and held between mated-nonlaying and virgin crickets (Table
1). Loher and Edson (1973) did not prevent mated *T. commodus* from laying eggs. The reason there is no difference between mated-nonlaying and virgin house crickets may be that there is no mechanism for dealing with a non-mated condition, and thus no provision for terminating oogenesis when mating does not occur. With control over oviposition, the female retains her eggs, so that, if and when she mates, she will lay viable eggs rather than unfertilized eggs that will not hatch.

**Prostaglandins**

Friedel and Gillott (1976) found an oviposition stimulant produced by the male accessory gland in *Melanoplus sanguinipes*. Prostaglandins and/or prostaglandin synthetase have been reported to be transferred from the male to the female during mating to regulate aspects of reproduction in some insects (Pickford *et al*., 1969; Destaphano and Brady, 1977). The enzyme prostaglandin cyclo-oxygenase and the rest of a series of enzymes in the biosynthetic pathway for prostaglandins are referred to collectively as prostaglandin synthetase (Brady, 1983). Destaphano and Brady (1977) injected prostaglandins into the body cavity, which is an unnatural location. They found PGE$_2$ and PGE$_2$ synthetase in the testes, seminal vesicles, and spermatophore of the male house cricket. In *A. domesticus*, PGE$_2$ and PGE$_2$ synthetase are injected
into the reproductive tract and into the spermatheca from the spermatophore. Loher et al. (1981) found that prostaglandin synthetase was passed along with the sperm in male T. commodus to stimulate egg laying in the female. They injected nanogram quantities of PGE₂ into the oviduct and obtained significant increases in the number of eggs laid over that of the controls. Destaphano and Brady, on the other hand, had to inject micrograms of PGE₂ into A. domesticus probably because they did not apply the prostaglandins directly into the females' reproductive system. These effects may be pharmacological due to the relatively large microgram doses of PGE₂ necessary to obtain stimulation of oviposition. My results show that PGE₂, as an ovipositional stimulant, is more effective when applied to the genital chamber than when injected into the hemocoel (Table 2). The shortlived effects of injected PGE₂ in the house cricket shown here agree with those of Destaphano and Brady (1977). Two days post injection, egg-laying activity was not significantly different from that of the controls (Table 2). Loher and Edson (1973) removed the spermatheca from mated females to check for persistence of PGE₂ stimulation. They found that without the PGE₂ synthetic capacity of the spermatheca, the egg-laying rate dropped and approached the premated levels. These findings indicate that a continuous supply of PGE₂ is needed as an ovipositional stimulant
in at least two species of cricket.

Loher and Edson (1973) testectomized *T. commodus*, and found that these males can make spermatophores that lack sperm. Testectomized males could court and mate, but they did not stimulate oviposition despite the transfer of a spermatophore. Testectomized *A. domesticus* also produced spermatophores that lacked sperm, but the results of mating these crickets were different. Testectomized house crickets court and mate apparently normally, but they are also capable of stimulating oviposition. There was no significant difference in the oviposition rate of females mated with testectomized males, and females mated with normal males. Since PGE$_2$ stimulated oviposition in both *T. commodus* and *A. domesticus*, it is obvious that PGE$_2$ synthetase is not in the spermatophores of testectomized *T. commodus* but is present in those produced by *A. domesticus*.

Leahy (1973a and b) found that it was the secretions of the male accessory gland complex, and not the sperm, that was responsible for the stimulation of oviposition in *Schistocerca gregaria*. Pickford et al. (1969) and Friedel and Gillott (1976) found that the ovipositional stimulant was associated with the accessory gland secretions in *M. sanguinipes*. Loher and Edson (1973) showed that the stimulatory factor produced
by *T. commodus* originated in the testes and not the accessory gland. Loher and Edson suggested that the stimulatory factor is PGE$_2$, produced in the spermatheca after the synthetase was transferred from the male. Destaphano and Brady (1977) determined that the oviposition-stimulating factor (PGE synthetase) was associated with the testes and seminal vesicles—and transported in the spermatophores—in *A. domesticus*. Murtaugh and Denlinger (1980) provided conflicting evidence that the actual ovipositional stimulant is the sperm rather than the PGE$_2$ synthetase in the seminal fluid.

*Teleogryllus commodus*, and *A. domesticus* are the only insects reported in which the male-transferred PG synthetase is used as an ovipositional stimulant, and it should be emphasized that it is the synthetase that is transferred and not simply prostaglandins (Brady, 1983). The single reported case where PG’s are the actual factors transferred is in *Bombyx mori* (Setty and Ramaiah, 1980). Destaphano and Brady (1977) found that 90% of the entire male reproductive-tract PGE$_2$ synthetase activity was present in the seminal vesicle, which remains in the male house cricket after testectomy. Perhaps in *T. commodus*, the testes are the only source of PGE synthetase, and this could explain the difference between the two crickets, *T. commodus* and *A. domesticus*, with regards to stimulation of
oviposition by testectomized males.

Hemiovariectomy

The remaining ovary in the hemiovariectomized crickets shows size compensation for the missing ovary. The remaining ovary in the hemiovariectomized cricket had 3/4 of the weight of eggs and 3/4 of the number of eggs of a single control ovary. It is suggested that in the absence of one ovary the other grows larger and produces more mature eggs because more space and nutrients are available. This points to the importance of space for developing eggs in determining the number of eggs and the rate of egg production. The space constraints on egg production caused by filling of the body with eggs (virgins) inhibits further egg production through pressure on the gut by limiting feeding (Clifford and Woodring, 1985a). When the rate of food consumption approaches, or even falls below, the amount required for maintenance, the production of eggs slows or even stops. Maintenance energy requirements appear to take precedence over demands for oogenesis.

Egg viability

The improvement in the percent of eggs that hatch in older females (54 to 68%), was partially due to the multiple mating of older females, which increased the supply and source of sperm available for fertilization.
Probably a more important factor in the improvement in the percent of eggs that hatch involved the rate of oviposition. Crickets will lay 200 or more eggs at the time when egg laying is initiated (Figure 4), occasionally depositing nearly 400 eggs at one time. At this rate of egg laying the eggs would pass through the genital chamber too fast for each to receive a sufficient dose of semen from the spermatheca via the fertilization spout in order to be fertilized. As the rate of oviposition decreases with age, the conditions for more efficient fertilization probably occur with the eggs remaining for a longer time in the genital chamber.

Resorption of eggs

Egg resorption occurs in representatives of several of the major orders of insects (Chapman, 1982). Starvation, enforced virginity, and aging, among other causes, may result in egg resorption (Bell and Bohm, 1975). Resorption of oocytes occurs in Periplaneta americana after 7-10 days of starvation (Bell, 1971). This may help explain how these cockroaches were able to withstand 30+ days of starvation. Schistocerca gregaria was also capable of resorbing oocytes when starved (Highnam et al., 1966). The ability to resorb oocytes provides a means by which some insects can survive times of inadequate food supply (Bell and Bohm, 1975). With few exceptions (Bell and Bohm, 1975),
oocytes in the ovarioles can be resorbed, whereas eggs, already ovulated and stored in the oviduct, cannot be resorbed. *A. domesticus* had comparatively little mass in terminal oocytes at any particular time, and their resorption would probably play little role in survival during starvation. The nutrients tied up in the already produced eggs appeared to be inaccessible to the cricket when starved (Table 5). There was also no evidence that a cricket could resorb some yolk from individual eggs as there were no differences in the average weight of an egg after starvation. Adequate energy stores for longer survival may be present in the cricket but the use of these stores may be blocked by the absence through depletion of certain necessary micronutrients.

**Juvenile hormone esterase**

Rotin et al. (1982) measured the activity of the juvenile hormone esterase (JHE) in mated female *Diploptera punctata* and found that JHE peaks two days after the JH peak (on day 5) and that this JHE peak occurs prior to the initiation of oviposition. They implied that JHE may reduce the level of active JH, which then terminates vitellogenesis and oogenesis when *Diploptera* is in the gestation period. JHE reduces the amount of active JH by converting it to JH acid. Repeated peaks of JH titre in the blood were reported by Renucci et al. (1984) during the first several days in
virgin *A. domesticus* at 33°C, and each peak was followed by a peak of JHE. JHE titres of about 3-5 nmoles/min/ml was found in the blood of *A. domesticus*, which was in the range shown by Renucci et al. (1984). The unvarying low titres of JHE in both the blood and tissues of adult virgins during the time that they filled with eggs and ceased oogenesis, and the same low titre in the blood of actively oogenic laying females, indicated no role for JHE in the control of oogenesis in *A. domesticus*.

The reproductive process in any animal has to be one of the most important for the individual and the species. Oogenesis in the house cricket seems to be initiated by JH release even when conditions such as those caused by starvation are inappropriate for successful reproduction. Egg production continues in virgin crickets until they become so full of eggs that space restrictions limit feeding, which slows oogenesis. Mating followed by egg laying allows continued feeding and oogenesis, thus more eggs can be made, but egg laying ends with the exhaustion of the PGE₂ synthetase supply in the spermatheca. Normally, oogenesis and oviposition continue until death. Once started, the only means to terminate oviposition (aside from death) is to stop mating and allow the supply of PGE₂ to become exhausted. Once the ovipositional stimulus is gone and oviposition has been terminated, the female's oviducts
expand as they fill with eggs to occupy most of the body cavity which will eventually stop oogenesis.
Acknowledgements—Many of these experiments required the performance of several tedious tasks. I appreciate the work of Gregory Moncada, Hoa Nguyen, Tom Perkins, Doyle Nolan, Pam duRousseau, Henry Rouquette, and the many others who helped in rearing and experimentation.
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Table 1. Effect of mating on egg production in 10-day and 15-day adult females.

<table>
<thead>
<tr>
<th></th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Number of Eggs</th>
<th>Egg Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mated 10-day</td>
<td>559±22(10)*</td>
<td>183±12*</td>
<td>434±32*</td>
<td>0.43±0.008*</td>
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<tr>
<td>Virgin 10-day</td>
<td>635±39(5)</td>
<td>196±12</td>
<td>442±53</td>
<td>0.45±0.014</td>
</tr>
<tr>
<td>Mated 15-day</td>
<td>646±30(9)*</td>
<td>223±14*</td>
<td>538±34*</td>
<td>0.42±0.01*</td>
</tr>
<tr>
<td>Virgin 15-day</td>
<td>622±34(6)</td>
<td>225±23</td>
<td>516±56</td>
<td>0.44±0.02</td>
</tr>
</tbody>
</table>

Females were not allowed to lay eggs by not providing an appropriate egg-laying medium. There were equal numbers of males and females in the experimental cartons. All weights are in milligrams. All values are expressed as means ± 1 S.E. and the number of observations is given in parentheses.

*--Not significantly different from virgins of the same age group.
Table 2. Effects of prostaglandin E₂ on oviposition activity when injected into the hemocoel vs. topically applied at the genital chamber of 15-day adult virgin house crickets

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of eggs laid first day post-application</th>
<th>Number of eggs laid second day post-application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemocoel injection</td>
<td>66±35(5)**</td>
<td>-0-</td>
</tr>
<tr>
<td>Injection control</td>
<td>-0- (5)</td>
<td>-0-</td>
</tr>
<tr>
<td>Topically applied at genital chamber</td>
<td>189±58(5)**</td>
<td>19.4±13</td>
</tr>
<tr>
<td>Adjuvant control</td>
<td>-0- (5)</td>
<td>-0-</td>
</tr>
<tr>
<td>Tactile control</td>
<td>-0- (10)</td>
<td>-0-</td>
</tr>
</tbody>
</table>

Prostaglandins initially dissolved in 100% ethanol and suspended in Freund's Incomplete Adjuvant for treatment. The dose (50ug) was either injected into the body cavity or topically applied at the opening of the genital chamber. The injection and adjuvant control contained adjuvant only.

**-significantly different (p<0.01) from the control(s).

+-significantly different (p<0.10) from the other treatment.

There were no significant differences (p>0.10) on the second day post-application.
Table 3. Duration of egg-laying period following removal of the male from mated female.

<table>
<thead>
<tr>
<th>Day</th>
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<th>C</th>
<th>D</th>
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<td>362</td>
<td>2134</td>
<td>1057</td>
<td>3210</td>
<td>1547</td>
</tr>
<tr>
<td></td>
<td>60 Day Total 1687</td>
<td>758</td>
<td>2718</td>
<td>1057</td>
<td>3308</td>
<td>1547</td>
</tr>
</tbody>
</table>

Each carton contained a male-female pair until the male was removed. Female in carton with male removed on day 9 escaped. Females typically lay eggs for at least 60 days.

*=Day on which the male was removed from the respective carton.

**=A new male added to each respective carton.

+=Cricket drowned.
Table 4. Response of adult female house cricket to hemiovariectomy.

<table>
<thead>
<tr>
<th></th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Number of Eggs</th>
<th>Egg Weight</th>
<th>Fat Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>627±27(11)</td>
<td>215±15</td>
<td>460±37</td>
<td>0.47±0.01</td>
<td>18±2</td>
</tr>
<tr>
<td>Hemiovariectomy</td>
<td>561±19(10)</td>
<td>156±12*</td>
<td>315±25*</td>
<td>0.49±0.01</td>
<td>15±3(4)</td>
</tr>
<tr>
<td>Half-Control</td>
<td>107</td>
<td>230</td>
<td>0.46</td>
<td>-----</td>
<td>---</td>
</tr>
</tbody>
</table>

Hemiovariectomy was performed on 1-day adults before the ovary began to develop, and dissected on day 16 when the ovary had reached a maximum, stable size. One half the control ovary weight and one half the control number of eggs is given for comparison. All weights are in milligrams. Values are expressed as means ± 1 S.E.

**—Significantly different (p<0.01) from the control values.**
Table 5. Effects of starvation on egg resorption and fat body use in adult female virgin crickets of matched initial weight.

<table>
<thead>
<tr>
<th></th>
<th>Initial Weight</th>
<th>Final Weight</th>
<th>Ovary Weight</th>
<th>Number of Eggs</th>
<th>Egg Weight</th>
<th>Fat Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved 5days</td>
<td>565 (10)</td>
<td>498 ±28 (10)</td>
<td>170 ±20</td>
<td>405 ±40</td>
<td>0.42 ±0.012</td>
<td>8.1 ±0.69</td>
</tr>
<tr>
<td>Control</td>
<td>552 (10)</td>
<td>565 ±30 (10)</td>
<td>190 ±13</td>
<td>413 ±31</td>
<td>0.46 ±0.007</td>
<td>14.2 ±2.1</td>
</tr>
</tbody>
</table>

The crickets were 15-day-old adults at the start of the experiment and they were starved for 5 days. Control crickets had food ad libitum. The matched initial weight groups were formed by taking a large number of 15-day adult crickets and weighing them out until two groupings of approximately the same total weight were obtained. All values (except the initial weights) are expressed as means ±1 S.E. and the number of observations is in parentheses. All weights are in milligrams.
<table>
<thead>
<tr>
<th></th>
<th>Body Weight</th>
<th>Ovary Weight</th>
<th>Fat Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved from day 0 adult to day 7 adult</td>
<td>$389\pm16(16)^*$</td>
<td>$21\pm3(16)^*$</td>
<td>$10.6\pm1.2(16)^*$</td>
</tr>
<tr>
<td>Starved from day 5 larvae to day 7 adult</td>
<td>$370\pm17(15)^*$</td>
<td>$10\pm1.4(15)^*$</td>
<td>$6.7\pm0.37(15)^*$</td>
</tr>
<tr>
<td>Normal day 7 adult</td>
<td>$521\pm14(30)$</td>
<td>$154\pm6(15)$</td>
<td>$23\pm3(6)$</td>
</tr>
</tbody>
</table>

Crickets were starved either from the first day after adult ecdysis (day 0) to day 7 of the adult or from the middle of the last instar through day 7 of the adult. Water was provided ad libitum. All weights are in milligrams. All values are expressed as means ± 1 S.E. and the number of observations is in parentheses.

*-Significantly different (p<0.01) from the normal day 7 values.
Figure 1. Female reproductive system of the house cricket, *Acheta domesticus*. A. Inner view of the right half of the posterior abdomen (modified from Snodgrass, 1933). B. Ventral detail of the posterior abdomen with internal parts of the genitalia shown by dashed lines. Abbreviations:

- C-cercus
- CG-cercal ganglion
- CN-cercal nerve
- CO-common oviduct
- DP-dorsal pouch of GC
- EP-epiproct
- FS-fertilization spout
- GC-genital chamber
- LO-lateral oviduct
- P-membranous pleuron
- PP-paraproct
- R-rectum
- SD-spermathecal duct
- SP-spermatheca
- S7-stermite 7
- S8-stermite 8
- T7,8,9-tergites
- VN-ventral nerve cord
- V1,2,3-valves of ovipositor
Figure 2. A. Top view of the house cricket spermatophore. B. Side view of the house cricket spermatophore. Abbreviations:

A-ampulla  P-papilla
C-cup       T-tube
H-handle
Figure 3. Change in the body weight (■), ovary weight (□), and number of eggs (♦) in adult female house crickets from day 1 to day 30 of adult life. Body weight in milligrams or actual number of eggs are shown on the same Y-axis. Error bars are ± 1 S.E. and may be smaller than the symbol. On day 5, the average egg weight is close to 1 mg but after this time the average egg weight decreases to about 0.5 mg, which explains the divergence of the ovary weight and the egg number lines on day 5.
Figure 4. Average egg production of adult female house crickets, *Acheta domesticus* L., from day 0 to 60. A peak of egg production is usually significantly different from an adjacent low point at the p<0.05 level. Error bars are not shown to avoid clutter.
Figure 5. Average adult food consumption of female house crickets from day 0 to 40. Error bars are not shown to avoid clutter.
Figure 6. Food consumption of unmated males \((m, 
\blacksquare\)\), virgin females \((f, 
\square\)\), and mated males and females \((m&f, 
\blackdiamond\)\) from the first day after adult ecdysis through day 15. There is a 375mg cumulative difference between the food consumption of the virgin males and females \((vm+v\text{f})\) and the food consumption of the mated males and females \((m&f)\). This amount is an indication of the mg of food needed to produce and lay the eggs. Error bars are ± 1 S.E. and may be smaller than the symbol.
PART 2.

The Effects of Enforced Virginity and Ovariectomy on Growth, Reserves, and Metabolism in the House Cricket, *Acheta domesticus* L.

KEYWORDS: *Acheta domesticus* L., enforced virginity, ovariectomy, egg production, oogenesis, fat body, oxygen consumption
ABSTRACT

The amount of feeding, growth, fat body reserves, and rate of oxygen consumed was affected by ovariectomy and enforced virginity in adult female *Acheta domesticus* L. from the start of the adult stage to day 18. Normal crickets had initiated oogenesis, mated and started egg laying by day 9 (day of adult ecdysis = day 0). Virgin crickets gained more weight than the mated or the ovariectomized crickets because they retained all of the eggs they produced. Mated and ovariectomized crickets gained about 125 mg in the first 6 days as they had very few or no eggs in storage. After 18 days, the mated females had the least amount of FB (8±0.6 mg) and the ovariectomized crickets had the most (38±3.0 mg). The amount of FB was inversely related to the number of eggs produced and mated crickets produced the most eggs. FB reserves, although only contributing a fraction of the nutrients for egg production, determined the length of survival when the crickets were starved. Feeding peaked between days 2-4 at about 80 mg/cricket/day and then decreased in the virgin and the ovariectomized crickets, but it remained at roughly 80 mg/cricket/day in the mated crickets due to the energy needs for the production and the laying of eggs. The rate of oxygen consumed was highest during the first days after the final ecdysis (1.5-1.7 ml O₂/g wet weight/hr) and
remained high in the ovariectomized crickets through day 18. A significant decrease occurred in the mated females, but the lowest value measured (0.68 ml O₂/g wet weight/hr) occurred in the virgin females on day 18. This decrease was a direct effect of the large mass of nonmetabolizing eggs in storage in the virgins. Eggs ovulated and sequestered in the female have not been fertilized and apparently do not consume measurable oxygen.
INTRODUCTION

The production of eggs represents the major metabolic sink for adult female insects. Although some somatic growth occurs in adult insects (Hill et al., 1968), most somatic growth occurs in the immature stages; most ovarial growth takes place in the adults. Enforced virginity caused the crickets to produce and maintain a maximum store of eggs, and ovariectomy prevented the crickets from producing any eggs at all. The fat body (FB) is the source of the precursors for egg production (Hagedorn and Kunkel, 1979). There are apparently no previous studies comparing ovarial growth, reserves, and metabolic rate in orthopteroid insects as affected by enforced virginity, mating, and ovariectomy. The effects of ovariectomy on various organs and physiological parameters has been studied in Melanoplus sanguinipes (Gillott and Elliott, 1976), Leucophaea maderae (Engelmann, 1978), Acheta domesticus L. (Bradley and Edwards, 1978; Bradley and Simpson, 1981), and Locusta migratoria (Mwangi and Goldsworthy, 1980). Virginity and its effect on corpus allatum activity in Periplaneta americana was studied by Weaver and Pratt (1977). The purpose of my work was to quantify how growth, metabolic rate (MR), and the fat body responded to ovariectomy and enforced virginity. This approach
will help to better define the changes associated with the initiation of reproduction.
METHODS

Rearing

The house crickets used in this study were reared according to the methods described by Clifford et al. (1977) at 30°C on a 12:12 LD cycle. All weights except for FB are wet weights and were determined, as were the other parameters, for days 0, 2, 6, 12, and 18 of the adult stage. Day 0 was the day on which the crickets molted and became adults.

Experimental groups

The experimental groups (reproductive states) in this study need to be clearly defined. Virgins were adult female crickets that were not mated, exposed to males since adult ecdysis, or allowed access to an ovipositional medium. Ovariectomized crickets (ovaries removed) were also never exposed to males as adults. Mated females always had access to males, but their ovipositional activity could be regulated by access to an ovipositional medium. The laying females always had access to ovipositional sites and laid eggs on a regular basis, whereas nonlaying females (not studied here) would not have access to ovipositional sites and did not lay eggs (Clifford and Woodring, 1985).
Ovariectomy

Crickets were ovariectomized on day 1 of the adult stage. A 2-3 mm slit was made dorsolaterally on each side of the insect between the 3rd and 4th abdominal segments. Gentle pressure around the slits caused the ovaries to protrude completely out of the slits and were then easily removed. Survival was total without either antiseptic procedures or postoperative antibiotic treatments (Clifford and Woodring, 1985).

FB assay

The fat body was washed out of the pinned-open cricket carcass (from which the gut had been removed) with a stream of water from a wash bottle to which a fine-pointed disposable pipette tip was attached. The pipette tip served to increase the force of the jet. A final inspection and picking at trapped FB masses was done prior to the last rinse. The wash water was collected in tared disposable plastic beakers and oven dried at 70°C for 48 hrs. This method of removal and estimation of the FB was simple and gave consistent results within experimental and control groups.

Oxygen consumption

Oxygen consumption was determined with a Gilson differential respirometer, 30% KOH being added to a
rolled-up paper wick in the sidearm of each 20 ml flask to absorb all expired CO\(_2\). All determinations were preceded by a 30-minute equilibration period, taken for at least 30 minutes, and corrected for STP.

**Food consumption**

Food consumption was determined by daily weighing of the food container from the rearing cartons, the difference being the amount of food consumed during that period. Control cartons without crickets were also weighed in order to detect possible humidity-induced weight changes of the food.

**Oviposition**

On the average oviposition began by day 9 (Clifford and Woodring, 1985). The number of eggs laid by the mated females was measured daily by placing a pan of moist sand in the carton. The eggs were washed from the sand, collected and counted. Although moist peatmoss is a more suitable oviposition medium because it retains moisture, sand was used since the eggs are more easily removed from sand than from peatmoss for counting. In order to determine the number of eggs remaining in a mated or virgin female, the ovary and oviduct with the mass of stored eggs was removed, opened, and counted.
RESULTS

Growth

There were no significant differences in the total body weight among the mated, ovariectomized, and virgin crickets until day 18 (Figure 1), when the virgin crickets were significantly (p<0.01) heavier than members of the other two groups.

Food consumption

Determination of food consumption for mated female crickets was complicated by the fact that there had to be a male present to fertilize the female, but inclusion of the food consumption of the male was not desired. To determine the food consumed by mated female crickets, the food consumption was measured for male and female pairs and then the food consumed by isolated males was subtracted from that for the pair (Table 1).

The amount of food consumed per day by all three groups was the same for the first three days of the adult stage (Table 2). By day 6, the daily food consumption rate of the mated crickets (Figure 2) was much greater than the daily consumption rate of either the virgins (Figure 3) or the ovariectomized females (Figure 4). This trend continued until the amount of food consumed daily by the virgins and the ovariectomized crickets had decreased to 15-30
mg/cricket/day.

FB reserves

Initially, the FB reserves of all three groups was the same (Table 3), and the reserves of the virgin and the mated crickets remained the same through the first 10 days of adult life. The FB reserves of virgin females dropped and leveled off at about 20 mg by day 6 (Figure 2). The FB reserves of mated female crickets, on the other hand, continued to decline until day 12 and leveled off at only 8 mg (Figure 3). The FB reserves of the ovariectomized crickets remained high with 38±3 mg still present by day 18 (Figure 4).

Starvation survival

Mated crickets starved from day 15 survived for 5.5±0.6 days (8). Ovariectomized crickets lived for 10.2±0.8 days (9) when starved at the same age.

Egg production

Naturally, there were no eggs produced by the ovariectomized crickets. Egg production for virgin and mated crickets was virtually the same through day 6, but by day 12 a significant difference developed (Figure 5). The virgin crickets continued to produce and store eggs to the extent that an 18-day virgin contained 248±14 mg of eggs. The mated females began laying on day 9, but
they contained fewer eggs once laying began than did virgin females. The average weight of eggs laid by the mated crickets from day 6 to day 12 was 215 mg, and the weight of eggs laid between day 12 and day 18 was 332 mg. The total weight of eggs produced and laid by mated females exceeded the weight of eggs produced and retained by virgins (Figure 5).

**Oxygen consumption**

The metabolic rate in the three groups was the same through day 2 (Table 4). The rate of oxygen consumption in the ovariectomized females remained high throughout the test period (Figure 4). The oxygen consumption of the mated females decreased to a very low level on day 6 and then increased to its day-2 level by day 12 and remained constant (Figure 2). The rate of oxygen consumption in the virgins steadily declined from the maximum on day 0 to a low on day 12, and it remained low for the remainder of the test period (Figure 3).

Fertilized, developing eggs consumed 0.26 ml O$_2$/g wet weight/hr, while the MR of 150 mg of unfertilized eggs (laid or unlaid) was unmeasurable after a 30 min reading in a respirometer.
DISCUSSION

Growth

After 12 days, the virgin females weighed more than either the ovariectomized or the mated females. This was the result of the fact that the virgins retained all the eggs they produced whereas mated crickets did not retain their eggs. The total food consumption of the mated crickets was greater than that of the other two groups (Table 2), because after 18 days the mated crickets had produced (contained and laid) significantly more eggs than the virgins (Figure 5). As more eggs were produced, more food was required to do so. The virgins produced as many eggs as they could hold and were forced to stop egg production because the egg-filled oviducts occupied most of the body cavity (Clifford and Woodring, 1985). The mated crickets did not retain the eggs for very long, but they fertilized the eggs with sperm from the spermatheca and then oviposited them. Thus the mated females produced more eggs overall than did the virgins. The ovariectomized crickets could not produce eggs, but they remained about the same weight as the mated crickets through 18 days (Figure 1). Although the ovariectomized females lacked eggs, they had about 30 mg more FB present than did mated females. In addition, the blood volume of ovariectomized crickets was about 25% greater than in
virgins (Clifford and Woodring, unpublished). This extra blood would account for another 20-25 mg (=20-25 ul) of the weight in the ovariectomized crickets.

**FB reserves**

The large amount of fat body (FB) present on day 0 in the adult female was produced and stored during the last larval stadium (Woodring et al., 1979). The source of material to make the eggs came predominately from the food eaten during vitellogenesis (Clifford and Woodring, 1985), but FB reserves were depleted to an extent determined by the number of eggs produced. The FB of mated and virgin females declined by the same amount through day 6. From day 6-12, the FB of virgins declined very little because their egg production tapered off to zero. The FB of the mated females declined faster to a lower level by day 12 because they started egg laying by day 9 and continued egg production. By day 18, the FB of virgins was double that of mated females. Since ovariectomized females produced no eggs, the FB reserves did not diminish over the test period. There was a significant increase in the weight of FB in ovariectomized *Melanoplus sanguinipes* over that found in the intact grasshoppers (Gillott and Elliott, 1976). They felt that the increase in FB weight after ovariectomy indicated that the FB was the main source of yolk precursors in
*M. sanguinipes*. In contrast, in *A. domesticus* there is a maintenance of FB in the ovariectomized crickets but no increase.

The FB mass in mated female *Acheta domesticus* more than 12 days old might represent the minimum FB mass necessary to process food to produce the egg material. It is curious that the FB reserves were gradually consumed even though ample food was available. What could be the function of the stored reserves in the FB when they represent such a small portion of the amount needed to produce the eggs? The 30 mg of FB used up from day 0 to day 18 in the mated females would only supply the nutrients for about 60 eggs. Any eggs produced after day 18 had to utilize nutrients from the food. The FB appears to store an immediate, though minimal, reserve for egg production but is itself the site of processing for the egg yolk precursors.

The FB may also serve as a means to survive short-term starvation. Oocyte or egg resorption does not occur in house crickets (*Clifford and Woodring, 1985*) and cannot, therefore, serve as an energy source during periods of starvation. Since the ovariectomized crickets survived twice as long as either the virgin or the mated crickets, it appears that the amount of FB is the prime determinant of length of survival when starved. Larval crickets, that also contain large FB reserves, survived more than ten days when starved.
Oxygen consumption

House crickets fertilize their eggs individually just as the eggs begin to enter the ovipositor (Clifford and Woodring, 1985). Unfertilized eggs did not consume any measurable amounts of oxygen during the test period. Therefore, the large stores of eggs in the oviducts of virgin females represented an essentially nonmetabolizing one-third of the total body weight. The metabolic rate of the virgin, mated, and ovariectomized crickets was the same for the first three days of adult life. With ovulation starting as early as day 5, the MR decreased in the virgins and the mated females because they accumulated a store of nonmetabolizing material (eggs). The MR of ovariectomized females remained constant throughout the 18 days. Thus the number of stored eggs appears to have considerable influence on the MR. If the 176 mg of eggs in the mated crickets were to be subtracted from the average weight for the 6-day mated female and the MR were based on the remaining tissue weight, then the MR of the mated crickets would be the same as that for the ovariectomized crickets (1.42 ml O₂/g wet weight/hr recalculated day-6 mated vs. 1.35 ml O₂/g wet weight/hr from Table 3). Thus the decreased MR of the virgin crickets was due to the large mass of eggs that they
contained. The MR of the virgin crickets continued to decline through day 18 because they were still producing and retaining eggs. By day 18, the virgins became filled to their limits with eggs. Because the mated females started egg laying on day 9 and thus reduced their store of nonmetabolizing eggs, by day 12 their MR approached that of the ovariectomized crickets.

Typically, MR is influenced by food consumption as has been reported in larval house crickets (Woodring et al., 1977) where the food consumed was used for somatic growth and the changes in MR followed food consumption. The food consumed by adult insects can be used for either somatic growth or for ovarian growth. If the food is used simply for somatic growth, then the MR rate should increase as in the larvae. If the food is used for ovarian growth, the MR does not increase for two reasons. Much of the ingested food is converted for egg production. Also, because MR is calculated on the basis of total body weight, oxygen consumption will be lowered by the mass of nonmetabolizing eggs stored in the body. Evidently, because the MR of the mated and ovariectomized crickets are more similar than that of the virgins, it is the presence of the eggs in the crickets that is most responsible for the differences in MR.

The FB in A. domesticus is the processor of the precursors for the production of eggs. However, the
actual amount of FB in virgins and mated females is much less than that found in last-larval-instar or ovariectomized crickets because in the latter two groups there is a considerable nutrient reserve in the FB. These reserves serve for short-term survival because the energy stored is insignificant for overall egg production. This indicates a dynamic cycling of food nutrients into egg precursors by way of the FB. The changes in MR are more understandable when recalculated and based on the actual quantity of actively metabolizing tissue. In fact there is no significant difference in the MR of the experimental groups when based on the body weight minus the weight of eggs. Eggs held in virgin crickets represent a wasted store of material if mating does not occur.
REFERENCES


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Physiol. 23, 207-212.

Woodring J.P., Clifford C.W. and Beckman B.R. (1979)  
Food utilization and metabolic efficiency in larval  
and adult house crickets. J. Insect Physiol. 25,  
903-912.
Table 1. Determination of food consumption by mated female house crickets on selected days.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Males + Females</th>
<th>Isolated Males</th>
<th>Mated Females*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>63±5(9)</td>
<td>23.0±1.3(8)</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>115±12(9)</td>
<td>31.6±2.8(8)</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>85±14(9)</td>
<td>14.8±1.1(8)</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>96±5(8)</td>
<td>15.7±1.6(8)</td>
<td>80</td>
</tr>
<tr>
<td>18</td>
<td>102±10(7)</td>
<td>16.2±2.4(8)</td>
<td>86</td>
</tr>
</tbody>
</table>

All values are expressed as mean weights in milligrams ±1 S.E. and the number of observations is in parentheses.

*Determined by subtraction.

Table 2. The effect of enforced virginity and ovariectomy on food consumption for selected days.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Virgin Females</th>
<th>Mated Females*</th>
<th>Ovariectomized Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>34±6(60)</td>
<td>40</td>
<td>36±3(15)</td>
</tr>
<tr>
<td>2</td>
<td>76±3(60)</td>
<td>83</td>
<td>85±5(14)</td>
</tr>
<tr>
<td>6</td>
<td>44±3(60)</td>
<td>70</td>
<td>32±2(20)</td>
</tr>
<tr>
<td>12</td>
<td>30±2(60)</td>
<td>80</td>
<td>28±2(20)</td>
</tr>
<tr>
<td>18</td>
<td>15±2(60)</td>
<td>86</td>
<td>26±2(15)</td>
</tr>
</tbody>
</table>

All values are expressed as mean weights in milligrams ±1 S.E. and the number of observations is in parentheses.

*From Table 1.

Table 3. The effect of enforced virginity and ovariectomy on fat body (FB) reserves.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Virgin Females</th>
<th>Mated Females</th>
<th>Ovariectomized Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>43±3(16)</td>
<td>40±3.0(6)</td>
<td>42±3(6)</td>
</tr>
<tr>
<td>2</td>
<td>38±3(16)</td>
<td>36±3.1(6)</td>
<td>34±4(6)</td>
</tr>
<tr>
<td>6</td>
<td>24±2(14)</td>
<td>23±3.0(6)</td>
<td>57±6(10)</td>
</tr>
<tr>
<td>12</td>
<td>20±2(14)</td>
<td>9±0.7(6)</td>
<td>40±4(9)</td>
</tr>
<tr>
<td>18</td>
<td>17±2(10)</td>
<td>8±0.6(6)</td>
<td>30±3(9)</td>
</tr>
</tbody>
</table>

All values are expressed as mean weights in milligrams ±1 S.E. and the number of observations is in parentheses.
Table 4. The effect of enforced virginity and ovariectomy on metabolic rate.

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Virgin Females</th>
<th>Mated Females</th>
<th>Ovariectomized Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.56±0.06(10)</td>
<td>1.63±0.06(6)</td>
<td>1.53±0.05(6)</td>
</tr>
<tr>
<td>2</td>
<td>1.39±0.05(10)</td>
<td>1.36±0.05(6)</td>
<td>1.43±0.05(6)</td>
</tr>
<tr>
<td>6</td>
<td>1.01±0.07(10)</td>
<td>0.95±0.05(6)</td>
<td>1.35±0.05(6)</td>
</tr>
<tr>
<td>12</td>
<td>0.76±0.07(10)</td>
<td>1.37±0.07(13)</td>
<td>1.58±0.02(6)</td>
</tr>
<tr>
<td>18</td>
<td>0.68±0.07(8)</td>
<td>1.30±0.05(6)</td>
<td>1.47±0.08(6)</td>
</tr>
</tbody>
</table>

All measurements are expressed as means in milliliters O₂/g wet-weight/hour ±1 S.E. and the number of measurements is in parentheses. Readings were calculated from 30-minute measurements taken at the same time of day and corrected for STP.
Figure 1. The effect of enforced virginity and ovariectomy on total body weight in milligrams as compared to mated females. Symbols used are as follows: ■ = mated females; □ = ovariectomized females; and ◆ = virgin females. Each point on the graph for the ovariectomized and virgin females represents at least 24 individuals and each point for the mated females represents at least 6 individuals. All points are ±1 S.E. and in some cases the plotting symbol may be larger than the S.E.
Figure 2. Mated females. Interrelationship of food consumption, FB reserves, and oxygen consumption during the first 18 days of the adult. Symbols used are as follows: □ = food consumption in milligrams; ■ = FB weight in milligrams; and ◆ = oxygen consumption in milliliters/gram wet weight cricket/hour. All points are ±1 S.E. and in some cases the plotting symbol may be larger than the S.E.
Figure 3. Virgin females. Interrelationship of food consumption, FB reserves, and oxygen consumption during the first 18 days of the adult. Symbols used are as follows: □ = food consumption in milligrams; ■ = FB weight in milligrams; and ◆ = oxygen consumption in milliliters/gram wet weight cricket/hour. All points are ±1 S.E. and in some cases the plotting symbol may be larger than the S.E.
Figure 4. Ovariectomized females. Interrelationship of food consumption, FB reserves and oxygen consumption during the first 18 days of the adult. Symbols used are as follows: □ = food consumption in milligrams; ■ = FB weight in milligrams; and ♦ = oxygen consumption in milliliters/gram wet weight cricket/hour. All points are ±1 S.E. and in some cases the plotting symbol may be larger than the S.E.
Figure 5. Comparison of egg production in milligrams in virgin and mated females. Symbols used are as follows: ◆ = milligrams of eggs produced and retained by the virgin females; ■ = milligrams of eggs contained by the mated females; and □ = milligrams of eggs laid plus the milligrams contained by the mated females. Egg laying normally began by day 9 and, by day 12, mated females had laid on the average 215 mg of eggs and, by day 18, had laid an additional average 332 mg of eggs. Each point on the graph for the virgin females represents at least 10 individuals and for the mated females at least 6 individuals.
PART 3.

Electrophoretic and Chromatographic Characterization of Vitellogenin and Its Variation in the House Cricket, *Acheta domesticus* L.

Keywords: *Acheta domesticus* L., vitellogenin, vitellin, chromatography, electrophoresis, ovariectomy.
ABSTRACT

The vitellogenin (Vg) of Acheta domesticus L. was isolated and purified and found to have a molecular weight (MW) of 400,000 daltons by gel filtration chromatography on Sepharose-6B. There are at least 2 polypeptide subunits with MW's of 112,000 and 40,000 daltons determined from SDS-polyacrylamide gel electrophoresis (PAGE). The native protein is a lipoprotein containing 9.6% lipid and 6.6% carbohydrate, and it has an isoelectric point (pI) of 5.7 to 6.4. The amino acid molar percents were determined for both vitellogenin (Vg) and vitellin (Vn), from which the sum of the differences (ΔAQ) of the amino acid molar percents between them and other Vg’s and Vn’s were calculated. An ΔAQ of 6.2 between the Vg and Vn of A. domesticus indicates closely related molecules. The calculated ΔAQ’s indicate that the Vg and Vn of A. domesticus are more closely related to those molecules in cockroaches than in locusts. The amino acid composition of the native Vg conformed to a predicted model of a nutritive protein.

Blood plasma or serum was run on SDS-PAGE, stained with Commassie Blue, and scanned with a densitometer to determine relative changes in the Vg concentration. The factors starvation, aging, and egg laying lowered the Vg titre more than these factors lowered the titre of the
other blood proteins while ovariectomy elevated the level of Vg in the blood as compared to the other blood proteins.
INTRODUCTION

All animals that lay eggs must supply the egg with the necessary nutrients for it to complete development. Insects produce these nutrients in the fat body and then transport them through the blood to the ovaries (Hagedorn and Kunkel, 1979). The egg protein made in the fat body (FB) and transported by the blood is called vitellogenin (Vg); when deposited in the oocyte at which time it is modified in some species, it is called vitellin (Vn). Insect plasma proteins were reviewed by Wyatt and Pan (1978). Reviews specifically on Vg and Vn were published by Hagedorn and Kunkel (1979) and by Engelmann (1979). The purification and properties of vitellogenin/vitellin in Locusta migratoria (Gellisen et al., 1976; Chen et al., 1978; Chinzei et al., 1981), in Leucophaea maderae (Dejmal and Brookes, 1968; Engelmann and Friedel, 1974; Engelmann et al., 1976; Masler and Ofengand, 1982), and in Periplaneta americana (Nielsen and Mills, 1968; Bell, 1970; Clore et al., 1978; Sams et al., 1980) illustrate some of the species studied.

Considerably less information is available on the house cricket, Acheta domesticus (Kunz and Petzelt, 1970; Bradley and Edwards, 1978). One purpose of this study was to characterize Vg and Vn in A. domesticus. The second aim was to understand the structure of Vg and Vn, and how closely related these molecules are to the Vg's
and Vn's in related orthopteroids. The final purpose was to monitor changes in Vg titre during oogenesis, and to determine the effects of starvation, ovariectomy, and egg laying.
METHODS

Rearing

Crickets were reared according to the methods of Clifford et al. (1977) at 30°C on 12:12 LD. The crickets were fed Purina cricket chow ad libitum. Newly molted females were isolated in groups of 10-12 and maintained in gallon cartons. Food and water were renewed every five days. Under these conditions, the lifespan of the adult female averaged 60 days.

Blood collection

Blood was collected by cutting off one or both front legs at mid-coxa from which 20-50 ul of blood per cricket could be obtained. Plasma was obtained by bleeding a cricket after a one-min immersion in 60°C water. The heat inactivated the hemocytes, which then did not rupture, thus preventing coagulation. The cells in the collected blood were removed by centrifugation. Serum was obtained by bleeding a cricket after a 10-sec immersion in 60°C water. The brief heat and stress intensified the clotting process, and the cells ruptured rapidly and the blood clotted thoroughly. The clot and cellular debris were removed by centrifugation at 8,000 xg for 5 min.
Separation of vitellogenin

Vitellogenin separation was accomplished using a slight modification of the low ionic strength precipitation method of Chinzei et al. (1981). After precipitation in 70% ammonium sulfate, the proteins are dissolved in distilled water. The protein solution is dialyzed in 4°C distilled water until a turbidity appears, which is then removed by centrifugation at 10,000 xg. The precipitate contains the Vg and is redissolved in distilled water. This method was used to isolate the two major lipoproteins, lipophorin and Vg, in the plasma of A. domesticus. The principal difficulty with this method was in the definition of turbidity. The final step requires anion-exchange chromatography (DEAE cellulose) to separate the two lipoproteins, with the Vg binding and the lipophorin passing through. Vitellogenin is eluted with 0.8 M KCl.

An alternative method was devised for the separation of Vg from other egg or blood lipoproteins prior to further purification. The isolation of Vg requires not only the removal of lipophorin, the lipid-carrier protein, but also the removal of several other proteins present in smaller quantities as well as of amino acids, carbohydrates, and lipids. Because lipophorin is also the principal component of a clot, a thorough clotting of the blood will remove most lipophorin. However, it appears that some Vg is also
lost in the clot. The speed and the degree of clotting increases directly as the concentration of blood cells increases. Immersion in 60°C water for 10 sec elevates the hemocyte count and immobilizes the cricket. Blood from these crickets was thoroughly clotted within a minute of collection. Centrifugation at 12,000 xg separates a clear, almost colorless serum. Vitellogenin is separated from all these other serum components by anion-exchange chromatography on a DEAE-cellulose column. The Vg alone binds to the column when serum is eluted with the 0.15 M phosphate buffer (pH 7.3). The Vg is released and collected by elution with 0.8 M KCl in phosphate buffer. The Vg is then desalted and concentrated with an M30 Amicon stirred Ultra Filter (MW cut off 30K). The final elution fraction was concentrated to about the same volume as the starting sample. The concentrated Vg in 0.15 M buffer is stable for many weeks and was used for gel filtration, SDS-PAGE, IEF, sugar, lipid, and amino acid analysis.

A better source of lipophorin is the adult male because the male does not produce Vg. Larval crickets also lack Vg, and their blood also has a larval-specific or storage protein. Both lipophorin and larval-specific protein are glycolipoproteins and their subunits share characteristics with Vg making separation of these proteins difficult. By using thoroughly clotted adult female blood, larval storage proteins are avoided and
most lipophorins are eliminated prior to DEAE-chromatography.

Vitellin can be isolated from the eggs in a manner similar to that used for isolating Vg from the blood. The eggs are homogenized with glass homogenizers with added 0.15 M phosphate buffer. Centrifugation at 12,000 xg produces three layers: cellular debris and chorions at the bottom, a middle solution, and a floating lipid layer. The middle solution is saved and stored at 4°C. Lipophorin and other proteins do not bind with DEAE-cellulose so that they pass—along with nonprotein solutes—through the ion-exchange column. Elution with 0.8 M KCl in phosphate buffer releases the bound vitellin and gives a very sharp elution profile.

Lipid and carbohydrate content

The tests used to determine the lipid and carbohydrate content of Vg were scaled down to a 600 ul final volume. The amount of protein in each concentrated sample (from the Amicon filter) was determined using a Biuret method (Gornall et al., 1949). The lipid content was determined with a sulphophosphovanillin method (Barnes and Blackstock, 1973) and the carbohydrate with a phenol-sulfuric acid method (Montgomery, 1957). The results are an estimation of the percent lipid and carbohydrate attached to the protein (w/w).
Amino acid composition of vitellogenin and vitellin

The amino acid composition of Vg and Vn were determined from samples separated by DEAE-chromatography and concentrated by ultrafiltration as described above. Approximately 0.5 mg of each protein was dried in a small test tube. Six-N HCl was added to each tube and the tube was flame sealed under a vacuum and then heated at 110° C for 24 hr. A subsample of the digest was placed on an amino acid analyzer. The plots were manually integrated and the molar concentration of each amino acid calculated. The molar concentrations were converted into molar percents, and the sum of the square of differences (S^2) between the amino acid compositions of different proteins was calculated according to the methods of Marchalonis and Weltman (1971).

Molecular weight determination

The molecular weight of native Vg and Vn was estimated by calibrated gel-filtration chromatography with Sepharose 6-B in a column 63 X 1 cm. A sample of 250 ul with 5% glycerol added was placed on the column and eluted with 0.15 M Na phosphate buffer, pH 7.3. Three-ml fractions were monitored at 280 nm via a flow-through cell in a Coleman UV spectrophotometer. Fractions containing peaks were concentrated via ultrafiltration for further analysis. The molecular
weight standards used were: Thyroglobulin-670,000; Catalase-230,000; Gammaglobulin-158,000; BSA-65,000; Ovalbumin-44,000; and Myoglobin-17,000.

**Densitometry**

Coomassie Blue-stained gels were read on a transmission densitometer (E-C Apparatus Corp., model EC910) to obtain a graphic picture of the bands and the area under the peaks through the densitometer integrator. The integration units were relative, but quantitative comparisons of protein titre are reliable if equal-sized samples are run on the same gel.

**Isoelectric Focusing**

Broad range pH (pH 4-9) isoelectric focusing (IEF) was performed using polyacrylamide gels in a flat bed electrophoresis cell. Gels 45 x 125 x 2 mm contained 5% polyacrylamide and 1.25% ampholytes (Biolytes 3/10, Biorad). A constant power of 4 watts was supplied by an ISCO electrophoresis power supply (model 494). Running temperature was approximately 24° C for 60 min following a pH-stabilizing period of 15 min. The pH gradient in the gel was measured with a micro-pH electrode.

**SDS-PAGE**

Denaturing (SDS) polyacrylamide gels were run according to the general techniques of Laemmli (1970) in
a vertical electrophoresis apparatus. Samples were heated for 5 min at 100° C in sample buffer containing 1% SDS + 0.1% mercaptoethanol and then applied to an 8% polyacrylamide gel at pH 8.8 containing 1% SDS. Running times at 30 mA constant current was determined by the bromphenol-blue dye front and was usually about 3 hr. SDS-PAGE and IEF gels were stained for 12 hrs in Coomassie Blue solution (3:1:6 95% ETOH:Glacial acetic acid:Water + 5 g CuSO₄/1 + 0.6 g Coomassie Blue/1) and destained for 24-48 hrs in 10% Isopropanol:10% Acetic acid. The molecular weight standards used were:
Myosin-194,000; Beta-galactosidase-116,000;
Phosphorylase-B-94,000; BSA-65,000; and
Ovalbumin-44,000.
RESULTS

Amino acid composition

Table 1 and 2 is a comparison of the mole percent for each of 16 amino acids of *Acheta domesticus* Vg and Vn compared to that of several other species. The SQ for Vg and Vn of *A. domesticus* was 6.2 (Tables 1 and 2) indicating a close relationship between the two molecules.

Molecular weight determination

The ratio of the elution volume to the void volume of the Sepharose 6B column was plotted against the log MW to make a standard curve (Figure 1). The formula for the regression line is \( Y = 4.20 + (-0.463 \times X) \). The \( V_e/V_o \) for the Vg was 1.61 (Figure 2) and the value for Vn was 1.55, which indicated that Vg has a molecular weight of approximately 400,000 and Vn a molecular weight of 540,000.

Isoelectric point

The Vg of *A. domesticus* is a lipoprotein with limited solubility in the IEF gel. It precipitated when not placed near its isoelectric point (pI). To estimate the pI, it was necessary to run many lanes of Vg and place the wicks in a "stairstep" fashion. The pI could
then be estimated from the pattern of movement around the different wicks that were located at different points of the pH field. Another method to facilitate movement of Vg in the gel was to mix the Vg with the gel solution before casting. The isoelectric point determined for Vg was 6.4 when the protein was mixed in the gel before casting and 5.7 when applied to the gel surface in the "stairstep" wicks.

**Lipid-carbohydrate content**

Vitellogenin contained (w/w) 6.6±1.2% (5) carbohydrate and 9.6±1.8% (5) lipid with the rest being protein and Vn contained (w/w) 8±1.0% (2) carbohydrate and 8.3±0.8% (2) lipid and the rest was protein.

**Subunits**

An electropherogram of the DEAE-cellulose column isolate from the Vg extraction procedure performed on adult male and female cricket plasma shows that Vg is lacking in the male sample (Figure 3A, lanes 1 and 2). A series of lanes from a gel run on the plasma of adult females aged from day 1-10 (Figure 3B) show that Vg is not present in the lanes for days 1 and 2, but appears by day 3 and persists thereafter. The nonoverlap of certain bands of Vg and lipophorin (Figure 3C) allows whole plasma to be run on SDS gels and to be scanned as a means of determining changes in the Vg titre.
Standards run on the SDS-PAGE (Figure 3A, lane 3) were scanned on the densitometer (Figure 4A). The Rf (ratio of band migration to solvent front migration) plotted against the log MW of the standards (Figure 4B) was used to estimate the molecular weight of the subunits of Vg. The formula for the regression line of the standard curve was \( Y = 6.322 + (-1.186 \times X) \).

There are at least two different bands associated with Vg and based on the standards these peaks have MW's of 112,000 and 40,000. The relative Vg subunit titre in 8-day-old ovariectomized females is higher than the Vg subunit titre in 8-day-old virgins (Figure 5), and there is no evidence of Vg subunits in the blood of the male.

**Variation in vitellogenin titre**

By comparing areas under the peaks (relative integration values) on densitometer scans of stained SDS-PAGE, and always comparing only those bands on the same gel, an estimation of the effects of age, egg laying, and starvation on the blood Vg and lipophorin titres was made (Table 3). Starvation of 20-day-old virgins caused a decrease in blood Vg titre when compared to nonstarved virgins of the same age. There was a corresponding decrease in one subunit associated with lipophorin. A ten-day female had a higher Vg titre than a 36-day female. There was a much greater decrease in Vg in the egg laying female than for lipophorins when
compared to virgin females. Laying eggs was about as effective as starvation in reducing the Vg titre (Table 3). By contrast, the effect of age on reducing the Vg titre was much less.
DISCUSSION

Amino acid composition

King and Jukes (1969) presented the case for non-Darwinian factors such as neutral mutations and genetic drift being most important in determining the amino acid composition and therefore the structure of most proteins. They calculated an "expected frequency" for each of the different amino acids based on the number of codons for the individual acids and corrected for the presence of the initiation and termination codons. Their most pertinent observation, after examining 53 different vertebrate polypeptides, was that all of the amino acids except arginine occur in the amounts predicted by the expected frequencies. Arginine is coded by six codons but four of these are rare in their distribution. The mole percents for the 16 amino acids determined for Vg and Vn are close to those values predicted by King and Jukes (1969).

Quantitation of the differences between proteins can be obtained by calculating such values as $S^Q$ (Marchalonis and Weltman, 1971) or MD (Manhattan Distance = sum of the differences of amino acid molar percents from 1 to 16; Farris, 1972). The $S^Q$ is most useful for finding differences between proteins as it emphasizes the differences by squaring them; the MD sums
the differences so as to determine the degree of differences (Hagedorn and Kunkel, 1979). Values for $S^0Q > 100$ indicate that the proteins are from completely different species. Values below 1 are indicative of identical proteins, whereas values between 1-4 usually indicate identical proteins that have been determined by different laboratories. Therefore, the value of 6.2 for the differences between Vg and Vn of *A. domesticus* indicates a close relationship between these two molecules which suggests little modification of Vg during incorporation into the oocyte. The $S^0Q$s are typically small within species between the Vg and Vn, and is as low as 0.12 for *Philosamia cynthia* (Chino et al., 1976). Between species, the values range from 24.0 to 80.4. The lowest value reported here for a cross-species comparison was for the Vn's of *Acheta domesticus* and *Locusta migratoria* (Table 2). Comparison of the Vg and Vn of *L. migratoria* determined in different labs gave an $S^0Q$ of 5.5.

The amino acid composition of Vg and Vn from the house cricket is similar to that determined for these molecules in other insects. Peled and Tietz (1975), Gellissen et al. (1976), Chen et al. (1978) and Gellissen and Emmerich (1980) all give reasonably consistent amino acid compositions for the Vg's of *Locusta migratoria*. They found high asparagine/aspartic acid, glutamine/glutamic acid, and leucine along with
low levels of histidine, glycine, and methionine. The same results were found for Leucophaea maderae by Dejmal and Brookes (1972) and by Engelmann and Friedal (1974). A lower glycine level is the only apparent difference in Vg's and Vn's compared to the amino acid composition of the predicted protein of King and Jukes (1969). These molecules as a whole, and most other large proteins, do not appear to differ significantly from the observed predicted protein amino acid composition because of selective pressures (Hagedorn and Kunkel, 1979). The selective forces affecting protein amino acid sequence are not as directed for a nutritive or storage protein as those affecting the sequence of an enzyme with its binding and allosteric sites or a structural protein with similar constraints. A nutritive protein need only contain a proportional mix of the amino acids (King and Jukes, 1969), and a protein such as Vg should be expected to have an amino acid composition very similar to the predicted protein. The amino acid analysis of lipophorin from the house cricket is not completed, but partial results reveal the absence of methionine. This would make lipophorin a less adequate nutritive protein than Vg. The selective forces that result in such a deletion in lipophorin may be related to its transport function.
Molecular weight and subunit structure

In this study the molecular weight for Vg was estimated to be 400,000 daltons and for Vn 537,000 daltons in *A. domesticus*. These values are higher than that determined by Bradley and his coworkers. The molecular weight of the two subunits of Vg (112,000 and 40,000 daltons) determined in this study are similar to the MW's reported by Bradley and Edwards (1978). There are indications of additional subunits for *A. domesticus*, but further work is required to obtain more purified preparations of Vg and Vn. The estimation of native Vg molecular weight in this study is based on gel filtration (Sepharose 6B) and that of Bradley and Edwards (1978) was based on native PAGE and the results are different.

Consistent results between labs were seen in determinations for *Locusta migratoria*. Gelissen et al. (1976) found a MW for Vn of 530,000 by sedimentation diffusion equilibrium techniques, while Chen et al. (1978) determined a MW for both Vg and Vn to be 550,000 with a sucrose gradient, gel exclusion chromatography, and gel electrophoresis. Chen et al. (1978) also determined that there were 8 subunits with molecular weights ranging from 140,000 to 52,000. *Periplaneta americana* native Vg was found to have a MW of 596,000 with sucrose and CsCl gradient techniques by Sams et
al. (1980), who also found three different polypeptides subunits weighing 123,000, 118,000, and 57,000, respectively, with a stoichiometry of 2:2:2. Gradient electrophoresis was used by Harnish and White (1982) with P. americana Vn to determine a MW of 440,000. They identified 4 subunits with MW's similar to those found in Vg. Bradley and coworkers have also found four subunits in house cricket Vg with respective weights of 130,000, 97,000, 49,000, and 47,300 MW (=323,000 total native MW) by SDS-PAGE (Bradley and Edwards, 1978) and they determined Vn to be of two types with MW's of 352,000 and 327,000 using native PAGE (Nicolaro and Bradley, 1980). Hagedorn and Kunkel (1979), in reviewing the literature on both Vg and Vn, found that these molecules in the orthopterans have MW's of 500,000 to 600,000.

Isoelectric point

Gellissen et al. (1976) reported a pI of 6.9 for L. migratoria whereas Chinzei et al. (1981) determined the pI to be 6.3 for the same species. Kunkel and Pan (1976) found the pI for the Vg to be 5.7 in Hyalophora cecropia and 5.0 in Blatella germanica. These values are in the same range as the pI of 5.7-6.4 determined for A. domesticus in this study. The pI is a result of the overall charge caused by the particular mixture of amino acids present. Vitellogenins as a group of
molecules are highly soluble in low ionic strength solutions at pH's above the pI (Engelmann, 1979).

**Lipid-carbohydrate content**

There are no previous estimates of lipid and carbohydrate content for the Vg or Vn of any cricket species. The lipid-carbohydrate content found in house cricket Vg is within the range of values reported for other orthopteroids. In their review, Hagedorn and Kunkel (1979) listed carbohydrate values of Vg ranging from 13.6% for *L. migratoria* to 1.0% for *Hyalophora cecropia* and lipid values of Vg ranging from 15.7% for *Blatella germanica* to 6.9% for *Leucophaea maderae*. The function of the lipid and carbohydrate component of Vg remains unclear. Engelmann (1979) suggested that the carbohydrate moiety might be involved in the recognition of the molecule during selective uptake by the oocytes. The hypothesis is presented here, that the lipid moiety of Vg might aid in uptake into the oocyte by reducing the solubility of the molecule. In the house cricket, it has been reported that Vg is only present at very low levels in the blood (Engelmann, 1979) and therefore uptake by the oocytes is against a gradient. Preliminary studies indicate that the pH of the egg interior is near 6, and the blood pH is 7.3 (unpublished). The pI of Vg is 5.7-6.4, therefore the pH of the oocyte is closer to the Vg pI and the Vg
would be less soluble. The decrease in pH from blood to oocyte might facilitate the removal of the Vg from solution thus reducing the gradient working against uptake. The lipid moiety also undoubtedly contributes to the overall lipid stores of the egg, though its contribution is minor compared to that supplied by lipophorin (Wyatt and Pan, 1978).

**Effect of age, starvation, and mating on Vg titre**

Comparative densitometric integration of stained subunits of Vg separated on SDS-PAGE offered a method to assay blood Vg titre. Two conditions had to be met: comparisons had to be on the same gel and the identity of the Vg bands confirmed. The bands identified as subunits of Vg appeared coincidentally with the release of JH on about day 3. The Vg bands were lacking in male and larval blood, thus they were adult female specific. The Vg bands were present in serum, and therefore were distinguishable from lipophorin subunits that were lacking in the serum.

Starvation, long-term egg production (aging), and egg laying all reduced the titre of Vg in the blood. Starvation, which reduced the titre of many blood proteins, lowered the level of the Vg to a greater extent. This is probably because, during starvation, Vg is used for energy in addition to oogenesis. Starved crickets do produce a few eggs. Thus, the dual drain on
Vg might explain why the Vg titre declines more than that of other proteins in response to starvation. Clifford and Woodring (1985a) found egg production in virgin females declined as the oviducts filled with eggs to eventually occupy most of the available body cavity. The filling of the body cavity compressed the gut and restricted the intake of food, which could be expected to lead to a reduced rate of vitellogenesis. The difference between the values of the gel run on the 10-day and the 36-day virgins shows a reduction of the Vg titre (Table 3), which supports the hypothesis that filling with eggs reduces vitellogenesis and reduces the Vg titre.

Mated crickets produced 300 mg more eggs by day 18 than virgin crickets (Clifford and Woodring, 1985b), which might explain why egg laying should reduce the blood Vg titre (Table 3). Mated crickets apparently had a higher turnover rate of Vg because of the much greater egg production, and therefore the Vg titre was lower than in the virgins. By day 18 virgins are filled with eggs and then produce additional eggs at a very slow rate. The turnover rate of Vg is low and it tends to accumulate to a higher titre than in mated females.

**Effect of ovariectomy on Vg titre**

Ovariectomy is reported to elevate the Vg blood titre in *A. domesticus* (Bradley and Edwards, 1978),
*M. sanguinipes* (Elliott and Gillott, 1979), and *L. migratoria* (Mwangi and Goldsworthy, 1980). My study confirms that Vg titre in *A. domesticus* increased following ovariectomy (Figure 5).

My study has produced some baseline data on Vg and Vn in *A. domesticus*, but I recognize that many questions remain and that additional studies must be completed. A more detailed analysis of the blood Vg profile during adult development is an essential next step. The effects of ovariectomy must be clarified. Studies on the regulation of the Vg synthesis, its transport in the blood, and its uptake by the oocytes, and the involvement of the endocrine system represent interesting areas of investigation.
Acknowledgements—I wish to thank Dr. Earnest W. Blakeney for the amino acid analysis. Thanks are also due to the many student workers who labored to supply the raw materials for my research. Special thanks are due to Greg Moncada for the preparation of many of the figures.
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Identification, purification, and characterization


Vitellogenin: Its structure, synthesis and 
processing in the cockroach Periplaneta americana. 

Table 1. Amino acid composition of vitellogenins from 5 species.

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Values are in mole percent. The numbers at the top of the columns indicate the species and source (see list below). The value at the bottom of each column is the $S^Q$ for a comparison between *A. domestica* and the species in that column.

1-*Acheta domestica*, this study.
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Values are in mole percent. The numbers at the top of each column indicates the species and source. The value at the bottom of each column is the $S^oQ$ for a comparison between *A. domesticus* and the species in that column.

1- *Acheta domesticus*, this study.
Table 3. Densitometer integration values of SDS-PAGE separations run on the whole blood of (1) virgin females vs. starved virgin female, (2) virgin females vs. mated females, and (3) 10-day virgin females vs. 36-day virgin females

<table>
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<th>Rf Fraction</th>
<th>Fed Virgin Female</th>
<th>Fed Starved Virgin Female</th>
<th>15-Day Virgin Female</th>
<th>15-Day Mated Female</th>
<th>10-Day Virgin Female</th>
<th>36-Day Virgin Female</th>
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<tr>
<td>LI</td>
<td>0.08±0.01</td>
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<td>35±4.7</td>
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<td>V1</td>
<td>0.24±0.01</td>
<td>6.4±2.2</td>
<td>0.4±0.35</td>
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<td>L2</td>
<td>0.43±0.02</td>
<td>16±0.8</td>
<td>17±1.7</td>
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<td>V2</td>
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<td>2.4±1.0</td>
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L1 and L2, and V1 and V2 refer to the bands identified as belonging to lipophorin and vitellogenin, respectively. Rf value is the distance migrated by a particular band divided by the distance migrated by the marker dye (solvent front). All values are in relative integration units, and if a S.E. is present, the value is a mean of at least 2 measurements. Each experimental comparison (1, 2, or 3) was always run on the same gel.

**-In experiment 1 the females were 20-days old at the start and the starved group was denied food for 7 days. Those surviving were tested on the day 27.

**-In experiment 2 both mated and virgin females were 15-days old.
Figure 1. Standard curve of $V_e/V_o$ vs. log MW for the determination of MW of a protein on a Sepharose-6B column. The elution volume ($V_e$) is the volume at which a particular component shows its peak absorbance. The void volume ($V_o$) is the elution volume of very large molecules that are distributed only in the mobile phase of the column because they are too large to enter the largest pores of the gel. $V_e/V_o$ is a means of normalizing elution data. Standards and their MW's are as follows: (1) Thyroglobulin (THY)-670,000; (2) Catalase (CAT)-230,000; (3) Gamma globulin (GLO)-158,000; (4) BSA-65,000; (5) Ovalbumin (OVA)-44,000; and (6) Myoglobin (MYO)-17,000;
Figure 2. Elution profile of sample of vitellogenin run on a Sepharose-6B column. $V_e/V_o$ is as stated for Figure 1 and was calculated to be 1.61.
Figure 3. (A) SDS gel of (1) final DEAE eluent of extracted blood from an adult male cricket, (2) final DEAE eluent of extracted blood from an adult female cricket, and (3) a set of MW standards run under conditions as stated in the methods.

(B) SDS gel of blood serum from a series of adult female house crickets from 1- to 10-day-old. Ten ul of blood from each cricket was used. The band marked with * includes the larval storage protein which is still present in the first 1-2 days of the adult.

(C) SDS gel of partially purified vitellogenin (1 and 2) and lipophorin (3 and 4). Bands labelled with L1 or L2 indicates that the band is associated with lipophorin. Bands marked with V1 or V2 belong to vitellogenin. Bands with an * are common to both vitellogenin, lipophorin, and possibly others.
FIGURE 3A

ORIGIN

MYS
BGA
PHB
BSA

OVA

1 2 3
FIGURE 3B

ORIGIN

ADULT AGE - DAYS

1 2 3 4 5 6 7 8 9 10

L→

UV

*→

UV

MIGRATION
FIGURE 3C

ORIGIN

MIGRATION

V→
Figure 4. (A) Densitometer scan of MW standards run on SDS-PAGE. Standards were as follows: (1) Myosin (MYS) - 194,000; (2) Beta-galactosidase (BGA) - 116,000; (3) Phosphorylase-B (PHB) - 94,000; (4) BSA - 66,000; and (5) Ovalbumin (OVA) - 43,000.

(B) Standard curve of Rf vs. log MW of the gel standards.
RELATIVE MIGRATION - Rf

FIGURE 4B
Figure 5. Comparison of densitometer scans of different lanes of the same SDS-PAGE run on plasma of (A) a virgin cricket, (B) a male cricket, and (C) an ovariecetomized cricket. All were 8-days old. Peaks marked with the * are those identified as subunits of vitellogenin. The peak marked 0 is the beginning of the running gel. Number under the peaks are the relative integrator units.
BIBLIOGRAPHY


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Leahy M.G. (1973b) Oviposition of virgin Schistocerca gregaria (Forskal) (Orthoptera:Acrididae) mated with males unable to transfer spermatophores. J. Ent. (A) 48(1), 79-84.


VITA

Craig William Clifford was born on 25 August 1950 at Touro Infirmary in New Orleans, Louisiana. While he was still very young, his family moved from New Orleans proper to the suburb of Metairie. He attended St. Christopher Elementary School and Archbishop Rummel High School, both parochial schools in Metairie.

After graduating from high school in 1968, Craig pursued the Bachelor of Science in the Department of Zoology and Physiology at Louisiana State University in Baton Rouge and was awarded that degree in 1972. He subsequently entered graduate school and continued in the Department where he earned a Master of Science degree in 1974. He is currently a candidate for the Ph.D. with a major in physiology and a minor in entomology.
EXAMINATION AND THESIS REPORT

Candidate: Craig W. Clifford

Major Field: Physiology

Title of Thesis: The Biology of Egg Production in the House Cricket, Acheta domesticus L.

Approved:

[Signatures]

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

[Signatures]

EXAMINATION AND THESIS REPORT

Date of Examination:

December 5, 1984