
Hormonal Regulation of Parental Care in Insects

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I. INTRODUCTION

No class of animals has evolved parental care more often than the insects. Parental care occurs in at least 12 orders, many of which include multiple origins of sub-social behavior (Wilson, 1971). For example, parental care occurs in at least 13 families in the order Hemiptera and in 17 families in the Coleoptera (Wilson, 1971; Kūdo, 1994). Even within a single family, parental care is often dispersed among several unrelated genera. This taxonomic diversity is matched by ecological and physiological diversity of care-giving insects. Representative insects exhibit oviparity, ovoviviparity, and viviparity as well as variation in who provides care (uniparental female, uniparental male, biparental, communal care). If one desires to begin a study of evolutionary physiology of parental and social behavior, the insects appear to be ideal subjects. However, the diversity of social behavior that has so inspired behavioral ecologists appears to daunt insect endocrinologists. Less attention has been given to the hormonal control of insect parental care than to any major group of vertebrates.

It is hoped that this chapter will demonstrate the potential of insects for hormonal studies of care. The diversity of parental care among insects can be exploited by both narrow and broad phylogenetic comparisons. In many cases, parental species are closely

related to nonparental species, providing clear examples of how the physiology of a nonparental ancestor might have been modified during the evolution of sub-social behavior. One key to comprehending the role of a hormone in parental care will be to understand its effects outside the parental period and its function in closely related species without care. Broad phylogenetic comparisons of taxa employing the same hormones and endocrine glands will provide answers to the question of whether convergent social behavior is based on convergent physiology. Among insects, there is striking variation in the level of juvenile hormone (JH) during parental care. JH is typically considered a gonadotropic hormone in adults (Koeppel *et al.*, 1985), having a role both in directing vitellogenesis in the fat body (Wigglesworth, 1936) and in facilitating the uptake of vitellogenin by oocytes (Davey, 1981). This gonadotropic role of JH appears to be present in most if not all care-giving species. However, in some insects with parental care, levels of JH are very high during care. The possible adaptive basis for this variation among species will be discussed. Adequate explanations of physiological variation will only be possible if the trend toward more detailed study of "model" species is balanced by investigation of less studied taxonomic groups.

Many of the advantages of working with insects are only now being exploited by endocrinologists studying

parental care. The short generation time of insects permits study of complete reproductive cycles and also makes possible artificial selection experiments. Although insects have been used as models for indirect genetic effects (Mousseau and Fox, 1998), care-giving species have rarely been chosen as experimental subjects. The small space requirements and the radically different sensory world of insects makes it feasible to conduct large-scale observational studies of natural behavior throughout the year. The ability to remove endocrine glands and to apply hormones noninvasively have been utilized for more than 50 years.

For those interested in insects as models of vertebrate social behavior, there are a number of intriguing parallels (Scharrer, 1987). Both the brain-cardiacum-allatum system of insects and the hypothalamic-hypophysial system of vertebrates employ structurally similar peptide hormones that can affect target organs (e.g., the ovary) directly or indirectly through an intervening endocrine gland. The ovary, in turn, exerts a positive feedback on this pathway in its immature state, but a negative feedback when oocytes are fully developed. In both invertebrate and vertebrate systems, the brain inhibits the reproductive system until appropriate sensory stimuli from the environment release the inhibition. Cycles of reproduction and parental care are dependent on cycles in the neuroendocrine apparatus (Scharrer, 1987). Insects provide both accessible models of the physiological control of parental investment and unique comparative opportunities for exploring parallel evolution of complex social behavior.

II. PREGNANCY IN COCKROACHES

A. Life History

There is considerable variation in reproduction among cockroaches (Roth and Willis, 1954; Roth, 1991). Oviparous species carry eggs externally in a hardened ootheca; false ovoviviparous species produce a soft ootheca that is at first extruded but then retracted into the uterus (brood sac); true ovoviviparous species deposit eggs directly into the brood sac without producing an ootheca; and the only well-studied viviparous species (*Diploptera punctata*) provides extensive nourishment to embryos within the brood sac

during a 60-day gestational period (Nalepa and Bell, 1997). Postzygotic investment by parents can vary extensively within each of these reproductive modes (Fig. 1). Among oviparous forms, for example, the ootheca may be formed, deposited, and abandoned in less than 36 hr (e.g., *Periplaneta americana* and *Supella longipalpa*). Alternatively, the ootheca may be carried by the mother throughout embryonic gestation (*Blatella germanica*). The longest parent-offspring association occurs in the oviparous *Cryptocercus punctulatus*, in which both parents stay with their young in rotting logs for several years (Cleveland *et al.*, 1934; Nalepa, 1984).

Despite the variation in reproductive modes and the multiple phyla that independently evolved ovoviviparity and viviparity, there is similarity in the hormonal control of the cockroach reproductive cycle. In all species examined, JH type 3 (JH III) is the principal form. JH is synthesized in the corpora allata (CA) and its titer in the hemolymph is largely determined by production within these paired endocrine glands (Fig. 2; Camps *et al.*, 1987). JH is necessary for oocyte development and normal reproductive cycles, and its biosynthesis can be modulated by neuronal input from the brain and by humoral factors (Fig. 2). The endocrinology of parental investment patterns are detailed for two species: *Blatella germanica* (oviparous) and *Diploptera punctata* (viviparous).

B. *Blatella germanica*

The German cockroach carries its ootheca for approximately 21 days and can produce up to nine broods, each brood hatching while still attached to the female (Roth and Stay, 1962). Because of the length of the parental investment period, *Blatella* is characterized by well-defined ovarian and endocrine cycles. The ootheca is partially retracted into the uterus. Its permeable surface permits the uptake of water (Roth and Willis, 1955) and perhaps nutrients (Nalepa and Bell, 1997). Thus, *Blatella* offers a possible transition between oviparity and ovoviviparity.

After emergence as an adult, JH synthesis in *Blatella* typically declines for several days and then begins to rise, peaking on day 7, just prior to oviposition. The increase in JH biosynthesis is correlated with an increase in CA volume caused by an increase in cell size within

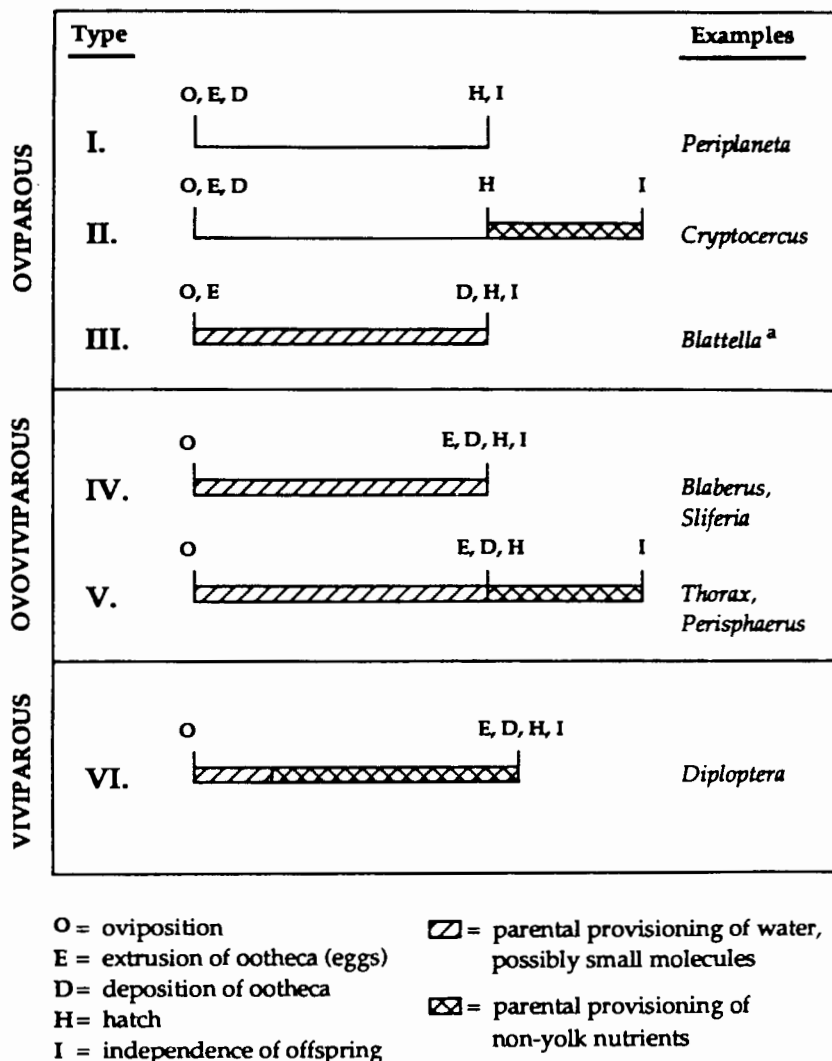


FIGURE 1 Diversity of postoviposition investment in cockroaches. ^aNeonates of *Blattella vaga* briefly feed on maternal secretions. (From Nalepa and Bell, 1997, with permission of Cambridge University Press).

the CA (Chiang *et al.*, 1996). The normal increase in JH titer and in oocyte development during the preoviposition period requires feeding, adequate nutrition, social stimulation, tactile cues, and mating (Engelmann, 1970; Gadot *et al.*, 1989; Schal *et al.*, 1993, 1997). Initially the maturing ovary exerts a stimulatory feedback on CA activity; removal of a young ovary inhibits JH synthesis in *Blattella* (Gadot *et al.*, 1991) as well as in other cockroaches (Stay and Tobe, 1978; Lanzrein *et al.*, 1981).

Classical endocrine techniques (removal of an endocrine gland and restoration of function with hormone

treatments) have shown that JH coordinates many reproductive events in *Blattella*. Allatectomy (removal of the CA) prevents normal sexual receptivity and pheromone production by females (Schal *et al.*, 1991). Allatectomy also disrupts the synthesis of oothecal proteins and development of accessory glands (Burns *et al.*, 1991), as well as the synthesis of vitellogenin (a yolk precursor) in the fat body (Kunkel, 1981; Don-Wheeler and Englemann, 1997).

During the prereproductive period, JH synthesis and oocyte development are initially inhibited by neural input from the brain. Transection of nervus corpus

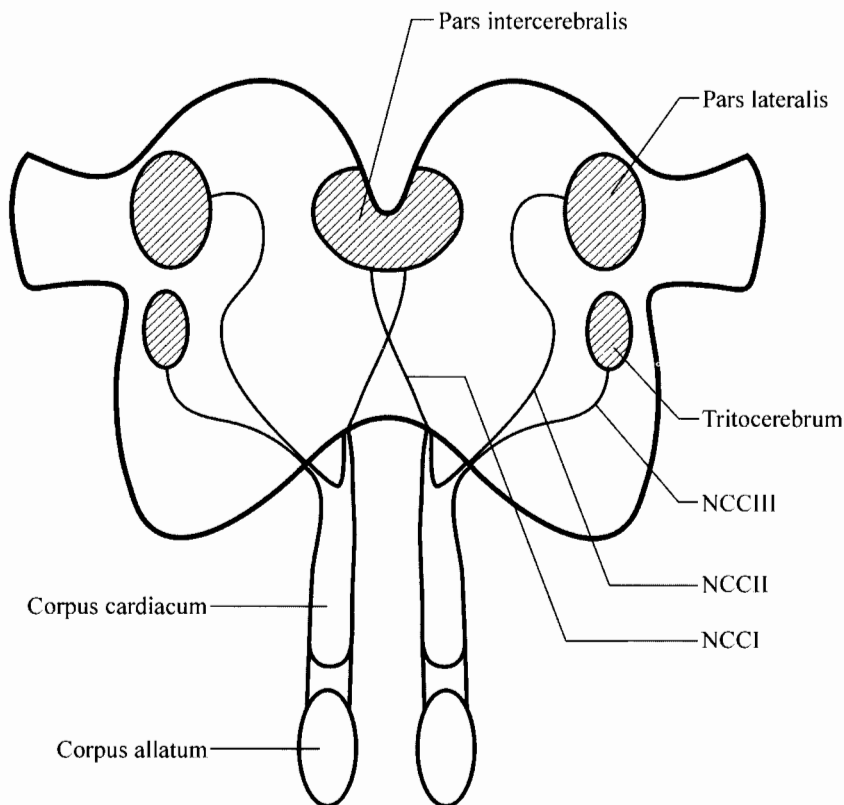


FIGURE 2 Schematic representation of the brain of an insect. NCCI/III, nervus corpus cardiacum I–III.

cardiacum I (NCC I) or II (NCC II) (neural connections are shown in Fig. 2) results in greater JH output by the CA, with the effect being more pronounced in starved females (Schal *et al.*, 1993). JH-related events, such as female calling behavior and oocyte development, are thereby accelerated. It therefore has been hypothesized that the brain produces allatostatic neuropeptides that inhibit the CA. Denervation of the CA causes allatostatins to accumulate at the proximal ends of the severed neurons (Scharrer, 1952). The presence of allatostatins in the hemolymph suggests that humoral, as well as neural, pathways may be involved (Vilaplano *et al.*, 1999). Environmental factors such as feeding, mating, and social stimulation are thought to release the CA from the inhibitory effect of brain allatostatins (Gadot *et al.*, 1989; Schal *et al.*, 1997; Holbrook *et al.*, 2000). Synthesis of JH declines prior to oviposition at the time eggs are being chorionated (Gadot *et al.*, 1989).

Pregnancy in *Blatella* is accompanied by a number of behavioral changes. Gravid females feed much less than

nongravid females; consequently, locomotory behavior is inhibited and metabolic activity is lower (Demark and Bennett, 1985; Hamilton and Schal, 1988). However, gravid females are more aggressive than nongravid females, presumably to protect the vulnerable ootheca (Breed *et al.*, 1975). The neural and hormonal changes responsible for these behavioral changes are not well understood.

A low titer of JH, inactive CA, and elevated levels of allatostatins are necessary for a normal pregnancy and to avoid premature deposition of the ootheca (Tobe and Stay, 1985; Vilaplano *et al.*, 1999). Several factors, working over different time frames, inhibit JH synthesis during pregnancy. The mature ovary which earlier had stimulated the CA now inhibits JH synthesis. Denervation of the CA will not completely stop the decline in CA activity, suggesting a humoral inhibitor. The ovaries produce ecdysteroids (Romaná *et al.*, 1995), which can inhibit the CA (Engelmann, 1959; Stay *et al.*, 1980). However, the inhibition is thought to

be temporary; ecdysteroid titers decline shortly after oviposition (Romaná *et al.*, 1995), while the CA remains inactive throughout pregnancy (Chiang *et al.*, 1991). The ootheca is a longer term inhibitor of the CA. Removal of the ootheca causes an increase in feeding behavior, followed by an increase in CA volume and activity (Osorio *et al.*, 1998). However, removal of the ootheca does not affect the CA in starved females, suggesting that the ootheca primarily inhibits feeding and, only indirectly, the CA. If a removed ootheca is replaced by a mechanical plug in fed females, the CA remains inhibited. A mechanical plug will not inhibit the CA, however, when the ventral nerve cord is severed, suggesting that neural signals from the uterus or vestibulum are important in maintaining low JH synthesis during pregnancy (Roth and Stay, 1959). While a mechanical plug is an effective long-term inhibitory substitute for the ootheca, even its effects are not indefinite. After the normal pregnancy period (approximately 21 days), a mechanical plug can no longer inhibit the CA, perhaps because mechanoreceptors become fatigued (Roth and Stay, 1962). In intact females at this time, the presence of an ovary with immature oocytes would likely stimulate renewed CA activity. It is apparent that multiple and sometimes redundant cues, active over different time frames, are employed both to maintain low JH titer during normal pregnancy and to allow for the increase in JH titer that occurs just prior to ootheca deposition and the initiation of a new reproductive cycle.

C. Diploptera punctata

Diploptera punctata is a viviparous cockroach that nourishes its young with brood sac secretions during a prolonged pregnancy (63 days at 27°C). Unlike *Blattella* and most other cockroaches, *Diploptera* females actively feed during pregnancy, doubling their body mass (Stay and Coop, 1974). Feeding is necessitated by severe nutritional demands; nymphs at parturition are 50 times heavier in mass than eggs. The female produces a nutrient-laden "milk" (45% protein, 5% free amino acids, 25% carbohydrates, 16 to 22% lipid) from epithelial tissue from the brood sac which is modified during embryogenesis for glandular secretion (Stay and Coop, 1973, 1974; Ingram *et al.*, 1977). Nymphs that do not maintain adequate contact with brood sac tissue are born underweight. One result of this maternal

investment is that nymphs of *Diploptera* are more advanced developmentally than any known cockroach, requiring fewer molts to adulthood (Nalepa and Bell, 1997).

Information on JH titer, JH synthesis, JH esterase activity, and oocyte development throughout the first reproductive cycle of *D. punctata* is available (Fig. 3). At adult emergence both JH titer and synthesis are low and rise through day 5 during a period of rapid increase in the length of basal oocytes. The peak in JH titer on day 5 is one of the highest levels of JH recorded for insects (Tobe and Stay, 1985). JH titer and synthesis decline during the 3 days leading up to oviposition, which occurs on day 8. Application of JH just after oviposition will lead to abortion of the embryos in the brood sac (Sutherland *et al.*, 2000). JH esterase activity reaches its peak on day 7 and then declines to moderate levels, which are maintained throughout pregnancy. The increase in JH esterase activity is apparently a response to rising levels of JH; the JH esterase peak is not recorded in allatectomized animals that have their source of JH removed (Rotin *et al.*, 1982). JH esterase released from the mature ovary may be necessary to degrade JH in and around the oocyte just prior to fertilization (Stoltzman *et al.*, 2000). JH titer and synthesis are very low throughout most of pregnancy, rising only in the few days prior to parturition and at the start of a new ovarian cycle. Rising and falling levels of JH biosynthesis are correlated with an increase in cell number in the CA (subject to neural inhibition) followed by autophagy which is nerve independent (Chiang *et al.*, 1996, 1999).

The normal cycle of JH synthesis requires mating and the presence of developing oocytes (Stay and Tobe, 1977; Stay *et al.*, 1983). As in *B. germanica*, the ovary initially exhibits a positive feedback on JH biosynthesis (Rankin and Stay, 1985a; Stay *et al.*, 1996). Rising titers of JH itself contribute to stimulating the CA during the preoviposition period (Tobe and Stay, 1979). Like *Blattella*, mating and presumably other cues are necessary to release the CA from inhibition from the brain. Transection of nerves connected to the CA abolishes the inhibition and results in increased JH biosynthesis (Tobe and Stay, 1980; Stay *et al.*, 1983). The mature ovary and high doses of JH may stimulate the brain to release inhibitory signals to prevent proliferation of cells in the CA (Chiang *et al.*, 1995). This inhibitory effect is not abolished by surgical implantation of an

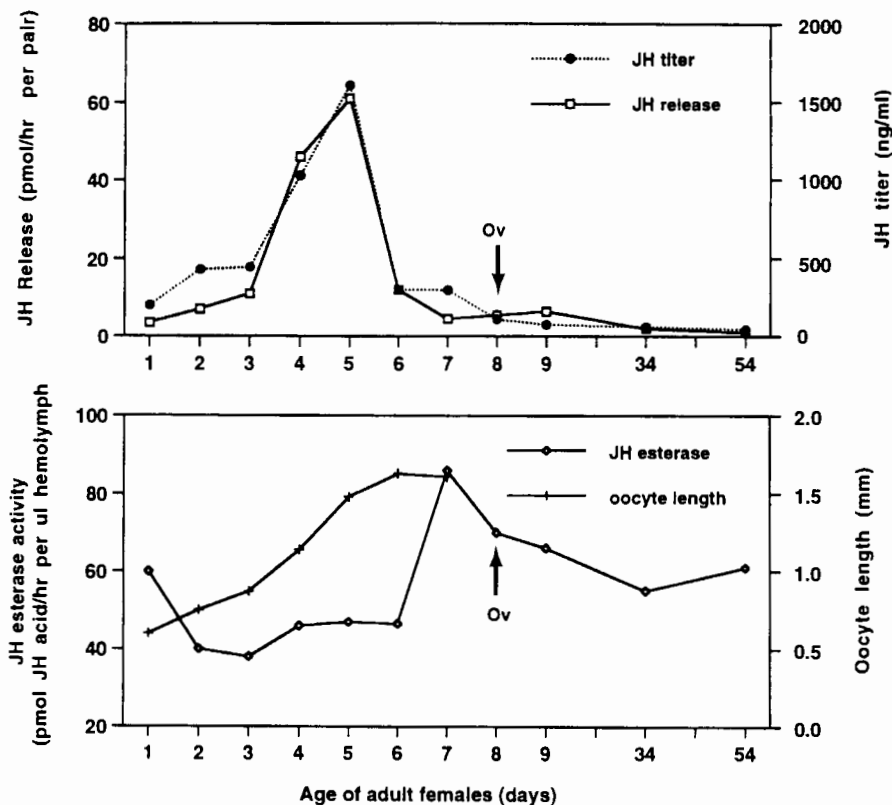


FIGURE 3 (Top) Mean hemolymph JH titer and JH release and (bottom) mean JH esterase activity and oocyte length in female *Diploptera punctata* from emergence as an adult through the first pregnancy cycle. Ov, oviposition. (Modified from Tobe *et al.*, 1985.)

isolated mature ovary into an ovariectomized female. These results suggest that humoral as well as neural inhibitory pathways are involved (Stay *et al.*, 1983).

A family of 13 structurally related allatostatins has been identified in *D. punctata* (Woodhead *et al.*, 1993; Tobe *et al.*, 2000). These allatostatins have the ability to inhibit CA output *in vitro*. Allatostatin concentration in the hemolymph changes during the reproductive cycle, peaking during early pregnancy (Yu *et al.*, 1993). The importance of hemolymph allatostatins during pregnancy is not clear. The degree of inhibition by allatostatins is best viewed as an interaction between allatostatin concentrations and changing sensitivity of the CA. The CA are very sensitive to allatostatins after vitellogenesis but much less sensitive during pregnancy even though this is a period of low CA output (Stay *et al.*, 1991). Changes in allatostatin receptors on the CA appear to be the mechanism for altering CA sensitivity (Cusson *et al.*, 1991, 1992). Taken together

these data suggest that brain-derived allatostatins may be the primary inhibitor of the CA at the end of vitellogenesis, whereas other long-term inhibitory factors become more important during pregnancy. Because allatostatins work quickly and their effects are reversible, their role may be most critical in regulating the rapid changes in CA output that occur during the period from adult emergence until oviposition (Woodhead *et al.*, 1994).

The locations of the inhibitory centers in the brain were first investigated more than 40 years ago by transection of nerves connected to the CA. In *Leucophaea maderae* (ovoviviparous) transection of NCC I but not NCC II led to a release of inhibition of the CA (Engelmann and Lüscher, 1957; see Fig. 2). In *D. punctata* destruction of the pars lateralis releases the CA from inhibition in virgin females but not pregnant or ovariectomized females. This suggests that additional factors, perhaps a stimulatory signal from the ovary, is

necessary to activate the CA (Ruegg *et al.*, 1983). Each corpus allatum is inhibited by the ipsilateral pars lateralis and the contralateral pars intercerebralis (Chiang *et al.*, 1999). Immunocytochemical localization of allatostatins has identified lateral neurosecretory cells of the brain as a site of allatostatin production (Stay *et al.*, 1993, 1996).

The release of allatostatins from the terminal connections with the CA also may be a stage at which JH synthesis can be regulated. The biogenic amine octopamine has an inhibitory effect on JH biosynthesis, most likely by modulating the release and uptake of inhibitory neuropeptides from terminals within the CA (Thompson *et al.*, 1990).

A normal gestational period and parturition require the maintenance of a physiological state conducive to parental investment. During pregnancy JH synthesis and titer are consistently very low (Rankin and Stay, 1985b; Fig. 3). Mitotic activity in the CA is undetectable during pregnancy (Chiang *et al.*, 1996). High JH titers disrupt normal pregnancy; application of JH analogs inhibits milk production and reverses the structural changes in the brood sac that occurred at the beginning of embryogenesis (Stay and Lin, 1981; Evans and Stay, 1995). It is not known whether JH acts directly on the milk glands or indirectly by altering the ovary or brain. It seems logical that high levels of JH would be strictly incompatible with care since ovarian development would constrain the mothers' ability to provide care internally. Stimulation of sensory hairs by embryos in the brood sac induces inhibition of the CA via neural signals transmitted by the ventral nerve cord (Engelmann, 1959). Since this inhibition is not completely abolished by denervation of the CA, additional humoral signals are thought to inhibit the CA during pregnancy. The relative importance of the different pathways inhibiting the CA during pregnancy is not known. During pregnancy the ovary remains small and incapable of inducing JH synthesis (Rankin and Stay, 1985a). Allatostatins in the hemolymph reach peak levels during pregnancy, but this occurs when the CA is less sensitive to the effects of these inhibitors (Yu *et al.*, 1993). JH esterase activity is moderately high during pregnancy (down from its peak following oviposition), but its role in maintaining low JH titer appears secondary as the CA is producing very little JH while embryos are being nourished (Rotin *et al.*, 1982; Fig. 3).

There are likely multiple, redundant signals (both neural and humoral) to inhibit the CA and to maintain a low JH titer during embryogenesis.

A few days prior to parturition the basal oocytes become vitellogenic in response to rising JH (Roth and Stay, 1962). The developing oocytes stimulate further JH synthesis, as occurred during the beginning of the reproductive cycle. The increasing JH titer likely inhibits milk production. Mitotic activity in the CA resumes and a new reproductive cycle is initiated (Chiang *et al.*, 1996).

D. Prospects

Variation in life history, especially the multiple independent origins of a prolonged period of parental investment, would seem to make cockroaches an ideal group for evolutionary study. Cockroaches that possess a prolonged gestation exhibit a number of similarities in the neuroendocrine control of the reproductive cycle. The limited number of taxa that have been studied endocrinologically makes it as yet impossible to determine where similarities are caused by common ancestry or by convergent adaptation.

One difference among cockroaches that has received very little speculation is the difference in JH titers among species. Although all species exhibit a similar pattern of JH increase and decrease concurrent with the vitellogenic and parental phases of the reproductive cycle, some species (*Diploptera punctata*, *Leucophaea maderae*) have much higher absolute levels of JH at each stage of the cycle (Schal *et al.*, 1997). Comparisons across phyla will be required to determine whether differences in JH titer are solely due to phylogenetic history or have adaptational significance. It will be necessary to investigate additional species to interpret physiological variation in an evolutionary context.

One promising but neglected area of research is investigation of postparturition physiology. Cockroaches of diverse life history (oviparous, ovoviviparous, viviparous) care for young outside the body after hatching (Nalepa and Bell, 1997; Fig. 1). It is almost certain that the parent-offspring association alters the parent's physiology; however, there is no obvious physiological reason for high JH levels to be incompatible with care outside the body. The ability to quantitatively manipulate the stimuli from offspring by altering brood size

should permit the testing of models for terminating care and to establish the physiological mechanisms controlling this decision. In addition, surgical manipulation of the CA, ovary and nerves, which are well established techniques for cockroaches, will provide insight into the control of familial and protective behavior during postparturition care.

The manner in which the life cycles of symbionts are tied to host reproductive cycles is another potential research area. Cockroaches contain fat body symbionts that return reusable nutrients to their nitrogen-limited hosts (Wrenn and Cochran, 1987). Virtually the same symbiotic associations are found in the termites, descendants of wood-feeding cockroaches (Potrikus and Breznak, 1981). Cockroaches also have an impressive array of gut symbionts which make it possible for these insects to exist on a variety of poor-quality food. It is known that gut symbionts undergo life stage changes (gametogenesis, encystment) in response to the hormonal/reproductive cycles of their host (Cleveland *et al.*, 1960). Study of symbionts during the gestational period of cockroaches will perhaps make these insects a model system for understanding how symbionts can facilitate parental investment of their hosts.

III. MATERNAL CARE IN EARWIGS

A. Life History

The earwigs (Dermaptera) are a sister group to the cockroaches within the loosely defined orthopteroid orders. Earwigs are hemimetabolous predacious or scavenging insects exhibiting oviparity or ovoviviparity (Lamb, 1976). All species studied have maternal care, which includes construction of a burrow, guarding and grooming of eggs, and defense of the brood (Shepard *et al.*, 1973; Lamb, 1976). Care giving is typically accompanied by fasting. Some species continue care for newly hatched nymphs and may provision the nest with food. A male is often found in the burrow with the female, but his parental contribution is uncertain (Shepard *et al.*, 1973; Lamb, 1976). Females abandon nymphs after a few days to resume feeding and will then produce a clutch in a new nest. Two species, *Labidura riparia* and *Euborellia annulipes*, have been the subject of extensive hormonal study. The reported differences

in endocrinological details between these two species are minor, and both species will be discussed together. Much of the information on *Labidura riparia* comes from the French literature (Caussanel, 1971; Caussanel, 1973a,b, 1978; Caussanel *et al.*, 1978, 1979) and has been reviewed (Baehr *et al.*, 1982; Rankin *et al.*, 1997).

After adult emergence *L. riparia* has a 5-day pre-reproductive period during which it feeds voraciously in preparation for oogenesis. Females undergo up to four or five ovarian cycles lasting approximately 10 days each, which alternate with care-giving periods, also of 10 days duration. During vitellogenesis females feed, increase in body mass, and mature 6 to 8 oocytes in each of 10 ovarioles. During the parental phase, females fast, lose body mass, and basal oocytes degenerate (in *E. annulipes*, females have less of a tendency to fast and oocytes are not resorbed during care giving; Jamet and Caussanel, 1995). Salivary glands also go through a regular cycle, decreasing in size during the nonfeeding parental phase (Rankin *et al.*, 1997). The suppression of feeding during parental care is similar to that in most cockroaches, including *Blattella germanica*, but unlike *Diploptera punctata* which must supply embryos with maternally derived nutrients.

The anatomy of endocrine structures differs from that of cockroaches. The corpus allatum is a singular medial gland that is closely associated with two neurohemal organs, the paired corpora cardiaca and the dorsal aorta (Awasthi, 1975; Juberthie and Claussanel, 1980). Although several forms of JH have been identified from earwigs, JHIII appears to be the gonadotropic hormone (Rankin *et al.*, 1997). Information on JH titer and ecdysteroid titer are shown for *Labidura riparia*, and JH biosynthesis and oocyte length for *Euborellia annulipes* (Fig. 4).

B. Endocrinology

The importance of the corpora allata for ovarian development in earwigs was first demonstrated by Ozeki (1951). After emergence as an adult, volume of the CA increases up to 15-fold during vitellogenesis, reflecting an increase in JH synthesis. Under the influence of JH, vitellogenesis is accompanied by structural changes in cells of the CA and in cells of the fat body (Sayah *et al.*, 1994). JH titer and synthesis increase steadily during the previtellogenic period. The increase in JH is

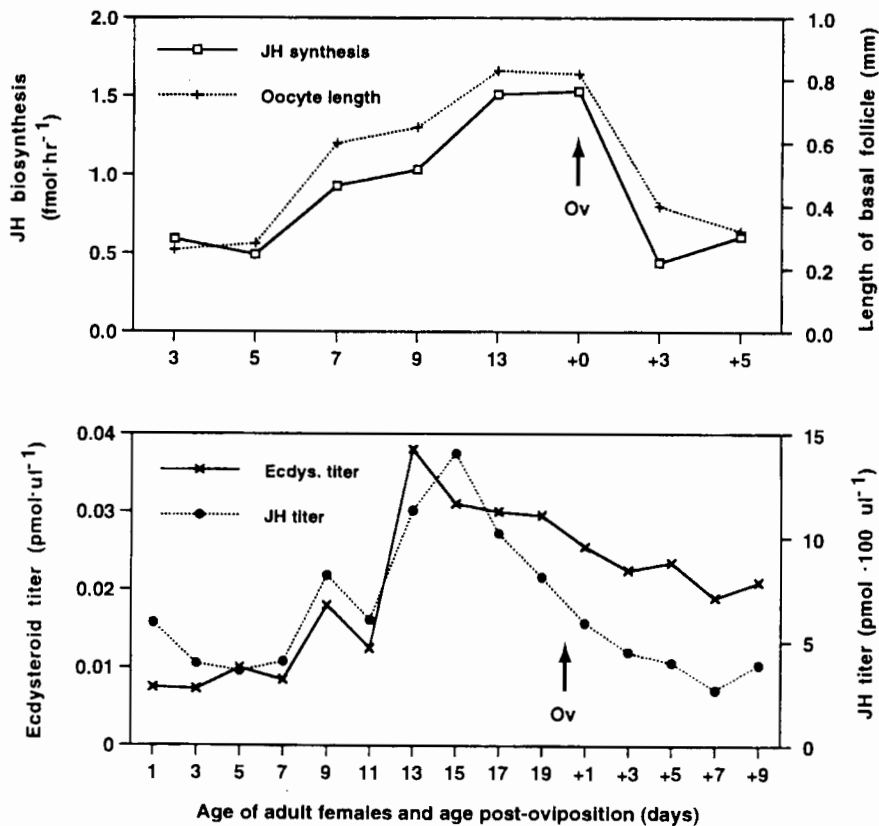


FIGURE 4 (Top) Mean JH biosynthesis and length of basal follicle in the earwig *Euborellia annulipes* through the first parental cycle. (Modified from Rankin, S. M., Palmer, J. O., Yagi, K. J. *et al.*, 1995. Biosynthesis and release of juvenile hormone during the reproductive cycle of the ring-legged earwig. *Comp. Biochem. Physiol. B* **110C**, 241–251.) (Bottom) Mean ecdysteroid titer and JH titer in *Labidura riparia*, from adult emergence through the first parental period. (Modified from Vancassel *et al.*, 1984.) Ov, oviposition.

not dependent on mating during the first (Vancassel *et al.*, 1984) or second gonadotropic cycle (Rankin *et al.*, 1995b). Likewise, application of JHIII to newly emerged females has little effect on mating behavior (Rankin *et al.*, 1995a). Adequate nutrition is necessary for normal JH biosynthesis and oocyte development. Poorly fed females do not undergo vitellogenesis, and the application of JH analogs enhances vitellogenesis in fed but not starved females (Rouland *et al.*, 1980, 1981). The importance of JH for vitellogenesis is also supported by allatectomy studies. Removal of the CA stops oocyte development, causes an increase in the fat body (presumably because resources are not being shunted to oocytes), and causes a drop in hemolymph vitellogenin. The administration of JH analogs subsequent to allatectomy restores normal oocyte develop-

ment and egg laying (Caussanel, 1973b; Baehr *et al.*, 1982).

The application of JHIII to normal previtellogenic females shortens the time to first oviposition. However, this was accompanied by a smaller clutch size and less hatching success than in nontreated females (Rankin *et al.*, 1995a). These detrimental effects on fitness can perhaps be viewed as stimulating young females into reproduction which have not had adequate time to feed, to store resources in the fat body, or to mature ovaries. Experiments employing such hormonal treatments are ideal for studying trade-offs among the different stages of life history (Ketterson and Nolan, 1992).

Unlike cockroaches, an immature ovary is not necessary to stimulate JH biosynthesis, nor is a mature ovary sufficient to inhibit synthesis. Ovariectomy during the

previtellogenic stage does not stop corpus allatum activity (Rankin *et al.*, 1999). In normal females, JH synthesis is maintained at high levels up to the time of oviposition, suggesting that a mature ovary cannot inhibit the corpus allatum (Rankin *et al.*, 1995c). It therefore appears that ecdysteroids derived from the ovary do not inhibit the corpus allatum, a finding that is supported by the concurrent high titers of JH and ecdysteroids rather late in vitellogenesis (Baehr *et al.*, 1982; see Fig. 4).

The role of the brain during vitellogenesis is not clear. Neurosecretory products from the brain and aorta reach a peak just before oviposition (Juberthie and Claussanel, 1980), and whole-brain extracts stimulate vitellogenesis. The pars lateralis apparently exerts an allatostatic effect in response to 20-hydroxyecdysone (Sayah *et al.*, 1995). Since specific allatotropins and allatostatins have not been identified for earwigs, extracts necessarily contain a mixture of molecules of various effects. Cautery of the pars intercerebralis in previtellogenic females blocks normal ovarian development (Souissi and Claussanel, 1981), most likely by inhibiting JH production (Baehr *et al.*, 1982). At this stage of the research program it can only be confidently stated that both allatotropic and allatostatic neurosecretory products originate in the earwig brain.

The mechanisms that control the duration of maternal behavior have been investigated by manipulation of environmental stimuli and by manipulation of the neuroendocrine system. Care-giving females normally move little, do not feed, and have low titers of both JH and ecdysteroids. Females prevented from mating prior to vitellogenesis will accept eggs and provide care, but the period of care is shortened in comparison with that of mated females (Rankin *et al.*, 1999). There appears to be a direct link between parental behavior and inhibition of feeding in earwigs, especially in *Labidura riparia* (Vancassel, 1984). Females continually supplied with food in the nest will eventually overcome their inhibition and begin to feed. This abnormal feeding results in a shortened period of care and an earlier onset of a second gonadotropic cycle (Vancassel *et al.*, 1984). However, such force-fed females do not cannibalize eggs as they would typically do outside the parental phase. This suggests that inhibition of cannibalism in these predacious insects is a key element of care; inhibitory centers in the pars intercerebralis are required to suppress cannibalism (Caussanel, 1973a).

Parental care in earwigs occurs outside the body. It is to be expected that changes in eggs and young larvae are potent stimuli for the mother. The mother appears to deposit secretions on the eggs during oviposition, which serves to keep the female in the nest and to inhibit the CA (Caussanel *et al.*, 1981; Oliver, 1983). If her clutch is replaced with younger eggs the period of care can be extended (Vancassel, 1977; Rankin *et al.*, 1996). The endocrine changes correlated with this manipulation have not been done but might suggest the physiological mechanisms that alter parental behavior. There may be an additional endogenous rhythm in earwigs that prevents a mother from staying in the nest indefinitely when new eggs are switched for old. If eggs are removed, all females lose their motivation to provide care within 72 hr (Vancassel *et al.*, 1984), and the onset of a second gonadotropic cycle is shortened (Rankin *et al.*, 1996). However, the removal of eggs does not produce an immediate effect on JH synthesis in ovariectomized females that are not fed.

Females allatectomized early in the parental phase will provide care for an extended period (Pierre, 1979). Conversely, administration of JHIII on the day of oviposition will shorten the parental period from 15 to 5 days and decrease the time to a second gonadotropic cycle (Rankin *et al.*, 1995a). Subsequent work (Rankin *et al.*, 1999) favors a hypothesis of indirect rather than direct inhibition of JH on maternal care. Although the parental phase is normally associated with low JH titer, high JH titers are not incompatible with care giving. Females that are ovariectomized during care have elevated JH synthesis but continue to stay with eggs (Rankin *et al.*, 1999). Similarly, Caussanel *et al.* (1978) found that females ovariectomized prior to vitellogenesis exhibit an increase in volume of the corpus allatum, but still exhibit parental care if provided eggs in a nest. These results suggest that rising levels of JH near the end of the parental phase (Baehr *et al.*, 1982) lead to feeding behavior and development of oocytes, which are the key stimuli for terminating care. In the absence of JH's effects on feeding and ovarian development, this hormone appears to have a minimal direct impact on care giving.

C. Prospects

The manner in which interactions among JH, feeding, and ovarian development regulate parental care

in earwigs is not completely understood. Two future experiments might be helpful. In one, JH could be administered to ovariectomized and sham-operated care givers in the absence of food to suggest whether it is the developing ovary or JH itself that terminates care. Care-giving *Labidura riparia* are normally reluctant to feed but will do so continually supplied with food. In a second experiment, it would be of interest to administer JH to ovariectomized and sham-operated care givers while such females are being presented with food. This design might reveal whether rising JH levels reduces the inhibition against feeding and whether a developing ovary alters that response. Such experimentation would likely elucidate the direct or indirect pathways by which JH decreases the motivation to provide care.

Manipulation of the number and age of offspring, accompanied by hormonal and behavioral studies, will reveal the effects of young on parents. Many organisms have been found to terminate care when there is severe brood reduction, presumably because of the low fitness payoffs of caring for a few young (Clutton-Brock, 1991; Trumbo, 1996). Still other organisms respond by producing a second, replacement clutch while continuing to provide care for the reduced first clutch. In an insect, this would likely require a surge in JH titer and vitellogenesis. The production of a replacement clutch while continuing to provide care seems unlikely in earwigs since vitellogenesis is closely tied to feeding away from the nest, a behavior that seems incompatible with care. We might thus expect earwigs either to produce a second clutch or to continue to care for the reduced first clutch, but not both (Rankin *et al.*, 1996). The difference among earwig species in the timing of oocyte resorption is of interest as to whether it relates ecologically to renesting potential and physiologically to changing JH levels. As outlined in the next section, burying beetles, which face extremely limited reproductive opportunities, are able to maintain high titers of JH, undergo vitellogenesis, and oviposit a second clutch, even when providing care for their initial brood.

IV. BIPARENTAL CARE IN BURYING BEETLES

A. Life History

Species of the genus *Nicrophorus* (Silphidae) prepare a small vertebrate carcass as a resource for their

young (Pukowski, 1933; but see Blouin-Demers and Weatherhead, 2000). After a carcass is discovered it is quickly buried or concealed, hair or feathers are removed, and the carcass is rounded into a ball (Fabre, 1949). Rapid preparation of the resource is necessary as the beetles compete with congeners, carrion flies, ants, and vertebrate scavengers. Eggs are oviposited in the surrounding soil within 24 hr and the resulting larvae eclose and move to the carcass 3 to 4 days later. A female, male, male–female pair, or occasionally a small communal group will care for altricial young by regurgitating liquefied carrion, maintaining the nest and defending against predators (Trumbo, 1996; Eggert and Müller, 1997; Scott, 1998). After the parent(s) leave the nest (usually the male first), they are reproductively competent and search for a new carrion resource to exploit (Trumbo, 1991; Scott and Gladstein, 1993). A carcass is an extremely valuable resource; most burying beetles will manage only one or two reproductive attempts in a season (Trumbo, 1991; Scott and Gladstein, 1993; Eggert and Müller, 1997). After a carcass has been discovered, an intruding congener may visit the nest, fight with the resident(s), kill any young in the nest, and attempt to use the resource for its own reproductive benefit (Trumbo, 1990; Robertson, 1993). An intruder will sometimes pair with the resident of the opposite sex to produce a new clutch.

Less is known about the neuroendocrine anatomy of burying beetles than the more well-studied cockroaches and earwigs. The heavy sclerotization and large muscles in the head make surgical procedures difficult. It has been possible to successfully remove the paired CA to assay JH biosynthesis, but mortality of experimental subjects has not allowed study of allatectomized individuals (Scott *et al.*, 2001). No studies of the brain or of neuropeptides have been undertaken.

B. Endocrinology

Data on JH synthesis, JH titer, JH esterase activity, and ovarian size from adult emergence through the completion of the first reproductive cycle have been obtained for *N. orbicollis* (Fig. 5). JHIII has been the only form of JH identified in this species (Trumbo *et al.*, 1995; Scott *et al.*, 2001). A newly emerged female exhibits low synthesis and titer of JH and has small, immature ovaries (Trumbo, 1997; Scott *et al.*, 2001). Reproductive competence is attained in 12 to 20 days, a period during

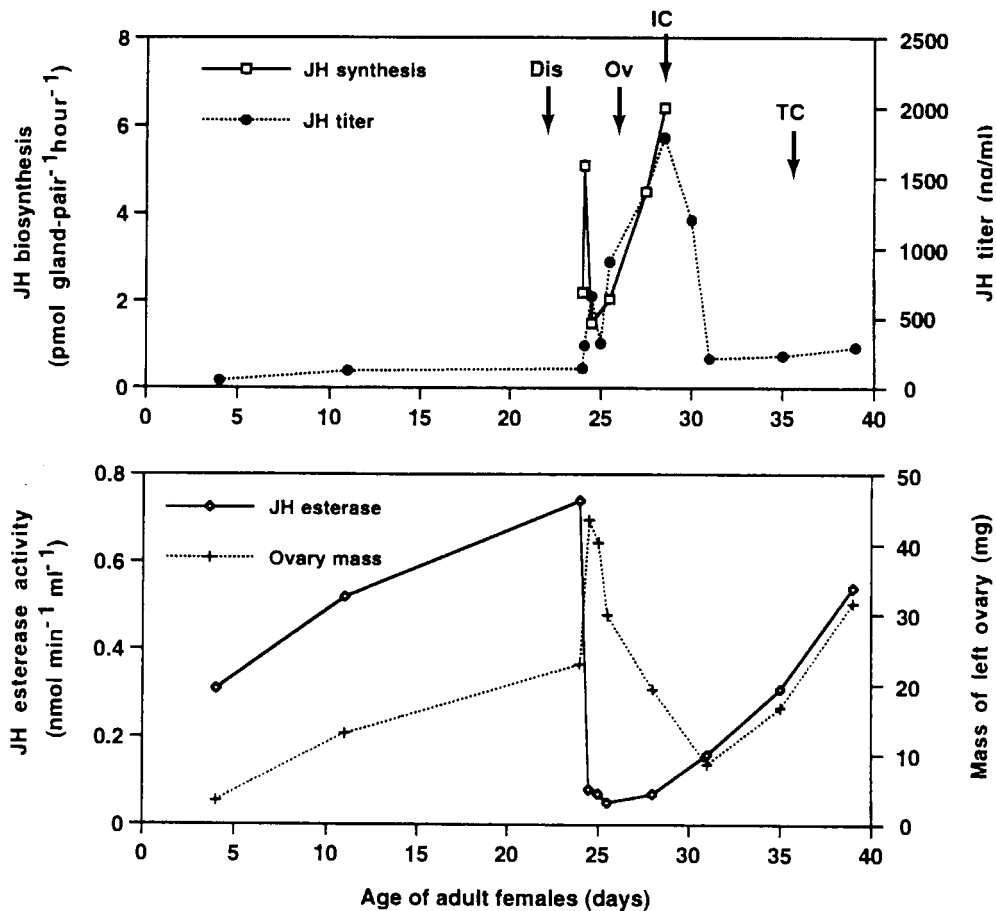


FIGURE 5 (Top) Mean JH biosynthesis and JH titer; (Bottom) JH esterase activity and mass of left ovary in *Nicrophorus orbicollis* from adult emergence through the first parental cycle. Dis, discovery of carcass; Ov, oviposition; IC, initiates care; TC, terminates care. [(Top) Reprinted from Trumbo, S. T., Borst, D. W., and Robinson, G. E., 1995. Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *J. Insect Physiol.* 41, 535–543. (Bottom) From Scott, M., Trumbo, S., Neese, P. *et al.*, 2001. Changes in biosynthesis and degradation of juvenile hormone during breeding by burying beetles: a reproductive or social role? *J. Insect Physiol.* 47, 295–302].

which the female feeds on carrion and on larvae of carrion insects. JH titer and ovarian size increase in concert and then plateau after approximately 20 days (Trumbo *et al.*, 1995). Poorly fed females maintain lower levels of JH and have smaller ovaries. Once reproductive competence is attained, the discovery of a usable small carcass induces rapid changes. Within 10 min of locating a carcass, four recognizable behaviors can be observed: palpating the carcass, lifting the carcass, circumambulating, and making forays away from the carcass while plowing the head through the soil. These behaviors are

thought to be related to assessment of the suitability of the carcass and the soil for a reproductive attempt (Trumbo *et al.*, 1995). Within 10 min of carcass discovery, an increase in JH titer occurs; this is the fastest JH response to a key stimulus recorded (Trumbo *et al.*, 1995). The rapid increase in JH titer is accompanied by an increase in JH synthesis, a decline in JH esterase activity, and a decline in hemolymph proteins (presumably because of vitellogenin uptake by oocytes) during the first 12 hr on the carcass (Scott *et al.*, 2001). Ecologically, the rapid hormonal and behavioral responses are

thought to be necessitated by the intense competition for a spatially unpredictable, protein-rich resource that deteriorates quickly.

In well-fed females the initial surge in JH titer occurs in the absence of feeding on the carcass or mating. However, poorly fed females begin to feed immediately upon discovering a carcass, compromising their ability to exploit the resource quickly. Poorly fed females take longer to oviposit, they oviposit fewer eggs, and the eggs are of smaller mass (S. T. Trumbo and G. E. Robinson, unpublished results, 2001). Well-fed females experience a two- to threefold increase in ovarian mass within 16 hr of discovering a carcass (Scott and Traniello, 1987). Under ideal nutritive, soil, and temperature conditions, oviposition occurs within 16 hr of discovery of a carcass.

As in cockroaches and earwigs, JH synthesis and titer decline by the onset of oviposition (Fig. 5). In a striking contrast, JH titer and synthesis in female burying beetles begin to increase during the nesting phase, reaching a peak on the day that first instar larvae arrive on the carcass. JH titers after oviposition are likely controlled primarily by synthesis since JH esterase activity is uniformly low until the termination of care (Scott *et al.*, 2001). The timing of the appearance of larvae on the carcass is critical to their acceptance by parents. Parents will accept their own or even congeneric young that eclose at the "expected" time. Larvae eclosing too early or late are rejected and cannibalized (Müller and Eggert, 1990). It is not known whether a hormonal change controls this behavioral change.

As larvae mature, they begin to feed exclusively from the carcass. Parents spend less time feeding large larvae and maintaining the nest but will still defend the brood against a predator or congeneric intruder (Fetherston *et al.*, 1990). JH titers fall as larvae develop and only rise again near the termination of the parental period (Trumbo, 1997).

Interestingly, changes in JH titers during the reproductive cycle are similar in males and females. JH titer and rates of synthesis increase in both sexes after discovery of a carcass, fall after oviposition, and reach a second peak on the day that larvae eclose (Scott *et al.*, 2001). However, this second JH peak, is much smaller in males than in females (S. T. Trumbo & G. E. Robinson, unpublished results, 2001). At this time, males are less active care givers than females (Fetherston *et al.*, 1994).

If the female is removed experimentally, the male will increase parental duties (more feeding of larvae and maintenance of the nest), and his JH titers will increase to a level approaching that of a female parent (S. T. Trumbo & G. E. Robinson, unpublished results, 2001).

Müller (1987) and Halffter *et al.* (1983) have clearly demonstrated that females can simultaneously provide care and develop a new clutch of eggs. If the initial brood of *N. vespilloides* is partially destroyed, some females will continue to care for this reduced brood while producing a smaller, replacement clutch (Müller, 1987). She will then care for both sets of these differently aged young. Nutritionally, this is possible because the nest includes a large, protein-rich resource that can be partially consumed and converted into a replacement clutch. Ecologically, the bonanza resources that burying beetles exploit are very rare (Wilson, 1971), and females are adapted to exploit any reproductive opportunity, even if this means producing a small clutch on a deteriorating resource. Unlike cockroaches and earwigs, therefore, parental care is not incompatible with a second round of vitellogenesis and oviposition.

C. Hypotheses to Explain the Role of JH in Burying Beetles

The most striking feature of burying beetle endocrinology is the second peak in JH, which occurs near the time larvae eclose and move to the carcass. High JH titers and rates of synthesis during a typical parental phase are in marked contrast to what has been reported for cockroaches and earwigs. Four hypotheses, not mutually exclusive, have been put forward to explain this second peak in JH. With the information presently available, none of these hypotheses is completely satisfactory.

Hypothesis 1: JH co-opted for a social role. Successful nesting by *Nicrophorus* pairs requires close coordination and communication between the male and female parent (Huerta and Halffter, 1992). The correlation between male and female JH titer and synthesis, the high levels of JH in nonvitellogenic males, and the peak in JH during parental care (when oocytes are immature) suggest that JH has more than a gonadotropic role in burying beetles. Application of JH or JH analogs to reproductively competent females will not induce the threefold increase in ovarian mass that occurs upon

discovery of a carcass (Scott *et al.*, 2001). Scott *et al.* (2001), therefore, propose that JH has been co-opted for a social role in burying beetles. Such a change in function for a chemical signal is not without precedent (Pener, 1985). In honeybees, laying queens have low levels of JH while JH in workers has the nonreproductive role of regulating division of labor (Fahrbach, 1997; Robinson and Vargo, 1997). In support of Scott *et al.*'s hypothesis, application of JH analogs to one burying beetle in a male–female pair at the onset of breeding prevents formation of the pair bond and increases aggression; application of JH to a nonbreeding beetle allows it to be accepted as a mate (M. P. Scott, personal communication, 2001). However, the social role hypothesis does not explain the different levels of JH in males and females on the day that larvae eclose, or the very typical increase and decrease in JH surrounding oviposition.

Hypothesis 2: JH is necessary during the most physiologically demanding stages of the adult life cycle. Juvenile hormone is known to affect many physiological processes; its role at the cellular level is typically to increase metabolism. This hypothesis suggests that the role of JH will evolve to regulate the onset of the most physiologically demanding stages of the life cycle. In most adult female insects, this stage is the production of mature eggs. In insects with active parental care, additional life stages may be equally demanding. This hypothesis accounts for the two peaks in JH, for the lower second peak in male burying beetles (which provide less care), and for the increase in JH in males when the female is prevented from providing care (Trumbo, 1997). However, comparative studies do not support this hypothesis. The cockroach *Diploptera punctata* provides substantial nutrients to developing embryos while maintaining low levels of JH throughout pregnancy (Section II.C), and the burrower bug apparently requires elevated levels of JH during a passive egg-guarding parental period (Section V; Kight, 1998).

Hypothesis 3: JH is a gonadotropic hormone that is necessary but not sufficient for ovarian maturation. This hypothesis suggests that JH is a gonadotropic hormone in burying beetles needed to achieve reproductive competence, but not sufficient to induce the rapid two- to threefold increase in ovarian mass that occurs upon discovery of a carcass. It might be expected that the rapid

ovarian response in females is dependent on key environmental stimuli as well as an increase in JH. A female that is provided a carcass for several hours, which is then removed, will experience a JH surge but will not oviposit. Her ovaries quickly return to the resting state. Ongoing stimulation from the resource, as well as high JH, may be necessary for the rapid ovarian response. Application of JH alone, then, would not be expected to lead to ovarian development. This hypothesis does not account for the second peak in JH in females or for the changing levels of JH in males.

Hypothesis 4: JH is a gonadotropic hormone, and females maintain high levels of JH up until the time that a replacement clutch might be produced. An extension of hypothesis 3, this hypothesis suggests that JH reaches a second peak after oviposition to allow the female to respond quickly to a brood failure (Trumbo, 1997). It is critical for burying beetles to exploit any possible reproductive opportunity because of the rarity of discovering a small, fresh carcass. This hypothesis suggests that after the initial oviposition and decline in JH, the JH levels again increase to facilitate the production of a replacement clutch, if needed. Replacement clutches are readily produced up to 48 hr after larvae arrive on the carcass (approximately 6 days after the first oviposition) (Robertson, 1993). In support of this hypothesis, JH begins to decline when a replacement clutch is no longer an option (once larvae begin to consume the carrion ball and open it up by feeding, the resource deteriorates quickly). Ovaries are typically small during parental care, but this has been examined in females with brood, not females that have oviposited and are waiting for their brood to eclose. After oviposition, the remaining oocytes in *Nicrophorus* are not immediately resorbed (Astorga, 1995). JH levels are variable in females during this period. It would be of interest to correlate JH variation with variation in ovarian development. A possible explanation for the correlation between male and female JH levels would be that the male's spermatogenesis and mating behavior should cycle with his partner's inclination to continue ovipositing, and that these phenomena are also under the control of JH. While the importance of JH for development of male reproductive function has been shown for other insects (Gäde *et al.*, 1997), there is no evidence for these conjectures on male physiology in burying beetles.

D. Prospects

Research on the endocrine control of burying beetle reproduction and parental care will be limited until a clear role for JH has been established. A number of experiments might reveal whether JH has a typical gonadotropic role in burying beetles. Promising studies would be to measure JH titers and oocyte development during the postoviposition, pre-eclosion stage, and to administer JH prior to experimentally manipulated brood failure to determine whether production of a replacement brood is facilitated. In addition, the role of JH following adult emergence could be investigated by topical application of JH to males and females in reproductive diapause (reared under short-day conditions) or to individuals that are poorly fed. It may be that JH has a permissive though not sufficient role in burying beetle reproduction. A useful study of JH esterase would be to measure activity in females whose carcass has been removed. This might reveal how females experiencing a surge in JH after discovery of a carcass are able to reverse ovarian development so quickly when the resource is lost.

The effect of JH on parental care and social interactions also merit continued study. A starting place is to examine whether administration of JH alters the quality or duration of care, and to measure JH when parents care for broods of different size, or when a parent is forced to care by itself because of mate desertion. The absence of a male parent is known to alter the parental behavior of the female, as well as her ability to breed successfully at a later time (Fetherston *et al.*, 1994; Jenkins *et al.*, 2000). We might also expect that key stimuli from the brood alter parents' behavior and physiology. Preliminary studies indicate that switching old larvae for young alters a parents' JH profile (S. T. Trumbo, unpublished results, 2001). Changing the age structure of the brood has been shown to alter parental behavior in earwigs and burrower bugs.

V. MATERNAL CARE IN BURROWER BUGS

The cockroaches, earwigs, and burying beetles are the three non-eusocial insect groups with extensive parental investment that have received the most en-

docrine study. Three other subsocial groups have been used for more limited endocrine work. Each of these systems represents an independent development of parental care and therefore will contribute to answering the question of whether convergent evolution of parental care has been accompanied by convergent mechanisms of physiological regulation.

Parental care is found in a number of families in the order Hemiptera, with two independent occurrences in the Cydnidae (Sites and McPherson, 1982; Tachikawa and Schaefer, 1985). The only cydnid to receive endocrine study is the burrower bug, *Sehirus cinctus*. *Sehirus cinctus* feeds on the fruits of various species of mints and nettles (Labiaceae) that are available for a brief period in spring. A female guards a clutch of 120 to 150 eggs for approximately 10 days, and then guards and provisions nymphs for several more days (Sites and McPherson, 1982). The small size of this species (less than 5 mg) would seem to preclude study of JH titer, JH synthesis, allatectomy, or brain dissection. Investigation has been limited to manipulation of stimuli eliciting care and to topical application of CA inhibitors and JH analogs. Kight (1996) has developed an assay of parental responsiveness by probing a guarding female with a dissecting needle. Guarding females tend to move toward the probe, nonguarding females away. As in earwigs, a parent burrower bug requires contact with its eggs to maintain maternal responsiveness. Separation of more than 48 hr abolishes the defensive response (Kight, 1996). The burrower bug also monitors cues related to hatching and the age of the brood. A mother given older eggs that hatch earlier than the "expected" time (truncated brooder) will provision the nest with food and provide care for fewer days than control females (Kight, 1997). A mother continually supplied with young eggs (older eggs removed so that hatching never occurs—extended brooding) will provide care for 2 to 3 additional days. Her maternal responsiveness will not continue indefinitely, however, suggesting that the appropriate duration of care in this species is influenced by both external cues (from eggs and nymphs) and internal cues (Kight, 1997). One internal cue may be an endogenous cycle related to hunger (Kight and Cseke, 1999). If the ambient temperature is warm and metabolic demand is presumably greater, the time before abandoning eggs during extended brooding is shortened. Additional

environmental cues also influence the duration of care. Females kept under a long photocycle (30-hr day) provide care for a longer absolute period than females under a 24-hr photocycle (Kight, 2000). This is the first demonstration that the number of light cycles is a proximate cue regulating the duration of care in an insect.

Application of an anti-corpora allatal compound (precocene II) depresses maternal responsiveness in a female with eggs. Treatment with methoprene (a JH analog) along with precocene restores parental defense of eggs (Kight, 1998). Therefore, JH appears to be necessary for normal maternal behavior in this species. It is not clear if titers of JH are higher during care or during vitellogenesis, but it is apparent that some level of JH is required for typical parental behavior. Elevated levels of JH during care in the burrower bug cannot serve a social function since this species is uniparental. Further, the assay was performed during a passive non-feeding phase of the parental cycle (during egg guarding) which therefore argues against the hypothesis that high JH may be necessary for metabolically demanding stages of the life cycle. Interpretation of these results is constrained by the lack of information on JH titers during different stages of the reproductive cycle. It is not even clear whether JH is elevated during care giving. One useful experiment would be to administer precocene and JH analogs to prereproductive females or to females between reproductive attempts. Such manipulations might answer whether onset of oviposition, clutch size, or mating behavior is altered by JH. It therefore will be possible to address whether JH also plays the expected role in oocyte development and has not been pre-empted for a solely parental function.

VI. THE MATERNAL OPTION IN THE LACE BUG

The endocrinology of parental care has recently been investigated in the hemipteran, *Gargaphia solani* (Tingidae). Female *G. solani* have two reproductive options, to produce and guard their own brood or to locate another guarding female and dump eggs with her (brood parasitism) (Monaco *et al.*, 1998). Dumpers produce eggs continually while guarders suspend egg production during the parental phase (Parr *et al.*, in press). Females able to dump eggs, therefore, produce

clutches at shorter intervals and will produce more eggs over a lifetime than females that guard (Tallamy and Brown, 1999). It is not surprising that when the opportunity exists, dumping is the preferred option. When no such opportunity can be found, females retain eggs internally for a period and then oviposit and care for their own eggs. It is hypothesized that feedback from the eggs and nymphs, mediated through the antennae, suppresses a new round of egg laying in guarders (Parr *et al.*, in press).

These clear differences in reproductive mode are reflected by physiology. Seventy-two hours after the first oviposition, guarders had fewer primary oocytes and a greater proportion of unchorionated eggs than did dumpers (Parr *et al.*, in press). Experiments employing a JH analog (methoprene) or precocene (an anti-JH compound that disrupts the CA) strongly suggest a role for JH in regulating the reproductive cycle. Females guarding eggs and treated with methoprene abandon their brood more quickly than control treated females; females looking for an opportunity to dump eggs can be induced to guard if treated with precocene (Tallamy and Monaco, in press). These manipulations indicate that, as in cockroaches and earwigs, JH levels increase prior to oviposition and JH levels are suppressed during the parental phase. It is not certain whether the effect of JH on behavior is direct, or whether JH indirectly affects behavior by altering sensitivity to cues from eggs and nymphs.

VII. PARENTAL CARE IN SCARAB BEETLES

A. Life History and Endocrine Correlates of Care

Members of the subfamily Scarabaeinae (dung beetles) feed on dung, carrion, or synthetic dung processed from leaves. Diverse nesting and parental patterns occur, including extended biparental care in underground galleries of a small number of brood balls, each containing one larva (Halffter and Edmonds, 1982). Extreme parental investment in the Scarabaeinae is reflected anatomically by the presence of only one functional ovary with one functional ovariole (Richter and Baker, 1974) (but see Martínez *et al.*, 1999). Cycles of

the ovary, CA, neurosecretion in the brain, and reproductive behavior are highly coordinated and reflect species differences in life history (Martínez, 1995).

After emergence as an adult, females feed and mate before beginning reproduction. *Phanaeus* and *Canthon* females prevented from mating do not construct brood balls nor oviposit (Halffter and Lopez, 1977; Martínez and Cruz, 1990). Insemination of *Canthon cyanellus* increases the CA volume and the release of neurosecretory granules from the pars intercerebralis (PI) (Martínez and Cruz, 1999). One of the longest periods of parental care among the scarabs occurs in *Copris incertus*. The male and female construct a nest together, and three to six eggs are oviposited, one per brood ball (Martínez and Huerta, 1997). Nesting attempts last for more than 100 days, the female parent only leaving when her young emerge as teneral adults. In *Copris*, the female normally does not feed after forming the brood balls and consuming leftover dung (Anduaga *et al.*, 1987). During care the basal oocyte is resorbed (Martínez and Huerta, 1997); the energy conserved by resorption is thought to be important for sustaining the female during the nest care period (Lopez-Guerrero, 1996). If a female is experimentally provided with additional dung early in the nesting cycle, she will construct more brood balls and raise more young. However, if she is provided additional dung after resorption of the basal oocyte, she cannot reverse the shutdown of her reproductive system and no more brood balls will be constructed (Tyndale-Biscoe, 1983). This change in response to additional resources corresponds to the timing of oocyte resorption, which likely is under the influence of the CA. In *Copris incertus* and *Canthon cyanellus*, CA volume and neurosecretory activity of the A cells of the PI peak before the peak in oocyte size and oviposition. Volume of the CA and neurosecretory activity decreases during parental care (when the oocyte is being resorbed), and only begins to increase near the termination of the nesting cycle (Martínez, 1995; Martínez and Huerta, 1997).

An interesting exception to the pattern of oocyte resorption and inhibition of feeding during care occurs in the biparental *Cephalodesmius*, an Australian species in which the female processes fermenting leaves to make a synthetic "dung" ball for each offspring (Monteith and Storey, 1981; Lopez-Guerrero, 1995). Throughout the nesting cycle the male provides new leaves to the fe-

male while she constructs brood balls in underground galleries and continues to feed and to maintain a mature oocyte (Lopez-Guerrero, 1995). This difference in reproductive (and presumably JH) pattern can best be explained by the ecological switch from a discrete and deteriorating resource (dung) to a renewable resource that is gathered continually (leaves) and converted to a stream of offspring. While producing eggs for newly constructed dung balls, the female continues hygienic behavior and progressively provisions larvae in dung balls. Parental care in *Cephalodesmius*, therefore, does not appear to be incompatible with JH synthesis or with ovarian development.

The maternal state in scarabs is maintained by feedback from developing young. In *Oniticellus cinctus*, a mother will abandon an empty brood ball, stimulating a new cycle of oocyte development (Klemperer, 1983). She will maintain care of an empty brood ball, however, if it is treated with dichloromethane extracts from viable larvae, suggesting a chemical cue (Klemperer, 1982). The presence of a stridulatory apparatus on larvae of *Cephalodesmius* suggests that acoustic interaction may be important for maintaining parental care in this group (Monteith and Storey, 1981).

In some but not all scarabs it has been demonstrated that the presence of a male parent affects female fecundity and brood success (Halffter and Lopez, 1977; Monteith and Storey, 1981; Sato, 1998). In biparental species, little is known about the changes in male physiology during the reproductive cycle. Spermatogenesis appears to be under the control of the PI and perhaps the CA in *Canthon cyanellus*; the accessory glands are affected by both the CA and the PI (Martínez, 1994). The cycles in CA volume and PI activity in male and female *Canthon* appear to be similar. It may be that the male and female hormonal cycles in scarabs are synchronized, as is true of burying beetles (Martínez, 1994).

B. Prospects

Direct measures of JH synthesis or of JH titer would be extremely helpful for understanding Scarabaeinae reproduction and parental care. While changes in CA volume have long been used to assess its activity, volume is not always a reliable indicator of synthesis, and lags between changes in volume and changes in synthesis may exist. Although assays of JH synthesis may

be difficult because of heavy sclerotization, assays of JH titer should be feasible because of the considerable volume of hemolymph in many large-bodied scarabs. The biparental scarabs offer a number of interesting possibilities for examining how parent–parent and parent–offspring interactions produce variation in parental care patterns. In *Cephalodesmius*, for example, there is a sexual division of labor; males gather leaves and protect the nest entrance, while females process the leaves into brood balls and clean the nest. However, if the male is not present, the female is fully capable of expressing the full range of behavior (Monteith and Storey, 1981). The neural and/or hormonal changes necessary for this facultative response would be of interest.

Recently, it has been shown that scarabs will respond to treatment with JH analogs. In *Onthophagus taurus* a hormonal switch in larvae related to body size is the key to whether a male will have large horns and be parental, or have short horns and adopt an alternative strategy of furtive matings. Experimental treatment of third instar larvae with JH analogs alters the normal horn size–body size relationship (Nijhout and Emlen, 1998). Artificial selection also can alter the horn size–body size relationship (Emlen, 1996), presumably by altering JH levels at a critical point in development. The ability to employ similar manipulations to study adult behavior and to explore the genetic basis of physiology and behavior will provide a powerful methodology for exploring the hormonal control of parental care in scarabs.

VIII. CONCLUSIONS

A. Broad Phylogenetic Comparisons

JH is the only hormone to have received extensive study in care-giving insects. There is a critical need to have study of other hormones and of biogenic amines. From the phylogenetic survey that has been made, seven tentative hypotheses regarding JH and subsocial reproduction can be made. (1) JH is a gonadotropic hormone in subsocial insects as in nonparental insects. (2) In insects that provide investment internally, high levels of JH and oocyte development will be incompatible with care. (3) In subsocial species that provide care outside the body, levels of JH will be low when care is accompanied by complete resorption of

oocytes and reproductive quiescence. (4) Where application of JH or its analogs inhibit care, the pathways will generally be indirect. (5) Levels of JH may be high during some stages of parental care in species that maintain developed oocytes during care, especially in species facing the ecological necessity of producing a replacement clutch quickly. (6) In species with biparental care, it is necessary to coordinate male and female behavior, especially events surrounding insemination and oviposition. Cycles of JH synthesis may be one mechanism that has been co-opted to facilitate male–female interaction. (7) JH esterase has an important role in clearing the hemolymph of JH near the time of oviposition. However, JH esterase is not a primary regulator of JH titer during the parental care period.

Because of the limited number of models studied, only hypothesis 1 can be considered to be well substantiated at present.

B. Models Examined

The cockroaches are our best model for understanding the role of JH in a group with internal parental investment. Numerous other lineages in insects exhibit “pregnancy” and can be examined to test hypothesis 2. One particularly interesting experiment would be to apply JH during pregnancy to ovariectomized females to evaluate whether the effects of JH on termination of care are direct or indirect (test of hypothesis 4).

In the earwigs there is no apparent ecological reason for oocyte development to occur during parental care. Feeding and care giving are mutually exclusive activities in many species. It has been suggested that cannibalism in earwigs is not consistent with care, and thus feeding must be inhibited. However, in other groups, both feeding and inhibition of cannibalism occur simultaneously during parental care. The lack of feeding in earwigs can best be explained by the mobile feeding behavior of earwigs being inconsistent with nest guarding. Under these circumstances it seems unlikely that the resources necessary for a second round of oogenesis would be available for care-giving females. The cycles of JH synthesis and JH titer in earwigs are understandably pronounced. In earwigs, JH has an inhibitory effect on parental care, although the pathway seems indirect.

One underutilized manipulation for earwigs and for all groups with care external to the body would be to examine the female's physiological and behavioral responses to severe brood reduction. This might suggest whether the separation of care from oogenesis and high JH is a physiological necessity or simply results from caring for a complete clutch while the reproductive system is shut down. Quantitative and qualitative manipulation of stimuli from young may indicate how social feedback coordinates the reproductive cycle. The inability to produce a supplementary clutch while providing care would be convincing evidence that oocyte maturation and high levels of JH are incompatible with care.

In burying beetles it is clear that high JH titer and synthesis occur during the most active parental phase (the day that larvae arrive on the carcass). The function of this JH peak is uncertain, however, because of a lack of information on the reproductive system during the postoviposition period, and on the effects of the male and female on each other. Hypotheses to explain the extraordinary second and higher peak in JH were detailed in Section IV.C.

In the scarab beetles that have been studied, periods of oogenesis and care giving are mostly distinct, and CA activity (as indicated by CA volume) appears to be low during the caring phase. Feeding also does not occur after the dung balls have been formed and the remnants of the dung pat consumed. In species in which several dung balls are made, it would be of interest to determine whether care for the initial dung ball continues during the latter part of the egg laying period. In species such as *Cephalodesmius* and *Lethrus*, which feed and nest for long periods underground while continuing to manufacture synthetic dung balls (Nikolayev, 1967; Monteith and Storey, 1981), it seems likely that there are periods of simultaneous high JH, egg laying, and parental care.

It is difficult to speculate about the burrower bug with the information at hand. It appears that at least some JH is necessary for normal parental care. Experiments to evaluate the expected gonadotropic role of JH in the burrower bug would be helpful. In total, these results suggest that JH has a typical gonadotropic role in subsocial insects but that JH itself is not incompatible with parental care. The possibility that JH has additional social functions is intriguing but unsubstantiated.

C. Narrow Phylogenetic Comparisons

More narrow phylogenetic comparisons will also be useful. Endocrinologists need to take advantage of the many insect groups in which parental and nonparental species occur in the same genus or family. In many cases, the endocrine background for nonparental species has already been done. For example, we know much about the endocrine control of mosquito reproduction (Klowden, 1997) but nothing about the hormonal control of an unusual care-giving mosquito (Lounibos, 1983; Lounibos and Machado-Allsion, 1983). Closely related species differing widely in the degree of parental investment occur in cockroaches, burying beetles, scarab beetles, burrower bugs, and many other groups. Narrow comparative study of such taxa will make it possible to determine how easily JH has been co-opted for different roles.

One tenet of behavioral ecology is that reproduction exacts costs on the individual. Species with parental investment are ideal for studying how hormones mediate the trade-offs among different stages of life history (Ketterson and Nolan, 1992). There are at least three potent techniques for manipulating trade-offs in insects. (1) Noninvasive application of hormones permits investigators to alter the intensity and duration of care in order to monitor correlated physiological parameters that affect fitness (reproductive output, fat body reserves, ability to survive starvation, susceptibility to infection). JH is known to have negative effects on some components of fitness, but these have been little investigated in parental insects. (2) Trade-offs between life history stages can be investigated by altering the physiological demands required during one particular stage. With parental insects this can be easily accomplished by altering the number and age of brood. (3) It also should be possible to examine trade-offs between life history stages by selecting for genetic lines that have enhanced or diminished parenting tendencies. Monitoring the correlated physiological changes will suggest a role for hormones in the regulation of parental care. Many insects are suitable for selection experiments. These have been underutilized in evolutionary and physiological studies of life history. The combination of comparative work, hormone applications, and selection experiments with traditional endocrine techniques will be a powerful methodology for

developing an evolutionary physiology of insect sub-social behavior.

Acknowledgments

This work was supported by NSF grants 9420985 and 9981381. I thank Scott Kight, Susan Rankin, Michelle P. Scott, and Sue Trumbo for review of earlier drafts of this paper. Many thanks to Gene Robinson, who introduced me to insect endocrinology.

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