Juvenile Hormone–Mediated Reproduction in Burying Beetles: From Behavior to Physiology

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The burying beetle, *Nicrophorus orbicollis*, is the first beetle exhibiting parental care for which endocrinological studies have been initiated. Burying beetles bury and prepare small vertebrate carcasses as a breeding resource for their offspring. After emergence as an adult, hemolymph titers of juvenile hormone and ovarian size increase in concert for 2–3 weeks, and both plateau until an appropriate resource is discovered. Upon finding a suitable carcass, titers of juvenile hormone increase extremely rapidly (<20 min), and within 18 h ovarian mass increases threefold and oviposition begins. This rapid reproductive development is hypothesized to be selected by the intense competition for these protein-rich but quickly deteriorating resources. Burying beetle females exhibit an additional juvenile hormone surge at the time young hatch. This peak in juvenile hormone is hypothesized to be associated with either the considerable behavioral demands which accompany care of young larvae or with a female's willingness to oviposit a replacement clutch should brood failure occur early in the care-giving phase. Parental care has evolved in at least 16 additional families within the Coleoptera. Comparative studies of nonparental and parental groups will be important for understanding how the physiology of ancestral groups affects the evolution of complex social behavior. Arch. Insect Biochem. Physiol. 35:479–490, 1997.   © 1997 Wiley-Liss, Inc.

INTRODUCTION

There is tremendous diversity of reproductive behavior among insects, which in many respects are the paradigmatic group for observing behavioral

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plasticity (Wilson, 1971; Thornhill and Alcock, 1983). While this behavioral diversity has attracted considerable experimental attention, the insights obtained by endocrinologists have not been readily integrated into the work of ecologists. Within the Coleoptera, numerous independent lineages occupying a broad range of ecological habitats have evolved parental and social behavior (Wilson, 1971). Since social behavior within these distinct phylogenetic groups is regulated by similar endocrine systems, interspecific comparisons may suggest how ancestral physiology affects the evolution of behavior. There are three potential contributions that physiological studies can make to insect behavioral ecology: 1) providing insights into the role of phylogeny in adaptive evolution, 2) providing tools for exploring the costs and benefits of life history alternatives, and 3) permitting the quantification of responses to key stimuli affecting behavior. The failure to adopt mechanistic approaches to the study of insect reproductive plasticity has not been due to the lack of available techniques. Endocrinologists have detailed the regulation of reproduction from the behavioral to the molecular level (Raabe, 1986; Koeppe et al., 1985; Epple et al., 1990). The analytical power of these techniques, however, has not come to bear forcefully on the behavioral variation that most intrigues ecologists. Endocrinological analyses of reproduction of social coleopterans will place functional studies of behavior on a more substantial foundation as well as provide behavioral ecologists with powerful tools for experimental manipulations.

Juvenile Hormone in Adult Beetles

Endocrinological studies of beetles that do not exhibit parental behavior suggest that juvenile hormone (JH) plays an important role in coleopteran reproduction (de Kort and Granger, 1981; Raabe, 1986). The corpora allata are the only known source of juvenile hormone in beetles, and titers of JH appear to be regulated primarily by signals from the brain (Feyereisen, 1985). In species from diverse orders, rising levels of juvenile hormone in the hemolymph are required for vitellogenesis and oviposition (Koeppe et al., 1985). The only confirmed juvenile hormone in adult coleopterans is juvenile hormone III, which acts as a gonadotropic hormone in *Letinotarsa decemlineata* and *Pterostichus nigrita* (Ferenz, 1981; Khan et al., 1982a, b; de Kort et al., 1982). Levels of juvenile hormone in the hemolymph, or the biosynthetic rate of juvenile hormone, are low in nonreproductive diapausing adults and high in postdiapause reproductives (Ferenz, 1981; de Kort et al., 1982). Administration of juvenile hormone or juvenile hormone analogs to diapausing beetles can induce vitellogenesis and oviposition (Bowers and Blickenstoff, 1966; Conin et al., 1967; Hodek et al., 1973; Okuda and Chinzei, 1988).

The importance of JH in the reproductive behavior of male beetles has received little study. JH does appear to be necessary for normal spermatogenesis in the scarab *Oryctes rhinoceris* (Jacob, 1992).

An investigation of the information cues that affect reproductive events and trigger behavioral responses is essential for understanding both the proximate causes of behavior and evolutionary direction. Exposure to long days has been found to be an important environmental cue for maintaining elevated synthesis and titers of JH (Kramer, 1978; Ferenz, 1981). Cues other

than photoperiod have received less study. Feeding but not mating was found to be necessary for high titers of JH in the Colorado potato beetle (*Leptinotarsa decemlineata*), which breeds shortly after adult emergence (Khan et al., 1982b; de Kort et al., 1985). Among noncoleopterans in which reproduction is triggered by an unpredictable event such as acquisition of a blood meal, individuals often attain reproductive competence and then remain in an arrested state until the appropriate stimuli are experienced (e.g., *Culex* spp.: Edman and Lynn, 1975; *Aedes* spp.: Klowden and Lea, 1979). Until recently, endocrinological studies of beetles had not been carried out on species that respond rapidly to a key environmental stimulus. Comparative studies promise to reveal variations in the role of JH in the control of coleopteran reproduction.

Social Behavior of Beetles

Within the Coleoptera, parental care is exhibited by at least one species in 17 different families (Wilson, 1971; Kudô, 1994). Each family likely represents at least one independent evolution of parental care, and several contain multiple such lineages. Among species providing parental care, social groupings vary from mother and offspring to biparental care to communal breeding with joint care of young. The ecological diversity of mating systems of caregiving insects has prompted extensive study. Wilson (1975) identified four prime movers that select for parental care: stable and structured habitats, harsh environments, predation, and highly valued resources. Each prime mover has been implicated as the primary selective cause of parental care in one or more groups of beetles. In the best studied examples, however, parental care has multiple functions, making it difficult to separate primary and secondary adaptations represented by parental care (*Bledius spectabilis*: Wyatt, 1986; Wyatt and Foster, 1989a, b; *Nicrophorus orbicollis*: Müller et al., 1990; Scott, 1990; Scott and Traniello, 1990; Trumbo, 1991. 1996; *Ips* spp.: Kirkendall, 1983). The ecological and phylogenetic diversity of parental behavior among beetles suggests that comparative studies of physiology will prove useful. Specific comparisons among closely related species differing in social behavior will provide insight into how altered physiology can accommodate the insertion of a parental phase into the reproductive cycle of a nonparental ancestor. Such comparisons will be facilitated where endocrinological studies have already been performed on nonparental species within families that also have species exhibiting care (e.g., Scarabaeidae, Tenebrionidae, Chrysomelidae, Carabidae). Broader phylogenetic comparison will provide insight into how physiology and ecology are related during evolution and whether physiology is flexible under selection for altered social behavior.

Burying Beetles

Burying beetles (*Nicrophorus* spp.) of the family Silphidae are the first group of care-giving beetles to be the subject of endocrinological study. After emerging as adults, males and females feed for 15–20 days on carrion and on insects of carrion until reproductive competence is obtained. Ovarian size initially increases during the feeding period but then plateaus until a resource suitable for reproduction is discovered. Females become sexually receptive

shortly after emergence and can store sperm for 3–4 weeks (Eggert, 1992). Within 24 h of discovery of a small vertebrate carcass by a competent female, ovarian mass increases two- to threefold, and oviposition begins in the surrounding soil (Wilson and Fudge, 1984; Scott and Traniello, 1987). Following discovery of the resource, males and females will fight with rivals, bury the resource, begin to remove hair or feathers, and deposit antifungal anal secretions to control the decomposition (Pukowski, 1933; Halffter et al., 1983). Preparation of the resource and oviposition is thought to occur rapidly because of intense competition from microbes, vertebrate scavengers, and diverse carrion-feeding insects.

Approximately 4 days after oviposition, larvae hatch and crawl to the nest to be cared for by the parent(s). During the first 2 days larvae are on the carcass, parents actively maintain the nest and supplement larval feeding by regurgitations to young. After this period, care becomes more passive (consisting primarily of guarding the nest) and larvae begin to feed exclusively from the prepared carrion ball (Fetherston et al., 1990). The male parent usually deserts the nest before the female. The duration of paternal care is quite flexible, however, and persists longer on larger carcasses, when the development of young is slower, when the female parent is absent (Trumbo, 1991), and when the density of competing beetles is high (Scott, personal communication). The resource can be taken over by burying beetle rivals at any time during the nesting cycle; the probability of a successful intrusion, however, declines markedly after the young have been on the carcass for 24 h (Robertson, 1993). The female parent normally leaves the nest near the time that mature larvae crawl from the nest and seek a place in the soil for pupation. Departing adults will then seek another reproductive opportunity.

EXPERIMENTAL STUDIES OF *NICROPHORUS ORBICOLLIS*

Endocrine Events Associated With Discovery of the Carcass

Trumbo et al. (1995) determined titers of JH in hemolymph samples collected from fed females (mealworm diet) 3, 6, 9, 12, 20, or 30 days after adult emergence and from starved females on days 3, 6, and 9. An additional group of female was fed for 29 days and on day 30 was given a 15–25 g mouse carcass for 24 h. Titers of JH were assayed using a radioimmunoassay (RIA) developed by David Borst (Hunnicutt et al., 1989; Huang et al., 1994; Huang and Robinson, 1995; Trumbo et al., 1995). This RIA requires minimal purification of hemolymph samples and is sensitive enough to be used on single individuals. It is therefore attractive for behavioral studies involving large numbers of individuals. Titers of JH as determined by the Borst RIA produce titer estimates comparable to the Strambi RIA (Strambi et al., 1984) and the Goodman RIA (Goodman et al., 1993), both of which have been validated with gas chromatography/mass spectroscopy (de Kort et al., 1985; Goodman et al., 1990).

In burying beetle adult females, titers of JH increased gradually over the first 20 days and then reached a resting state, closely paralleling ovarian condition (Fig. 1). Within 24 h of finding a carcass, ovarian mass and JH titer
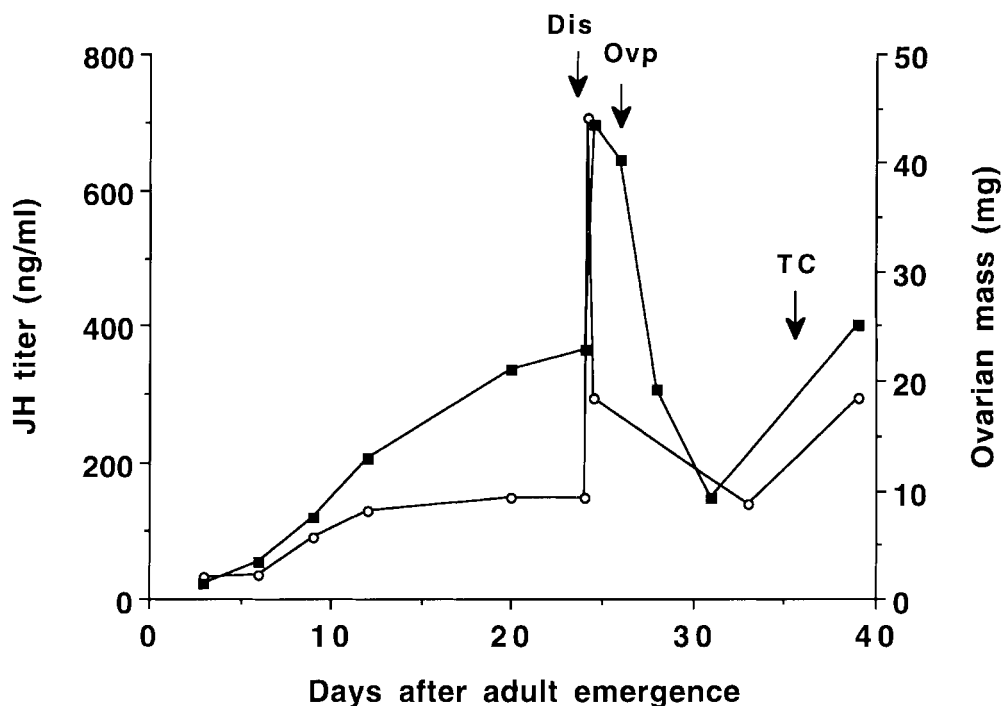
Fig. 1.   Hemoloymph titers of juvenile hormone (open circles) and ovarian mass (closed squares) over the adult lifespan of female *Nicrophorus orbicollis*. Dis = discovery of carcass; Ovp = oviposition, TC = terminate care. Figure modified from Trumbo et al. (1995) and from unpublished results.

increased two- to threefold. These titers, which are some of the highest levels of JH measured, have been confirmed by qualitative analyses using high performance liquid chromatography (Trumbo et al., 1995).

Hemolymph samples were taken from females 2, 10, 20, and 60 min following discovery of a small mouse carcass and from control females without a carcass in order to examine the JH response to carcass discovery more finely. Females exhibited extremely rapid behavioral and hormonal responses. Juvenile hormone titers increased significantly (+100%) within 10 min, perhaps the fastest response of JH to a specific stimulus recorded. Titers of JH continued to increase during the 60 min following discovery and then fell by the time oviposition began (24 h) (Trumbo et al., 1995).

During the 10 min following carcass discovery, females were observed to perform four behaviors which appeared to be related to assessment of resource quality. These were as follows:

palpate: the beetle moves its mouthparts back and forth over the surface of the carcass but does not puncture the skin;
lift: the beetle moves under the carcass, gets into a supine position, and pushes the carcass off the soil surface using its legs;
circumambulate: the beetle walks completely around the midsection of the carcass or walks the length of the carcass from head to tail; and

foray: the beetle leaves the carcass and uses its head to plow through the soil and then returns to the carcass.

Although functions of these behaviors have not been determined, we hypothesize that assessment occurs in three stages: determining whether the resource is carrion (palpating), determining whether the resource is of proper size and in a suitable state of decomposition (lifting and circumambulating), and determining whether the substrate is suitable for burial and oviposition (forays).

Scott and Traniello (1987) found that handling a carcass, rather than feeding or mating, was the cue that triggered ovarian maturation in response to resource discovery. A similar statement can be applied to the JH increase. In our experiments, there was no feeding and no possibility of mating (males were not present) in the 10 min following carcass discovery. Thus, the rapid increase in JH appears related to information obtained during assessment of the resource rather than a response to mating, feeding or extensive preparation of the carcass. Reproductive maturation in burying beetles, then, is regulated by a complex interaction of environmental, behavioral, and endocrine factors in a manner analogous to many vertebrates (Lehrman, 1965).

Juvenile Hormone During the Parental Phase

Mated females of *Nicrophorus orbicollis* were provided mouse carcasses and sampled during the parental cycle to investigate JH correlates of the parental cycle. Titers of JH initially declined following the oviposition period. A second peak in JH titer, however, occurred at the time young arrive on the carcass (Fig. 2). It should be noted that this JH peak was not associated with an increase in ovarian mass. During the first 48 h young are on the carcass, parents are active in maintaining the nest and regurgitating liquefied carrion to larvae (Fetherston et al., 1990). As young grow, they become independent of the parent and begin to feed exclusively from the carcass. Titers of JH decline during the latter part of the parental cycle, before increasing near the time parents desert the nest (Fig. 2).

There are at least two possible explanations for high levels of JH during the first 2 days of parental care (Trumbo, 1996). The first hypothesis (parental care) is that elevated JH is related to active care-giving (parents rarely rest during the first 48 h larvae are present on the carcass). A second hypothesis (clutch replacement) is that JH remains elevated in females to facilitate a second attempt at exploiting the valuable resource should a brood failure occur. Females that lose their brood will often attempt to reuse the carrion resource if it has not been severely depleted (Müller, 1987). Similarly, dung beetles are reproductively prepared to reoviposit in brood balls in the event of brood mortality (Klemperer, 1983; Edwards and Aschenborn, 1989). Among burying beetles in the field, brood failure occurs primarily because a conspecific takes over the resource and commits infanticide. If the intruder is a male, he will attempt to mate with the resident female, thereby manipulating the female into initiating a new brood that he has sired (Trumbo, 1990; Scott, 1990,1994). Successful takeovers usually occur before the developing young have consumed much of the resource (Robertson, 1993). Later in the parental
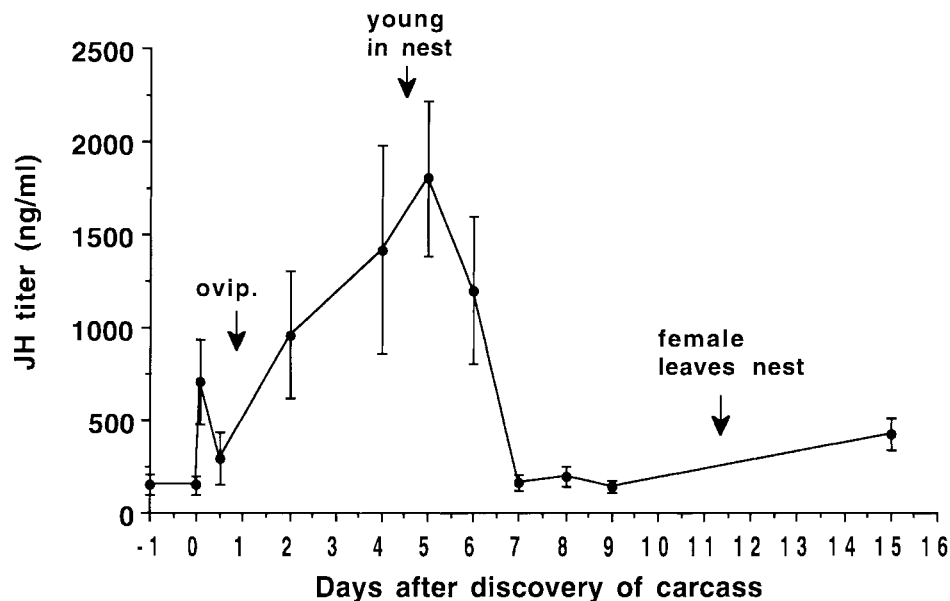
Fig. 2.   Mean (± SE) titers of JH in females during the parental phase of the reproductive cycle (N = 6–10 per data point). Day 0 = day of carcass discovery.

cycle, females will not oviposit a replacement clutch because the resource has deteriorated. Under the clutch replacement hypothesis, JH is maintained at high levels as long as the female will reinitiate reproduction in response to brood failure. Declining JH later in the parental cycle would then be associated with unwillingness to oviposit near a resource that can no longer support a new brood (late in the parental cycle, JH levels are lower than in reproductively competent females without a carcass). Neither the parental care nor clutch replacement hypothesis for the second JH peak in burying beetles can be ruled out with the available evidence.

Preliminary work indicates that JH titer again increases after the female parent leaves the nest, returning to levels associated with reproductively competent females which are without a carcass. Some indication of this can be seen in Figure 2. This increase in JH is associated with an increase in ovarian size, further suggesting a primary role for JH in regulating the cyclical bouts of reproduction in burying beetles.

DISCUSSION

The integration of endocrinology and insect ecology will enhance behavioral studies by suggesting how phylogeny affects social evolution, by providing tools to examine the costs and benefits of life history alternatives, and by the use of hormonal assays to monitor organisms' responses to critical stimuli. The multiple origins of parental behavior within the insects will allow for two types of comparative tests. First, broad phylogenetic comparisons can be made to examine whether convergent behavior is necessarily based on convergent physiology. Although JH is undoubtedly important in

regulating reproduction, it is not known whether the role of JH in organizing the reproductive cycle is the same in each group. Phylogenetic comparisons may make it possible to attribute variation in the role of JH in reproduction to ancestry or to variation in ecology. Such tests will provide insights into how the physiology of nonparental ancestors constrained and influenced the subsequent evolution of social behavior in descendants. More narrow phylogenetic comparisons will provide specific examples of evolutionary transitions from a nonsocial to a social state. For example, the parental *Nicrophorus* is closely related to the nonparental *Ptomascopus* which likewise utilizes small carrion as a breeding resource. *Ptomascopus*, however, does not interact with developing young and abandons the resource shortly after preparing it (Peck, 1982). Hormonal comparisons of these two genera will likely provide insight into the role of JH in species differing in sociality. Similarly, the endocrinology of well-studied nonsocial species can be used as a basis for comparison when future studies of closely related social species are completed. The integration of physiological and ecological studies employing such groups will elucidate how physiology has been altered when a parental phase becomes inserted within the reproductive cycle of a nonsocial ancestor. In turn, an evolutionary perspective will provide endocrinologists with a framework for organizing the variable roles of JH which are being uncovered.

Hormones play the principal role in allocating organisms' resources to nonreproductive, mating, gamete-producing, and parenting phases of the life cycle (Finch and Rose, 1995). Hormonal manipulations can allow experimenters to alter the time and resources spent on these competing needs and thus provide powerful tests of adaptationist hypotheses derived from ecological studies. Such manipulations have been utilized by vertebrate biologists (Marler and Moore, 1988; Ketterson et al., 1992); intriguing possibilities exist for students of insects. For example, in the dung beetle, *Onthophagus bimodis*, males that complete pupal development above a critical body size pursue a parental strategy, locating dung pats and assisting females with brood care. Smaller-bodied males, however, attempt sneak copulations and never provide care (Cook, 1990; Emlen, 1994). Such size-dependent life history switches are thought to be selected in response to strong intrasexual competition along with a variable feeding environment for larvae (Eberhard, 1982). Unfortunately, very little is known of the hormonal control of adult male behavior in *Onthophagus*. Manipulations of larvae and/or adults which alter the strict body size–reproductive strategy relationship may permit testing of functional hypotheses of alternative life histories.

When an organism makes life history decisions, it typically monitors critical environmental stimuli. The stimuli which a species has evolved to rely upon may influence subsequent life history evolution. In biparental burying beetles (*Nicrophorus* spp.), for example, a female will spend more time performing energetically demanding care-giving activities such as feeding young and maintaining the nest when her mating partner abandons the brood (Fetherston et al., 1994). Is her response dependent on stimuli associated with the condition of young, with behavioral demands by the young, with the presence/absence of her mate, or with parental effort of her mate? Conflicts between parents over the appropriate level of care as well as limits to adap-

tive decision-making by parents will not be understood until we know which cues parents monitor selectively. Endocrinological analyses may permit easily quantifiable, sensitive probes for determining when an organism perceives environmental stimuli. These analyses may be especially useful when the behavioral effects are subthreshold and therefore cannot be detected by traditional behavioral techniques.

Studies of insect social behavior which jointly apply endocrinological and ecological techniques need to be undertaken. Comparative studies provide a logical starting point. Phylogenetic comparisons have already uncovered numerous ecological correlates of variation in social behavior. There are no formidable barriers to extending the analysis to the level of physiology.

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