

Social and nonsocial stimuli and juvenile hormone titer in a male burying beetle, *Nicrophorus orbicollis*

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Abstract

We investigated the interaction of social and nonsocial stimuli on juvenile hormone (JH) titer in male burying beetles (*Nicrophorus orbicollis*). The initial JH response to discovery of a carcass was substantial (10–15-fold increase over controls) and rapid (<1 h), and occurred whether or not a female was present. By 3 h after discovery, JH titers were declining, the decline being more pronounced when a female was not present. We also tested the effect of larval stimulation on JH titer in care-giving males by removing a male's brood and replacing it with a brood of first or third instar larvae. Males initially providing care for begging first instar larvae continued to maintain high titers of JH when the replacement broods were first but not third instars. Males caring for third instar larvae (normally low JH titers) maintained low levels of JH regardless of the developmental stage of the replacement brood. This suggests that once males begin to care for nutritionally independent third instar larvae, JH titers remain low regardless of subsequent larval stimulation. Burying beetles are socially and hormonally complex organisms in which stimuli from a breeding resource, mating partners, rivals and young interact to alter the JH profile of breeding adults.

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1. Introduction

The intricate interplay of hormones, behavior and environment, long recognized as important in vertebrates (Lehrman, 1958; Ketterson and Nolan, 1992), is a perspective that is increasingly necessary to understand social invertebrates. Environmental regulation of juvenile hormone (JH) titer is particularly complex. In various insect species, JH titer in adults is affected by nonsocial and social stimuli such as photoperiod, food, mating, young, and dominance interactions (Wyatt and Davey, 1996; Scott and Panaitof, 2004). JH may then regulate ovarian development, vitellogenesis, locomotion, sexual behavior, pheromone release, aggression, migration, termination of parental care and longevity (Koeppel et al., 1985; Cusson, 1989; Kight, 1998; Nijhout and Emlen, 1998; Tatar, 1999; Belgacem and Martin, 2002). Some JH effects (e.g.,

breaking reproductive diapause) may be rapid (Khan et al., 1983) while others (e.g., worker development in honey bees) work gradually and persist over a lifetime (Bloch et al., 2000). The varied effects of JH, many seemingly unrelated at the behavioral level, suggest that our understanding of JH action is inadequate (Wheeler and Nijhout, 2003; Flatt et al., 2005).

A fuller understanding of JH effects may be realized with three approaches. Comparative study has demonstrated that JH may have opposite effects on the same behavior in different species indicating, for example, that JH is not an 'aggression' hormone *per se* because of its varying effects on social dominance across taxa (Röseler, 1985; Brent et al., 2006), nor strictly even a 'gonadotropic' hormone because of the lack of such effects in some social insects (Robinson and Vargo, 1997). Cellular and molecular approaches have shown that JH alters metabolism (Novak, 1966), oxygen consumption (Watanabe and Tanaka, 2000) and mitochondrial activity (Stepien et al., 1988), actions that may link some of the diverse behavioral and

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morphological effects. Concentrated examination of multiple effects of JH in a single model (male, female, juvenile, behavior, physiology, morphology) may reveal an underlying pattern of JH effects that is not apparent in one species—one trait studies. Within a single species, JH levels can respond to multiple stimuli, and, in turn, be the cause of a variety of morphological and behavioral effects (Robinson and Vargo, 1997). In burying beetles, by example, high titers of JH are correlated with a female's discovery of a vertebrate carcass used for breeding, the presence of a rival, carcass preparation, and periods of active care (nest maintenance, feeding of dependent young) (Trumbo, 1996, 1997, 2002; Scott and Panaitof, 2004; Scott, 2006a). JH supplementation is known to increase begging by larvae (Crook et al., 2008), injuries among competing females (Scott, 2006b), egg production in unpaired females, and mortality rates in starved females (Trumbo and Robinson, 2004). A thorough understanding of JH is unlikely to be achieved until the response to stimuli, and the effects of changing levels of JH, are understood for larvae, adult females and adult males. The interaction of hormones and environmental stimuli is especially poorly understood for most male insects (but see Loher, 1960; Pener, 1985) including burying beetles. High JH titers in burying beetle males are correlated with both discovery of a carcass in the presence of a female (Scott and Panaitof, 2004) and with paternal care (Panaitof et al., 2004).

In the present study we examine the interaction of social stimuli (female partner, young) and a nonsocial stimulus (carcass) on JH titer in male *Nicrophorus orbicollis* Say. Males are particularly appropriate subjects for correlating JH and changing activity levels because male behavior following carcass discovery and during parental care is more variable than female's (Fetherston et al., 1994; Rauter and Moore, 2004). Further, while a female will complete carcass preparation and initiate reproduction with or without a male, a male's behavior after carcass discovery is highly dependent on female behavior (Pukowski, 1933). (1) The JH response to discovery of a carcass, known to be rapid in females (Trumbo et al., 1995), was examined in males with and without a partner. Scott and Panaitof (2004) did not find a JH response to a carcass when a female was not present, but JH titers were not examined immediately after discovery when males are most active in assessing the resource. (2) We evaluated the effect of larval stimulation on JH titer in single males caring for a brood. Both care-giving female and single male *N. orbicollis* have high titers of JH during the first 48 h of care, and JH titers decline rapidly thereafter (Panaitof et al., 2004; Scott and Panaitof, 2004, Trumbo and Robinson, unpublished result). Scott and Panaitof (2004) found that in females, continual exposure to young (first instar) larvae prolonged the period of high JH titer during care-giving. Young larvae actively beg for food, and in *N. orbicollis* will not survive without parental regurgitation (Trumbo, 1992). Begging by larvae and regurgitations by parents decline

markedly after the onset of the third instar as the larvae reach nutritional independence (Fetherston et al., 1990; Smiseth et al., 2003). By abruptly switching first for third instar larvae, and vice versa, we tested the hypothesis that the decline in JH titer, characteristic of the latter stages of parental care, is triggered by the presence of less dependent third instar larvae in the nest. We hypothesize that a common feature of rising levels of JH in burying beetles is an increase in energy-intensive behavior.

2. Materials and methods

2.1. Study organism

A laboratory colony of *N. orbicollis*, derived from wild-caught beetles in northern Michigan, USA, was maintained at 21 °C, 15L:9D in mixed-sex groups of 8–12, and fed scraps of chicken liver in containers (8 × 15 × 30 cm) without soil to inhibit reproduction. For breeding experiments, 25–35 day old beetles were presented a thawed 24–30 g mouse carcass in a breeding container two-thirds filled with soil.

2.2. Carcass discovery

Males were isolated two days prior to experimental trials. At the beginning of the active period (lights out) males were presented only a carcass, a carcass and a female, or neither (control) in a breeding container ($N = 8$ –13 per treatment). Contact with the resource was assured by placing it along one edge of the container and confirming discovery by observation. A hemolymph sample (2–6 µl) was taken at 1, 3, 5 or 10 h after discovery of the resource (and at similar times for controls) by severing the hind legs and collecting with a micropipette. An additional set of control males was sampled 2 h prior to the active period. Each male was sampled once. Hemolymph samples were assayed for JH titer as in Trumbo et al. (1995) using a chiral-specific radioimmunoassay (RIA) (Hunnicuttt et al., 1989).

2.3. Caring for larvae

To examine the effect of larval developmental stage on JH levels of care-giving males, male–female pairs were established with a mouse carcass in a breeding container. The female was removed 4 days after carcass preparation (before larvae made their way to the carcass). The nest was checked every 12 h until larvae were present and the male was observed feeding young. For the first two treatments (1st → 1st, 1st → 3rd), the experimental manipulation occurred at this stage (first instar), and for the latter two treatments (3rd → 1st, 3rd → 3rd) the manipulation occurred two days later (young third instar). In all treatments ($N = 10$) the male's brood was removed and replaced with a brood of 12–15 first or third instar larvae taken from another nest. Supernumerary nests were maintained to

ensure an adequate supply of larvae. Ten hours after the switch, brief observations were made to confirm that males fed young, and a hemolymph sample was taken.

2.4. Statistical analysis

To compare JH levels between two samples, titers were log-transformed and evaluated using *t* tests. For the carcass discovery experiment, planned comparisons were JH titers for males on a carcass with and without a female at 1, 3, 5 and 10 h. For multiple unplanned comparisons, Tukey–Kramer tests for all combinations of pairs ($\alpha = 0.05$) were employed (SAS, 2000). Titers were back-transformed for graphical presentation.

3. Results

3.1. Carcass discovery

In response to discovery of a carcass, JH titer increased rapidly in male *N. orbicollis*, as occurs in females (Trumbo et al., 1995). One hour after discovery JH titer in males with a carcass was 10–15 times the level of control males without a carcass (Fig. 1, Tukey–Kramer test). The presence of a female partner did not affect the response at 1 h ($t = 1.74$, 16 df, $p = 0.10$). After 1 h, JH titer began to decline for males with a carcass. The decline was steeper for males without a female such that titers were lower at 3 h ($t = 3.69$, 16 df, $p = 0.002$), 5 h ($t = 3.54$, 14 df, $p = 0.002$) and at 10 h ($t = 2.04$, 16 df, $p = 0.052$) compared to males

with a carcass and a female. JH titers in males with a carcass but no female were significantly elevated over controls at 3 h and 5 h, but not at 10 h (Tukey–Kramer test).

3.2. Caring for larvae

Scott and Panaitof (2004) demonstrated that in parental females, high levels of JH could be prolonged by continually removing developing larvae and replacing them with first instar larvae. We performed the opposite manipulation with males, removing first instar larvae and replacing them with third instar larvae. We found that males given third instar larvae had JH titers characteristic of the later stages of parental care despite having provided care for less than 36 h total (Fig. 2, Tukey–Kramer test). Mean JH titers were nine-fold greater in males on the first day of care when their replacement brood was first instar larvae as compared to males feeding third instar larvae ($t = 5.81$, 18 df, $p < 0.001$). By Day 3 of care, JH titers were consistently low whether single males were given first or third instar larvae as a replacement brood (Fig. 2, Tukey–Kramer test).

4. Discussion

JH titer in male *N. orbicollis* responded to several classes of stimuli. The initial response to discovery of a carcass was rapid (10–15-fold) in the first hour and occurred whether or not a female was present. Scott and Panaitof (2004) did not

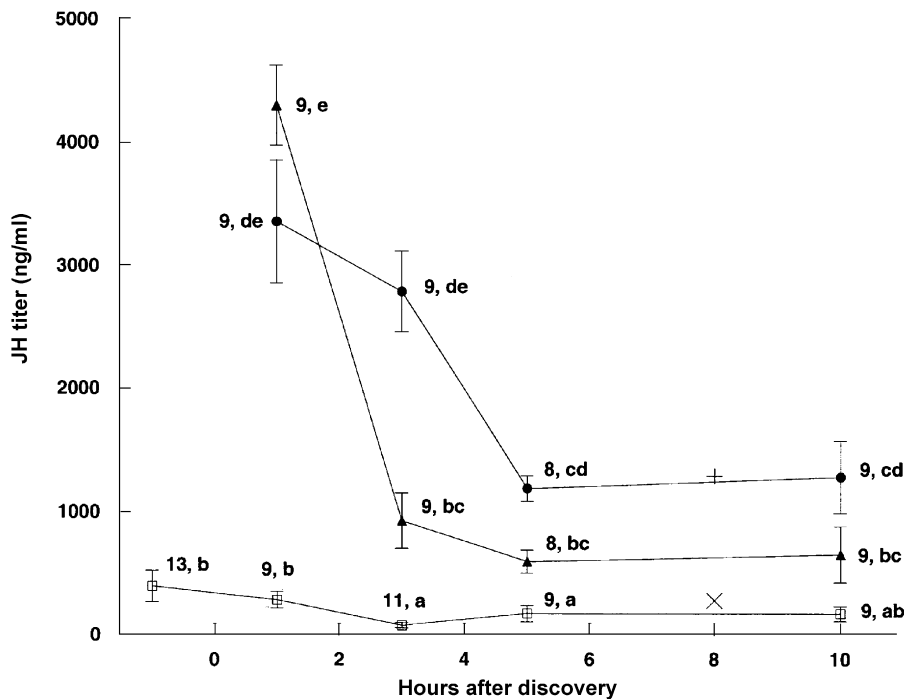


Fig. 1. JH titer (mean \pm se) following discovery of a carcass in males with a carcass (solid triangles), males with a carcass and a female (solid circles), and control males without a carcass (open squares). Sample size is shown next to each point. Different letters represent statistically significant differences (Tukey–Kramer test). The + and x represent mean values for results from Scott and Panaitof (2004) for males on a carcass with and without a female, respectively.

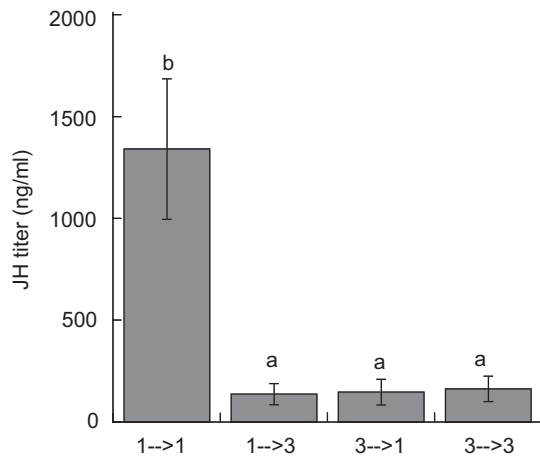


Fig. 2. JH titer (mean \pm se) for unpaired males whose first or third instar brood was removed and replaced with a brood of first or third instar larvae. Different letters represent statistically significant differences (Tukey–Kramer test, $N = 10$ per treatment).

find a JH titer increase in response to carcass discovery when a female was not present, but they took their first measurement at 8 h. They speculated that it was unlikely that JH titer initially peaked then fell by 8 h because variation in JH titer was not correlated with variation in male behavior during this period. We found, however, that with or without a female, JH titer quickly peaked and then declined. The decline in JH was more pronounced when a female was not present. The relationship between JH titer in males and presence of a female needs to be examined. After discovery of a carcass, single males will partially bury a carcass and explore the surrounding soil. They quickly adopt a pheromone-emitting posture in the absence of a female, becoming immobile for long periods (Pukowski, 1933). Paired males, on the other hand, continue carcass preparation behaviors for a sustained period and also mate frequently with the female (mean of 29 matings in 24 h in *Nicrophorus vespilloides*, Müller and Eggert, 1989). How differences in the time profiles of JH titer in males are related to these behavioral differences, and for acceptance by a female partner (Steiger et al., 2007), have not been determined. The interaction of social (female) and non-social (carcass) stimuli and the change in this interaction over time indicates that the complexity of hormonal regulation in this insect approaches that of many vertebrates (Lehrman, 1958; Crews, 1975).

A second important social stimulus affecting JH titer in burying beetles is care of larvae. Scott and Panaitof (2004) found that care-giving *N. orbicollis* females that were continually supplied with first instar larvae had a prolonged period of high JH titer characteristic of the early parental period while females naturally caring for third instar larvae had low levels of JH. We found that JH titer in unpaired males caring for third instar larvae could not be manipulated by providing them with first instar larvae for which to care. Although it is possible that there is a sex difference in the parental response, we feel that the

most likely difference between the studies is methodological. Scott and Panaitof (2004) never provided first instar larvae to females that had previously cared for third instars, as in our study. The third (and last) instar is hypothesized to be an important signal that the period of active care is nearing an end. Regurgitations to third instar larvae occur after the molt but then rapidly decline (Fetherston et al., 1990). Scott and Panaitof (2004) found high JH titers in breeding females that were not allowed to care for any young suggesting that the JH decline during parental care is brought about by stimuli from third instar larvae, not from the absence of regurgitation itself. This conclusion is consistent with our finding that males given third instar larvae experienced a rapid decline in JH titer despite having provided care for less than 36 h, and also consistent with Smiseth et al. (2003) who found that food allocation in the transition to larval independence is largely controlled by offspring.

While exposure to third instar larvae may cause JH titer in parents to drop precipitously, JH titer during the first and second instar may be related to the intensity of care. Parental care in *N. vespilloides* has a positive effect on both survival and growth of larvae (Eggert et al., 1998). Females provide more care than paired males (Fetherston et al., 1990), and single males provide more care than paired males (Fetherston et al., 1994; Rauter and Moore, 1999, 2004; Smiseth et al., 2005). Both examples of greater care are associated with higher JH titers in *N. orbicollis* (Panaitof et al., 2004). In addition, paired males but not females provide more care with a large as compared to a small brood, likely because paired females work close to their maximum rate with a small brood (Rauter and Moore, 2004). In *Nicrophorus pustulatus*, males but not females have higher JH titers on larger carcasses (> 100 g) with larger broods than on smaller carcasses. On large carcasses JH titers in paired males equaled that of females (Trumbo and Scott, unpublished result). Taken together, these results suggest that high JH titers correspond or anticipate periods of active parental care and that titers decline as soon as stimuli (third instar larvae) indicate a less intense period of parental care.

Despite the remarkable correlations of JH titer and active parental care, attempts to qualitatively manipulate parental behavior by treatment with fluvastatin (inhibitor of JH biosynthesis), OTFP (JH esterase inhibitor) and methoprene (a JH analog) have produced no striking results (Panaitof, 2006; Trumbo, unpublished result). Similarly, JH or JH analog treatment prior to carcass discovery had only a marginal effect on increasing egg production (Trumbo and Robinson, 2004), and no effect on the outcome of dominance interactions (Scott, 2006b; Trumbo, 2007). JH treatment has been shown to increase begging by larvae (Crook et al., 2008), increase injuries in dominance interactions (Scott, 2006b), and decrease longevity in starved beetles (Trumbo and Robinson, 2004). There remain obvious gaps in our knowledge of the role of JH in adult burying beetles. It would not be unprecedented

for a hormone to be tightly correlated with behaviors that it did not directly control. Sullivan et al. (2000, 2003) found that in worker honey bees JH did not activate foraging, a behavior associated with high JH titer, but JH did affect the age of onset of foraging by accelerating the pace of development. Allatectomy (removal of the corpora allata, the source of JH) delayed foraging and impaired flight by lowering metabolic rate. In various insects JH is known to alter metabolic activities including cytochrome oxidase activity (Stepien et al., 1988), respiratory metabolism (Novak, 1966) and oxygen consumption (Watanabe and Tanaka, 2000).

In burying beetles the peak in JH titer after carcass discovery, the higher peak when a rival is present (Scott, 2006a), and the highest peak during early parental care are all associated with the onset of a sustained period of activity that does not involve a diurnal quiescent period. A direct effect of JH might be to increase metabolic rates associated with transitions to more active behavioral states in adult burying beetles. The JH peak during active parental care (first instar larvae) in female *N. pustulatus* is associated with a 150% increase in CO₂ production, an indicator of higher metabolic rates (Rauter, unpublished data). Treatments to lower JH titer during early parental care do not terminate care (Panaitof, 2006) suggesting that JH does not control this behavior directly. Rather, high JH titer in burying beetles may provide the physiological conditions for this and other energy-intensive behavior to be sustained or performed closer to optimality without incurring related physiological deficits. The multiple roles for JH and the complex interplay of hormones, behavior and environmental stimuli, apparent in burying beetles, suggest that a simple cause-and-effect relationship of JH and behavior might not occur in this group.

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References

- Belgacem, Y.H., Martin, J.R., 2002. Neuroendocrine control of a sexually dimorphic behavior by a few neurons of the pars intercerebralis in *Drosophila*. Proceedings of the National Academy of Sciences 99, 15154–15158.
- Bloch, G., Borst, D.W., Huang, Z.-Y., Robinson, G.E., Cnaani, J., Hefetz, A., 2000. Juvenile hormone titers, juvenile hormone biosynthesis, ovarian development and social environment in *Bombus terrestris*. Journal of Insect Physiology 46, 47–57.
- Brent, C., Peeters, C., Dietmann, V., Crewe, R., Vargo, E., 2006. Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*. Journal of Comparative Physiology, A 192, 315–320.
- Crews, D., 1975. Psychobiology of reptilian reproduction. Science 189, 1059–1065.
- Crook, T.C., Flatt, T., Smiseth, P.T., 2008. Hormonal modulation of larval begging and growth in the burying beetle *Nicrophorus vespilloides*. Animal Behaviour 75, 71–77.
- Cusson, M., 1989. Involvement of juvenile hormone in the regulation of pheromone release activities in a moth. Science 243, 210–212.
- Eggert, A.-K., Reinking, M., Müller, J.K., 1998. Parental care improves offspring survival and growth in burying beetles. Animal Behaviour 55, 97–107.
- Fetherston, I.A., Scott, M.P., Traniello, J.F.A., 1990. Parental care in burying beetles: the organization of male and female brood-care behavior. Ethology 85, 177–190.
- Fetherston, I.A., Scott, M.P., Traniello, J.F.A., 1994. Behavioural compensation for mate loss in the burying beetle *Nicrophorus orbicollis*. Animal Behaviour 47, 777–785.
- Flatt, T., Tu, M.P., Tatar, M., 2005. Hormonal pleiotropy and the juvenile hormone regulation of *Drosophila* development and life history. Bioessays 27, 999–1010.
- Hunnicut, D., Toong, Y.C., Borst, D.W., 1989. A chiral specific antiserum for juvenile hormone. American Zoologist 29, 48a.
- Ketterson, E.D., Nolan, V.J., 1992. Hormones and life histories: an integrative approach. American Naturalist 140, 533–562.
- Khan, M.A., Koopmanschap, A.B., deKort, C.A.D., 1983. The relative importance of nervous and humoral pathways for control of corpus allatum activity in the adult Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say). General and Comparative Endocrinology 52, 214–221.
- Kight, S.L., 1998. Prococene II modifies maternal responsiveness in the burrower bug, *Sehirus cinctus* (Heteroptera). Physiological Entomology 23, 38–42.
- Koeppel, J.K., Fuchs, M., Chen, T.T., Hunt, L.M., Kovalick, G.E., Briers, T., 1985. The role of juvenile hormone in reproduction. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology, vol. 8. Pergamon Press, Oxford, pp. 165–204.
- Lehrman, D.S., 1958. Induction of broodiness by participation in courtship and nest-building in the ring dove (*Streptopelia risoria*). Journal of Comparative Physiology and Psychology 51, 32–36.
- Loher, W., 1960. The chemical acceleration of the maturation process and its hormonal control in the male of the desert locust. Proceedings of the Royal Society Series B - Biological Sciences 153, 380–397.
- Müller, J.K., Eggert, A.-K., 1989. Paternity assurance by "helpful" males: adaptations to sperm competition in burying beetles. Behavioral Ecology and Sociobiology 24, 245–249.
- Nijhout, H., Emlen, D., 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Sciences 95, 3685–3689.
- Novak, V.J.A., 1966. Insect Hormones. Methuen and Co., Ltd., London.
- Panaitof, S.C., 2006. Physiological Bases of Parental Care in the Burying Beetles, *Nicrophorus orbicollis*. University of New Hampshire, Durham, 145 pp.
- Panaitof, S.C., Scott, M.P., Borst, D.W., 2004. Plasticity in juvenile hormone in male burying beetles during breeding: physiological consequences of the loss of a mate. Journal of Insect Physiology 50, 715–724.
- Pener, M.P., 1985. Hormonal effects on flight and migration. In: Kerkut, G.A., Gilbert, L.I. (Eds.), Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Oxford, pp. 441–490.
- Pukowski, E., 1933. Ökologische untersuchungen an *Necrophorus* F. Zeitschrift für Morphologie und Ökologie der Tiere 27, 518–586.
- Rauter, C.M., Moore, A.J., 1999. Do honest signalling models of offspring solicitation apply to insects? Proceedings of the Royal Society Series B - Biological Sciences 266, 1691–1696.
- Rauter, C.M., Moore, A.J., 2004. Time constraints and trade-offs among parental care behaviors: effects of brood size, sex and loss of mate. Animal Behaviour 68, 695–702.
- Robinson, G., Vargo, E., 1997. Juvenile hormone in adult eusocial Hymenoptera: gonadotropin and behavioral pacemaker. Archives of Insect Biochemistry and Physiology 35, 559–583.

- Röseler, P.F., 1985. Endocrine basis of dominance and reproduction in polistine wasps. In: Hölldobler, B., Lindauer, M. (Eds.), *Experimental Behavioral Ecology and Sociobiology*. Sinauer, Sunderland, Massachusetts, pp. 259–272.
- SAS, 2000. *JMP Statistics and Graphics Guide v.4*. SAS Institute, Inc., Cary, North Carolina.
- Scott, M.P., 2006a. Resource defense and juvenile hormone: the “challenge hypothesis” extended to insects. *Hormones and Behavior* 49, 276–281.
- Scott, M.P., 2006b. The role of juvenile hormone in competition and cooperation by burying beetles. *Journal of Insect Physiology* 52, 1005–1011.
- Scott, M.P., Panaitof, S.C., 2004. Social stimuli affect juvenile hormone during breeding in biparental burying beetles (Silphidae: *Nicrophorus*). *Hormones and Behavior* 45, 159–167.
- Smiseth, P.T., Darwell, C.T., Moore, A.J., 2003. Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society Series B—Biological Sciences* 270, 1773–1777.
- Smiseth, P.T., Dawson, C., Varley, E., Moore, A.J., 2005. How do caring parents respond to mate loss? Differential response by males and females. *Animal Behaviour* 69, 551–559.
- Steiger, S., Peschke, K., Francke, W., Müller, J.K., 2007. The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. *Proceedings of the Royal Society Series B—Biological Sciences* 274, 2211–2220.
- Stepien, G., Renaud, M., Savre, I., Durand, R., 1988. Juvenile hormone increases mitochondrial activities in *Drosophila* cells. *Insect Biochemistry* 18, 313–321.
- Sullivan, J.P., Jassim, O., Fahrbach, S.E., Robinson, G.E., 2000. Juvenile hormone paces behavioral development in the adult worker honey bee. *Hormones and Behavior* 37, 1–14.
- Sullivan, J.P., Fahrbach, S.E., Harrison, J.F., Capaldi, E.A., Fewell, J.H., Robinson, G.E., 2003. Juvenile hormone and division of labor in honey bee colonies: effects of allatectomy on flight behavior and metabolism. *Journal of Experimental Biology* 206, 2287–2296.
- Tatar, M., 1999. Evolution of senescence: longevity and the expression of heat shock proteins. *American Zoologist* 39, 920–927.
- Trumbo, S.T., 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). *Ecological Entomology* 17, 289–298.
- Trumbo, S.T., 1996. Parental care in invertebrates. *Advances in the Study of Behavior* 25, 3–51.
- Trumbo, S.T., 1997. Juvenile hormone-mediated reproduction in burying beetles: from behavior to physiology. *Archives of Insect Biochemistry and Physiology* 35, 479–490.
- Trumbo, S.T., 2002. Hormonal regulation of parental care in insects. In: Pfaff, D.W., Arnold, A.P., Etgen, A.M., Fahrbach, S.E., Moss, R.L., Rubin, R.R. (Eds.), *Hormones, Brain and Behavior*. Academic Press, New York, pp. 115–139.
- Trumbo, S.T., 2007. Can the challenge hypothesis be applied to insects? *Hormones and Behavior* 51, 281–285.
- Trumbo, S.T., Robinson, G.E., 2004. Nutrition, hormones and life history in burying beetles. *Journal of Insect Physiology* 50, 383–391.
- Trumbo, S.T., Borst, D.W., Robinson, G.E., 1995. Rapid elevation of juvenile hormone titer during behavioral assessment of the breeding resource by the burying beetle, *Nicrophorus orbicollis*. *Journal of Insect Physiology* 41, 535–543.
- Watanabe, M., Tanaka, K., 2000. Hormonal control of diapause and overwintering traits in a leaf beetle, *Aulacophora nigripennis*. *Physiological Entomology* 25, 337–345.
- Wheeler, D.E., Nijhout, H.F., 2003. A perspective for understanding the modes of juvenile hormone action as a lipid signaling system. *Bioessays* 25, 994–1002.
- Wyatt, G.R., Davey, K.G., 1996. Cellular and molecular actions of juvenile hormone. 2. Roles of juvenile hormone in adult insects. *Advances in Insect Physiology* 26, 1–155.