

Can the “challenge hypothesis” be applied to insects?

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Abstract

The challenge hypothesis, conceived for testosterone and vertebrates, has recently been applied to juvenile hormone (JH) and insects. Scott [Scott, M.P., 2006a. Resource defense and juvenile hormone: the “challenge hypothesis” extended to insects. *Horm. Behav.* 49, 276–281] found that JH in the burying beetle *Nicrophorus orbicollis* increased in response to a social challenge in the presence of a breeding resource, while there was no such JH response in the purported brood parasite *Nicrophorus pustulatus*. Two important implications of the challenge hypothesis that need to be tested are whether JH affects dominance and why there are interspecific differences in the JH response to a social challenge. The effect of JH on dominance in burying beetles was examined by topical application of JH III to one of two competing females at 24 h and again at 1 h prior to presentation of a breeding resource (mouse carcass). JH supplementation had no effect on dominance in intraspecific interactions in *N. orbicollis*, as measured by possession of the carcass on Day 1, 3 or 7. Similarly, JH did not alter carcass ownership during competition between *N. orbicollis* and *N. pustulatus* nor did it affect reproductive success. Irrespective of JH supplementation, *N. pustulatus* became increasingly dominant as the trials progressed, rarely occupying the carcass on Day 1, but excluding *N. orbicollis* in nearly half the trials by Day 7. These findings, and a brief review of the burying beetle literature, suggest that the challenge hypothesis, as applied to testosterone and vertebrates, does not yet have an analogous model for JH and insects.

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The challenge hypothesis proposes that the production of hormones that regulate aggression and dominance will be influenced by the social environment (Wingfield, 1984; Wingfield et al., 1990). As conceived, the vertebrate hormone testosterone would increase seasonally when dominance interactions, especially interactions related to territorial aggression, were likely (Wingfield, 2004; Wingfield et al., 1987), and might further increase in response to a social challenge (Wingfield, 1985). In monogamous birds, by example, testosterone in males is expected to increase at the start of breeding and decrease after egg laying and during paternal care. If monogamous males are challenged socially, however, testosterone is expected to remain high throughout the nesting period, a pattern more similar to polygynous males (Parisot et al., 2005; Vleck and Brown, 1999; Wingfield et al., 1990). Production of hormones that promote breeding and dominance

behavior can be costly. It is not unexpected that high levels of these hormones would be limited to contexts in which increased production results in a fitness advantage. While testosterone can promote or help to maintain dominance (Wingfield, 2004; Wingfield et al., 1987), it can also decrease paternal care (Ketterson and Nolan, 1992; Silverin, 1980) and survival (Dufty, 1989).

Juvenile hormone (JH) is known to have a broad array of effects in insects, even broader than that of testosterone in vertebrates. Most JH effects are unrelated to aggression or dominance. In different species, JH is known to affect larval development, adult diapause, reproduction, migratory behavior, temporal polyethism in social insects, learning and memory, pheromone emission, wing length polyphenism, aging, immune function, nestmate recognition, the termination of parental care, and, in burying beetles, the maintenance of parental care (Panaitof et al., 2004; Robinson and Vargo, 1997; Tatar et al., 2001) (reviews in Flatt et al., 2005; Wheeler and Nijhout, 2003; Wyatt and Davey, 1996). While JH supplementation promotes

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dominance in polistine wasps (Barth et al., 1975; Röseler, 1985), JH supplementation has no such effect in other species (e.g., Adamo et al., 1994; Bloch et al., 2000) and even causes a loss of dominance in a queenless ponerine ant (Brent et al., 2006). The multitude of JH effects and the opposite direction of JH behavioral effects in different species with similar social behavior, suggest a complex and not fully understood role for this hormone (Flatt et al., 2005; Wheeler and Nijhout, 2003).

Recently, Scott (2006a) extended the challenge hypothesis to insects using a burying beetle, *Nicrophorus orbicollis* Say, as a model system (also see Fahrbach, 2006). Like monogamous birds, burying beetles are biparental, compete intensely for breeding opportunities, and may or may not be challenged by rivals during the care-giving period (Pukowski, 1933). Burying beetles locate a small vertebrate carcass and prepare it as a food resource for young (Pukowski, 1933). Males often fight with males, and females with females (Bartlett, 1988; Otronen, 1988) although the time course over which aggression is intense varies with species (Suzuki, 2000). Eggs are oviposited in the soil near the buried carcass and after 3–4 days, larvae hatch and make their way to the nest where they are cared for by both parents. Defeated females attempt to parasitize the brood (Bartlett, 1988; Müller et al., 1990).

In place of testosterone in vertebrates, Scott (2006a) proposes that juvenile hormone in burying beetles would play similar roles, promoting breeding, altering dominance, incurring costs, and increasing when a social challenge is likely. JH production and JH titers are highly correlated in *N. orbicollis*, and predictably change with phases of the reproductive cycle. JH levels increase within 10 min of discovery of a carcass and exhibit a peak (500–1000 ng/ml) in females prior to oviposition. JH levels then decline but increase again and reach a second, higher peak (1500–3000 ng/ml) in females during the first 2 days of care for larvae (Scott et al., 2001; Trumbo, 1997). JH levels during parental care are higher in females than in males, and differences in JH have been related to the intensity of parental care (Panaitof et al., 2004; Scott, 2006a). Scott (2006a) found that when a male or female *N. orbicollis* discovered a carcass, the typical increase in JH is accentuated if a same-sex rival is present. In *Nicrophorus pustulatus* Herschel, by contrast, no such social effect on JH was noted. Scott (2006a) attributed the interspecific difference to *N. pustulatus* being ‘nonparental’ because *N. pustulatus* can act as a brood parasite (Trumbo, 1994) and can utilize a resource that requires less preparation (Blouin-Demers and Weatherhead, 2000).

The challenge hypothesis generates a number of intraspecific and interspecific predictions (Hirschenhauser et al., 2003; Wingfield, 2004; Wingfield et al., 1990) that can be tested with burying beetles. Table 1 summarizes ten predictions that might apply to burying beetles if JH acts as does testosterone in vertebrates. Although only two of these predictions (A and I) are met, several of the most critical predictions have not been tested. These are that JH supplementation promotes dominance and reproductive success in competitive contexts (predictions B and C), and that species and sex differences in the JH response to a challenge are consistent with differences in life history (prediction J). I use JH supplementation experiments, and a

Table 1

Predictions of the challenge hypothesis (Hirschenhauser et al., 2003; Wingfield et al., 1990) and relevant findings for burying beetles based on JH supplementation and correlation experiments

Predictions of the challenge hypothesis	Findings for JH and burying beetles
A. Hormone increases during competitive social interactions	Yes (correlation, Scott, 2006)
B. Hormone promotes dominance	? (Supplementation, this paper)
C. Hormone increases reproductive success in competitive contexts	? (Supplementation, this paper)
D. Hormone is higher during periods of social instability	? (Correlation, reviewed in this paper)
E. Hormone is higher in the more competitive sex	No, males are more competitive but females reach higher levels of JH (correlation, Panaitof et al., 2004; Scott et al., 2001; Trumbo, 2006)
F. Hormone has little or a negative effect on reproductive success in non-competitive contexts	No, supplementation increases egg production and decreases latency to oviposition in females (Trumbo and Robinson, 2004)
G. Hormone is higher during nest initiation than during parental care	No (correlation, Scott et al., 2001; Trumbo, 1997)
H. Hormone decreases when parental care (non-guarding) increases	No (correlation, Panaitof et al., 2004)
I. Hormone decreases survival	Yes (supplementation, Trumbo and Robinson, 2004)
J. Hormone levels will be higher in species experiencing social challenges during reproduction	? (Correlation, reviewed in this paper)

brief review of the burying beetle literature, to test these predictions. I focus on females because although males are more competitive (Trumbo, 2006), females have higher levels of JH than males, females engage in intra- and interspecific competition for carcasses at discovery while males delay aggression until a female is present (Bartlett, 1988), and most importantly, Scott (2006a) found a clear JH response to a social challenge in female *N. orbicollis*.

Methods

General procedures

All beetles were from laboratory colonies maintained on a 15L:9D photoperiod at 21 ± 1 °C and fed scraps of chicken liver for 25–30 days after emerging as adults. A high dose of JH III was selected for supplementation for three reasons: (1) burying beetles naturally exhibit high peak levels of JH III; (2) previous work using a radioimmunoassay indicated that this dose would increase JH III titers in the hemolymph to the high end of the physiological range (Trumbo et al., 1995, unpublished result); and (3) JH supplementation at these and slightly lower levels produces clear biological effects including increasing egg production, decreasing latency to oviposition (prediction F), reducing survival under a starvation regime (prediction I, Trumbo and Robinson, 2004) and, more recently, increasing larval begging (T.C. Crook, T. Flatt, P.T. Smiseth, unpublished data on JH analog supplementation).

Experimental protocol

To examine the effects of JH on dominance, two uninjured *N. orbicollis* females were matched for age and size (pronotal width and body mass). One of the two females, chosen at random, was treated with 400 µg of JH III (Sigma-Aldrich) in 2 µl of hexane at 24 h and again at 1 h prior to presentation of a 22–25 g mouse (*Mus musculus* L.) carcass ($N=40$ intraspecific pairs). Control

females were given 2 μ l of hexane. Both females were marked by clipping the lower edge of one elytron. The treated and control *N. orbicollis* female were held on the mouse carcass for 20 s and gently released underneath. The carcass lay on top of soil in a 30 \times 18 \times 11-cm container. The container was checked once per day on Days 1, 3 and 7, and scored as: JH-treated beetle only on the carcass, control beetle only on the carcass, or other (neither or both beetles on the carcass). Mortality of females was determined on Day 7.

A similar procedure was used to examine interspecific encounters. Female *N. orbicollis* were paired with female *N. pustulatus* of the same pronotal width. Either the *N. orbicollis* or *N. pustulatus* was supplemented with JH as outlined above. The second female was given 2 μ l of hexane. There were a total of 64 pairs of females, 32 pairs in which *N. orbicollis* was treated with JH and *N. pustulatus* with hexane and 32 pairs in which *N. pustulatus* was treated with JH and *N. orbicollis* with hexane. Pairs were presented a 21–24 g mouse carcass. Containers were checked on Days 1, 3 and 7. Dispersing larvae were placed into pupating containers and emerging adults were counted and weighed, by species.

Statistical analysis

Statistical analysis followed SAS (2000). Frequency data for dominance (sole possession of a mouse carcass) were placed into 2 \times 2 tables and analyzed using Fisher's Exact Tests (two-tailed). Measures of reproductive success were not normally distributed and between-treatment variances were typically not homogeneous. Nonparametric measures (Kruskal–Wallis) of treatment differences were therefore employed.

Results

JH supplementation had no effect on dominance for *N. orbicollis* in intraspecific encounters. Hormone treatment did not alter the ability of a female to be in sole possession of a mouse carcass (Fig. 1A; $P > 0.20$ Fisher's Exact Test for each of Days 1, 3 and 7). Mortality did not differ between hormone-treated and control beetles ($P > 0.20$, Fisher's Exact Test) although low mortality (six hormone treated and three control beetles) limited the power of the statistical test.

JH supplementation also did not affect dominance in interspecific encounters (Figs. 1B, C). The probability that *N. orbicollis* or *N. pustulatus* possessed the carcass on Days 1, 3 and 7 did not depend on which female was treated with JH (Figs. 1B, C; $P > 0.20$ for each of Days 1, 3 and 7, Fisher's

Exact Tests). Carcass ownership by *N. orbicollis* during interspecific encounters declined between Days 1 and 7 ($P = 0.005$; Fisher's Exact Test, data for *orbicollis*-treated and *pustulatus*-treated trials combined, $N = 64$). *N. orbicollis* was clearly being replaced by *N. pustulatus*, which was more likely to possess the mouse carcass on Day 7 compared to Day 1 ($P < 0.0001$; Fisher's Exact Test, data for *orbicollis*-treated and *pustulatus*-treated trials combined, $N = 64$). Both *N. pustulatus* and *N. orbicollis* were ultimately aggressive (13 *N. orbicollis* and six *N. pustulatus* were killed; $P > 0.20$, Fisher's Exact Test). It was apparent, however, that while *N. orbicollis* was aggressive from the start, *N. pustulatus* only became highly aggressive near the time larvae began arriving on the carcass (see Suzuki (2000) for a similar competitive shift between *Nicrophorus vespilloides* and *N. quadripunctatus* during the breeding attempt).

On Days 3 and 7 there were a few trials in which neither or both females were on the mouse carcass (4/64 trials on Day 3, 3/64 trials on Day 7). On Day 1 there were 12/64 such trials, significantly higher than on Days 3 and 7 ($P = 0.002$, Fisher's Exact Test), likely reflecting the absence of the dominant female while ovipositing in the surrounding soil. Exactly half of these 12 were in the *orbicollis*-treated and *pustulatus*-treated trials ($P = 0.99$).

Possession of the carcass on Day 7 was strongly related to reproductive success in interspecific encounters. In 64 trials, there were 56 females that both were in sole possession of the carcass on Day 7 and produced broods. In 51 of those trials, the resident female produced a greater brood mass than the excluded beetle ($P < 0.0001$; Binomial test). In three trials in which *N. orbicollis* controlled the carcass, and in two trials in which *N. pustulatus* controlled the carcass on Day 7, the defeated female produced a greater brood mass, indicating highly successful brood parasitism. There was no difference in reproductive success between *orbicollis*-treated and *pustulatus*-treated trials for *N. pustulatus* (Fig. 2; $N = 32$ per treatment; brood mass: $S = 1054$, $P > 0.20$; number of larvae: $S = 1051$, $P > 0.20$; Kruskal–Wallis) or for *N. orbicollis* ($N = 32$ per treatment;

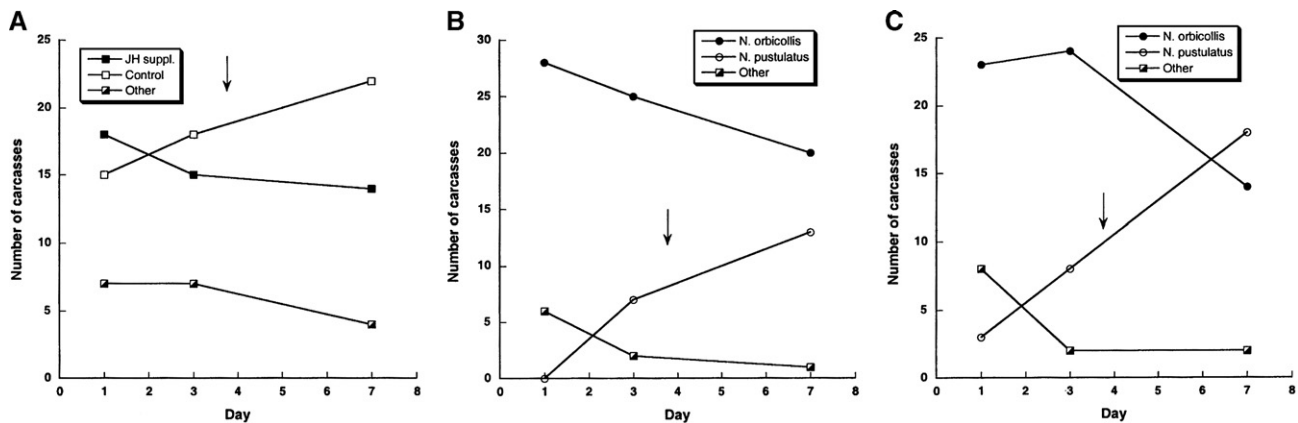


Fig. 1. (A) The number of carcasses ($N = 40$) occupied solely by the JH-treated *N. orbicollis* female, solely by the control-treated *N. orbicollis* female, or other (neither or both females on the carcass) during intraspecific encounters. (B and C) The number of carcasses occupied during interspecific encounters solely by *N. orbicollis*, solely by *N. pustulatus*, or other (neither or both females on the carcass) when (B) *N. orbicollis* was treated with JH ($N = 32$) and when (C) *N. pustulatus* was treated with JH ($N = 32$). The arrows indicate the approximate time larvae arrive on the carcass.

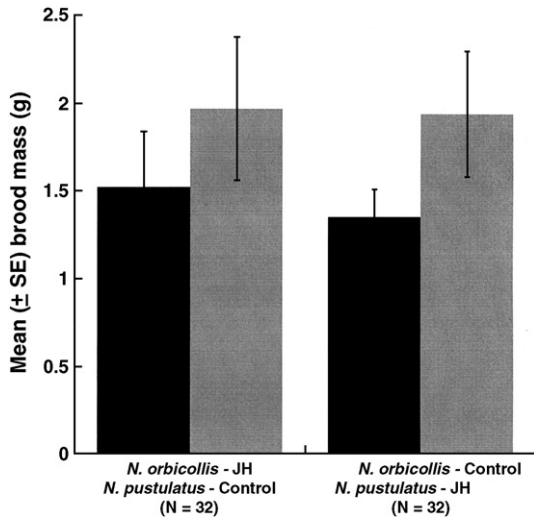


Fig. 2. Brood mass produced by *N. orbicollis* (black bars) and *N. pustulatus* (gray bars) during interspecific competition when *N. orbicollis*.

brood mass: $S=1010$, $P>0.20$; number of larvae: $S=1022.5$, $P>0.20$; Kruskal–Wallis).

Discussion

Scott (2006a) proposed that the burying beetle might be the first insect model to satisfy the requirements of the challenge hypothesis, as originally envisioned for testosterone in male vertebrates (Wingfield, 1985). There are a number of difficulties. Of ten predictions generated by the challenge hypothesis (Table 1), only two were clearly met for JH and burying beetles. These are Scott's (2006a) demonstration that JH is up-regulated in *N. orbicollis* in response to a social challenge (prediction A), and the finding that JH has survival costs (prediction I, Trumbo and Robinson, 2004). The present study provides critical evidence that does not support the application of the challenge hypothesis to burying beetles and JH. In both intra- and interspecific interactions among unfamiliar individuals, JH did not affect dominance as would be predicted if JH in burying beetles has a similar role to testosterone in vertebrates (prediction B). The challenge hypothesis further predicts that high levels of hormones related to aggression will be adaptive in competition, and might be expected to increase reproductive success in those contexts. In the present study, JH supplementation had no effect on reproductive success in interspecific interactions between *N. orbicollis* and *N. pustulatus* (prediction C). Recently, Scott (2006b) reported that application of a JH analog, methoprene, increased injuries in burying beetles but had no effect on dominance 2 days after presentation of a carcass or on reproductive success among competing conspecific females. These findings further suggest that JH in burying beetles fails to satisfy many of the criteria of the challenge hypothesis. Although body size, sex, residency and species identity affect dominance in burying beetle encounters (Otronen, 1988; Suzuki, 2000; Trumbo, 2006), we still lack knowledge about a physiological basis for this trait.

There are additional difficulties with applying the challenge hypothesis to JH and burying beetles (Table 1). In birds, testosterone is higher in males than females, and higher still when males do not provide parental care (Ketterson and Nolan, 1992). In burying beetles, the highest JH levels are found in females during an intense period of parental feeding (prediction G, Panaitof et al., 2004). JH is much lower in parental males despite the fact that they spend more time guarding the nest than females (Fetherston et al., 1990; Smiseth and Moore, 2004) and are specialized for brood defense (prediction E, Trumbo, 2006). If the female parent is removed, the male spends less time guarding and more time feeding (Fetherston et al., 1994; Smiseth et al., 2005). Contrary to prediction H of the challenge hypothesis, its JH levels spike upward (Panaitof et al., 2004). In burying beetles, periods of social instability (when nest takeovers are likely) may have either low JH levels (following oviposition and before care) or high JH levels (before oviposition, during care) (prediction D, Panaitof et al., 2004; Scott et al., 2001; Trumbo, 1997). Parents also remain highly aggressive toward intruders late in the parental cycle when JH levels are quite low.

The lack of a JH response to a social challenge in *N. pustulatus* cannot be due to its being 'nonparental' (Scott, 2006a) because both male and female *N. pustulatus* regurgitate to larvae and defend the brood (Robertson, 1992; Trumbo, 1992). Scott (2006a) noted that *N. pustulatus* can be a brood parasite (Trumbo, 1994) and suggested that this might explain their lack of JH response to a social challenge. All burying beetle species that have been examined, however, exhibit brood parasitism (Müller et al., 1990; Suzuki, 2000); it is unlikely that this trait can explain interspecific differences. Scott (2006a) also found that *N. pustulatus* did not elevate JH titers when confronted by a competitor 4 days after discovery of a carcass, at a time that the present study indicates that *N. pustulatus* is highly aggressive. This result is contrary to prediction A of the challenge hypothesis.

Because of the diverse effects of JH on insect behavior and physiology (Flatt et al., 2005; Wheeler and Nijhout, 2003), it can only be speculated why JH titers increased in *N. orbicollis* in response to rivals in Scott's (2006a) study. JH in adult burying beetles increases in response to three very different cues: discovery of a carcass, a challenge by a rival (in some species), and the presence of young larvae on a carcass. In each case, the relevant cue indicates a period of heightened and sustained future activity. JH may be required to facilitate such energetically demanding physiology or behavior, but increased aggression may or may not be involved. Sustained competitive interaction is one of only many behavioral and life history contexts that may be correlated with increasing levels of JH, and when JH levels do increase, there is minimal effect on dominance and competitive ability. The challenge hypothesis will require an alternative model system if it is to apply to JH and insects.

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