ANIMAL BEHAVIOUR, 2008, 75, 71–77
doi:10.1016/j.anbehav.2007.04.009

Available online at www.sciencedirect.com
ScienceDirect

Hormonal modulation of larval begging and growth in the burying beetle *Nicrophorus vespilloides*

TARA C. CROOK*, THOMAS FLATT† & PER T. SMISETH*
*Faculty of Life Sciences, The University of Manchester
†Department of Ecology and Evolutionary Biology, Brown University

(Received 19 January 2007; initial acceptance 14 March 2007; final acceptance 2 April 2007; published online 25 September 2007; MS. number: 9246)

Recent studies on birds show that two steroid hormones, testosterone and corticosterone, stimulate nestling begging and growth. Here, we seek to investigate whether juvenile hormone, a major regulatory insect hormone, has similar effects on larval begging and growth in insects. To this end, we experimentally elevated larval juvenile hormone levels by topical application of methoprene, a potent and stable synthetic juvenile hormone analogue, and monitored effects on larval begging and growth in the burying beetle *Nicrophorus vespilloides*. In this species, larvae feed partly by begging for predigested carrion from parents and partly by self-feeding. We showed that elevated juvenile hormone levels stimulate larval begging, suggesting that juvenile hormone in insects could have a similar function to that of testosterone and corticosterone in birds. We also showed that elevated juvenile hormone levels have a negative effect on larval growth and that this negative effect occurs regardless of whether larvae forage by begging or by self-feeding. This finding shows that the effects of juvenile hormone on larval growth are independent of the effects on begging, suggesting that the mechanisms by which juvenile hormone affects offspring growth in insects differ from those by which testosterone and corticosterone affect growth in birds.

Keywords: begging; burying beetle; growth; insects; juvenile hormone; *Nicrophorus vespilloides*; signalling of need

In many animal species, offspring solicit resources from their parents by engaging in conspicuous begging displays (Kilner & Johnstone 1997). Game theory suggests that conspicuous offspring begging signals provide an evolutionarily stable resolution of within-family conflicts of interest over resource allocation because such signals are costly and therefore provide parents with reliable information on offspring need (Godfray 1991, 1995). Empirical studies in diverse taxa suggest that begging levels reflect offspring hunger and that parents provide resources in relation to begging levels (Kilner & Johnstone 1997). Recent studies on different birds show that hormones play an important role in stimulating nestling begging effort, thereby enhancing offspring growth (e.g. Schwabl 1996; Kitaysky et al. 2001; Eising & Groothius 2003; Goodship & Buchanan 2006). Here, we seek to investigate whether the endocrine system in insects has a similar effect on larval begging.

In birds, testosterone and corticosterone, two steroid hormones, stimulate nestling begging behaviour. For example, in canaries, *Serinus canaria*, and black-headed gulls, *Larus ridibindus*, levels of maternally derived testosterone in the egg yolk are positively correlated with begging and growth after hatching (Schwabl 1996; Eising & Groothius 2003). In pied flycatchers, *Ficedula hypoleuca*, levels of circulating endogenous testosterone in the nestlings correlate positively with begging and fledging success (Goodship & Buchanan 2006), and in black-legged kittiwakes, *Rissa tridactyla*, experimentally elevated levels of corticosterone stimulate begging (Kitaysky et al. 2001). By contrast, levels of maternally derived testosterone in the egg yolk of European starlings, *Sturnus vulgaris*, correlate positively with growth but negatively with begging at hatching (Pilz et al. 2004). Thus, while there is good evidence that hormones affect begging and growth in birds,

Correspondence: P. T. Smiseth, Faculty of Life Sciences, Michael Smith Building, The University of Manchester, Oxford Road, Manchester M13 9PT, U.K. (email: per.t.smiseth@manchester.ac.uk). T. Flatt is at the Department of Ecology and Evolutionary Biology, Division of Biology and Medicine, Brown University, Box G-W, 80 Waterman Street, Providence, RI 02912, U.S.A.

0003–3472/08/$30.00/0

the detailed causal relationship between circulating hormones, begging, and growth shows interspecific variation.

To date, studies on the hormonal modulation of offspring begging and growth have exclusively focused on birds. This is surprising given that offspring begging occurs in a wide range of animal taxa, including some insects (Rauter & Moore 1999; Smiseth & Moore 2002; Kaptein et al. 2005; Kölliker et al. 2006). Insects offer a particularly interesting model for studying hormonal modulation of begging and growth because insects possess a hormone system quite distinct from that of vertebrates (Nijhout 1994). Besides many specialized peptide neurohormones, insects have two major regulatory hormones (1) juvenile hormone, a lipid-like sesquiterpenoid, and (2) 20-hydroxy-ecdysone, a steroid, both of which are involved in the regulation of numerous aspects of physiology, development, life history, and behaviour (Nijhout 1994; Flatt et al. 2005).

Here we focus on the potential role of juvenile hormone in modulating larval begging and growth in the burying beetle *Nicrophorus vespilloides*. This species, like other members of the same genus, breeds on carcasses of small vertebrates (Eggert & Müller 1997; Scott 1998). Larvae forage partly by begging for predigested carrion from the parents and partly by self-feeding from the carcass (Smiseth & Moore 2002; Smiseth et al. 2003). Juvenile hormone is a versatile hormone that plays a regulatory function in numerous contexts in insect larvae, including the onset of metamorphosis (Williams 1956; Nijhout 1994), growth of imaginal tissues (Emlen & Allen 2003), metabolic activity (Sláma & Hodková 1975) and immune function (Rantala et al. 2003). In other insects, juvenile hormone titers reflect offspring nutritional state (e.g. Schal et al. 1997). This makes juvenile hormone a particularly attractive candidate hormone for regulating begging and growth because, in *N. vespilloides*, begging levels reflect nutritional state (Smiseth & Moore 2004), and begging has a positive effect on growth (Lock et al. 2004). In adult burying beetles, juvenile hormone is positively correlated with parental care towards begging larvae (Panaitof et al. 2004; Scott & Panaitof 2004; Trumbo & Robinson 2004). However, whether juvenile hormone also plays a role in modulating larval begging and growth is unknown.

The aim of our study was to investigate whether juvenile hormone could play a similar role in modulating larval begging and growth in insects as testosterone and corticosterone in birds. A recent study shows that juvenile hormone can play a similar role in insects as testosterone and corticosterone in vertebrates at least with respect to effects on immune function (Rantala et al. 2003). However, juvenile hormone is structurally different from testosterone and corticosterone and appears to play a regulatory role in a multitude of contexts in insects, which in vertebrates are regulated by different hormones (Nijhout 1994). If juvenile hormone plays a similar role in insect larvae as testosterone and corticosterone in nestling birds, we expect that elevated juvenile hormone levels stimulate begging and enhance growth. To test this expectation, we experimentally elevated juvenile hormone levels of *N. vespilloides* larvae and monitored effects on larval begging and growth. We next investigated the causal relationship between juvenile hormone levels, begging, and growth. In birds, testosterone and corticosterone might enhance nestling growth and survival by stimulating begging (Schwabl 1996; Kitaysky et al. 2001) or by inhibiting ineffective and costly begging (Pilz et al. 2004). If juvenile hormone has a similar effect as testosterone and corticosterone in birds, we expect that increased hormone levels affect growth when larvae beg for resources from the parents but not when they forage by self-feeding. To test this expectation, we manipulated the presence or absence of parents and monitored the effects of juvenile hormone on growth.

METHODS

General Procedures

Adult beetles originated from an outbred laboratory population. For use in the experiments, we randomly selected pairs of nonsibling virgin male and female beetles. Once pairs had been selected, they were moved to a transparent container (17 × 12 cm and 6 cm high) filled with a 2 cm layer of moist soil. We provided each pair with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, U.K.), with a mean size ± SD of 24.5 ± 2.1 g ($N = 65$).

Approximately 60 h after a pair had been set up for breeding, the female was transferred to a new container along with the carcass. The male parent was removed at this stage because male care has no effect on offspring growth or survival under laboratory conditions (Smiseth et al. 2005). The eggs were left to hatch in the old containers, which were searched for any newly hatched larvae four times each day. We used newly hatched larvae to generate standardized broods of 20 same-aged larvae. This brood size is well within the natural variation for this species (mean ± SD: 21 ± 10 larvae; range 2–47 larvae; Smiseth & Moore 2002). We standardized brood size and age composition because begging is affected by variation in both brood size (Smiseth & Moore 2002) and age (Smiseth et al. 2003).

Juvenile Hormone Treatment

To investigate whether juvenile hormone plays a role in modulating larval begging and growth in *N. vespilloides*, we experimentally elevated larval juvenile hormone levels by topical application of methoprene (Sigma-Aldrich Company Ltd, Poole, Dorset, U.K.) to the back of the larvae just below the head using a pipette. Methoprene is a potent and stable synthetic juvenile hormone analogue that is widely used in insect endocrinology because it reliably mimics juvenile hormone action (Wilson 2004). The manipulations were always conducted 24 h after hatching, that is, during the second larval instar, corresponding to the age of the peak in begging and food provisioning (Smiseth et al. 2003).

To determine which concentrations of methoprene to use in our experiments, we first conducted a pilot experiment in which we randomly divided larvae of 11 broods into four within-brood treatment groups: (1) 50 μg of