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Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae)

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Abstract. In nature, larvae of the dung beetle *Onthophagus taurus* (Schreber 1759) are confronted with significant variation in the availability of food without the option of locating new resources. Here we explore how variation in feeding conditions during the final larval instar affects larval growth and the timing of pupation. We found that larvae respond to food deprivation with a reduction in the length of the instar and premature pupation, leading to the early eclosion of a small adult. To achieve pupation, larvae required access to food for at least the first 5 days of the final instar (= 30% of mean third-instar duration in control individuals), and had to exceed a weight of 0.08 g (= 58% of mean peak weight in control individuals). Larvae that were allowed to feed longer exhibited higher pupation success, but increased larval weight at the time of food deprivation did not result in increased pupation success except for larvae weighing > 0.14 g. Larvae responded to food deprivation by initiating and undergoing the same sequence of developmental events, requiring the same amount of time, as *ad libitum*-fed larvae once those had reached their natural peak weight. Our results reveal a striking degree of flexibility in the dynamics and timing of larval development in *O. taurus*. They also suggest that premature exhaustion of a larva's food supply can serve as a cue for the initiation of metamorphosis. Premature metamorphosis in response to food deprivation has been documented in amphibians, but this is, to the best of our knowledge, the first time such a behaviour has been documented for a holometabolous insect. We discuss our findings in the context of the natural history and behavioural ecology of onthophagine beetles.

Key words. Developmental cues, dung beetle, larval development, *Onthophagus*, regulation of pupation, timing of metamorphosis.

Introduction

The duration of larval growth and the timing of pupation are critical components of postembryonic development of holometabolous insects (Nijhout, 1994). Both have received early attention by biologists (e.g. Riley, 1883; Wodsedalek, 1917) and continue to be the focus of many developmental, physiological and evolutionary studies (e.g. Riddiford & Ashburner, 1991; Nijhout, 1994, 1999; Zera & Denno, 1997; Greene, 1999). However, with the exception of a few model systems (Nijhout, 1994), little is known about how holometabolous insects determine when to halt larval growth and when to make the transition to the pupal stage (e.g. Lafont, 1994). The internal or external cues that holometabolous insects use to regulate the duration of larval growth and the timing of pupation likewise remain a mystery (see e.g. Riddiford, 1996; Nijhout, 1999).

In the regulation of the duration of larval growth and the timing of pupation, some insects appear to rely at least in part on a pre-programmed critical size threshold and their ability to somehow assess their own size, or some close correlate thereof, as they grow (e.g. Nijhout, 1981). Transition to the pupal stage requires, among other things, that individuals

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exceed this pre-programmed size threshold prior to the next moult (Nijhout, 1999). Consequently, if deprived of food before attaining their threshold size, critical-size controlled insects will not pupate. However, many retain the ability to reach their critical size at a later point in time by means of extending the last larval instar (e.g. Bradshaw & Johnson, 1995; Petersen *et al*., 2000), or by moulting into supernumery instars (see below). In the sphinx moth *Manduca sexta*, for example, final-instar caterpillars deprived of food prior to attaining their critical weight can moult into a supernumery instar, which in nature presumably allows the individual to locate additional food, resume feeding, and to eventually pass the critical weight prior to the next moult (Nijhout, 1975). Also, several species in the dermestid beetles genus *Trogoderma* undergo multiple moults into progressively smaller supernumery instars if deprived of food for extended periods of time, and resume feeding and growth when food once more becomes available (Riley, 1883; Wodsedalek, 1917; Beck, 1971a, b; for similar findings in other taxa see e.g. Beck, 1950; Allegret, 1964; Blakley & Goodner, 1978; Nijhout, 1979, 1981; Blakley, 1981; Mieczyslawa & Szolajska, 1995; but see Hill & Goldsworthy, 1970).

For some holometabolous insects, extending larval development to locate additional food sources may not always be an option. For example, many dung beetle larvae are largely immobile and develop in underground chambers that contain a finite amount of resource provided by their parents (Halffter & Edmonds, 1982; Halffter, 1997). Once this resource is used up, locating additional food sources is impossible. In such instances, completion of larval development would require either correct assessment of the initial resource size and quality by the parents (e.g. Moczek, 1998) or the ability to terminate larval development prematurely and undergo pupation and metamorphosis at a smaller body size once the food supply is exhausted. Here we investigate how larvae of the scarab dung beetle *Onthophagus taurus* (Schreber 1759) respond to variation in food availability, and examine the consequences of variation in food provisioning for the duration of larval growth and the timing of pupation.

Onthophagus taurus is a common dung beetle in pastures of the Eastern U.S. Adult *O. taurus* reproduce in tunnels dug underneath horse and cattle dung pads, where they provision dung fragments for their offspring in the form of so-called brood balls. Larvae are largely immobile and ill-equipped to dig through soil and hence are unable to reach other brood balls after they have consumed their own (Moczek, unpublished data, see also Moczek & Emlen, 1999). Each brood ball therefore constitutes the total amount of resource available to a single larva (Moczek, 1996, 1998; Moczek & Emlen, 1999; for information on the natural history of *O. taurus* see Goidanich & Malan, 1962, 1964; Moczek, 1996, 1998, 1999; Moczek & Emlen, 1999, 2000).

Earlier studies on this species demonstrated that the amount of food available to larvae, the brood ball mass, determines adult body size, probably by affecting some component of larval growth and development (Moczek, 1998). Brood ball masses produced by females in the laboratory vary considerably (mean = 2.57 g, s.d. = 0.79 g, range: 0.80–4.22; $n = 124$; see Moczek, 1998), and this variation is sufficient to generate a variation in body sizes comparable to natural populations (Hunt & Simmons, 1997; Moczek, 1998; Moczek & Emlen, 1999). This suggests that significant variation in larval feeding conditions is probably commonplace in nature, and it appears that larvae possess a mechanism that permits them to complete larval development and metamorphosis despite variation in their food supply.

Most of the larval growth of *O. taurus* occurs during the final, third instar (A. P. Moczek, unpublished data; see also Emlen & Nijhout, 1999). Larvae spend about 70% of their growth period as third instars and increase their initial weight by approximately 500% over the course of the third instar alone (A. P. Moczek, unpublished data). Any effects of food availability on larval growth should therefore be most obvious during the third instar. Here, we focus on *O. taurus* third-instar larvae and monitor larval development under experimentally manipulated feeding conditions and explore the effects of variation in larval feeding conditions on the duration of larval development and the timing of pupation.

Materials and methods

Breeding and rearing

We used a laboratory colony of *O. taurus* to obtain adults for the production of larvae. The colony was derived from over 1500 individuals collected from pastures in Durham County, NC. Beetles were originally collected in August 1997 by hand from whole dung pad samples (see Moczek & Emlen, 1999 for details on sampling methods). The colony was past its sixth generation in the laboratory at the time of our experiments (February to May 1999) with a colony size of over 1000 individuals per generation, and was kept in a constant temperature room at 26°C and 60% relative humidity (RH) under a LD 16 : 8 h photocycle.

We obtained larvae by allowing five pairs of adult beetles, selected at random from the colony, to breed in plastic containers (25 cm tall, 20 cm diameter) filled with a moist sand-soil mixture and provided with 0.5 L of cow manure enriched with 4% wheat germ and 1% torula yeast. Breeding containers were kept at 26°C and 60% RH under a LD 16 : 8 h photocycle. A total of approximately 190 containers and 950 beetle pairs was used to produce over 5300 brood balls over the course of this study. Approximately half of the brood balls was used to maintain the laboratory colony through subsequent generations, whereas the remainder was allocated at random to several experiments including those described here. For the present study brood balls were collected 7 days after adults had been introduced to the breeding containers. By this time most larvae had already hatched and passed through the first instar. Brood balls were carefully opened and second-instar larvae were transferred into artificial growth containers.

Growth containers were made of 12-well tissue culture plates (Costar™). Each well (1.5 cm deep, 1.8 cm diameter) was filled with cow manure that had previously been squeezed by hand through a cheese cloth to remove excess moisture and

to obtain a consistency similar to that of brood balls naturally produced by adult beetles. Earlier work showed that the removal of excess moisture substantially improves larval growth and reduces the likelihood of fungal infections (A. P. Moczek, unpublished observation). Dung was pressed tightly into each well and a small chamber was carved into the centre to provide room for a larva. Each well contained a single larva. The amount of dung in each well was in excess (> 5 g) of the amount typically provisioned by adult beetles. In addition we added dung to the well over the course of larval development to minimize the likelihood that larvae would run out of food prior to the intended day of food removal. The larval rearing plates were kept in a constant temperature room at 26°C, 60% RH, and in complete darkness except for a brief daily examination.

Monitoring larval development

Larvae were assigned at random to either control or experimental groups. Control animals had unlimited access to food for their entire larval development. Experimental animals were removed from their food source at days 2, 3, 4, 5, 6, 7 and 10 of the third larval instar by transferring larvae into wells that contained a moist sand soil mixture but no manure. We initially assigned five larvae to each food removal day. No individual survived if food deprivation began prior to day 5 (see below). Because the main goal of this study was to examine the effects of food deprivation on the duration of larval development and the timing of pupation we therefore assigned the remaining larvae preferentially to days 5, 6, 7 and 10, when larvae had a chance to survive to the pupal stage. All experimental and control larvae were checked daily. During the third instar, each larva was weighed daily to the nearest 0.001 g using a high sensitivity balance (Mettler AE50^{TM}). We also recorded the day at which larvae reached their peak weight or were removed from food, the day at which larvae purged their gut and entered the prepupal stage, the day at which individuals pupated and eclosed as adults, and if and at what stage an animal died. Furthermore, we measured the width of the thorax of eclosing adults as an estimate of body size (see Emlen, 1994a, b, for justification, and Moczek & Emlen, 1999, for a detailed description of measurement procedures).

Statistical analyses – pupation success

To examine the effects of larval weight and the time of food removal on larval ability to reach the pupal stage we used linear regression analyses of pupation success on larval weight class or day of food removal, respectively. We initially included all experimental animals in this analyses. However, no individual survived prior to day 5, or below a weight of 0.08 g, suggesting the existence of an age and weight threshold below which larvae are physiologically incapable of pupation. To examine whether larval weight gain beyond 0.08 g, or access to food beyond day 5, further increase the probability of larvae to reach the pupal stage, we repeated our analyses excluding those animals that were deprived of food prior to day 5, or weighed < 0.08 g at the time of food deprivation. To test for the robustness of these analysis we re-analysed our data using multiple logistic regressions.

Duration of larval and pupal development

No individual survived if food deprivation began prior to day 5 of the third instar, and mortality remained high in larvae starved on days 5, 6 and 7. To increase sample size and statistical power we therefore pooled larvae starved on days 5, 6 and 7 into one group (early food deprivation). We then examined the effects of food deprivation on the duration of larval and pupal development and adult body size by comparing three groups of animals: those that were deprived of food on days 5 to 7 (early food deprivation), those that were removed from food on day 10 (late food deprivation), and animals that had unlimited access to food (controls). We used one-way analysis of variance (ANOVA) to test for significant differences between these three treatment groups. When ANOVA indicated significant differences, we used *t*-tests (two-tailed) for pairwise contrasts (Sachs, 1992; Sokal & Rohlf, 1995). We used multiple *t*-tests (two-tailed, assuming unequal variance) to contrast adult body sizes of field-collected, control and experimental animals, and used sequential Bonferroni correction procedures to correct for multiple comparisons (Sachs, 1992; Sokal & Rohlf, 1995). Body sizes of field-collected individuals were taken from an earlier study (Moczek & Emlen, 2000) and were derived from 810 individuals collected from pastures in Durham County from May to July 1995. All data are presented as means ± standard errors unless otherwise noted.

Results

Equivalence of control and experimental animals prior to food deprivation

Control and experimental animals did not differ in their weights on the first day of the third larval instar ($t = 1.32$ d.f. = 40, $P > 0.1$). Control and experimental animals, likewise, did not differ significantly in their weight gains over the first 5 days of the third instar ($t = 1.75$, d.f. = 42, $P > 0.05$; animals that were food deprived prior to day 5 were excluded from this analysis).

Food deprivation and survivorship

When all experimental animals were included in the analysis, both the day at which larvae were removed from food, and larval weight at the time of food deprivation, significantly affected larval ability to survive to the pupal stage (day of food removal: $F = 55.44$, $P < 0.001$, $r^2 = 0.92$, $n = 7$; larval weight: $F = 31.04$, $P < 0.01$, $r^2 = 0.89$, $n = 4$). However,

larvae that were removed from food at different times during the third instar experienced a threshold effect of food access on survivorship (Fig. 1). No individual starved prior to day 5 survived to pupation, and survivorship increased from 0% to about 30% on day 5. If individuals starved prior to day 5 were excluded from the analysis, day of food deprivation continued to significantly affect survivorship ($F = 20.39$, $P = 0.04$, $r^2 = 0.91$, $n = 4$).

Larval weight at the time of food deprivation also had to exceed a threshold, and larvae weighing less than of 0.08 g at the time of food deprivation (= 58% of mean peak weight of control individuals) never managed to pupate (Fig. 2). Larvae starved between 0.08 g and 0.14 g experienced an approximately ~50% survivorship, whereas in larvae starved at weights > 0.14 g survivorship increased to 80% (Fig. 2). When larvae weighing < 0.08 g were excluded from the analysis, larval weight no longer significantly affected pupation success ($F = 4.70$, $P = 0.16$), except when pupation success of the largest weight class was compared to that of the three previous weight classes combined ($\chi^2 = 4.23$, d.f. = 1, $P < 0.05$, Fig. 2). Re-analysing our data using multiple logistic regressions confirmed the robustness of our findings in all cases.

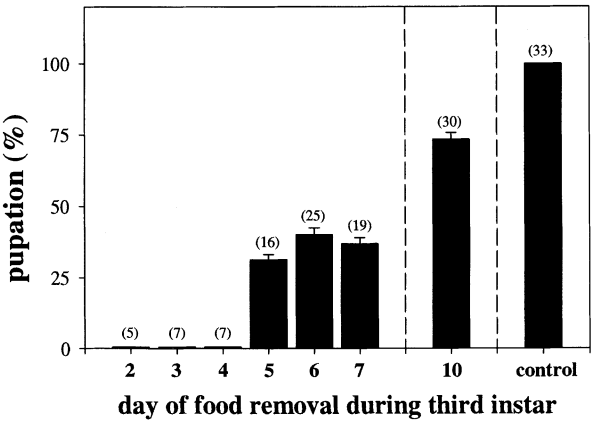

Fig. 1. Pupation success of *O. taurus* larvae as a function of food access during the final instar. Day of food removal indicates the day of the third instar when food deprivation began. Control animals had unlimited access to food during the entire instar. Data are means ± standard errors. Sample sizes are given in parentheses.

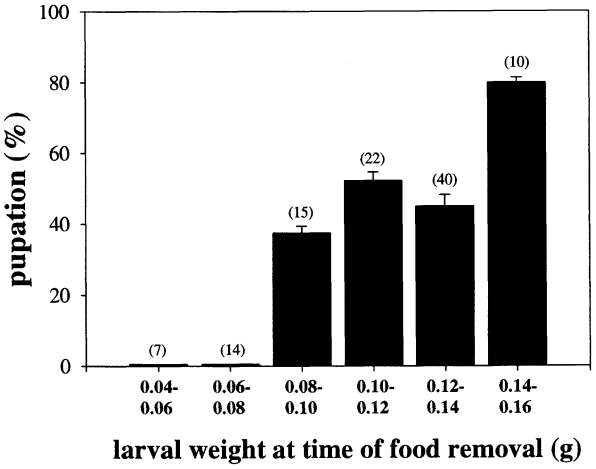

Fig. 2. Pupation success of all experimental *O. taurus* larvae combined as a function of larval weight at the time of food removal. Data are means ± standard errors. Sample sizes are given in parentheses.

Growth and timing of pupation

Control individuals reached their peak weight 9.4 ± 0.31 days after moulting to the third instar ($n = 20$) and remained active, continued feeding and responded aggressively to stimulation by forceps for an additional 3 days. Larval activity gradually decreased, and larvae entered the prepupal stage 4.2 ± 0.36 days after reaching peak weight, and pupated 7.6 ± 0.34 days after reaching peak weight ($n = 20$, Fig. 3a).

Experimental animals reached their peak weight by default on the day at which they were removed from food. Such animals remained active for about 3 days after removal from food, regardless of whether they were removed from their food source early or late during the instar. Food-deprived larvae also responded aggressively to stimulation with forceps during this 3-day period. Activity levels decreased following the third day and larvae entered the prepupal stage 4.9 ± 0.18 days after removal from food ($n = 20$; data are for late food-deprived animals only), and pupated on average on day 7 or 8 post food removal (early food deprivation: 7.95 ± 0.39, $n = 22$; late food deprivation: 7.0 ± 0.21, $n = 22$, Fig. 3a). There was no significant difference between control and both experimental groups with respect to how long it took individuals to reach the prepupal and pupal stage once they had attained their peak weight (one-way ANOVA, $F = 2.33$, $P > 0.1$; Fig. 3a). As a consequence, experimental and control groups differed highly significantly in the total length of the third larval instar, measured as the number of days from moult to the third instar to pupation ($F = 26.07$, $P < 0.001$; Fig. 3b). Control individuals pupated after 17.0 ± 0.31 days ($n = 20$), whereas late food-deprived animals required only 16.1 ± 0.24 days ($n = 23$), and early food-deprived animals required 13.9 ± 0.35 days ($n = 22$). The length of the third larval instar exhibited a strong tendency to decrease the earlier larvae were removed from their food source (Kendall's Tau, $n = 3$, $P = 0.059$; Fig. 3b).

There was no difference in the duration of the pupal stage between experimental and control animals ($F = 2.31$, $P > 0.1$; Fig. 3c). Consequently, animals deprived of food early in their third instar eclosed to adulthood on average 3 days earlier than late food-deprived or control animals of the same cohort.

Body sizes of eclosing adult control individuals were slightly but not significantly smaller than body sizes of field-collected individuals (t = 1.09, d.f. = 24, $P > 0.2$; controls: 4.93 ± 0.053 mm, $n = 23$; field: 4.99 ± 0.013 mm, $n = 810$; Fig. 4). Body sizes > 5.25 mm were common among field-collected individuals, but absent among control individuals,

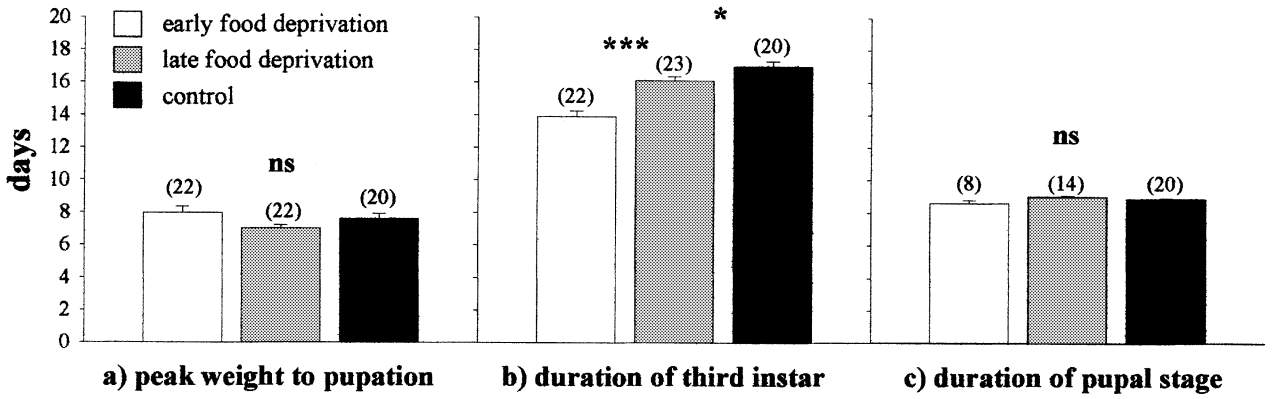

Fig. 3. Effects of early and late food deprivation on the duration of larval and pupal development in *O. taurus*. (a) number of days from the first day of food deprivation (experimental animals) or peak weight (control animals) to pupation. (b) duration of the entire third instar measured in number of days from the moult to the third instar to pupation. (c) duration of pupal stage (***$P<0.001$, *$P<0.05$, ns = not significant, *t*-test). Control animals had unlimited access to food during the entire instar. Data are means ± standard errors. Sample sizes are given in parentheses.

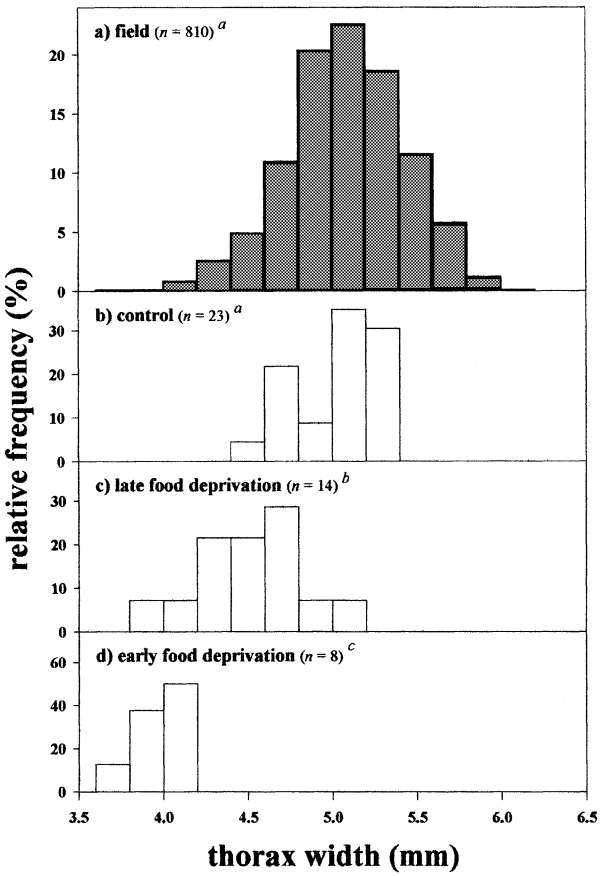

Fig. 4. Body size ranges of adult *O. taurus:* (a) collected from pastures near Durham, North Carolina, (b) reared in the laboratory with unrestricted access to food (control), (c) reared in the laboratory and deprived of food late in larval development, (d) reared in the laboratory and deprived of food early in larval development. Samples not sharing the same letter in the exponent are significantly different ($P<0.001$; multiple *t*-tests including sequential Bonferroni correction procedures to correct for multiple comparisons).

possibly due to the additional stress caused by daily weighing during larval development in the latter group. Late food-deprived larvae metamorphosed into adults with body sizes approximately 1.6 standard deviations smaller than the mean size in field populations (4.41 ± 0.078 mm, $n=14$), whereas early food-deprived larvae expressed adult body sizes similar to the smallest body sizes measured in natural populations (3.92 ± 0.052 mm, $n=8$; Fig. 4). The differences in body sizes between field-collected individuals and late and early food-deprived larvae were highly significant (field vs. late food-deprived individuals: $t=7.30$, d.f. = 14, $P<0.001$; field vs. early food-deprived individuals: $t=19.94$, d.f. = 8, $P<0.001$), as were the differences between control individuals and early and late food-deprived individuals (control vs. late food-deprived animals: $t=5.49$, d.f. = 25, $P<0.001$); control vs. early food-deprived animals: $t=13.53$, d.f. = 22, $P<0.001$), and the differences between early and late food-deprived animals ($t=5.16$, d.f. = 20, $P<0.001$).

Discussion

The larval stage constitutes the primary feeding stage of holometabolous insects, and serves, among other things, to maximize the accumulation of reserves to fuel pupation and metamorphosis into adulthood. Not surprisingly, food availability plays a pivotal role in the regulation of larval development and the timing of subsequent developmental events (e.g. Greene, 1999). Several insect groups have evolved mechanisms that allow them to respond to inadequate larval nutrition, primarily by delaying progressive development until sufficient resources have been secured (e.g. Riley, 1883; Wodsedalek, 1917; Beck, 1950, 1971a, b; Allegret, 1964; Nijhout & Williams, 1974; Nijhout, 1975, 1979, 1999; Blakley & Goodner, 1978; Blakley, 1981; Mieczyslawa & Szolajska, 1995).

Here we identify a different type of response to food deprivation. The earlier larvae of *O. taurus* depleted their

resources during the third instar, the earlier they initiated a stereotypic sequence of events leading first to premature pupation and finally to the early eclosion of a small adult. In contrast to critical-size controlled insects, *O. taurus* larvae reduce rather than extend the duration of the larval stage. The behaviour of larvae after being removed from their food source was highly repeatable and stereotypic. The timing of subsequent events such as the gut purge and pupation was independent of whether larvae were deprived of food early or late during the instar, and occurred after constant intervals very similar to larvae fed *ad libitum* once those had naturally reached their peak weight. Starving individuals at the same day effectively synchronized their subsequent development. These results suggest that, in nature, larvae may use food depletion as a cue to time when to end the third instar and when to initiate pupation.

Successful pupation was not completely independent of growth conditions during the third instar. Larvae removed from their food source prior to day 5 never managed to pupate ($n = 13$). Once larvae passed this 5 day threshold (= 30% of the instar relative to a mean of 17 days in control individuals), pupation became possible, and survival to pupation increased significantly the longer larvae were allowed to feed. In contrast, increased larval weight beyond the minimum necessary for pupation did not significantly increase pupation success, with the possible exception of the largest weight class. These results suggest that unrestricted development during the early part of the third instar is crucial for reaching the pupal stage, whereas the weight gained during the latter two-thirds of the instar is less important for pupation success and instead primarily affects the body size of emerging adults.

Body size-independent pupation and its adaptive significance

The response of *O. taurus* larvae to food deprivation is contrary to what has been reported for other insects, and reveals a striking degree of plasticity in the duration and dynamic of larval development for this species (Riley, 1883; Wodsedalek, 1917; Beck, 1950; Beck, 1971a, b; Allegret, 1964; Blakley & Goodner, 1978; Nijhout, 1979, 1981, 1999; Blakley, 1981; Mieczyslawa & Szolajska, 1995; for a possible exception see Hill & Goldsworthy, 1970). To the best of our knowledge this is the first demonstration that a holometabolous insect can terminate larval growth in a flexible fashion and progress to the pupal stage without delay at a wide range of larval weights once its food supply is exhausted. In another group of organisms, however, a very similar phenomenon is common and has been well studied. Many species of amphibians adjust the duration of the tadpole stage depending on certain qualities of their growth environment, such as the availability of water in the pond in which they develop (Crump, 1989; Tejedo & Reques, 1994a,b), crowding (Wilbur & Collins, 1973) or food availability (Alford & Harris, 1988). Once conditions become less favourable, tadpoles can terminate larval development and metamorphose at smaller body sizes. Tadpoles are usually unable to move to other ponds to escape unfavourable growth conditions. Therefore, the ability to terminate the tadpole stage once crucial resources are used up has generally been interpreted as an adaptation that permits individuals to complete larval development under a wide range of largely unpredictable environmental conditions (Wilbur & Collins, 1973). *Onthophagus taurus* larvae, likewise, are faced with considerable variation in larval growth conditions. Earlier studies have shown that brood ball mass, i.e. the total amount of resource available to a larva, can vary substantially as a function of dung quality (Moczek, 1998), parent size (Hunt & Simmons, 2000) and soil temperature and moisture (Sowig, 1996a,b; A. P. Moczek, unpublished observations). Hence, *O. taurus* larvae have to cope with a substantial and unpredictable range of nutritional environments. Whereas larvae of many holometabolous insects are confronted with the same problem and solve it by actively searching for additional resources, *O. taurus* larvae are confined to their subterranean brood ball, and equipped with a larval morphology that precludes the possibility to locate alternative food sources. It therefore appears adaptive that larvae initiate pupation once food availability has ceased, as it allows an individual to reach adulthood, and hence maintain the possibility of reproduction (see below). As many holometabolous insect larvae probably face a similarly restrictive resource environment, size-independent pupation and metamorphosis may be more common than currently recognized (e.g. Lafont, 1994).

Size-independent pupation and male dimorphism

Small body size due to reduced larval food availability probably reduces fecundity in females (Hunt & Simmons, 2000). However, the fitness costs – if any – for small-sized males are more difficult to predict. Like many onthophagine species, male *O. taurus* express two alternative phenotypes: horned and hornless (Paulian, 1935). Males that exceed a critical body size develop a pair of spectacular horns on their heads, whereas males smaller than this threshold develop only rudimentary horns, or no horns at all (Hunt & Simmons, 1997; Moczek, 1998, 1999). Male horn morphology covaries with male reproductive behaviour. Large, horned males rely exclusively on aggressive head-to-head combat to access and defend breeding tunnels, whereas small, hornless males employ a set of sneaking behaviours to access females (Moczek & Emlen, 2000). When both morphs compete for access to females, hornless males regularly manage to circumvent horned males and mate with females (Moczek & Emlen, 2000). Males that eclosed after starvation treatment were small and hornless without exception. Whereas in a monomorphic species this would have put them at a severe disadvantage when competing for mating opportunities with larger males, the specialized reproductive behaviour utilized by small, hornless *O. taurus* males may actually allow them to sire a significant portion of a female's offspring, although this remains to be tested experimentally. In an earlier study Eberhard (1982a, b) showed that in *Podischnus agenor* – another horn dimorphic scarab beetle – small, hornless males tended to emerge earlier in the season than large, horned

males, and proposed that early emergence itself may provide hornless males with a competitive advantage over horned males. Our data do not allow us to examine the adaptive significance of early emergence of small, hornless males, but suggest that its developmental basis may be as simple as reduced food availability during larval development.

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