Evolutionary Ecology Research, 2006, **8**: 1129–1137

Are beetle horns costly to produce?

Yutaka Iguchi*

Laboratory of Biology, Yamashita-cho 1-10-6, Okaya City, Nagano 395-0005, Japan

ABSTRACT

Question: Do male beetles pay costs of producing horns before becoming adults?

Hypotheses: (1) Smaller males emerge earlier in the season. (2) Smaller males pupate earlier in the season. (3) Males require longer pupal duration than females of the same size. (4) Horn size affects pupal duration independently of body size. (5) Males require more final-instar mass to become adults than females of the same size. (6) Horn size depends on final-instar mass independently of body size.

Organism: The Japanese horned beetle *Trypoxylus dichotomus septentrionalis* (Kono) (Coleoptera: Scarabaeidae). Males produce horns, but females do not.

Methods: I reared larval beetles under nearly natural conditions until they became adults. I recorded final-instar mass, pupation date, pupal duration, adult emergence date, adult body size, and horn size.

Results: (1) Larger males emerged earlier in the season. (2) Larger males pupated earlier in the season. (3) Males required only the same pupal duration as females of the same size. (4) Horn size did not affect pupal duration independently of body size. (5) Males required less final-instar mass than females of the same size. (6) Horn size increased with increasing final-instar mass independently of body size.

Conclusions: The results provide support for the sixth hypothesis, but not the other hypotheses. Beetle horns are not so costly to produce as entomologists expect them to be.

Keywords: adult emergence, body size, horned beetle, horn cost, horn size, larval mass, pupal duration, pupation, *Trypoxylus dichotomus septentrionalis*.

INTRODUCTION

Secondary sexual traits, such as the antlers of deer, the tails of peacocks, and the horns of beetles, are very important life-history characters of animals. Such traits are generally used as ornaments in courtship or as armaments in combat in the adult stage. Therefore, individuals with more elaborate or larger traits are expected to receive greater reproductive benefit. However, they may be costly to produce. Many theoretical and empirical studies on life histories have examined trade-offs between body size and development time (for a review, see Roff, 2000), but very few studies have examined whether secondary sexual traits require some developmental costs independently of body size before the adult stage (Arnqvist, 1994;

---

* e-mail: bio-igu@f8.dion.ne.jp

Kotiaho, 2001). This question is of considerable interest in the context of the evolution of secondary sexual traits. In this article, I explore the developmental costs of beetle horns.

In horned beetles, males generally have horns but vary widely in body size and horn size between small males and large males (reviewed by Emlen and Nijhout, 2000). In contrast, females have small horns or no horns. Males frequently fight for access to feeding sites or females. In such fights, large males beat small males by using their large body and large horns (Eberhard, 1980; Siva-Jothy, 1987). Therefore, entomologists have assumed that large male beetles accrue reproductive benefits (Rasmussen, 1994; Emlen, 2000; Moczek and Emlen, 2000). Recently, Hunt and Simmons (2001) measured reproductive success and demonstrated this advantage of horns in *Onthophagus taurus*.

However, to be larger as adults could be costly for males. For example, several studies on insect life histories have suggested that larger adults require longer growth periods in the pupal and larval stages (for reviews, see Roff, 1980; Klingenberg and Spence, 1997; Nylin and Gotthard, 1998). This appears also to be true for horned beetles. For example, Eberhard (1982) observed adult emergence in the horned beetle *Podischnus agenor* and reported that smaller males emerged earlier in the emergence season of 1977. Based on his observation, many later studies assumed that smaller male horned beetles emerged earlier in the season (Siva-Jothy, 1987; Rasmussen, 1994), and several researchers reported that small males develop faster than large males in the laboratory (Emlen and Nijhout, 1999; Moczek and Emlen, 2000; Shafiei *et al.*, 2001). However, this assumption has yet to be examined for other horned beetles. In addition, it could be that *P. agenor* had another emergence pattern: small males emerged both early and late in the emergence season of 1976 (Eberhard, 1982). Therefore, it remains unclear whether smaller males of horned beetles generally emerge earlier in the season. Moreover, it is unclear whether earlier emergence results from earlier pupation or from shorter pupal duration.

Recently, Hunt and Simmons (1997) compared male development periods with female development periods in the horned beetle *Onthophagus taurus*. As a result, they suggested that horn production was costly in terms of delayed development of males. However, whether horn production is the cause of this developmental delay remains unclear, since horn size is highly correlated with body size, and large body sizes also can require extended developmental periods. Moreover, another study on *O. taurus* suggested that by the end of the first larval instar, individual mass was correlated with horn growth (Emlen and Nijhout, 1999). However, the separate effects of horn size and body size remain to be elucidated.

The present study focuses on the Japanese horned beetle *Trypoxylus dichotomus septentrionalis* (Kono) (= former *Allomyrina dichotoma septentrionalis*) (Coleoptera: Scarabaeidae). In this beetle, males have horns, but females do not. This beetle belongs to the same subfamily Dynastinae as *P. agenor* and shows male dimorphism in horn size like *P. agenor* (Siva-Jothy, 1987; Iguchi, 1998). These two species are ecologically similar in the following three respects [Eberhard (1982) for *P. agenor*; my personal observations for *T. d. septentrionalis*]. First, the life cycle lasts about one year. Second, beetles spend their egg, larval, and pupal stages underground. Third, larvae feed on humus and adults feed on juice from plants (sugar cane for *P. agenor* and oak trees for *T. d. septentrionalis*).

In this study, I explore the possible developmental costs of horn growth in *T. d. septentrionalis* by rearing larval beetles under approximately normal conditions and measuring the durations of the respective life stages as well as total development time. If horn growth delays development, then I predict that smaller males will emerge earlier in the season than larger males (hypothesis 1). If this delay is due to prolongation of the larval period, then I predict that smaller males will pupate earlier in the season than larger males (hypothesis 2).

If this developmental delay is due to prolongation of the pupal period, then I predict that males with horns will require longer pupal durations than females of the same size (hypothesis 3). Moreover, if the developmental delay is due to prolongation of the pupal period, then I predict that when horn size and body size are analysed together, horn size will be found to affect pupal duration independent of the effects of body size (hypothesis 4). Similarly, if horn growth utilizes resources that could otherwise have been devoted to body size, then for a given final-instar larval mass, I predict that males (due to the added costs of horn growth) will mature at smaller body sizes than females (hypothesis 5). Finally, I test whether final-instar larval mass, or final adult body size, are effective predictors of adult horn size (hypothesis 6).

MATERIALS AND METHODS

Life history

Trypoxylus dichotomus septentrionalis is a common beetle in Japan. Adults of this beetle usually emerge in July and August in Okaya City, Nagano Prefecture, where this study was conducted. They lay eggs on humus in the soil in July and August. Larvae hatch out in about 10 days. They feed on humus and develop to the third (final) instar before winter. The final instars overwinter and pupate in the soil in June and July the following year. The pupae become adults in about 20 days.

Experimental procedure

This study was conducted in 1999 and 2001. In 1999, I conducted a rearing experiment to measure larval, pupal, and adult sizes of individuals, and to determine the dates at which animals pupated and subsequently emerged as adults. Because much of the biology of *T. d. septentrionalis* is related to season, I collected partially developed animals from the wild and reared them in separate containers that were kept outdoors. For this experiment, I collected 71 final instars in the soil near a forest of assorted trees in the western part of Tatsuno-machi, Kamiina-gun, Nagano Prefecture, Japan on 5 June. The soil was dark, soft, moist humus that contained many chips of decayed wood. The body mass of each larva was immediately measured, and then each larva was put in a transparent glass bottle (9 cm diameter and 16 cm high). Each bottle was filled with the soil (12 cm deep) in which the larva had lived, and then it was covered with a metallic lid. All the bottles were placed together outdoors in the shade of a building. No further humus or soil was added during the experiment, but water was sprinkled on the soil to keep it moist.

In 2001, I planned to observe seasonal changes in sizes of more adults. For this experiment, I collected 200 final instars at the above-mentioned site on 5 May. The larvae were placed in five opaque plastic boxes (40 larvae in each box) of the same size (37 cm wide × 70 cm long × 30 cm high) with the soil (28 cm deep) in which they had lived. Each box was covered with an opaque plastic board and placed outdoors. No further humus or soil was added during the experiment, but water was sprinkled on the soil to keep it moist.

In both years, I observed larvae and pupae daily. For each newly emerged adult, the emergence date and sex were recorded. In addition, for each adult, body length and horn length were measured in a straight line with a slide caliper. In 1999, the pupation date and pupal duration (the time from pupation to eclosion) of each larva were also recorded.

Statistical analyses

I used correlation analysis to determine whether male size increases in the season as a whole. I used regression analysis to control for body length and compare male pupal duration with female pupal duration. In addition, I used regression analysis to control for final-instar mass and compare male body length with female body length. I used partial correlation analysis (Zar, 1996) to remove the effect of body length and estimate the independent effect of horn length on pupal duration or final-instar mass. Therefore, I calculated relative horn length, relative pupal duration, and relative final-instar mass as residuals from regressions of these traits on body size. Using these relative values, I calculated the partial correlations between horn length and pupal duration and between horn length and final-instar mass.

In all the correlation analyses, I used Spearman's rank correlation. In this article, Spearman's coefficients are denoted by r_s.

RESULTS

Figure 1 shows seasonal variations in body length and horn length of newly emerged males. In 1999, adult size became significantly smaller as the season progressed (body length: $r_s = -0.69$, $n = 30$, $P < 0.001$; horn length: $r_s = -0.59$, $n = 30$, $P < 0.001$). Similarly, in 2001,

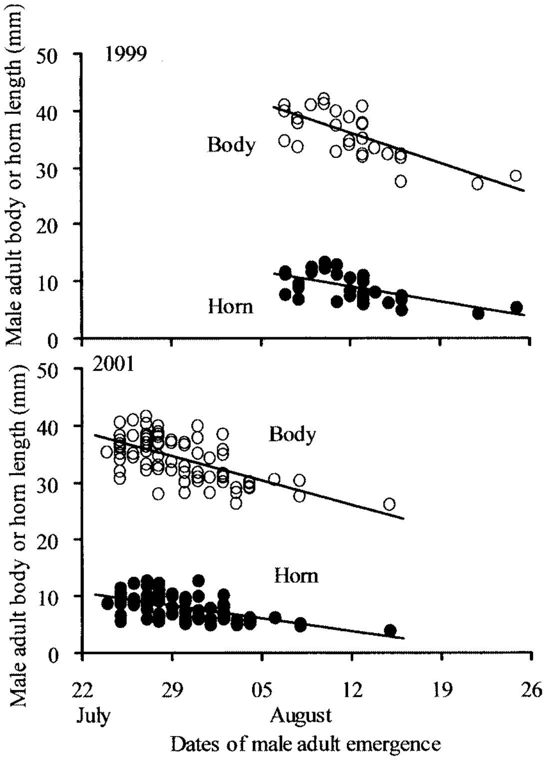

Fig. 1. Seasonal changes in body length and horn length of newly emerged males in 1999 ($n = 30$) and 2001 ($n = 77$). The lines were calculated by the method of least squares. These lines indicate a decrease in male size in both years.

adult size became significantly smaller as the season progressed (body length: $r_s = -0.59$, $n = 77$, $P < 0.001$; horn length: $r_s = -0.56$, $n = 77$, $P < 0.001$).

Figure 2 shows the relationship between pupation dates and adult sizes in males. The correlation was significantly negative both for body length ($r_s = -0.78$, $n = 30$, $P < 0.001$) and for horn length ($r_s = -0.76$, $n = 30$, $P < 0.001$).

Figure 3 shows the regression of pupal duration on adult body length in both sexes. This regression was significantly positive both for males ($F_{1,28} = 27.85$, $P < 0.001$) and for females ($F_{1,32} = 6.01$, $P < 0.001$). Neither the slope ($F_{1,60} = 0.088$, $P > 0.7$) nor the elevation ($F_{1,61} = 0.019$, $P > 0.8$) differed significantly between the sexes. As a result, the two regression lines were statistically identical.

Figure 4 shows the simple correlation and partial correlation between horn length and male pupal duration. The simple correlation, which included the effect of body size, was positive and highly significant ($r_s = 0.73$, $n = 30$, $P < 0.0001$). The partial correlation, which excluded the effect of body size, was also positive, but somewhat weaker than the simple correlation and statistically non-significant ($r_s = 0.32$, $n = 30$, $P > 0.05$). This result indicates

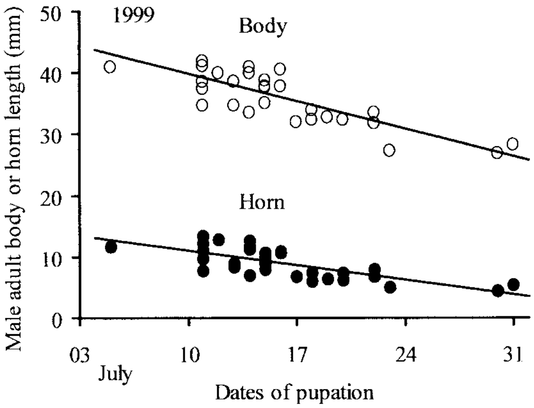

Fig. 2. Relationships between pupation dates and adult sizes in males in 1999 ($n = 30$). The lines were calculated by the method of least squares. These lines indicate a decrease in male size.

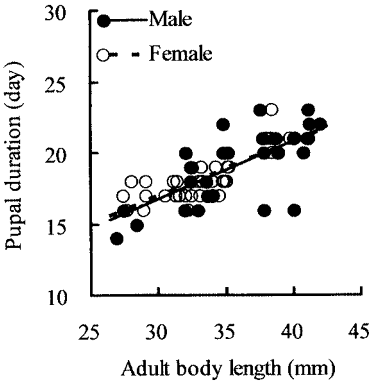

Fig. 3. Relationships between adult body length and pupal duration for 30 males and 31 females observed in 1999.

that although the effect of horn length may have been ecologically significant, body size had a greater effect on male pupal duration.

Figure 5 shows the regression of adult body length on final-instar mass in both sexes. This regression was significantly positive both for males ($F_{1,28} = 25.76$, $P < 0.001$) and for females ($F_{1,32} = 94.86$, $P < 0.001$). The slope was steeper in females than in males, but the difference was not significant ($F_{1,60} = 2.30$, $P > 0.1$). However, the elevation differed significantly between the sexes ($F_{1,61} = 5.53$, $P < 0.05$). As a result, the two regression lines were statistically parallel and the regression line for males lay above that for females.

Figure 6 shows the simple correlation and partial correlation between horn length and male final-instar mass. The simple correlation was positive and highly significant ($r_s = 0.68$, $n = 30$, $P < 0.0001$). The partial correlation was weaker, but remained significantly positive ($r_s = 0.42$, $n = 30$, $P < 0.05$). This result indicates that horn length had a significant independent effect on male final-instar mass.

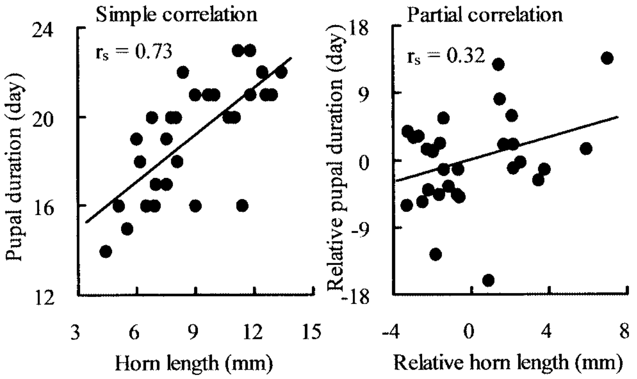

Fig. 4. The simple correlation and partial correlation between horn length and male pupal duration ($n = 30$). Relative horn length and relative pupal duration were calculated as residuals from regressions of these traits on body size. Thus, the partial correlation excluded the effect of body size from the simple correlation.

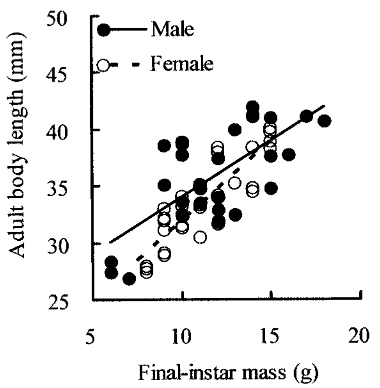

Fig. 5. Relationship between final-instar mass and adult body length for 30 males and 31 females observed in 1999.

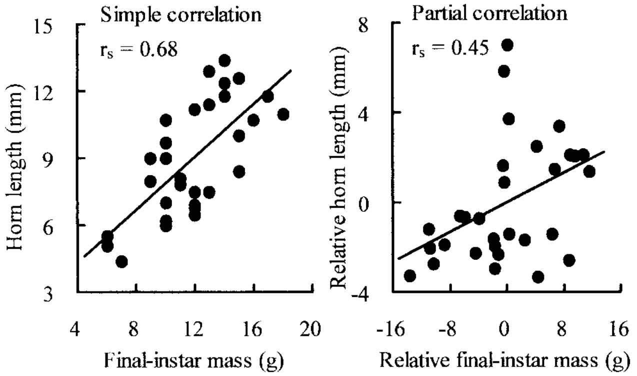

Fig. 6. The simple correlation and partial correlation between male final-instar mass and horn length ($n = 30$). Relative final-instar mass and relative horn length were calculated as residuals from regressions of these traits on body size. Thus, the partial correlation excluded the effect of body size from the simple correlation.

DISCUSSION

In 1999 and 2001, males showed the same emergence pattern. That is, larger males emerged earlier in the season. This result contradicts the main result of Eberhard (1982), because he showed that in *P. agenor* smaller males emerged earlier in the season. However, he also showed another emergence pattern. That is, in 1976, small males emerged both early and late in the season and the frequency of the largest males was highest in the middle of the season (see Figure 2 in Eberhard, 1982). This second result of Eberhard (1982) and my result contradict the first hypothesis that smaller males emerge earlier in the season. This hypothesis has frequently been employed in life-history theories (Klingenberg and Spence, 1997). However, recent studies on other insects have shown that a decrease in size does not always lead to a shorter development time (Klingenberg and Spence, 1997; Roff, 2000). Therefore, it would appear that smaller male beetles do not always emerge earlier in the season.

In this study, larger males pupated earlier in the season than smaller males. This result is inconsistent with a cost of horn growth that prolongs the duration of the larval period. Thus, I reject the second hypothesis. As far as I am aware, this is the first report of earlier pupation of larger males in horned beetles.

Both male and female pupal durations increased with body size and these relationships did not differ either in slope or in intercept. This result suggests that although only males produce horns in this beetle species, males and females require the same pupal duration for any body size. Thus, I reject the third hypothesis. Male pupal duration also appeared to increase with horn size. However, this effect was largely due to body size, not to horn size itself. Thus, I reject the fourth hypothesis. Consequently, the rejection of the third and fourth hypotheses is inconsistent with a cost of horn growth that prolongs the duration of the pupal period.

Finally, the relationship between final larval mass and adult body size did not differ between males and females, suggesting that horn growth did not have a negative impact on final body size. Thus, I reject the fifth hypothesis.

Thus, this study rejects the first five hypotheses and all patterns appear inconsistent with a developmental cost of horn growth. This finding contradicts the suggestion of Hunt and

Simmons (1997) that horn production prolongs male development time. However, Hunt and Simmons (1997) did not conduct partial correlation analyses that separate the effects of horn size and body size on pupal development time. Therefore, it is possible that their effects were attributable to body size as well.

Note that the timing of adult emergence (Fig. 1) depended on two contradictory factors, the timing of pupation (Fig. 2) and pupal duration (Fig. 3). In this study, larger male adults emerged earlier in the season. However, if larger males require extremely long pupal duration, then they may emerge later in the season. Therefore, I predict that the timing of emergence of small males and large males may be variable. This could be the reason for the two different emergence patterns of *P. agenor* (Eberhard, 1982).

The partial correlation analysis of final-instar mass showed that horn size increased significantly with increasing final-instar mass independently of body size. Thus, I accept the sixth hypothesis. This result suggests that horn size and body size may depend on different developmental mechanisms in the final-instar stage. As shown in Fig. 5, the regression of adult body size on final-instar mass differed slightly in slope between the sexes. This result may also suggest an independent effect of male horns on final-instar mass, although the regression analysis showed no significant difference between the sexes. The present study did not reveal the developmental mechanisms of horns and body. However, recent studies on other beetles have revealed that only male larvae exceeding a specific final-instar mass become adults with fully developed horns (Emlen and Nijhout, 1999, 2001; Moczek, 2002). A similar mechanism may affect horn development in *T. d. septentrionalis*.

Several studies have stressed the costs of beetle horns (Kawano, 1995; Nijhout and Emlen, 1998; Emlen, 2000, 2001). These studies showed sufficient evidence for trade-offs – that is, negative correlations between horns and other morphological traits such as wings and eyes. However, I tested the six hypotheses regarding the costs of horn production from the larval stage through the adult emergence stage. The results support the sixth hypothesis, but contradict the other five hypotheses. Thus, I conclude that horn production is not as costly in the larval, pupal, and adult emergence stages as entomologists believe.

ACKNOWLEDGEMENTS

I thank Charles Fox, Douglas Emlen, and an anonymous reviewer for valuable comments on the first draft of the manuscript.

REFERENCES

Arnqvist, G. 1994. The cost of male secondary sexual traits: developmental constraints during ontogeny in a sexually dimorphic water strider. *Am. Nat.*, **144**: 119–132.

Eberhard, W.G. 1980. Horned beetles. *Sci. Am.*, **242**: 124–131.

Eberhard, W.G. 1982. Beetle horn dimorphism: making the best of a bad lot. *Am. Nat.*, **119**: 420–426.

Emlen, D.J. 2000. Beetle horn evolution. *Bioscience*, **50**: 403–418.

Emlen, D.J. 2001. Costs and the diversification of exaggerated animal structures. *Science*, **264**: 567–574.

Emlen, D.J. and Nijhout, H.F. 1999. Hormonal control of male horn length dimorphism in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J. Insect Physiol.*, **45**: 45–53.

Emlen, D.J. and Nijhout, H.F. 2000. The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.*, **45**: 661–708.

Emlen, D.J. and Nijhout, H.F. 2001. Hormonal control of male horn length dimorphism in *Onthophagus taurus* (Coleoptera: Scarabaeidae): a second critical period of sensitivity to juvenile hormone. *J. Insect Physiol.*, **47**: 1045–1054.

Hunt, J. and Simmons, L.W. 1997. Patterns of fluctuating asymmetry in beetle horns: an experimental examination of the honest signaling hypothesis. *Behav. Ecol. Sociobiol.*, **41**: 109–114.

Hunt, J. and Simmons, L.W. 2001. Status-dependent selection in the dimorphic beetle *Onthophagus taurus. Proc. R. Soc. Lond. B*, **268**: 2409–2414.

Iguchi, Y. 1998. Horn dimorphism of *Allomyrina dichotoma septentrionalis* (Coleoptera: Scarabaeidae) affected by larval nutrition. *Ann. Entomol. Soc. Am.*, **91**: 845–847.

Kawano, K. 1995. Horn and wing allometry and male dimorphism in giant rhinoceros beetles (Coleoptera: Scarabaeidae) of tropical Asia and America. *Ann. Entomol. Soc. Am.*, **88**: 92–99.

Klingenberg, C.P. and Spence, J.R. 1997. On the role of body size for life-history evolution. *Ecol. Entomol.*, **22**: 55–68.

Kotiaho, J.S. 2001. Costs of sexual traits: a mismatch between theoretical considerations and empirical evidence. *Biol. Rev.*, **76**: 365–376.

Moczek, A.P. 2002. Allometric plasticity in a polyphenic beetle. *Ecol. Entomol.*, **27**: 58–67.

Moczek, A.P. and Emlen, D.J. 2000. Male horn dimorphism in the scarab beetle, *Onthophagus taurus*: do alternative reproductive tactics favor alternative phenotypes? *Anim. Behav.*, **59**: 459–466.

Nijhout, H.F. and Emlen, D.J. 1998. Competition among body parts in the development and evolution of insects. *Proc. Natl. Acad. Sci. USA*, **95**: 3685–3689.

Nylin, S. and Gotthard, K. 1998. Plasticity in life-history traits. *Annu. Rev. Entomol.*, **43**: 63–83.

Rasmussen, J.L. 1994. The influence of horn and body size on the reproductive behavior of the horned rainbow scarab beetle *Phanaeus difformis* (Coleoptera: Scarabaeidae). *J. Insect Behav.*, **7**: 67–82.

Roff, D.A. 1980. Optimizing development time in a seasonal environment: the ‘ups and downs’ of clinal variation. *Oecologia*, **45**: 202–208.

Roff, D.A. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *J. Evol. Biol.*, **13**: 434–445.

Shafiei, M., Moczek, A.P. and Nijhout, H.F. 2001. Food availability controls the onset of metamorphosis in the dung beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *Phys. Entomol.*, **26**: 173–180.

Siva-Jothy, M.T. 1987. Mate securing tactics and the cost of fighting in the Japanese horned beetle, *Allomyrina dichotoma* L. (Scarabaeidae). *J. Ethol.*, **5**: 165–172.

Zar, J.H. 1996. *Biostatistical Analysis*, 3rd edn. Upper Saddle River, NJ: Prentice-Hall.