The effect of a seasonal time constraint on development time, body size, condition, and morph determination in the horned beetle *Allomyrina dichotoma* L. (Coleoptera: Scarabaeidae)

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Abstract. 1. In horned beetles selection favours males that adjust their investment in horn development in relation to cues that predict adult body size. Here it is shown that in the Japanese horned beetle, *Allomyrina dichotoma*. There is a significant discontinuity in the horn length body size allometry. This can be described as a linear relationship that is shifted towards an increased horn length to body length ratio in males with horns longer than 16 mm.

2. Larval nutrition explains morph determination in *A. dichotoma*. However, unlike other species, variation in larval nutrition was the result of a seasonal time constraint that limits the time available for feeding prior to the onset of winter diapause.

3. Even when eggs were reared with an *ad libitum* food supply, minor morphs were still observed. Individuals that were oviposited later in the season had less time to feed, shorter development times, eclosed as smaller individuals and, in the case of males, were more likely to be hornless. Major morphs, minor morphs, and females all reduced their body size in response to seasonal time constraints in the same way. However, males that were laid later in the season had faster development times than females laid at the same time, but showed no reduction in their size relative to females, suggesting seasonal time constraints increase growth rates in males but not in females.

4. No evidence was found that seasonal time constraints resulted in a reduction of size-corrected fat reserves at eclosion, or that minor morphs gained any developmental advantage by reducing investment in horn length.

Key words. *Allomyrina dichotoma*, development rate, fat reserves, horned beetle, polyphenism, seasonality, sexual dimorphism.

Introduction

Adaptive phenotypic plasticity refers to the ability of an organism to modify its phenotype in response to current or future environmental conditions (Levins, 1963, 1968;

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Bradshaw, 1965). Polyphenisms arise when organisms within the same population produce irreversible discrete morphs in different environments (West-Eberhard, 2003). Examples include caste polyphenisms in social insects (Wheeler & Nijhout, 1983), predator-induced polyphenisms (Lively, 1986a, b), dispersal polyphenisms (Zera & Denno, 1997), seasonal polyphenisms in butterflies (Kingsolver, 1995), and the many examples of male alternative morphologies derived from male : male

competition (Eberhard & Gutierrez, 1991; Radwan, 1993).

In many species of horned beetle, horn size and body length allometry is discontinuous, and often characteristically sigmoidal in shape resulting in the production of horned and hornless males (Eberhard, 1982; Siva-Jothy, 1987; Eberhard & Gutierrez, 1991; Emlen, 1994, 1997a; Iguchi, 1998; Moczek et al., 2002). Larger males are typically better fighters than smaller males (Eberhard, 1982; Siva-Jothy, 1987; Emlen, 1997b; Moczek & Emlen, 2000). Consequently, the benefit of developing horns decreases as males get smaller, creating a selection pressure for males that are able to adjust their investment in horn length in relation to their predicted future body size (Eberhard, 1982). In dung beetles of the genus *Onthophagus*, the mechanistic basis underlying the polyphenism has been studied extensively. Parents provision their offspring with a brood ball, the size of which determines the size of the offspring (Moczek, 1998). Once the brood ball has been consumed, the cessation of feeding triggers metamorphosis (Shafiei et al., 2001). In the laboratory, development is direct and takes approximately 30 days (Emlen & Nijhout, 1999; Shafiei et al., 2001). Males that exceed a heritable critical size/condition threshold develop horns, whereas males below this threshold remain hornless (Emlen, 1994; Hunt & Simmons, 1998; Moczek & Emlen, 1999). A similar mechanism is often assumed to operate in other species of beetle outside of the *Onthophagus* genus, yet only one other species has been studied. Iguchi (1998) showed that in the Dynastine beetle, *Allomyrina dichtoma septentrionalis*, morph determination also appears to be related to larval nutrition. However, Iguchi (1998) did not demonstrate a statistical discontinuity in horn to body length allometry *sensu* Kotiaho and Tomkins (2001) and the cause of variation in larval nutrition was not explained.

In *Allomyrina dichotoma*, parents do not provision offspring with a brood ball and, because larvae feed in humus or other wood-based detritus, the availability of resources is often unlikely to be limiting. However, the time available to consume resources may be limiting as *A. dichotoma* is a seasonal species. During the first two instars, the time available for larval feeding is constrained by the onset of a 5-month winter diapause (November to March), and in the final post-diapause instar, the time available for larval feeding is constrained by the onset of the reproductive season (July to September). Seasonal time constraints have previously been shown to impact on fitness in a number of ways. Time-constrained individuals are often forced to reduce body size at maturation in order to speed up development (Nylin & Gotthard, 1998; Johansson & Rowe, 1999; Plaistow & Siva-Jothy, 1999). The need to grow faster can lead to increased feeding rates and an increased risk of predation (Lima & Dill, 1991). Time constraints can also lead to increased developmental errors (Sibly & Calow, 1986; Arendt, 1997), reduced starvation resistance (Stockhoff, 1991; Gotthard et al., 1994), reduced energy stores (Chippendale et al., 1996)

and compromised immune functioning and cell function (Arendt, 1997; Rolff et al., 2004).

In this study, the hypothesis that season-induced time constraints explain variation in larval nutrition in *A. dichotoma*, and therefore influence morph determination in this species, was tested. It was predicted that eggs that were laid later in the reproductive season would (1) eclose as smaller individuals; (2) develop at a faster rate; (3) eclose in worse physiological condition; and (4) have a higher probability of developing as a minor morph compared with eggs laid earlier in the season. These predictions were tested by rearing two generations of *A. dichotoma* under semi-natural conditions. The average mass of all larvae was tracked over the developmental period in order to examine how diapause influences growth patterns. Evidence of a discontinuity in horn to body length allometries was statistically tested for and characterised using the methodologies proposed by Kotiaho and Tomkins (2001).

Methods

Biology of the species

In this species the life cycle lasts approximately 1 year (Iguchi, 1998). After hatching, larvae feed on humus and develop to the third instar before overwintering. Larvae continue to feed in the spring before pupating in around June–July. In this species both males and females show size variation (Siva-Jothy, 1987). Females have no horns whatsoever whereas males possess a long stout head horn and a shorter prothoracic horn. There is a characteristically sigmoidal allometry between horn length and body size (Siva-Jothy, 1987). Major morphs use their horns in contests over localised sap feeding sites, while minor morphs typically avoid contests with conspecifics after an initial assessment. Minor morphs also arrive at feeding sites earlier than most major morphs and females and spend less time feeding when they are there (Siva-Jothy, 1987).

Rearing larvae

Larvae used in the experiment were all collected from pairings between laboratory-reared virgin adults. In total eight virgin pairs were measured for body size (elytra length) and horn length before being placed into separate plastic containers full of leaf litter. The containers were searched once a week and approximately 15 eggs (mean ± SE, 15.579 ± 0.965, range 7–20) were removed for the purposes of the experiment. The number of eggs selected from each search varied because some larvae had already hatched in some broods and could therefore not be used. The duration of the egg stage in laboratory-reared *A. dichtoma* is approximately 7 days (K. Tsuchida, pers. obs.). Consequently, age variation within each brood should be 1 week or less. Each pair produced two or three broods between 19 July and 2 September. In total the

experiment used 296 individuals from 22 broods. Upon hatching, each brood of larvae were placed into semi-transparent plastic containers (48 cm × 33 cm × 28 cm) containing 30–35 litres of leaf litter and two semi-rotten logs ($\approx$ 5 cm in diameter and 40 cm in length). Each container was covered with a nylon mesh (1 mm), and placed outside on a veranda away from direct sunlight. The depth of leaf litter and dead wood in the containers decreased as it was consumed. Consequently containers were continually topped up with a mixture of leaf litter and dead wood such that they were all maintained at a constant depth of 14 cm, thus aiming to provide *ad libitum* food conditions throughout the course of development. During the hot season (August to September), water was sprayed on the containers once a day to maintain conditions of high soil humidity. Spraying was reduced to once a week in all other months except winter (December to February) when no water was used. The condition and weight of the larvae was checked five times (1 October 1996, 14 November 1996, 28 December 1996, 1 March 1997, and 30 April 1997) during the course of development and the average weight of larvae in each brood was calculated. On the 30 April individual larvae were transferred to a leaf litter filled plastic pot (20 cm depth × 20 cm diameter) and allowed to make a pupation cell. From mid July 1997 onwards all containers were checked every day for the presence of newly eclosed beetles. Total development time was recorded as the number of days between oviposition and eclosion. Digital calipers (Mitutoyo^{TM}, Kawasaki, Japan) were used to measure the elytra length (mm) of males and females and the horn length (mm) of all eclosing males. A subset of 50 newly eclosed male beetles that included all the size variation produced were then killed and freeze dried for fat analysis. Each specimen was weighed to the nearest 0.001 mg using a Mettler AE160 balance. In order to avoid measuring the non-utilisable fat associated with nervous tissue in the brain we removed the cephalothoraxes of all specimens. Fat extractions were based on the method of Plaistow and Siva-Jothy (1996). Following extraction the thorax (including the elytra), abdomen, and legs were re-weighed to obtain the dry fatless mass. Total fat content was calculated from the difference between the dry mass and the dry fatless mass.

Male horn dimorphism

Examination of the relationship between body size and horn length in *A. dichotoma* revealed a significant overlap in body size between males that had large horns and males that did not. Therefore in order to characterise and test for a significant dimorphism in *A. dichotoma*, the aim was to find the optimal horn length switch point and then test for a significant discontinuity in the body size of the two groups (Kotiaho & Tomkins, 2001) using the following model:

$$X = \alpha + \beta_1 Y + \beta_2 (Y - Y_D)D + \beta_3 D + \varepsilon, \quad (1)$$

where X is elytra length (mm); Y is horn length (mm); Y_D is the proposed switch point; $D = 0$ if $Y < Y_D$, $D = 1$ if $Y \geq Y_D$; α is a constant, β are regression coefficients, and ε is the random component. To determine the best switch point we iterated values of Y_D in order to find the model that gave the best fit (highest R^2 value). Using the best switch-point value we determined the significance of any discontinuity in the relationship between horn length and elytra length by testing whether the regression coefficient for β_3 in equation 1 differed significantly from zero; differences in slope were tested for by examining whether β_2 also differed from zero (Eberhard & Gutierrez, 1991; Kotiaho & Tomkins, 2001).

Statistical analysis

All analyses were carried out on the mean values of traits for major morphs, minor morphs, and females that eclosed from each bucket in order to correct for the non-independence of samples. Any effect of Family was corrected for by using linear mixed-effects models with Family entered as a random effect (Crawley, 2002). The analysis was carried out in R (Ihaka & Gentleman, 1996; R Development Core Team). Day of oviposition, and larval density were entered as continuous covariates, with Type entered as a three-level categorical variable (Major morph, Minor morph, Female). For the subset of males for which stored fat reserves were measured we were interested in how the size-corrected fat reserves of major and minor morphs varied in response to day of oviposition. Consequently, a linear mixed-effects model was fitted with Day of oviposition and Elytra length fitted as covariates, Morph fitted as a two-level categorical variable (Major, Minor), and Family included as a random effect. The analysis was carried out in R (Ihaka & Gentleman, 1996; R Development Core Team, 2004) using the nlme package (Pinheiro & Bates, 2002). In all cases, a full model was initially fitted to the data and then a backwards stepwise procedure was used to remove higher order terms that were not significant (Crawley, 2002). Finally, the effect of Day of oviposition and Final larval mass on the probability of eclosing as a minor morph was examined using a generalised linear mixed effects model (GLMM) using a logit link function and a binomial error structure. Day of oviposition and Final larval mass were entered as continuous explanatory variables and Family was entered as a random term. The analysis was carried out in R (Ihaka & Gentleman, 1996; R Development Core Team, 2004), using the glmmPQL package within the MASS library (Venables & Ripley, 2002).

Results

Male horn dimorphism

The best fitting switch point (Y_D value) for *A. dichotoma* using model 1 occurred at a horn length of 16 mm and explained 71.8% of the overall variation in elytra length.

Table 1. Statistical test for dimorphism in *Allomyrina dichotoma* using model 1 with a best switch point value ($Y_D = 16.0$). Elytra length (mm) represents the dependent variable with Horn length (mm), $(Y - Y_D)D$, and D entered as predictors. The $(Y - Y_D)D$ tests the change in the slope, and D tests the discontinuity at the switch point Y_D.

Multiple regression

Source	β	SE of β	t	P-value
Constant	13.756	2.275	6.05	<0.0001
Horn length	0.8996	0.1684	5.34	<0.0001
$(Y - Y_D)D$	−0.4595	0.1714	−2.68	0.008
D	−1.9447	0.5941	−3.27	0.001

ANOVA

Source	d.f.	MS	F	P-value
Regression	3	122.11	116.55	<0.0001
Residual	137	1.05		
Total	140			

The dimorphism was best characterised as being discontinuous (β_3 differed significantly from 0, Table 1, Fig. 1) with a significant change in slope (β_2 also differed from 0, Table 1, Fig. 1).

Body size and development time

Figure 2 examines how the mass of larvae changed during the course of the experiment and clearly demonstrates that the majority of growth is achieved prior to the onset of the winter diapause in November/December. Both larval density and day of oviposition were negatively correlated with body size at eclosion (Table 2, Fig. 3a). These effects were the same for all individuals irrespective of sex and morph (Table 2, Fig. 3a). However, major morphs were significantly larger than minor morphs and females that were laid at the same time (Table 2, Fig. 3a). Larval crowding increased the development time of major morphs, minor morphs, and females in a comparable manner (Table 2). However, time-constrained males reduced their development time more than that of females laid on the same day (Table 2, Fig. 3b).

Male fat reserves at eclosion

The fat reserves of newly eclosed males was strongly positively correlated with elytra length (body size) in both major and minor morphs (Elytra length, $F_{1,47} = 137.89$,
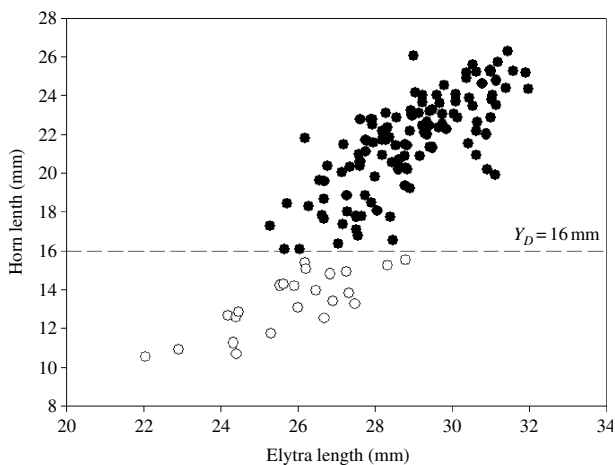
Fig. 1. Graphical fit of the model used to test for a significant discontinuity between horn length and body size at the 'switch point' (Y_D). All males occurring below the switch point are termed as 'Minor' morphs and are indicated as clear circles, while all the males above the switch point are termed 'Major' morphs and are indicated by filled circles (see equation 2 for details).
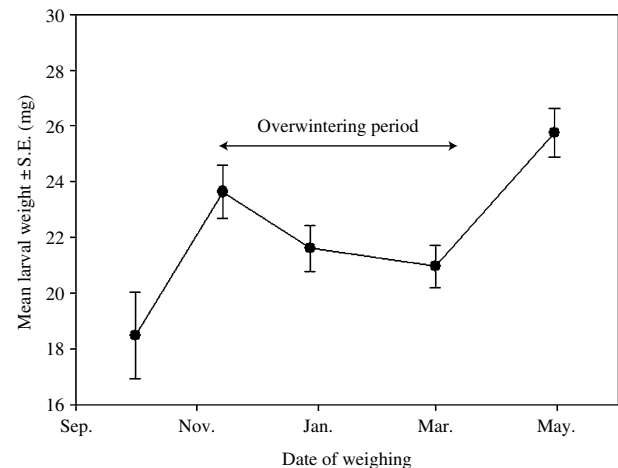
Fig. 2. Changes in the mean mass ± SE of larvae (males and females) throughout the course of the study. All larvae were weighed on 3 October 1996, 14 November 1996, 28 December 1996, 1 March 1997, and 30 April 1997. The most significant increase in larval mass occurs as larvae fed prior to over wintering. Larvae weight then declines during the winter diapause (November to March) before increasing again during the spring.

Table 2. Linear mixed effects models examining how seasonal time constraints, larval crowding and type influence size at eclosion (mm) and total development time (days). All analyses were carried out on the mean values of traits for major morphs, minor morphs, and females that eclosed from each bucket in order to correct for the non-independence of samples. Any effect of Family was corrected for by using linear mixed-effects models with Family entered as a random effect.

Source	d.f.	Adj. SS	Adj. MS	F	P-value
Elytra length					
Day of oviposition	1	7.65	7.65	11.18	0.002
Larval density	1	3.05	3.05	4.45	0.042
Type	2	53.03	26.51	38.77	<0.001
Family	7	9.74	1.39	2.03	0.079
Error	34	23.26	0.68		
Total	45	116.31			
Development time					
Day of oviposition	1	2305.26	2305.26	215.76	<0.001
Larval density	1	2.49	2.49	0.23	0.632
Type	2	2.54	1.27	0.12	0.889
Type × Day of oviposition	2	78.46	39.23	3.67	0.037
Family	7	344.5	49.22	4.61	0.001
Error	32	341.90	10.68		
Total	45	4254.60			

$P < 0.0001$, Fig. 4). Although, the increase in fat reserves with increasing body size was greater in major morphs than in minor morphs (Morph × Elytra length, $F_{1,47} = 4.02$, $P = 0.05$, Fig. 4). No influence of either Family ($F_{7,47} = 0.73$, $P = 0.65$) or Day of oviposition ($F_{1,47} = 0.09$, $P = 0.763$) on the size-corrected fat reserves of major and minor morphs was found.

*Factors influencing the probability of being a minor morph*

The probability of eclosing as a minor morph in this study was strongly negatively correlated with final larval weight (coefficient $= -0.571$, $t_{132} = -6.80$, $P < 0.001$, Fig. 5). The effect of larval weight on the probability of eclosing as a minor morph was the same irrespective of the time in the season that the larva had been oviposited (coefficient $= -0.004$, $t_{130} = -0.719$, $P = 0.4737$). Once variation in larval weight was accounted for, no evidence that day of oviposition had any influence on the probability of eclosing as a minor morph was found (coefficient $= -0.0578$, $t_{131} = -1.39$, $P = 0.166$).

Discussion

These results confirm previous suggestions that male *A. dichotoma* are polyphenic (Siva-Jothy, 1987; Iguchi, 1998). The horn length body size allometry is best described as a linear relationship that is shifted towards a higher horn length to body length ratio in males with horns longer than 16 mm. These findings correspond exactly with a previous study of *A. dichotoma* from the same study site, which estimated a switch point of 16 mm by eye (Siva-Jothy,

1987). There is a considerable overlap in the body size of the two morphs in *A. dichotoma* (Fig. 2). Eberhard and Gutierrez (1991) proposed that a similar overlap in the body sizes of dimorphic earwigs may arise because male cerci have multiple functions, and may therefore be under a more complex selective regime. Alternatively, the overlap may arise because the fitness consequences of making a developmental mistake are reduced in these species. In the *Onthophagus* genus, small males that develop horns are selected against because horns inhibit sneaking behaviour in tunnels (Moczek & Emlen, 2000). This cost may be greatly reduced in non-tunnel dwelling species such as *A. dichotoma*. Otherwise, the overlap might simply result from imperfect developmental fine tuning of the allometric relationship between horn length and body size, or even genetic variation in the size thresholds responsible for determining whether a male develops as a major morph or a minor morph (Emlen, 1996; Moczek *et al.*, 2002).

As with all other beetle horn polyphenism studies to date (Emlen, 1994; Hunt & Simmons, 1997, 1998; Iguchi, 1998; Emlen & Nijhout, 1999; Moczek & Emlen, 1999), morph determination in *A. dichotoma* was governed by larval nutrition (Fig. 5). However, variation in resource accumulation in *A. dichotoma* appears to be the result of feeding time available prior to the onset of a winter diapause, rather than the availability of food *per se*. Figure 2 shows that the majority of larval mass gain occurs prior to the onset of the winter diapause in November. Consequently, eggs laid later in the season have less time to feed. This was confirmed by the finding in this study that development time was negatively correlated with day of oviposition (Fig. 3b). Development time and body size were also negatively influenced by larval density suggesting that attempts to feed larvae *ad libitum* food in this study were unsuccessful.

(a)

(b)
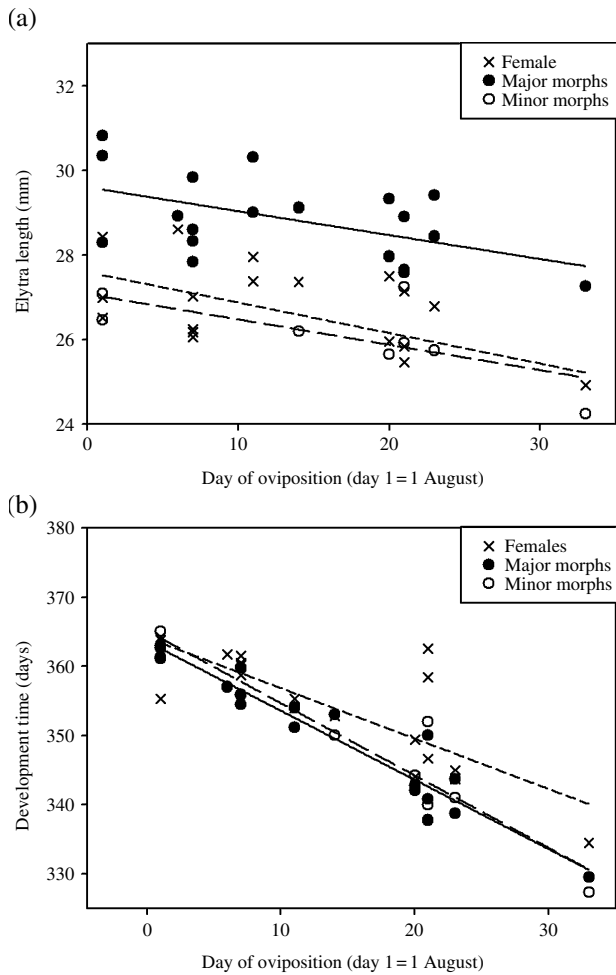
Fig. 3. The relationship between the day of oviposition and (a) the mean body size (elytra length) ± SE for major morphs (●, solid line), minor morphs (○, long dash line), and females (×, short dash line) eclosing from each bucket; (b) development time ± SE, for major morphs, minor morphs, and females eclosing from each bucket. Day 1 refers to 1 August 1996, the day that the first eggs in the study were laid.

However, the effects of larval crowding were less important than the effects of day of oviposition (Table 2). Individuals that were laid later in the season had eclosed as smaller individuals (Fig. 3a). However, once the effect that day of oviposition has on feeding time and body size was accounted for, no further effect of day of oviposition on the probability of eclosing as a minor morph was found (Fig. 5).

Reductions in development time and body size are typical responses to time constraints in species with complex life histories (Leimar, 1996; Nylin & Gotthard, 1998; Johansson & Rowe, 1999; Plaistow & Siva-Jothy, 1999; Blankenhorn, 2000) and are generally considered to be the most important link between larval stress and adult fitness (Rowe & Ludwig, 1991; Abrams *et al.*, 1996; Day & Rowe, 2002). However, there is also some evidence that food stress and time constraints can influence physiological traits that
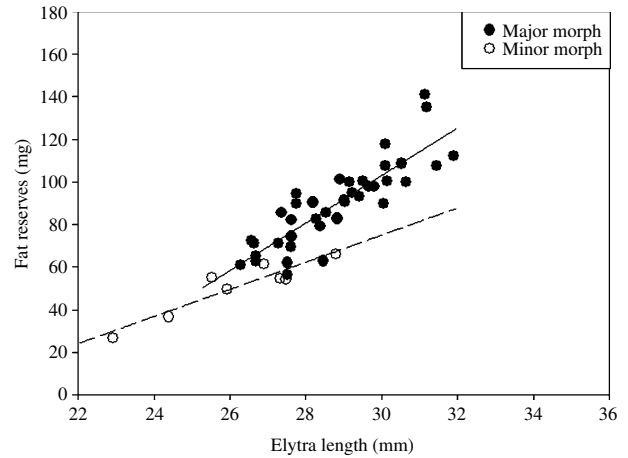
Fig. 4. The relationship between elytra length (mm) and stored fat reserves of major morphs and minor morphs at eclosion.

are independent from changes in age and size at maturity. Rolff *et al.* (2004) found that in *Lestes* damselflies, time constraints had no influence on fat reserves but did significantly reduce immune functioning. As immune functioning was not measured in this experiment, the possibility that time constraint during development induces a physiological cost cannot be ruled out. However, after correcting for body size, no effect of day of oviposition on the stored fat reserves of either major or minor morphs was found, suggesting that in *A. dichotoma* reductions in body size are sufficient to compensate for reductions in development times without inducing further costs.

Males and females both showed a reduction in development time in response to seasonal time constraints, but the response was stronger for males (Table 2, Fig. 3b). The most likely explanation for this observation is that males are
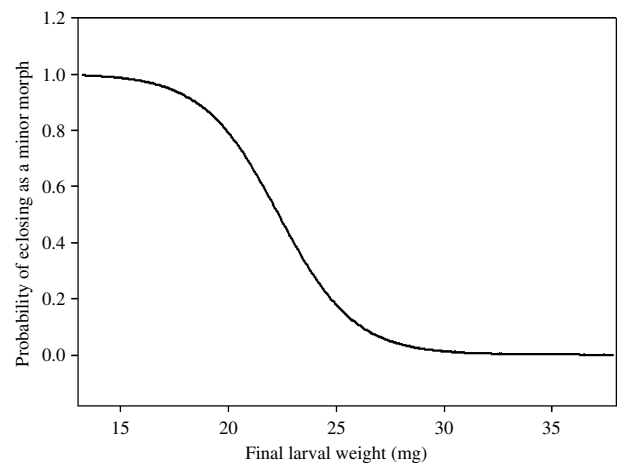
Fig. 5. The relationship between final larval weight and the probability that a male ecloses as a minor morph if it was laid on 11 August (the median day of oviposition for all individuals in this study).

protandrous in *A. dichotoma*. However, as well as having shorter development times, major morphs were also considerably larger than females that developed over the same time period (Fig. 3a), suggesting that males have higher growth rates than females. This hypothesis is supported by the observation that the final larval mass of major morphs is considerably higher than that of females (Major morphs, 30.127 ± 0.407, $n = 118$; Females, 21.551 ± 0.325, $n = 96$; Minor morphs, 23.09 ± 1.02, $n = 23$). Sex-specific growth responses have recently been demonstrated in a number of studies (Nylin *et al.*, 1993; Post *et al.*, 1999; Crowley, 2000; Bedhomme *et al.*, 2003; Plaistow *et al.*, 2004), and are thought to evolve when selection on size related life-history trade-offs operates differently in the two sexes (Badyaev, 2002). In the case of *A. dichotoma*, the male biased sexual size dimorphism is almost certainly a reflection of the importance of body size in determining the outcome of contests for territories in this species (Siva-Jothy, 1987).

As minor morphs do not develop horns, or develop horns that are greatly reduced in size, studies of other horned beetle polyphenisms suggest that growth responses might also be morph specific. For example, Hunt and Simmons (1997) found that in *Onthophagus taurus*, developing horns increases development time, although this finding was not repeatable (Moczek & Nijhout, 2002). No difference was found in the development time of major and minor morphs that were oviposited at the same time (Table 2, Fig. 3b). Similarly there was no evidence that minor morphs have higher stored fat reserves as a result of not developing a horn (Fig. 4). So it seems that there is little evidence of a developmental benefit associated with being hornless in *A. dichotoma*. However, one cannot rule out the possibility that developing a horn reduces investment in some other traits that were not measured such as eyes or wings, as has been found in some other horned beetle species (Kawano, 1997; Nijhout & Emlen, 1998). Alternatively, it may be that in *A. dichotoma*, the cost of possessing a horn is ecological rather than developmental, e.g. Moczek and Emlen (2000).

Acknowledgements

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