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Larval nutritional environment determines adult size in  
Japanese horned beetles *Allomyrina dichotoma*

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The effects of larval nutrition and parental size on offspring horn (male) and body size (male and female) were examined in the Japanese horned beetle *Allomyrina dichotoma* L.

(Coleoptera: Scarabaeidae). Offspring-parent regressions for both horn size and body size of males show no heritable effect, and the magnitudes of these traits were primarily determined by the larval nutritional condition. Male *A. dichotoma* also displayed dimorphic horn size-body size allometry, i.e., larger males had longer horns relative to their body size and vice versa. Because it has been suggested that males of different body sizes adopt different reproductive tactics, the dimorphic horn size-body size allometry and male reproductive tactics are also a result of the larval environment. Similarly, female body size was determined by larval nutrition, and, thus, larval condition may influence future female fecundity.

Females under low nutrition treatment spent longer duration of the third larval instar than females under high nutrition. Probably females under poor nutrition attempted to be larger as much as possible by the extent of larval duration. Since horn and/or body sizes of males and females affect their fitness, this suggests the evolution of female choice for better oviposition site.

**Key words:** Horned beetles; trait size; nutritional environment; heritability; larval duration.

## Introduction

It is well known that some beetles exhibit remarkable sexual dimorphism, e.g., males possess longer horn(s) or larger mandibles but females have none or only smaller such traits (Darwin 1871; Andersson 1994). These male horns or mandibles are often used as weapons in male-male competition (Eberhard 1982; Andersson 1994). In general, males with larger horns are better competitors in intermale combat for access to females and/or other resources (Conner 1988, 1989; Emlen 1997b). Horn size often varies among individuals and it usually increases sigmoidally with male body size (Eberhard 1982; Eberhard & Gutierrez 1991; Emlen 1994; Moczek 2002; Hongo 2003).

It has been shown in some horned beetles that male horn and body sizes are determined by larval nutritional condition (Emlen 1994, 1997a; Moczek 1998, 2002; Moczek & Emlen 1999). For example, Emlen (1994) documented that horn length of the dung beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae) was primarily determined by food quantity during the larval period, but not by paternal horn size. In these dung beetles, females dig vertical tunnels beneath dung and form oval-shaped 'brood balls' using dung pieces at the end of the tunnels (Emlen 1994; Moczek 1998). Females spawn a single egg in a brood ball, which is subsequently consumed by the hatched larva. Therefore, the larval nutritional condition may be constrained by the size of the brood ball.

In contrast to dung beetles, in the Japanese horned beetle *Allomyrina dichotoma* L. (Coleoptera, Scarabaeidae, Dynastinae), females lay their eggs in the soil in a scattered fashion, and

their larvae feed freely on the humus around them (Iguchi 1998). Hence, many factors, such as the quality and quantity of humus, number of food competitors, and the amount of litter supplement from trees, may affect the larval nutrition of *A. dichotoma*. Iguchi (1998) examined the relationship between nutritional condition at larval period and male horn/body sizes, and found males with good larval nutrition had larger bodies and horns than those with poor nutrition. However, this experiment could not exclude genetic effects on male size, because the specimens were randomly collected from the natural habitat (Iguchi 1998). Moreover, the two nutrition treatment groups were obtained and reared in different years, hence involved different cohorts (Iguchi 1998). It is also possible that the difference in male size between the two groups resulted from other factors, such as a difference in humus quantity and quality between the years.

In the present study, we examined the influence of both the genetic component and nutritional environment during larval period on adult male horn and body sizes in *A. dichotoma* using parent-offspring regressions and nutritional manipulation experiments. Although female body size in insects usually influences their fitness component via fecundity (Clutton-Brock 1988), very little information is available on the determinant factors of female body size in horned beetles (e.g., Hunt & Simmons 2000). In order to clarify the importance of maternal body size and larval nutritional condition on female body size, we also performed a similar experiment for females. In addition, since Shafiei *et al.* (2001) have demonstrated the effect of nutritional condition

on larval duration in the dung beetle *Onthophagus taurus*, we examine the influence of nutrition treatment on larval duration also in *A. dichotoma*. Considering with the factors determining male and female trait sizes, we discuss the importance of larval environment on sexual and natural selection and possible adaptive parental behavior.

## **Methods**

### ***Insects***

Female *A. dichotoma* spawn their eggs into humus in the soil during July-September. Larvae feed on the surrounding humus, develop to the third instar and pupate in the soil during June-July of the next year (Iguchi 1998). Adults usually emerge from late June until September. Thus, *A. dichotoma* has a 1-year life cycle.

Male *A. dichotoma* have horns protruding from both the head and prothorax, with the head horn being longer (Siva-Jothy 1987). In this experiment, we used head horn length as an indicator of horn size, and elytra length as an indicator of body size. Both characteristics were measured to the nearest 0.05 mm with a vernier caliper. Male horn length varied sigmoidally with elytra length (Fig. 1; also see Hongo 2003 in detailed analysis of male dimorphism) as observed in other horned beetles having horn dimorphism (e.g., Eberhard 1982; Emlen 1994; Moczek 2002). Since females of this species have no horns, we measured only elytra length for females.

### ***Experimental procedures***

We collected third instar larvae from a forest in Ueda, Nagano, central part of Japan, in April 1998 and 1999. These larvae were reared individually to adulthood in the laboratory. After measuring horn and/or elytra lengths, 29 adult males (elytra length; 18.40-33.40 mm) and 29 adult females (elytra length; 23.50-25.55 mm) were chosen as parents for the experiment. We set a pair of a given male and female and placed them in separate plastic aquaria (36 x 21 x 25 cm) filled to about 18 cm depth with humus. Adults were fed apples as food and sprayed with water once per day. They were maintained in their aquaria at 26-28°C until natural death. By maintaining isolated parental pairs, we could easily obtain offspring from the same parents. Individual offspring were reared separately from the first or second instar until the end of the experiment in small cages (13 x 21 x 25 cm) in order to avoid cannibalism and competition for food.

Offspring were randomly divided into the two nutrition treatments: high nutrition (HN) condition and low nutrition (LN). Larvae from the HN group were reared in cages fully filled with humus, and those from the LN group were reared in cages containing humus and the soil consisted by red earth (Green Tec Inc.) in a 1:1 ratio. We replaced the humus and red earth with the new ones once a month for both groups. We used humus (Japan Tobacco Inc.) which consisted of only *Quercus acutissima* (Fagales: Fagaceae) litter to maintain constant

food quality. We measured the duration of the third larval instar in number of days from the moult to the third instar to pupation for each individual. After all individuals emerged as adults, male head horn length and elytra lengths of males and females were recorded.

### ***Statistical analysis***

From 17 parental pairs, we obtained 1-9 male siblings for each treatment group. Similarly, from 20 parental pairs, we obtained 2-10 female siblings for each treatment group. We took the mean horn and/or elytra lengths from each treatment group within each parental pair brood for further analyses. All data showed the normal distribution (Kolmogorov-Smirnov one-sample test,  $P > 0.2$ ), so parametric statistics were used. In order to clarify any heritable effect, father-son and mother-daughter regressions were conducted for each treatment group separately. Significant regressions would suggest a heritable component for variation in offspring horn and body sizes, and the heritability ( $h^2$ ) can be estimated by twice the regression slope when one parent is considered (Falconer & Mackay 1996). In addition, we examined offspring-parent regressions by using a mid-parent value; in this analysis, the heritability can be estimated as the slope of the regression (Falconer & Mackay 1996). To examine the effect of nutritional condition on offspring horn and body sizes, we conducted paired  $t$ -test (two-tailed) between the mean values of for both nutrition groups for siblings within both sexes.

In order to examine the effect of nutritional condition on larval duration, we conducted ANOVA with the duration of the third larval instar as the dependent variable and with strain (parents), nutrition treatment and sex of offspring as dependent variables. We conducted this analysis only for 8 broods in which both male and female offspring could survive until adulthood.

## Results

Father-son regressions showed no significant relationships in either nutrition groups for both horn size and body size (Table 1; Fig. 2). When mean values of all male siblings from each parental pair were examined regardless of nutrition groups, heritable variation was also not detected for both horn length and body size (Table 1). We also performed a regression analysis using mid-parent body size, but the regressions were not significantly different from 0 for HN or LN groups, or combined data (Table 1). Thus, no heritable contribution to male horn and body sizes could be detected in this experiment.

On the other hand, the larval nutrition treatment remarkably affected male horn and body sizes (Fig. 2). Horn length of the HN group (mean  $\pm$  SD =  $19.46 \pm 3.80$  mm,  $n = 17$ ) was significantly longer than that of the LN group ( $11.76 \pm 2.98$  mm,  $n = 17$ ; paired  $t$ -test,  $t = 5.88$ ,  $P < 0.001$ ). Male elytra length of the HN treatment ( $25.96 \pm 1.32$  mm,  $n = 17$ ) was also much greater than that of the LN treatment ( $22.24 \pm$



1.72 mm,  $n = 17$ ; paired  $t$ -test,  $t = 6.56$ ,  $P < 0.001$ ).

Similarly, a maternal heritable effect on female body size could not be detected. Mother-daughter regressions were not significant both in HN and LN treatments (Table 1; Fig. 3), or combined data (Table 1). Even using mid-parent value, significant regressions were not found in either the HN, LN or combined data (Table 1).

Female body size was also primarily determined by larval nutrition treatment. Females reared under the HN treatment had longer elytra (mean  $\pm$  SD =  $24.53 \pm 1.77$  mm,  $n = 20$ ) than those from the LN treatment ( $22.23 \pm 2.07$  mm,  $n = 20$ ; paired- $t$  test,  $t = -4.73$ ,  $P < 0.001$ ; Fig. 3).

These results indicate that the determinant factor of both male and female trait sizes is the nutritional condition of the larval environment rather than a genetic effect in *A. dichotoma*.

ANOVA demonstrated the significant effect of strain on the duration of the third larval instar (Table 2). Therefore, larval duration may be determined by genetically to a certain degree. Effects of nutrition treatment, sex and the interaction between nutritional condition and sex on larval duration were also significant (Table 2). Females spent longer larval duration as the third instar than males (Fig. 4). Individuals under the LN treatment spent longer larval duration than those of the HN treatment (Fig. 4). Nevertheless, the significant effect of nutritional condition disappeared when ANOVA was conducted for data only from males (strain,  $F_{7, 40} = 6.2$ ,  $P < 0.001$ ; nutritional condition,  $F_{1, 40} = 0.7$ ,  $P = 0.42$ ; strain  $\times$  nutritional condition,  $F_{7, 40} = 0.6$ ,  $P = 0.74$ ;

Fig. 4). Contrarily, when ANOVA was done for data from females, the effect of nutrition treatment on larval duration remained significantly (strain,  $F_{7, 53} = 7.9$ ,  $P < 0.001$ ; nutritional condition,  $F_{1, 53} = 20.2$ ,  $P < 0.001$ , strain x nutritional condition,  $F_{7, 53} = 1.2$ ,  $P = 0.30$ ; Fig. 4). This indicates that females alter their larval duration in accordance with their nutritional condition, whereas males did not.

## Discussion

The present study clarified that the expressed horn and body sizes of male *A. dichotoma* were primarily determined by the nutritional environment during the larval period. For many horned beetles, it has been suggested that smaller males with shorter horns have lower competitive abilities and they adopt alternative reproductive tactics such as satellite and sneaking behavior (Eberhard 1982; Rasmussen 1994; Emlen 1997b). Alternative tactics of males with smaller body size and shorter horns have been also suggested for *A. dichotoma* (Siva-Jothy 1987; Setsuda et al. 1999; but also see Hongo 2003). Smaller males never escalate the fight with larger conspecific males in order to avoid serious damage (Siva-Jothy 1987; Hongo 2003), and behave from the early time of the day when larger males are scarce (Siva-Jothy 1987). Because larval environment determines adult male size, as revealed in this study, expressed male reproductive tactics may be determined by condition-dependent effects, i.e., by the larval nutritional environment and size at adulthood. If larval environment, such

as the number of food competitors and the amount of litter supplement from trees, would be unpredictable or highly variable, the phenotypic plasticity in both male reproductive tactics and male horn size-body size allometry may be adaptive.

Horn size in male *A. dichotoma* was dimorphic according to body size; males with smaller body size had much shorter horns, but larger males possessed much longer horns relative to their body size (Fig. 1; Hongo 2003). This tendency was also found in other horned beetles (Eberhard 1982, 1987; Eberhard & Gutierrez 1991; Emlen 1994a, 1996; Moczek 2002). This male horn dimorphism may reflect reproductive tactics dependent upon male body size. As mentioned above, because larger males often fight with other males to compete for females or food resources, they should have longer horns for weapons. In contrast, smaller males may avoid fights with larger opponents and should reduce the energy allocation to build horns that may be unnecessary for their tactics. In *A. dichotoma*, horn size is more important than body size in outcome of male-male combat (our unpublished data). Thus, larger males should invest much more energy to build longer horns. The switch of energy allocation from building relatively shorter horns in smaller males to building relatively longer horns in larger ones may also be determined by larval environment (also see Moczek 2002).

In the dung beetle *Onthophagus taurus*, larvae under poor nutrition reduce their larval duration and become small adults (Shafiei *et al.* 2001). In contrast to the dung beetle, female *A. dichotoma* under low nutritional condition spent longer larval duration than females under high nutritional condition.

It is well known that body sizes of females directly influence their reproductive output in many insects (cf. Clutton-Brock 1988). Female *A. dichotoma* under poor nutrition may extend their larval period to compensate their smaller body sizes and fecundity by being larger as much as possible. On the other hand, larval duration did not differ between males from low and high nutrition treatments. Smaller adult males can mate with females by alternative reproductive tactics as mentioned above, although their reproductive success may be lower than larger males (Siva-Jothy 1987). Smaller males often behave not only from the early time of the day (Siva-Jothy 1987) but also from the early reproductive season (our unpublished data) similar to *Podischnus agenor* (Eberhard 1982), because larger males are scarce in the early time of the day and the early season. Therefore, males under poor nutrition may conduct a trade-off between benefits of prolonged larval duration for increment of body size and costs of the delay of their eclosion, such as the higher probability of direct conflict with larger males in the later season (Eberhard 1982).

This study revealed that male horn size and body size of males and females were primarily determined by the nutritional environment during the larval period. These results are quite similar to those seen in previous studies of dung beetles (Emlen 1994a, 1997a; Moczek 1998; Moczek & Emlen 1999; Hunt & Simmons 2000). However, the nutritional condition of dung beetle larvae is constrained by the size and quality of their brood balls, which are provisioned by their parents. Given that there is only one larva per brood ball, competition for food among larvae, at least within the brood ball, is absent.

On the other hand, food competition among larvae, even among siblings, would be expected in *A. dichotoma*, because females lay their eggs in a scattered fashion in humus and larvae freely feed on humus. Therefore, the female ability to detect high larval nutrition spawning sites as well as the number of females spawning at a single site, i.e., the number of future rivals of their offspring, is important for horn and/or body sizes of their progeny. Since effects of horn sizes of males and body sizes of males and females on their reproductive successes are expected (Siva-Jothy 1987; Clutton-Block 1988), the fitness of both males via sexual selection and females via natural selection may be determined by larval environment. Therefore, the evolution of oviposition site choice for a high-quality environment with few competitors is predicted in female *A. dichotoma*. Further research on female oviposition site choice and spawning tactics (e.g., oviposition at early or late of the season, the number of eggs spawned at a single site and so on) is recommended.

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## Figure legends

**Fig. 1.** Allometric relationship between horn length and body size (elytra length) for 255 males collected from Tokyo in 1999. The equation to describe the average relationship between body size and horn length is indicated by solid line;  $\text{horn length} = 22.67 / \{1 + \exp[-(\text{body size} - 26.88) / 2.04]\} + 9.69$ ,  $r^2 = 0.92$ ,  $P < 0.001$ .

**Fig. 2.** Effect of larval nutritional condition and paternal size on male horn length (a) and body size (b). Each dot shows the mean value of siblings for each nutritional condition. Solid and broken lines indicate linear regression lines for high and low nutrition treatments, respectively.

**Fig. 3.** Effect of larval nutritional condition and maternal body size on female body size. Each dot shows the mean value of female siblings for each nutritional condition. Solid and broken lines indicate linear regression lines for high and low nutrition treatments, respectively.

**Fig. 4.** Effect of nutrition treatment on the duration of the third larval instar in male and female *A. dichotoma*. Means are given  $\pm$  SD.

**Table 1** Heritability estimates ( $h^2$ ) of male horn size and body sizes of males and females

Traits	$n_p$	$n_o$	$h^2 \pm \text{SE}$	$P$
Horn size				
Father - son				
HN group	17	62	$0.05 \pm 0.19$	0.92
LN group	17	43	$0.05 \pm 0.15$	0.92
Combined data	17	105	$0.01 \pm 0.11$	0.98
Body size				
Father - son				
HN group	17	62	$0.17 \pm 0.13$	0.74
LN group	17	43	$0.27 \pm 0.17$	0.60
Combined data	17	105	$0.12 \pm 0.10$	0.82
Mid-parent - son				
HN group	17	62	$0.11 \pm 0.13$	0.67
LN group	17	43	$0.14 \pm 0.17$	0.61
Combined data	17	105	$0.04 \pm 0.10$	0.87
Mother - daughter				
HN group	20	52	$0.22 \pm 0.10$	0.65
LN group	20	46	$0.05 \pm 0.12$	0.91
Combined data	20	98	$0.17 \pm 0.10$	0.73
Mid-parent - daughter				
HN group	20	52	$0.11 \pm 0.10$	0.63
LN group	20	46	$0.03 \pm 0.12$	0.91
Combined data	20	98	$0.08 \pm 0.10$	0.73

$n_p$ , number of parental pairs;  $n_o$ , total number of offspring.

**Table 2** Results from ANOVA for the duration of the third larval instar in *A. dichotoma*

Independent variables	d.f.	Mean square	<i>F</i>	<i>P</i>
Strain	7	1241.8	11.6	< 0.001
Nutritional condition	1	1230.9	11.5	0.001
Sex	1	609.3	5.7	0.02
Strain x nutritional condition	7	179.7	1.7	0.12
Strain x sex	7	108.9	1.0	0.42
Nutritional condition x sex	1	446.4	4.2	0.04
Strain x nutritional condition x sex	7	12.5	0.1	0.82
Residual	93			

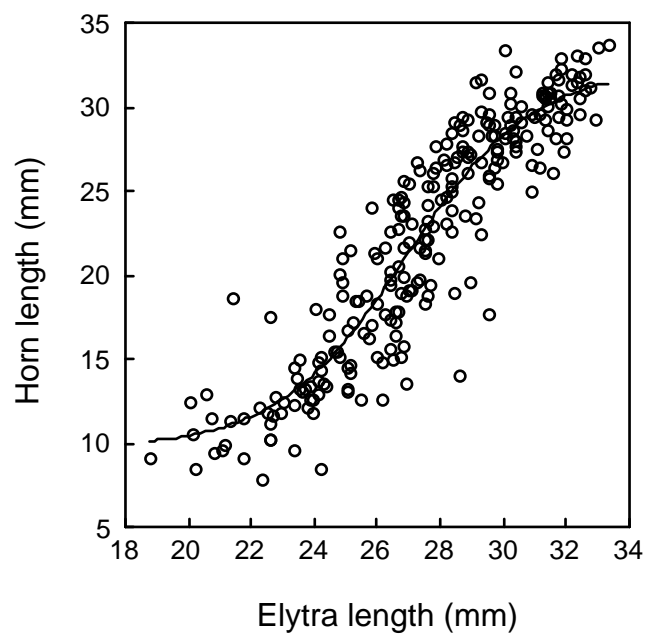


Fig. 1.

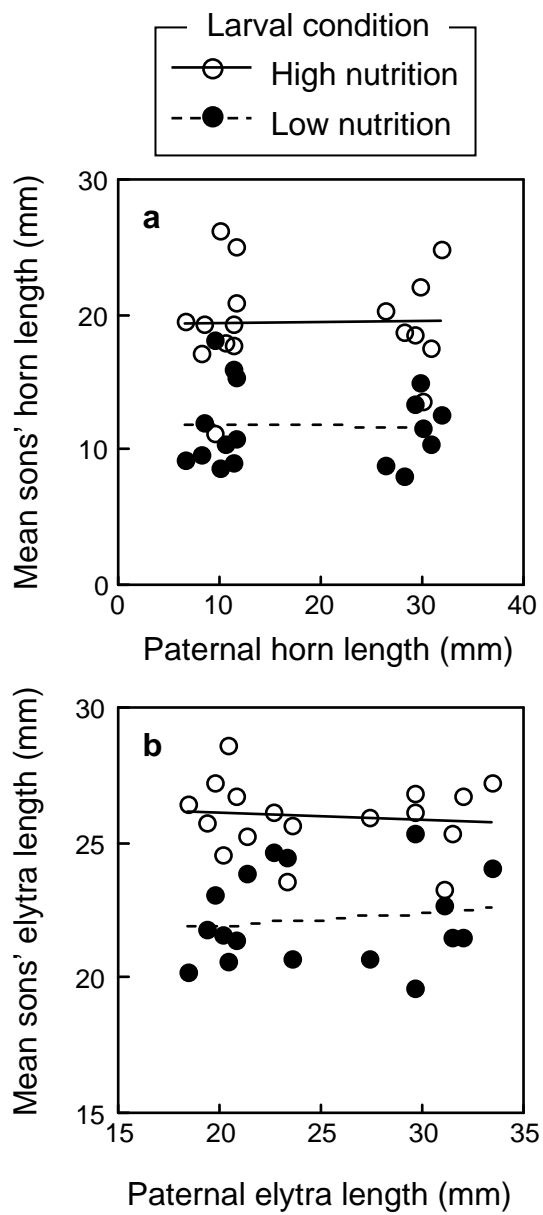


Fig. 2.

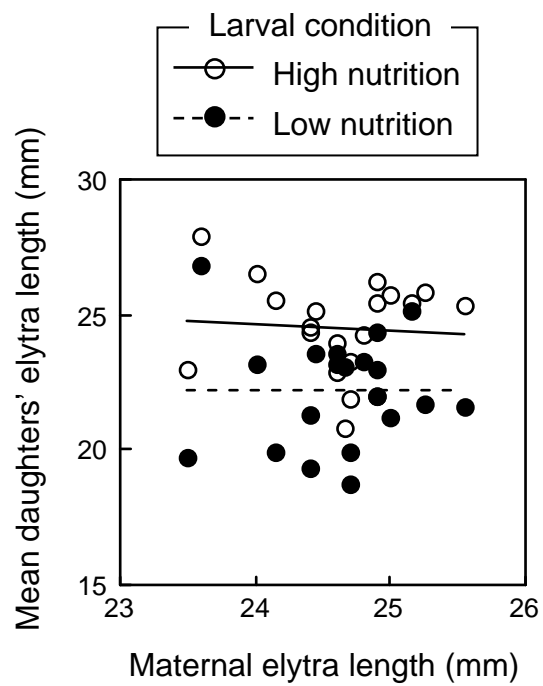


Fig. 3.

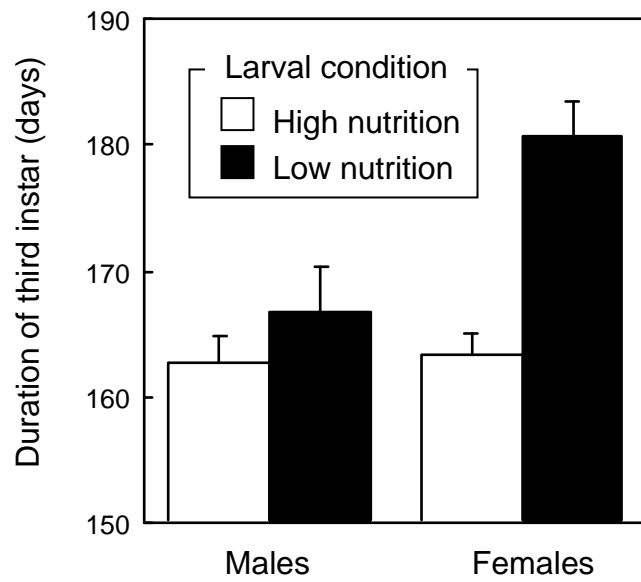


Fig. 4.