Allometric Patterns of Heads and Genitalia in the Stag Beetle *Lucanus maculifemoratus* (Coleoptera: Lucanidae)

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ABSTRACT   Males of *Lucanus maculifemoratus* Motschulsky are markedly variable in size of body and heads. In this study, we investigated the possible causes that affect the development of the male heads and genitalia by comparing variability of weight among body parts and allometry in each part to body size. Genitalia varied least in weight among males, and the frequency distribution of their weight was approximately normal. In contrast, the frequency distribution of head weight exhibited a conspicuous skew. Significant positive allometry was found for heads, whereas genitalia showed significant negative allometry. Heads that are used for fighting are allometrically highly variable, whereas genitalia are highly stable, suggesting variable strategies for obtaining mates while maintaining equally sexually functional genitalia. The low variability and low allometric coefficient with body size for genitalia may indicate that the development mechanism for genitalia is separated from that for other body parts, as a result of sexual selection.

KEY WORDS   Lucanidae, allometry, head, genitalia, lock-and-key hypothesis, sexual selection hypothesis

IN MANY SPECIES of stag beetles, large bodies and large heads with well-developed mandibles are advantageous for male-male combats (Hayashi 1987). Males with small heads and bodies are likely less able to find a beneficial habitat and females to mate. If males with larger heads and bodies are more favored in intersexual competition, size of heads will tend to become steadily larger because of directional selection. However, where the cost of evolving large heads is inevitable (e.g., Kawano 1997), head size may be subject to stabilizing selection, which leads to negative allometry. Nevertheless, one can expect positive allometry if males with heads of different size adopt different reproductive strategies.

Three main hypotheses have been advanced to explain the evolution of genitalic characters: (1) the lock-and-key hypothesis (Eberhard 1985, 1996; Shapiro and Porter 1989), (2) the pleiotropy hypothesis (Mayr 1963), and (3) the sexual selection hypothesis (Thornhill 1983; Eberhard 1985, 1994, 1996). Although many empirical studies have tackled the evolutionary mechanisms responsible for complexity of genitalia in insects, it is difficult to examine which hypothesis is most plausible because insemination and fertilization proceed in the internal organs of females so that differences in reproductive success among males cannot be observed directly.

Comparing variation of genitalic and nongenitalic characters may provide an insight into the patterns of selection on both character sets (e.g., Arnqvist 1997). Insects that exhibit intrasexual polymorphism in fighting apparatus will be useful for assessing the factors that are responsible for genitalic variation. If genital size is influenced by sexual selection like the size of heads and bodies, or is determined by pleiotropic effects of the same genes, then the pattern of variation and the allometric relationship to body size in genitalia would be similar to that in heads. Otherwise, it may be pertinent to consider other constraints that render the variation of genitalia independent. For example, under the lock-and-key hypothesis, genitalia might be selected for constant size resulting in low variation and low allometry coefficient relative to head size.

The aim of this study was to consider possible effects operating on male heads and genitalia by comparing patterns of variation of allometry, using males of the stag beetle *Lucanus maculifemoratus* Motschulsky that vary conspicuously in the size of body and head.

Materials and Methods

In total, 47 *L. maculifemoratus* males from several localities in Hokkaido, northern Japan were used for the analyses. All specimens were collected in 1997 and preserved in 80% ethanol. The allometric relationships between characters were examined focusing on weight, because the use of dry weight rather than dimensions can be seen as an indication of the investment of resources distributed to each body part during development. Each specimen was cut into four parts at membranous joints: head, prothorax, fused seg-

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ments of mesothorax-abdomen, and genitalia (Fig. 1). Each cut part was dried at 65°C for 24 h using a drying-oven (DX-38, Yamato Industry, Gumma, Japan) and weighed on the 1.0×10^{-5} g scale using a fine electric balance (ISO9001, Sartorius, Tokyo). For an index of the general concept of body size, we did not use the total body weight because it includes the weight of each part and is not independent of the weight of the part under examination. Thus, the segments of mesothorax-abdomen weight, which contributed most to the total weight and exhibited the highest correlation with the total weight, was taken as an index of body size.

The coefficient of variation (CV) was computed for the weight of each body part to compare variability among body parts with different means. Pearson's product-moment correlations were calculated between body parts. The significance level of correlation coefficients was adjusted by the sequential Bonferroni method (Rice 1989). In addition, we applied the Shapiro-Wilks test (Shapiro and Wilk 1965) to each body part to examine whether the weight of each part follows the normal distribution.

The allometric relationship between two characters can be described by the ordinary allometric model, $y = bx^k$ (Huxley 1931, 1932) or a full allometric model, $y = a + bx^k$ (Huxley 1932, Bales 1996) where x and y are the observations of the characters, and a, b, and k are constant. To examine whether a full allometric model explains the data better than does an ordinary allometric model, we calculated the following F-statistic,

$$F = \frac{SSR_{ordinary} - SSR_{full}}{SSR_{full} / (n\text{-}3)},$$

where $SSR_{ordinary}$ and SSR_{full} are the sum of squared residuals for ordinary and full allometric equations, respectively, and n is the number of observations. The statistic follows the F-distribution when normality is satisfied for the distribution of residuals in both models (Gallant 1987). When F is greater than $F_\alpha = F^{-1}$ $(1 - \alpha;1,n - 3)$, where α is a critical value, the full allometric model is more appropriate. Otherwise, it does not matter which model is used for allometric analysis. We tested the advantage of the full model by estimating allometric equations and their SSRs using Marquardt's algorithm (Marquardt 1963) in the SAS (SAS Institute 1988) procedure TNLIN (the Marquardt option), but there was no significant improvement ($F = 1.335$, $P = 0.254$ for head; $F = 1.276$, $P = 0.265$ for prothorax; $F = 0.929$, $P = 0.340$ for genitalia). Thus, we used only ordinary estimation.

Although the developmental relationship between two characters has often been analyzed by linear ordinary least squares regression (type I regression: Sokal and Rohlf 1995), like the above analyses, it has been pointed out that the type II regression (Pagel and Harvey 1988, Sokal and Rohlf 1995) is preferable when the two variables are measured with error (Green 1999) (but the justification for using type II regression is questionable when a causality relation exists between variables, Sokal and Rohlf 1995, Eberhard et al. 1999). Thus, the major axis regression (Sokal and Rohlf 1995), which is one of the type II regressions, has also been used for assessing allometric slope. The significant level of allometry was assessed by t-test for results of type I regression. We also estimated 95% confidence intervals for each regression slope to compare the results of the different types of methods.

All statistical calculations described above were performed using the SAS program package (SAS Institute 1988) and BIOMstat for windows (Rohlf and Slice 2000).

Results

Of the four body parts examined, genitalia had the smallest coefficient variation (Table 1), suggesting that their variability is much restricted. The distribution of genital weight did not differ significantly from the normal distribution (Fig. 1). However, the distributions of the other parts deviated significantly from normality (Fig. 1), and those of head and segments of mesothorax-abdomen exhibited conspicuous right-hand skew. The correlations between body parts were all positive, and strong correlations of >0.8 were found between nongenital parts. The correlation between genitalia and segments of mesothorax-abdomen was the lowest, even though the coefficient was significant (Table 1).

There was no difference in the estimates of allometric relationships of either heads or genitalia with body size (segments of mesothorax-abdomen weight) between type I and type II regression, from comparison of 95% confidence intervals (Table 2). The relative growth of heads increases disproportionately as body size becomes greater (t-test, $t = 2.47$, df = 46, $P < 0.05$), and that of genitalia decreases disproportionately as body size increases (t-test, $t = 11.82$, df = 46, $P < 0.001$). There was a tendency toward negative allometry of prothorax but the results varied, depending on the method of estimating the regression slope: the confidence interval for slope includes unity in the major axis regression, whereas it does not include it in the ordinary regression (Table 2). Coefficients of type I regression slopes were consistently lower than those of type II regression slopes, a result similar to Green (1999).

Discussion

The frequency distribution of body mass (segments of mesothorax-abdomen) did not conform to a bimodal distribution, as often observed in species whose males have weapons (Inukai 1924, Eberhard and Gutierrez 1991). The fact that the pattern of size distribution of *L. maculifemoratus* was unimodal may imply that males exhibit no clear difference in mating tactics. The positive allometry of head against body mass implies that heads, including the mandibles that are used as weapons, acquire a disproportionately large allocation of resources with increasing total resources during development. It has been reported that
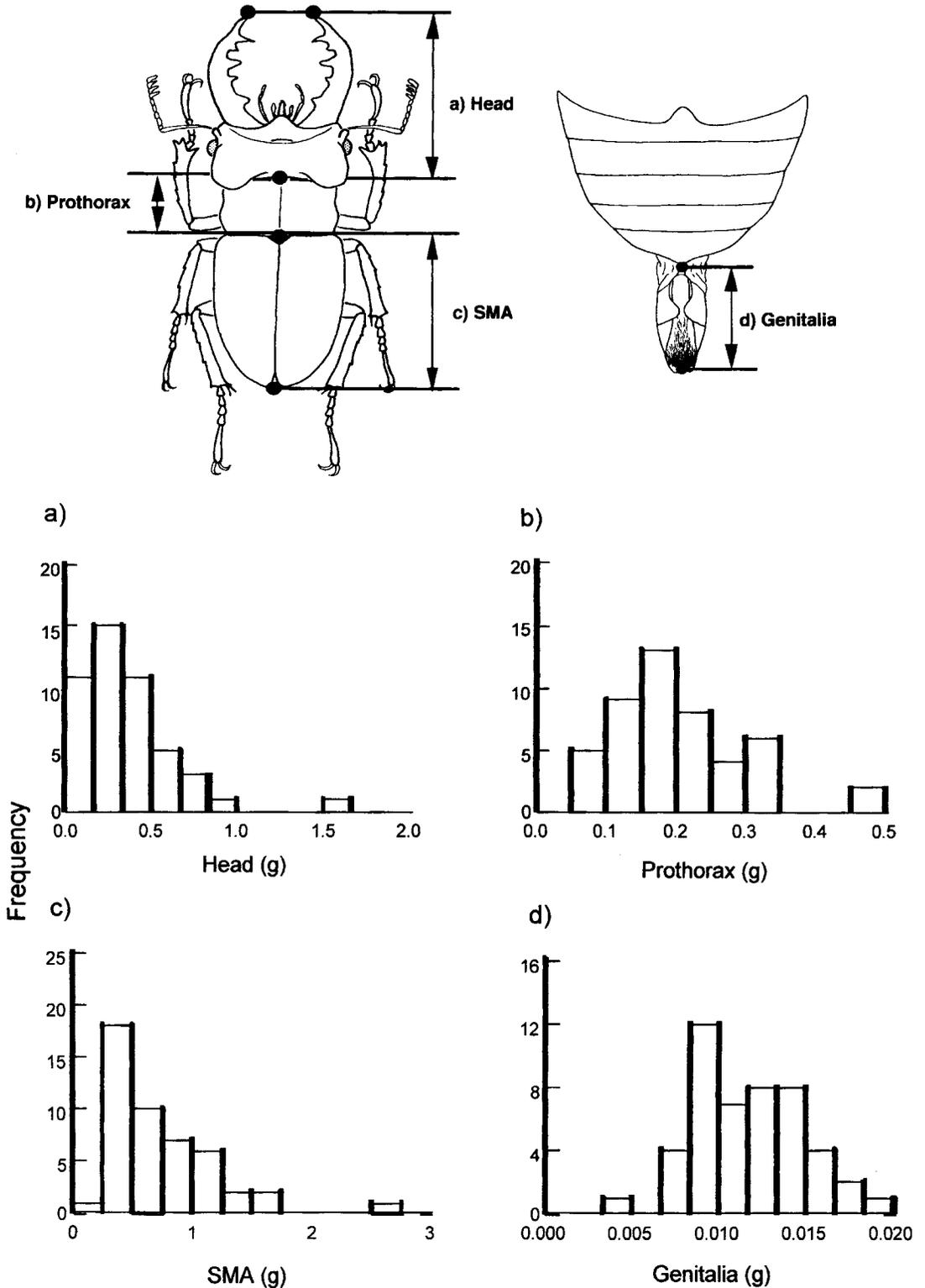
Fig. 1. Measurements of four body parts in *Lucanus maculifemoratus* and frequency distributions of each body part. Statistic of Shapiro-Wilks test (abbreviated as W) was calculated to examine whether or not the weight of each part follows the normal distribution. (a) Head: $W = 0.817$, $P < 0.0001$. (b) Prothorax: $W = 0.942$, $P = 0.033$. (c) Segments of mesothorax-abdomen (SMA): $W = 0.841$, $P < 0.0001$. (d) Genitalia: $W = 0.979$, $P = 0.721$.

Table 1.   Means and standard deviations of four male characters

Character	Mean ± SD, g	CV, %	Head	Prothorax r^a	SMA	Genitalia
Head	0.370 ± 0.266	72.082	1.000			
Prothorax	0.201 ± 0.091	45.391	0.870**	1.000		
SMA	0.745 ± 0.457	61.361	0.963**	0.831**	1.000	
Genitalia	0.012 ± 0.003	25.696	0.513*	0.704**	0.478*	1.000

*, $P < 0.01$; **, $P < 0.001$. SMA, estimate of body size.
[a] Pearson's product-moment correlation coefficients between characters. Significance is adjusted by Bonferroni procedure.

the body size of male *L. maculifemoratus* is greatly influenced by food conditions in the larval stage (Hayashi 1987). Although no genetic studies have been done on this species, much evidence from horned beetles and other insects suggests that phenotypic variance in size is mostly composed of environmental variance (Emlen 1994, 1997; Arnqvist and Thornhill 1998). If this is the case in *L. maculifemoratus,* the pattern of relative allocation to head can be viewed as a norm of reaction (Stearns 1992), in which resources in excess of a threshold are largely invested in heads. This pattern may reflect the selective advantage of larger heads in the acquisition of territories and in intrasexual competition for mates (Eberhard 1982). However, because evidence has been provided that there is an obvious tradeoff between resource allocations to mandibles and wing structures in stag beetles, including *L. maculifemoratus* (Kawano 1997), stabilizing selection on heads can be expected because the benefit of growing larger heads is balanced by the developmental costs of heads. Nevertheless, the developmental cost would not necessarily lead to selective disadvantage on heads if there are different strategies in reproduction between males with large heads and males with small ones (Kawano 1997). Thus, significant positive allometry in heads suggests that the optimal allocation of resources to heads, including mandibles, increases as the overall resource availability increases. This is to be expected when a male with few resources overall will do best to grow as large as possible, whereas a male with more resources can gain more by investing a higher proportion in weapons.

The lower variability and normal distribution of genital weight suggest that the development of male genitalia is restricted to a certain size range. The re-

striction of variability in genitalia is completely contrary to the expectations of the pleiotropy hypothesis. Provided that an increase in the amount of food resources could affect the development of an entire set of body parts, the smaller variation in genital weight requires an explanation. Based on the knowledge of the distribution of lucanid beetles in the area we have investigated, there is no implication that other closely related species have been sympatric with *L. maculifemoratus.* Furthermore, because the genitalia of Lucanidae are adjustable in total length by sliding and protruding at the time of copulation, and the apical part (ejaculation duct which actually enters the female vagina) is very pliable (K. Kawano, personal communication) and there is no character that averts mechanical fitting between different species, such as the copulatory piece in carabid beetles (Sota and Kubota 1998), it is highly improbable that parts of genitalia contribute to prezygotic reproductive isolation in *L. maculifemoratus.* Therefore, there needs to be another explanation for the mechanism by which males advance the development of genitalia separately from the development processes of other body parts.

One possibility is that differences in male reproductive success may be based on cryptic female choice, in which females favor a particular size and shape of male genitalia (Eberhard 1996, Arnqvist et al. 1997). Although we considered the weight of male genitalia only, the results obtained suggest that cryptic female choice, which operates as a form of stabilizing selection, may be responsible for the low variability of male genitalia. Another possibility is that there is no genetic variation in genitalic characters of *L. maculifemoratus* because of a low frequency of mutation (cf., Aguade et al. 1992) or genes that code for genitalic characters are tightly linked to ones that themselves are under stabilizing selection as a result of natural or sexual selection. However, this explains only low variability, not the low allometry coefficient. Further experimental work, including selective gradient analysis (Lande and Arnold 1983) and estimates of genetic covariance between genitalic and nongenitalic characters, is needed to determine the detailed picture of possible forces keeping the size of male genitalia constant.

Table 2.   Allometric coefficients for each character with the estimate of body size (SMA)

Character	Type I		Type II	
	F^a	Slope	r^b	Slope
Head	250.459***	1.185	0.921***	1.315
(CI)[c]		(1.034, 1.336)		(1.160, 1.497)
Prothorax	114.217***	0.730	0.847***	0.839
(CI)		(0.592, 0.867)		(0.692, 1.011)
Genitalia	15.214***	0.248	0.503***	0.299
(CI)		(0.120, 0.376)		(0.149, 0.461)

***, $P < 0.001$.
[a] F-statistic for assessing significance of regression.
[b] Pearson product-moment correlation between the estimate of body size (SMA) and each character.
[c] 95% confidential interval for regression slope.

Acknowledgments

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