Developmental Stability and Adaptive Variability of Male Genitalia in Sexually Dimorphic Beetles

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ABSTRACT: Animal genitalia often show distinct developmental and
evolutionary relationships with other parts of the body. Morphological observations of 29 sexually dimorphic and monomorphic beetle
species in 16 genera of families Scarabaeidae and Lucanidae, Coleoptera, in 53 locations revealed that male genitalia size was consistently and distinctly less variable than that of other body parts within
the same population, while it differentiated more readily among
different populations than other body parts. The most noticeable
genitalia size differentiation occurred in populations that coexisted
with morphologically and ecologically similar congeneric species.
Such differentiation may indicate selection for reproductive isolation.
These characteristics of genitalia morphology may have been instrumental in generating the speciation pattern seen in most beetles.

Keywords: genitalia variability, character displacement, reproductive
isolation, Coleoptera.

Animal genitalia often show a distinct developmental relationship with other parts of the body (Eberhard 1990;
Eberhard et al. 1998) and sometimes exhibit unusual patterns of evolution (Eberhard 1985; Shapiro and Porter
1989). Even before Darwin's time, genitalia morphology
has been an important topic of biology as a key to preventing interspecific hybridization through the lock-and-key hypothesis and, after Darwin, as a form of reproductive
character displacement evolved through natural selection
toward interspecific reproductive isolation (Dufour 1844;
Jordan 1896 [both summarized in Shapiro and Porter
1989]). Male genital morphology of beetles can be highly

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sensitive to interspecific interaction (Eberhard 1990). In
giant rhinoceros beetle species of genus *Chalcosoma*, the
penis length is 22%–35% of the body length, and a 24%
difference in penis length appears to be sufficient for sexually isolating two species in sympatry (Kawano 2002). In
stag beetle species of genus *Odontolabis*, the corresponding
figures are 24%–36% and 20%, respectively (Kawano
2003). Despite the obviously important relation of genitalia to reproductive isolation as such, few studies have
quantitatively dealt with the variability of genitalia size
within and among mating populations. The question of
variability of genitalia as compared with other traits, the
degree of developmental independence of genitalic variation from nongenitalic variation, and the stability of genitalia size in changing environments is to be addressed.
This would lead to answering the important question of
relative degree of intra- versus interpopulational variability
in genitalic morphology and the occurrence of genitalia
character displacement caused by interaction with other
species that has largely remained unanswered (Shapiro and
Porter 1989).

Sexually dimorphic beetles are primarily distinguished
by the remarkable morphological disparity between the
males and females and include some of the more striking
insects of the world (Darwin 1874; Beebe 1944; Endrödi
1985; Mizunuma and Nagai 1994). Most males of sexually
dimorphic species have conspicuously elongated horns,
mandibles, or tibiae, which are considered to have evolved
through sexual selection as fighting apparatus for securing
mating females (Darwin 1874; Otte and Stayman 1979).
These traits considered to be the product of sexual selection show positive allometric relationships with body size
(the size of trait being disproportionately large in larger
body individuals) and, consequently, unusually high variability (Eberhard 1980; Mizunuma and Nagai 1994; Kawano 1995, 1997). If male genitalia are associated with
sexual selection through female choice (Eberhard 1985),
then a positive allometric relationship of male genitalia
size with body size may be expected (Lloyd 1979; Howden
and Gill 1993). However, some studies suggest (though
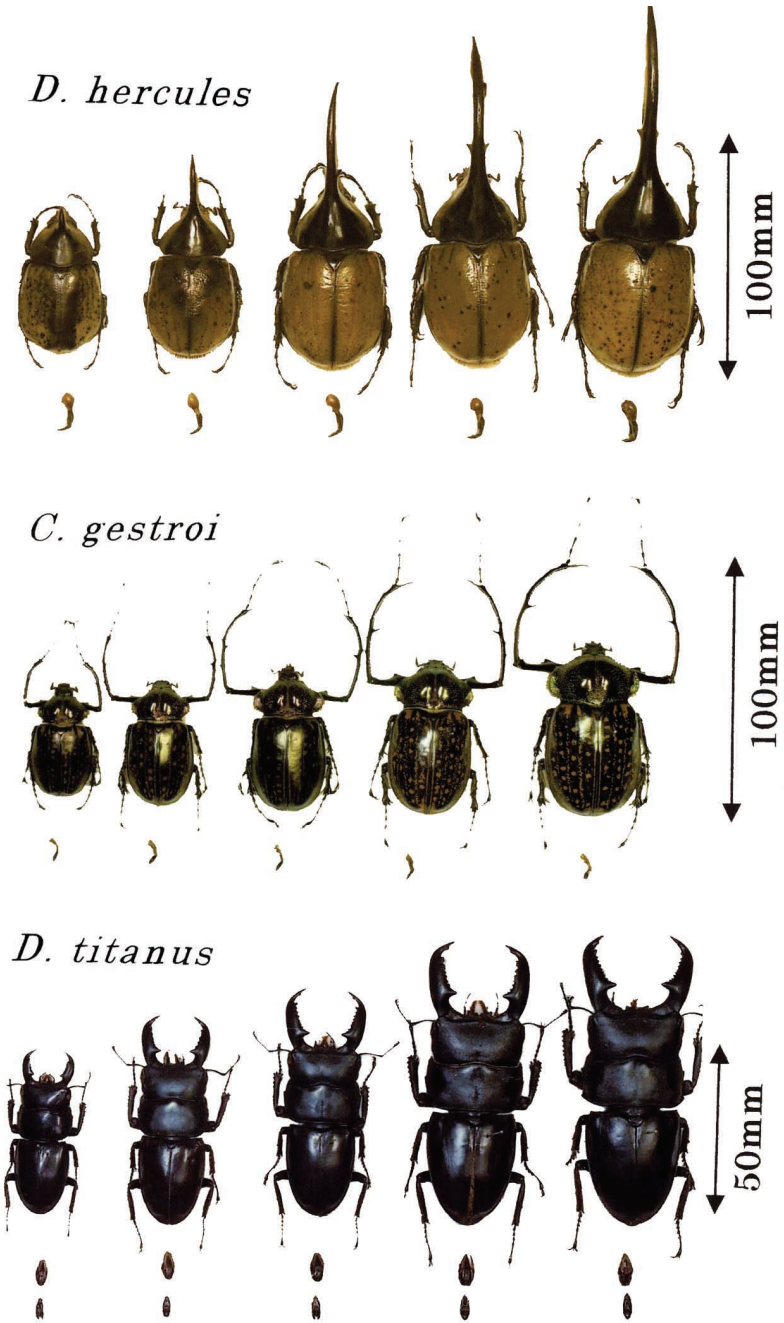not altogether conclusively by some critiques; see Green

Figure 1: Within-population, intralocation variation among males of *Dynastes hercules* in Santander, Colombia (*top*), *Cheirotonus gestroi* in Khao Yai, central Thailand (*middle*), and *Dorcus titanus* in Wiangpapao, north Thailand (*bottom*). Side views of genitalia are shown immediately below the bodies in *D. hercules* and *C. gestroi*. In *D. titanus,* the exterior cover of the genitalia and the genitalia (penis) are shown.

1999) negative allometry to body size and smaller variability of male genitalia size than other body parts in some species of insects (Wheeler et al. 1993; Eberhard et al. 1998). This background justifies studying the relative variability of genitalia based on a large data set covering wide taxonomic orders and broad geographic areas.

All species of the tribe Dynastini of subfamily Dynastinae, family Scarabaeidae, are known to be "true" rhinoceros beetles in which the male has a large head horn (and often a thorax horn or horns as well; fig. 1; Endrödi 1985). Males of all species in the subfamily Euchirinae of the family Scarabaeidae are commonly known as long hand beetles and are characterized by overly elongated tibiae (Mizunuma 1999). Most species of the subfamily Cetoninae, family Scarabaeidae, are "common normal" beetles that have no allometrically developed secondary sex traits and are considered to be sexually monomorphic (Ek-Amnuay 2002). Males of most species of the family Lucanidae have highly elongated mandibles and are known as stag beetles (Mizunuma and Nagai 1994). Several species of Dynastini and Lucanidae are distributed extensively over a broad range of geographical areas. The stag beetle species *Dorcus titanus* (Boisduval), for example, occurs throughout the area encompassing virtually all Asian tropics, subtropics, and temperate areas from South Sumatra to North Korea and from southeast Mindanao to northeast India (Mizunuma and Nagai 1994). In many locations, several species of the same genus and several genera of the same family occur sympatrically. This provides an excellent opportunity to analyze the effects of geography and coexisting species on the variation of genitalia size.

I analyzed three kinds of variation scheme: intrapopulation variation, interpopulation variation, and interspecific interaction. For intrapopulation variation of each trait, I used the following three measures: coefficient of variation (CV), allometric index of the trait in relation to body size, and correlation coefficient (r) between the trait and body size after logarithmic transformation. The CV is a general measure for the relative magnitude of variation in each trait. Allometric index, that is, the slope of the allometric line (Huxley 1931; Peters 1983; LaBarbera 1989), represents a "design feature" of the developmental relationships between traits (Eberhard et al. 1998). When the allometric index of a trait to body size is 1, the variation of the trait is parallel to that of body size (isometry). When it is 0, the growth and the resulting adult variation of the trait are independent of the body growth and variation (no allometry). When it is between 0 and 1, the size of the trait is disproportionately small in large individuals (negative allometry). When it is >1, the size of the trait is disproportionately large in larger individuals (positive allometry). The third measure r, the correlation coefficient, is added to determine whether a low or high CV is caused by the developmental relationship or the absence of it. When the correlation between the trait and body size is high and significant, the allometric slope primarily decides the magnitude of CV; that is, a low allometric index relates to low CV and vice versa. However, when the correlation is low and insignificant or in a "reduced developmental control" (Pomiankousky and Moller 1995), the CV can be large regardless of the allometric index (Eberhard et al. 1998; Green 1999). Twelve species of Dynastini, three species of Euchirinae, 11 species of Lucanidae, and four species of sexually monomorphic Cetoninae were used for this analysis.

In analyzing interpopulation variation, three species of Dynastini and six species of Lucanidae that are widely distributed were observed in a total of 48 locations throughout Asia. For the analysis of interspecific interaction, sympatric species of the same genus and those of different genera in the same family, subfamily, or tribe group were compared in 10 locations in Dynastini, one location in Euchirinae, one location in Cetoninae, and 15 locations in Lucanidae. A total of 28 sympatric species pairs of the same genus and 58 pairs of different genera were analyzed. The objective of this article is to describe the developmental stability and the adaptive variability of male genitalia using a large data set from some of the most variable beetle species and determine the causes for the stability and variability.

Material and Methods

Species Studied and Sample Locations

Twelve rhinoceros beetle species of Dynastini, three species of Euchirinae, four species of Cetoninae, and 11 species of Lucanidae, their sample locations, and the number of male individuals sampled and used for intraspecific, intralocation analyses are listed in appendix A, table A1 in the online edition of the *American Naturalist*. The three species of Dynastinae and six species of Lucanidae used for analyzing the geographic variation are listed in table A2 in the online edition of the *American Naturalist*, along with their sampling locations and the number of individuals sampled in each location. Species identification of Dynastini and Euchirinae followed a monograph by Mizunuma (1999), and that of Lucanidae followed a monograph by Mizunuma and Nagai (1994). In most locations, collections were conducted at several sites within an area of <10 km radius in the same mountain range. Sample individuals were collected on the trees as well as by bait and light traps. The samples were collected from 1985 to 2002.

Morphological Measurement

I measured the samples with a slide caliper to the nearest 0.1 mm for body length (the distance from the front of the head [excluding horn or mandibles] to the tip of the elytron along the center line of the body), hind wing length, elytron length (the distance from the tip of the elytron to the posterior point of the scutellum), elytron width (the distance across the elytron at its widest point), pronotum width (the width of the pronotum at its widest point), tibia length, head horn length (the straight-line distance from the base to the tip), and mandible length (from the base to the tip). I extracted the male copulating organ (penis, the part chitinously hardened; in Lucanidae, the part chitinously hardened inside the male copulating organ; fig. A1 in the online edition of the *American Naturalist*) from fresh or water-softened samples and measured the length of the straightened penis under a low-magnifying microscope with a slide caliper to the nearest 0.1 mm. I treated this as genitalia length. In most species of Dynastini, Euchirinae, and Cetoninae, the genitalia length was >10 mm; hence, this simple method yielded sufficiently accurate measurements. In Lucanidae where the genitalia length was <10 mm, the relative magnitude of error may not be negligent; however, the number of samples in each species was sufficiently large to contain the relative effect of measurement error at a minimal level.

Handling of Quantitative Data

I used the coefficient of variation (CV; standard deviation divided by the mean) in each character in each location in each species to describe the magnitude of intrapopulation variation. I also used the CV of the location means in each species in each character for a measurement of interpopulation variability.

The degree of allometry (or isometry) can be measured by $Y = aX^{\alpha}$, where X is the measurement of a basic, independent character such as body length, Y is the measurement of a dependent character whose allometric index α is to be determined, and a is a constant (Huxley 1931; Peters 1983; LaBarbera 1989). I obtained allometric indices of male genitalia length and other characters of body length through regression analyses (conventional regression analysis by Model I [Peters 1983]); $\ln Y = \alpha \ln X + \ln a$ (natural logarithmic conversion). The appropriateness of this Model I analysis will be evaluated and discussed with the results obtained.

Body size is determined by the interaction of the genetic capacity for growth with the nutritional condition of the environment, in other words, a reflection of all the growth factors accumulated to the adult stage. It is appropriate to use body size as the basis of allometric analysis. As an indicator of overall body size, one measure of a nongenitalic body part, such as elytron length (e.g., Clark 1977; Goldsmith 1985), elytron width (e.g., Eberhard 1980; Cook 1987), or pronotum width (or thorax width; e.g., Eberhard et al. 1998) has been used. However, in sexually dimorphic beetle species equipped with allometrically developed fighting apparatus, elytron length and width are negatively allometric to body length (Kawano 1995, 1997; results of this study), and pronotum width is positively allometric to body length, especially in Euchirinae and Lucanidae (Kawano 1997; results of this study). Thus, these traits are not well suited to represent general body size in sexually dimorphic beetle species. Body length as defined in this study can be treated as the most direct and simplest representation of overall body size (Kawano 1995, 1997, 2000; e.g., for other insect species, Gaston 1988; Wilkinson 1993). In addition, the materials in this study provided accurate measurement and large variation in body length as the independent variable, and they showed nearly normal distribution of body length in logarithmic transformation, thus satisfying some of the important conditions necessary for a conventional regression analysis based on body length to be meaningful in allometric description (Peters 1983).

I calculated the correlation coefficient (r) of the relation between body length and each of the other traits (in logarithmic transformation) to estimate the closeness (strict developmental control) or the looseness (reduced developmental control) of the allometric relationship of body size with other characters. Statistical significance of the deviation of the allometric index (slope) from 0 (no allometric relation) or 1.0 (isometry) in genitalia length, and from 1.0 (both directions) in other characters, was obtained by the *t*-test for the regression coefficient (slope) of allometric computation

Results

Intrapopulation Variability

The CVs of male body length ranged between 10% and 20% in most sexually dimorphic species (Dynastini, Euchirinae, and Lucanidae), and those in sexually monomorphic species (Cetoninae) ranged between 5% and 10% (table 1). The CVs of genitalia length were consistently and distinctly smaller than those of body length in all the species in all the groups (statistically highly significant in each species by *F*-test for the difference in variance with trait values adjusted to the trait mean). The CVs of the most conspicuous secondary sexual trait, that is, head horn length in Dynastini species, tibia length in Euchirinae species, and mandible length in Lucanidae species, were highly significantly larger than those of body length. The CVs of

Table 1: Coefficient of variability (%) of key morphological characters in males of sexually dimorphic and monomorphic species

Species	Body length	Genitalia length	Wing length	Elytra length	Elytra width	Pronotum width	Tibia length	Head horn length	Mandible length
Dynastini:									
Allomyrina dichotoma	15.1	8.2	13.6	14.1	15.4	18.1	17.2	33.9	…
Augosoma centaurus	10.8	2.4	7.3	9.1	8.7	10.8	10.4	54.1	…
Chalcosoma atlas	10.1	5.8	8.4	10.4	9.4	10.4	12.0	42.9	…
Chalcosoma caucasus	12.7	4.8	10.8	11.0	11.1	13.8	13.8	48.9	…
Chalcosoma moellenkampi	11.2	5.5	9.2	9.9	9.9	12.5	12.1	44.7	…
Dynastes hercules	12.8	5.1	10.0	12.2	12.6	13.1	14.1	42.7	…
Dynastes neptunus	13.2	4.0	10.4	12.6	12.6	13.3	17.2	38.7	…
Eupatorus gracilicornis	12.0	3.8	9.7	11.8	13.0	16.1	14.9	55.2	…
Eupatorus siamensis	10.7	4.4	9.3	10.1	10.5	12.5	15.2	48.5	…
Megasoma janus	10.5	4.1	7.6	10.5	10.1	11.9	13.5	26.8	
Pachyoryctes solides	10.8	5.6	8.3	9.0	8.4	10.6	11.4	51.8	…
Xylotrupes gideon	16.0	5.1	14.5	14.2	13.8	15.9	18.2	52.7	…
Euchirinae:									
Cheirotonus gestroi	11.3	5.3	10.2	12.0	13.2	16.5	23.4	…	…
Cheirotonus parryi	11.2	4.5	8.9	11.0	11.8	15.7	24.0	…	…
Euchirus dupontianus	20.2	7.6	17.0	20.3	19.8	25.1	39.5	…	…
Protaetia brevitarsis	9.5	2.8	9.6	10.3	9.4	10.0	10.6	…	…
Protaetia lenzi	7.8	3.2	8.2	8.9	8.0	7.6	6.6	…	…
Protaetia orientalis	7.0	3.6	6.9	7.4	7.6	7.3	8.4	…	…
Rhomborrhina japonica	5.6	3.0	5.6	5.8	6.0	5.7	5.9	…	…
Lucanidae:									
Cyclommatus elaphus	19.6	7.9	16.8	17.7	18.1	22.0	21.1	…	49.7
Dorcus antaeus	24.9	7.9	19.6	20.9	25.6	31.9	24.2	…	52.2
Dorcus parryi	17.7	5.4	13.1	13.5	16.1	21.1	16.3	…	39.6
Dorcus reichei	20.8	4.1	16.1	17.2	20.6	22.1	19.6	…	43.2
Dorcus titanus	19.3	6.3	17.0	17.2	20.2	22.4	18.6	…	27.9
Lucanus planeti	14.6	4.1	13.1	13.7	14.8	14.7	15.5	…	28.8
Neolucanus parryi	9.3	4.8	8.9	8.6	8.6	9.9	10.0	…	14.1
Odontolabis cuvera	15.4	5.2	13.0	13.2	15.1	16.8	15.6	…	41.3
Odontolabis mouhoti	14.1	5.9	12.5	12.7	12.9	15.0	14.2	…	42.4
Prosopocoilus astacoides	24.3	4.7	19.2	21.0	22.5	24.6	20.8	…	47.4
Prosopocoilus giraffa	17.2	6.0	15.8	16.2	17.6	21.7	18.1	…	31.5

other traits were similar to that of body length in each species in all the groups.

The allometric indices of secondary sexual traits in sexually dimorphic species were highly significantly >1.0 (table 2). With respect to the relationship of body length to the secondary sexual trait, the males of these sexually dimorphic species showed either a discrete dimorphism (DD), an indiscrete dimorphism (ID), or a linear relationship (L) (table A3 in the online edition of the *American Naturalist*). The determination of male dimorphism followed the method practiced in a previous article (Kawano 2000). Even in discretely dimorphic species observed in this study, much of the body-length relationship with the secondary sexual trait could be accounted for by the primary linear allometry as evidenced by the high values of multiple correlation coefficient (R or r^2) or the generally

small proportion of residual variation ($1 - r^2$) in the allometry regression (see the right-hand columns of table A3).

The allometric indices of pronotum width were highly significantly >1.0 in all the species of Euchirinae, and those in Lucanidae tended to be slightly higher than 1.0. Those of elytron length and width were slightly lower than 1.0 in Dinastini and Lucanidae, and those of (hind) wing length were consistently and significantly lower than 1.0 in all the groups except Cetoninae. In sexually monomorphic Cetoninae species, the allometric indices of these traits (head horn and mandible lengths being absent or not considered) did not deviate significantly from 1.0 in most samples. In most of these analyses, the correlation coefficients between character measurement and body length in logarithm (r for the allometric indices presented

Table 2: Allometry index of key morphological characters to body length in males of sexually dimorphic and monomorphic species

Species	Genitalia length (r^a)	Wing length	Elytra length	Elytra width	Pronotum width	Tibia length	Head horn length	Mandible length
Dynastini:								
Allomyrina dichotoma	.50** (.94**)	.87**	.92**	1.01	1.21**	1.09**	2.57**	…
Augosoma centaurus	.18** (.80**)	.67**	.81**	.75**	.96	.89	5.42**	…
Chalcosoma atlas	.45** (.77**)	.80**	.96	.91*	.97	1.14**	3.96**	…
Chalcosoma caucasus	.27** (.79**)	.84**	.84**	.85**	.99	1.06	4.02**	…
Chalcosoma moellenkampi	.46** (.96**)	.80**	.88**	.87**	1.10	1.07	3.87**	…
Dynastes hercules	.34** (.86**)	.77**	.90**	.95**	.97	1.04	3.96**	…
Dynastes neptunus	.22** (.75**)	.77**	.91**	.94*	.99	1.18*	8.75**	…
Eupatorus gracilicornis	.28** (.86**)	.81**	.96	1.06	1.31**	1.21*	5.01**	…
Eupatorus siamensis	.34** (.81**)	.86**	.85**	.96	1.15**	1.32**	4.61**	…
Megasoma janus	.34** (.89**)	.70**	.97	.94	1.12*	1.24**	2.55**	…
Pachyoryctes solides	.46** (.89**)	.75**	.78**	.73**	.89	.96	4.89**	…
Euchirinae:								
Xylotrupes gideon	.25** (.80**)	.89**	.87**	.85**	.99	1.12*	4.14**	…
Cheirotonus gestroi	.39** (.86**)	.89*	1.05	1.13*	1.50**	2.27**	…	…
Cheirotonus parryi	.34** (.87**)	.74**	.93	1.01	1.38**	2.12**	…	…
Euchirus dupontianus	.34** (.92**)	.83**	.99	.95	1.16**	2.05**	…	…
Protaetia brevitarsis	.21** (.64**)	1.00	1.08	.98	1.04	1.10	…	…
Protaetia lenzi	.24** (.56**)	1.02	1.06	.99	.93	.79**	…	…
Protaetia orientalis	.50** (.80**)	.97	.95	.93	.99	1.10	…	…
Rhomborrhina japonica	.44** (.73**)	.99	.92	.96	.95	.94	…	…
Lucanidae:								
Cyclommatus elaphus	.36** (.90**)	.81**	.89**	.91**	1.13**	1.03	…	2.72**
Dorcus antaeus	.29** (.93**)	.84**	.89**	1.04	1.48**	.91	…	2.46**
Dorcus parryi	.21** (.71**)	.82**	.73**	.88*	1.18**	.90	…	2.62**
Dorcus reichei	.15** (.80**)	.78**	.82**	.97	1.22**	.99	…	2.31**
Dorcus titanus	.28** (.87**)	.88**	.88**	.98	1.23**	.99	…	1.69**
Lucanus planeti	.25** (.91**)	.76**	.90**	1.00	1.00	1.03	…	2.11**
Neolucanus parryi	.27** (.89**)	.94	.91	.91	1.04	1.05	…	1.40**
Odontolabis cuvera	.26** (.78**)	.84**	.85**	.99	1.21**	1.07	…	3.32**
Odontolabis mouhoti	.38** (.86**)	.85**	.87**	1.01	1.27**	1.08	…	3.43**
Prosopocoilus astacoides	.15** (.81**)	.79**	.88**	.99	1.18**	1.01	…	2.23**
Prosopocoilus giraffa	.31** (.91**)	.85**	.93**	1.02	1.28**	1.04	…	2.00**

[a] Correlation coefficient between body and genitalia lengths in logarithm.

* Statistically significant at .05. For genitalia length >0 and <1.0; for other characters <1.0 or >1.0.

** Statistically significant at .01. For genitalia length >0 and <1.0; for other characters <1.0 or >1.0.

in table 2; actual *r* values, being universally high, are not presented except for genitalia length) were >0.9 (the mean and the confidence interval [CI] by $t_{0.05}$ of *r* being 0.969 ± 0.014).

The allometric indices of male genitalia length (table 2), however, were invariably and distinctly lower than those of any other trait (statistically highly significant in each species by *t*-test for regression coefficient). The correlation coefficients of genitalia length with body length were somewhat lower than those of other characters, yet most of them were >0.8 (the mean and the CI by $t_{0.05}$ of *r* being

0.829 ± 0.095). The CI (at the 0.01 probability by *t* distribution) of the lowest allometric index (*Dorcus reichei*) was 0.152 ± 0.061, indicating that the low allometric indices of genitalia length were not caused by error.

Figure 2 illustrates the relationship of body length to genitalia length, elytra width, and a secondary sexual trait (head horn or mandible length) in *Chalcosoma atlas*, a typical discrete male dimorphic species, and *Cyclommatus elaphus*, where the mandible length was linearly allometric to body length. In both examples, genitalia length was highly stable throughout the whole range of body-length
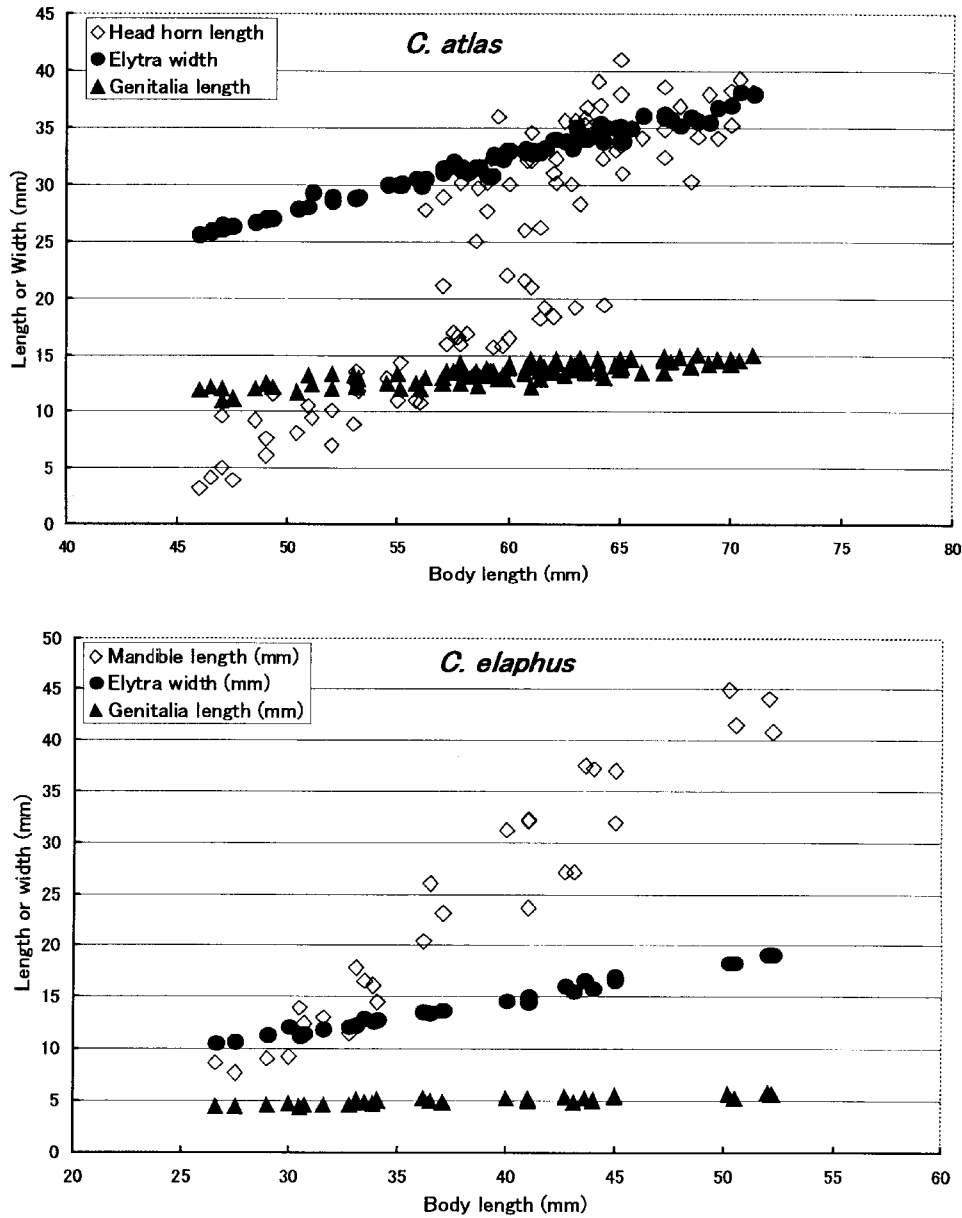
Figure 2: Relationship of body length with genitalia length, elytra width, and a secondary sexual trait in *Chalcosoma atlas* (*top*), a typical discrete male dimorphic species, and *Cyclommatus elaphus* (*bottom*), where the mandible is linearly allometric to body length.

variation, elytra width was isometric to body length, and the secondary sexual trait was highly variable and positively allometric.

Interpopulation Variability

As was expected, the variability of genitalia length within a given location was consistently lower than that of body length in every location and every species of Dynastini and Lucanidae observed. This was seen in the mean genitalia-length CV over locations compared with that of body-length CV (table 3). The difference among the means of locations was highly significant in both body and genitalia lengths in all the species. Contrary to the extrapolation from the intrapopulation variability, the magnitude of interlocation variation (given as CV of the location means) of genitalia length was greater than that of body length in seven out of the total nine species observed. When the

Table 3: Intra- and interlocational coefficient of variability (CV) in male body and genitalia length in three rhinoceros and six stag beetles species

Species	Intralocation CV (%)[a]		Interlocation CV (%)[b]	
	Body length	Genitalia length	Body length	Genitalia length
Chalcosoma atlas[c]	9.6	5.2	5.2**	8.1**
Chalcosoma caucasus	10.2	3.9	3.1**	5.5**
Xylotrupes gideon	12.4	4.5	7.1**	8.3**
Dorcus parryi	16.3	5.6	4.6**	5.1**
Dorcus reichei	18.4	5.4	7.1**	13.6**
Dorcus titanus	18.6	7.9	9.2**	12.5**
Odontolabis cuvera	11.8	4.3	5.4**	4.7**
Odontolabis mouhoti	12.8	5.4	3.1**	3.9**
Prosopocoilus giraffa	18.6	5.1	5.7**	4.7**

[a] Mean of coefficients of variability in all locations.

[b] CV of location means.

** Mean values being highly significantly different ($P < .01$) among locations by ANOVA.

locational difference was expressed in values relative to the intrapopulation variability, the variation of genitalia size attributable to geographic variation was far greater than the variation of body size in all species.

Interspecific Interaction

I compared the difference in body length and genitalia length between two sympatric species in all combinations. There were 22 locations where more than one species pair from the same group (Dynastini, Euchirinae, Cetoninae, and Lucanidae) were sampled. A total of 86 sympatric species pairs within the same group were available. From this, 58 species pairs were combinations between two species belonging to different genera (intergeneric comparison), and 28 were between species belonging to the same genus (congeneric comparison). The intergeneric and congeneric species pairs at each location are listed in table A4 in the online edition of the *American Naturalist*. These species pairs resulted without any prior selection or arrangement. Nevertheless, because of the abundance and/or broad distribution of some species, certain species appeared repeatedly in species pairs (e.g., *Dorcus titanus* 14 times in intergeneric and 13 times in congeneric species pairs). In body length, mean congeneric species pair difference (11.7 mm) was slightly greater than the mean intergeneric species pair difference (10.6 mm), but the difference between the means was not statistically significant. The result was similar when I made the same comparison using relative values (% to paired species mean). Body size difference between species appeared to occur randomly regardless of whether the species pair is from different genera or congeneric (fig. 3).

In genitalia length, mean congeneric species pair difference (2.05 mm) was statistically significantly greater

than the mean intergeneric species pair difference (1.57 mm). The same comparison using relative values gave a similar result, but the overall difference was not statistically significant. More importantly, the genitalia lengths of only one congeneric species pair was within the difference of <0.5 mm and only three species pairs within the difference of <1.0 mm, while the genitalia lengths of nine intergeneric species pairs and another nine intergeneric species pairs had their genitalia lengths within the difference of <0.5 mm and 1.0 mm, respectively (fig. 4, *top*). Expressed in relative value, no congeneric species pair had their genitalia lengths within the difference of <5% and only three species pairs within the difference of 10%, while seven and seven intergeneric species pairs had their genitalia lengths within the difference of <5% and 10%, respectively (fig. 4, *bottom*).

In species pairs of different genera, genitalia size difference between the species appeared to occur randomly. It commonly occurred that body and genitalia lengths of two sympatric species belonging to different genera completely overlapped with each other (fig. 5). Between sympatric species pairs of the same genus, however, genitalia sizes tended to differentiate, which prevented overlapping, even when the body sizes of the two species overlapped (fig. 6). *Protaetia brevitarsis* and *Protaetia orientalis* are very similar in their external morphology to the extent that their identities had been often mistaken or confused even though they are common, conspicuous species (Nakane 1955). The larvae of these two species were found in the same compost mounds, and the adult beetles of the two species emerged at the same time of year and fed on the pollen of the same trees (mostly cultivated chestnut) from the end of May to the end of June and on the tree sap of the same trees (mostly of oak) from the end of June to the beginning of September at the relatively small com-
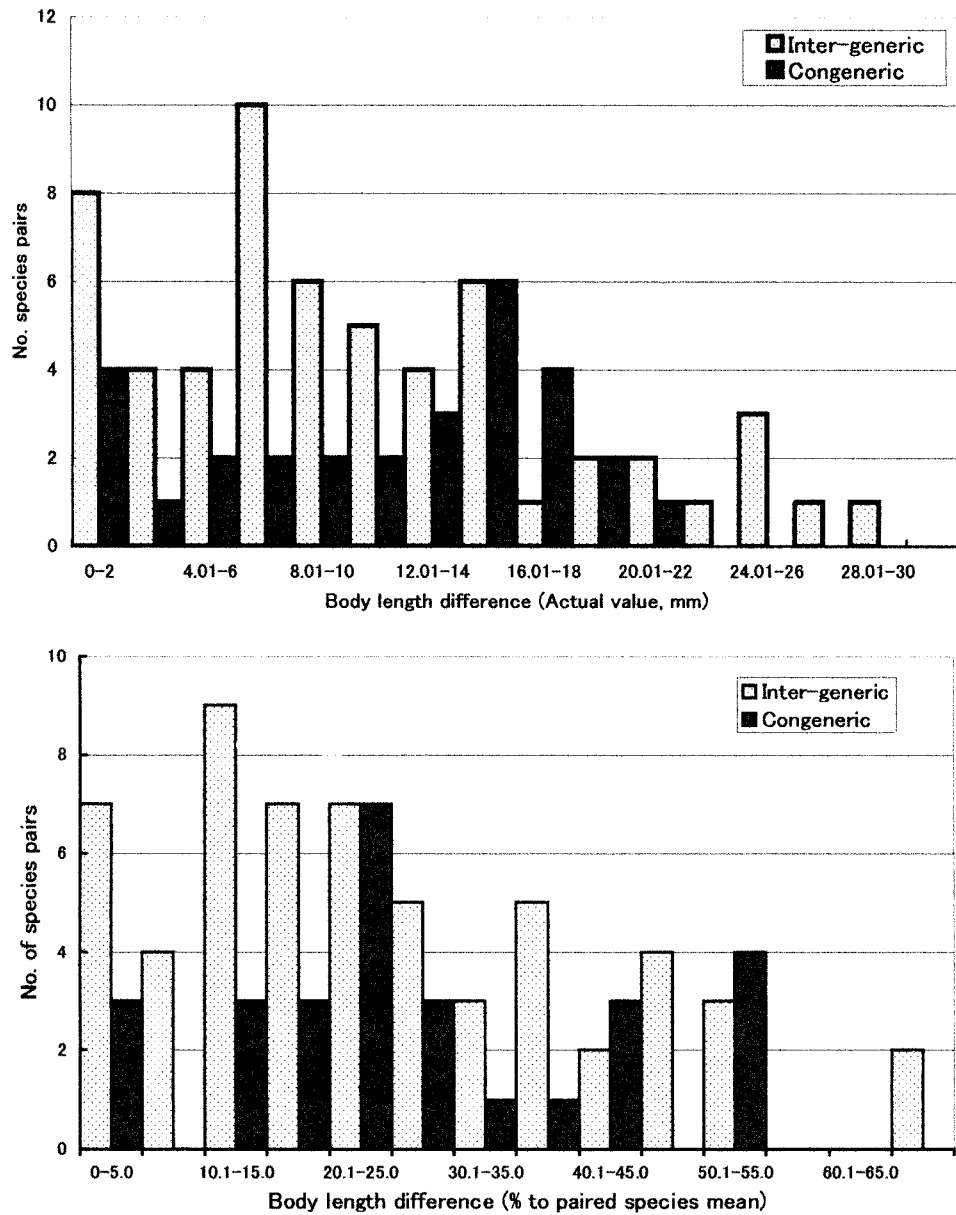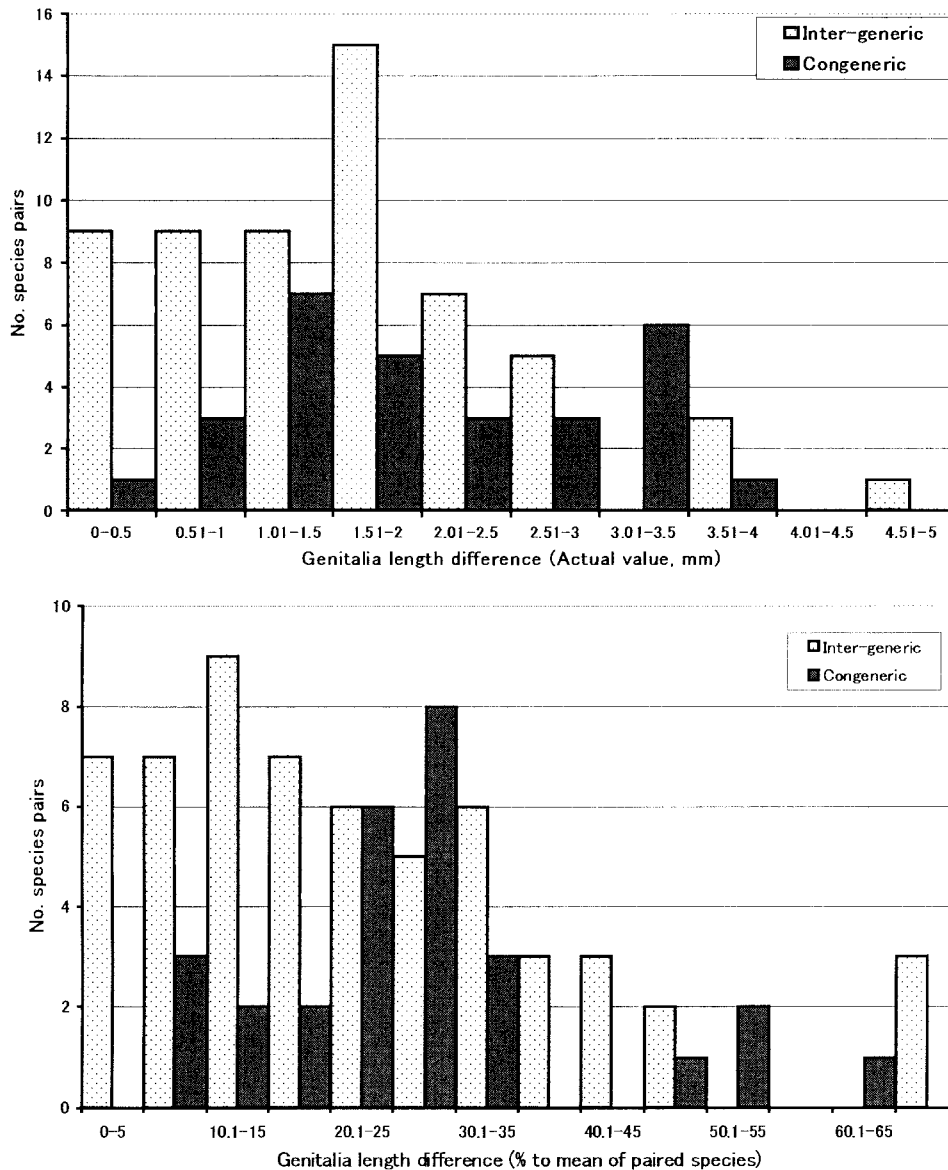
Figure 3: Frequency distribution of body-length difference between two sympatric species of different genera (intergeneric comparison: *light bars*) and that between two sympatric species of the same genus (congeneric comparison: *dark bars*), shown in actual value at the top and in relative value at the bottom.

pound of the Kobe University Experimental Farm in Kasai (approximately 40 ha). Their body size nearly completely overlapped with each other, and they could not be distinguished easily by their external morphology. However, their male genitalia sizes were completely different from each other, and there was no overlap between the two species (fig. 6). Many congeneric species pairs in sympatry followed this pattern, while no intergeneric species pairs showed this pattern.

Discussion

Three measurements, that is, the CV of each trait, the allometric index (α) of the trait to body length, and the correlation coefficient (r) between the trait and body length, are to be simultaneously considered for describing the variability of a trait and the interrelationship of the trait with other traits. In all the traits except genitalia

Figure 4: Frequency distribution of genitalia-length difference between two sympatric species of different genera (intergeneric comparison: *light bars*) and that between two sympatric species of the same genus (congeneric comparison: *dark bars*), shown in actual value at the top and in relative value at the bottom.

length, *r* was very close to 1.0, suggesting a virtually error-free description of body-length allometry with these traits. In this situation, ordinary least squares method is most appropriate for allometry analysis (Sokal and Rohlf 1995; Eberhard et al. 1999). The intrapopulation variability of these characters reflects the allometric relationship of body size to each character.

In genitalia length, *r* was somewhat lower than in other traits, although it was statistically highly significant in all cases. The α of genitalia length in this measurement could

be underestimated compared with the true allometric slope. Even allowing that the true allometric slope may fall between α (allometric slope obtained by ordinary least squares regression [Model I; Peters 1983]) and α/*r* (allometric slope obtained by reduced major axis; LaBarbera 1989; Green 1999), the highest possible true allometric slope would still be lower than α of any other trait (statistically highly significantly different by *t*-test for regression coefficient; table 3). Thus, genitalia size clearly defies the allometric scheme of the other traits.
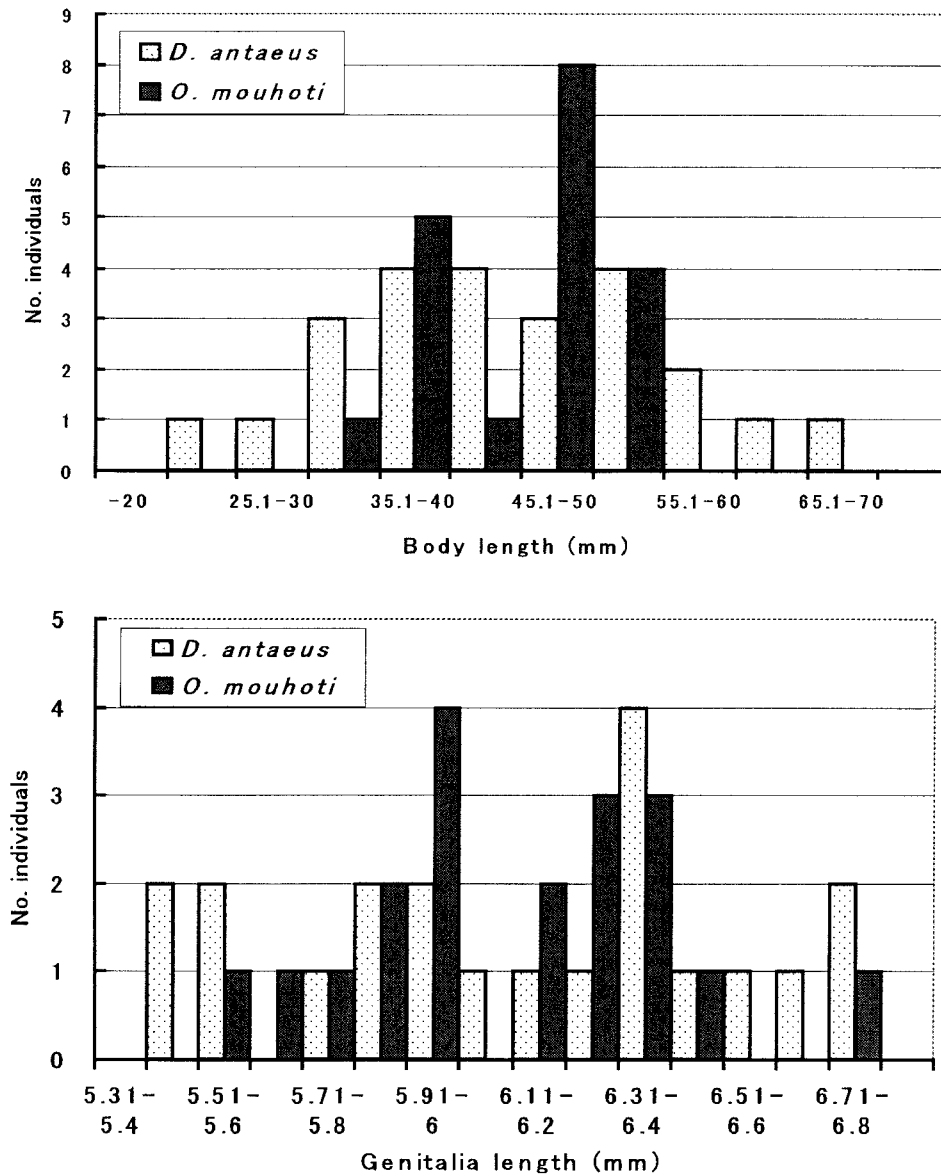
Figure 5: Body length (*top*) and genitalia length (*bottom*) of *Dorcus antaeus* (*light bars*) and *Odontolabis mouhoti* (*dark bars*) in Wiangpapao, north Thailand. Both body lengths and genitalia lengths of the two species overlap completely.

In beetles where adult morphology is fixed once they emerge from the pupa, body size represents the genotype as influenced by environmental condition (of which nutrition is the primary component). Furthermore, adult variation range of the same species is largely a reflection of the species' ontogenetic program (Kawano 2000; Emlen and Nihout 2001). The present phenotypic variation of body size and other sexually dimorphic traits may reflect the ontogenetic relationship of body size with sexually dimorphic traits. This ontogenetic program must have

been formed primarily by sexual selection for larger head horn, tibia, or mandibles and by secondary selection for shorter hind wings and elytra as a cost of evolving exaggerated fighting apparatus (Kawano 1997; Emlen 2001). This ontogenetic relationship, once evolved, is considered to be evolutionarily highly conservative (Emlen 1996; Kawano 1998, 2000).

Thus, genitalia size clearly differs from the allometric scheme of the other traits, and the ontogenetic relationship of genitalia with other body parts appears to be different
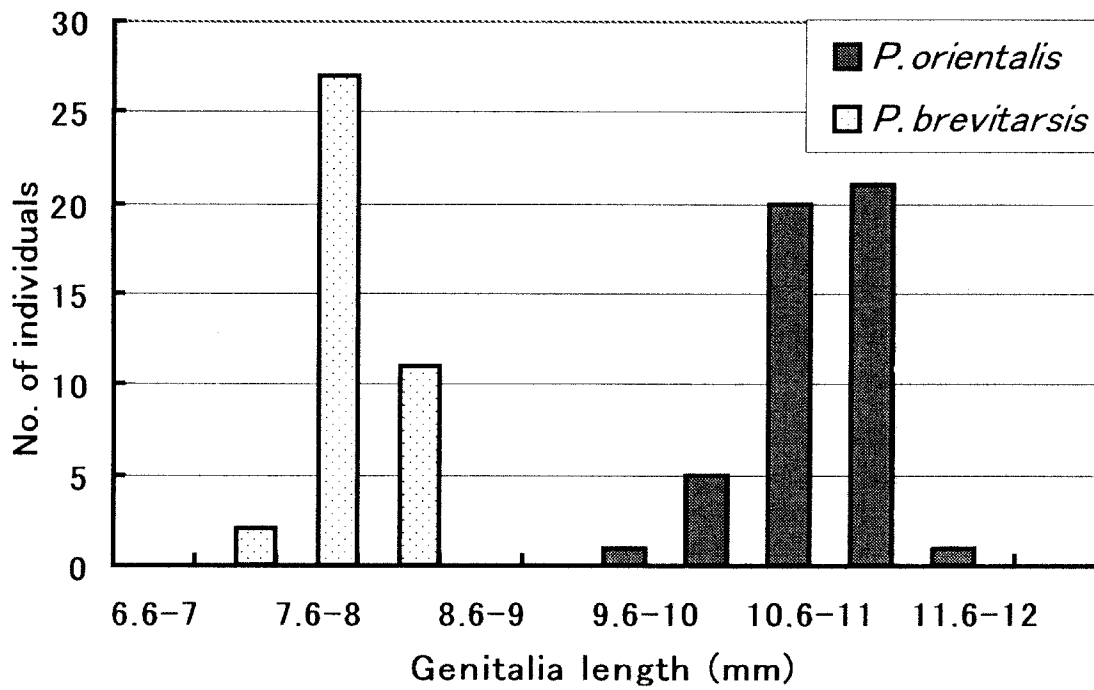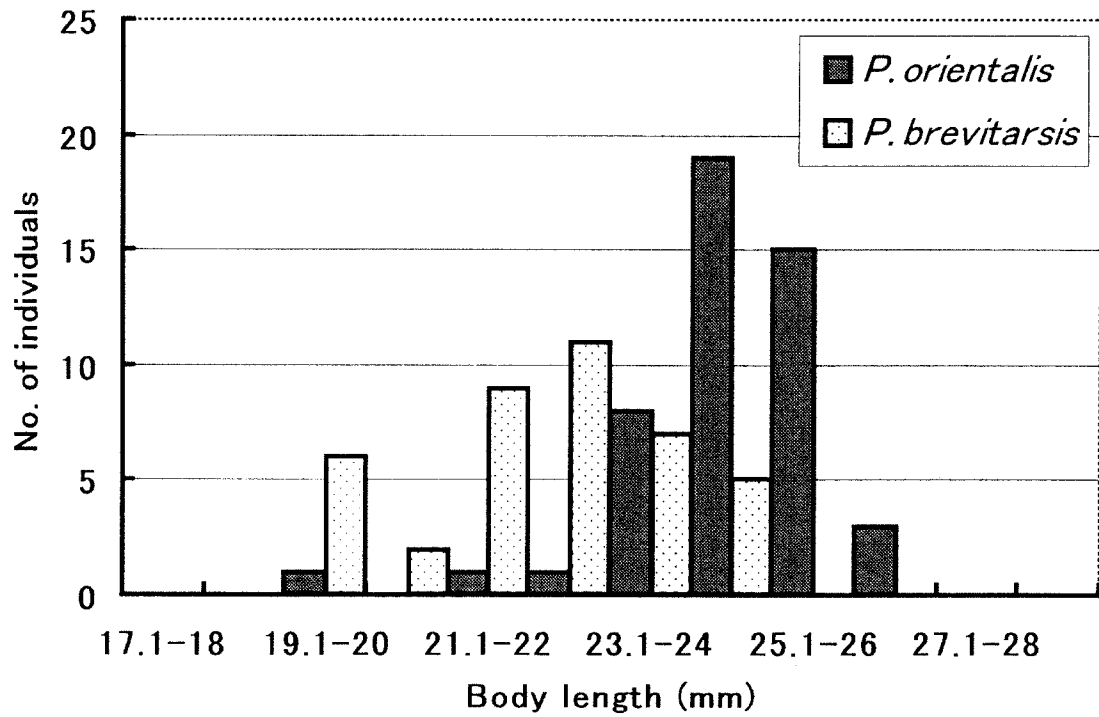
Figure 6: Body length (*top*) and genitalia length (*bottom*) of *Protaetia brevitarsis* (*light bars*) and *Protaetia orientalis* (*dark bars*) in Kasai, central Japan. Body lengths of the two species overlap, but genitalia lengths do not.

from relationships among other body parts. The low variability, or high stability, of genitalia size within a population seems to be caused by its low ontogenetic correlation with other characters. The effects of past selection on genitalia possibly have been very different from those on other traits.

Sympatric character displacement appears to be caused by resource use competition (Losos 2000) and/or stronger mating discrimination or reinforcement (Noor 1999). If genitalic morphology diverges at the species level as an adaptive response to enhance reproductive isolation (reinforcement), we would expect that the genitalic morphology of closely related taxa would be more dissimilar in sympatry than in allopatry (Shapiro and Porter 1989). Moreover, if reinforcement is more important for sympatric coexistence than resource use stratification, we would expect a larger degree of character displacement in genitalic morphology than in body size and other traits. Eberhard (1985) could not detect cases of convincing character displacement in genitalic morphology, and an extensive search for this by Shapiro and Porter (1989) did not yield clear occurrences of genitalia morphology displacement.

In a previous article (Kawano 2002), a comparison between a congeneric, morphologically similar rhinoceros beetle species (tribe Dynastini, Coleoptera) pair of *Chalcosoma atlas* L. and *Chalcosoma caucasus* F. in allopatric and sympatric locations demonstrated a clear case of sympatric character displacement caused by interaction between two species where the degree of displacement was greater in genitalia size than body size. Largely the same sympatric character displacement was detected with a congeneric, morphologically similar stag beetle (family Lucanidae) species pair of *Odontolabis mouhoti* Parry and *Odontolabis cuvera* Hope, where the degree of displacement was also greater in genitalia size than body size (Kawano 2003). The present result also suggests that sympatric genitalia size displacement may be taking place more readily between species of the same genus with similar morphology and behavior than between species of different genera. Phylogenetic analyses using most advanced molecular methods are not available with the species observed in these studies. Yet, following the conventional understandings, interspecific interaction must have been more recent and more intensive between congeneric species with similar ecological behavior than between species belonging to different genera. The need for enhancing reproductive isolation would be stronger between closely related species than between unrelated species. The present intergeneric versus congeneric comparison (figs. 3, 4) is based on naturally available species pairs but may not necessarily represent all the phylogenetic situations, and the accompanying examples of two species pairs (figs. 5, 6) may be representing only a relatively narrow taxonomic variation. Thus, further analyses with larger sample populations covering many phylogenetic groups would be necessary to determine how general and how widespread is the sympatric genitalia size displacement.

In "Homage to Santa Rosalia or why are there so many kinds of animals?" Hutchinson (1959) introduced a hopeful notion that a roughly 1.3 ratio of body size difference persists between co-occuring species. This would be the case of ecological niche divergence but has been challenged by many researchers as unsubstantiated (e.g., Horn and May 1977; Simberloff and Boecklen 1981; Boecklen and NeSmith 1985). The present result also did not show a clear case of ecological body size displacement. Nevertheless, Hutchinson's conviction that there must be a limit to similarity between coexisting species and the question of how similar coexisting species can be does not cease to be relevant. The present result offers an example of sympatric genitalia size displacement, although they may be more related to reproductive isolation than to ecological niche divergence. Santa Rosalia may live on in reinforcement of beetles' genitalia differentiation.

The present findings suggest that genitalia size is less variable within a mating population than among populations of a species. Intrapopulation variability in this group of beetle species is caused mostly by developmental response of general body size to varying nutritional conditions of the habitat and allometric growth of individual characters (Kawano 2000; Emlen and Nihout 2001). The low allometric relation of genitalia size to body size, or the relative developmental independence of genitalia from the growth of other body parts, leads to high stability of genitalia size within the same mating population. Genitalia size stability may secure reproductive isolation of individual species. Interpopulation variability, however, results from adaptation to different environmental situations, of which interaction with closely related species is a major factor. Sympatric character displacement is caused by adaptation to the presence of competing species. Genitalia size may differentiate more readily because its variability can be independent from the conservative ontogenetic network that constrains the genetic variability of other characters.

This nature of developmental genitalia size stability sustains the stability and stasis of established species, and that of adaptive variability facilitates the rapid differentiations of species. Since this genitalia size variation scheme is seen consistently in a broad taxonomic group encompassing at least two major families, its evolutionary history must be as old as most of the present beetle families themselves.

Acknowledgment

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