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Male mandible trimorphism in the stag beetle *Dorcus rectus* (Coleoptera: Lucanidae)

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Abstract. Males in many beetles show horn or mandible dimorphism. The stag beetle *Dorcus rectus* was assumed to have dimorphic males, but in this species there are not two but three visually distinct mandibular phenotypes. The aim of this study is to determine if the three phenotypes represent an allometric trimorphism in this species by studying the scaling relationship between body length and mandible length in 148 adult males and 31 adult females. The relationship was fitted using the following four statistical models; a simple line, a logistic curve, a continuous segmented line and a discontinuous line. The best-fitting model for the males was a discontinuous line with two break points and that for the females a simple line. The male data was also well fitted by a logistic curve. The discontinuous linear model divided the males into three groups that were in good agreement with the three phenotypes. These results suggest that a subtle allometric trimorphism possibly exists in males of this species. Small males of this species have no distinct teeth on their mandibles, whereas small males of *D. striatipennis* and *D. curvidens*, which are closely related to *D. rectus*, have a pair of distinct teeth on their mandibles. The phylogenetic relationship of these *Dorcus* species suggests that the trimorphism in *D. rectus* may have arisen through the loss of teeth in small males.

INTRODUCTION

Males in many beetle species show great variation in the expression of secondary sexual traits such as horns or mandibles. Such variation in males is often discontinuous and males are either large or small with respect to their horn or mandible size (Inukai, 1924; Clark, 1977; Eberhard, 1982; Goldsmith, 1985; Cook, 1987; Rasmussen, 1994; Siva-Jothy, 1987; Eberhard & Gutierrez, 1991; Kawano, 1995, 1997; Iguchi, 1998; Emlen & Nijhout, 2000; Moczek & Emlen, 2000; Shiokawa & Iwahashi, 2000; Tatsuta et al., 2001, 2004). Recently, however, Rowland & Emlen (2009) indicated that several families of the Coleoptera (e.g., Scarabaeidae and Lucanidae) considered to be male dimorphic actually contain species that are trimorphic in terms of their horns or mandibles. Nevertheless, there are very few reports of such trimorphism in beetles (but see Eberhard et al., 2000; Iguchi, 2002).

Males of *Dorcus rectus* (Motschulsky) are considered to be dimorphic with respect to mandible length (Iguchi, 2001a). However, they also show three mandible types with respect to the number of teeth (Fig. 1a–c; Kurosawa et al., 1988; Iguchi, 1992), whereas females have only one type of mandible (Fig. 1d). As mandible length increases, the number of teeth seems to increase in males (Iguchi, 1992). However, the relationship between mandible length and mandible shape has not yet been examined in detail. Moreover, there are no morphometric studies on females of this species.

This paper uses the following four models for the scaling relationship between body length and mandible length in *D. rectus*; a simple line, a logistic curve (Fig. 2a), a continuous segmented line (Fig. 2b) and a discontinuous line (Fig. 2c). These models have been used to determine horn or mandible dimorphism. However, there are very few studies that compare these models in detail (Knell, 2009). In this paper the scaling relationship is determined using these models and the possibility of male allometric trimorphism in *D. rectus* discussed. Rowland & Emlen (2009) suggest that trimorphism will be difficult to detect using current methods. However, this paper shows that the comparison of these models, especially the use of the discontinuous model, might help detect a subtle allometric trimorphism. This paper presents the best-fitting models for males and females and then discusses the evolution of their mandibles on the basis of the phylogenetic relationship of the species of *Dorcus*.

MATERIAL AND METHODS

Sampling and measurement

A total of 148 adult males and 31 adult females of *D. rectus* were collected at sap sites on oak trees on 22 days between 20 July and 5 September 2002 in Nirasaki City, Yamanashi Prefecture, Japan. For each living male and female, body length (from the front of the head excluding the mandibles to the tip of the elytra) and mandible length (the greatest length of the left or right mandible measured in a straight line) were measured to the nearest 0.1 mm with a slide caliper in the laboratory. Voucher specimens are deposited in the Laboratory of Biology, Okaya City, Japan.

Classification of the mandible types

The mandibles of large males differ in shape from those of small males (Fig. 1). Males can be visually divided into three types, as suggested in previous studies (Kurosawa et al., 1988; Iguchi, 1992); males with two pairs of teeth (Fig. 1a; $n = 43$), males with one pair (Fig. 1b; $n = 75$) and males with no teeth on their mandibles (Fig. 1c; $n = 30$). On the other hand, females only ever have one pair of teeth on their mandibles (Fig. 1d; $n = 31$).

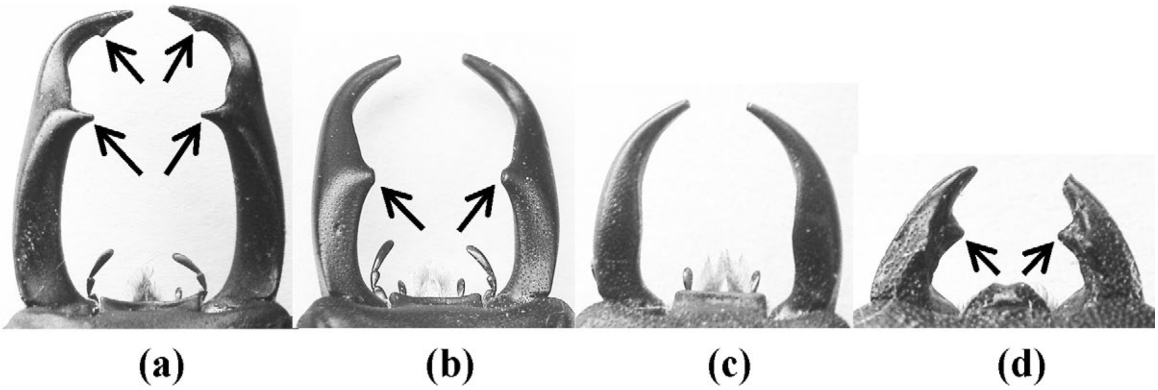

Fig. 1. Mandibles of *Dorcus rectus* of different shapes. (a) Male mandibles with two pairs of teeth (arrows). (b) Male mandibles with one pair of teeth (arrows). (c) Male mandibles with no teeth. (d) Female mandibles with one pair of teeth (arrows).

Data analyses

This study examined the following four statistical models, where x is body length, y is mandible length and a, b, c and d are parameters.

(a) Simple line

$$y = ax + b$$

(b) Logistic curve (Fig. 2a)

$$y = \frac{a}{1+b\exp(-cx)}$$

The curve is divided into three parts (I, II and III) by two "break" points (B_1 and B_2), in which the magnitude of the second derivative is largest, i.e. the slope changes fastest.

(c) Continuous segmented line with one or two break points (Fig. 2b)

This model follows Eberhard & Gutierrez's (1991) model 1. The model with one break point is expressed as

$$y = a + bx + c(x - B_1)D,$$

where $D = 0$ when $x < B_1$ and $D = 1$ when $x \geq B_1$. In this model, the slope changes at the break point $x = B_1$, but the fitted line is continuous. The model with two break points is defined analogously.

(d) Discontinuous line with one or two break points (Fig. 2c)

This model follows Eberhard & Gutierrez's (1991) model 2. The model with one break point is expressed as

$$y = a + bx + c(x - B_1)D + dD,$$

where $D = 0$ when $x < B_1$ and $D = 1$ when $x \geq B_1$. In this model, the parameter d expresses the magnitude of discontinuity at the break point $x = B_1$. Therefore, when d is significantly different from zero, it is concluded that the regression line is discontinuous at $x = B_1$. The model with two break points is defined analogously.

The models were fitted by least-squares regression, i.e., by minimizing the sum of squares of residuals (SSR) from the line or curve, in the Maxima and Gnuplot programs (freely available at http://maxima.sourceforge.net and http://www.gnuplot.info).

In the logistic model, the x-values of the two "break" points were determined by differentiating the fitted function with respect to x. When the two "break" points fell within the range of the data, the relationship between body length and mandible length was called sigmoidal (S-shaped).

In the continuous segmented and discontinuous models, SSR was repeatedly calculated by changing the x-values of the break points every 0.01 mm in the range of the data. In this way, the break points were determined by the smallest SSR. For all the models, the normality of residuals was confirmed by the Shapiro-Wilk test ($P > 0.3$).

Finally, the corrected Akaike's information criterion (AICc) was calculated for each model as

$$\mathrm{AICc} = n\ln\left(\frac{\mathrm{SSR}}{n}\right) + \frac{2k(k+1)}{n-k-1}$$

where n is the number of data points, k is the number of parameters plus one and ln is the natural logarithm (Motulsky & Christopoulos, 2004). AICc compensates for the difference in number of parameters in statistical models and the model with the lowest AICc value is considered to be the best descriptor of the data (Motulsky & Christopoulos, 2004; Knell, 2009). Models

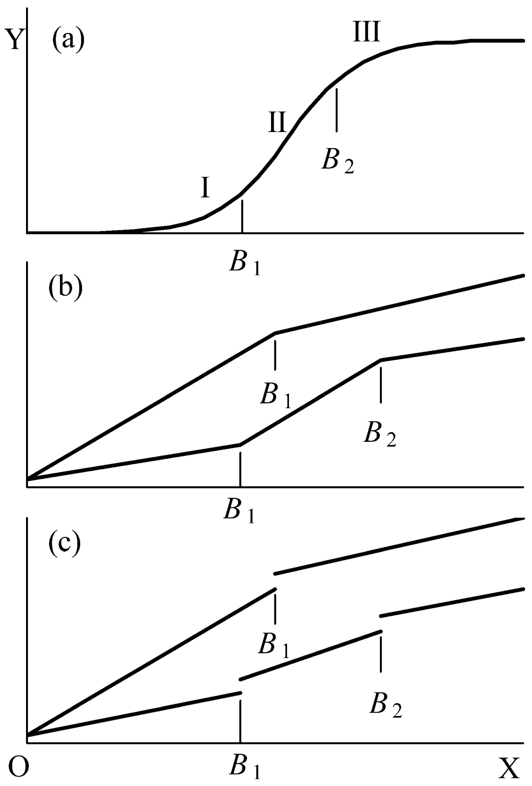

Fig. 2. Statistical models. (a) Logistic curve. This is divided into the three parts (I, II and III) by two break points (B_1 and B_2), at which the slope changes fastest. (b) Continuous segmented line with one or two break points. (c) Discontinuous line with one or two break points.

Table 1. Basic statistics of body length and mandible length.

Sex	n	Body length (mm)			Mandible length (mm)		
		Mean	SD	Range	Mean	SD	Range
Male	148	24.1	3.2	17.8–32.4	7.5	2.7	2.6–13.3
Female	31	22.9	2.0	20.1–27.8	2.4	0.3	1.9–3.2

presented in this study differ in the number of parameters. Therefore, AICc was used to choose the best-fitting model.

The evidence ratio is also calculated. It is the relative likelihood of a model being more correct than the best-fitting model (Burnham & Anderson, 2002). For example, a model with an evidence ratio of 2 is half as likely to be correct as the best-fitting model. In other words, the best-fitting model is twice as strongly supported when the evidence ratio is 2. Models with evidence ratios < 2.7 are considered to have substantial support (Burnham & Anderson, 2002).

Degree of agreement between the classifications of males based on the best-fitting model and the shape of their mandibles

Fleiss' kappa statistic *k* (Fleiss, 1971, 1981) was used to determine the degree of agreement between the classifications of males using the best-fit model and that based on mandible shape. This statistic *k* varies between 1 (perfect agreement) and –1 (perfect disagreement), and $k = 0$ indicates that the agreement can be explained solely by chance. Generally, $k > 0.75$ denotes excellent agreement, $0.40 < k < 0.75$ fair to good agreement and $k < 0.40$ poor agreement (Fleiss, 1981).

RESULTS

The basic statistics of body length and mandible length are shown in Table 1.

The data for males were fitted best by the discontinuous line with two break points (Fig. 3a and Table 2). The logistic curve model also had substantial support (evidence ratio 2.4) and suggested an S-shaped relationship between body length and mandible length with two "break" points (Fig. 3b). In contrast, the best-fitting model for the female data was a simple line (Fig. 3c) and no other model had substantial support (Table 2).

Table 2. The sum of squares of residuals (SSR), corrected Akaike's information criterion (AICc) and evidence ratio (ER) for the relationship between body length and mandible length in male and female *Dorcus rectus*. The ER values were compared with the lowest AICc (in bold).

Sex	Model	SSR	AICc	ER
Male	Simple line	37.63	–196.5	$2.2 \cdot 10^7$
	Logistic curve	29.87	–228.6	2.4
	Continuous line with 1 break point	31.14	–220.3	155.9
	Continuous line with 2 break points	29.82	–222.3	56.2
	Discontin. line with 1 break point	29.31	–227.0	5.3
	Discontin. line with 2 break points	27.39	**–230.4**	1
Female	Simple line	1.03	**–98.7**	1
	Logistic curve	1.01	–96.5	3.0
	Continuous line with 1 break point	0.93	–96.5	3.0
	Continuous line with 2 break points	0.89	–91.2	42.0
	Discontin. line with 1 break point	0.90	–94.4	8.7
	Discontin. line with 2 break points	0.75	–88.6	152.8

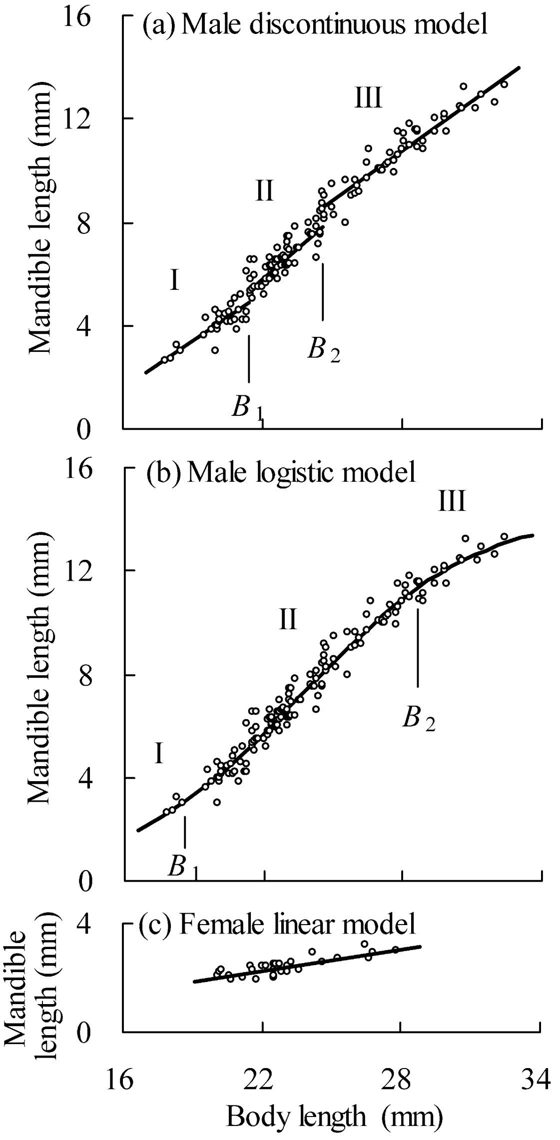

Fig. 3. (a) Discontinuous line with two break points fitted to the male data. I: $y = 0.61x–8.3$. II: $y = 0.77x–11.2$. III: $y = 0.64x–7.2$. (b) Logistic curve fitted to the male data. $y = 14.3/[1+489\exp(–0.263x)]$. B_1 and B_2 show the break points, at which the slope changes fastest. (c) Single line fitted to the female data. $y = 0.13x–0.62$.

In the discontinuous linear model for the males (Fig. 3a), the parameter *d* was significantly different from zero at both break points, B_1 ($t = 3.03$, df = 90, $P = 0.003$) and B_2 ($t = 4.76$, df = 115, $P < 0.0001$), showing that the three regression lines were discontinuous.

Kappa statistic showed excellent agreement ($k = 0.81$, P < 0.001) between the classification of males on the basis of mandible shape and that using the discontinuous linear model (Table 3).

DISCUSSION

Beetles with dimorphic males in which intermediate sized males tend to be rare often exhibit sigmoidal scaling relationships between body length and horn or mandible

TABLE 3. Comparison of the classification of males by mandible shape (Fig. 1) and using the discontinuous linear model (Fig. 3a).

Mandible shape	Discontinuous model		
	I	II	III
No teeth	26	4	0
One pair of teeth	3	61	11
Two pairs of teeth	0	0	43

length (Eberhard & Gutierrez, 1991; Kawano, 1995; Moczek et al., 2002; Emlen et al., 2005a). However, Rowland & Emlen (2009) showed that beetles with trimorphic males, such as the dung beetle *Oxysternon conspicillatum* and stag beetle *Odontolabis cuvera*, in which intermediate-sized males are abundant, also exhibit sigmoidal scaling relationships.

Unlike in these beetles, there is not a clear sigmoidal scaling relationship between body length and mandible length in male *D. rectus*. Polynomial functions have often been used to detect dimorphism in beetles (Eberhard et al., 2000; Hongo, 2003; Tomkins et al., 2005; Harvey & Gange, 2006). However, the results presented suggest that the fitting and comparison of a larger set of models may be a better way of detecting and distinguishing between monomorphism, dimorphism and polymorphism in scaling relationships. A subtle sigmoidal scaling relationship for male *D. rectus*, for which there is abundant data for intermediate-sized males, received some support in the statistical analyses carried out in this paper.

The best-fitting model for the males was a discontinuous line with two break points. Moreover, the classification of the males by the discontinuous linear model was in excellent agreement with that based on mandible shape. These results suggest that subtle allometric trimorphism possibly exists in male *D. rectus*. On the other hand, as expected, the best-fitting model for females was a linear model. This is not surprising, but there are very few studies providing such statistical evidence for a linear model. As shown in Fig. 3c, body length and mandible length varies little in females. Therefore, it is difficult to describe accurately the scaling relationship between them.

Many studies on dimorphic beetles have shown that there are behavioural differences between large and small males. For example, large males fight with other males for possession of females and food, whereas small males avoid fighting with other males and use other tactics (Eberhard, 1982; Goldsmith, 1987; Siva-Jothy, 1987; Rasmussen, 1994; Emlen, 1997; Moczek & Emlen, 2000; Iguchi, 2001b; Hongo, 2003). On the other hand, Rowland & Emlen (2009) suggest that male trimorphism may be related to three reproductive tactics; a dominant (fight and guard) tactic, a subordinate (sneak) tactic, and a female-mimicry tactic. Unfortunately, there are no detailed studies on the behaviour of males of *D. rectus* that could be used to evaluate this hypothesis.

Inukai (1924) examined the mandibles of the stag beetles *Cladognathus inclinatus* and *Lucanus maculifemoratus* and suggests that the female form is the primary one from which the male form was derived. Rowland & Emlen (2009) also indicate that the smallest male forms in the beetle families Scarabaeidae, Lucanidae and Curculionidae resemble females. In *D. rectus*, however, the mandibles of females do not resemble those of small males but those of intermediate-sized males (Fig. 1).

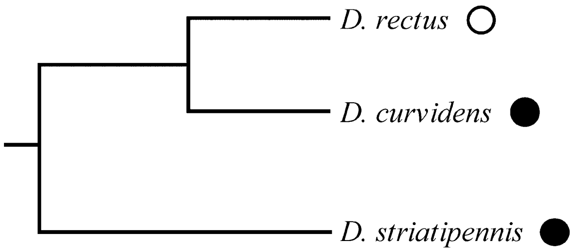

Fig. 4. Phylogenetic relationships between *D. rectus*, *D. curvidens* and *D. striatipennis* based on the mitochondrial DNA study of Hosoya et al. (2003). Small males of *D. rectus* have no distinct teeth on their mandibles (open circle), whereas small males of *D. curvidens* and *D. striatipennis* have one pair of distinct teeth on their mandibles (filled circles).

Interestingly, even small males of *D. striatipennis* and *D. curvidens*, two species closely related to *D. rectus*, have, like females, one pair of distinct teeth on their mandibles (Kurosawa et al., 1988; Iguchi, pers. observ.). The phylogenetic relationships between these three species (Fig. 4; Hosoya et al., 2003) suggest that for small males the ancestral condition may have been one pair of teeth on the mandibles. Consequently, the trimorphism in *D. rectus* may have arisen from the loss of the teeth in small males. Several studies have shown that horned beetles repeatedly gained and lost their horns during evolution (Moczek, 2005; Emlen et al., 2005b, 2007). However, the evolution of the teeth on the mandibles of stag beetles remains to be explored.

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