

SHORT COMMUNICATION

Morph-dependent form of asymmetry in mandibles of the stag beetle *Prosopocoilus inclinatus* (Coleoptera: Lucanidae)

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Abstract. 1. The form of asymmetry in bilateral organs usually follows the same pattern within single populations. However, some exceptions may occur when a population consists of different phenotypes that are from different ontogenic backgrounds and under different selective pressures. We investigated the asymmetric patterns of mandibles of larvae, females, and males in the stag beetle *Prosopocoilus inclinatus*.

2. Larval mandibles exhibited directional asymmetry both in length and cross direction, whereas female mandibles showed directional asymmetry in cross direction. These asymmetric structures might be more effective in cutting wood fibres.

3. For the relation of male mandible length to body size, a model with a switch point showed a better fit to the data than a convex curve model. This shows that the males are dimorphic with two distinct morphs.

4. The form of asymmetry in male mandible length differed between the morphs. The smaller males exhibited left-biased directional asymmetry in common with larvae, whereas the larger males exhibited fluctuating asymmetry.

5. This is a novel finding of a morph-dependent asymmetry. The morph-dependent asymmetry in males may be as a result of different selection on each morph or a developmental constraint from larval mandibles to adult ones.

Key words. Condition-dependent asymmetry, directional asymmetry, fluctuating asymmetry, mandibles, phenotypic plasticity.

Introduction

Bilateral organs often exhibit deviations from symmetric forms. The forms of asymmetry generally fall into one of the following three categories: fluctuating asymmetry (FA), antisymmetry (AS), and directional asymmetry (DA). FA is characterised by random deviations from symmetry and is assumed to be attributable to environmental or genetic stresses on development (Van Valen, 1962; Palmer & Strobeck, 1986). Some studies have reported that FA is a good indicator of male quality (Møller, 1990;

Møller & Swaddle, 1997; Martin & Lopez, 2000); however, other studies have questioned such an interpretation of FA (Tomkins & Simmons, 1995; Björkstén *et al.*, 2000). Thus, there is controversy surrounding the interpretation of FA (Polak, 2003; Van Dongen, 2006). DA means that all the individuals in a population exhibit either left or right-handedness. In contrast, antisymmetry (AS) refers to a state where left-handed or right-handed individuals are equally common within a population (Palmer & Strobeck, 1986; Palmer, 1996). The frequency distribution of the levels of asymmetry (L–R) is bimodal in AS, whereas it is monomodal around zero in FA (Palmer & Strobeck, 1986). Generally, DA and AS are adaptive (Van Dongen, 2006).

In general, the forms of asymmetry within a single species fall into one of the above three categories (Møller, 1990, 1992; Radesäter & Halldórsdóttir, 1993; but see Rowe *et al.*, 1997). However, different forms of asymmetry may arise within the same species when it exhibits condition-dependent alternative morphologies (i.e. polyphenism) that differ in functional requirements and

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developmental backgrounds (West-Eberhard, 1989). One of the best examples is the male dimorphism in ornamented beetles (Eberhard, 1982; Emlen, 1997; Hongo, 2003). Recent physiological studies suggest that different male morphs have different ontogenetic backgrounds (Emlen & Nijhout, 1999, 2001).

The stag beetle *Prosopocoilus inclinatus* Motschulsky (Coleoptera: Lucanidae), exhibits a male dimorphism that is represented by bimodal size-frequency distribution (Inukai, 1924) associated with the existence of an allometric switch point (this study). In male–male combats, *P. inclinatus* males hold their opponents by the mandibles, then attempt to raise and throw them (Unno & Takashima, 2003) using their mandibles like *sugar tongs*. It is predicted that symmetric mandibles are favoured in combat as they are to gain a tight hold of the opponent, particularly in the larger males that engage frequently in combats. In contrast, larvae and females that generally do not engage in combats do not use their mandibles in a *holding* manner. Instead, they cross their mandibles like a *pair of scissors* in order to cut damp wood fibres for feeding and oviposition. For an accurate crossing of mandibles, directional asymmetry is expected for the mandibles of females and larvae. In this study, we investigated the asymmetry of mandible morphology (length, structure, and cross direction) in two male morphs, females and larvae of *P. inclinatus*. Functional meanings of the asymmetry in each class were discussed.

Materials and methods

Adult beetles that flew to street lights were collected in Hobetsu, Hokkaido, Japan (N42.5, E142.1) and their offspring were reared in the laboratory. Males of *P. inclinatus* exhibit bimodal size-frequency distribution (Inukai, 1924) suggesting that they show intra-sexual dimorphism. Large males possess large, thick mandibles, whereas small males have tiny, reduced mandibles (Fig. 1c). As in horned beetles (Emlen, 1994), the quality and amount of food at the larval stage determines the male morph in *P. inclinatus* (Y. Okada, pers. obs.).

Judging from the scatter plots between mandible size and body size (Fig. 1c), the allometric relationship between them is likely to be represented by a convex curve (convex curve model; see Knell *et al.*, 2004), two straight lines of different slope meeting at a switch point (continuous switch point model, e.g. Eberhard & Gutierrez, 1991; Kotiaho & Tomkins, 2001), or two straight lines of different slope and intercept depending on the size of the beetle (discontinuous switch point model; e.g. Eberhard & Gutierrez, 1991; Kotiaho & Tomkins, 2001). As a convex curve may not necessarily support a dimorphism (Knell *et al.*, 2004; Tomkins *et al.*, 2005) a model selection was performed using a convex curve model, continuous switch point model, and discontinuous switch point model using Akaike information criteria (AIC, Akaike, 1974).

For a convex curve model, a second order polynomial regression was conducted on the log-transformed data.

$$\log_{10} Y = \alpha_0 + \alpha_1 \log_{10} X + \alpha_2 (\log_{10} X)^2$$

where X is the elytra length, Y is the average mandible length, and α_i are the regression coefficients. For a continuous switch point model, two linear regressions meeting at a switch point (X_D) were fitted to the log-transformed data.

$$\log_{10} Y = \beta_0 + \beta_1 \log_{10} X + \beta_2 (\log_{10} X - \log_{10} X_D) D,$$

$$(D = 0 \text{ if } X < X_D \text{ and } D = 1 \text{ if } X \geq X_D)$$

where X is the elytra length, Y is the average mandible length, and β_i are the regression coefficients. For a discontinuous switch point model, two linear regressions separated at a switch point (X_D) were fitted to the log-transformed data.

$$\log_{10} Y = \gamma_0 + \gamma_1 \log_{10} X \quad (\text{if } X < X_D)$$

$$\gamma_0^* + \gamma_1^* \log_{10} X \quad (\text{if } X \geq X_D)$$

where X is the elytra length, Y is the average mandible length, γ_i and γ_i^* are the regression coefficients below (γ_i) and above (γ_i^*) the switch point X_D . In these two models, the switch point was estimated as the value that gave the best fit as indicated by the log likelihood (Crawley, 2005). In order to choose the better model, the convex curve model and two switch point models were evaluated using AIC (Crawley, 2005) in which the number of parameters used in these models was taken into account. The convex curve model has four parameters, that are three coefficients and one variance parameter. The discontinuous switch point model has seven parameters, which are four coefficients, two variance parameters and one parameter of switch point, and the continuous switch point model has six parameters, which are three coefficients, two variance parameters and one parameter of switch point. We selected one of these models that had the smallest AIC. If either of the switch point models is selected, we can conclude that males show a dimorphism.

We examined the cross direction of mandibles (i.e. whether the left or right mandible comes to rest on the upper side when the mandibles are crossed) and the number of teeth. After dissection, the mandible length (from tip to the lateral base) of adult males, females, and third instar larvae was measured to the nearest 0.01 mm using digital calipers. Elytra widths of adults and head capsule widths of larvae were measured as an index of body size, and the length of left and right mandibles were averaged to represent individual trait values. Sixty-three males, 48 females and 12 larvae were used for the measurements.

Left minus right mandible length (L–R) was plotted against body size in order to represent the relationship between the form of asymmetry and body size (Rowe *et al.*, 1997). Mandible length was log-transformed before statistical analysis. The parameter estimations and AIC evaluations were conducted using the statistical package R (ver. 2.5.1).

Results

Larvae

In larvae, the left mandible (4.56 mm \pm 0.43 SD) was always longer than the right mandible (4.35 mm \pm 0.42 SD), indicating strong directional asymmetry (Fig. 1a, (L–R) = 0.203 mm \pm 0.11; paired t -test, $t_{11} = 6.1$, $P < 0.001$). The left and right mandibles also differed in inner tooth morphology (Fig. 1e). Larvae always crossed their mandibles with the left mandible to the

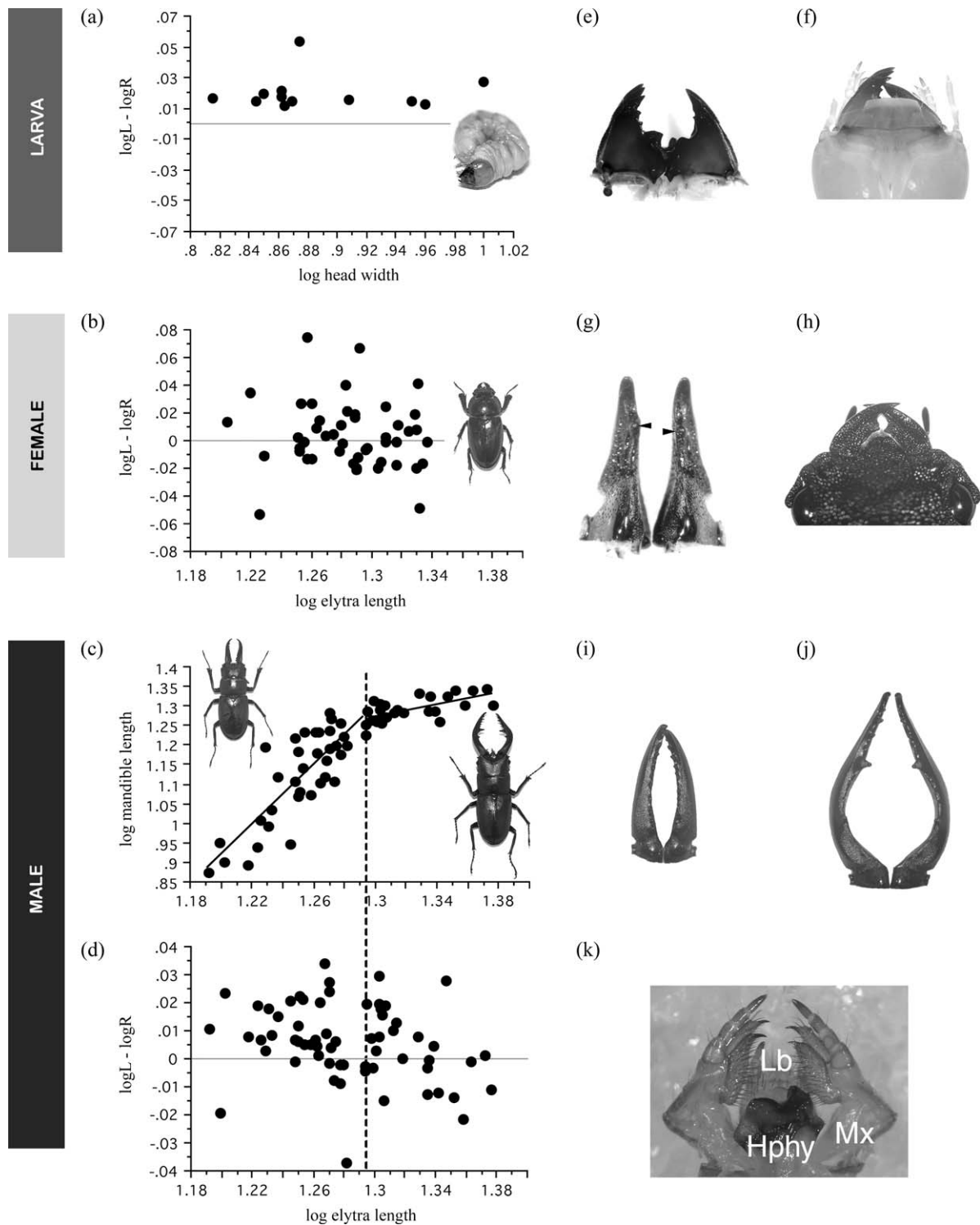


Fig. 1. Mandible length asymmetry in *Prosopocoilus inclinatus*. (a, b, d) The difference between left and right mandible length (left minus right) as a function of body size in males ($n = 63$), females ($n = 49$), and larvae ($n = 12$). (c) Allometric relationship between elytra length and mandible length in males. Broken line shows the dimorphic switch point between the smaller (left, $n = 37$) and the larger males (right, $n = 26$). (e) Dissected larval mandibles. (f) Cross direction of larval mandibles. (g) Dissected female mandibles viewed from the inner lateral side. Note that the tooth on the left side is located at a more forward position (arrowheads). (h) Cross direction of female mandibles. (i, j) Mandibles of smaller and larger males. (k) Distinctively asymmetric larval hypopharynx (Hphy) (dorsal view). Lb, labium; Mx, maxilla.

upper side (Fig. 1f). In addition, distinctive asymmetric sclerotisation was observed in the larval hypopharynx just below the mandible base (Fig. 1k).

Females

In females, the mandibles were $3.57 \text{ mm} \pm 0.35 \text{ SD}$ (left) and $3.54 \text{ mm} \pm 0.35 \text{ SD}$ (right) in length and exhibited an FA pattern [Fig. 1b, (L–R) = $0.026 \text{ mm} \pm 0.19$; paired *t*-test, $t_{47} = 0.90$, $P = 0.37$]. Left and right mandibles had single inner teeth, and the tooth on the left mandible was located at a more forward position (Fig. 1g). All females crossed their mandibles with the left mandible to the upper side (48/48, Fig. 1h).

Males

The AIC of the discontinuous switch point model [(switch point $X_D = 19.7$ (mm)] was the smallest (AIC = -214.2), i.e. the best among three models. The estimates of parameters were given as,

$$\log_{10} Y = -3.65 + 3.81 \log_{10} X \quad (\text{if } X < 19.7) \\ 0.44 + 0.64 \log_{10} X \quad (\text{if } X \geq 19.7)$$

The AIC difference between the discontinuous switch point model and the continuous switch point model [$\log_{10} Y = -3.53 + 3.71 \log_{10} X - 3.16 (\log_{10} X - \log_{10} 19.8) D$, $D = 0$ if $X < 19.8$ and $D = 1$ if $X \geq 19.8$, $X_D = 19.8$ (mm), AIC = -209.2] was five, whereas the difference between the discontinuous switch point model and the convex curve model [$\log_{10} Y = -30.23 + 46.56 \log_{10} X - 17.16 (\log_{10} X)^2$, AIC = -184.6] was 29.6, suggesting that neither models differ greatly; however, the discontinuous switch point model was slightly better than the other models. The calculated switch point corresponded to Inukai's (1924) criteria that discriminates the morphs by size-frequency distribution. Therefore, we concluded that males of *P. inclinatus* show a dimorphism and classified them into either larger males ($n = 26$) or smaller males ($n = 37$) based on the switch point evaluated by the discontinuous model.

In smaller males, the left mandibles were usually longer than the right mandibles [Fig. 1d, left: $13.82 \text{ mm} \pm 3.35 \text{ SD}$, right: $13.63 \text{ mm} \pm 3.41$, (L–R) = $0.191 \text{ mm} \pm 0.43$; paired *t*-test,

$t_{36} = 3.14$, $P = 0.003$] indicating DA in mandible length. In contrast, the asymmetry of the mandible length differed between individuals in larger males, and the mean difference was not significantly different from zero [Fig. 1d, left: $19.80 \text{ mm} \pm 1.21$, right: $19.62 \text{ mm} \pm 1.35$, (L–R) = $0.180 \text{ mm} \pm 0.62$, paired *t*-test, $t_{25} = 1.53$, $P = 0.14$], suggesting FA. The number of inner teeth on the male mandibles ranged from 5 to 17, the number being larger in the smaller males. The number of inner teeth differed between the left and right mandibles in 48 out of 63 males. However, the occurrence of this asymmetry was independent of morph (smaller males: 25/37, larger males: 17/26; Fisher's exact probability test, $P > 0.9$).

The cross direction of the mandibles was flexible in males, i.e. both left and right mandibles can come to rest on the upper side within the same individual.

Discussion

Directional asymmetry in larvae and females

In contrast to males, larvae and females had uniformly designed mandible forms. Larval mandibles exhibited distinctive DA in length, inner tooth patterns and cross direction (Table 1). The larvae of *P. inclinatus*, in common with other stag beetle larvae, dig tunnels in damp wood upon which they feed. The difference in the length may help digging behaviour because the shorter right mandible would not impede the longer left mandible to strike into the wood. The mandibles with the fixed cross direction may be used like a pair of scissors to cut and grind hard wood fibres. In larvae, an asymmetric hypopharynx structure would effectively support the grinding function.

Females also exhibited asymmetric design in the cross direction and tooth location on the mandibles. Stag beetle females dig holes in woods for oviposition; thus, females also use their mandibles like scissors to cut wood fibres. However, female mandibles exhibit FA in length (Table 1). It is probable that female mandibles are less specialized than those of larvae, the latter of which must be strongly specialized to make long tunnels while females just dig holes to lay eggs.

Morph-dependent asymmetry forms in males

The better fit of the switch point models indicated an intra-sexual dimorphism in males. The larger and smaller males of *P. inclinatus* differed in the form of asymmetry (Table 1); the smaller males exhibited left-biased DA, whereas the larger males exhibited random deviations from symmetry (FA). In male–male combat, *P. inclinatus* males hold their opponents by the mandibles and attempt to raise and throw them (Unno & Takashima, 2003). During this combat, males use their mandibles like sugar tongs in order to hold their opponents. Unlike females and larvae, males do not cross their mandibles like scissors. When using mandibles as a sugar tong, symmetric mandibles would be favoured to hold competitors tightly. The lack of a fixed asymmetry in the cross direction of male mandibles may be as a result of the loss of cutting function in them. In contrast

Table 1. Mandible asymmetry patterns in *Prosopocoilus inclinatus*.

| | Length | Cross direction | Teeth structure | Functions |
|--------------|--------|-----------------|-----------------|-----------------------------------|
| Larger male | FA | Flexible | FA | Male–male combat, Sugar tong |
| Smaller male | DA | Flexible | FA | Unknown |
| Female | FA | DA | DA | Cut and dig wood fibres, Scissors |
| Larva | DA | DA | DA | Cut and dig wood fibres, Scissors |

to *P. inclinatus*, large males have directionally asymmetric mandibles in *Agathidium* beetles (Miller & Wheeler, 2005). In these beetles, the left mandible has a horn, which is used in *thrusting* behaviour during a combat; therefore, DA is functional (Miller & Wheeler, 2005). The required asymmetry may depend on the manner of combats in ornamented beetle species.

The smaller males of *P. inclinatus* tend to avoid direct combats, a trait observed in several male-dimorphic beetle species (Eberhard, 1982; Emlen, 1997; Hongo, 2003). Therefore, in contrast to the larger males, the mandibles of the smaller males would be less selected as a weapon. Consequently, relaxed selection may have caused the loss of symmetry in smaller males. Some studies indicate that the size and FA of ornaments signal male conditions (Møller, 1990; Møller & Swaddle, 1997); however, no female preference for male mandible length was detected in *P. inclinatus* (Okada & Hasegawa, 2005). The morph-dependent form of asymmetry may be as a result of the functional difference between the morphs.

Directional asymmetry in smaller males

In eusocial thrips that exhibit wing polyphenism, wing-reduced soldiers exhibit greater wing FA than the fully winged disperser morph, indicating that canalisation of development is relaxed in the absence of selection on functionality (Crespi & Vanderkist, 1997). The mandibles of the smaller males may be a vestigial trait; however, they did not exhibit larger FA but displayed DA. This difference could be because of a developmental constraint during mandible development or some specific functions as discussed below.

In insect mouthparts as well as in other appendages, adult structures differentiate from the epidermis of equivalent larval structures after larval-pupal apolysis (Snodgrass, 1959; Heming, 2003). In stag beetles, adult mandible primordia develop in the cuticle of larval mandibles (Y. Okada, H. Goto & T. Miura, unpubl. data); therefore, the size of the mandible primordia could be affected by the larval mandible size where the left mandible is distinctively longer than the right one. While the mandibles of the larger males are greatly exaggerated for a functional purpose and completely modified from larval ones, the modification may be to a lesser degree in the smaller males. The less modified mandibles of smaller males may reflect an asymmetric larval form. The developmental and genetic correlation of larval and adult asymmetry should be tested in future studies.

There may be an adaptive function of directionally asymmetric mandibles of smaller males. In the closely related species *P. dissimilis*, male mandibles are used not only in fighting, but also in stripping the branch bark to exude sap (Hongo, 2005). If the asymmetric mandibles are effective in stripping the bark and smaller males are more adapted to the stripping behaviour than fighting, morph-specific DA can occur. Directionally asymmetric morphologies have been found in species that prey on snails (water-scavenger larvae; Inoda *et al.*, 2003, snakes; Hosono *et al.*, 2007) or with specific combat behaviour (antlers of a deer; Alvarez, 1995, mandibles of *Agathidium* beetles; Miller & Wheeler, 2005). We need to investigate further whether DA in the smaller male is adaptive or not.

Phenotypic plasticity and forms of asymmetry

The current view on FA is that small random perturbations cause the developmental pathway to deviate from its expected trajectory (Van Dongen, 2006). However, FA and developmental instability (DI) are not always correlated; therefore, the reason for this inconsistency is open to debate (Van Dongen, 1998, 2006; Whitlock, 1998). Under the phenotypic plasticity that creates diverse morphology, the expected developmental pathway is not single, and therefore FA may not be a good indicator of the perturbation of a specific developmental event. The unusual asymmetric pattern in *P. inclinatus* is a good example of such a case. The existence of phenotypic plasticity may contribute to understanding the observed inconsistency in the FA–DI relationship.

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