

Sexual selection in a moth: effect of symmetry on male mating success in the wild

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Sexual selection is generally caused by female choice and male–male competition. In female choice process, female preference is favored indirectly and/or directly by sexual selection. In indirect selection, females expressing the preference might gain indirect genetic benefits. In direct selection, females expressing the preference might gain direct benefits or avoid male-imposed costs. The white-tailed zygaenid moth *Elcysma westwoodii* is monandrous, and males often gather around a female to mate with her, suggesting a high opportunity for sexual selection on male traits. We quantified phenotypic selection on male morphology in this species in the field. The morphological characters analyzed included body weight, antenna length, forewing length, hind wing length, hind wing tail length, genital clasper length, and the fluctuating asymmetry (FA) of these bilateral traits. In *E. westwoodii*, selection favored males with more symmetric genital claspers, as well as longer and more symmetrical hind wings and antennae. Negative correlations between FA and size were also detected in the clasper and the antenna. Our results suggest that FAs of male traits, in particular the genital clasper, may have indirect and direct influences on mating success. During a copulatory attempt, an *E. westwoodii* male will try to grasp the female's abdominal tip with his claspers but often fail to do so because of the female's reluctance to mate. The female abdominal tips are smooth and strongly sclerotized and could thus be difficult for males to grasp. We hypothesize that more symmetrical male claspers are more efficient in overcoming female reluctance. *Key words*: fluctuating asymmetry, genitalia, Lepidoptera, phenotypic selection, sexual conflict, sexual selection. [*Behav Ecol*]

Sexual selection is generally caused by 2 processes: competition between members of one sex for members of the opposite sex (usually male–male competition) and preference by members of one sex for certain members of the opposite sex (usually female choice). These 2 processes often act together (Andersson 1994). Any trait that enhances mating and fertilization opportunities in either of these 2 processes will be favored by sexual selection. In female choice process, female preference is favored indirectly and/or directly by sexual selection. In indirect selection, females expressing the preference might gain indirect genetic benefits through Fisher's runaway process or "good gene" process. In direct selection, females expressing the preference might gain resources or other direct benefits or avoid male-imposed costs such as reduced longevity. Such female avoidance or minimization of direct cost imposed by males can evolve under sexual conflict or sexually antagonistic coevolution (Holland and Rice 1998; Chapman et al. 2003; Arnqvist and Rowe 2005). Sexual conflict occurs when the genetic interests of males and females differ with each other. Females often suffer significant direct costs of matings (Arnqvist and Rowe 2005). Therefore, female traits that facilitate resistance to unwanted matings evolve, and as a result, exaggerated male traits also evolve to overcome resistant females (i.e., chase-away sexual selection; Holland and Rice 1998).

Female resistance is also interpreted from the perspective of indirect selection instead of sexual conflict; it evolved and is maintained as a result of selection to screen among males of different genetic quality (Eberhard 1996; Cordero and Eberhard 2003), although such indirect genetic benefits are

expected to be a weak force because of the costs of resistance (Chapman et al. 2003). In the elephant seal *Mirounga angustirostris*, for example, females increase the probability of mating with a high-ranking male by simply rejecting all copulatory attempts during early estrus and thus inciting male competition (Cox and Le Boeuf 1977). Such female incitation of male aggression has also been reported in several insect species (Thornhill and Alcock 1983).

The white-tailed zygaenid moth *Elcysma westwoodii* has a monandrous mating system. The male has a pair of extended forceps-like claspers of the genitalia (see Figure 1c). Forceps-like claspers are also known in the closely related species, *Elcysma dohertyi* (Elwes 1890; Horie K, personal communications) as well as in several species of the related genera, *Achelura* and *Agalope* (Owada 1992; Owada et al. 1999). Such grasping devices are common among insects and other animals such as amphibians, and sexual conflict may have played an important role in the evolution of these devices (see Arnqvist and Rowe 2005). In *E. westwoodii*, courting males try to grasp the female abdominal tip with their claspers while fluttering their wings, but females are often reluctant to mate and refuse male attempts by bending their abdomens and walking away (Koshio and Hidaka 1995). Many males sometimes gather around a single female to copulate, suggesting a strong opportunity for sexual selection. It is likely that the female's resistance behavior is relevant to sexual selection favoring males with the ability to overcome reluctant females (Koshio and Hidaka 1995). Here we try to understand sexual selection on male morphological traits including fluctuating asymmetry (FA).

FA is defined as small, random deviations from perfect symmetry of bilateral traits (Van Valen 1962; Markow 1995). FA is thought to indicate an individual's ability to buffer against genetic or environmental stress during development and thus to be an index of the individual's developmental stability (Palmer and Strobeck 1986, 1992; Palmer 1994). Several

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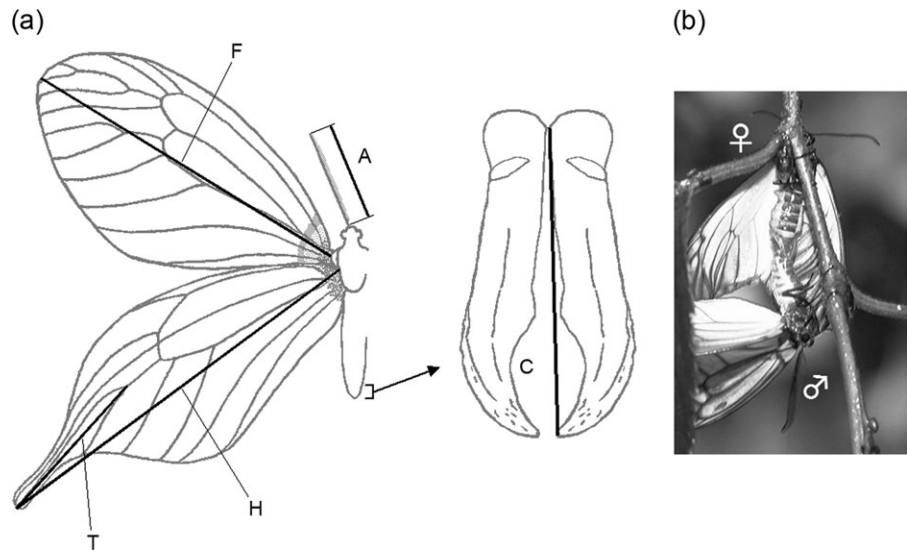


Figure 1
Measurements made for the sexual selection analysis (a): forewing (F), hind wing (H), hind wing tail (T), antennae (A), and clasper length (C). Ventral view of a copulating pair (b): a male grasps the female abdomen with his claspers.

reviews refer to the relationship between FA and fitness indicators especially in the context of sexual selection (see Swaddle 2003). The role of FA in the sexual selection process, however, is a controversial topic and the subject of considerable debate (Møller and Swaddle 1997; Palmer 1999; Swaddle 2003). In many cases, relationships between FA and fitness are argued on the basis of the association between FA and developmental instability related to genetic quality. By contrast, some of the known relationships between fitness and FA arise from the direct influences of asymmetry (see Swaddle 2003). In male dung flies, for example, symmetrical forelegs enhance mating success possibly due to mechanically constraining their ability to grip resistant females (Allen and Simmons 1996). In birds, asymmetry in wings and tails has aerodynamic costs (Balmford et al. 1993; Thomas 1993; Evans et al. 1994).

In the present study, we quantify phenotypic selection on males of the white-tailed zygænid moth *E. westwoodii* by targeting morphological traits, including FA, during mating episodes using selection gradient analysis (Lande and Arnold 1983; Brodie et al. 1995). In examining phenotypic selection in natural populations, this method is particularly effective for identifying target characters of selection and quantifying the mode and degree of selection when selection acts simultaneously on a set of characters.

MATERIALS AND METHODS

Insects

Elcysma westwoodii is usually monandrous (Koshio and Hidaka 1995) and protandrous (Koshio C, Muraji M, Kudo S, unpublished data). It is univoltine, and larvae depend on plants belonging to the family Rosaceae, for example, cherries, plums, and peaches. Adult emergence and breeding occur from late September to early October. Adults are diurnal; pheromone release by females settled on leaves, male searching flights, and mating all occur in the early morning (Koshio and Hidaka 1995). Copulation usually lasts for several hours after which the females takeoff to search for oviposition sites. Oviposition occurs in the afternoon (Koshio 1996).

Mating experiments

Our field experiments were carried out in Tokushima city (34°4'N, 134°34'E), Tokushima Prefecture, Shikoku, Japan. *Elcysma westwoodii* breeds every year in this area, which is host to many cherry trees.

Despite the high density of moths, finding naturally emerged virgin females calling males on cherry trees is very difficult. Thus, we conducted female-setting experiments in the field to observe sufficient mating events for analysis.

We collected pupae in September 1997 in and around the study area and kept them in plastic cages under natural conditions until emergence. After emergence, we kept males and females separately. Males were released into the field the following morning, whereas females were reared on water until the mating experiments were started.

The mating experiments were carried out between 0530 and 0900 h from 21 September until 8 October 1997; this covered almost the entire mating period. Our experiments were designed to analyze sexual selection only after males had landed on leaves with females and started a copulatory attempt. Thus, selection during the male searching flight and orientation to females were not the subjects of this study. We placed virgin females (21.4 ± 11.7 [standard deviation] females per day, $n = 364$ in total) on the underside of host leaves in the observation area. These females always stayed on the leaves, probably calling males. No male came to approach to 171 out of 364 females. The remaining 193 females were approached by, on average, 1.61 ± 1.22 males. First arriving males did not always mate females. Females approached by males were collected irrespective of their mating status at the end of mating period in that morning. We collected all males that approached and attempted to copulate with the females (for mating behavior, see Koshio and Hidaka 1995). The males were divided into 2 classes: males that achieved copulation and males that failed to copulate. Nonmating males were caught by net as they flew away from females. Once copulation was established, we kept the pair in a plastic cage until the completion of copulation. Both mating and nonmating males were killed by freezing and kept for trait measurements at -20°C .

Measurement of traits

For the sexual selection analysis, we measured 6 traits on mating and nonmating males: body weight and the lengths of antennae, of forewings and hind wings, of hind wing tails, and of claspers, respectively (Figure 1). Males were weighed just after collection using an electric balance with an accuracy of 0.01 mg (Sartorius AG, Goettingen, Germany). Wings, antennae, and claspers were detached from the body before measurement. Wings and antennae were carefully extended

and held between 2 transparent sheets to be unfolded and measured using calipers with an accuracy of 0.01 mm. Forewing length was measured from the small wart on the base to the distalmost end of the wing (F: Figure 1a). Hind wing length was also measured from the base of the frenulum, a projection that couples with the forewing, to the distal end of the tail (H: Figure 1a). Tail length was measured from the junction of the M_2 and M_3 veins to the distal end of the tail (T: Figure 1a). Clasper lengths were measured from the base of inner side to the tip (C: Figure 1a) using a stereoscopic microscope (10 \times) with micrometer.

We also investigated the degree of FA on 5 bilateral traits: antennae, forewings and hind wings, tail of hind wings, and claspers. To quantify measurement errors (see below), all individuals were measured 3 times. We did not reposition them between repeated measures to avoid damaging them. For each individual paired trait, we calculated signed asymmetry as the mean value of 3 measures of left minus right value ($L - R$).

FA analysis

FA is, by definition, small, and it can therefore have a magnitude similar to that of measurement errors. Thus, we evaluated measurement errors according to Palmer and Strobeck (1986) and Palmer (1994). A mixed-model 2-way analysis of variance (ANOVA) was performed for all individuals that were measured 3 times, with “side” as a fixed factor and “individuals” as a random factor. The side mean square was divided by the interaction mean square to obtain an F value indicating whether significant directional asymmetry was present. The interaction mean square divided by the error mean square yielded F and P values, indicating whether the asymmetry was significantly larger than the measurement error. Kurtosis was calculated to test for the presence of platykurtosis, an indication of antisymmetry.

The results of the ANOVA indicated that each asymmetry was measured with sufficient precision for the subsequent analysis (antenna FA: $F_{297,1192} = 3.87$; forewing FA: $F_{307,1232} = 4.48$; hind wing FA: $F_{286,1148} = 7.15$; tail FA: $F_{284,1140} = 373.74$; clasper FA: $F_{308,1236} = 40.02$; $P < 0.001$ for all traits). Directional asymmetry, the side effect in the ANOVA, was not significant for any trait. Kurtosis was positive for all traits, indicating no evidence of antisymmetry. Therefore, we used the asymmetry (a mean value of the 3 $R - L$ measures) of these 5 bilateral traits for subsequent selection analysis as true FA.

The Spearman's rank correlation coefficients among 11 traits, including 6 trait sizes and 5 unsigned FAs, were calculated for the entire male sample. The significance levels of all coefficients were adjusted using the sequential Bonferroni method (Rice 1989).

Trait allometry

The allometric slopes were estimated by the ordinary least square (OLS) regression and the major axis (MA) regression methods (Sokal and Rohlf 1995), regressing the \log_{10} -transformed value of the traits including forewing, hind wing, antenna, clasper, and hind wing tail on the \log_{10} -transformed value of the cube root of body weight as an indicator of body size. Those 99% confidential intervals were estimated by bootstrap resampling procedure (10 000 random resamplings with replacement).

Measurement of sexual selection

Sexual selection was estimated using the fitness-regression approach developed by Lande and Arnold (1983) (see also

Mitchell-Olds and Shaw 1987; Brodie et al. 1995). After males with damaged traits were excluded, 274 males out of 309 were used in the following analysis. Male mating success was used as a fitness component in the selection analysis. We assigned absolute fitness values of 1 to mating males and 0 to nonmating males. Individual measures of the fitness component were transformed to relative values scaled to mean 1, dividing each individual's value by the overall mean.

The values of all traits were log transformed and standardized to a mean 0, variance 1, and thus the selection differentials and gradients described below represent standardized values. Each unsigned FA, that is, an absolute value of $R - L$ ($|FA|$), was transformed using the Box-Cox method before standardization (Swaddle et al. 1994).

Linear directional selection differentials (s'), describing changes in trait means, and nonlinear selection differentials (c'), describing changes in trait variance, were calculated as the covariance of trait values with relative fitness components. Significance testing of selection differentials was made with Spearman's rank correlation tests.

Selection gradients estimate the direct effects of a particular trait on fitness by controlling for other traits. We estimated linear selection gradients (β') for all 11 traits. We also estimated quadratic selection gradients (γ') for all traits except their FAs because it is difficult to interpret the nonlinear effects of unsigned FA on fitness components. The dependent variable (i.e., fitness component) is binary, and thus, we used logistic regression analysis for significance tests for single partial regression coefficients (Mitchell-Olds and Shaw 1987; Janzen and Stern 1998).

To test the temporal change in selection regimes throughout the mating period, we included the interaction term “trait by date” in the logistic regression models. No significant interactions were detected for any trait in these tests ($P > 0.5$ in both linear and quadratic models, after sequential Bonferroni correction), implying no significant temporal effects on sexual selection. All data from the entire mating period were, therefore, pooled for the sexual selection analysis.

All statistical analyses were performed using SPSS 11.0 (SPSS Inc. 2001), statistical program R 2.2.1 (R Development Core Team 2005), and JMP 5.1 (SAS Institute 2004).

RESULTS

Phenotypic correlations and allometry

The mean values of the 6 trait sizes (body weight and antenna, forewing and hind wing, hind wing tail, and clasper lengths) as well as 5 unsigned FAs and 5 relative unsigned FAs of males approaching to females are summarized in Table 1. Variance of relative signed FAs among traits was significantly different (Bartlett's test: $P < 0.0001$).

The allometric slopes of forewing and clasper length on the cube root of body weight were lower than 1.0 and their confidence intervals did not include 1.0 in either regression method (coefficient and 99% confidence interval of forewing—OLS: 0.71, 0.65–0.76, MA: 0.78, 0.72–0.85; clasper—OLS: 0.41, 0.34–0.47, MA: 0.49, 0.42–0.57), although those of other traits were inconsistent (antenna—OLS: 0.64, 0.55–0.74, MA: 0.86, 0.73–1.02; hind wing—OLS: 0.90, 0.83–0.96, MA: 1.02, 0.94–1.11; hind wing tail—OLS: 1.10, 1.00–1.20, MA: 1.41, 1.26–1.57).

The 6 trait sizes were highly positively correlated to each other (Table 2). Positive correlations were also found between hind wing $|FA|$ and antenna $|FA|$, antenna $|FA|$ and clasper $|FA|$, and forewing $|FA|$ and clasper $|FA|$. Negative correlations between a trait size and its $|FA|$ were found in the antenna and the clasper.

Table 1
Summary of morphological traits of all males coming to females

Traits	N	Mean \pm standard deviation
Size		
Body weight (mg)	309	86.92 \pm 22.02
Antenna (mm)	298	13.52 \pm 1.04
Forewing (mm)	309	28.43 \pm 1.98
Hind wing (mm)	287	31.59 \pm 2.82
Tail (mm)	285	13.65 \pm 1.57
Clasper (mm)	309	4.32 \pm 0.23
[FA] (mm)		
Antenna	298	0.22 \pm 0.31
Forewing	309	0.23 \pm 0.36
Hind wing	283	0.36 \pm 0.42
Tail	285	0.34 \pm 0.29
Clasper	309	0.02 \pm 0.05
Relative [FA] (%)		
Antenna	298	1.68 \pm 2.67
Forewing	309	0.83 \pm 1.34
Hind wing	283	1.17 \pm 1.50
Tail	285	2.58 \pm 2.29
Clasper	309	0.55 \pm 1.19

For detailed explanations of traits, see Figure 1. [FA]: the unsigned mean of 3 measures of the left minus the right value of each bilateral trait. Relative [FA]: the unsigned FA divided by size of each trait.

Sexual selection regimes

Linear selection estimates, differentials and gradients, are shown in Table 3. The numbers of mating and nonmating males were 159 and 115, respectively, after males with damaged traits were excluded. The directional selection differential was positive for all trait sizes, whereas that for [FA], except tail [FA], was always negative.

The coefficient of determination of the multivariate linear model was highly significant (Table 3). Negative selection gradients were detected for 3 FAs: antenna [FA], hind wing [FA], and clasper [FA]. However, no significant directional selection gradients were found for any of the 6 trait sizes, despite significant estimates for these selection differentials. This lack of effect of size may be due to problems with multicollinearity, especially because phenotypic correlations across traits were high (Table 2). We thus adopted a stepwise regression approach with backward elimination using likelihood ratio in order to

reduce the influence of the multicollinearity (Mitchell-Olds and Shaw 1987). After choosing variables by the stepwise method, significant directional selection gradients were detected in 4 traits: positive selection on hind wing size and negative selection on antenna [FA], hind wing [FA], and clasper [FA].

The nonlinear selection differential was negative but not significant for any trait size (c' ranged from -0.087 to -0.177 , $0.160 < P < 0.786$). The coefficient of determination of the multivariate model including quadratic and correlational terms was also not significant (linear regression model: $R^2 = 0.106$, $F_{27,246} = 1.085$, $P = 0.36$; logistic regression model: $\chi^2 = 31.23$, $LR = 31.22$, $P = 0.26$).

DISCUSSION

The selection gradient analysis revealed that selection for successful mating favored *E. westwoodii* males with more symmetrical claspers and more symmetrical antennae (Table 3). The magnitude of FA in male traits has often been shown to be important in precopulatory sexual selection in some insects (e.g., Thornhill 1992a, 1992b; McLachlan and Cant 1995; Møller and Zamora-Muñoz 1997), although the relationship between selection and morphological asymmetry is often controversial in animal taxa (e.g., Markow 1995; Clarke 1997; Palmer 1999; Tomkins and Simmons 2003). As well as in *E. westwoodii*, males of a cerambycid beetle with symmetric antennae enjoy a mating advantage (Møller and Zamora-Muñoz 1997). However, the causal relationship between antennal symmetry and sexual selection remains unknown in the both species.

There have been many studies on the relationship between indirect benefits and FA (Swaddle 2003). Such indirect mechanism could operate in mate choice or intrasexual competition if the asymmetry is related to some unidentified property of an individual. In these cases, FA in secondary sexual traits should reliably signal male phenotypic condition (Møller 1990; Møller and Pomiankowski 1993; Møller 1997). For example, FAs are negatively correlated with male longevity in ladybird beetles (Ueno 1994), forest tent caterpillar moths (Naugler and Leech 1994), sphragis-bearing butterflies (Tsubaki and Matsumoto 1998), and water boatmen (Nosil and Reimchen 2001), whereas no such relationships have been detected in other species (Hunt and Simmons 1997; Leung and Forbes 1997; Windig 1998; Woods et al. 2002).

If males with more elaborate secondary sexual traits are of better phenotypic condition, and the degree of FA signals this phenotypic condition, then FA should be negatively related to

Table 2
Phenotypic correlations between traits (Spearman's rank correlation coefficient: r_s)

	Forewing	Hind wing	Antenna	Clasper	Tail	Forewing [FA]	Hind wing [FA]	Antenna [FA]	Clasper [FA]	Tail [FA]
Body Weight	0.862**	0.872**	0.721**	0.653**	0.817**	-0.025	-0.090	-0.116*	-0.143	-0.042
Forewing		0.963**	0.793**	0.742**	0.887**	-0.089	-0.160*	-0.128*	-0.135	-0.071
Hind wing			0.810**	0.745**	0.938**	-0.082	-0.171*	-0.136*	-0.118	-0.090
Antenna				0.653**	0.776**	-0.018	-0.185*	-0.186**	-0.168*	-0.011
Clasper					0.674**	-0.084	-0.101	-0.077	-0.190**	-0.073
Tail						0.104	-0.154	-0.094	-0.121	-0.097
Forewing [FA]							0.067	0.121*	0.219**	0.070
Hind wing [FA]								0.211**	0.175*	0.113
Antenna [FA]									0.353**	-0.075
Clasper [FA]										0.075

For an explanation of [FA], see Table 1.

* $P < 0.05$.

** $P < 0.05$ after the sequential Bonferroni correction.

Table 3
Standardized directional selection differentials (s') and gradients (β') using male mating success as a fitness component

	s'	P	$\beta' \pm SE$	P	$\beta' \pm SE^a$	P^a
Body weight	0.151	0.004	0.047 \pm 0.097	0.694		
Antenna	0.143	0.006	-0.118 \pm 0.078	0.121	-0.127 \pm 0.074	0.089
Forewing	0.163	0.002	-0.034 \pm 0.190	0.780		
Hind wing	0.177	0.001	0.137 \pm 0.243	0.525	0.210 \pm 0.073	0.008
Tail	0.170	0.003	0.042 \pm 0.130	0.717		
Clasper	0.160	0.004	0.018 \pm 0.070	0.704		
Antenna [FA]	-0.294	<0.001	-0.171 \pm 0.048	0.002	-0.171 \pm 0.046	0.002
Forewing [FA]	-0.159	0.002	-0.077 \pm 0.045	0.135		
Hind wing [FA]	-0.212	<0.001	-0.116 \pm 0.047	0.029	-0.117 \pm 0.045	0.027
Tail [FA]	-0.070	0.242	-0.028 \pm 0.045	0.832		
Clasper [FA]	-0.394	<0.001	-0.319 \pm 0.047	<0.001	-0.335 \pm 0.045	<0.001
Linear regression model			$R^2 = 0.322$, $F_{11,262} = 11.318$, $P < 0.001$		$R^2 = 0.311$, $F_{5,268} = 24.25$, $P < 0.001^a$	
Logistic regression model ^b			$\chi^2 = 103.37$, LR = 269.38, $P < 0.001$		$\chi^2 = 100.39$, LR = 272.35, $P < 0.001^a$	

Significance tests of single partial regression coefficients are made using logistic regression analysis. For an explanation of [FA], see Table 1. SE, standard error.

^a After stepwise regression with backward elimination using likelihood ratio.

^b LR represents $-2 \log$ likelihood ratio.

trait size in secondary sexual traits, whereas it should be U shaped or flat in other traits (Møller and Pomiankowski 1993; Blanckenhorn et al. 1998; Møller and Cuervo 2003). In *E. westwoodii*, correlations between each trait size and the magnitude of its FA were always negative but were statistically significant only in secondary sexual traits, the antenna and the clasper (Table 2), the FAs of which were under directional selection (Table 3).

If the FA reflects developmental instability and thus individual phenotypic condition, an organism-wide indication of FAs and among-trait correlations in FAs within the same individual may be expected (Polak et al. 2003). Nevertheless, there is little evidence to support this prediction, and recently several explanations for the lack of among-trait correlations have been developed (Swaddle 2003). In *E. westwoodii*, correlations among FAs were always positive, except for that between tail and antenna, and some correlations were significant even after the sequential Bonferroni correction (Table 2).

Concerning the role of FA as an indicator in sexual selection, Blanckenhorn et al. (1998) addressed other assumptions and predictions; FA should be heritable; FA of male secondary sexual traits is greater than that of homologous female traits; FA of sexually selected traits is greater than that of less costly nonsexual traits. We have no available data on the former two in *E. westwoodii*. For the last issue, we could not detect any such trends in *E. westwoodii* (Table 1); although the relative FAs among male traits were significantly different, those of male secondary sexual traits, antennae and claspers, were not consistently greater than those of the forewings and hind wings. Blanckenhorn et al. (1998) also predicted that FA as an indicator of male genetic quality should be related to fitness components other than mating success, such as mate fecundity or offspring survivorship. In *E. westwoodii*, however, FA of male morphological traits did not affect either female fecundity or the hatching rates of their eggs (Koshio C, Muraji M, Kudo S, unpublished data).

In summary, the negative correlations between size and FA and the positive correlations between FAs suggest that the degrees of both genital clasper and antenna FA may be indicators of male phenotypic condition in *E. westwoodii*. However, some other hypotheses have been proposed to interpret the negative correlations between size and FA. One interpretation is that individuals developing more exaggerated traits are constrained to more symmetrical development (Evans 1993;

Evans and Hatchwell 1993). This hypothesis predicts that the cost of a given asymmetry will increase with increasing ornament size and that the rate of increase in cost as ornament size increases should be greater when asymmetrical than when symmetrical (Evans 1993). There are no available data on these points in *E. westwoodii*. Polak and Starmer (2005) proposed an alternative hypothesis, the environmental heterogeneity hypothesis, to explain the negative correlations between size and FA in natural populations. This hypothesis states that negative size-FA correlations arise because of environmental heterogeneity instead of heterogeneity of male genetic quality. Further evidence, therefore, is needed to explicitly test the indicator hypothesis.

Recent studies have shown that asymmetry can directly influence mating success (see Swaddle 2003). In the shore crab *Carcinus maenas*, males with more symmetrical fifth pereopods are advantaged in male-male antagonistic interactions, probably due to their greater physical stability during pushing contests (Sneddon and Swaddle 1999). The mating advantage of periodical cicada males with symmetrical forelegs is also explained by the functional hypothesis; more symmetrical forelegs might advance mobility or maneuverability during male-male competition (Cooley 2004). In the yellow dung fly, *Scatophaga stercoraria*, males with more symmetrical wings and hind tibia enjoy greater mating success, probably due to the greater competitive ability of those males in the male-male competition (Liggett et al. 1993). In another dung fly *Sepsis cynipsea*, it has been suggested that males with symmetrical fore tibia have a functional advantage in coping with precopulatory struggling by females (Allen and Simmons 1996; Blanckenhorn et al. 1998). The effect of asymmetry on mating success, however, varies among populations in dung flies (Blanckenhorn et al. 2003, 2004).

In *E. westwoodii*, the male clasper has an essential function during mating behavior: grasping the female abdominal tip (Koshio and Hidaka 1995). It is, therefore, more likely that symmetrical claspers have a functional advantage. During a copulatory attempt, males try to grasp a female's abdominal tip with their claspers but often fail to do so because of female reluctance to mate (Koshio and Hidaka 1995). Furthermore, female morphology may be associated with a male's failure; the surfaces of the seventh and eighth segments of the female abdomen are smooth and strongly sclerotized, and thus, it seems to be difficult for males to grasp them during copulatory

attempts. A male's ability to grasp a female's abdomen with his claspers is essential for successful mating, and it is most likely that males with more symmetrical claspers are better able to control females. Smooth and sclerotized female abdominal tips are also observed in the related species with forceps-like male genital claspers (Elwes 1890; Owada 1992; Owada et al. 1999).

In a few animal taxa, directional asymmetry is also detected in grasping apparatus, which are usually used to manipulate asymmetrical objects (e.g., Smith and Palmer 1994). In water-scavenger *Hydrophilus acuminatus*, for example, larvae have asymmetric mandibles, which are effective in holding and manipulating right-handed snails (Inoda et al. 2003). However, this is not the case in genital claspers of *E. westwoodii*; female abdominal tips are not only smooth and sclerotized but also symmetrical (Koshio C, Muraji M, Kudo S, unpublished data).

Female reluctance and their smooth and sclerotized abdomens are likely to have evolved as a response to male copulatory attempts using their forceps-like claspers. For such female resistance, 2 nonmutually exclusive explanations have been proposed, a mating-rate reduction process and a male screening process (Chapman et al. 2003; Cordero and Eberhard 2003; Arnqvist and Rowe 2005), both of which may work simultaneously or sequentially (Cordero and Eberhard 2005). The former especially is widely regarded as sexual conflict, broadly defined as differences in the evolutionary interests between males and females (Parker 1979; Chapman et al. 2003), or the chase-away process (Holland and Rice 1998). In water striders, in which multiple matings occur but are actually costly for females, males have a grasping apparatus to control reluctant females, and the length of this apparatus is important for their mating success (Arnqvist 1989). Females show morphological counteradaptations to these male devices (Arnqvist 1997; Arnqvist and Rowe 2002). Many theoretical models, however, have been developed to understand the evolution of sexual conflict over mating rate, assuming potentially polyandrous females (Gavrilets et al. 2001; Gavrilets and Hayashi 2006; Lessells 2005; Rowe et al. 2005). The mating system of *E. westwoodii* is monogamous (Koshio and Hidaka 1995), and thus, it is unlikely that sexual conflict over mating rate is the driving force to evolve male genital claspers and female sclerotized abdomens.

On the other hand, under such a strict monandrous system, females should choose higher quality males. Therefore, female reluctance and smooth and sclerotized abdominal tips in *E. westwoodii* may have been under indirect selection for genetic benefits by screening males of different qualities (Eberhard 2002). If FAs of male traits are reliable indicators of genetic quality in this species, reluctant females could obtain dual indirect benefits from sons with not only good ability to control females but also good genetic qualities.

Different modes of selection have implications for trait allometry, particularly when dealing with sexually selected traits (see Hosken and Stockley 2004). Positive allometry, the tendency for large individuals to have relatively larger morphological traits, is observed more frequently in secondary sexual traits than in other traits because of directional sexual selection (Petrie 1992; Simmons and Tomkins 1996; see also Hosken and Stockley 2004). In male genitalia, in contrast, negative allometry probably due to stabilizing selection is common in arthropods (Eberhard et al. 1998; Tatsuta et al. 2001; Bernstein S and Bernstein R 2002; Ohno et al. 2003). In *E. westwoodii*, the allometric slopes of forewing and clasper length were below 1.0 using both OLS and MA regressions and that of hind wing tail was higher than 1.0 using MA regression, although neither directional nor quadratic selection was detected in these traits (Table 3). The allometric slope of genital clasper, furthermore, was sufficiently lower than those of other

traits, suggesting developmental constraints preventing the elongation of male genital claspers in this species.

Here we analyzed sexual selection on males in the mating event after starting a copulatory attempt. Our results indicated that males with symmetrical claspers were advantageous in this episode. However, selection in other episodes, such as the location and detection of females by males, remains unknown. Deinert et al. (1994) showed the opposing selective force in the 2 distinct selective mating sequence events in *Heliconius hewitsoni*. To clarify the whole picture of sexual selection on male morphology in *E. westwoodii*, further study on the selective forces in different mating episodes will be required.

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