Genital and body allometry in two species of noctuid moths (Lepidoptera: Noctuidae)

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One-size-fits-all and related hypotheses predict that static allometry slopes for male genitalia will be consistently lower than 1.0 and lower than the slopes for most other body parts (somatic traits). We examined the allometry of genitalic and somatic morphological traits in males and females of two species of noctuid moths, *Spodoptera exigua* (Hübner, [1808]) and *Helicoverpa armigera* (Hübner, [1808]). The relationship between genitalic traits and body size was generally strongly negative-allometric in males but with no significant differences from 1.00 in females of the two species examined. However, in females, the slope of genital traits was also lower than the slopes for somatic traits. The relationship between somatic traits and the body size indicator was approximately isometric in most cases in males, except in four traits in *S. exigua*, in which the slopes showed slight negative allometry, and the hind tibia in *H. armigera*, in which the slope had positive allometry. However, in females, some somatic traits showed isometric and some other showed negative allometry in both species. The coefficients of variation (CV) for all structures in the males were low, not exceeding 10%. Genitalic traits showed significantly lower CV than somatic traits in males. In females, somatic traits showed lower CV than genitalic traits but with no significant difference in the *H. armigera*. Our observations of strongly negative allometry for genitalic traits in males are consistent with stabilizing selection on genital size and we suggest that male performance in interactions with females is the source of selection on male genital allometry. The difference in the degree of phenotypic variation between genitalic and somatic traits in the two studied species is attributed to the different developmental-genetic architectures of these traits. Female genitalia showed a similar trend to the males, although the difference between genital and somatic traits was not significant in females. This finding suggests that selection is acting differently on male and female genitalia. Positive allometry of hind tibia in *H. armigera* may be a result of secondary sexual function. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **116**, 183–196.

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INTRODUCTION

Huxley (1932) was first to employ the term 'allometry' to define the differential analysis of size relationships between different body parts. This is still a powerful quantitative approach, used to examine how selection impacts the relationship between morphology and performance. The observed allometric slope (a slope in log-log regression on an indicator of body size) is presumably a consequence of selection

that favours one slope over others (Eberhard *et al.*, 1998). Static allometry is the relationship between a body part and body size among conspecific individuals at a particular developmental stage. If the slope exceeds 1.0, the trait is said to be positively allometric, such that larger individuals have relatively larger traits. Allometric slopes around 1.0 show isometry, where relative trait size remains constant across the range of body sizes. Slopes significantly lower than 1.0 indicate negative allometry, where larger individuals express relatively smaller traits. Some sexually-selected morphological characters

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used as weapons, or as visual advertisements, exhibit positive static allometries when scaled against other body parts (Vencl, 2004). Selection acts in three general ways: directional, disruptive or stabilizing. Stabilizing selection favours phenotypes with traits of intermediate size (Hosken & Stockley, 2004). Hypotheses based on selection pressures suggest that an allometric relationship will have a slope of one when selection on the morphological character is the same as on overall body size and a slope other than one when selection acts differently on the two traits (Gould, 1966; Bondursky & Day, 2003). Genitalic allometries might be important for female choice because large male body size may be associated with superior abilities to accumulate resources and to survive (Andersson, 1994). The idea that sexual selection influences genital evolution has been widely developed in the context of postcopulatory sexual selection. Eberhard *et al.* (1998; Eberhard, 2009) studied the allometry of the sizes of male and female genitalia and other body parts of many species of arthropods (including different orders of insects and several species of other arthropods(and found that allometric slopes for male genitalia were consistently lower than 1.0 and lower than the slopes for the other body parts. Based on the findings, the one-size-fits-all hypothesis was proposed, which posits that, within a species, sexual selection favours males with genitalia of average size, and suggests that approximate size-invariance of genitalia is achieved by shallow static allometry. Eberhard *et al.* (1998) suggested that females typically 'perceive a male's genitalia at close range by more or less precisely aligned touch', so that visual assessment is unlikely to play a major role. Eberhard (2009) suggested a more general explanation, whereby low allometric slopes in arthropods reflect selection for both mechanical fit and stimulation. Subsequent to Eberhard *et al.* (1998), several studies on beetles, dipterans, moths, and spiders have similarly detected negative allometry for male genital size (Palestrini, Rolando & Laiolo, 2000; Schmitz, Reinhold & Wagner, 2000; Uhl & Vollrath, 2000; Iwahashi, 2001; Iwahashi & Routhier, 2001; Tatsuta, Mizota & Akimoto, 2001; Bernstein & Bernstein, 2002; Eberhard, 2002; Ohno *et al.*, 2003; Mutanen & Kaitala, 2006; Mutanen, Kaitala & MÖnkkÖnen, 2006). Low allometric slopes for male genital structures therefore appear to be common among insects and spiders. However, some exceptions to this general pattern have also been observed (Johnson, 1995; Cayetano *et al.*, 2011; Nava-Bolaños *et al.*, 2014). This suggests that genital traits may evolve via different kinds of selective pressures in different species, and perhaps within species. Further research is thus required to gain a better understanding of the diverse functional roles of genitalic traits, and the selective pressures driving their evolution.

Green (1999) challenged Eberhard's idea, arguing that the appropriate technique for estimating such lines of 'organic correlation' is Model II regression (RMA) rather than ordinary least square regression (OLS), and emphasizing that Model II regression yields substantially steeper allometries for genitalic traits. In response, Eberhard, Huber & Rodriguez (1999) suggested that the weak correlations between genitalia and somalic structures may have biological significance in that they may indicate the unreliability of genitalia size in providing information to the female about overall body size of the male. Nonetheless, genitalic allometries might be important for female evaluation of male abilities to accumulate resources and to survive (Andersson, 1994). According to the 'good viability genes' model, females use male genitalia to assess male' heritable quality. Strong negative allometry between body size and genital size is proposed by some other hypotheses, such as the lock-and-key hypothesis and the cryptic female choice hypothesis (Eberhard *et al.*, 1998; Eberhard, 2009), and may be produced by other mechanisms of sexual selection as well (Bondursky & Day, 2003). According to the cryptic female choice hypothesis, sperm of males with superior stimulating ability during copulation is preferred by females. Although Eberhard *et al.* (1998) and Eberhard (2009) found that female genitalia may show the same patterns of variation as male genitalia, previous studies that investigate the allometry relationships in insects have focused almost exclusively on males and set females aside (Palestrini *et al.*, 2000; Schmitz *et al.*, 2000; Tatsuta *et al.*, 2001; Bernstein & Bernstein, 2002; Eberhard, 2002; Ohno *et al.*, 2003; Mutanen & Kaitala, 2006; Mutanen *et al.*, 2006; Al-Wathiqui & Rodriguez, 2011; Nava-Bolaños *et al.*, 2014). Comparison of static allometries of male and female traits could be used to determine whether male genitalia are really distinctive from other types of traits in their shallow static allometries: if selection favours shallow allometry in male genitalia because of sexual selection, then female genitalic traits would not be expected to exhibit similarly shallow allometries. Rather, female genitalia should be similar to somatic traits in their allometries. Conversely, if both male and female genitalic traits have similarly shallow allometries, then this would suggest that genitalia have shallow static allometry slopes for reasons unrelated to sexual selection.

According to Eberhard (1985, 1996), the male genitalic traits of many animals function as copulatory courtship devices that mechanically stimulate females in a specific way during copulation, in contrast to the male ornaments that stimulate females

visually. Eberhard *et al.* (1998) proposed that sexual selection can cause low allometry for male genital sizes, as follows. Because females assess male genitalia at close range by touch, rather than visually, selection may favour males with average-sized genitalia because such genitalia may be most effective at stimulating average-sized females (i.e. the most common class of females in the population). If male body size varies considerably but selection favours a relatively invariant, average genital size across the range of male body size, a negative static allometry in male genitalic traits is expected to evolve.

To determine whether genitalic traits exhibit strong negative allometry, consistent with stabilizing selection, in noctuid moths, we examined the allometry of genitalic and somatic morphological traits in males and females of two pest species, the beet armyworm, *Spodoptera exigua* (Hübner, 1808) (Noctuidae: Xyleninae) and the cotton bollworm, *Helicoverpa armigera* (Hübner, 1808) (Noctuidae: Heliothinae). According to Fibiger & Lafontaine (2005), subfamilies Heliothinae and Xyleninae are considered as two sister-groups and are included in the 'pestclade' of Mitchell, Mitter & Regier (2006). The studied species belong to different subfamilies and investigation of both species allowed us to determine whether similar patterns occur in these distinct noctuid lineages. They differ in the male genitalic structure: one species has a valvae elaborated by morphological structures (*S. exigua*) and the other has a rather simple valval structure (*H. armigera*).

For similar reasons, we also predicted that genitalic traits would exhibit lower variability [quantified as the coefficient of variation (CV) and standard error of estimate (SEE)] than somatic traits.

MATERIAL AND METHODS
INSECTS

The study species, *S. exigua* and *H. armigera*, are common agricultural pests in Iran (Fibiger & Hacker, 2007; Matov, Zahiri & Holloway, 2008). Sample sizes (34 males and 34 females for *S. exigua* and 31 males and 31 females for *H. armigera*) of adults were collected during 2010–2011, from agricultural fields around Mashhad city, north-eastern Iran. Night samplings were carried out by using light traps. Materials were deposited in the Insect and Mite Collection of Ahvaz, Plant Protection Department, Shahid Chamran University of Ahvaz, Iran.

PREPARATION PROCEDURE

The body parts were first boiled in water and then washed with ethanol. After cleaning their scales and setae with a camel's hair brush, they were then prepared between microscope slides in Canada balsam fixative. The abdomen was first removed and preserved in 10% caustic potash for 24 h and then washed with water. Genitalia of both sexes were then removed from the softened surrounding tissues, dehydrated with ethanol, and mounted on Euparal between the microscope slides and cover slips. After preparation, the genitalia were photographed through a microscope using a C-5050ZOOM digital camera (Olympus).

MORPHOLOGICAL TRAITS AND MEASUREMENTS

We examined both male and female adults; 19 (10 somatic and nine genitalic) traits in males and 12 (10 somatic and two genitalic) traits in females of *S. exigua*, as well as 10 (five somatic and five genitalic) traits in both sexes of *H. armigera* were measured in the present study. In all parts of the body, the left one was measured. In *S. exigua*, the ten measured somatic traits in both sexes were the lengths of the body parts: forewing, hindwing, fore femur, fore tibia, mid femur, mid tibia, hind femur, hind tibia, and the first and second segments of the labial palp (Fig. 1). In *S. exigua*, the measured nine genitalic traits of males were the lengths of the genitalic parts: valva, sacculus, clasper, aedeagus, cornutus of vesica, uncus, ampulla, tegumen, and diameter of aedeagus (Fig. 1). In the female genitalia of this species, the lengths of the papilla analis and apophysis posterior were measured (Fig. 1). In *H. armigera*, the five measured somatic traits in both sexes were the lengths of the body parts: forewing, fore femur, fore tibia, hind femur, and hind tibia (Fig. 2). The five measured genitalic traits in *H. armigera* males, were the lengths of the body parts: valva, aedeagus, uncus, tegumen, and the diameter of aedeagus (Fig. 2). In the female genitalia, the measured lengths of the genitalic parts were: papilla analis, apophysis posterioris, apophysis anterioris, bursa copulatrix, and ostium bursa diameter (Fig. 2). All the measurements were performed using TPSDIG, version 2.16 (Rohlf, 2004).

MEASUREMENT ERROR

To evaluate measurement error, three measurements of each measured trait were repeated nonconsecutively. The percentage measurement error was calculated as (Yezerinac, Lougheed & Handford, 1992):

$$\%\mathrm{ME} = \frac{S^2_{\mathrm{within}}}{S^2_{\mathrm{within}} + S^2_{\mathrm{among}}} \times 100$$

The percentage measurement error values were mostly less than 1% of the total variance (mean 0.15,
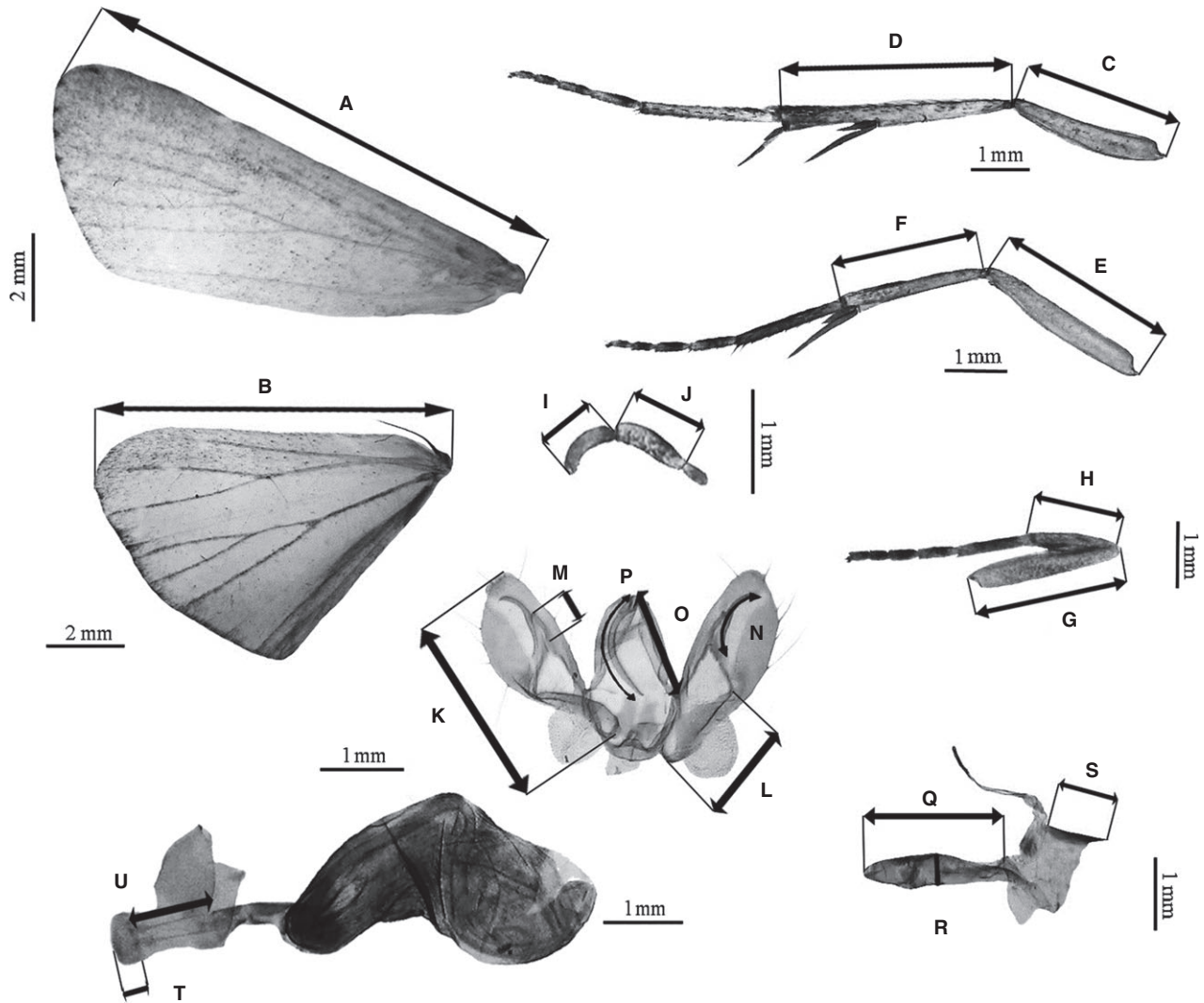
Figure 1. Measured body parts of *Spodoptera exigua* male and female (A–J: somatic, K–U: genitalic). A, forewing; B, hindwing; C, hind femur; D, hind tibia; E, mid femur; F, mid tibia; G, fore femur; H, fore tibia; I; J, first and second segments of labial palp; K, valve length; L, sacculus length; M, ampulla length; N, clasper length; O, tegumen length; P, uncus length; Q, length of aedeagus; R, diameter of aedeagus; S, length of spine of vesica; T, papilla analis; U, apophysis posterioris.

range 0.00017–1.83) and did not differ significantly between the two species (Mann–Whitney *U*-test, $P = 0.946$) and between genitalic and somatic trait groups in both sexes (Mann–Whitney *U*-test, $P = 0.383$). For these reasons, variables for further analyses were calculated by averaging the repeated measurements.

Estimation of allometric slope and other features of morphological variation

We used principal component analysis (PCA), conducted separately for each species/sex, to examine the covariation structure of the trait matrix (Figs 3 and 4). Several studies on genital allometry used a single somatic trait as the indicator of body size, although the use of a different body-size indicator may give rise to different results (Green, 1999). We selected forewing, which loads most strongly on PC1, for use as index of body size and to calculate all of the allometric slopes. Using PC1scores as body size indicator is problematic because all traits are included in the PCA, and the *x* and *y* variables in the regressions are thus not independent.

Correlations between body size and the morphological traits were then calculated one by one using
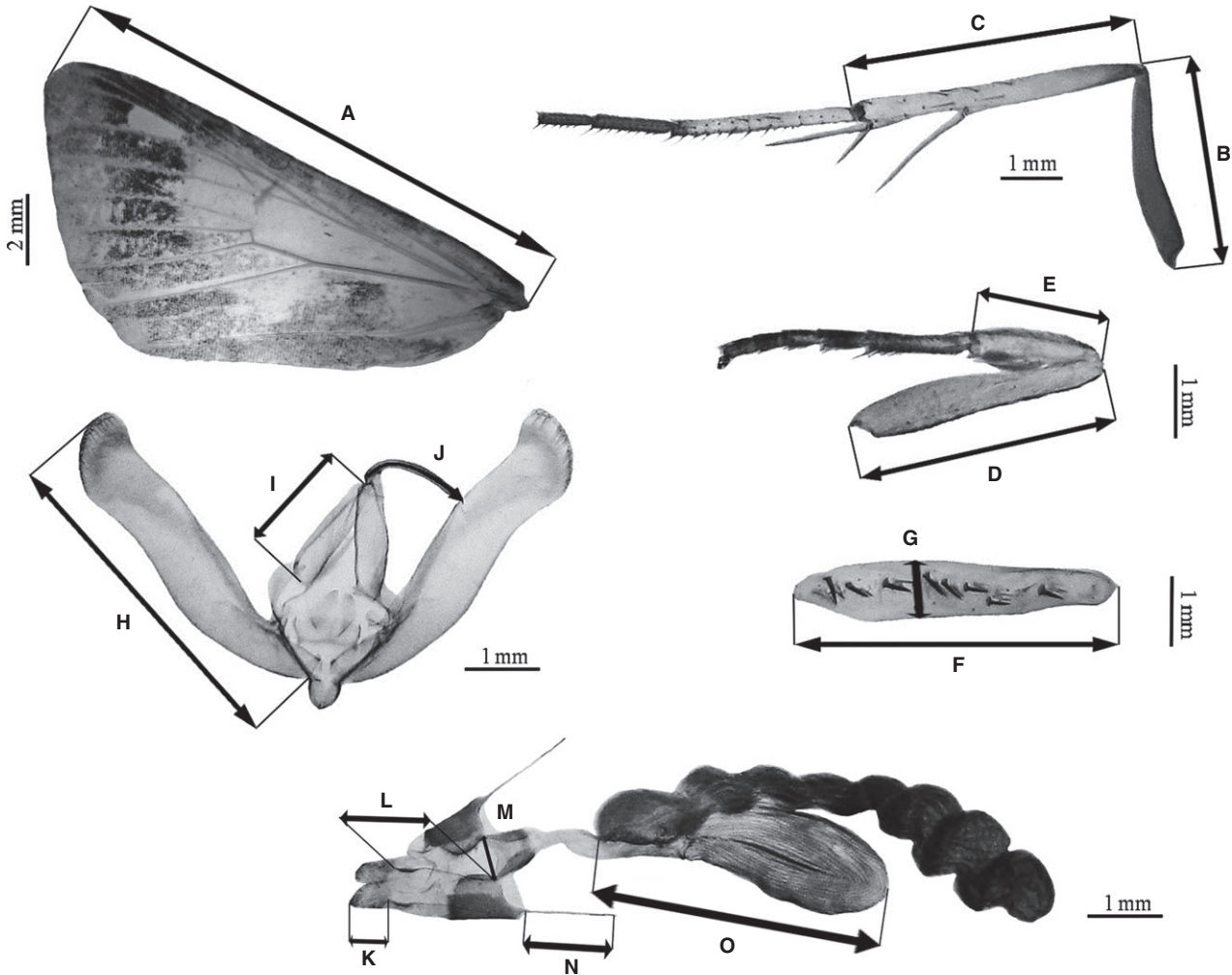
Figure 2. Measured body parts of *Helicoverpa armigera* male and female (A–E: somatic, F–O: genitalic). A, forewing; B, hind femur; C, hind tibia; D, fore femur; E, fore tibia; F, length of aedeagus; G, diameter of aedeagus; H, valve length; I, tegumen length; J, uncus length; K, papilla analis; L, apophysis posterioris; M, ostium bursa diameter; N, apophysis anterioris; O, bursa copulatrix.

Pearson's product–moment correlation coefficient. For each trait, the allometric slope was estimated by linear regression analysis of the $\log_{10}$-transformed value of the trait on the $\log_{10}$-transformed forewing length values.

We used type I (OLS) regression analysis (Eberhard *et al.*, 1998, 1999; Cuervo & Møller, 2001; Al-Wathiqui & Rodriguez, 2011). Because OLS regression assumes that the values in *x* are determined without error, it is generally expected to underestimate slopes unless the error in *x* is much smaller than in *y* (Sokal & Rohlf, 1981). The absolute values of OLS slope estimates should therefore be interpreted with caution, although Al-Wathiqui & Rodriguez (2011) concluded that OLS regression is relatively robust to measurement error in *x*. However, in the present study, we

are mainly interested in comparing slopes between genitalic and somatic traits and between male and female traits, rather than in estimating the absolute values of these slopes. Because all slopes are calculated in a similar way (using OLS regression with forewing as the body size index), the tendency of OLS regression to yield shallower slope estimates than RMA regression is therefore unlikely to lead to biased results in our analysis. In addition, the use of RMA regression suffers from problems of interpretability (Cuervo & Møller, 2001; Ohno *et al.*, 2003; Al-Wathiqui & Rodriguez, 2011). In particular, RMA slopes should not be used when the correlation between trait size and body size is nonsignificant (Warton *et al.*, 2006), as is often the case for genitalic traits. Therefore, we use slopes from OLS regression
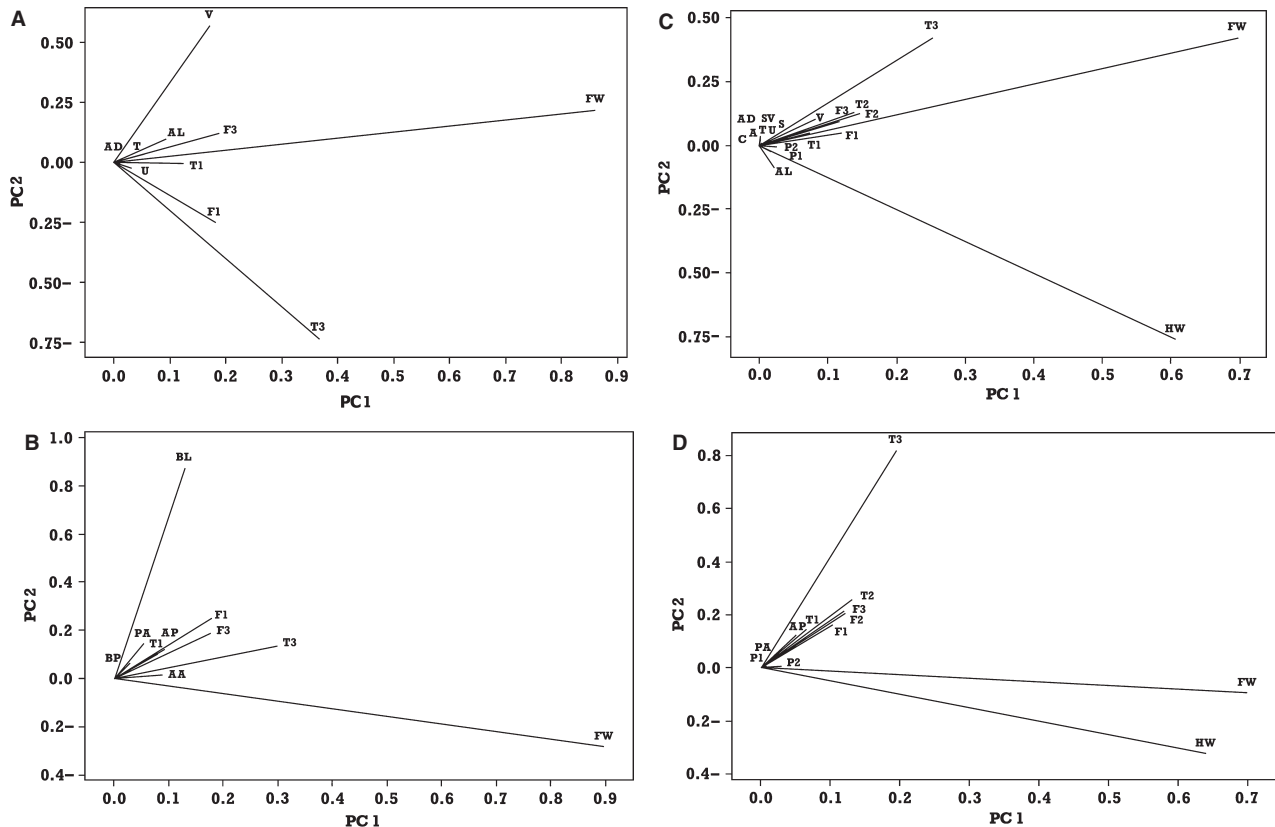
Figure 3. Principal component (PC) analysis plots of trait loadings on PC1 vs. PC2 showing the covariation structure in the trait matrix of the males and females. A, *Helicoverpa armigera* male. B, *H. armigera* female. C, *Spodoptera exigua* male. D, *S. exigua* female. FW, forewing; HW, hindwing; F1, fore femur; F2, mid femur; F3, hind femur; T1, fore tibia; T2, mid tibia; T3, hind tibia; P1; P2, first and second segments of labial palp; AL, aedeagus length; AD, aedeagus diameter; V, valve; U, uncus; SV, spine of vesica; T, tegumen; S, sacculus; A, ampulla; C, clasper.

throughout (Table 1) (Cuervo & Møller, 2001). However, for comparison, we provide a table including the allometric slopes of morphometric traits in the two examined species using three regression methods OLS, RMA and MA (see Supporting information, Table S1). The null hypothesis of $\beta = 1$ was tested by *t*-tests, and by examining 95% confidence intervals (Tables 1, 2).

Another, potentially independent source of information about trait variation is the CV, which is calculated as the SD/mean. The CV denotes the relative amount of variation in the size of a body part, independent of the magnitude of the mean. A high variance, as shown by a high CV value, is considered to be an important indicator of sexual selection compared to measures of overall body size (Vencl, 2004).

The difference in CV values among traits is affected by the degree of dispersion of data points around the allometric line, in addition to the allometric slope (Eberhard *et al.*, 1998). To estimate the degree of dispersion of points around the line, we calculated the CV'. Because CV' is invalid when *r* is not

significant (Eberhard *et al.*, 1998; Cuervo & Møller, 2001), we also calculated SEE, which is another measure of the degree of dispersion of points (Cuervo & Møller, 2001). SEE is unaffected by *r*, although it has the disadvantage of not being dimensionless (Eberhard *et al.*, 1998). The allometric slope, CV, CV', and SEE were respectively compared between trait categories (genitalic vs. somatic) in two sexes by Mann–Whitney *U*-tests within each species.

RESULTS
PCA ANALYSIS

In *H. armigera* males, the first component of the PCA explained 92.3% (eigenvalue 0.63) and the second explained 2.6% (eigenvalue 0.018) of the total variance. In *H. armigera* females, the first component of the PCA explained 93.4% (eigenvalue 1.01) and the second explained 3.2% (eigenvalue 0.034) of the total variance. For *H. armigera*, plots of trait loadings on PC1 vs. PC2 that reveal the covariation
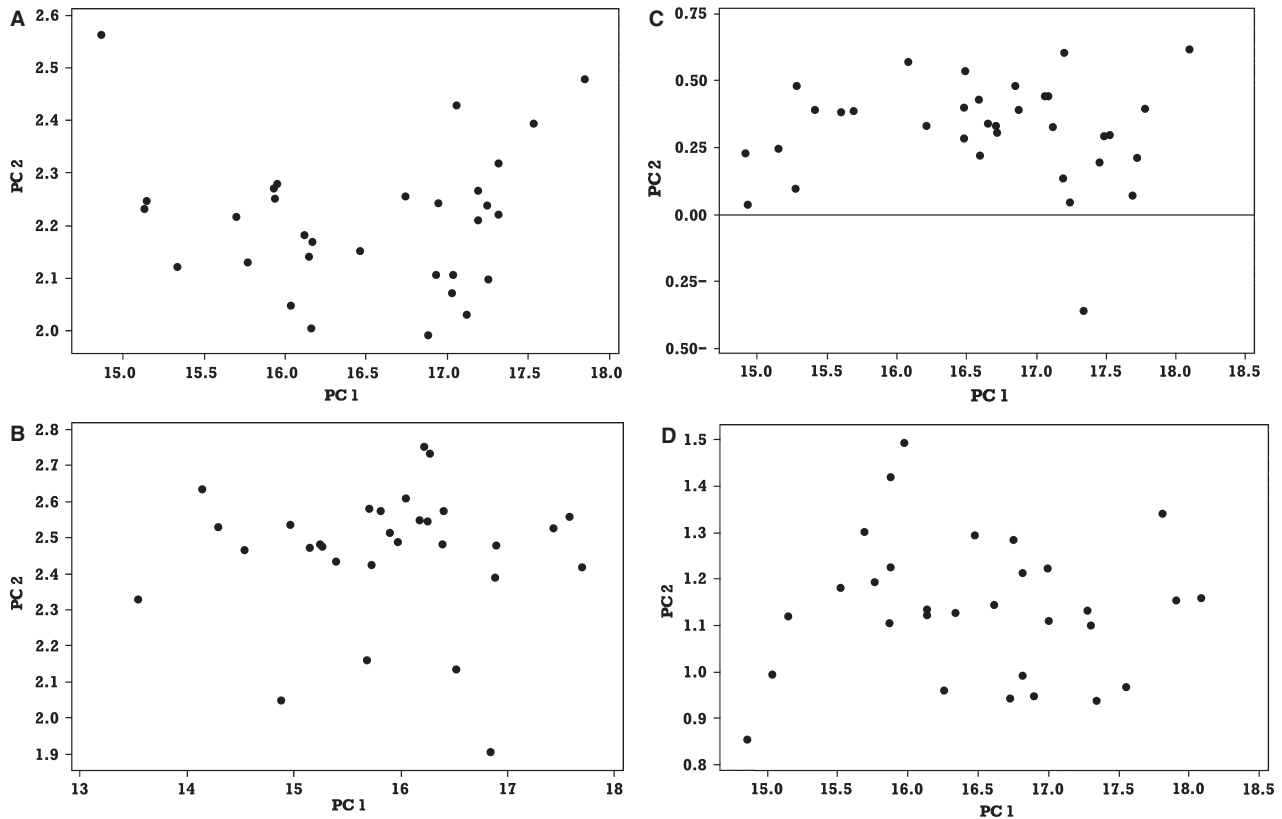
Figure 4. Matrix scatterplots of two examined species that illustrate the allometric relationships between all measurements. A, *Helicoverpa armigera* male. B, *H. armigera* female. C, *Spodoptera exigua* male. D, *S. exigua* female. PC, principal component.

structure in the trait matrix are shown in Figure 3A, B. In *S. exigua* males, the first component of the PCA explained 86.0% (eigenvalue 0.78) and the second explained 0.041% (eigenvalue 0.037) of the total variance. In *S. exigua* females, the first component of the PCA explained 93.3% (eigenvalue 0.72) and the second explained 2.09% (eigenvalue 0.022) of the total variance. For *S. exigua*, plots of trait loadings on PC1 vs. PC2 that reveal the covariation structure in the trait matrix are shown in Figure 3C, D. For examined species/sexes, matrix scatterplots that illustrate the allometric relationships between all measurements are given (Fig. 4).

CORRELATION BETWEEN BODY AND EXAMINED TRAITS SIZES

All somatic traits in each species/sex were significantly correlated with body size ($r = 0.249–0.970$, all $P < 0.01$) (Tables 1, 2, 3, 4). In *H. armigera*, all genitalic traits in males and females were also significantly correlated with body size ($r = 0.521–0.761$, all $P < 0.01$). However, in *S. exigua*, only two out of 11 genitalic traits of males and females had significant correlations ($r = 0.54–0.74$, $P < 0.01$). When a correlation

was observed, it was statistically highly significant ($P < 0.01$ in all cases).

TEST OF ISOMETRY

The relationship between male genitalic traits and the body size indicator was in general strongly negatively allometric (i.e. all of the slopes in both species were significantly less than 1.0) (Tables 1, 3). In females, three genitalic traits showed strongly negatively allometric relationship with body size and four traits showed isometry (Tables 2, 4). The relationship between somatic traits and the body size indicator did not differ significantly from one (isometry) in 15 cases ($N = 26$). In both species, most of the allometric slopes of somatic traits in both sexes showed allometric values equal to or slightly less than 1.0. However, in *S. exigua*, the allometric slope of labial palps showed a strong negatively allometric relationship with the body size indicator in three out of four cases (Tables 1, 2). However, in four traits in *S. exigua* males and four traits in *S. exigua* females, the slopes showed significant negative allometry, whereas two traits in *H. armigera* females showed significant negative allometry and the hind tibia in

Table 1. Allometric slopes and other features for 19 morphometric traits of *Spodoptera exigua* males

Traits	Mean†	CV‡	Slope§	95% intervals¶	r**	CV'††	SEE‡‡	H_o: $\beta = 1$§§
Somatic								
Forewing	11.59	5.43						
Hindwing	9.62	6.6	0.96[NS]	0.80–1.12	0.906*	2.49	0.0109	(=)¶¶
Femur1	2.39	5.73	0.77[NS]	0.58–1.00	0.825*	2.89	0.0128	(=)
Femur2	2.98	5.09	0.78*	0.61–0.97	0.828*	2.64	0.0127	(−)
Femur3	2.67	4.7	0.67*	0.48–0.89	0.790*	2.64	0.0127	(−)
Tibia1	1.38	6.54	0.83[NS]	0.52–1.13	0.697*	4.53	0.0207	(=)
Tibia2	2.36	6.41	0.90[NS]	0.63–1.17	0.771*	3.68	0.0182	(=)
Tibia3	3.62	7.55	1.99[NS]	0.77–1.43	0.764*	4.42	0.0226	(=)
Palp1	0.54	6.06	0.40*	0.02–0.78	0.357*	5.45	0.0256	(−)
Palp2	0.73	5.25	0.53*	0.29–0.76	0.630*	4.03	0.0158	(−)
Genitalic								
Aedeagus length	1.84	3.94	0.18*	−0.07–0.43	0.249[NS]	3.79	0.0171	(−)
Aedeagus diameter	0.35	4.97	0.09*	−0.23–0.41	0.098[NS]	4.94	0.0217	(−)
Spine of vesica	0.87	6.05	0.04*	−0.36–0.43	0.034[NS]	6.05	0.0268	(−)
Uncus	1	4.42	0.17*	−0.11–0.45	0.211[NS]	4.29	0.0190	(−)
Valve	2.33	4.09	0.56*	0.38–0.74	0.740*	2.65	0.0123	(−)
Tegumen	1.07	3.63	0.18*	−0.05–0.41	0.273[NS]	3.49	0.0155	(−)
Ampulla	0.33	3.53	0.13*	−0.10–0.35	0.195[NS]	3.46	0.0153	(−)
Sacculus	1.13	4.56	0.36*	0.15–0.56	0.539*	4.38	0.0136	(−)
Clasper	0.62	4.24	0.06*	−0.23–0.34	0.071[NS]	4.23	0.0189	(−)

*$P < 0.01$; NS, not significant.

†Mean in millimetres.

‡CV, coefficient of variance (calculated as SD / mean × 100).

§The slope, b, of the ordinary least square regression line.

¶95% intervals, L_1 and L_2, are lower and upper 95% confidence intervals of the difference.

**Pearson product–moment correlation coefficient between a trait and body size.

††A measure of the degree of dispersion of data points around the regression line, expressed as CV $(1 - r^2)^{1/2}$.

‡‡SEE, standard error of estimate, which is the square root of the residual mean square from the regression line.

§§Null hypothesis is that 95% confidence intervals of slope embrace isometric slope of 1.

¶¶(−) = slope significantly < 1.

H. armigera males showed significant positive allometry (Tables 1, 2, 3, 4).

COMPARISON OF ALLOMETRY SLOPES IN DIFFERENT TRAIT TYPES

The mean slope for the nine genitalic traits was significantly lower than the mean slope for the nine somatic traits in *S. exigua* males (Mann–Whitney *U*-test, $P < 0.01$). Similarly, the mean slope for the five genitalic traits was significantly lower than the mean slope for the four somatic traits in *H. armigera* males (Mann–Whitney *U*-test, $P < 0.01$). In the females, although the mean slope of genital traits was lower than the mean slope for somatic traits, the difference was not significant (Mann–Whitney *U*-test for *S. exigua*, $P = 0.085$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.172$). The slopes for somatic traits did not differ significantly between males and females in either species (Mann–Whitney *U*-test for *S. exigua*,

$P = 0.545$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.173$). The slopes for sexual traits of the males were significantly lower than the slopes for sexual traits of the females in each species (Mann–Whitney *U*-test for S. exigua, $P < 0.05$; Mann–Whitney *U*-test for H. armigera, $P < 0.05$). Note that different regression models give different slope estimates in several cases (see Supporting information, Table S1).

CV MEASUREMENTS

Dispersion around the mean, as measured by the CV, is one possible indicator of sexual selection that was calculated for all of the traits measured in the two species (Tables 1, 2, 3 4). The CVs for all structures were low, not exceeding 10%. In *S. exigua* males, genitalic traits showed significantly lower CV than somatic traits (Mann–Whitney *U*-test, $P < 0.01$). Similarly, in *H. armigera* males, genitalic traits showed significantly lower CV than somatic traits (Mann–Whitney

Table 2. Allometric slopes and other features for 12 morphometric traits of *Spodoptera exigua* females

Traits	Mean†	CV‡	Slope§	95% intervals¶	r**	CV'††	SEE‡‡	H_o: β = 1§§
Somatic								
Forewing	11.86	5.04						
Hindwing	9.59	5.73	1.09^{NS}	0.99–1.2	0.970*	1.39	0.0061	(=)¶¶
Femur1	2.26	4.5	0.74*	0.55–0.93	0.831*	2.50	0.0110	(−)
Femur2	2.94	3.88	0.67*	0.53–0.81	0.876*	1.87	0.0083	(−)
Femur3	2.63	4.28	0.73*	0.58–0.89	0.872*	2.10	0.0092	(−)
Tibia1	1.31	5.58	0.81^{NS}	0.51–1.10	0.727*	3.83	0.0170	(=)
Tibia2	2.31	5.49	0.95^{NS}	0.74–1.15	0.869*	2.72	0.0120	(=)
Tibia3	3.59	5.83	0.90^{NS}	0.62–1.19	0.773*	3.70	0.0166	(=)
Palp1	0.52	4.11	0.20*	−0.10–0.51	0.249*	3.98	0.0176	(−)
Palp2	0.71	6.14	0.64^{NS}	0.23–1.05	0.514*	5.27	0.0239	(=)
Genitalic								
Papilla analis	0.35	7.13	0.34*	−0.19–0.87	0.239^{NS}	6.92	0.0308	(−)
Apophysis posterior	1.16	8.16	0.65^{NS}	0.09–1.2	0.410^{NS}	7.44	0.0322	(=)

*$P < 0.01$; NS, not significant.
†Mean in millimetres.
‡CV is the coefficient of variance, calculated as SD / mean × 100.
§The slope, b, of the ordinary least square regression line.
¶95% intervals, L_1 and L_2, are lower and upper 95% confidence intervals of the difference.
**Pearson product–moment correlation coefficient between a trait and body size.
††A measure of the degree of dispersion of data points around the regression line, expressed as CV $(1 − r^2)^{1/2}$.
‡‡SEE, standard error of estimate, which is the square root of the residual mean square from the regression line.
§§Null hypothesis is that 95% confidence intervals of slope embrace isometric slope of 1.
¶¶(−) = slope significantly < 1.

Table 3. Allometric slopes and other features for 10 morphometric traits of *Helicoverpa armigera* males

Traits	Mean†	CV‡	Slope§	95% intervals¶	r**	CV'††	SEE‡‡	H_o: β = 1§§
Somatic								
Forewing	13.98	4.89						
Femur1	3.32	5.03	0.86^{NS}	0.64–1.08	0.829*	2.60	0.0125	(=)¶¶
Femur3	3.27	5.03	0.91^{NS}	0.72–1.1	0.874*	2.19	0.0110	(=)
Tibia1	1.75	6.2	1.13^{NS}	0.91–1.36	0.886*	2.61	0.0129	(=)
Tibia3	4.63	6.72	1.29*	1.07–1.50	0.913*	2.33	0.0125	(+)
Genitalic								
Aedeagus length	4.63	2.34	0.32*	0.19–0.46	0.674*	1.70	0.0077	(−)
Aedeagus diameter	0.84	1.7	0.20*	0.1–0.31	0.588*	1.38	0.0061	(−)
Uncus	1.35	3.3	0.35*	0.13–0.56	0.521*	2.75	0.0124	(−)
Valve	4.53	3.79	0.59*	0.40–0.78	0.762*	2.37	0.0109	(−)
Tegumen	1.63	4.19	0.51*	0.25–0.77	0.591*	3.35	0.0151	(−)

*$P < 0.01$; NS, not significant.
†Mean in millimetres.
‡CV is the coefficient of variance, calculated as SD / mean × 100.
§The slope, b, of the ordinary least square regression line.
¶95% intervals, are lower and upper 95% confidence intervals of the difference.
**Pearson product–moment correlation coefficient between a trait and body size.
††A measure of the degree of dispersion of data points around the regression line, expressed as CV $(1 − r^2)^{1/2}$.
‡‡SEE, standard error of estimate, which is the square root of the residual mean square from the regression line.
§§Null hypothesis is that 95% confidence intervals of slope embrace isometric slope of 1.
¶¶(+) = slope significantly > 1, (−) = slope significantly < 1.

Table 4. Allometric slopes and other features for 10 morphometric traits of *Helicoverpa armigera* females

Traits	Mean†	CV‡	Slope§	95% intervals¶	r**	CV'††	SEE‡‡	H_o: β = 1§§
Somatic								
Forewing	13.75	6.58						
Femur1	3.16	6.19	0.84[NS]	0.68–1.00	0.896*	2.75	0.0122	(=)¶¶
Femur3	3.22	5.8	0.81*	0.69–0.93	0.932*	2.10	0.0093	(−)
Tibia1	1.68	6.04	0.70*	0.49–0.92	0.784*	3.75	0.0164	(−)
Tibia3	4.52	6.9	0.99[NS]	0.85–1.12	0.944*	2.27	0.0101	(=)
Genitalic								
Papilla analis	1.3	5.71	0.61*	0.37–0.84	0.699*	4.08	0.0182	(−)
Apophysis posterioris	1.39	9.16	0.98[NS]	0.61–1.35	0.713*	6.42	0.0284	(=)
Apophysis anterioris	1.33	9.08	0.98[NS]	0.62–1.34	0.724*	6.26	0.0276	(=)
Ostium bursa diameter	0.64	7.49	0.68[NS]	0.33–1.32	0.601*	5.99	0.0267	(=)
Bursa copulatrix length	4.43	4.78	0.42*	0.19–0.65	0.573*	3.92	0.0176	(−)

*$P < 0.01$; NS, not significant.
†Mean in millimetres.
‡CV is the coefficient of variance, calculated as SD / mean × 100.
§The slope, *b*, of the ordinary least square regression line.
¶95% intervals, are lower and upper 95% confidence intervals of the difference.
**Pearson product–moment correlation coefficient between a trait and body size.
††A measure of the degree of dispersion of data points around the regression line, expressed as CV $(1 - r^2)^{1/2}$.
‡‡SEE, standard error of estimate, which is the square root of the residual mean square from the regression line.
§§Null hypothesis is that 95% confidence intervals of slope embrace isometric slope of 1.
¶¶(+) = slope significantly > 1, (−) = slope significantly < 1.

U-test, $P < 0.01$) (Tables 1, 3). However, in the females of both species, somatic traits showed lower CV than genitalic traits. This difference was not significant in *H. armigera* (Mann–Whitney *U*-test for *S. exigua*: $P < 0.05$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.602$) (Tables 2, 4). CV of genitalic traits in the males were significantly lower than CV of genitalic traits in the females of both species (Mann–Whitney *U*-test for *S. exigua*, $P < 0.05$; Mann–Whitney *U*-test for *H. armigera*, $P < 0.05$). The differences in CV of somatic traits between two sexes was not significant in the both species (Mann–Whitney *U*-test for *S. exigua*, $P = 0.054$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.249$).

CV' AND SEE MEASUREMENTS

The measure of dispersion of points around the allometric line, CV', did not differ significantly between the trait categories (somatic vs. genitalic) in *S. exigua* males (Mann–Whitney *U*-test, $P = 0.22$) or in *H. armigera* males (Mann–Whitney *U*-test, $P = 0.60$) (Tables 1, 3). In the females of both species, CV' of somatic traits were significantly lower than CV' of genitalic traits (Mann–Whitney *U*-test for *S. exigua*, $P < 0.05$; Mann–Whitney *U*-test for *H. armigera*, $P < 0.01$) (Tables 2, 4). CV' of genitalic traits in the males were significantly lower than CV' of genitalic traits in the females (Mann–Whitney

U-test for *S. exigua*: $P < 0.05$; Mann–Whitney *U*-test for *H. armigera*: $P < 0.01$) and the difference in CV' of somatic traits between two sexes was not significant in each species (Mann–Whitney *U*-test for *S. exigua*, $P = 0.175$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.602$).

SEE, another measure of dispersion of points around the allometric line, did not differ significantly between the trait categories (somatic vs. genitalic) in either *S. exigua* males (Mann–Whitney *U*-test, $P = 0.252$) or *H. armigera* males (Mann–Whitney *U*-test, $P = 0.602$) (Tables 1, 3). In the females of both species, SEE of somatic traits were significantly lower than SEE of genitalic traits (Mann–Whitney *U*-test for *S. exigua*, $P < 0.05$; Mann–Whitney *U*-test for *H. armigera*, $P < 0.01$) (Tables 2, 4). SEE of genitalic traits in the males were significantly lower than SEE of genitalic traits in the females (Mann–Whitney *U*-test for *S. exigua*, $P < 0.05$, Mann–Whitney *U*-test for *H. armigera*, $P < 0.01$) and the difference in SEE of somatic traits between two sexes was not significant in each species (Mann–Whitney *U*-test for *S. exigua*, $P = 0.364$; Mann–Whitney *U*-test for *H. armigera*, $P = 0.754$).

DISCUSSION

Although most studies on genitalic allometry have examined only one or two traits, and have typically

limited their analysis to males, the present study compared static allometries and several measures of variation for a large number of genitalic and somatic traits within both sexes of two insect species. Our study therefore furnishes an unusually powerful and comprehensive comparison of genitalic and somatic traits in these species. According to our results, male genitalic traits of *S. exigua* and *H. armigera* showed negative allometry, whereas the allometric slopes of most somatic traits in the males were near-isometric (equal to or slightly less than 1.0 but greater than 1.0 in one case). We confirm that, in *S. exigua* and *H. armigera*, male genitalia exhibited a similar pattern to the results reported by Eberhard *et al.* (1998) and Eberhard (2009). This suggests that, in these species, males with intermediate sized genitalia, regardless of overall body size, apparently left more surviving offspring than males with relatively small or large genitalia. Some of our somatic traits showed negative allometry relationships with body size. Negative allometry is typical for most traits, although it is not clear, in most cases, why nonsexual traits vary in static allometry slope. Further research is required to determine the reasons for this finding.

Our conclusion that the shallow static allometry slopes of male genitalic traits reflect functional roles associated with sexual competition is supported by the finding that the static allometry slopes of female genitalic traits were more similar to the slopes for somatic traits than was the case in males. Female homologues of male genital traits can serve as a useful control and, in this case, the study of female genitalia allowed us to test the idea that male genital components are under selection for low static allometry slopes. Because female genitalia were more similar than male genitalia to somatic traits, we may conclude that male genitalia are under selection for shallow static allometry as a result of their role in mating and sexual competition, as assumed by the functional hypotheses discussed above.

As Eberhard *et al.* (1998) stated, with a given distribution of body sizes, both a higher allometric value and a greater dispersion of points around the allometric line will result in a larger CV. Eberhard *et al.* (1998) referred to the allometric slope as a 'design feature' of the organism, a manifestation of the developmental-genetic programme that evolves under selection favouring a particular scaling relationship. The degree of dispersion, on the other hand, may be related to various causes, including genetic differences among individuals, differences in factors such as hormone titres that affect the size of the structure, variation in environmental factors during particular stages of growth, and imprecision in developmental programmes.

In our results, the degree of dispersion of data points around the allometric lines did not significantly differ between genitalic and somatic traits in the males. However, the CV that combines the effects of the slope and the SEE exhibited a weaker but nevertheless significant trend towards smaller values in genitalic than in somatic characters in males of each of the two species. Because the genitalic traits showed less phenotypic variation than somatic traits, these findings show that, in *S. exigua* and *H. armigera*, the size of male genitalia is more stable than that of somatic parts against changes in the body size, and the difference in the degree of phenotypic variation between genitalic and somatic traits is attributed to the difference in allometric slopes. The same conclusion can be drawn from the ordination plots for males of the two species (Fig. 3): the genitalic traits exhibit much smaller loadings on PC1 (reflecting weaker correlations with body size) than the somatic traits. The present findings are congruent with those of previous studies that analyzed both the allometric slope and the dispersion of points around the allometric line for male genitalia and other body parts (Eberhard *et al.*, 1998; Palestrini *et al.*, 2000; Ohno *et al.*, 2003). Because the shape of male genitalia is conspicuously diversified among taxa in insects and spiders (Eberhard, 1985), it is particularly interesting that male genital size is stable within single populations of diverse taxa. This may suggest that a common evolutionary force has stabilized the male genital size within various species of insects and spiders. Interestingly, Pomiankowski & Møller (1995), in their survey, found that CVs above 20% were common for sexually-selected characters, whereas those of nonsexually-selected characters averaged almost 8%. It was argued that sexual selection favours alleles that reduce the degree of developmental control of quantitative secondary-sexual traits, with the result that they might be expected to have higher CVs than somatic traits. This suggests that genitalic traits differ markedly in their development (and, presumably, in the selective regime that shapes the developmental-genetic architecture) from secondary sexual structures, despite the role of genitalic traits in sexual interactions and empirical evidence of sexual selection on genitalia in some species (Bertin & Fairbairn, 2007).

Our findings suggest that such stabilizing sexual selection may operate on genital size in noctuid moths. However, apart from cryptic female choice, low allometric values may also result from sperm competition strategies that exert stabilizing selection on male genitalic size. Male genitalic size might be optimized for sperm removal, quick sperm transfer or sperm displacement. In all of these cases, males may have an advantage if their genitalia fit best to

the most typical female. Stabilizing selection on male genitalic size through selection on sperm competition capability is thus as plausible as stabilizing selection through cryptic female choice (Schmitz *et al.*, 2000).

Allometric slopes for genitalia of the females in several species examined by Eberhard *et al.* (1998) and Eberhard (2009) were lower than the median allometric slope for somatic traits for the same species. In accordance with their results, they stated that female genitalia may show the same patterns of variation as male genitalia (Eberhard *et al.*, 1998; Eberhard, 2009). Eberhard *et al.* (1998) reported that, just as in males, the sizes of female genitalia were slightly less variable than those of somatic characters. In our results, somatic traits of females showed lower CV than genitalic traits and this difference was significant in *S. exigua*. Moreover, CV of genitalic traits in the males was significantly lower than CV of genitalic traits in the females in each species. Also, Eberhard *et al.* (1998) showed that the SEE was larger for the allometric line of genitalia than the median for nongenitalia in 11 of 12 genitalic traits. In our results, CV' and SEE of somatic traits were significantly lower than CV' and SEE of genitalic traits in the females of both species. According to our results, the differences in the CV of genitalic and somatic traits of the females are related to the differences of CV' and SEE. As Eberhard *et al.* (1998) stated, the degree of dispersion may be related to various causes including genetic differences among individuals.

According to the results of the present study, female genitalia showed a similar trend to the males (especially in terms of relatively low static allometry slopes, relative to somatic traits), although the difference between genital and somatic traits was not significant in females. This finding suggests that selection is acting differently on male and female genitalia.

Eberhard *et al.* (1998) concluded that selection on females may favour intermediate, standard sizes of genitalic structures that are contacted by males. In the present study, the papilla analis showed strong negative allometry in both species (Tables 2, 4). This structure is the terminal part of the female's genitalia in the moths and is assumed to be contacted by males during copulation. Other genitalic structures in the females showed isometric relationships with body size, with the exception of bursa copulatrix length in the *H. armigera*, which showed negative allometry. This part of the female genitalia receives the vesica of the male aedeagus during copulation. In the Noctuidae, the aedeagus and vesica (penis) in the males, and the bursa copulatrix in the females, together form a lock-and-key mechanism (Mikkola, 2008). Our results showed the same allometric relationship with body size for the aedeagus in the males and the bursa copulatrix in the females of *H. armigera*, supporting the presence of the lock-and-key mechanism in this species as well. Such a mechanism is consistent with the presence of selection (via both mechanical fit and stimulation) for low allometric slopes in arthropods (Eberhard, 2009).

The hind tibia in *H. armigera* males showed positive allometry with body size. This result suggests selection for larger hind tibia in large *armigera* males and/or relatively smaller hind tibia in small *armigera* males. If secondary sexual trait sizes are under directional sexual selection, a large relative trait size can yield high mating success, although viability costs may limit secondary sexual trait expression in small individuals, resulting in positive allometry (Bonduriansky, 2007). It has also been suggested that positive allometry evolves to amplify differences in body size, which may be important in male–male interactions, or female assessment of male mate quality. The hind tibia in *H. armigera* females showed an isometric relationship with body size (Table 4). Therefore, positive allometry of hind tibia in *H. armigera* may be a result of secondary sexual function. However, positive allometry has also been observed in nonsexual somatic traits, and may be especially common in locomotory structures (Bonduriansky, 2007). The function of the hind tibia in this species remains to be determined.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Allometric slopes of morphometric traits in two species *Spodoptera exigua* and *Helicoverpa armigera*, using three regression methods.