

Relationship between size and shape in the sexually dimorphic beetle *Prosopocoilus inclinatus* (Coleoptera: Lucanidae)

HARUKI TATSUTA^{1*}, KOJI MIZOTA² and SHIN-ICHI AKIMOTO³

¹Centre for Biodiversity and Conservation, School of Biology, The University of Leeds, Leeds LS2 9JT, UK

²Environmental Education Centre, Miyagi University of Education, 980–0845 Sendai, Japan

³Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo, 060–8589 Japan

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Male characters that are used for male–male combat are often developed and exaggerated, whereas female equivalent characters are vestigial or vanished. In order to assess whether the characters common to both sexes share the same phenotypic variability due to common genetic architecture, we compared males and females of the stag beetle *Prosopocoilus inclinatus* using recently developed geometric morphometric methods. Elliptic Fourier analysis was used to compare shape variation between male characters (including exaggerated mandibles) and developmentally homologous female characters. A significant positive correlation was found between the size or between the weight of different body parts in both sexes, but a conspicuous difference was detected in the frequency distribution of the weight of all the body parts. Elliptic Fourier analysis demonstrated that there was marked discontinuous variation in mandibles in males, whereas such a discontinuity was not clear in females. The shape of a character in males exhibited some similarity with that of other characters, but this was not found in females. In a character, growth trajectory of shape was significantly affected by both size and weight in males, whereas size and shape tended to vary independently in female characters. These results support the hypothesis that a large sexual dimorphism in variation in shape is due to alleles accumulating in tight linkage with a sex-determining gene. © 2004 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2004, **81**, 219–233

ADDITIONAL KEYWORDS: elliptic Fourier analysis – exaggerated character – morphometrics – multivariate allometry – phenotypic variability – stag beetle.

INTRODUCTION

Exaggerated male characters such as horns or mandibles in insects are used for intrasexual combat (Tatsuta, Mizota & Akimoto, 2001). Male behavioural strategies are often associated with the size of exaggerated characters. Shiokawa & Iwahashi (2000) reported that in the stag beetle *Prosopocoilus dissimilis okinawanus*, the diurnal pattern of mating activity differs completely between males with large and small

mandibles. Such behavioural differences contribute to reducing competition between large and small males, resulting in an increase in the fitness of both types (Eberhard, 1982). Although developmentally homologous mandibles in females have received comparatively little attention, these could have a similar phenotypic variation if the development of the characters in males and females is more or less controlled by a common genetic architecture. If the development of homologous characters differs between the sexes, this might be due to sex-linked genes resulting from sex-limiting selective pressures.

If the selective optima of characters are different for males and females, conflicting selection on the alleles at particular loci tends to reduce the genetic correlation between the sexes, and finally promotes sexual

*Corresponding author. Current address: Laboratory of Ecological Risk Assessment, Research Project for Biodiversity and Conservation, National Institute for Environmental Studies, 16–1, Onogawa, Tsukuba, Ibaraki, 305–8506, Japan. E-mail: htatsuta@nies.go.jp

dimorphism (Rice, 1992). For example, sexual dimorphism in the relative distance of eyespan in a stalked fly, *Cyrtodiopsis dalmanni*, results from sex-limiting selection (Wolfenbarger & Wilkinson, 2001). In contrast, when the function of a male character is similar to that of a female homologous character, there should be no conflicting selection, leading to convergence in the variability of the characters. Hence, comparing phenotypic variation between homologous characters of the sexes would provide an insight into the context of selective pressures in natural environments.

We conducted several morphometric analyses to examine the amount of variation between different characters in males and females the stag beetle, *Prosopocoilus inclinatus* (Motschulsky) and compare fine shape variability in homologous characters between them. This species, distributed widely in Japan, exhibits its conspicuous sexual dimorphism in the size of body and mandibles (Inukai, 1924), and thus is suitable for assessing the sexual selection hypothesis. Elliptic Fourier analysis was performed for the analysis of outlines of body parts because simple measurements such as the distance between landmarks are not sufficient to permit detailed analysis of fine-scale morphological variation. Recent studies show that the shape of subtle and tiny structures as well as the size of particular characters plays an important role in the context of selection (Johnson & Black, 2000; Walker & Bell, 2000) and hence size and shape are expected to coevolve responding to selective agents. The elliptic Fourier method is feasible for describing the contour of particular characters and has been used for examining complex variation in shape (McLellan & Endler, 1998; Cannon & Manos, 2001), especially when few, if any, distinctive landmarks are available (Monti, Baylac & Lalanne-Cassou, 2001). Although there is a problem in the recognition of homology for a particular point in different organisms and in the biological interpretation of harmonics (Bookstein *et al.*, 1982), its mathematical characteristics and handling are superior to those of other similar techniques (Rohlf & Archie, 1984).

MATERIAL AND METHODS

STUDY SITE AND DATASET

In total, 49 males and 55 females of *P. inclinatus* from several localities in Hokkaido, northern Japan were used for the analysis. All specimens were collected in 1998. We eliminated damaged body parts from the subsequent analysis. Prior to weighing of body parts, each specimen was cut into four parts at membranous joints: left and right mandible, head, prothorax, fused segments of mesothorax-abdomen (SMA: after Tat-

suta *et al.*, 2001). Each cut part was dried at 65°C for 24 h using a drying oven and weighed on a 1.0×10^{-5} g scale using a fine electric balance (ISO9001, Sartorius, Japan). The dry weight of each character was regarded as an indication of resource allocation to the body part during development (Tatsuta *et al.*, 2001). The coefficient of variation (CV) was computed for the weight of each body part to compare variability among body parts with different means. Pearson's product-moment correlations were calculated between the weight of body parts. The significance level of correlation coefficients was adjusted by the sequential Bonferroni method (Rice, 1989). In addition, Kolmogorov-Smirnov tests (Sokal & Rohlf, 1995) were applied to each body part to examine whether the weight of each part follows the normal distribution. These calculations were conducted using SAS (SAS Institute Inc., 1988).

Images for each body part were captured using a digital camera (PDMC-2, Polaroid, USA) coupled with a macro lens (AF Micro-Nikkor 60 mm, Nikon, Japan). For all characters, taken images were magnified to be almost the same size on computer screen to reduce measurement errors due to original character size. The *x* and *y* coordinates of each outline were estimated by TPSDIG ver. 1.26 (Rohlf, 1998) software. All contours started at the first landmark (Fig. 1), defined as type I according to Bookstein (1991), and were digitized clockwise. A total of 310 points was scored for each of left and right mandibles, and 350 points for each of other body parts.

DESCRIPTION OF OUTLINES OF EACH BODY PART

There are several different techniques to describe outlines of closed contours in a two dimensional plane (see Rohlf & Archie, 1984; Rohlf, 1990). We used the elliptic Fourier method as originally described by Kuhl & Giardina (1982) for the description of shape of body parts. In this method, a curve is decomposed into a sum of harmonically related ellipses. Since Fourier decompositions are very sensitive to the location, size, and orientation of objects, the setting of each specimen is important when different specimens are to be compared. For comparison between different pictures, Fourier coefficients were normalized based on the method advocated by Kuhl & Giardina (1982). Before decomposition in Fourier series, we rotated the outline so that the long axis defined by the first harmonic was parallel to the horizontal axis (the phase angle of the first harmonic was set at zero), and set the initial point at the end of the long axis defined by the first harmonic. We took radius of ellipse of the first harmonic as an indicator of dimensions of characters (hereafter morphometric size) and then calculated Pearson's correlation coefficients between the charac-

ters. Size was standardized by dividing the coordinates by the square root of the area of the ellipse defined by the first harmonic. These treatments resulted in the degeneration of the first three Fourier coefficients. Thus for N harmonics, there are $4N-3$ non-trivial normalized coefficients (Rohlf & Archie, 1984; Ferson, Rolf & Koehn, 1985).

We estimated 30 harmonics and so acquired 117 meaningful Fourier coefficients for analysing the shape of each body part; preliminary studies showed that 30 harmonics were sufficient to express fine scale structures (e.g. Monti *et al.*, 2001). Elliptic Fourier coefficients were calculated using NTSYS-pc (Rohlf, 2000).

MULTIVARIATE ANALYSES FOR SHAPE VARIATION

Overall difference in shape was analysed by principal component analysis (PCA) based on the covariance matrix among estimated standardized Fourier coefficients. Each set of 117 coefficients was treated as a multivariate point representing the outline of a character. To investigate whether there is a discontinuity in the shape characteristic of each body part on a particular morphometric plane, density contours of plots using a multivariate kernel density estimator (Simonoﬀ, 1996; SPSS Inc., 1998) were overlaid on the plane. The Kolmogorov–Smirnov test for normality was performed to test whether each shape characteristic (PC) was normally distributed. To visually clarify features of shape variation explained by each PC, we reconstructed three types of imaginary shape; one had mean principal score along each principal axis (PC1, 3), one mean plus two standard deviation, and one mean minus two, respectively, and set other components as zero. Fourier coefficients of each reconstructed shape were estimated by inverse Fourier transformation (Rohlf & Archie, 1984). Reconstructed characters were overlaid on figures of frequency distribution of PCs for each body part.

Associations between the shapes of different body parts were evaluated by the similarity of Euclidean distance matrices among individuals, one for each body part characterized by the 117 Fourier coefficients. Overall similarity between the morphometric distance matrices for two different body parts was evaluated statistically using a Mantel test (Mantel, 1967). The significance was tested by comparing the observed Mantel statistic with the distribution of the statistic obtained from 10 000 randomizations of the elements of the distance matrix. The significance level of the Mantel statistic was adjusted by the sequential Bonferroni method (Rice, 1989). Likewise, to investigate association between shape components (PCs) of different body parts, Kendall's rank correlation between characters was calculated for each of the

first three PCs, respectively. Multivariate analyses described above were performed using SAS (SAS Institute Inc., 1988), SYSTAT (SPSS Inc., 1998) and NTSYS-pc (Rohlf, 2000).

MULTIVARIATE ALLOMETRY BETWEEN SIZE AND SHAPE COMPONENTS

To examine how the growth trajectory of the overall shape of each body part changes as the size and weight of the character increase, we performed multiple-regression analysis, in which the scores of morphometric size (radii of the first ellipse) and weight of each character were taken as dependent variables, and estimated shape components (PC1–4) as independent, according to the method advocated by Mosimann & James (1979). Overall significance of multiple-regression model was assessed by an F -test. Since the weight is directly related to the volume of the parts, all measurements of body mass were transformed to cubic roots to equalize the dimension with that of other variables.

RESULTS

VARIATION IN BODY MASS

Of the five body parts examined (Fig. 1), the weight distributions of all parts deviated significantly from normality in males, whereas none of them deviated significantly from normality in females (Fig. 1; Table 1). Male mandibles showed conspicuous right-hand skew. A large sexual difference in the variability of body mass was found especially in the weight of left and right mandibles and head: they exhibited conspicuous large variation in males, whereas the variation in females was kept almost equal to that in other body parts. A large coefficient of variation (CV) mainly due to large variation in bending (Figs 2, 3) was observed in the weight of mandibles and heads in males. The correlations between morphometric size (radii of the first ellipse), between weight (Table 1) and between size and weight (Table 2) were all significantly positive between different body parts, and strong correlations (>0.61 between morphometric size, >0.65 between weight, and >0.43 between the size and weight) were found both in males and in females, indicating that the dimensions of any body part increase with increasing weight.

VARIATION IN THE SHAPE OF CHARACTERS

The first three PC loadings based on 117 elliptic Fourier coefficients for each character in total explained 62–91% of the total variation of shape in males, and 58–86% in females. PC analysis detected

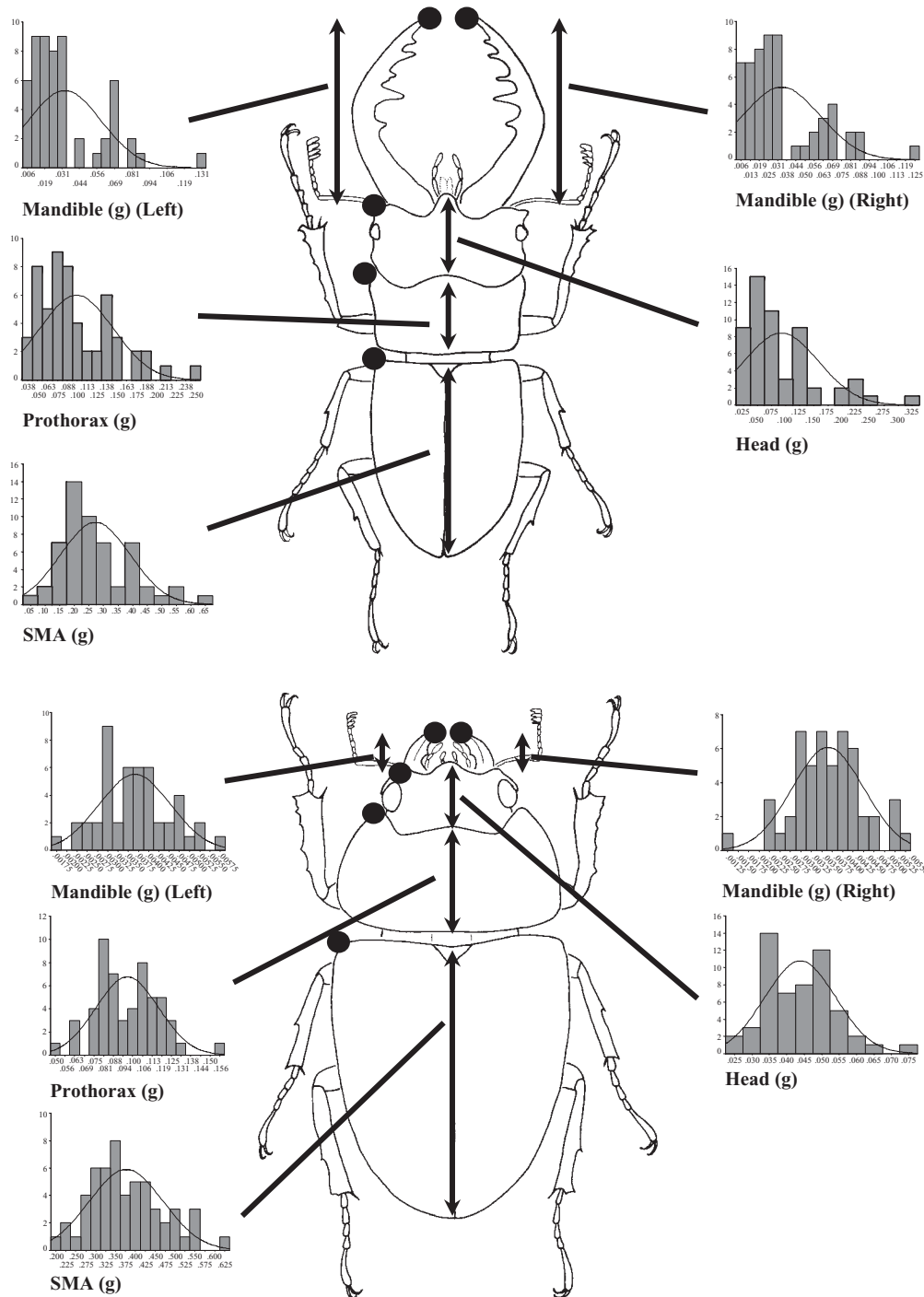


Figure 1. Measurements of five body parts in males and females of *Prosopocoilus inclinatus* and frequency distributions of each body part. Normal curve is fitted to each distribution. The starting point for taking coordinates is also shown as a black circle.

conspicuous and discontinuous polymorphism in the shape of male mandibles (PCs 1, 2, PCs 1, 3), whereas such a polymorphism was not clear in any body parts in females (Figs 2–6). Each PC distin-

guished a contrasting character state of rounded outlines vs. fine and angular structures. For male mandibles, two or three types, straight or crooked, were distinguished using kernel density contours.

Table 1. Means, standard errors and coefficients of variation for each character. All correlation coefficients were significant at 1% level. *Abbreviations:* ML = mandible left; MR = mandible right; Pro = prothorax; CV = coefficient of variation; SMA = fused segments of mesothorax-abdomen

	<i>N</i>	Mean, SE	D_{max}^a	CV	ML	MR	r^b Head	Pro	SMA
MALES									
ML	49	0.0329, 0.0038	0.2202**	81.911	1	0.996	0.970	0.647	0.911
MR	49	0.0339, 0.0039	0.2251**	80.407	0.932	1	0.970	0.642	0.909
Head	49	0.0954, 0.0099	0.1665**	72.742	0.659	0.703	1	0.650	0.939
Pro	49	0.1004, 0.0069	0.1462*	47.969	0.903	0.959	0.753	1	0.618
SMA	49	0.2789, 0.0177	0.1265*	44.428	0.856	0.904	0.740	0.965	1
FEMALES									
ML	55	0.0037, 0.0001	0.0690	22.493	1	0.812	0.863	0.820	0.874
MR	55	0.0037, 0.0001	0.0610	24.245	0.764	1	0.778	0.716	0.770
Head	55	0.0439, 0.0014	0.0993	23.140	0.830	0.865	1	0.928	0.932
Pro	55	0.0966, 0.0027	0.0868	20.982	0.952	0.845	0.904	1	0.934
SMA	55	0.3774, 0.0125	0.0932	24.592	0.900	0.666	0.720	0.881	1

^aStatistic of Kolmogorov–Smirnov test for normality.

^bPearson's product moment correlation between characters for morphometric size and body mass (upper and lower diagonal, respectively).

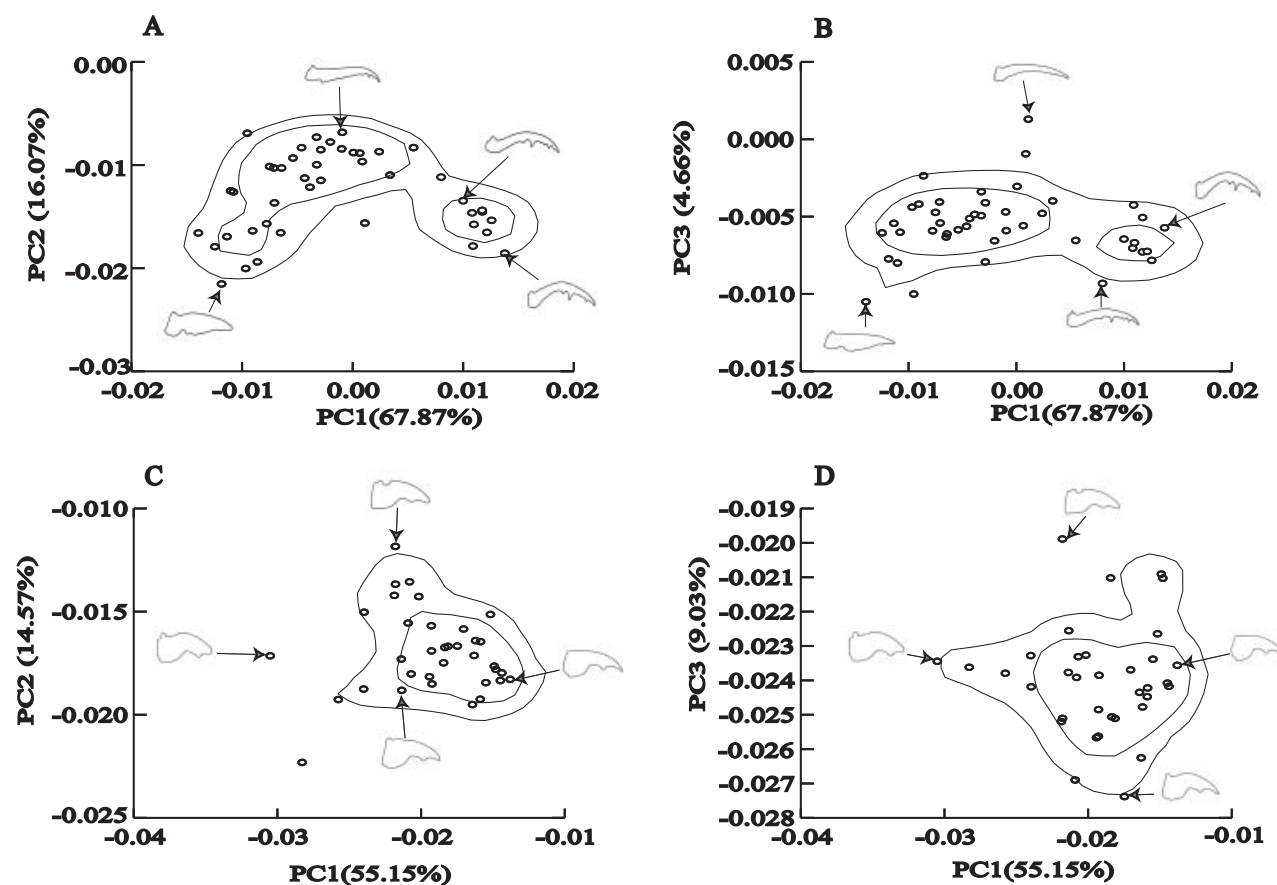


Figure 2. Principal component analysis (PCA) of Fourier coefficients in left mandible. PCs 1, 2: A, males, C, females. PCs 1, 3: B, males, D, females. Here and in Figs 3–6 the proportion of the contribution of each PC to the total variance in parentheses and some extreme characters are shown. Kernel density contours (see text) are overlaid on the plane.

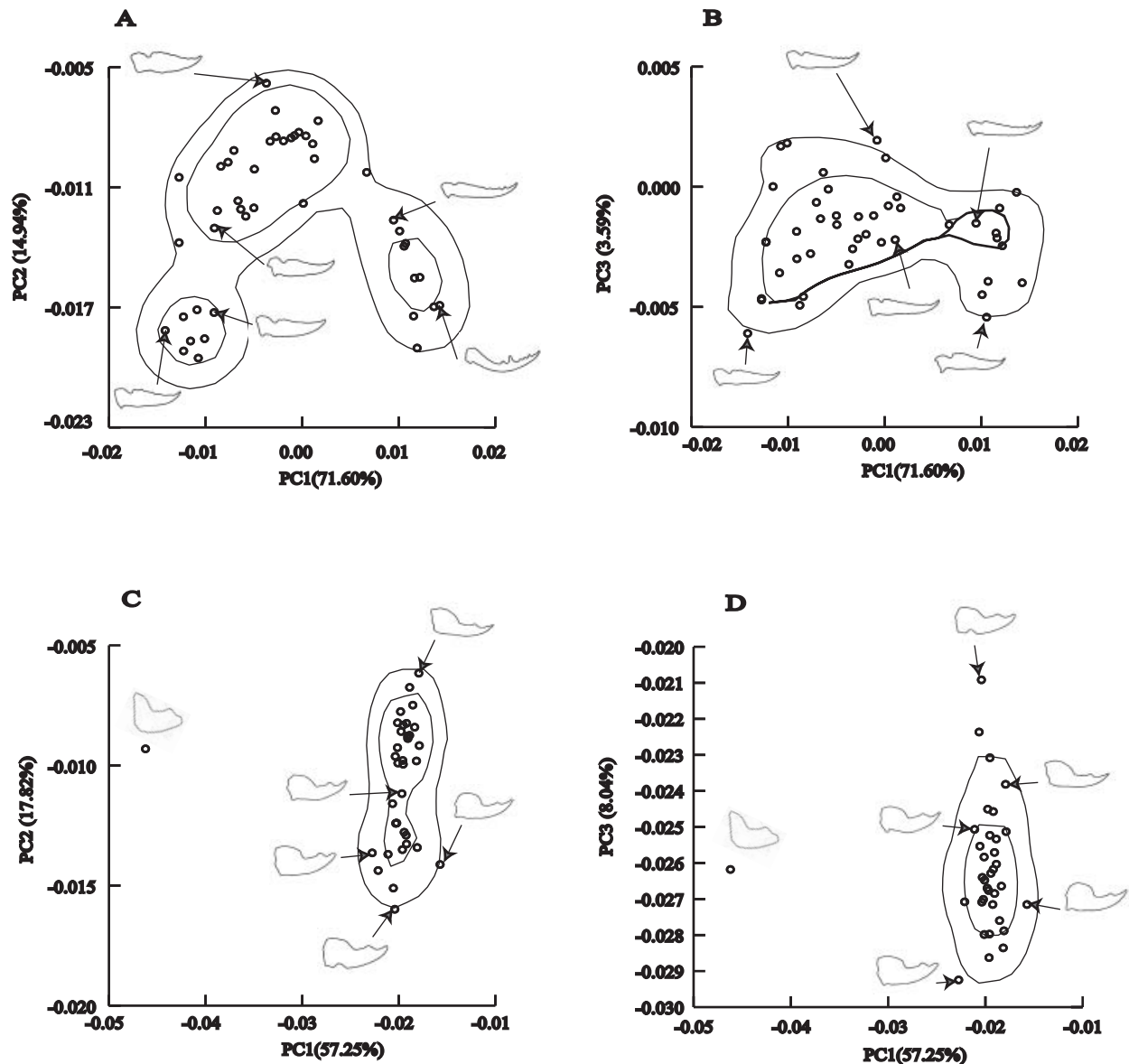


Figure 3. PCA of Fourier coefficients in right mandible. PCs 1, 2: A, males, C, females. PCs 1, 3: B, males, D, females.

Interestingly, the patterns of the kernel contours were slightly different between left and right mandibles (Figs 2, 3).

In both sexes, the pattern of frequency distribution of each shape component (PCs 1, 3) for a character was different from that of the body mass (Figs 1, 7, 8). In males, some but not all shape components (PCs 1 and 2 in right mandible, PC1 in prothorax, and PC1 in SMA) showed significantly skewed distributions (Fig. 7). In females, although no character showed significantly skewed distribution in the weight of body parts, three shape components (PCs 1 and 2 in right mandible and PC2 in head) deviated significantly from normal (Fig. 8).

SHAPE SIMILARITY BETWEEN CHARACTERS

The associations between the shapes of different characters were positive and significant in males except for the combination of head and SMA (Table 3). In contrast, the associations were weak and not significant in almost all combinations of characters in females. The largest, positive correlation coefficient was found in the combination of left and right mandibles in both sexes (Table 3), as expected from the bilaterally symmetrical relationship. Furthermore, at least one shape component of any male character showed significant correlation with at least one component of another character, whereas only a few characters, except for

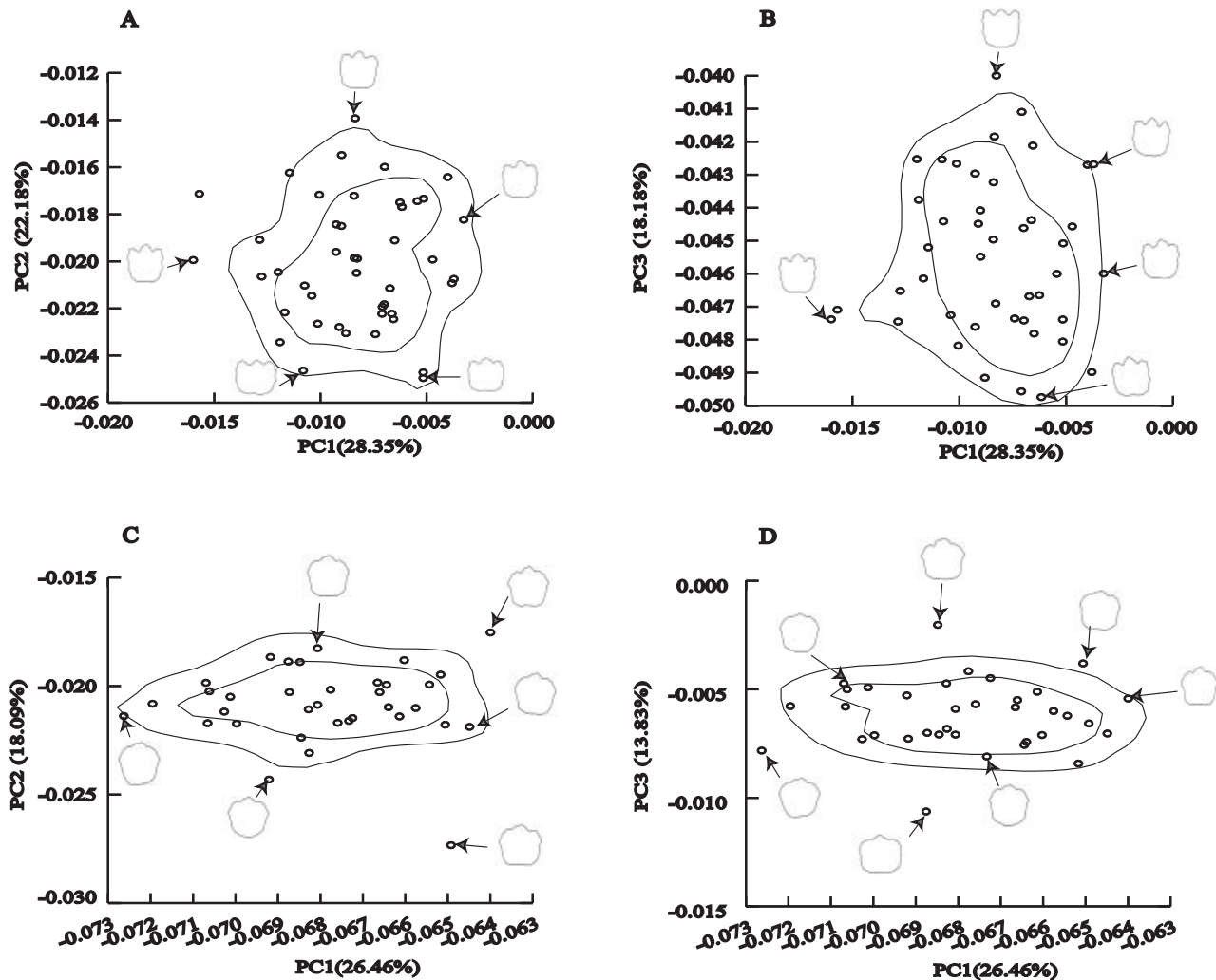


Figure 4. PCA of Fourier coefficients in head. PCs 1, 2: A, males, C, females. PCs 1, 3: B, males, D, females.

Table 2. Correlation between body mass and morphometric size in each character. All coefficients are significant at 1% level. Abbreviations per Table 1

	ML	MR	Head	Pro	SMA
MALES					
ML	0.938				
MR	0.937	0.858			
Head	0.961	0.869	0.582		
Pro	0.669	0.627	0.438	0.661	
SMA	0.887	0.814	0.507	0.866	0.770
FEMALES					
ML	0.825				
MR	0.665	0.875			
Head	0.911	0.928	0.761		
Pro	0.906	0.893	0.746	0.863	
SMA	0.939	0.919	0.815	0.908	0.715

mandibles, showed significant correlation with other characters in females (Table 4), suggesting a well integrated pattern of shape transformation between characters in males and a less well integrated one in females.

MULTIVARIATE ALLOMETRY

In males, all allometric relationships between morphometric size (radii of the first ellipse) and different shape components (PC1–4), in which the size was standardized, were significant according to the *F*-test, as were those between weight and shape, except for a weight–shape relationship in SMA. In contrast, only two relationships (size–shape in the prothorax and weight–shape in the SMA) were significant in females (Table 5). These results again imply the existence of a well integrated shape transformation with increasing

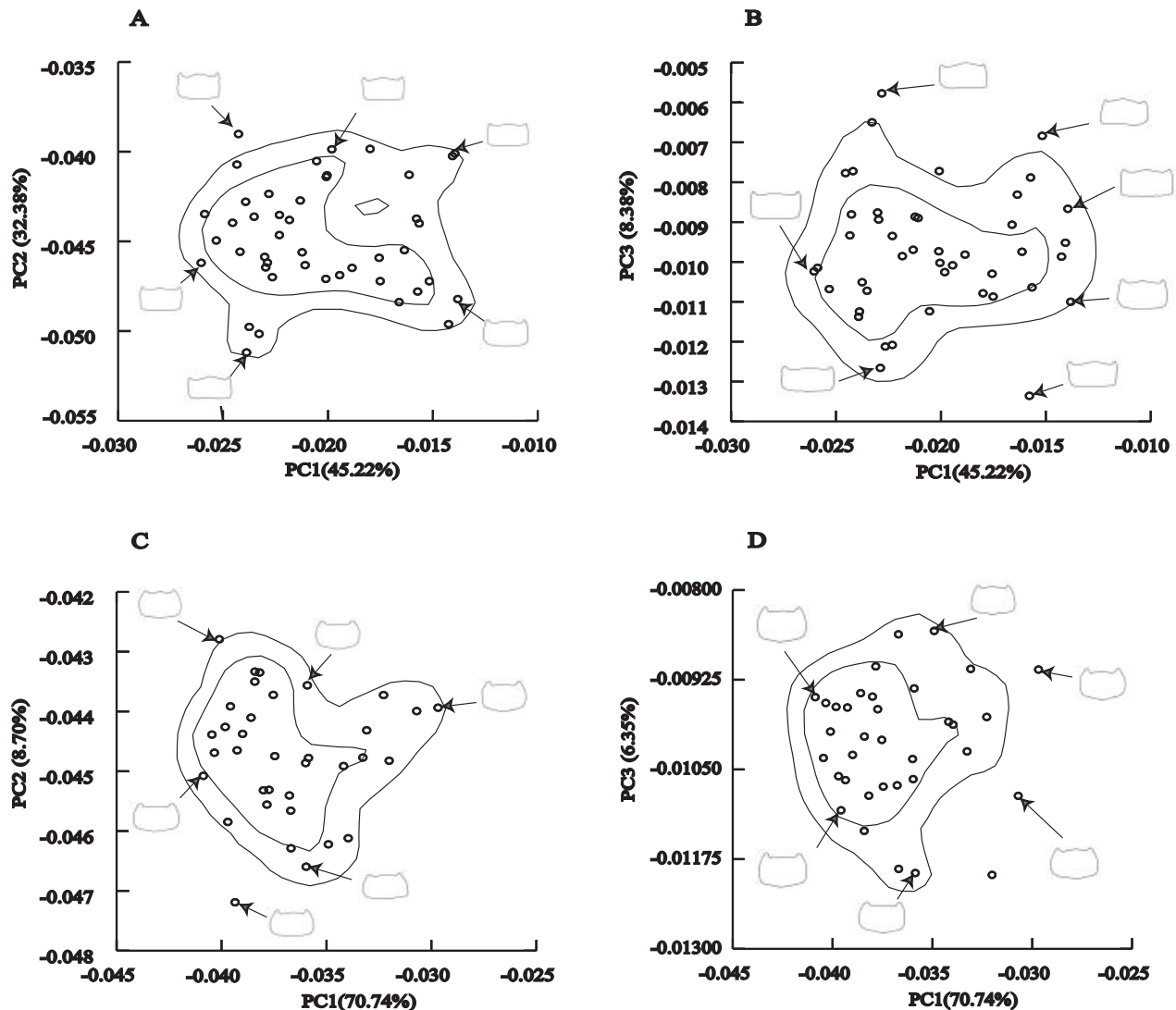


Figure 5. PCA of Fourier coefficients in prothorax. PCs 1, 2: A, males, C, females. PCs 1, 3: B, males, D, females.

size and weight of body parts in males and a less well integrated pattern in females. In male mandibles, straight vs. bending features were tightly associated with both size and weight of the characters (Table 5, PC1 in Fig. 7), but such features as fine structures of inner teeth and shape of membrane joint were significantly affected by size alone (Table 5, PCs 2, 3 in Fig. 7). Generally, in both males and females, even though a shape component in a character was significantly affected by size, it was not always affected by weight, and vice versa (Figs 7, 8; Table 5).

DISCUSSION

As shown in Inukai (1924), there was a conspicuous sexual dimorphism in the weight and shape of the body parts. The pattern of frequency distribution of

weight varied among characters in males: bimodality was much stronger in mandibles and heads than in other body parts. In contrast, there was no such difference in female characters. This kind of bimodality can be observed in exaggerated characters in several kinds of insect species (Emlen & Nijhout, 2000). Mathematical models predict that sexual selection favours a steep and positive allometry of exaggerated characters against body size (Choe & Crespi, 1997), leading to the occurrence of dimorphism in the size and shape of characters. In most cases, because exaggerated characters play a role in competition for access to reproduction (i.e. sexual selection: Emlen & Nijhout, 2000), more reproductive output is expected in males possessing more exaggerated characters. Thus, positive selective pressures are exerted on the character and ultimately affect its developmental pattern.

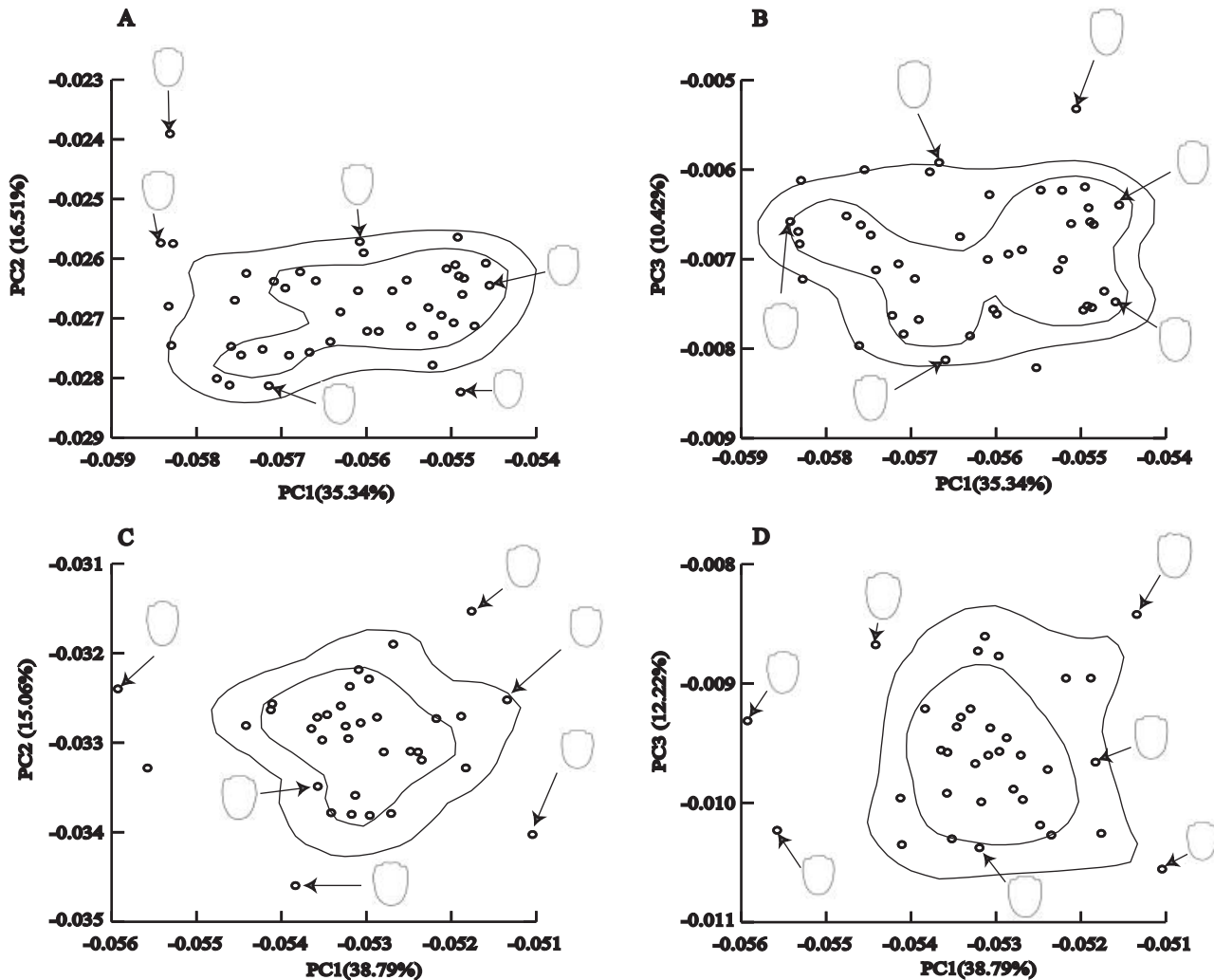


Figure 6. PCA of Fourier coefficients in SMA. PCs 1, 2: A, males, C, females. PCs 1, 3: B, males, D, females.

Table 3. Correlation coefficient (standardized Mantel statistic) on the similarity of shape between characters. Abbreviations per Table 1

	ML	MR	Head	Pro	SMA
MALES					
ML	1				
MR	0.920**	1			
Head	0.286**	0.263**	1		
Pro	0.435**	0.407**	0.149*	1	
SMA	0.276**	0.311**	0.014	0.209**	1
FEMALES					
ML	1				
MR	0.560**	1			
Head	-0.026	-0.025	1		
Pro	-0.007	0.064	-0.075	1	
SMA	-0.027	0.038	0.002	-0.124	1

* $P < 0.05$; ** $P < 0.01$.

This study revealed two new phenomena concerning sexual dimorphism in size and shape variation. First, the shapes of different characters were more strongly associated with one another in males than in females. This suggests that the shape characteristic of a character can be expected to some degree to conform to that of another character in males, but not in females. In particular, the correlation of shape between left and right mandibles was the highest among all character pairs in both sexes. It seems plausible that these symmetrical characters are used for the same purpose and thus are subject to the same selective agents, leading to the same allometry between the two. Nevertheless, subtle differences were observed between left and right mandibles in males. Generally, such a small difference in bilateral characters has been explained in the context of fluctuating asymmetry (Møller & Swaddle, 1997), which sometimes affects reproductive episodes, but the dif-

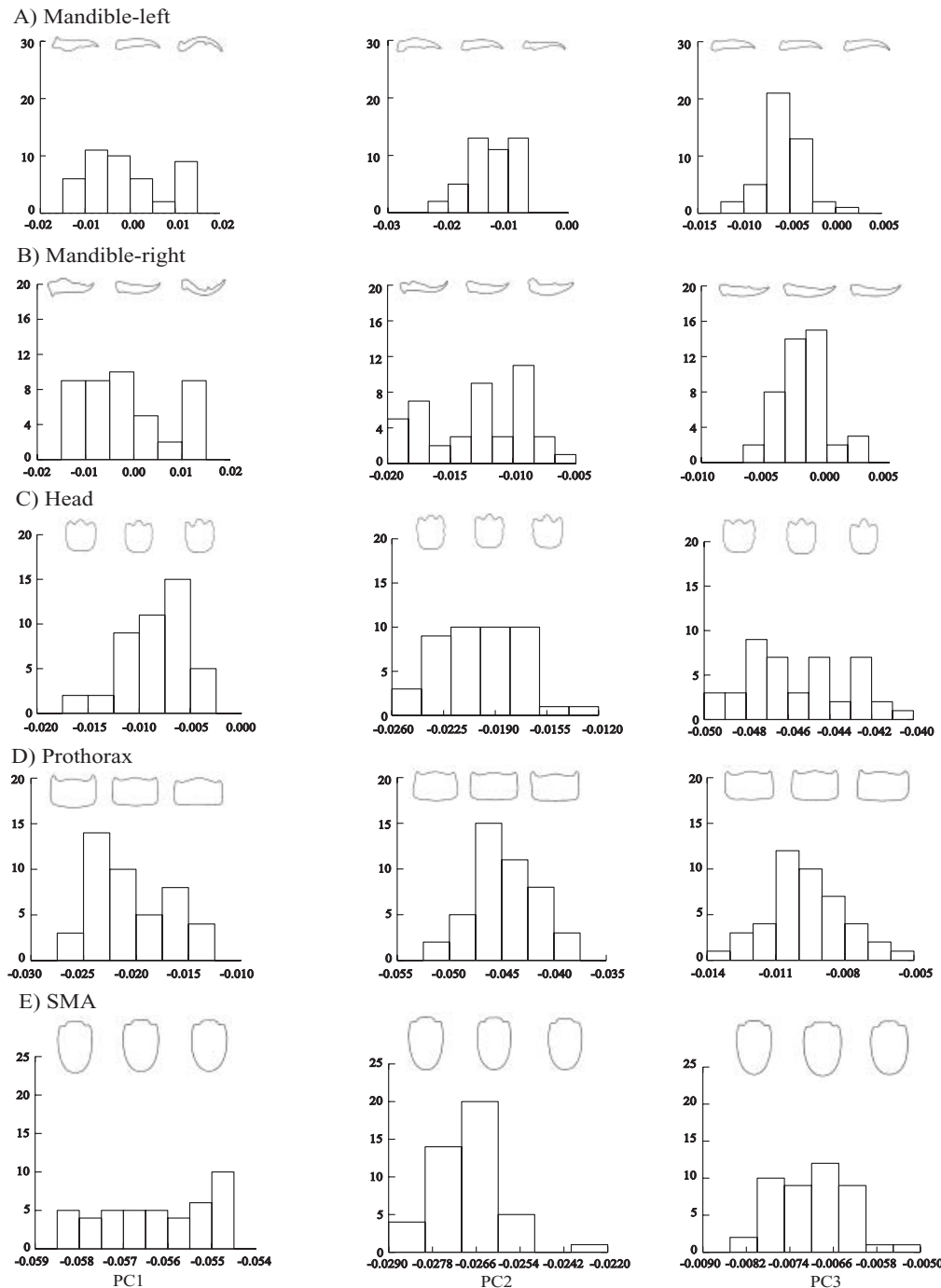


Figure 7. Frequency distributions of the first three PCs calculated from decomposition of covariance matrices of standardized Fourier coefficients in each male character. Reconstructed three characters are overlaid on the plane: average (middle), average + 2 SD (right), and average – 2 SD (left) along each principal axis and zero along all other axes. Significant deviation from normality was observed by Kolmogorov–Smirnov in the right mandible (PC1: $D_{max} = 0.129$, $P < 0.05$) (PC2: $D_{max} = 0.130$, $P < 0.05$), prothorax (PC1: $D_{max} = 0.133$, $P < 0.05$) and SMA (PC1: $D_{max} = 0.135$, $P < 0.05$).

ference found in this study might also be due to a functional constraint: mandibles possessing a slightly asymmetrical shape might be beneficial for dealing with matters of reproduction or competition. Second,

some male characters tended to move towards a specific shape with increasing body mass, whereas the association between size and shape was quite weak in females. The correlations between the weight of the

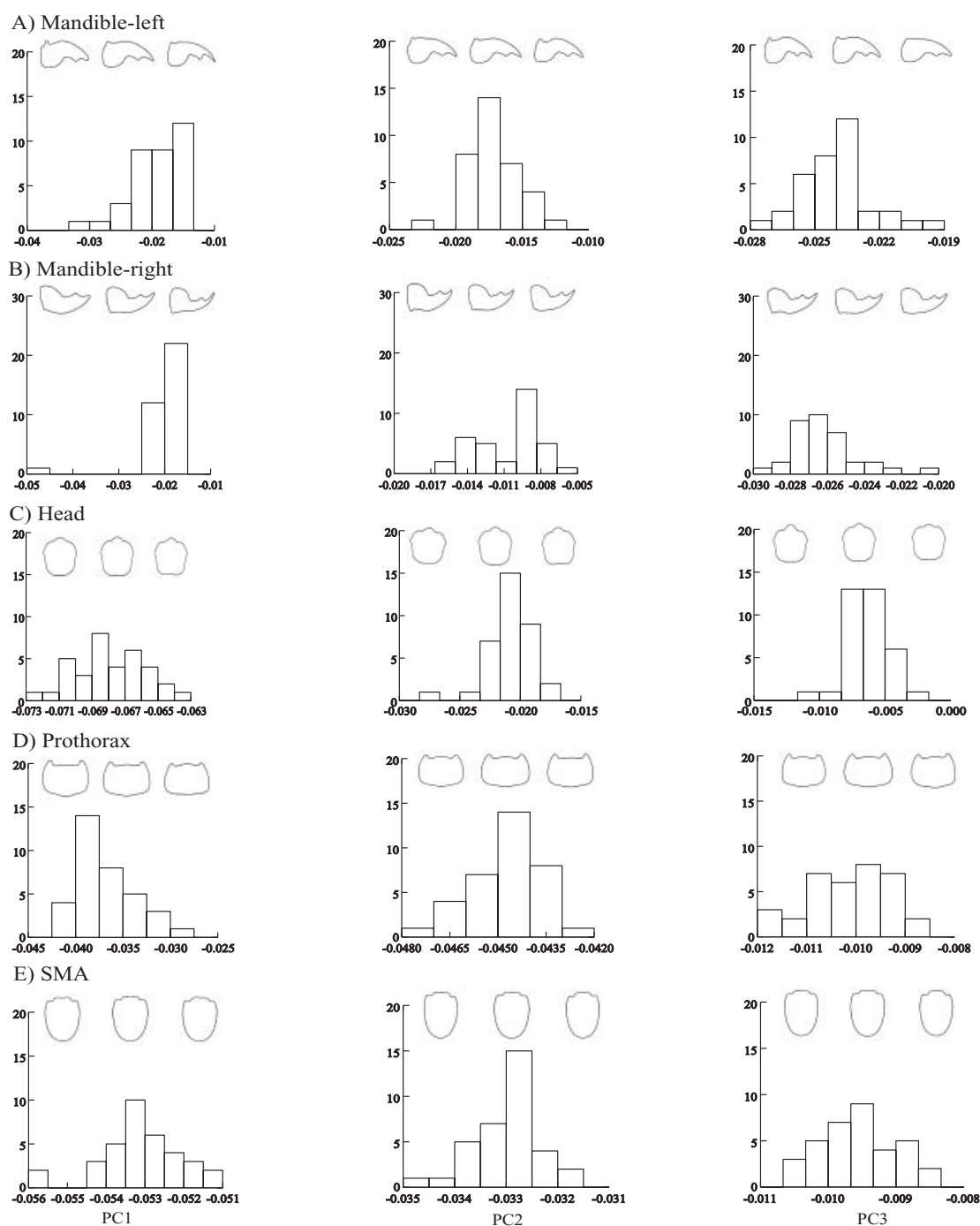


Figure 8. Frequency distributions of the first three PCs calculated from decomposition of covariance matrices of standardized Fourier coefficients in each female character. Reconstructed three characters are overlaid on the plane: average (middle), average + 2 SD (right), and average - 2 SD (left) along each principal axis and zero along all other axes. Significant deviation from normality was observed by Kolmogorov–Smirnov in the right mandible (PC1: $D_{max} = 0.356$, $P < 0.001$; PC2: $D_{max} = 0.180$, $P < 0.01$) and head (PC2: $D_{max} = 0.165$, $P < 0.05$).

body parts were positive and significant in both sexes, implying that energy resources allotted for development increase in all characters as the total body size increases. However, the response of shape

to an increase in body mass differed greatly between the sexes. It seems possible to explain sexual dimorphism in the developmental pattern of characters from the environmental and genetic points of view.

Table 4. Kendall's rank correlation between principal component loadings of each character. Abbreviations per Table 1

ML				MR			Head			Pro		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
MALES												
MR	PC1	0.843***	0.057	0.020								
	PC2	0.119	0.658***	0.214*								
	PC3	0.03	-0.069	0.532***								
Head	PC1	0.193*	-0.041	-0.169	0.147	-0.045	-0.100					
	PC2	0.402***	0.060	0.054	0.365***	0.118	0.005					
	PC3	0.319**	0.197*	-0.001	0.354***	0.200*	-0.078					
Pro	PC1	0.022	-0.085	-0.116	-0.011	-0.107	-0.199*	0.097	0.026	-0.004		
	PC2	0.695***	0.147	-0.056	0.687***	0.152	-0.065	0.214*	0.345***	0.379***		
	PC3	-0.053	0.005	-0.225*	-0.054	-0.070	-0.117	0.085	0.105	0.008		
SMA	PC1	-0.294**	-0.057	0.236*	-0.321**	-0.084	0.157	-0.129	-0.170	-0.141	-0.061	-0.388***
	PC2	0.123	0.102	-0.118	0.124	0.002	-0.204*	-0.041	0.124	0.016	0.016	-0.022
	PC3	-0.108	-0.386***	-0.002	-0.099	-0.367***	0.108	-0.064	-0.022	-0.315**	0.065	-0.213*
FEMALES												
MR	PC1	0.288**	-0.014	-0.196*								
	PC2	0.207*	0.214*	-0.163								
	PC3	0.181	0.191	0.498***								
Head	PC1	0.030	-0.122	-0.010	-0.056	-0.163	-0.048					
	PC2	-0.163	0.081	-0.081	-0.111	-0.082	-0.078					
	PC3	-0.081	0.000	0.047	-0.093	-0.047	-0.058					
Pro	PC1	-0.074	0.101	-0.118	0.009	-0.174	-0.027	-0.073	0.084	-0.049		
	PC2	-0.126	0.286**	0.037	0.096	0.232*	0.147	-0.280**	0.070	-0.046		
	PC3	0.010	0.058	-0.051	-0.029	-0.020	0.078	-0.017	-0.060	-0.178		
SMA	PC1	-0.089	-0.041	-0.167	-0.064	-0.044	-0.305**	-0.043	0.107	-0.026	0.026	-0.176
	PC2	0.155	-0.073	0.107	0.144	-0.045	0.125	-0.118	-0.021	-0.088	-0.026	0.094
	PC3	0.110	-0.164	0.134	0.096	-0.019	0.022	-0.048	-0.260*	-0.041	-0.116	0.079
											0.019	0.136
											-0.026	0.094
											-0.116	0.079
												-0.174

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 5. Association between size and shape components and between weight and shape in each character. Abbreviations per Table 1

	Character	Shape component ^a	Size-shape b^b	Size-shape F^c	Weight-shape b^d	Weight-shape F^e
MALES	ML	PC1(67.87%)	0.944***		0.938***	
		PC2(16.07%)	0.225***	179.08***	0.003	192.82***
		PC3(4.66%)	-0.025		0.054	
		PC4(2.00%)	0.077*		0.061	
	MR	PC1(71.60%)	0.944***		0.823***	
		PC2(14.94%)	0.234***	209.34***	-0.006	63.17***
		PC3(3.59%)	-0.092*		0.126	
		PC4(1.74%)	0.024		0.099	
	Head	PC1(28.35%)	0.269**		0.083	
		PC2(22.18%)	0.504***	20.81***	0.276**	14.69***
		PC3(18.18%)	0.589***		0.204	
		PC4(6.14%)	0.085		0.473***	
	Pro	PC1(45.22%)	0.216		0.824***	
		PC2(32.38%)	0.636***	8.51***	0.127	48.42***
		PC3(8.38%)	0.063		-0.355***	
		PC4(3.33%)	0.107		0.200	
	SMA	PC1(35.34%)	-0.445***		-0.220	
		PC2(16.51%)	0.300**	16.46***	-0.102	1.72
		PC3(10.42%)	-0.139		-0.073	
		PC4(8.93%)	0.566***		0.191	
FEMALES	ML	PC1(55.15%)	0.307		-0.067	
		PC2(14.57%)	0.011	1.54	0.005	0.41
		PC3(9.03%)	0.230		-0.168	
		PC4(4.21%)	0.150		-0.058	
	MR	PC1(57.25%)	0.451**		-0.314*	
		PC2(17.82%)	-0.196	2.50	0.295	1.97
		PC3(8.04%)	0.088		0.288	
		PC4(4.45%)	0.001		-0.197	
	Head	PC1(26.46%)	0.352*		0.040	
		PC2(18.09%)	-0.046	2.02	0.033	1.44
		PC3(13.83%)	0.113		-0.215	
		PC4(9.57%)	-0.271		-0.213	
	Pro	PC1(70.74%)	-0.231		-0.005	
		PC2(8.70%)	-0.455**	3.31*	-0.202	0.50
		PC3(6.35%)	-0.211		0.116	
		PC4(3.72%)	-0.037		-0.103	
	SMA	PC1(38.79%)	0.215		0.194	
		PC2(15.06%)	-0.112	1.85	-0.351*	2.80*
		PC3(12.22%)	0.361*		-0.054	
		PC4(6.89%)	-0.094		-0.233	

^aPCs, taken as independent variables in multiple-regression analysis, calculated from elliptic Fourier analysis for analysing shape of characters. Proportion of the contribution of each PC to the total variance is shown in parentheses.

^bStandardized partial regression coefficients when size (radii of the first ellipse in the Fourier analysis) of body parts was taken as dependent variable.

^c F -statistic for testing overall significance of multiple-regression model for assessing allometric relationship between size and shape components.

^dStandardized partial regression coefficients when weight of body parts was taken as dependent variable.

^e F -statistic for testing overall significance of multiple-regression model for assessing allometric relationship between weight and shape components.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Development of characters is known to be affected by nutritional conditions early in life. Several studies have demonstrated that single genotypes can potentially generate a wide range of morphology (Emlen & Nijhout, 2000). The exaggerated size of horns in the males of two closely related beetle species, *Onthophagus acuminatus* and *O. taurus*, can be altered by varying the amount of food available during early developmental stages, suggesting that every genotype has the potential to generate the full range of possible horn lengths (Emlen, 1994). Emlen & Nijhout (2000) pointed out that such developmental plasticity may be common in other species of insects as well. The difference in habitat usage (e.g. rich vs. poor nutrient habitat) between sexes would engender sex-limited variation; however, this hypothesis is less likely because the habitat usage between sexes is almost the same.

Another possibility is that the sexual difference in the pattern of genetic correlation is responsible for the development of shape. In general, males and females are expected to share a common genetic background in the expression of developmentally homologous characters and thus the evolution of dimorphism most likely requires selective pressures leading to genetic divergence between males and females (Fisher, 1958; Lande, 1980). Recent theoretical and empirical observations indicate that sexual dimorphism occurs when alleles with antagonistic effects on the sexes accumulate in a tight linkage with a sex-determining gene (Rice, 1992; Rhen, 2000). When some sex-limited loci on chromosomes in one sex (e.g. X-chromosome, Wolfenbarger & Wilkinson, 2001) are under the control of sexual selection and influence the shape of different characters pleiotropically, the overall developmental pattern of characters in that sex would be well integrated. If particular male characters such as mandibles are under strong directional selection, evolutionary change in alleles that code other characters will be constrained by the genetic correlation. In contrast, longer mandibles may not be favoured in females, because fighting is quite rare and because they are likely to be less adaptive for digging holes in the bark to lay eggs. Thus, contrasting selective agents due to different optima of the trait may act antagonistically on sex-specific loci, leading to sexual dimorphism in the shape of characters. However, it is still not enough to explain why size and shape vary independently in female characters. If males do not discriminate for mating purposes and thus selective pressures do not cause genetic variation in females (Wu, Johnson & Palapoli, 1996; Tatsuta & Akimoto, 1998), greater genetic variation in alleles of sex-specific loci may remain, resulting in lower genetic and phenotypic correlation between female characters. These predictions remain to be confirmed by quanti-

tative genetics and measurement of the strength of selective pressures.

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REFERENCES

- Bookstein FL. 1991.** *Morphometric tools for landmark data. Geometry and biology*. Cambridge: Cambridge University Press.
- Bookstein FL, Strauss RE, Humphries JM, Chernoff BC, Elder RL, Smith GR. 1982.** A comment upon the uses of Fourier methods in systematics. *Systematic Zoology* **31**: 85–92.
- Cannon CH, Manos PS. 2001.** Combining and comparing morphometric shape descriptors with a molecular phylogeny: the case of fruit type evolution in Bornean *Lithocarpus* (Fagaceae). *Systematic Biology* **50**: 860–880.
- Choe JC, Crespi BJ. 1997.** *The evolution of mating systems in insects and arachnids*. Cambridge: Cambridge University Press.
- Eberhard WG. 1982.** Beetle horn dimorphism: making the best of a bad lot. *The American Naturalist* **119**: 420–426.
- Emlen DJ. 1994.** Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society of London Series B* **256**: 131–136.
- Emlen DJ, Nijhout HF. 2000.** The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* **45**: 661–708.
- Ferson S, Rohlf FJ, Koehn RK. 1985.** Measuring shape variation of two-dimensional outlines. *Systematic Zoology* **34**: 59–68.
- Fisher RA. 1958.** *The genetic theory of natural selection*. Oxford: Oxford University Press.
- Inukai T. 1924.** Statistical studies on the variation of stagbeetles. *Transactions of Sapporo Natural History Society* **6**: 77–91.
- Johnson MS, Black R. 2000.** Associations with habitat versus geographic cohesiveness: size and shape of *Bembicium vittatum* Phillipi (Gastropoda: Littorinidae) in the Houtman Abrolhos Islands. *Biological Journal of the Linnean Society* **71**: 563–580.

- Kuhl FP, Giardina GR. 1982.** Elliptic Fourier features of a closed contour. *Computer Graphics and Image Processing* **18**: 236–258.
- Lande R. 1980.** Sexual dimorphism sexual selection and adaptation in polygenic characters. *Evolution* **34**: 292–305.
- Mantel N. 1967.** The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.
- McLellan T, Endler JA. 1998.** The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology* **47**: 264–281.
- Møller AP, Swaddle JP. 1997.** *Asymmetry, developmental stability, and evolution*. Oxford: Oxford University Press.
- Monti L, Baylac M, Lalanne-Cassou B. 2001.** Elliptic Fourier analysis of the form of genitalia in two *Spodoptera* species and their hybrids (Lepidoptera: Noctuidae). *Biological Journal of the Linnean Society* **72**: 391–400.
- Mosimann JE, James FC. 1979.** New statistical methods for allometry with application to Florida red-winged blackbirds. *Evolution* **33**: 444–459.
- Rhen T. 2000.** Sex-limited mutations and the evolution of sexual dimorphism. *Evolution* **54**: 37–43.
- Rice WR. 1989.** Analyzing tables of statistical tests. *Evolution* **43**: 223–225.
- Rice WR. 1992.** Sexually antagonistic genes: experimental evidence. *Science* **256**: 1436–1439.
- Rohlf FJ. 1990.** Fitting curves to outlines. In: Rohlf FJ, Bookstein FL, eds. *Proceedings of the Michigan morphometrics workshop, the university of Michigan museum of zoology*, 167–177.
- Rohlf FJ. 1998.** *TpsDIG, Version 1.26*. Ecology and Evolution, State University of New York at Stony Brook.
- Rohlf FJ. 2000.** *NTSYS-pc, Version 2.1. Numerical taxonomy and multivariate analysis system*. New York: Exeter Software.
- Rohlf FJ, Archie JW. 1984.** A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Systematic Zoology* **33**: 302–317.
- SAS Institute Inc. 1988.** *SAS/STAT user's guide*, 6.03 ed. Cary: SAS Institute Inc.
- Shiokawa T, Iwahashi O. 2000.** Mating success of small sized males of Japanese stag beetle *Prosopocoilus dissimilis okinawanus* Nomura. *Japanese Journal of Entomology (New Series)* **3**: 157–165 [In Japanese with English summary].
- Simonoff JS. 1996.** *Smoothing methods in statistics*. New York: Springer.
- Sokal RR, Rohlf FJ. 1995.** *Biometry*, 3rd edn. New York: Freeman.
- SPSS Inc. 1998.** *SYSTAT 8.0 Statistics*. Chicago: SPSS.
- Tatsuta H, Akimoto S. 1998.** Sexual differences in the pattern of spatial variation in the brachypterous grasshopper *Podisma sapporensis* (Orthoptera: Podisminae). *Canadian Journal of Zoology* **76**: 1450–1455.
- Tatsuta H, Mizota K, Akimoto S. 2001.** Allometric patterns of heads and genitalia in the stag beetle *Lucanus maculifemoratus* (Coleoptera: Lucanidae). *Annals of the Entomological Society of America* **94**: 462–466.
- Walker JA, Bell MA. 2000.** Net evolutionary trajectories of body shape evolution within a microgeographic radiation of threespine sticklebacks (*Gasterosteus aculeatus*). *Journal of Zoology, London* **252**: 293–302.
- Wolfenbarger LL, Wilkinson GS. 2001.** Sex-linked expression of a sexually selected trait in the stalk-eyed fly *Cyrtodiopsis dalmanni*. *Evolution* **55**: 103–110.
- Wu C-I, Johnson NA, Palopoli MF. 1996.** Haldane's rule and its legacy: why are there so many sterile males? *Trends in Ecology and Evolution* **11**: 281–284.