# Morphological variation of an ornament expressed in both sexes of the mosquito *Sabethes cyaneus*

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## ABSTRACT

**Question:** Do elaborate ornaments expressed in both sexes show patterns of phenotypic variation consistent with selection via mutual mate choice?

**Data description:** Detailed morphometric data on the striking leg 'paddle' ornament of the sabethine mosquito *Sabethes cyaneus*: ornament size and shape and size of general morphological traits. Data derive from 80 males and 80 females from a wild-type laboratory colony established with individuals collected in Panama.

**Search method:** Shape variation was analysed using geometric morphometric methods (elliptic Fourier analyses). We investigated sex differences in the relationships between body size on the one hand and leg length, ornament size, and ornament shape on the other, using general linear models. We also explored morphological variation in asymmetry, allometry, and in the magnitude of phenotypic variation across traits.

**Conclusions:** These ornaments showed many of the classic hallmarks of a sexually selected and condition-dependent ornament: (i) phenotypic variation in size was much greater than for any other trait; (ii) the size of the major part of the paddle showed positive allometry; and (iii) the degree of fluctuating asymmetry in one component of the shape of the leg paddles decreased with body size. Remarkably, these patterns were more pronounced in females and sexual dimorphism in size and shape of the leg paddle ornament was slight. Although data on the current pattern of morphological variation alone does not allow firm conclusions about past selection, our results are consistent with the maintenance of these ornaments in both sexes by sexual selection via mutual mate choice.

*Keywords*: allometry, Diptera, elliptic Fourier analysis, sexual selection, signal, variation.

## INTRODUCTION

The strikingly elaborate ornaments (defined as exaggerated or novel structures used to visually attract mates) possessed by many animal species have inspired much research into the evolution of signals via sexual selection. Most of these studies have been conducted in avian taxa with a particular focus on the elongated, curled, and often brightly coloured tail feathers (Darwin, 1871; Jennions, 1993; Andersson, 1994; Cuervo and Møller, 1999). Factors that affect the

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intensity of sexual selection include gamete investment, mating investment, parental investment, and variance in mate quality (see Andersson, 1994; Shuster and Wade, 2003). In the majority of avian species, females invest a relatively higher amount into gamete production (Trivers, 1972). Moreover, both females and males are known to mate multiply and seek extra-pair copulations, further increasing the variance in male fitness and intensifying the strength of sexual selection (Ligon, 1999). This characterizes the common scenario of relatively indiscriminate males competing for mating opportunities with choosy females, which forms the general basis for our understanding of the evolution of ornaments in males by female mate choice (Ligon, 1999; Dunn et al., 2001).

Our understanding of ornament evolution in insects is much more restricted. There are many descriptions of exaggerated male structures that represent putative ornaments, as they are displayed during courtship (see Thornhill and Alcock, 1983; Sivinski, 1997). However, it generally remains unknown which factors have contributed to the evolution of these ornaments. One notable exception is the evolution of antlers and eye stalks in flies. Although female preference for these ornaments has been demonstrated (Burkhardt and de la Motte, 1988; Wilkinson and Reillo, 1994; Hingle et al., 2001; Cotton et al., 2006), they are employed in male–male competition and intrasexual selection clearly plays a key role in their evolution (Wilkinson and Dodson, 1997). In general, scramble competition polygyny is considered to be widespread in many insect taxa (Thornhill and Alcock, 1983). This has contributed to the widely held view that there is little opportunity for female mate assessment and direct female choice in insects (Alexander et al., 1997), although indirect female choice (sensu Wiley and Poston, 1996) is common. Yet, there is growing evidence that female insects indeed assess males and show direct mate choice [e.g. burying beetles (Beeler et al., 2002), stalk-eyed flies (Burkhardt and de la Motte, 1988), wax moths (Jang and Greenfield, 1996, 1998), and field crickets (Simmons, 1986)]. In most insects, both males and females mate multiply and exhibit no parental care (Thornhill and Alcock, 1983; Arnqvist and Nilsson, 2000). Here, the selective pressures acting upon ornaments may be largely understood in terms of differential investment into gamete production. However, in other insects the mating system and pattern of ornamentation is more atypical, and these systems can provide valuable insights into ornament evolution by sexual selection.

Our study organism, the sabethine mosquito *Sabethes cyaneus* (Diptera: Culicidae), possesses elaborate paddle-like ornaments consisting of elongated and iridescent scales on the mid-leg tarsus and tibia (Fig. 1). Four facts point to a central role for sexual selection in the evolution of these visually striking paddles. First, unlike most mosquitoes, sabethine
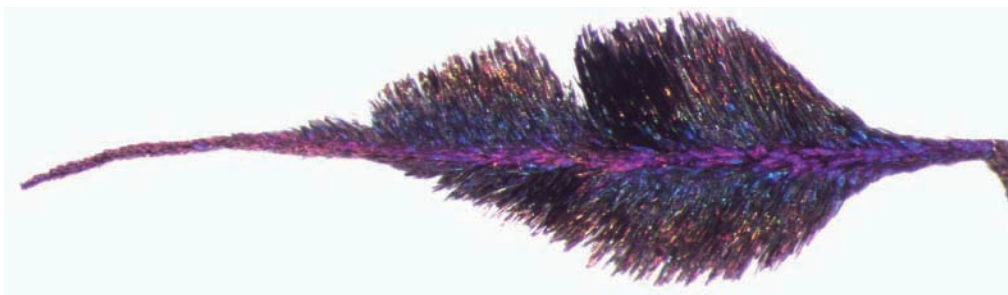


**Fig. 1.** The leg paddle of *Sabethes cyaneus*. The tibial (right) and tarsal (left) component of this ornament are separated by a small indentation.

mosquitoes are diurnal and show unique visual adaptations in the optical structure of their eyes (Land *et al.*, 1999). The fact that their visual ornaments are also unique among culicid mosquitoes (Sivinski, 1997) is therefore consistent with a signal function. Second, there is large variation in paddle number, shape, and coloration, but not in general morphological traits, across closely related sympatric sabethine species (Harbach and Petersen, 1992; Judd, 1996). Such a pattern of rapid morphological divergence is a general hallmark of sexual selection (Coyne and Orr, 2004). Third, complex male courtship behaviours, during which these mid-leg ornaments are prominently displayed in front of the female, have been described in all four species of sabethines studied to date, including our study system, *S. cyaneus* (Okazawa *et al.*, 1986; Hancock *et al.*, 1990a; Philips *et al.*, 1996; Zsemlye *et al.*, 2005). Fourth, direct phenotypic engineering experiments (i.e. removal of the ornaments) have shown that this ornament affects mating success in *S. cyaneus*, yet flight and oviposition behaviours remain unaffected (Hancock *et al.*, 1990b). Notably, this effect was stronger, and only statistically significant, in females.

*Sabethes cyaneus* provides a particularly intriguing model system. This is not only because the ornaments are extraordinarily striking (Shannon, 1931), but also because the ornaments appear to be sexually monomorphic to the naked human eye and because females are strictly monandrous while males are polygynous (South and Arnqvist, 2008; S.H. South *et al.*, submitted). In addition, males and females adopt traditional sex roles, with males searching for females perched on horizontal branches before aligning to perform a highly stereotyped and vigorous courtship dance in which the ornaments are displayed in front of the female (Hancock *et al.*, 1990a). Furthermore, females are generally reluctant to mate, there is no parental care by either sex, and there is no evidence of nutritional gifts being transferred to females with the male seminal fluid. Given the mating system of *S. cyaneus*, the occurrence of male ornaments (henceforth referred to as 'leg paddles') is entirely consistent with evolutionary theory. However, it is unclear why we should see the expression of ornamental leg paddles in females.

There are two main classes of hypotheses that aim to explain the expression of elaborate ornaments in both sexes (for a review, see Kraaijeveld *et al.*, 2007). The first posits that male and female expression of the ornament is due to direct selection on the ornament in both sexes. Direct selection may result from (i) selection for sexual ambiguity in females (Burley, 1981; Langmore and Bennett, 1999), (ii) female contest competition (West-Eberhard, 1983; Gwynne, 1991), and/or (iii) mutual mate choice (Huxley, 1914; Kraaijeveld *et al.*, 2004). The second group of hypotheses suggests that the expression of the trait in females is a non-adaptive result of an intersexual genetic correlation, such that the female ornament is merely a correlated response to selection for the ornament in males (Darwin, 1871; Lande, 1980, 1987; Lande and Arnold, 1985). By simply considering what is already known of the morphology and mating system of our model system, several of these hypotheses can be rejected.

Selection for sexual ambiguity could occur when being male brings social benefits (Burley, 1981) or reduces sexual harassment of females (Cook *et al.*, 1994; Forbes *et al.*, 1997; Van Gossum *et al.*, 2007). It is highly unlikely that there are any social benefits such as access to food sites that could select for sexual ambiguity in *S. cyaneus*, simply because mosquitoes do not exhibit social behaviour. A reduction in the amount of sexual harassment is also unlikely, as males of *S. cyaneus* court males as eagerly as females (Hancock *et al.*, 1990a; personal observation), as do males of many insect species,. More importantly, experimental removal of the leg paddles in females actually led to a reduction in courtship by males (Hancock *et al.*, 1990b). These facts strongly suggest that the maintenance of these ornaments in females cannot be accounted for by selection for sexual ambiguity. Furthermore, no aspect of the ecology of this species

could generate female contest competition. Sites for oviposition (plant-held waters), mating (horizontal branches), and feeding (primate hosts, flowers) are abundant in natural populations, females do not actively seek males, and females have never been observed to engage in contests. Thus, female contest competition is very unlikely to contribute to the elaboration of these ornaments in females.

The remaining applicable hypotheses that may account for the maintenance of these ornaments in females are direct selection via mutual mate choice or non-adaptive expression due to an intersexual genetic correlation. Theory has shown that mutual mate choice can evolve under a range of conditions and studies of monogamous birds support the presence of selection by mutual mate choice (Jones and Hunter, 1993, 1999; Kraaijeveld *et al.*, 2004; Hooper and Miller, 2008). However, *S. cyaneus* adopts 'traditional' sex roles: male polygyny/female monandry, in combination with male mate searching and male courtship/female mate rejection. Nonetheless, there is growing empirical support for male/mutual mate choice also in polygynous systems (for reviews, see Amundsen, 2000; Bondurianksy, 2001; Kraaijeveld *et al.*, 2007), which has sparked recent theoretical interest in this area (Servedio and Lande, 2006; Servedio, 2007). It is also possible that the occurrence of female paddles in *S. cyaneus* is due solely to a correlated expression in females of sexual selection for paddles in males. To our knowledge, there are no known cases of non-adaptive expression of elaborate ornaments to the extent that they are basically sexually monomorphic. This fact has been accredited to the costs associated with ornament expression in females, such as increased conspicuousness to predators (Godin and McDonough, 2003), impaired ability and energetic costs during flight (Evans, 2004; Allen and Levinton, 2007), and production costs during ontogeny (Munoz *et al.*, 2008). However, it remains possible that the costs associated with expression of these paddles in *S. cyaneus* females are low enough to permit such an extreme and intriguing case of non-adaptive expression. We note, however, that female paddles do affect female attractiveness to and/or recognition by males, as their removal results in a drastic reduction in female mating rate and courtship attempts by males (Hancock *et al.*, 1990b).

One potentially useful route to gain insights into the evolution of ornaments in any taxa is to study the pattern of ornament expression. Here, studies comparing the variability of ornaments and other body parts are valuable (e.g. Schluter and Price, 1993), as are studies of static allometry of ornaments (e.g. Kodric-Brown *et al.*, 2006). This approach is particularly suitable for adult holometabolous insects, because they grow solely during egg, larval, and pupal stages (Klingenberg and Zimmermann, 1992; Emlen, 1994; Kemp and Rutowski, 2007). In this study, we explore the pattern of phenotypic variation of the leg paddle ornament in *S. cyaneus* by assessing variability as well as the relationships between ornaments and size in both sexes. We employ geometric morphometric methods, which allow for powerful analyses of variation in size and shape (Klingenberg, 1996).

One of our goals is to assess those facets of morphological variation and allometry that may help discriminate between direct selection via mutual mate choice and an intersexual genetic correlation. We begin by exploring sexual dimorphism in general body measures as well as ornament shape and size. Under mutual mate choice, we expect directional selection (see below) in both sexes to lead to similar expression in males and females, even if sex-specific factors are likely to affect expression to some extent (Kimball and Ligon, 1999). As discussed above, however, a non-adaptive genetic correlation may also lead to similar expression in males and females, provided that there are no costs of expressing the ornament in females. We then proceed to explore the following three classic hallmarks of sexual selection. First, sexual ornaments are expected to be more phenotypically variable

than non-sexual traits (Schluter and Price, 1993; Rowe and Houle, 1996; Cuervo and Møller, 1999; Badyaev, 2004). Second, the degree of bilateral asymmetry in sexual ornaments may decrease with body size, as a result of condition-dependent expression (e.g. Swaddle, 2003; but see Cotton *et al.*, 2004). Third, sexual ornaments often, but not always (Bondurianksy, 2007), show positive static allometry (i.e. increase with overall body size at a rate > 1.0) presumably as a reflection of differential trade-offs in resource allocation during ontogeny (Grafen, 1990a, 1990b; Green, 1992; Petrie, 1992; Emlen and Nijhout, 2000; Knell *et al.*, 2004; Kodric-Brown *et al.*, 2006).

Although several models have suggested that sexually selected traits should show positive allometry (see references above), the generality of this prediction was critically appraised by Bonduriansky and Day (2003). They showed that the classic sexual selection scenario does not necessarily result in positive allometry. A recent reappraisal by Bonduriansky (2007) delineated the conditions that should lead to positive allometry and we suggest that those conditions are met in *S. cyaneus*. First, the ornament is truly a 'dedicated' secondary sexual character that does not have any known viability-related function (see above). Second, there is no known alternative male mating tactic that may favour small ornaments and result in disruptive selection. Third, paddle expression does not appear to affect flight ability or oviposition behaviour (see above; Hancock *et al.*, 1990b) and it thus is unlikely that intermediate trait sizes are favoured for such reasons. Fourth, it is very reasonable to assume that the relative viability costs of expressing this ornament diminishes with body size (see Discussion).

## MATERIALS AND METHODS

We used a strain of *S. cyaneus* established by R.G. Hancock and W.A. Foster in December 1988 from a sample of mated females collected at the Isla de Maje, Lago Bayano, Panama, Republic of Panama. This colony was maintained at Ohio State University, USA at a population size of approximately 200–300 individuals. Our colony has been housed at Uppsala University, Sweden since April 2006 at $26 \pm 1°C$, 78–82% relative humidity, and a 12L : 12D photoperiod, at a population size of approximately 400 individuals. Larvae were reared in plastic trays ($21.5 \times 14.5 \times 5$ cm) filled to 2.5 cm with deionized water, which was changed weekly. They were fed an *ad libitum* diet of crushed fish flake food. Pupae were collected in small dishes (diameter 8 cm; height 2.5 cm) and these were placed in terraria ($29 \times 17.5 \times 18$ cm). An *ad libitum* supply of honey-soaked sponges and deionized water wicks was provided in these terraria.

We collected and froze adults within 24 h of emergence to avoid variation in leg paddles that is due to wear and/or damage. Individuals were sampled across three generations all raised under the rearing regime described above. We then collected morphometric information using a digitizing tablet (Summasketch® III) placed under a side-mounted camera lucida attached to a dissecting microscope (Leica® MZ8). The following traits were measured in a sample of 80 females and 80 males on the left side of the body: wing length; thorax width; proboscis length; antenna length; fore-leg femur, tibia, and tarsus length; mid-leg femur, tibia, and tarsus length; hind-leg femur, tibia, and tarsus length. Wing length was used as an integrative measure of body size (Siegel *et al.*, 1992; Armbruster and Hutchinson, 2002). We also captured the outline of both the left and right leg paddles in two separate segments: tibial paddle and tarsal paddle. All measurements were taken twice to enable an estimation of the repeatability of our measures in a subset of 30 males and 30 females.

All length measurements were converted to millimetres before analysis. The leg paddle outlines were analysed using elliptic Fourier analysis (EFA) (Ferson *et al.*, 1985; Rohlf, 1992). The outlines of all paddles for all individuals were included in a common EFA, using the software Morpheus et al. (Slice, 2002), which was also used to extract the area of all paddles. The EFA itself was made invariant of size, position, and rotation, and used 12 harmonics (yielding 48 Fourier coefficients). These functions provided a near perfect fit to all paddle outlines. To reduce the dimensionality of our shape descriptors, the 48 Fourier coefficients for each leg paddle segment were treated as variables in principal component analyses performed on the covariance matrix (Rohlf and Archie, 1984). The first five principal components (PCs) from each leg paddle were retained for subsequent analyses. These five PCs collectively described 88.79% and 87.53% of the shape variation in tibial and tarsal paddles respectively (see Appendix 1).

The relationship between paddle area and length measurements within and between the sexes was investigated using full analyses of covariance (ANCOVAs) with sex as a fixed factor and length measurements as a continuous covariate. Analogously, paddle shape was investigated using multivariate analyses of covariance (MANCOVAs) with the five PCs as dependent variables, sex as a fixed effect factor, and body size as a covariate.

We compared the magnitude of phenotypic variation in traits presumed to be under sexual selection (leg paddle area and the length of the leg segments the paddles are located on – mid-leg tarsus and tibia) and other representative body traits (thorax, wing, fore-leg femur, tibia, and tarsus) in two different ways. First, we compared the coefficients of variation across traits. Second, following a transformation where all values were divided by the trait mean, we compared the proportional variances across traits. We note that the latter method gives all traits a mean value of one and a variance that is proportional across traits (Sokal and Rohlf, 1995).

We calculated leg paddle asymmetry simply by subtracting the left-hand paddle value from the right-hand paddle value for each individual and for all measures of paddle morphology, in both tibial and tarsal paddles. We assessed whether the pattern of asymmetry for all leg paddle variables was consistent with directional asymmetry [as opposed to fluctuating asymmetry (Swaddle, 2003)] by conducting *t*-tests of $H_0$: $\mu = 0$ (where $\mu$ is the mean signed asymmetry), both for the entire sample and for males and females separately. The relationship between body size and unsigned asymmetry was then assessed by Spearman rank correlations between the absolute value of asymmetry and body size. Any unsigned asymmetry variables with a significant correlation were then further investigated using ANCOVAs with sex as a fixed factor and body size as a covariate.

The allometric slopes were estimated using Reduced Major Axis regression [RMA, Model II regression or Geometric Mean regression (Ricker, 1984; McKinney and McNamara, 1991)] of body size against the paddle size and leg measurements, using the software MODEL II (Sawada, 1999). For paddles, the area measurements were first linearized using an ellipse transformation as the paddle resembles an ellipse. We used the equation describing the area of an ellipse ($A = \pi \times a \times b$, where $a$ and $b$ are the lengths of the ellipse's semi-major and semi-minor axes). We approximated $a$ to be $2 \times b$ and used this to solve for the length of the major axis. We henceforth denote this linearized measure of paddle area as 'paddle size'. This linearized value of paddle area was then converted to millimetres to make it directly comparable to other length measurements. Before the RMA regressions, all data were log $(1 + x)$ transformed.

## RESULTS

Most of our measurements were highly repeatable ($r > 0.70$; Appendix 2). It is noteworthy that this was also true for our multivariate measures of paddle shape and for measures of asymmetry. Thorax width, proboscis length, and antennal length measurements showed a slightly lower repeatability than wing length and will not be used in the subsequent analyses. We note that females were larger than males ($t_{144} = 9.353$, $P < 0.001$, based on separate variances).

We investigated the relationship between tibial paddle size and body size within and between the sexes. Tibial paddle size increased with body size in both sexes ($F_{1,156} = 35.3$, $P < 0.001$) (Fig. 2a), but there was no main effect of sex ($F_{1,156} = 0.6$, $P = 0.442$) and no significant interaction between tibial paddle size and body size ($F_{1,156} = 0.7$, $P = 0.421$). In contrast, the relationship between body size and tarsal paddle size differed significantly between the sexes (Fig. 2b). As for tibial paddle size, tarsal paddle size increased with body size ($F_{1,156} = 16.7$, $P < 0.001$), but there was also a main effect of sex ($F_{1,156} = 4.4$, $P = 0.039$) and, most importantly, an interaction between tarsal paddle size and body size ($F_{1,156} = 5.6$, $P = 0.019$). This relationship was steeper in females than males, revealing that paddles were proportionately larger in females (Fig. 2b).

The relationship between body size and mid-leg tibia and tarsus length was similar to that observed with the part of the paddles situated on each corresponding segment. That is, the relationship between body size and mid-leg tibia did not differ between the sexes, whereas that between body size and mid-leg tarsus did (Table 1), with females having proportionately longer tarsi. The fore-leg tibia and tarsus length showed a similar pattern to the mid-leg tibia and tarsus: female fore-leg segments were proportionately longer than those of males (Table 1). Notably, the hind-leg tibia and tarsus length differed from this pattern of proportionately larger female paddles: although the relationship differed between the sexes (Table 1), both male hind-leg tibia and tarsus length were proportionately longer than for females.

The relationship between leg paddle shape and leg paddle size differed between the sexes (see Table 2) and, in this sense, this ornament is sexually dimorphic. These effects were larger for the tarsal components of the leg paddles, and a closer analysis using univariate $F$-tests of each of the tarsal paddle shape PCs showed that the main difference between the sexes falls on the first principal component, PC1 (sex: $F_{1,156} = 8.4$, $P = 0.004$; tarsal paddle area: $F_{1,156} = 242.2$, $P < 0.001$; sex × tarsal paddle area: $F_{1,156} = 32.6$, $P < 0.001$). Visualizations of this result showed that paddles change shape more rapidly with paddle size in males than in females (Fig. 3a). For the first PC of tarsal paddle shape, male paddles tend to be slightly broader at the widest point than do female paddles and the breadth of the paddle increases with paddle area (Fig. 3b). In summary, although these analyses show that paddle shape differs significantly between the sexes, the differences are very slight in absolute magnitude.

We tested for equality of variances across all traits (using variables transformed to show proportional variances – see Methods) and found significant differences in variability across traits (Bartlett's test: $\chi^2 = 1259.3$, $P < 0.001$). Visual examination of the data showed that paddle size was more variable than any other trait (Fig. 4). Interestingly, although the variation in the leg segments that carry the paddles is also relatively high, they do not show the same strikingly high levels of variation as the ornaments themselves (see Fig. 4). Additionally, for many traits females showed significantly higher phenotypic variation than
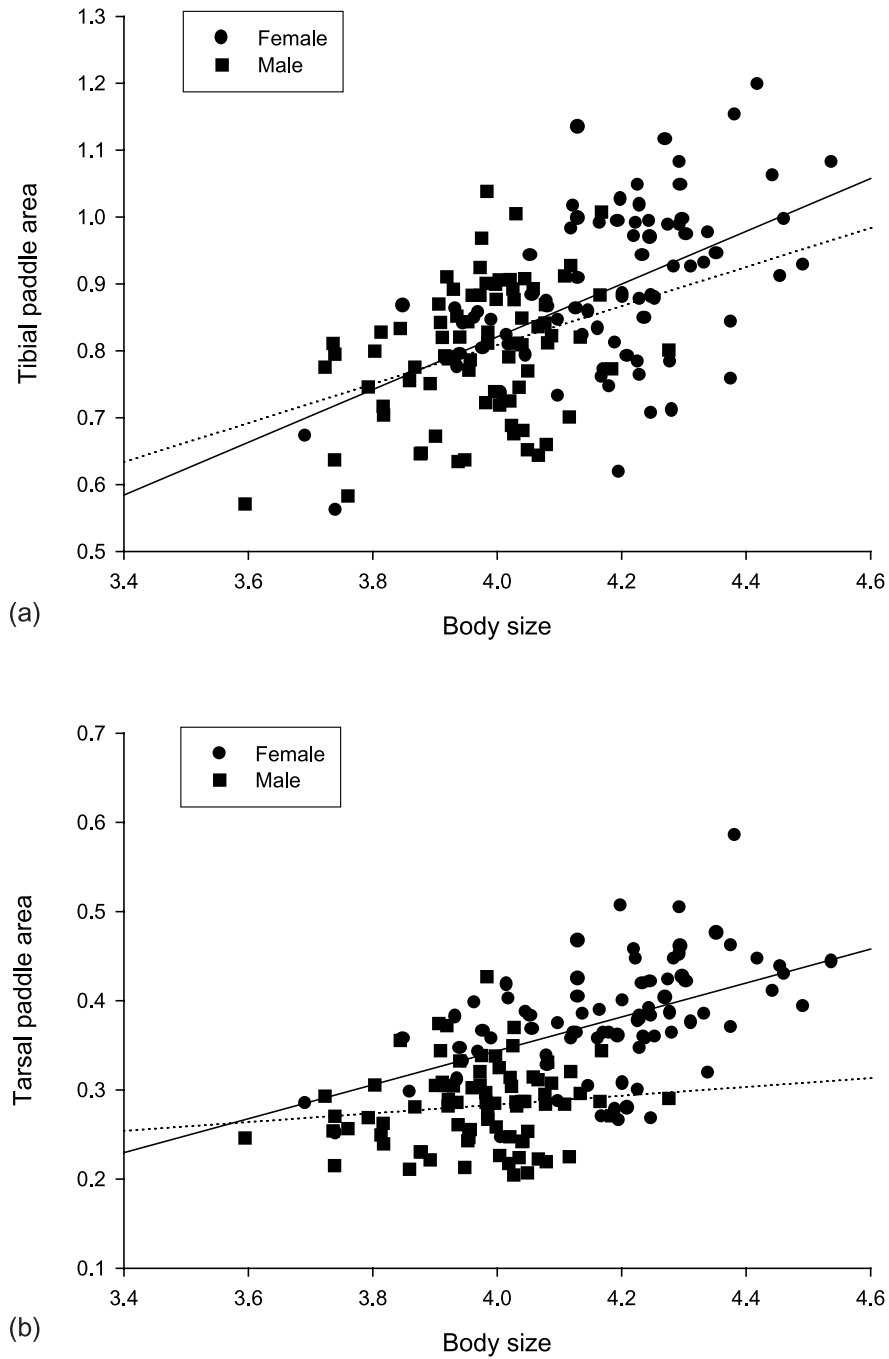
**Fig. 2.** The relationship between body size and (a) tibial paddle area and (b) tarsal paddle area. Lines (solid: females; dotted: males) represent ordinary linear regressions.

**Table 1.** Analyses of covariance of the relationship between body size and leg segment lengths in the two sexes

|  | F | d.f | P |
|---|---|---|---|
| **Mid tibia length** | | | |
| Sex | 3.841 | 1, 156 | 0.052 |
| Body size | 61.953 | 1, 156 | 0.000 |
| Sex × body size | 3.914 | 1, 156 | 0.050 |
| **Mid tarsus length** | | | |
| Sex | 5.466 | 1, 155 | 0.020 |
| Body size | 36.942 | 1, 155 | 0.000 |
| Sex × body size | 9.171 | 1, 155 | 0.003 |
| **Fore tibia length** | | | |
| Sex | 15.923 | 1, 156 | 0.000 |
| Body size | 111.077 | 1, 156 | 0.000 |
| Sex × body size | 14.334 | 1, 156 | 0.000 |
| **Fore tarsus length** | | | |
| Sex | 5.356 | 1, 156 | 0.022 |
| Body size | 62.873 | 1, 156 | 0.000 |
| Sex × body size | 5.307 | 1, 156 | 0.023 |
| **Hind tibia length** | | | |
| Sex | 11.852 | 1, 156 | 0.001 |
| Body size | 112.720 | 1, 156 | 0.000 |
| Sex × body size | 8.862 | 1, 156 | 0.003 |
| **Hind tarsus length** | | | |
| Sex | 9.475 | 1, 156 | 0.002 |
| Body size | 75.339 | 1, 156 | 0.000 |
| Sex × body size | 8.638 | 1, 156 | 0.004 |

**Table 2.** Multivariate analyses of covariance of paddle shape

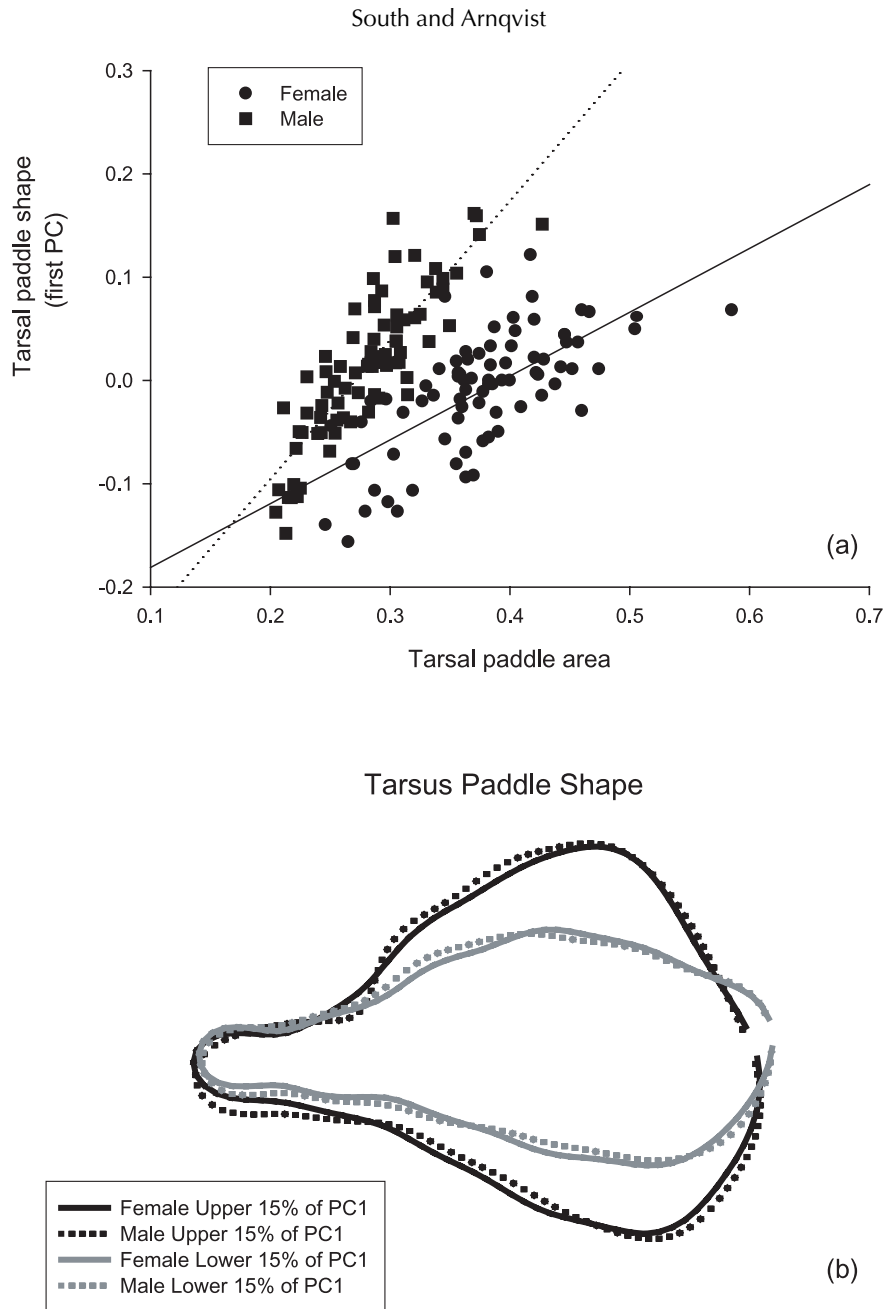|  | Wilks' λ | F | d.f | P |
|---|---|---|---|---|
| **Tibial paddle** | | | | |
| Sex | 0.889 | 1.842 | 10, 147 | 0.058 |
| Tibial paddle area | 44.519 | 44.519 | 10, 147 | 0.000 |
| Sex × tibial paddle area | 0.794 | 3.804 | 10, 147 | 0.000 |
| **Tarsal paddle** | | | | |
| Sex | 0.873 | 2.144 | 10, 147 | 0.024 |
| Tarsal paddle area | 0.297 | 34.767 | 10, 147 | 0.000 |
| Sex × tarsal paddle area | 0.774 | 4.287 | 10, 147 | 0.000 |

**Fig. 3.** (a) The relationship between tarsal paddle area and shape, represented by the first principal component of variation in tarsal paddle shape. Lines represent ordinary linear regressions. (b) The upper and lower extremes of the first principal component of tarsal paddle shape. For further details, see text.

males. These patterns were very similar whether variability was measured as proportional variance or as the coefficient of variation (see Fig. 4). We note (i) that the elevated variation seen in paddle size was clearly not due to inflated measurement error, as the repeatabilities
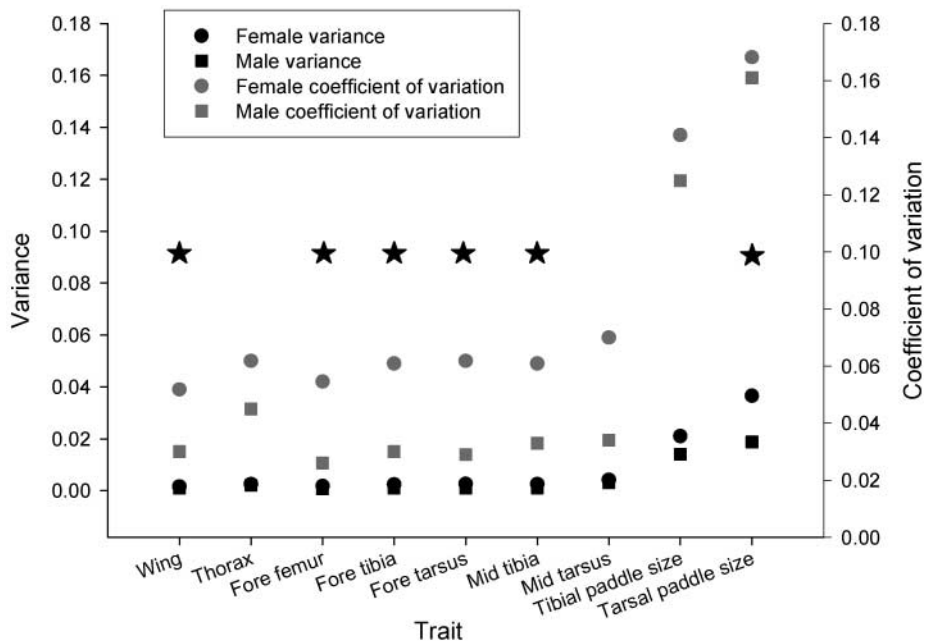
**Fig. 4.** Phenotypic variation of morphological traits, shown both as proportional variances and coefficients of variation (see text). Traits for which the proportional variance differed significantly between the sexes ($P < 0.005$) are indicated with a star.

for ornament size (range: 0.90–0.98) were actually higher than for most other traits (see Appendix 2), and that (ii) the exact same pattern is seen when analysing phenotypic variation in paddle area rather than paddle size.

The only measure of asymmetry of leg paddle morphology that showed a significant relationship with body size was PC2 of tarsal paddle shape [$r_s = -0.253$, $P < 0.05$, using false discovery rate compensation (Storey, 2003)]. An ANCOVA confirmed the negative relationship between the unsigned asymmetry of PC2 and body size ($F_{1,156} = 7.6$, $P = 0.007$), and showed that the sexes did not differ significantly in this respect (sex: $F_{1,156} = 1.5$, $P = 0.217$; sex × body size: $F_{1,156} = 1.5$, $P = 0.223$). Hence, although no other measure of paddle shape and size showed a significant correlation with body size, these analyses show that paddles do grow slightly more symmetrical with size. We also note that the pattern of asymmetry in leg paddles was consistent with fluctuating, rather than directional, asymmetry (Appendix 3).

We found positive allometry for tibial paddle size in both males and females (see Table 3). There was, however, no significant difference from isometry for tarsal paddle size. Notably, tarsal leg segment length (carrying the tarsal component of the paddle) did show positive allometry in both sexes. In contrast, there was little evidence for deviations from isometry for fore- and hind-leg segments.

## DISCUSSION

Studies of the pattern of morphological variation of sexual ornaments have a long history (Huxley, 1932), and many authors have suggested that one can gain insights into development,

**Table 3.** Estimates of allometric scaling coefficients of leg paddles and leg segment lengths in the two sexes

| Trait | Sex | Slope | SE | $r_p$ | $t$ |
|---|---|---|---|---|---|
| Tibial paddle size | Female | 1.931 | 0.183 | 0.528 | **5.080** |
|  | Male | 2.164 | 0.226 | 0.360 | **5.155** |
| Tarsal paddle size | Female | 0.890 | 0.087 | 0.494 | 1.270 |
|  | Male | 0.931 | 0.103 | 0.136 | 0.674 |
| Fore femur | Female | 0.992 | 0.068 | 0.791 | 0.110 |
|  | Male | 0.820 | 0.072 | 0.624 | 2.512 |
| Fore tibia | Female | 1.125 | 0.076 | 0.800 | 1.661 |
|  | Male | 0.919 | 0.092 | 0.445 | 0.874 |
| Fore tarsus | Female | 1.219 | 0.105 | 0.637 | 2.087 |
|  | Male | 0.933 | 0.094 | 0.443 | 0.717 |
| Hind femur | Female | 1.067 | 0.076 | 0.768 | 0.880 |
|  | Male | 0.913 | 0.088 | 0.511 | 0.997 |
| Hind tibia | Female | 1.107 | 0.077 | 0.782 | 1.388 |
|  | Male | 0.915 | 0.088 | 0.504 | 0.960 |
| Hind tarsus | Female | 1.786 | 0.149 | 0.666 | **5.276** |
|  | Male | 1.223 | 0.121 | 0.470 | 1.845 |
| Mid femur | Female | 1.078 | 0.072 | 0.801 | 1.085 |
|  | Male | 0.889 | 0.081 | 0.579 | 1.363 |
| Mid tibia | Female | 1.110 | 0.094 | 0.653 | 1.172 |
|  | Male | 0.982 | 0.099 | 0.435 | 0.187 |
| Mid tarsus | Female | 1.469 | 0.135 | 0.573 | **3.485** |
|  | Male | 1.700 | 0.189 | 0.088 | **3.697** |

*Note*: Traits that show significant allometry (i.e. $\beta \neq 1$; $t$-test of $H_0$: $\beta = 1$) are indicated in **bold**. SE = standard error.

past selection, and evolutionary history from the extant pattern of trait variation alone (see McKinney and McNamara, 1991). However, it is now clear that the strength of such inferences is somewhat limited. For example, several models have predicted that ornaments should often show positive allometry (Grafen, 1990a, 1990b; Kodric-Brown *et al.*, 2006). Yet, as outlined in the Introduction, Bonduriansky and Day (2003) clearly showed that the classic sexual selection scenario does not necessarily result in positive allometry. Nevertheless, positive allometry is a characteristic of ornamental traits used to visually attract mates in many taxa (see Kodric-Brown *et al.*, 2006) and a number of important insights have been gained from studies of phenotypic variation of ornamental traits. In insects, studies of variation and allometry have been key in understanding the evolution and maintenance of sexually selected 'ornamental' traits in, for example, stalk-eyed flies (Wilkinson and Dodson, 1997), horned beetles (Emlen *et al.*, 2007), earwigs (Simmons and Tomkins, 1996), and butterflies (Kemp, 2006). Below, we first ask whether the pattern of trait variation in the leg paddles of both sexes of *S. cyaneus* conforms to the predictions under mutual mate choice. We then discuss how compatible our results are with the possibility that ornament expression in females is the result of an

intersexual genetic correlation, and finish with what we consider to be the most important implications of our findings.

The ornamental leg paddles of *S. cyaneus* showed many of the classic hallmarks of a condition-dependent (*sensu* Rowe and Houle, 1996) ornament under directional sexual selection in both sexes. First, variation in the size of the leg paddles was much greater than in general traits. While the latter showed a degree of phenotypic variation typical for morphological traits (CV: 2–7%), the leg paddles showed a variation (CV: 12–17%) that was even greater than the typical ornament in birds (Cuervo and Møller, 1999). Second, the degree of fluctuating asymmetry of one aspect of the shape of the tarsal paddle was negatively related to size, as might be expected for condition-dependent ornaments (Watson and Thornhill, 1994; Swaddle, 2003; Polak and Starmer, 2005). Third, in contrast to most general traits, the major part of the ornamental leg paddles showed a strongly positive allometric scaling coefficient (~2).

However, we note that our results are not fully in accordance with the predicted patterns for a sexually selected ornament. First, the smaller of the two leg paddle components, the tarsal paddle, did not show positive allometry. However, the length of the tarsal segment upon which this ornament component is situated did show positive allometry. As this segment is itself an integrated part of the ornament (see Fig. 1), it is reasonable to nevertheless conclude that the ornament as an integrated structure showed positive allometry. Second, a trait that we do not consider to be under sexual selection, the length of the hind tarsus in females, also showed positive allometry. It is worth noting that this pattern was again seen on the tarsus, albeit of a leg that does not carry the ornament. We suggest that this may result from developmental integration of tarsi across legs. Indeed, regulation of tarsal development in *Drosophila* has been shown to be dependent on a molecular mechanism that is distinct to the other leg segments (Campbell and Tomlinson, 1998; Rauskolb, 2001). Third, as mentioned in the previous paragraph, only the tarsal paddle showed a significant negative relationship between fluctuating asymmetry and size and this was only true for an aspect of paddle shape that describes a sub-dominant form of paddle shape variation (i.e. PC2). Unfortunately, we did not explore environmental effects on trait expression and thus we are restricted in our ability to draw detailed inferences as to the presence or lack of fluctuating asymmetry in particular aspects of ornament morphology (Polak and Starmer, 2005). We note, however, that no aspect of fluctuating asymmetry of the ornament showed a positive relationship with size.

Despite these departures from ideal conformity with predictions, the results are generally consistent with the hypothesis that the leg paddles of both males and females are sexually selected. This is further supported by the fact that the ornament varied little in size and shape between the sexes and had similar allometric scaling coefficients in males and females. If anything, leg paddles tended to be proportionally larger and more variable in females and leg paddle area increased more rapidly with body size in females than in males. We stress that the pattern we document here is strikingly different from that of most other taxa, and we know of no comparable findings in any taxa. In birds with some female expression of male ornaments that are assumed to be under sexual selection only in males, females show less variation and a much lower allometric scaling coefficient than do males (see Cuervo and Møller, 1999). Thus, our results suggest that the expression of this ornament is at least as condition dependent in females as it is in males. Unfortunately, we know of no other detailed morphometric analyses of ornaments expressed in both sexes that are thought to be used in mutual mate choice.

Two facets of our results are particularly difficult to reconcile with the hypothesis that the

expression of leg paddles in *S. cyaneus* females represents a non-adaptive correlated response to sexual selection in males only. First, the pattern of ornament variation in females showed the classic hallmarks of directional sexual selection to a greater, rather than lesser, extent compared with males. This is in stark contrast to bird systems where female ornament expression is thought to be a non-adaptive correlated response to sexual selection in males (Cuervo and Møller, 1999). Second, the fact that the leg paddle ornament was basically sexually monomorphic would only be predicted if trait expression was cost-free in females. However, there should be at least some size-dependent viability costs of this ornament, because both costs of growth (allocation of cuticular material during metamorphosis) and maintenance [much time is spent grooming (personal observation)] of leg paddles should be condition dependent to some extent. In addition to the present results, the fact that experimental removal of the paddles has a larger, rather than smaller, effect on mating rate in females than males (Hancock *et al.*, 1990b) is clearly not expected if the ornament is only under sexual selection in males. Although these considerations imply that it is very unlikely that an intersexual genetic correlation is responsible for ornament expression in female *S. cyaneus*, it remains theoretically possible that this is the case. If, for example, the costs of ornament expression are much lower in females, sex-specific natural selection could interact with a correlated response and result in proportionately larger and more variable leg paddles in females. However, if this was true, our model system would provide the most extreme case of an ornament being expressed in females that was primarily under selection in males and would challenge current intra-locus sexual conflict theory (Fisher, 1930; Lande, 1980, 1987; Bonduriansky and Rowe, 2005), considering that ornament expression is certainly associated with at least some costs in females.

Although there is growing empirical evidence for male mate choice in polygynous species (for reviews, see Amundsen, 2000; Bonduriansky, 2001; Kraaijeveld *et al.*, 2007), the theoretical basis for this phenomenon is not trivial because the operational sex ratio is often likely to be male biased and females are likely to have an equal mating success in such systems. South and colleagues (submitted) explored the possibility that male *S. cyaneus* may be investing heavily into courtship and mating and thus be expected to be choosy (Gwynne, 1991, 1993; Johnson and Burley, 1997). They found longevity costs due to courtship and copulation that would decrease a male's future mating success, but whether these costs are large enough to maintain male mate choice in this system is unclear. Servedio and Lande (2006) recently showed that male choosiness can be maintained under polygyny even with modest male mating investment. This can occur when, for example, female signals are not arbitrary but correlate with fecundity or genetic quality. In *S. cyaneus*, there is a positive relationship between female paddle area and body size, which is generally correlated with fecundity in insects (Bonduriansky, 2001). In addition, paddle size may signal female age and mating status. Paddle size decreases markedly with age as a result of wear (personal observation) and female lifespan is remarkably long both in the laboratory (South and Arnqvist, 2008) and in the field (Dégallier *et al.*, 1998). Young, and thus also virgin, females should therefore have on average larger paddles. An additional possibility is that males are under selection to direct courtship towards conspecifics to avoid costly heterospecific matings (Servedio, 2007). *Sabethes cyaneus* is sympatric with several congeners throughout most of its distribution, and there is striking variation across species in the number of legs that carry ornaments as well as in their size and coloration (Lane and Cerqueira, 1942). Thus, males that direct their courtship efforts towards females with large paddles are not only likely to court more fecund females, but also unmated (i.e. receptive) females of the right species.

We acknowledge that mutual mate choice should, ideally, be demonstrated by detailed experimental studies of the role of paddle size during interactions between the sexes. Such studies are underway in this system and may provide direct behavioural evidence for mutual mate choice. Nonetheless, by applying geometric morphometrics to explore patterns of variation and sexual dimorphism in *S. cyaneus*, we have gained valuable insights into the likely selective pressures that act upon the leg paddle ornament in this insect species. Although the data presented here are not entirely conclusive (cf. Bonduriansky and Day, 2003), they present a rare and important case in a comparative sense and provide a benchmark for future research on systems with sexually monomorphic ornaments.

## ACKNOWLEDGEMENTS

## REFERENCES

Alexander, R.D., Marshall, D.C. and Cooley, J.R. 1997. Evolutionary perspectives on insect mating. In *The Evolution of Mating Systems in Insects and Arachnids* (J.C. Choe and B.J. Crespi, eds.), pp. 4–31. Cambridge: Cambridge University Press.

Allen, B.J. and Levinton, J.S. 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. *Funct. Ecol.*, **21**: 154–161.

Amundsen, T. 2000. Why are female birds ornamented? *Trends Ecol. Evol.*, **15**: 149–155.

Andersson, M. 1994. *Sexual Selection*. Princeton, NJ: Princeton University Press.

Armbruster, P. and Hutchinson, R.A. 2002. Pupal mass and wing length as indicators of fecundity in *Aedes albopictus* and *Aedes geniculatus*. *J. Med. Entomol.*, **39**: 699–704.

Arnqvist, G. and Nilsson, T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim. Behav.*, **60**: 145–164.

Badyaev, A.V. 2004. Developmental perspective on the evolution of sexual ornaments. *Evol. Ecol. Res.*, **6**: 975–991.

Beeler, A.E., Rauter, C.M. and Moore, A.J. 2002. Mate discrimination by females in the burying beetle *Nicrophorus orbicollis*: the influence of male size on attractiveness to females. *Ecol. Entomol.*, **27**: 1–6.

Bonduriansky, R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biol. Rev.*, **76**: 305–339.

Bonduriansky, R. 2007. Sexual selection and allometry: a critical reappraisal of the evidence and ideas. *Evolution*, **61**: 838–849.

Bonduriansky, R. and Day, T. 2003. The evolution of static allometry in sexually selected traits. *Evolution*, **57**: 2450–2458.

Bonduriansky, R. and Rowe, L. 2005. Intralocus sexual conflict and the genetic architecture of sexually dimorphic traits in *Prochyliza xanthostoma* (Diptera: Piophilidae). *Evolution*, **59**: 1965–1975.

Burkhardt, D. and de la Motte, I. 1988. Big 'antlers' are favoured: female choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. *J. Comp. Physiol A: Sensory Neural Behav. Physiol.*, **162**: 649–652.

Burley, N. 1981. The evolution of sexual indistinguishability. In *Natural Selection and Social Behaviour: Recent Research and New Theory* (R.D. Alexander and D.W. Tinkle, eds.), pp. 121–137. New York: Chiron Press.

Campbell, G. and Tomlinson, A. 1998. The roles of the homeobox genes *aristaless* and *Distal-less* in patterning the legs and wings of *Drosophila*. *Development*, **125**: 4483–4493.

Cook, S.E., Vernon, J.G., Bateson, M. and Guilford, T. 1994. Mate choice in the polymorphic African swallowtail butterfly, *Papilio dardanus* – male-like females may avoid sexual harassment. *Anim. Behav.*, **47**: 389–397.

Cotton, S., Fowler, K. and Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependence expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B*, **271**: 771–783.

Cotton, S., Rogers, D.W., Small, J., Pomiankowski, A. and Fowler, K. 2006. Variation in preference for a male ornament is positively associated with female eyespan in the stalk-eyed fly *Diasemopsis meigenii. Proc. R. Soc. Lond. B*, **273**: 1287–1292.

Coyne, J.A. and Orr, H.A. 2004. *Speciation*. Sunderland, MA: Sinauer Associates.

Cuervo, J.J. and Møller, A.P. 1999. Phenotypic variation and fluctuating asymmetry in sexually dimorphic feather ornaments in relation to sex and mating system. *Biol. J. Linn. Soc.*, **68**: 505-529.

Darwin, C. 1871. *The Descent of Man, and Sexual Selection in Relation to Sex*. London: John Murray.

Dégallier, N., Sa, G.C., Monteiro, H.A.O., Castro, F.C., Da Silva, O.V., Brandao, R.C.F. *et al.* 1998. Release–recapture experiments with canopy mosquitoes in the genera *Haemagogus* and *Sabethes* (Diptera: Culicidae) in Brazilian Amazonia. *J. Med. Entomol.*, **35**: 931–936.

Dunn, P.O., Whittingham, L.A. and Pitcher, T.E. 2001. Mating systems, sperm competition, and the evolution of sexual dimorphism in birds. *Evolution*, **55**: 161–175.

Emlen, D.J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond. B*, **256**: 131–136.

Emlen, D.J. and Nijhout, H.F. 2000. The development and evolution of exaggerated morphologies in insects. *Annu. Rev. Entomol.*, **45**: 661–708.

Emlen, D.J., Lavine, L.C. and Ewen-Campen, B. 2007. On the origin and evolutionary diversification of beetle horns. *Proc. Natl. Acad. Sci. USA*, **104**: 8661–8668.

Evans, M.R. 2004. Limits on the evolution of tail ornamentation in birds. *Am. Nat.*, **163**: 341–357.

Ferson, S., Rohlf, F.J. and Koehn, R.K. 1985. Measuring shape variation of two-dimensional outlines. *Syst. Zool.*, **34**: 59–68.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.

Forbes, M.R., Schalk, G., Miller, J.G. and Richardson, J.M.L. 1997. Male–female morph interactions in the damselfly *Nehalennia irene* (Hagen). *Can. J. Zool.*, **75**: 253–260.

Godin, J.G.J. and McDonough, H.E. 2003. Predator preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behav. Ecol.*, **14**: 194–200.

Grafen, A. 1990a. Biological signals as handicaps. *J. Theor. Biol.*, **144**: 517–546.

Grafen, A. 1990b. Sexual selection unhandicapped by the Fisher process. *J. Theor. Biol.*, **144**: 475–516.

Green, A.J. 1992. Positive allometry is likely with mate choice, competitive display and other functions. *Anim. Behav.*, **43**: 170–172.

Gwynne, D.T. 1991. Sexual competition among females: what causes courtship-role reversal? *Trends Ecol. Evol.*, **6**: 118–121.

Gwynne, D.T. 1993. Food quality controls sexual selection in mormon crickets by altering male mating investment. *Ecology*, **74**: 1406–1413.

Hancock, R.G., Foster, W.A. and Yee, W.L. 1990a. Courtship behavior of the mosquito *Sabethes cyaneus* (Diptera: Culicidae). *J. Insect Behav.*, **3**: 401–416.

Hancock, R.G., Yee, W.L. and Foster, W.A. 1990b. Tests of *Sabethes cyaneus* leg paddle function in mating and flight. *J. Am. Mosquito Control Assoc.*, **6**: 733–735.

Harbach, R.E. and Petersen, J.L. 1992. Two species previously confused under the concept of *Sabethes tarsopus* in Central America (Diptera: Culicidae). *Mosquito Syst.*, **24**: 102–124.

Hingle, A., Fowler, K. and Pomiankowski, A. 2001. Size-dependent female mate preference in the stalk-eyed fly, *Cyrtodiopsis dalmanni. Anim. Behav.*, **61**: 589–595.

Hooper, P.L. and Miller, G.F. 2008. Mutual mate choice can drive costly signaling even under perfect monogamy. *Adaptive Behav.*, **16**: 53–69.

Huxley, J.S. 1914. The courtship habits of the great crested grebe (*Podiceps cristatus*); with and addition to the theory of sexual selection. *Proc. Zool. Soc. Lond.*, **35**: 491–562.

Huxley, J.S. 1932. *Problems of Relative Growth*. New York: Lincoln Mac Veagh – The Dial Press.

Jang, Y. and Greenfield, M.D. 1996. Ultrasonic communication and sexual selection in wax moths: female choice based on energy and asynchrony of male signals. *Anim. Behav.*, **51**: 1095–1106.

Jang, Y. and Greenfield, M.D. 1998. Absolute versus relative measurements of sexual selection: assessing the contributions of ultrasonic signal characters to mate attraction in lesser wax moths, *Achroia grisella* (Lepidoptera: Pyralidae). *Evolution*, **52**: 1383–1393.

Jennions, M.D. 1993. Female choice in birds and the cost of long tails. *Trends Ecol. Evol.*, **8**: 230–232.

Johnson, K. and Burley, N.T. 1997. Mating tactics and mating systems of birds. *Ornithol. Monogr.*, **1997**: 21–60.

Jones, I.L. and Hunter, F.M. 1993. Mutual sexual selection in a monogamous seabird. *Nature*, **362**: 238–239.

Jones, I.L. and Hunter, F.M. 1999. Experimental evidence for mutual inter- and intrasexual selection favouring a crested auklet ornament. *Anim. Behav.*, **57**: 521–528.

Judd, D.D. 1996. Review of the systematics and phylogenetic relationships of Sabethini (Diptera: Culicidae). *Syst. Entomol.*, **21**: 129–150.

Kemp, D.J. 2006. Heightened phenotypic variation and age-based fading of ultraviolet butterfly wing coloration. *Evol. Ecol. Res.*, **8**: 515–527.

Kemp, D.J. and Rutowski, R.L. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly colorations. *Evolution*, **61**: 168–183.

Kimball, R.T. and Ligon, J.D. 1999. Evolution of avian plumage dichromatism from a proximate perspective. *Am. Nat.*, **154**: 182–193.

Klingenberg, C.P. 1996. Multivariate allometry. In *Advances in Morphometrics* (L.F. Marcus, M. Corti, A. Loy, G.J.P. Naylor, and D.E. Slice, eds.), pp. 23–49. New York: Plenum Press.

Klingenberg, C.P. and Zimmermann, M. 1992. Static, ontogenetic, and evolutionary allometry: a multivariate comparison in nine species of water striders. *Am. Nat.*, **140**: 601–620.

Knell, R.J., Pomfret, J.C. and Tomkins, J.L. 2004. The limits of elaboration: curved allometries reveal the constraints on mandible size in stag beetles. *Proc. R. Soc. Lond. B*, **271**: 523–528.

Kodric-Brown, A., Sibly, R.M. and Brown, J.H. 2006. The allometry of ornaments and weapons. *Proc. Natl. Acad. Sci. USA*, **103**: 8733–8738.

Kraaijeveld, K., Gregurke, J., Hall, C., Komdeur, J. and Mulder, R.A. 2004. Mutual ornamentation, sexual selection, and social dominance in the black swan. *Behav. Ecol.*, **15**: 380–389.

Kraaijeveld, K., Kraaijeveld, F.J.L. and Komdeur, J. 2007. The evolution of mutual ornamentation. *Anim. Behav.*, **74**: 657–677.

Land, M.F., Gibson, G., Horwood, J. and Zeil, J. 1999. Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *J. Comp. Physiol A: Sensory Neural Behav. Physiol.*, **185**: 91–103.

Lande, R. 1980. Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution*, **34**: 292–305.

Lande, R. 1987. Genetic correlations between the sexes in the evolution of sexual dimorphism and mating preferences. In *Sexual Selection: Testing the Alternatives* (J.W. Bradbury and M.B. Andersson, eds.), pp. 83–95. Chichester: Wiley.

Lande, R. and Arnold, S.J. 1985. Evolution of mating preference and sexual dimorphism. *J. Theor. Biol.*, **117**: 651–664.

Lane, J. and Cerqueira, N.L. 1942. Os sabetineos da América (Diptera: Culicidae). *Arquivos de Zoologia*, **3**: 473–849.

Langmore, N.E. and Bennett, A.T.D. 1999. Strategic concealment of sexual identity in an estrildid finch. *Proc. R. Soc. Lond. B*, **266**: 543–550.

Ligon, J.D. 1999. *Evolution of Avian Breeding Systems*. Oxford: Oxford University Press.

McKinney, M.L. and McNamara, K.J. 1991. *Heterochrony: Evolution of Ontogeny*. New York: Plenum Press.

Munoz, A., Aparicio, J.M. and Bonal, R. 2008. Male barn swallows use differential resource allocation rules to produce ornamental tail feathers. *Behav. Ecol.*, **19**: 404–409.

Okazawa, T., Horio, M., Suzuki, H. and Mogi, M. 1986. Colonization and laboratory bionomics of *Topomyia yanbarensis* (Diptera: Culicidae). *J. Med. Entomol.*, **23**: 493–501.

Petrie, M. 1992. Are all secondary sexual display structures positively allometric and, if so, why? *Anim. Behav.*, **43**: 173–175.

Philips, T.K., Hancock, R.G. and Foster, W.A. 1996. Epigamic display and unique mating position in *Wyeomyia arthrostigma* (Diptera: Culicidae). *J. Insect Behav.*, **9**: 739–753.

Polak, M. and Starmer, W.T. 2005. Environmental origins of sexually selected variation and a critique of the fluctuating asymmetry-sexual selection hypothesis. *Evolution*, **59**: 577–585.

Rauskolb, C. 2001. The establishment of segmentation in the *Drosophila* leg. *Development*, **128**: 4511–4521.

Ricker, W.E. 1984. Computation and uses of general trend lines. *Can. J. Zool.*, **62**: 1897–1905.

Rohlf, F.J. 1992. The analysis of shape variation using ordinations of fitted functions. In *Ordination in the Study of Morphology, Evolution and Systematics of Insects* (J.T. Sorensen and R. Foottit, eds.), pp. 95–112. Amsterdam: Elsevier.

Rohlf, F.J. and Archie, J.A. 1984. Comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). *Syst. Zool.*, **33**: 302–317.

Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. R. Soc. Lond. B*, **263**: 1415–1421.

Sawada, M. 1999. *ModelII: Geometric Mean Regression Add-In for Excel '97*. Freeware by M. Sawada, University of Ottawa.

Schluter, D. and Price, T. 1993. Honesty, perception and population divergence in sexually selected traits. *Proc. R. Soc. Lond. B*, **253**: 117–122.

Servedio, M.R. 2007. Male versus female mate choice: sexual selection and the evolution of species recognition via reinforcement. *Evolution*, **61**: 2772–2789.

Servedio, M.R. and Lande, R. 2006. Population genetic models of male and mutual mate choice. *Evolution*, **60**: 674–685.

Shannon, R.C. 1931. On the classification of Brazilian Culicidae with special reference to those capable of harboring the yellow fever virus. *Proc. Entomol. Soc. Wash.*, **33**: 125–164.

Shuster, S.M. and Wade, M.J. 2003. *Mating Systems and Strategies*. Princeton, NJ: Princeton University Press.

Siegel, J.P., Novak, R.J., Lampman, R.L. and Steinly, B.A. 1992. Statistical appraisal of the weight–wing length relationship of mosquitos. *J. Med. Entomol.*, **29**: 711–714.

Simmons, L.W. 1986. Female choice in the field cricket *Gryllus bimaculatus* (De Geer). *Anim. Behav.*, **34**: 1463–1470.

Simmons, L.W. and Tomkins, J.L. 1996. Sexual selection and the allometry of earwig forceps. *Evol. Ecol.*, **10**: 97–104.

Sivinski, J. 1997. Ornaments in the Diptera. *Florida Entomol.*, **80**: 142–164.

Slice, D.E. 2002. *Morpheus et al. – Multiplatform Software for Morphometrics Analysis*.

Sokal, R.R. and Rohlf, F.J. 1995. *Biometry: The Principles and Practice of Statistics in Biological Research*. New York: W.H. Freeman.

South, S.H. and Arnqvist, G. 2008. Evidence of monandry in a mosquito (*Sabethes cyaneus*) with elaborate ornaments in both sexes. *J. Insect Behav.*, **21**: 451–459.

Storey, J.D. 2003. The positive false discovery rate: A Bayesian interpretation and the q-value. *Ann. Stat.*, **31**: 2013–2035.

Swaddle, J.P. 2003. Fluctuating asymmetry, animal behavior, and evolution. *Adv. Study Behav.*, **32**: 169–205.

Thornhill, R. and Alcock, J. 1983. *The Evolution of Insect Mating Systems*. Cambridge, MA: Harvard University Press.

Trivers, R.L. 1972. Parental investment and sexual selection. In *Sexual Selection and the Descent of Man* (B. Campbell, ed.), pp. 136–179. London: Heinemann.

Van Gossum, H., Beirinckx, K., Forbes, M.R. and Sherratt, T.N. 2007. Do current hypotheses explain continental and seasonal variation in female morph frequencies of the damselfly, *Nehalennia irene*? *Biol. J. Linn. Soc.*, **90**: 501–508.

Watson, P.J. and Thornhill, R. 1994. Fluctuating asymmetry and sexual selection. *Trends Ecol. Evol.*, **9**: 21–24.

West-Eberhard, M.J. 1983. Sexual selection, social competition, and speciation. *Q. Rev. Biol.*, **58**: 155–183.

Wiley, R.H. and Poston, J. 1996. Indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution*, **50**: 1371–1381.

Wilkinson, G.S. and Dodson, G.N. 1997. Function and evolution of antlers and eye-stalks in flies. In *Mating Systems in Insects and Arachnids* (J.C. Choe and B.J. Crespi, eds.), pp. 310–328. Cambridge: Cambridge University Press.

Wilkinson, G.S. and Reillo, P.R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. *Proc. R. Soc. Lond. B*, **255**: 1–6.

Zsemlye, J.L., Hancock, R.G. and Foster, W.A. 2005. Analysis of a complex vertical copulatory-courtship display in the yellow fever vector *Sabethes chloropterus*. *Med. Vet. Entomol.*, **19**: 276–285.

## APPENDICES

**Appendix 1.** The percentage of variance explained by each of the 10 first principal components of variation in leg paddle shape

| | Principal component | Percentage of variance explained |
|---|---|---|
| Tibial paddle | 1 | 57.610 |
| | 2 | 13.015 |
| | 3 | 9.145 |
| | 4 | 4.806 |
| | 5 | 4.210 |
| | 6 | 2.809 |
| | 7 | 2.481 |
| | 8 | 1.619 |
| | 9 | 1.047 |
| | 10 | 0.616 |
| Tarsal paddle | 1 | 52.278 |
| | 2 | 16.107 |
| | 3 | 9.584 |
| | 4 | 5.421 |
| | 5 | 4.139 |
| | 6 | 2.255 |
| | 7 | 1.914 |
| | 8 | 1.627 |
| | 9 | 1.290 |
| | 10 | 0.884 |

**Appendix 2.** Repeatabilities, estimated as Pearson's correlation coefficients, of all measurements taken

| Trait | Side | Female | Male | Joint |
|---|---|---|---|---|
| Wing | Left | 0.87 | 0.70 | 0.87 |
| Thorax | Left | 0.62 | 0.73 | 0.66 |
| Proboscis | Left | 0.87 | 0.60 | 0.78 |
| Antenna | Left | 0.66 | 0.64 | 0.87 |
| Fore femur | Left | 0.89 | 0.81 | 0.87 |
| Fore tibia | Left | 0.93 | 0.77 | 0.87 |
| Fore tarsus | Left | 0.96 | 0.87 | 0.95 |
| Hind femur | Left | 0.94 | 0.70 | 0.90 |
| Hind tibia | Left | 0.91 | 0.73 | 0.88 |
| Hind tarsus | Left | 0.99 | 0.74 | 0.94 |
| Mid femur | Left | 0.96 | 0.76 | 0.92 |
| Mid tibia | Left | 0.81 | 0.67 | 0.82 |
| Mid tarsus | Left | 0.73 | 0.64 | 0.93 |
| Tibial paddle | | | | |
| *Area* | Left | 0.98 | 0.97 | 0.98 |
| *Shape PC1* | Left | 0.98 | 0.97 | 0.98 |
| *Shape PC2* | Left | 0.76 | 0.92 | 0.85 |
| *Shape PC3* | Left | 0.78 | 0.91 | 0.88 |
| *Shape PC4* | Left | 0.80 | 0.66 | 0.78 |
| *Shape PC5* | Left | 0.51 | 0.70 | 0.59 |
| *Area* | Right | 0.98 | 0.90 | 0.97 |
| *Shape PC1* | Right | 0.98 | 0.99 | 0.98 |
| *Shape PC2* | Right | 0.92 | 0.83 | 0.88 |
| *Shape PC3* | Right | 0.80 | 0.96 | 0.93 |
| *Shape PC4* | Right | 0.71 | 0.96 | 0.91 |
| *Shape PC5* | Right | 0.72 | 0.88 | 0.80 |
| *Asymmetry Area* | N/A | 0.97 | 0.91 | 0.94 |
| *Asymmetry Shape PC1* | N/A | 0.97 | 0.97 | 0.97 |
| *Asymmetry Shape PC2* | N/A | 0.72 | 0.77 | 0.74 |
| *Asymmetry Shape PC3* | N/A | 0.82 | 0.94 | 0.89 |
| *Asymmetry Shape PC4* | N/A | 0.80 | 0.93 | 0.89 |
| *Asymmetry Shape PC5* | N/A | 0.67 | 0.75 | 0.72 |
| Tarsal paddle | | | | |
| *Area* | Left | 0.94 | 0.92 | 0.96 |
| *Shape PC1* | Left | 0.90 | 0.95 | 0.92 |
| *Shape PC2* | Left | 0.70 | 0.88 | 0.84 |
| *Shape PC3* | Left | 0.68 | 0.34 | 0.47 |
| *Shape PC4* | Left | 0.58 | 0.59 | 0.62 |
| *Shape PC5* | Left | 0.72 | 0.81 | 0.76 |
| *Area* | Right | 0.97 | 0.90 | 0.97 |
| *Shape PC1* | Right | 0.96 | 0.96 | 0.96 |
| *Shape PC2* | Right | 0.86 | 0.86 | 0.88 |
| *Shape PC3* | Right | 0.78 | 0.76 | 0.78 |
| *Shape PC4* | Right | 0.63 | 0.67 | 0.66 |
| *Shape PC5* | Right | 0.78 | 0.87 | 0.84 |
| *Asymmetry Area* | N/A | 0.79 | 0.76 | 0.78 |
| *Asymmetry Shape PC1* | N/A | 0.87 | 0.88 | 0.87 |
| *Asymmetry Shape PC2* | N/A | 0.84 | 0.87 | 0.85 |
| *Asymmetry Shape PC3* | N/A | 0.59 | 0.48 | 0.51 |
| *Asymmetry Shape PC4* | N/A | 0.39 | 0.58 | 0.54 |
| *Asymmetry Shape PC5* | N/A | 0.68 | 0.86 | 0.78 |

**Appendix 3.** Tests of directional asymmetry in components of leg paddle morphology

| Trait | Sex | Mean | $t$ | d.f. | $P$ |
|---|---|---|---|---|---|
| Tibial paddle | | | | | |
| *Area* | Joint | 0.002 | 0.267 | 159 | 0.790 |
| *Shape PC1* | Joint | −0.002 | −0.256 | 159 | 0.800 |
| *Shape PC2* | Joint | −0.001 | −0.354 | 159 | 0.724 |
| *Shape PC3* | Joint | −0.004 | −1.162 | 159 | 0.247 |
| *Shape PC4* | Joint | 0.003 | 1.325 | 159 | 0.187 |
| *Shape PC5* | Joint | 0.001 | 0.556 | 159 | 0.579 |
| Tarsal paddle | | | | | |
| *Area* | Joint | 0.004 | 0.859 | 159 | 0.391 |
| *Shape PC1* | Joint | 0.008 | 1.230 | 159 | 0.220 |
| *Shape PC2* | Joint | −0.001 | −0.178 | 159 | 0.859 |
| *Shape PC3* | Joint | −0.001 | −0.291 | 159 | 0.771 |
| *Shape PC4* | Joint | 0.005 | 2.119 | 159 | **0.036** |
| *Shape PC5* | Joint | −0.004 | −1.765 | 159 | 0.080 |
| Tibial paddle | | | | | |
| *Area* | Female | −0.006 | −0.463 | 79 | 0.645 |
| *Shape PC1* | Female | 0.015 | 1.195 | 79 | 0.236 |
| *Shape PC2* | Female | 0.001 | 0.116 | 79 | 0.908 |
| *Shape PC3* | Female | −0.007 | −1.670 | 79 | 0.099 |
| *Shape PC4* | Female | 0.003 | 0.929 | 79 | 0.356 |
| *Shape PC5* | Female | 0.003 | 0.838 | 79 | 0.405 |
| Tarsal paddle | | | | | |
| *Area* | Female | −0.004 | −0.568 | 79 | 0.571 |
| *Shape PC1* | Female | 0.001 | 0.085 | 79 | 0.933 |
| *Shape PC2* | Female | 0.007 | 1.318 | 79 | 0.191 |
| *Shape PC3* | Female | −0.006 | −1.235 | 79 | 0.221 |
| *Shape PC4* | Female | 0.006 | 1.786 | 79 | 0.078 |
| *Shape PC5* | Female | −0.003 | −1.165 | 79 | 0.247 |
| Tibial paddle | | | | | |
| *Area* | Male | 0.011 | 0.942 | 79 | 0.349 |
| *Shape PC1* | Male | −0.020 | −1.699 | 79 | 0.093 |
| *Shape PC2* | Male | −0.003 | −0.715 | 79 | 0.477 |
| *Shape PC3* | Male | −0.000 | −0.070 | 79 | 0.945 |
| *Shape PC4* | Male | 0.004 | 0.967 | 79 | 0.337 |
| *Shape PC5* | Male | −0.000 | −0.044 | 79 | 0.965 |
| Tarsal paddle | | | | | |
| *Area* | Male | 0.011 | 2.193 | 79 | **0.031** |
| *Shape PC1* | Male | 0.015 | 1.584 | 79 | 0.117 |
| *Shape PC2* | Male | −0.008 | −1.157 | 79 | 0.250 |
| *Shape PC3* | Male | 0.004 | 0.741 | 79 | 0.461 |
| *Shape PC4* | Male | 0.004 | 1.243 | 79 | 0.218 |
| *Shape PC5* | Male | −0.005 | −1.319 | 79 | 0.191 |

*Note*: Significant values ($P = 0.05$; no compensation for multiple tests) are highlighted in **bold**.