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Size matters, but so does shape: quantifying complex shape changes in a sexually selected trait in stalk-eyed flies (Diptera: Diopsidae)

AMY M. WORTHINGTON[1]*, CHELSEA M. BERNS[1] and JOHN G. SWALLOW[2]

[1]*Department of Ecology, Evolution, and Organismal Biology, Iowa State University, Ames, IA 50011, USA*
[2]*Department of Biology, The University of South Dakota, Vermillion, SD 57069, USA*

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The elaborate morphologies of sexually selected ornaments are ubiquitous across the animal kingdom. In studies investigating these traits, ornament size is frequently the focus, and empirical evidence supports its positive correlation with fitness. Yet shape plays an important role and, surprisingly, is often overlooked. Shape frequently changes with size, influenced by biomechanical, developmental, or performance constraints. Therefore, shape can provide additional insights into the morphological differences between individuals and the potential limits on sexual trait exaggeration. Here, we used landmark-based geometric morphometric methods on a sexually dimorphic species of stalk-eyed fly (*Teleopsis dalmanni*) to examine patterns of sexual shape dimorphism. Our analyses reveal a significant difference in head shape between the sexes, with males exhibiting smaller eye bulbs, thinner stalks, and smaller heads than females. Additionally, as eyestalk length increases within each sex, a similar pattern of shape change was observed as that observed between sexes. This pattern of shape change may be the result of constraints acting against further ornament exaggeration, and we suggest that this may significantly impact the whole-organism performance in stalk-eyed flies. © 2012 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **106**, 104–113.

ADDITIONAL KEYWORDS: constraint – geometric morphometrics – sexual dimorphism – sexual selection – stalk-eyed flies – *Teleopsis dalmanni*.

INTRODUCTION

Sexual selection is a pervasive process known to create some of the most bizarre and extreme morphologies observed in the animal kingdom. This array of conspicuous signals, ornaments, and armaments provide their bearer with a competitive advantage over conspecifics, and increase mate acquisition abilities, ultimately leading to greater offspring production (Andersson, 1994). However, these same traits can be associated with negative fitness effects, preventing trait evolution without limits. Indeed, these costs are required in many theoretical models of sexual selection (Fisher, 1930; Zahavi, 1975; Grafen, 1990; Kotiaho, 2001). Costs and trade-offs associated with producing and maintaining elaborate secondary structures are becoming increasingly well documented. These include direct survivorship costs associated with an increased risk of predation (Stuart-Fox *et al*., 2003; Basolo & Wagner, 2004), injury in male–male competition (Bean & Cook, 2001), and parasitism (e.g. Zuk & Kolluru, 1998). There may also be indirect costs associated with physiological and/or energetic trade-offs (e.g. Vehrencamp, Bradbury & Gibson, 1989; Kotiaho *et al*., 1998), locomotive disadvantages (reviewed in Kotiaho, 2001; Oufiero & Garland, 2007), or production and/or maintenance costs (Nijhout & Emlen, 1998; Emlen, 2001; Basolo & Alcaraz, 2003; Allen & Levinton, 2007).

Despite these non-trivial costs, individuals bearing more exaggerated structures are predicted to be rewarded with increased reproductive success. Many

*Corresponding author. E-mail: aworthin@iastate.edu

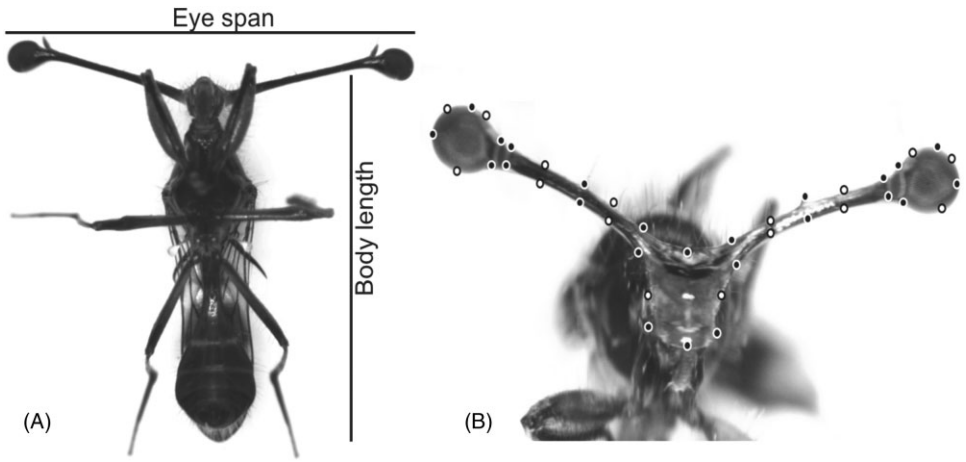


Figure 1. A, linear measurements of eye span and body length. B, locations of the landmarks used in the geometric morphometric analysis of stalk-eyed fly head shape. ●, biologically homologous landmarks; ○, sliding semi-landmarks.

studies have observed the positive relationship between the size of sexually selected ornaments and some aspect of reproductive fitness (Møller *et al*., 1998; Hingle, Fowler & Pomiankowski, 2001). When females also bear ornaments as a result of correlated selection, they are expected to be closer to the optimum set by natural selection (Haas, 1976; Lande, 1980; Anholt, 1997), and hence are smaller in magnitude. Therefore, the sexual size dimorphisms of these traits and their association with sexual dimorphism in the performance of ecologically relevant tasks are often used to investigate performance costs. Although the size of sexually selected traits is of obvious importance, shape plays an important role as well (Hendry *et al*., 2006; Butler, Sawyer & Losos, 2007), and until recently, patterns of sexual shape dimorphism have been largely neglected (Lande & Arnold, 1983; Gidaszewski, Baylac & Klingenberg, 2009).

Influenced by biomechanical, developmental, or performance constraints, the shape of sexually selected traits often changes with size, resulting in phenotypic and genetic correlations that may inhibit the production of exaggerated morphologies. The allocation of limited resources during development may cause size and shape trade-offs as a result of the differential investment of finite energy resources (Emlen, 2001). For example, dung beetle horns positioned at different locations on the body directly trade off with nearby components (Nijhout & Emlen, 1998). Large horns at the front of the head result in beetles with small antennae, horns in the middle of the head trade-off against eye area, and horns at the base of the head are negatively correlated with wing size (Emlen, 2001). These correlations between body parts may represent a developmental source of constraint and may regulate the size of those parts. Biomechanical constraints may also be responsible for correlations between size and shape. For example, head shape has significant impact on the force generated by jaw closure in some amphibians (Adams & Rohlf, 2000) and lizards (Herrel, DeGrauw & Lemos-Espinal, 2001). Lastly, performance may impose constraints on shape because it is tightly correlated with fitness (Arnold, 1983). Any trait that is detrimental to performance capacity is likely to be constrained to certain shapes and sizes. For flying organisms, there are significant aerodynamic costs associated with exaggerated traits (Evans & Thomas, 1992; Møller, de Lope & Saino, 1995). The shape characteristics of sexually selected traits, such as mass, distance from the body, and level of drag, could greatly affect flight performance. For example, male barn swallows have ornamented tails used to attract females. Aerodynamic drag increases with tail size, but the shape of the outermost feathers minimizes this flight cost by being narrower at the tips (Møller, de Lope & Saino, 1995). These sources of constraint may regulate the size and shape of sexually selected traits, preventing further exaggeration or changing the structure of them to minimize these trade-offs.

Stalk-eyed flies (Diopsidae) are ideal model organisms for the study of sexually selected traits. All species in this family exhibit hypercephaly, a type of extreme head morphology in which the eyes and antennae are displaced laterally on long peduncles (Fig. 1A; Schillito, 1971). Both males and females share this morphological characteristic; however, in sexually dimorphic species the male eye span greatly exceeds that of the female (Wilkinson & Dodson, 1997), and often exceeds the total body length (Baker & Wilkinson, 2001). Eye span is both highly variable and heritable (Wilkinson & Taper, 1999), and hence is

subject to both natural and sexual selection (Wilkinson, 1993). Together, female preference for males exhibiting longer stalks (Burkhardt & de la Motte, 1988; Wilkinson, Kahler & Baker, 1998; Hingle *et al*., 2001; Cotton *et al*., 2006) and male–male competition over copulation sites (Burkhardt & de la Motte, 1985; Panhuis & Wilkinson, 1999; Small *et al*., 2009) have played a role in the evolution and maintenance of this extravagant ornament.

Historically, studies on stalk-eyed flies have used univariate measures of eye span (e.g. ratios of linear measurements and mass), but such measurements may be inaccurate if there is variation in the angle at which the eyestalks are attached to the head. An individual with longer stalks and a lesser angle of attachment between the stalks could yield the same linear measurement of eye span as an individual with shorter eyestalks and a larger angle of attachment. Other empirical examples of discrepancies between measurement methods exist. A study on hummingbird bills compared several univariate and geometric morphometric methods in their ability to detect sexual shape dimorphism, and concluded that univariate approaches can provide conflicting results, even when based on the same data (Berns & Adams, 2010). Furthermore, valuable information on ornament shape is lost, resulting in analyses that ignore substantial variation present between individuals. Changes in the size and shape of the eyes, eyestalks, and head will provide more accurate information for estimating whole-organism performance and the associated constraints of the ornament.

In this study, we calculated univariate measurements of size and quantified overall head shape in a sexually dimorphic species of stalk-eyed fly (*Teleopsis dalmanni*) to compare patterns of sexual shape and size dimorphism. Additionally, we investigated patterns of shape change within each sex to determine how components of head shape vary with increasing size of the eyestalks. We hypothesize that in addition to the well-documented size dimorphism in eye span, males and females will differ significantly in multiple components of head shape. As size of ornament increases within each sex, we also expect to see correlated changes in shape, which may indicate constraints preventing further elaboration of these ornaments.

MATERIAL AND METHODS

Study organism

Male and female *T. dalmanni* were descendants of pupae obtained from a large stock population currently maintained at the University of Maryland at College Park. Flies were housed together in $40 \times 20 \times 22$ cm clear plastic containers lined using moist cotton and blotting paper, and kept at 80% humidity and 26 °C on a 12 h light/12 h dark cycle. The flies were reared in age cohorts based on eclosion date and were provided pureed corn supplemented by Ward's *Drosophila* medium *ad libitum*. *Teleopsis dalmanni* reaches maturity after 1 week, but peaks at 4 weeks after eclosion (Baker *et al*., 2003). We exclusively used mature flies 4–8 weeks post-eclosion in this experiment.

Univariate morphological measurements

Morphological measurements were performed on 40 male and 40 female flies. One female fly was removed from analysis because of a morphological abnormality of the eyestalks, resulting in only 39 female flies. We anaesthetized each fly using CO_2 and measured total body mass in a sealed pre-weighed 1.5 mL plastic tube. Mass was measured to the nearest 0.01 mg using an electronic microbalance (Mettler, MT5). We then took a scaled planform image of the fly lying on its thoracic spines under a digital camera mounted on a dissection microscope at a magnification of 15–20× and a resolution of 100 pixels per mm. From these digital images, we recorded the standard linear measurements used in previous stalk-eyed fly studies (e.g. Wilkinson, 1993), including eye span (the distance from the outermost edges of the two eyes; Fig. 1A) and total body length (the distance from the front of the head to the tip of the folded wing) to the nearest 0.01 mm (following Ribak, Egge & Swallow, 2009a) using ImageJ (National Institutes of Health). We then placed the flies in individually marked 1.5 mL plastic tubes and froze them at –20 °C until the more detailed photographs required for landmark-based geometric morphometrics could be taken.

Geometric morphometrics

To quantify head shape, we finely positioned flies in modelling clay and used a mounted Nikon D-90 digital camera to obtain high-quality digital photographs of the heads of each of the 79 specimens. Images of frontal views of the heads were used in order to quantify shape and size of the eyestalks from the perspective that the flies use when competing with conspecifics and choosing mates. Because of the difficulty of positioning these delicate insects, the bodies of most specimens were damaged, preventing further dry mass measurements of individual body components. A previous study has published these data on *T. dalmanni* (Ribak & Swallow, 2007), and we will be referring to them as accurate indices of the sex-specific mass of body components.

From the images, head shape was quantified using landmark-based geometric morphometric methods (Rohlf & Marcus, 1993; Adams, Rohlf & Slice, 2004). These methods allow a rigorous quantification of shape after the effects of non-shape variation have been mathematically held constant. Our quantification procedure followed that of Berns & Adams (2010). We identified the locations of 24 biologically homologous landmarks for each specimen. These landmarks were placed as follows: the outermost edge of each eye bulb, the outer vertical bristle, the dorsal and ventral bases of each eye bulb, the connection of each eye bulb to the eyestalk, the inner vertical bristle and the eyestalk directly ventral to it, the dorsal and ventral base of each eyestalk, the midline of the dorsal and ventral head, and the outermost portions of the ventral head (closed circles in Fig. 1B). Additionally, 16 sliding semi-landmarks were placed in the areas between homologous landmarks to capture detailed curvature (open circles in Fig. 1B). These landmarks were then digitized using TPSDIG2 (Rohlf, 2010). When the landmarks of an individual were combined, they formed a collection of 40 *x–y* coordinates that quantified the size and curvature of the head and eyestalks, representing the shape of the overall structure.

These coordinates were then subjected to a Generalized Procrustes Analysis (Rohlf & Slice, 1990) to remove all non-shape variation (i.e. position, orientation, and size). This technique aligns all specimens to a common coordinate system using the centroid (geometric centre) of each individual's coordinates as the origin. Each individual's coordinates are then scaled to a common unit of size while maintaining the proportional distance between the coordinates. Finally, all 40 coordinates of each individual are rotated to minimize the squared differences between corresponding landmarks of different individuals (Gower, 1975; Rohlf & Slice, 1990). During this procedure, semi-landmarks mathematically slide along their tangential directions (Bookstein *et al*., 1999; Gunz, Mitteroecker & Bookstein, 2005) in order to minimize the Procrustes distance (e.g. Serb *et al*., 2011). From these configurations of each individual, a mean configuration is computed to represent the mean shape of all individuals included in the analysis. The differences in the coordinates of corresponding landmarks of individuals and their means can then be treated as a multivariate data set representing shape. This data set was used for further analyses of shape variation and covariation (e.g. Adams, West & Collyer, 2007; Adams, 2010; Adams & Nistri, 2010).

Statistical analyses

To assess differences between the sexes based on univariate measurements (eye span, residual eye span, body length, and mass), summary statistics were generated and two-sample Student's *t*-tests were conducted. Because of the tight correlation between eye span and body size (Burkhardt & de la Motte, 1987), these analyses were conducted to ensure that the majority of the differences between the sexes were in eye span and not in overall body size. As is customary, residual eye span was used for all comparisons with shape to account for this correlation between stalk length and body size, with large residuals representing individuals exhibiting larger eye spans than would be expected for their body size.

To quantify sexual shape dimorphism, we performed a permutational multivariate analysis of covariance (MANCOVA) between the sexes using the landmark variables (40 *x–y* coordinates per individual) as response variables representing shape. We tested the relationship of sex (dependent variable) and shape (response variables) with size as a covariate for 9999 iterations to compare the observed values with the predicted values obtained through this residual randomization. We tested for a relationship between shape and residual eye span size (i.e. allometry) between sexes using a factorial multivariate analysis of variance (MANOVA) with the factors shape, sex, and size, as well as the interaction terms size*sex and shape*sex. We tested for allometry within the sexes with a linear regression model using the factors shape and size for each sex separately. All analyses were performed in R 2.11.1 (R Development Core Team, 2010).

To visualize patterns of head shape variation, we performed a principal components analysis on the 40 *x–y* coordinates obtained from the General Procrustes Analysis representing overall head shape after non-shape variation had been removed. Thin-plate spline deformation grids of the phenotypic means of males and females were mathematically generated from the differences in landmark locations, allowing the depiction of head shapes to facilitate biological interpretation of these differences. Finally, to visually compare the change in shape with increasing residual eye span within and between the sexes, thin-plate splines of the males and females that had the largest, median, and smallest eye spans were created.

RESULTS

Univariate morphological measurements

We found a significant difference between the sexes in eye span (males = 8.380 ± 0.094 mm; females = 5.807 ± 0.029 mm; $P < 0.0001$) and residual eye span (males = 1.209 ± 0.006 mm; females = 0.859 ± 0.002 mm; $P < 0.0001$). Body length (males = 6.923 ± 0.052 mm; females = 6.758 ± 0.031 mm; $P =$

0.0840) and mass (males = 6.647 ± 0.157 g; females = 6.604 ± 0.176 g; $P = 0.8544$) differences between the sexes remained non-significant.

Geometric morphometrics

We found significant overall head shape differences between the sexes (approximate $F_{1,77} = 91.765$; $P < 0.0001$). The differences between head shapes were clear in a principal components plot, in which PC1 (sex) accounted for 56.59% of the variation and PC2 (shape) accounted for 19.66% of the variation, totalling 76.25% of the overall variation between males and females. PC3 was examined as well, but accounted for only a small portion of the variation (5.2%), and was thus biologically uninformative. Thin-plate spline deformation grids (Fig. 2) allowed visualization of the differences between male and female overall head shapes. Specifically, these revealed that the mean head shape of females is much larger than that of the males. Additionally, females had much thicker, shorter eyestalks with larger, rounder eye bulbs compared with the mean, whereas males had longer, thinner eyestalks with smaller, more elliptical eye bulbs.

We found a significant change in overall head shape as residual eyestalk length increased. This pattern was significant between the sexes (approximate $F = 126.05$; $P < 0.0001$) as well as within females (approximate $F = 1.375$; $P = 0.0392$) and within males (approximate $F = 1.766$; $P = 0.0275$). Thin-plate spline deformation grids revealed that females with smaller eye span had more downward-curved, thicker eyestalks attached at a greater angle to the head compared with females with larger eye span, which had thinner, straighter stalks attached at a lesser angle to the head. Males had a similar pattern, in which the smallest males showed more downward-curved eyestalks and a greater angle of attachment compared with the largest males. The smallest males also

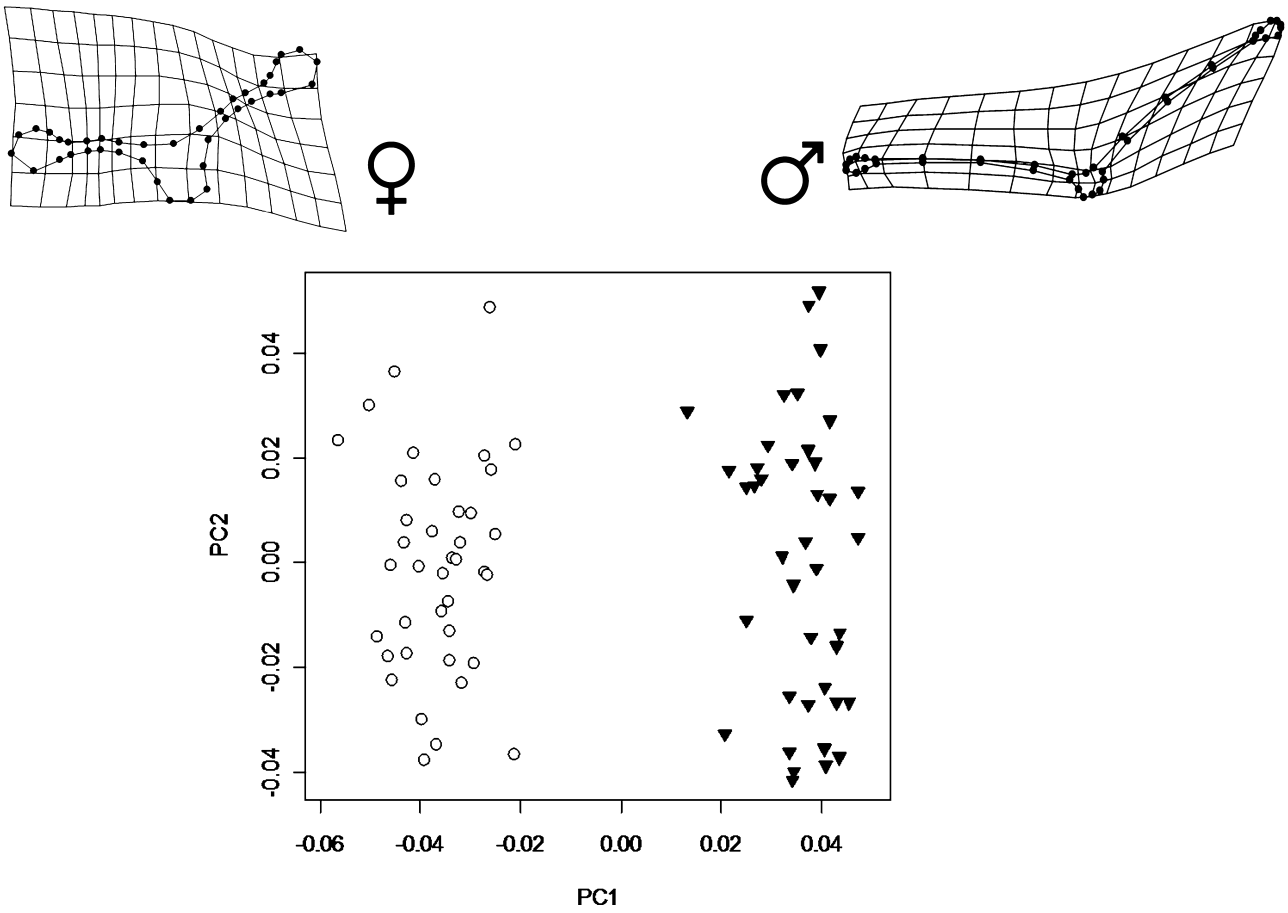


Figure 2. Results of principal component analysis of male and female shape: PC1 = 56.59% variation; PC2 = 19.66%, accounting for 76.25% of the overall variation. ○, females; ▲, males. Also shown are the thin-plate spline deformation grids of female and male mean head shapes that visualize the patterns of shape variation. These have been accentuated by a factor of two to enhance interpretation.

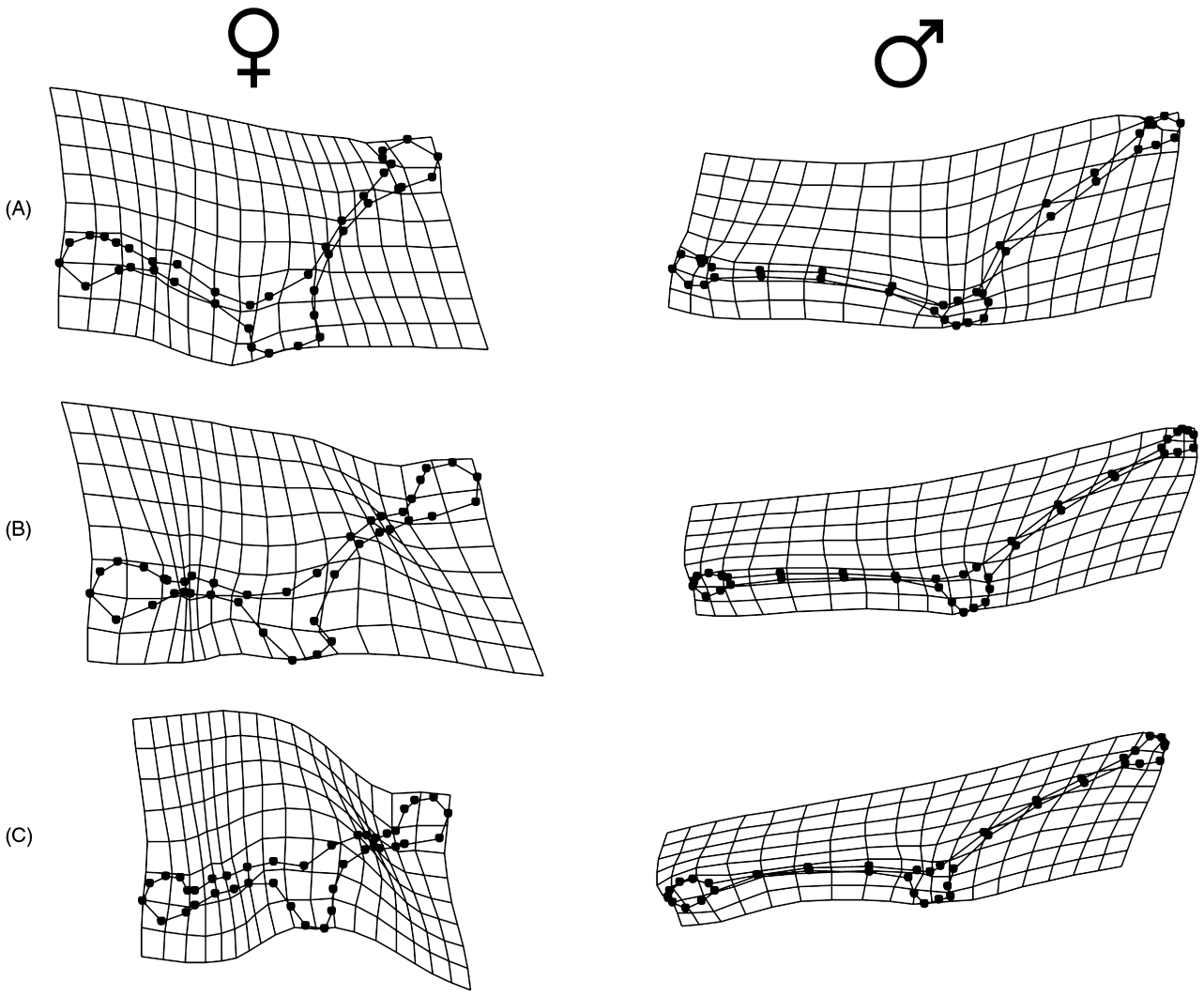


Figure 3. Thin-plate spline deformation grids visualizing the change in shape with the change in size for females and males with: (A) the largest eye span; (B) the median eye span; and (C) the smallest eye span. These have been accentuated by a factor of two to enhance interpretation.

exhibited a larger head than that seen in the largest males (Fig. 3).

DISCUSSION

Stalk-eyed flies of the species *T. dalmanni* exhibit significant sexual dimorphism in overall head size and shape. Females have large, round eye bulbs and short, thick stalks attached to a large head, whereas males have small, elliptical eye bulbs and long, thin stalks attached to a small head. These data show that the sexes differ not only in the length of their eyestalks, but also in the size and shape of the individual components (i.e. eyes, stalks, and head) that compose the overall head morphology. Stalk-eyed flies can now be added to the list of species in which sexual shape dimorphism has been rigorously quantified using geometric morphometric methods, which includes tree weta (Kelly & Adams, 2010), hummingbirds (Berns & Adams, 2010), and turtles (Valenzuela *et al*., 2004), among others. In addition to differences between the sexes, we found a significant change in shape as residual eye span increased within each sex, with larger-stalked flies exhibiting a lesser angle of eyestalk attachment to the head and straighter stalks compared with those of smaller flies. Moreover, eye bulb size decreased with increasing eye span and eyestalks became thinner as they became more elongated. This significant allometry is likely to play an important role in determining the costs of eyestalks on whole-organism performance, as these changes in shape may significantly reduce the aerodynamic costs

associated with the structure. Additionally, the change in the attachment angle of the eyestalks provides further evidence that linear measurements can inaccurately estimate trait size, non-proportionally underestimating the length of the eyestalks in flies with larger eye spans relative to flies with smaller eye spans.

The changes we observed in the size of discrete portions of the head with increasing eye span are supported by previous studies. Ribak & Swallow (2007) weighed the head and its component parts (eyes, stalks, and head minus the eyes and stalks) of male and female *T. dalmanni*. Mass measurements indicate that males and females do no differ significantly in overall head mass, even though males have significantly longer eyestalks than do females. Male eyestalks weighed significantly more than female eyestalks, yet overall the head mass was not affected because females had marginally heavier heads than males. In a within-sex comparison of *T. dalmanni* artificially selected for large and small eye spans, the eye bulb area decreased significantly with increasing eye span in both sexes (Fry, 2006). Moreover, males also showed a significant decline in stalk diameter as eyestalks lengthened.

These morphological differences within and between the sexes could be indicative of developmental constraints on eyestalk elongation. Because they are holometabolous insects, stalk-eyed flies acquire resources during the larval phase, whereas the growth and maturation of adult structures occurs after pupation when feeding has ceased (Nijhout, 1994). Upon eclosion, the exoskeleton hardens quickly, resulting in structures that are fixed in size. This closed system of development results in traits competing for a finite pool of resources, leading to size trade-offs in structures that are in close proximity to each other (Nijhout & Wheeler, 1996; Nijhout & Emlen, 1998). The eyes, eyestalks, and antennae all develop from the eye-antennal imaginal disc (Buschbeck, Roosevelt & Hoy, 2001; Hurley *et al*., 2002). As more resources are invested into eyestalk elongation, fewer resources may be available for eye bulb development, potentially reducing visual acuity and constraining further elongation of the eyestalks. Trade-offs between these structures are no doubt occurring at some developmental level; however, this cannot account for the changes in shape we observed in the curvature and angle of eyestalk attachment.

The patterns in the shape differences we have observed are concordant with the concept that entire suites of characters evolve to maximize whole-organism performance (Arnold, 1983; Lande & Arnold, 1983; Irschick *et al*., 2008). The changes in shape with increasing eye span that we observed can potentially be added to the already documented coevolution of other body parts with eyestalk exaggeration. Studies investigating the flight performance trade-offs between males and females have found minor or no differences in aerial performance (Swallow, Wilkinson & Marden, 2000; Ribak & Swallow, 2007), despite predictions that males should suffer from decreased aerial maneuverability compared with females (Swallow *et al*., 2000). Substantiating these results on performance, in staged laboratory interactions with a predator, indicate that males exhibit higher survival when facing a predator than do females (Worthington & Swallow, 2010; Worthington & Swallow, 2011). Reasons for this lack of a trade-off have largely been attributed to morphological compensation by flight musculature and wing size, with males exhibiting significantly larger thoracic masses (Swallow *et al*., 2000; Ribak & Swallow, 2007) and wing sizes (Ribak *et al*., 2009b; Husak *et al*., 2011; Husak & Swallow, 2011) than females. In addition to these compensations, in this study we found significant changes within the sexually selected ornament itself, which may further reduce the aerodynamic costs associated with the eyestalks.

Changes in ornament and head shape are likely to play a large role in determining the moment of inertia (MOI), and therefore may greatly affect flight performance. MOI is a measure of the resistance the body offers to any change in its angular velocity (Schaum & van der Merwe, 1961). Its calculation relies on body mass and the distribution of that mass relative to the axis of rotation, causing mass components a greater distance from the axis to have an amplified effect on the MOI (Ribak *et al*., 2009b). This suggests that even small changes in the mass of the eye bulbs or the lateral placement of the stalks would have a significant effect on MOI, and could therefore greatly affect flight agility and maneuverability (Ribak *et al*., 2009b). We found that both males and females with larger eye spans have smaller eye bulbs, reduced stalk thickness, and lower stalk mass (for mass, see Ribak & Swallow, 2007), compared with individuals of smaller eye spans. Additionally, we found that the angle in which the eyestalks are attached to the head is also altered, bringing the eye bulbs closer to the axis of rotation as the eye span increases. How this affects flight performance is not yet empirically known, but just as ice skaters fold in their arms to spin faster, this may have a similar effect in stalk-eyed flies, resulting in a reduced MOI and enhanced turning performance. Thus, the angle of eyestalk attachment, eye bulb size, and stalk thickness may be important morphological compensations for the increased performance constraints that their additional mass and length impose.

An alternative, untested, hypothesis for the observed trends in allometry relies not on constraints

per se, but rather on selection pressures. It remains possible that female preference for eyestalk shape, in addition to the well-documented preference for size, may play an important role in the evolution of this sexually selected trait. As the use of geometric morphometric methods becomes more widespread, we will be able to determine whether shape is acted upon by natural and sexual selection. This includes determining whether shape is heritable, whether there is female preference for shape, and whether shape can be manipulated for experimental studies. Research on the evolution of shape differences in eyestalks will open many new avenues of research and help us to further understand sexual selection in this model species.

This is the first study to use landmark-based geometric morphometric methods to explore correlations of ornament shape and size in stalk-eyed flies. Our significant results demonstrate the importance of integrating geometric morphometric methods into future studies examining stalk-eyed flies. An important next step will be to expand on this analysis to include many species of stalk-eyed flies from diverse lineages to determine if these patterns of shape change are consistent across taxa. Furthermore, the inclusion of monomorphic species, in which males and females have comparable eye spans, will provide additional insight into whether these differences in shape are linked to sex alone or are more broadly a consequence of eyestalk elongation. Incorporating flies from wild populations, artificially selected lines, and larvae reared on high- and low-quality diets in future studies will also further prove the importance of the correlation between shape and size.

Finally, landmark-based geometric morphometric methods are less constrained than the more commonly used univariate approaches in quantifying the size and shape of morphological features. Geometric morphometrics quantify the complex details that a variable such as shape exhibits, providing an integrated picture of the overall variation present within a structure, rather than a data set of disparate size and mass measurements. Our results indicate that simple bivariate relationships between linear size measurements of an ornament and some proxy for fitness (e.g. performance and/or survival) may be insufficient to detect the costs of ornaments. We advocate that future studies investigating sexually selected traits use geometric morphometric methods in combination with univariate measurements in order to provide a deeper appreciation of the effect that shape may play on performance and fitness.

CONCLUSION

Stalk-eyed flies show significant intra- and intersexual shape dimorphism in head morphology, implying several constraints on eyestalk elongation. Whereas many studies have investigated sexual shape dimorphism, this is the first study to use geometric morphometrics to explore correlations of shape and size in this sexually selected trait. We have shown that, although the use of linear eye span measurements used in the past may be a common method to quantify eyestalk size, it reduces a complex structure into a single measurement, causing the loss of significant amounts of information on the morphological variation between individuals, sexes, and species. Furthermore, because of the differences in the curvature of the eyestalks and angle that they attach to the head, linear measurements may sometimes provide an inaccurate estimate of ornament size. Thus, it is possible that excluding measurements of shape differences between individuals has added to the difficulty in demonstrating the costs of this sexually selected trait. The patterns in shape we observed may have been affected by several factors, including developmental and/or performance constraints. In examining sexual shape and size dimorphism in a species of stalk-eyed fly, we have enhanced the understanding of possible constraints limiting the evolution of eyestalk elongation, and suggest that the shape patterns observed could significantly reduce the flight performance costs associated with the structures composing the head.

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