

Effects of ornamentation and phylogeny on the evolution of wing shape in stalk-eyed flies (Diopsidae)

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Abstract

Exaggerated male ornaments are predicted to be costly to their bearers, but these negative effects may be offset by the correlated evolution of compensatory traits. However, when locomotor systems, such as wings in flying species, evolve to decrease such costs, it remains unclear whether functional changes across related species are achieved via the same morphological route or via alternate changes that have similar function. We conducted a comparative analysis of wing shape in relation to eye-stalk elongation across 24 species of stalk-eyed flies, using geometric morphometrics to determine how species with increased eye span, a sexually selected trait, have modified wing morphology as a compensatory mechanism. Using traditional and phylogenetically informed multivariate analyses of shape in combination with phenotypic trajectory analysis, we found a strong phylogenetic signal in wing shape. However, dimorphic species possessed shifted wing veins with the result of lengthening and narrowing wings compared to monomorphic species. Dimorphic species also had changes that seem unrelated to wing size, but instead may govern wing flexion. Nevertheless, the lack of a uniform, compensatory pattern suggests that stalk-eyed flies used alternative modifications in wing structure to increase wing area and aspect ratio, thus taking divergent morphological routes to compensate for exaggerated eye stalks.

Introduction

Locomotion is of key importance to fitness in many taxa, and locomotor ability is expected to exhibit correlated evolution with other aspects of the phenotype that do not constitute the primary locomotion system. Locomotor ability of individuals in a population is thus likely to represent a balance between natural and sexual selection acting on locomotion directly, as well as indirectly due to selection on other aspects of the

phenotype that may influence locomotion (Dickinson *et al.*, 2000; Irschick & Garland, 2001; Husak & Fox, 2008; Irschick *et al.*, 2008). Typically, locomotion, and the lower level traits that underlie it, is thought of in terms of how natural selection selects against those individuals too slow to escape predators or acquire food (Irschick *et al.*, 2008). However, recent studies suggest that better locomotor performance may also directly increase mating success (Husak *et al.*, 2006) and be under the direct influence of sexual selection (Lailvaux & Irschick, 2006; Husak & Fox, 2008; Byers *et al.*, 2010). On the other hand, sexual selection may have indirect negative effects on locomotion, via the direct detrimental effects of sexually selected traits on locomotor abilities (reviewed in Oufiero & Garland, 2007; Husak & Swallow, 2011). Indeed, negative effects

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associated with bearing sexually selected signals, ornaments and weapons are required in many theoretical models of sexual selection (Zahavi, 1975; Grafen, 1990; Kotiaho, 2001; but see review by Számádo, 2011), and this burden has often been hypothesized to take the form of reduced locomotor performance (reviewed in Kotiaho, 2001; Oufiero & Garland, 2007) because of its presumed link to individual fitness. Nevertheless, selection should also favour, within some constraints, traits that mitigate the detrimental performance effects of sexually selected traits (reviewed in Oufiero & Garland, 2007; Husak & Swallow, 2011). Despite many decades of theoretical and empirical studies on sexually selected traits, however, many key questions about how their costs may be ameliorated remain unresolved. What morphological changes allow functional changes in locomotion to compensate for the detrimental effects of sexually selected traits? Does phylogenetic history influence the evolution of compensatory traits, thus limiting or constraining morphological change? Does evolution proceed through different combinations of morphological changes that arrive at similar functional compensation? A more general way to phrase these questions in a comparative framework is to ask whether related taxa facing similar selective pressures (i.e. reduced locomotor ability due to similar sexually selected traits) evolve a functional solution via similar morphological changes or via different morphological routes to the same functional end.

As the last question suggests, studying the evolution of morphological traits in a comparative analysis can be complicated by the presence of multiple morphological 'solutions' to a given functional 'problem'. Such many-to-one mappings of form to function have been documented in multiple functional systems for numerous taxa (Wainwright *et al.*, 2005; Wainwright, 2007; Losos, 2011). For example, the 4-bar linkage system in the oral jaws of labrid fishes allows for the evolution of multiple 4-bar shapes that produce similar jaw movement and function (e.g. Alfaro *et al.*, 2004, 2005; Wainwright *et al.*, 2005). Sprint speed of *Anolis* lizard species is determined, in general, by hindlimb length and muscle mass, but similar sprint speeds can be obtained in different species with various combinations of limb segment dimensions and muscle mass (Vanhooydonck *et al.*, 2006). Similarly, *Drosophila subobscura* populations along a geographical gradient evolved convergent wing size, but this convergence in size, which functions to increase force production, was accomplished via changes in different parts of the wing (Huey *et al.*, 2000). Specifically, North American and European flies both increased wing length over evolutionary time with increasing latitude, but they did so by increasing different segments of the wing. These examples emphasize that when similar selective pressures favour a specific functional capacity, evolutionary change may take a variety of routes. Conversely, there are numerous cases

where similar selective pressures have resulted in convergent changes in morphology. For example, convergence in wing shape has been shown in previous studies to result from similarity in migration distance (Marchetti *et al.*, 1995; Voelker, 2001; Johansson *et al.*, 2009), foraging strategies (Bullen & McKenzie, 2007; Kaboli *et al.*, 2007) and mating displays (Hedenström & Møller, 1992). Because much of this work has been conducted on bird species, generalization to the most species-rich group of flying animals (insects) remains unclear (Dudley, 2000; Johansson *et al.*, 2009). Also, little remains known about how selective pressures resulting from bearing an ornament affect flight performance and morphological components of the flight apparatus (reviewed in Husak & Swallow, 2011). Multiple studies have shown that tail ornament length in birds is positively correlated with wing size across species (Andersson & Andersson, 1994; Balmford *et al.*, 1994), but whether wing size is increased via lengthening of the same morphological features in all species remains unknown.

Stalk-eyed flies (Diopsidae) represent an ideal system with which to test hypotheses about morphological and functional adaptations to compensate for ornaments (Ribak *et al.*, 2009; Husak *et al.*, 2011a,b). Stalk-eyed flies have their eyes displaced laterally from the sides of their heads on stalks, and the males of many species have eye stalks that far exceed that of females, with the male eye span of some species greatly exceeding body length (Wilkinson & Dodson, 1997). The family Diopsidae is a species-rich lineage of flies with 200–300 described species within 10–14 genera (Feijen, 1989) and marked variation in the degree of hypercephaly (Wilkinson & Dodson, 1997; Baker & Wilkinson, 2001). This exaggerated male ornament is under both intersexual selection via female choice (Burkhardt & de la Motte, 1988; Wilkinson *et al.*, 1998; Hingle *et al.*, 2001; Cotton *et al.*, 2006) and intra-sexual selection during male–male competition (Burkhardt & de la Motte, 1985; Panhuis & Wilkinson, 1999; Small *et al.*, 2009). Even though the significantly increased moment of inertia of the head of males (Ribak & Swallow, 2007) is predicted to decrease aerial turning performance compared with females, flight performance trials have failed to consistently detect flight performance decrements in males (Swallow *et al.*, 2000; Ribak & Swallow, 2007). The best supported hypothesis for the apparent lack of a flight performance cost in males is that they have evolved compensatory mechanisms to offset such costs. In eye-span dimorphic species, male stalk-eyed flies have larger wings than females (Ribak *et al.*, 2009; Husak *et al.*, 2011a). Looking only at males, those with relatively longer eye stalks have relatively larger wings, both within (Husak *et al.*, 2011a) and among dimorphic species (Ribak *et al.*, 2009). Finally, as dimorphism in eye span increases across species, so too does dimorphism in wing length and area, as well as aspect ratio

(Ribak *et al.*, 2009; Husak *et al.*, 2011b). Since wings do not appear to be under direct sexual selection in males of dimorphic stalk-eyed fly species (Burkhardt & de la Motte, 1985; Panhuis & Wilkinson, 1999; Egge *et al.*, 2011), the increase in wing size that is associated with increased eye span is unlikely to be due to direct sexual selection on wing size or function. The finding that the aspect ratio of wings may be affected by male ornamentation suggests that shape differences between males and females exist. Aspect ratio is a shape property (non-dimensional) affecting the aerodynamic performance (i.e. function) of a wing with a given area. However, it only describes the ratio between wing length and average width (chord), thereby providing only a coarse measure of wing shape. Since increasing wing area and/or aspect ratio can be accomplished in many different morphological ways, we used geometric morphometric techniques to examine the finer details of change in wing shape across stalk-eyed fly species to determine how dimorphism in ornamentation specifically alters wing shape.

Our objective was to determine if stalk-eyed fly species increase wing size and aspect ratio in association with eye-span dimorphism via similar or divergent morphological changes in wing shape. We studied wing shape in 24 species of stalk-eyed flies in four genera, using phylogenetic comparative methods to determine which aspects of wing shape, if any, are evolutionarily correlated with dimorphism in eye span and whether there are phylogenetic patterns in wing shape unrelated to dimorphism in eye span. If species have taken similar morphological routes to increase wing size, then we predicted that there would be a strong relationship between dimorphism in eye span and dimorphism in wing shape. Conversely, if there are divergent morphological solutions to increase wing size, then we predicted that there would be weak or no evolutionary relationships across species between dimorphism in eye span and dimorphism in shape variables. Finally, we examined changes in shape within genera to determine if there is concordance or

discordance in morphological changes associated with dimorphism in eye span.

Materials and methods

We studied 24 species of stalk-eyed flies, obtaining data on average male and female eye span and body length from Baker & Wilkinson (2001) and Ribak *et al.* (2009). We obtained wing images from subsets of individuals used in previous studies: *Diasemopsis aethiopica*, *D. dubia*, *D. meigenii*, *D. signata*, *Diopsis apicalis*, *Sphyracephala beccarii*, *Teleopsis dalmanni*, *T. quinqueguttata* and *T. thaii* were obtained from flies used in Ribak *et al.* (2009); *Diasemopsis* species' wings were from flies used in Baker & Wilkinson (2003); *T. quadriguttata* are from flies used in Wilkinson & Taper (1999); and the remaining species were from flies used in Baker & Wilkinson (2001). We refer readers to those papers for details of laboratory rearing of flies and specimen preparation. Previous work has shown considerable variation in eye-stalk dimorphism (Baker & Wilkinson, 2001, 2003), and we follow those studies of eye-stalk allometry and their classifications of dimorphic and monomorphic species for analyses.

We used tpsDig v. 2.1 (Rohlf, 2006) to obtain coordinates for 14 landmarks (Fig. 1) on each wing ($n = 1031$ individuals; sample sizes provided in Fig. 2). With one exception, landmarks chosen represent the intersections of wing veins and are comparable to landmarks used in other studies of fly wing shape (e.g. Pélabon *et al.*, 2010). Landmark 3 was defined as the intersection of the trailing edge of the wing with the straight line defined by landmarks 4 and 5. Vein intersections that were more proximal to the wing hinge than landmarks 1 and 2 were not reliably present in many individuals. Although some methodological details in preparing wings for photographs differed, the various techniques did not affect obtaining reliable or comparable landmark data from the images (see Supporting Information available online).

We used a custom-written (Bush *et al.*, 2002) program for SAS (9.1, SAS Institute Inc., Cary, NC, USA)

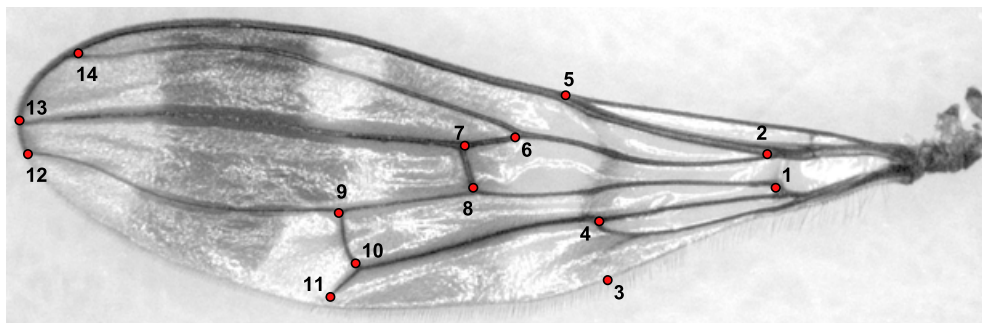


Fig. 1 Landmarks used in analysis of wing shape. Image is of a *Teleopsis dalmanni* wing.

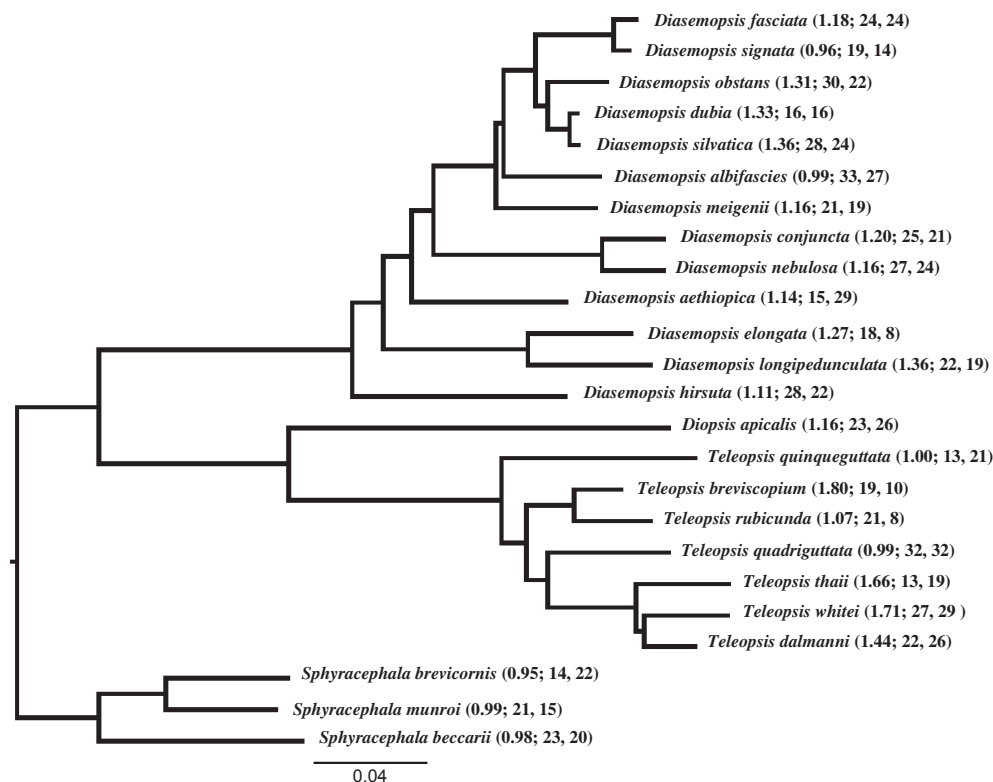


Fig. 2 Phylogenetic relationships among the stalk-eyed fly species studied. Dimorphism indices and male and female sample sizes, respectively, are given in parentheses. The phylogeny is from Baker *et al.* (2001), with the addition of *T. thalii*. See Materials and methods for details on tree construction.

to perform a generalized least-squares Procrustes analysis (GPA) on landmark coordinates. GPA rotates, translates and scales coordinates to remove positioning and isometric size effects in landmark data (Rohlf & Slice, 1990; Bookstein, 1991; Marcus *et al.*, 1996). Orthogonally projected tangent coordinates were obtained for all individuals (Slice, 2001), and these coordinates were entered into a principal components analysis (PCA). We then analysed wing shape and dimorphism of shape using two different approaches, each of which answers different questions. First, we examined differences among species and between the sexes, using multivariate analysis of variance (MANOVA), as well as by examining dimorphism indices of wing shape (see below). Second, we analysed the differences in magnitude and direction (i.e. angles) of phenotypic trajectories (Collyer & Adams, 2007; Adams & Collyer, 2009). Although the first approach examines how specific segments of the wing differ among species, the second considers how sexual shape dimorphism varied among species in multidimensional morphospace (see below). We also visually examined changes in shape with thin-plate splines (using tpsSpline v. 1.20; Rohlf, 2004) within genera, qualitatively comparing deformation grids of average

male consensus shapes of species monomorphic for eye span to those dimorphic for eye span within *Diasemopsis*, *Sphyracephala* and *Teleopsis*.

We conducted a two-way ANOVA to examine the effects of sex and species on centroid size (Gidaszewski *et al.*, 2009). We then conducted a two-way multivariate analysis of variance (MANOVA) with principal component scores as response variables to examine the effects of sex and species on wing shape. This allowed us to examine specific differences among species considering individual variation within species and analysing the sexes simultaneously. We complimented this analysis with phylogenetic PCA (Revell, 2009; see below), which considers species as data points.

We then calculated dimorphism indices for each variable of interest. For eye-span dimorphism, we used species averages from Baker & Wilkinson (2001) and Ribak *et al.* (2009); for centroid size, we used values obtained from GPA. The dimorphism index for eye span and centroid size was the male average divided by the female average, which is desirable for this variable due to the marked variation in body size among the stalk-eyed fly species studied (Baker & Wilkinson, 2001). To examine dimorphism in shape, we computed the

Euclidean distance in tangent space (hereafter, 'shape dimorphism') between the mean shapes of the males and females of each species (Dryden & Mardia, 1998; Gidaszewski *et al.*, 2009). We note that this measure of shape dimorphism is equivalent to the magnitude of shape change in the phenotypic trajectory analysis described below.

We used phenotypic trajectory analysis (Collyer & Adams, 2007; Adams & Collyer, 2009) to more fully understand effects of the interaction between species and sex on wing shape. Briefly, this method examines phenotypic evolution as phenotypic change vectors between two evolutionary units (the two sexes in our case), comparing the vectors across pairs of taxa. The magnitude of each vector represents the level of phenotypic change (or difference) between the two units, whereas the direction (or orientation) of the vector represents the way in which the phenotype differs between the two units (Collyer & Adams, 2007; Adams & Collyer, 2009). When comparing the vectors of two species, differences between the magnitudes indicate differences in dimorphism, whereas differences in directions (i.e. the angle between vectors) describe the difference in the evolutionary trajectories between the units across the taxa compared. For magnitudes and directions, the smaller the differences between taxa, the more similar are the levels of sexual dimorphism and evolutionary trajectories respectively. This method allowed us to examine whether sex-based differences in wing shape are concordant across monomorphic and dimorphic species, how patterns of sexual shape dimorphism differ within and between genera and whether species differ predominantly in the direction of change or the magnitude of change. Specifically, we calculated a magnitude and direction of change between the least-squares means of male and female individuals for each species to generate the endpoints of the species vector. We then tested for concordance across species vectors by calculating pairwise differences in magnitude and direction (i.e. angles) between vectors and testing for statistical significance using a residual randomization procedure (9999 iterations) (ter Braak, 1992; Gonzalez & Manly, 1998). To adjust for Type I error rates, sequential Bonferroni corrections (Holm, 1979; Rice, 1989) were applied to all pairwise tests. In addition, we assessed the relationship between phenotypic trajectories and phylogenetic relatedness. As vector direction in multi-dimensional space is only interpretable as the angle between two vectors, we used Mantel correlations to determine the relationship between the angle between two species' vectors and the patristic distance between the two taxa. Mantel correlations were also used to compare magnitude and patristic distance, and for a comparison of magnitudes and angles between taxa. These statistical analyses were conducted using R (R Development Core Team, 2011).

Because common ancestry makes species averages nonindependent of each other (e.g. Felsenstein, 1985), we tested for phylogenetic signal in our data and performed correlation analyses with phylogeny taken into account. The phylogeny used for our analyses was generated from the nucleotide matrix consisting of three mitochondrial genes (CO II, 12S and 16S), and three nuclear genes (*Elongation factor-1alpha*, *wingless* and *white*) that was constructed in Baker *et al.* (2001). Twelve taxa for which we had no morphological measurements were pruned from the matrix and DNA sequence for 4 genes (CO II, 16S, *wingless* and *white*) from a new species, *T. thaii* (Földvári *et al.*, 2007), was added to the matrix. A partitioned maximum likelihood (ML) search, with 100 bootstrap replicates, using six distinct models with joint branch length optimization and an estimated Gamma model of rate heterogeneity, was conducted in RAxML (Stamatakis *et al.*, 2005). The ML tree was converted to ultrametric in Tree Edit (<http://tree.bio.ed.ac.uk/software/treededit/>) using the nonparametric rate smoothing option.

We conducted phylogenetic PCA (Revell, 2009) on males and females separately using the 'phytools' package (Revell, 2012) of R (R Development Core Team, 2011). Phylogenetic PCA uses species averages as data points input into a PCA that takes phylogeny into account when computing eigenvalues, eigenvectors, component loadings and component scores (Revell, 2009). We obtained principal component scores for males and females, separately, for each species. This analysis does not give scores that are 'phylogenetically corrected,' but they are adjusted to reduce type I error rates, compared with nonphylogenetic procedures, when further analysed. We then conducted ANOVA on each of the first four principal components (eigenvalues ≥ 3) for males and females separately, testing for differences among genera. *Diopsis* was included in the phylogenetic PCA, but not in the subsequent ANOVAs, because we only had data for one species of *Diopsis*. When ANOVAs were significant, we used Tukey Honestly Significant Difference *post hoc* tests to determine which genera differed from each other statistically in each of the principal components. These analyses allowed us to test whether genera differed in wing morphology using a technique that gives us more confidence in our hypothesis tests.

We tested for phylogenetic signal in all dimorphism variables using a randomization test implemented in the 'picante' package (Kembel *et al.*, 2010) of R. The K-statistic gauges the amount of phylogenetic signal relative to the amount expected for a character undergoing Brownian motion evolution along the specified topology and branch lengths (Blomberg *et al.*, 2003), and a significant K-value means there is statistically significant phylogenetic signal for the variable being tested.

We then used the PDAP:PDTREE module (Garland *et al.*, 1999; Midford *et al.*, 2005) in Mesquite v. 2.72 (Maddison & Maddison, 2009) to calculate standardized phylogenetically independent contrasts (Felsenstein, 1985) for species and sex averages of dimorphism in eye span, centroid size and wing shape. None of the dimorphism indices showed significant correlations between the absolute value of the contrast and the standard deviation of the contrast (eye-span dimorphism, 2-tailed $P = 0.22$; centroid dimorphism, 2-tailed $P = 0.33$; shape dimorphism, 2-tailed $P = 0.67$), so contrast analyses should perform equally well to alternative methods of analysis (Garland *et al.*, 1992; Revell, 2010). To examine how increases in eye span might influence changes in wing size and shape, we used independent contrasts in Pearson correlation analyses, testing for correlations between eye-span dimorphism and dimorphism in both centroid size and wing shape.

To examine how species monomorphic and dimorphic for eye span differ in wing size and shape, we used the data set of 24 species to conduct both a standard (nonphylogenetic) and phylogenetic analysis of variance (ANOVA), following Garland *et al.* (1993), with species averages as data points, centroid size, wing shape and dimorphism indices as dependent variables, and eye-span dimorphism/monomorphism (following Baker & Wilkinson, 2001; Ribak *et al.*, 2009; Husak *et al.*, 2011b) as the main effect. This offers a compliment to our analysis of independent contrasts by considering eye-span dimorphism as a categorical variable instead of as a continuous variable, and allows us to test for average differences in wing size and shape between monomorphic and dimorphic species. Our phylogenetic ANOVA used 1000 simulations with a Brownian motion model to produce the null distribution of F -statistics against which we tested the empirical F -statistic (Garland *et al.*, 1993). Males and females were analysed both separately and combined for centroid size, since we were interested in how the sexes individually may be affected by the evolution of eye-span dimorphism. Although the standard and phylogenetic ANOVAs have different assumptions, for completeness we present the results from both. ANOVAs were conducted in the 'geiger' (Harmon *et al.*, 2008) package of R.

Results

Wing shape and dimorphism

The first four principal components from the PCA described 83% of the variation in shape, while subsequent component axes each explained less than 5% of the variation. The ANOVA including all individuals of all species revealed a significant species*sex interaction ($F_{23,983} = 4.55$, $P < 0.001$) for centroid size (species effect: $F_{23,983} = 148.24$, $P < 0.001$; sex effect: $F_{1,983} = 0.22$, $P = 0.646$). The MANOVA with PC scores that

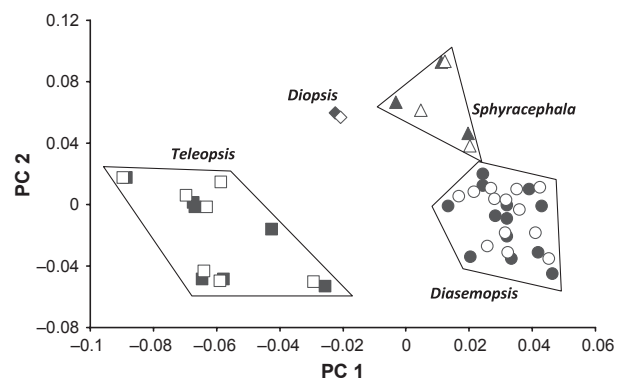


Fig. 3 Species averages of principal component (PC) 2 scores plotted against PC 1 scores for female (open symbols) and male (filled symbols) stalk-eyed flies from a principal components analysis of tangent coordinates from a procrustes analysis of wing landmarks (see Fig. 1). Polygons are drawn around the genera included in this study.

included all individuals of all species revealed a significant species*sex interaction ($F_{552,22586} = 2.88$, $P < 0.001$), in addition to significant species ($F_{552,22586} = 41.70$, $P < 0.001$) and sex ($F_{24,960} = 28.74$, $P < 0.001$) main effects, for wing shape. These differences can be seen in a plot of the first two principal components (Fig. 3), which explain the most variation in wing shape (67.4%). Genera are distinctly separated from each other in the first two dimensions of morphospace.

The phylogenetic PCA showed similar results as the nonphylogenetic PCA. For females, ANOVA on each of the first 4 principal components (eigenvalues = 8.20, 4.77, 3.46, 3.21 respectively), revealed that genera differed from each other along all four principal component axes, with *Teleopsis* being most different from the other genera (Table 1; Fig. 4). For males, ANOVA on each of the first 4 principal components (eigenvalues = 9.99, 4.37, 3.51, 2.96 respectively), revealed that genera differed from each other along the first three, but not the fourth, principal component axes, with *Teleopsis* being most different from the other genera (Table 1; Fig. 4).

Wing centroid size had significant phylogenetic signal whether males and females were examined separately (Males: $K = 0.625$, $P = 0.001$; Females: $K = 0.786$, $P = 0.001$) or together ($K = 0.716$, $P = 0.001$). Centroid dimorphism did not have significant phylogenetic signal ($K = 0.297$, $P = 0.34$), nor did shape dimorphism ($K = 0.312$, $P = 0.19$), but eye-span dimorphism approached significance ($K = 0.409$, $P = 0.088$). The standard and phylogenetic ANOVAs revealed that males of eye-span dimorphic species had larger wings than males of monomorphic species, but this was not the case for females (Table 1). Species dimorphic for eye span significantly differed in eye-span dimorphism and centroid dimorphism; however, species dimorphic for eye span did not differ from species monomorphic for

Table 1 Results of standard (nonphylogenetic) ANOVAs testing for differences among genera in the first four principal components from a phylogenetic PCA (see text for details). For significant ANOVAs, results from Tukey's Honestly Significant Difference tests are shown, comparing specific genera. Males and females were analysed separately, and the genus *Diopsis* was not included, because it was represented by only one species. Significant differences are bolded.

	$F_{2,20}$	P	<i>Diasemopsis</i> – <i>Sphyracephala</i>	<i>Diasemopsis</i> – <i>Teleopsis</i>	<i>Sphyracephala</i> – <i>Teleopsis</i>
Females					
PC 1	7.96	0.003	0.004	0.86	0.003
PC 2	9.60	0.001	0.12	0.001	0.60
PC 3	36.86	< 0.001	0.16	< 0.001	0.002
PC 4	40.23	< 0.001	0.04	< 0.001	< 0.001
Males					
PC 1	12.25	< 0.001	< 0.001	0.99	0.001
PC 2	43.18	< 0.001	0.53	< 0.001	< 0.001
PC 3	27.14	< 0.001	0.33	< 0.001	< 0.001
PC 4	1.33	0.29	–	–	–

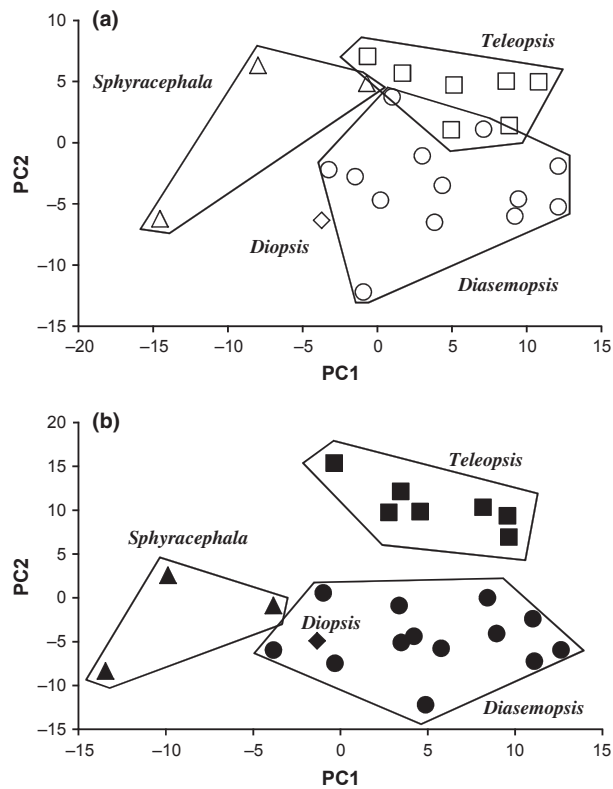


Fig. 4 Species averages of phylogenetic principal component (PC) 2 scores plotted against phylogenetic PC 1 scores for female (open symbols) and male (filled symbols) stalk-eyed flies from a phylogenetic principal components analysis of tangent coordinates from a procrustes analysis of wing landmarks (see Fig. 1). Polygons are drawn around the genera included in this study.

eye span in the magnitude of wing shape dimorphism (Table 2).

When analysing phylogenetically independent contrasts of dimorphism indices, we found that eye-span dimorphism was positively correlated across species with centroid dimorphism ($r = 0.73$, $P < 0.001$; Fig. 5a) and shape dimorphism ($r = 0.52$, $P = 0.012$; Fig. 5b).

Table 2 Results of standard (nonphylogenetic) and phylogenetic ANOVAs testing for differences in wing centroid size and dimorphism indices (see text for details) between species of stalk-eyed flies monomorphic and dimorphic for eye span. Males and females were analysed separately for centroid size. Significant differences are bolded.

		Standard P	Phylogenetic P
Centroid size			
Males	$F_{1,22} = 8.08$	0.009	0.008
Females	$F_{1,22} = 2.33$	0.14	0.16
Eye-span dimorphism	$F_{1,22} = 8.37$	0.008	0.014
Centroid dimorphism	$F_{1,22} = 8.96$	0.007	0.019
Shape dimorphism	$F_{1,22} = 1.08$	0.28	0.43

Shape dimorphism was not significantly correlated with centroid dimorphism ($r = 0.39$, $P = 0.07$).

In our qualitative assessment of how eye-span dimorphism is associated with wing shape, deformation grids that compared consensus (i.e. average) shapes of species monomorphic for eye span and those dimorphic for eye span within each genus showed dissimilar patterns of morphological differences among genera when monomorphic and dimorphic males were compared (Fig. 6). Whereas in both *Diasemopsis* and *Teleopsis* dimorphic species appear to increase wing size with longer, narrower wings, the specific sections of the wing that change differ between these two genera (Fig. 6).

Phenotypic trajectory analysis

When examining the magnitude and direction (angle) of change between the least-squares means of male and female individuals for each species vector, we found that sexual shape dimorphism differed in magnitude among species more often than in direction (Table 3). The lowest percentage of significant differences within or among genera occurred between species monomorphic for eye span, and this was true for both the magnitude and direction of change (Table 3). When considering only differences in magnitude, the largest percentage of significant differences occurred between

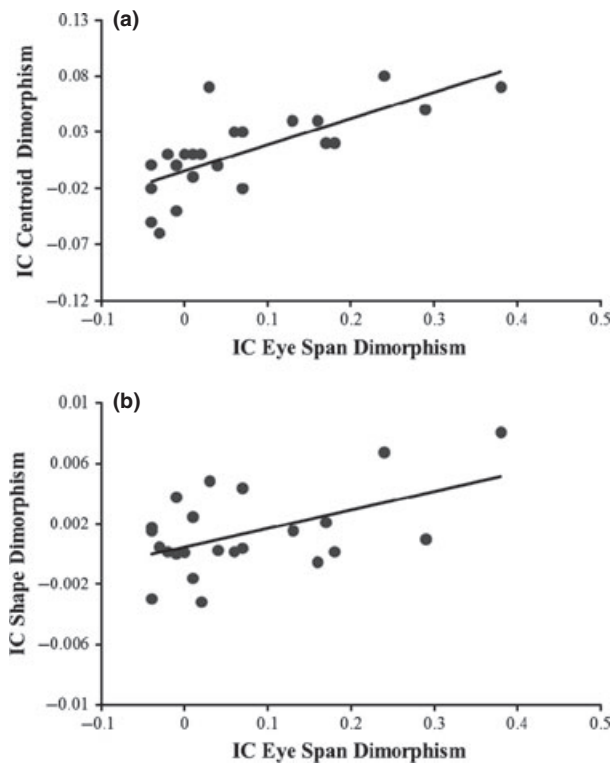


Fig. 5 Relationships between (a) wing centroid size dimorphism and eye-span dimorphism and (b) wing shape dimorphism and eye-span dimorphism across species of stalk-eyed flies (Diopsidae) taking phylogenetic relationships into account with independent contrasts. Shape dimorphism is the tangent distance between the mean shapes of the males and females for each species (see text for details). Least-squares regression lines are shown for visual indication of statistical significance.

species dimorphic for eye span, and differences were more often significant when comparing species within a genus than between genera (Table 3). However, when comparing species dimorphic for eye span to those monomorphic for eye span, differences were more often significant when comparing species between genera than within a genus (Table 3). When considering the direction (angle) of change, differences between species dimorphic for eye span were more often significant within a given genus than between genera, and this was also true when comparing species dimorphic for eye

Table 3 Summary of phenotypic trajectory analysis of sexual shape dimorphism, showing the proportion of significant tests (and after correction with sequential Bonferroni adjustment) when comparing the magnitude and direction (angle) of change between the least-squares means of male and female individuals for each species vector. Comparisons were made between species dimorphic for eye span (DM vs. DM, $N = 153$ comparisons), species monomorphic for eye span (MM vs. MM, $N = 15$ comparisons), and between species dimorphic for eye span and monomorphic for eye span (DM vs. MM, $N = 108$ comparisons), as well as within (congeneric, $N = 102$ comparisons) and between genera (intergeneric, $N = 174$ comparisons).

	DM vs. DM	MM vs. MM	DM vs. MM	All
Magnitude				
All	49.7 (16.3)	13.3 (0.0)	38.0 (6.5)	43.1 (11.6)
Congeneric	50.8 (18.5)	0.0 (0.0)	32.4 (2.9)	43.1 (12.7)
Intergeneric	48.9 (14.8)	16.7 (0.0)	40.5 (8.1)	43.1 (10.9)
Direction (angle)				
All	4.6 (0.7)	0.0 (0.0)	3.7 (1.9)	4.0 (1.1)
Congeneric	7.7 (1.5)	0.0 (0.0)	5.9 (2.9)	6.9 (2.0)
Intergeneric	2.3 (0.0)	0.0 (0.0)	2.7 (1.4)	2.3 (0.6)

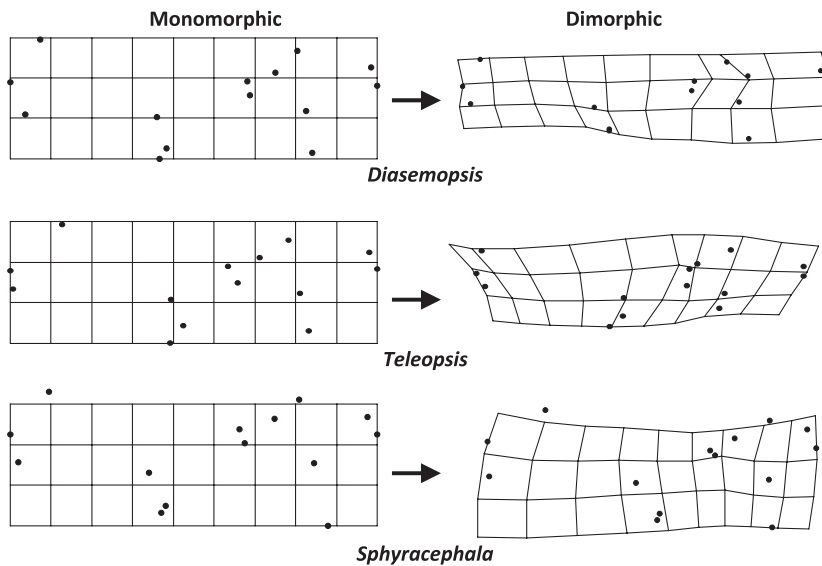


Fig. 6 Thin-plate spline deformation grids of males within each genus, showing comparison of wing shape between species monomorphic versus dimorphic for eye span. Deformation grids have been accentuated by a factor of three to enhance visual interpretation.

span to those monomorphic for eye span (Table 3). Taken together, species differed predominantly in the magnitude of shape change, and less so in the direction. Most of the differences resulted from the comparisons between species from the same genera that are dimorphic for eye span. For example, 18% of the of the 55 species comparisons of eye-span dimorphic *Diasemopsis* species differed in the magnitude of shape dimorphism, and one (1.8%) of the comparisons was significant for the direction of shape dimorphism. Similarly, 20% of the of the 10 species comparisons of eye-span dimorphic *Teleopsis* species differed in the magnitude of shape dimorphism, and none of the comparisons were significant for the direction of shape dimorphism. On the other hand, only 22% of the 55 species comparisons between *Teleopsis* and *Diasemopsis* species dimorphic for eye span differed in the magnitude of shape dimorphism, and none of the comparisons were significant for the direction (angle) of shape dimorphism (see also Supporting Information available on-line). None of the comparisons between monomorphic species in magnitude or direction of change were significant.

Mantel correlations to assess the relationship between phenotypic trajectories and phylogenetic relatedness revealed a significant relationship between patristic distance and difference in angle between vectors ($r = 0.22$, $P = 0.006$), but there was no such relationship detected between patristic distance and difference in magnitude of vectors ($r = 0.10$, $P = 0.09$). There was also no correlation between the difference in angle between vectors and the difference in magnitude of vectors ($r = -0.01$, $P = 0.54$).

Discussion

Stalk-eyed flies illustrate how morphological traits that control locomotion (wing shape and size) can display correlated evolution with a sexually selected ornament (eye span). Males of species dimorphic in eye span have larger wings compared to females, and wing shape differs among species in association with eye-span dimorphism (Ribak *et al.*, 2009; Husak *et al.*, 2011b; this study). Our finding of a positive correlation between eye-span dimorphism and two measures of wing dimorphism (centroid size and wing shape) is consistent with these previous findings. Thus, our analyses of dimorphism indices and phenotypic vectors indicate that eye-span dimorphism may have affected wing shape evolution in at least some consistent ways across all species examined. Moreover, we show that wing shape is also affected by phylogeny; much of the variation in wing shape (i.e. the first two principal components) grouped members according to genus (Fig. 3), even when phylogeny had been accounted for (Fig. 4), and the direction of the sex-difference in shape dimorphism increased with phylogenetic distance between taxa. Thus, our results reveal a complicated picture for the

evolution of wing size and shape in stalk-eyed flies. It appears that compensatory increases in wing size are a common feature of all dimorphic males, but there are some differences among genera in the way wing shape has changed between monomorphic and dimorphic species. Our results provide some evidence that compensatory increases in wing size have occurred via alternative morphological routes consisting of different structural wing changes among the genera. The fact that we found dimorphism effects overlaid on the phylogenetic component of wing shape further corroborates that stalk-eyed fly wings are under strong indirect sexual selection pressure from the presence of a sexually selected ornament (Husak & Swallow, 2011) that may otherwise reduce flight performance if not for the compensatory adjustment of the wings.

Examination of deformation grids comparing average consensus shapes of species monomorphic for eye span and those dimorphic for eye span within each genus revealed that morphological differences between males of monomorphic and dimorphic species are dissimilar among genera (Fig. 6; see also Figs. 3–4). This was supported by the phenotypic trajectory analysis, which revealed that the greatest number of significant differences between eye-span dimorphic and eye-span monomorphic species was between rather than within genera. In both *Diasemopsis* and *Teleopsis*, dimorphic species appear to increase wing size with longer, narrower wings. However, the specific sections of the wing that change differ between these genera (Fig. 6). In *Diasemopsis*, the distal section of the wing is narrowed, with the trailing distal edge extended. Conversely, in *Teleopsis*, the proximal section of the wing is narrowed, with the leading distal edge extended. Dimorphic *Sphyracephala* species exhibit a completely different pattern of shape change compared to the other genera, with increases in wing width along much of the wing length. The phylogenetic PCA supported these findings, as species within a genus grouped in morphospace. In the phenotypic trajectory analysis, 18% of the comparisons between eye-span dimorphic *Sphyracephala* and *Diasemopsis* were significantly different for direction of change, and 20% comparing *Sphyracephala* to *Teleopsis* (though none remained significant after Bonferroni correction). Numerous studies have now shown that eye-stalk elongation is evolutionarily associated with increases in wing area and changes in some measures of wing shape (Ribak *et al.*, 2009; Husak *et al.*, 2011a,b), but the results of our current study suggest that the general interspecies trend is in fact comprised of genera-specific changes in wing architecture converging into a similar functional means of compensation, thus representing another example of many-to-one mapping of form to function (Wainwright *et al.*, 2005; Wainwright, 2007).

Some care should be taken when interpreting the phylogenetic ANOVA results since most of the dimorphic species are *Diasemopsis* and *Teleopsis* species, whereas

two of the three *Sphyracephala* species are monomorphic (and *S. beccarii* is only moderately dimorphic, with substantially smaller eye stalks than most *Diasemopsis* or *Teleopsis* species). Thus, our phylogenetic ANOVA may still have been somewhat influenced by phylogeny despite taking it into account, because two of the five monomorphic species came from one genus (*Sphyracephala*), and there were only three species from that genus in the analysis. Regardless, the lack of a significant difference in the phylogenetic ANOVA between monomorphic and dimorphic species in wing shape dimorphism did not match with our correlation analysis of wing size and shape dimorphism indices relative to eye-span dimorphism. Although phylogenetic effects may partly explain this disparity, another reason for our somewhat discordant results is that classifying species as 'monomorphic' or 'dimorphic' in the phylogenetic ANOVA does not completely capture the large range of variation across species in eye-span dimorphism (Baker & Wilkinson, 2001) as well as the correlation analysis did. Nevertheless, the lack of a general difference between 'monomorphic' and 'dimorphic' species with phylogenetic ANOVAs reinforces the notion that the genera have, to some degree, attained wing size and shape dimorphism via different morphological routes. Moreover, we did find significant differences in eye-span dimorphism (as would be expected), as well as centroid dimorphism. These results, taken together, provide support to the notion that species dimorphic for eye span have larger wings, but have done so via different shape changes.

One surprising finding in our data was that many of the differences among genera when comparing monomorphic and dimorphic species seem related to internal movement of wing vein intersections and due less to changes in landmarks that would signify differences in wing length, area, or aspect ratio (see Fig. 6). This may be due to phylogenetic constraints coupled with isometric increases in wing size within a species, instead of dramatic shape changes between the sexes to compensate for eye-span elongation (Ribak *et al.*, 2009). With the data in hand we can only postulate on the functional significance of evolutionary changes in wing morphology across stalk-eyed fly species. Many of the differences among genera appear related to positioning of wing veins (see Fig. 1) that are either proximal-anterior (landmarks 5 and 6) or define cross-veins that connect longitudinal veins (landmarks 7–10). Movement of landmarks 9 and 10 affects the size and shape of the basal medial – distal medial cell. Similarly, movement of landmarks 7 and 8 affects the size and shape of the basal-radial cell in the wing. Note that these landmarks (5–10) form a diagonal band traversing from the leading edge at approximately 1/3 of the wing length (measured from the hinge) to the trailing edge at approximately 2/3 the wing length (Fig. 1). Since these landmarks represent the most distal intersections of the wing veins (excluding the wing tip) their specific positions should have important roles in

structurally stiffening and controlling deformation of the wing area. When aerodynamic load and wing inertia act on the flapping wing they will tend to bend it, and, since the bending moment is highest at the hinge, the proximal section of the wing is reinforced with thicker veins that are closer to one another. The thickness and positioning of wing veins along the leading edge determines wing stiffness along the wing span during flight (Combes & Daniel, 2003). The trailing edge has a reduced number of veins and these are much thinner (e.g. see Fig. 1). As a result, the trailing edge is less rigid and has a higher tendency to elastically deform during flapping. We postulate that the diagonal line of landmarks 5–11 can serve as a reinforcement of the wing structure for limiting wing deflection beyond the distal 1/3 of the wing and controlling wing camber. If this presumption is correct, then even small changes in the positions of landmarks 6–10 can have an effect on the elastic deformation of the wing during flapping (Ennos, 1989; Wootton, 1992). Ennos (1989) showed that ventral flexion of dipteran wings can allow force vectors to be changed (and hence maneuvers to be performed) with little to no body angle changes and that such flexion reduces stress on the wing articulation (Ennos, 1988, 1989; Wootton, 1992). Obviously, the functional implications of the wing vein intersection changes found in our study, and why different patterns of wing shape are associated with certain lineages, require confirmation from empirical studies. We hope that our findings and functional interpretations catalyse future work on the subtleties of wing veins and how they affect flight mechanics.

The phenotypic trajectory analysis was in general agreement with our other analyses in that it emphasized that most differences in wing shape dimorphism among species are due to the magnitude of shape change between the sexes and not the direction of shape change. As in our other analyses, there was a significant phylogenetic effect, with greater differences in the direction of shape change occurring between more distantly related species. Despite this phylogenetic effect on wing shape, we still detected numerous shape dimorphism differences among species, and even within genera. This finding suggests that many species within and among genera have similarities in wing shape, but that there are also strong differences, likely related to dimorphism in eye span as indicated by the significant relationship between eye-span dimorphism and shape dimorphism (Fig. 5). The variety of morphological solutions across species to compensate for exaggerated eye stalks is consistent with eye-span dimorphism and monomorphism having evolved multiple times in stalk-eyed flies (Baker & Wilkinson, 2001). Unlike the other analyses we used, the phenotypic trajectory analysis allowed us to divide dimorphism into magnitude and direction. Doing so revealed that patterns of dimorphism are dominated by differences in magnitude, not direction. Further, Mantel correlations revealed phylogenetic effects on direction of change, but not

magnitude. We found no phylogenetic signal in wing shape dimorphism indices using the K-statistic, but those indices indicate only the magnitude of dimorphism. Taken together, these results suggest that, not only is there phylogenetic signal in wing shape but also there is phylogenetic signal in the direction of morphological change between the sexes. We would not have detected this effect if we had not examined phenotypic trajectories.

Although considerable research has been conducted on insect wing form and function (Wootton, 1992; Grodnitsky, 1999; Stark *et al.*, 1999; Dudley, 2000; Combes & Daniel, 2003), even specifically within flies (Ennos, 1989), most of this work has examined functional differences among widely disparate taxa with highly divergent wing morphology and venation patterns. Although we have speculated on some of the potential functional consequences of smaller scale changes in wing shape, actual studies that examine functional differences at the magnitude of morphological divergence that we observed within diopsid flies are greatly needed. Such studies could be done with correlational biomechanical analyses of closely related species or with robotics (e.g. Shang *et al.*, 2009). Nevertheless, our comparative geometric morphometric analysis of wing shape allows for an examination of specific wing form changes that might be expected at the microevolutionary scale in response to selection. Our study also emphasizes the strong indirect effects that sexual selection may have on morphology, in addition to the direct effects on ornaments and other traits that directly enhance mating success. Testing whether the detrimental effects of sexually selected traits, in general, are ameliorated by the evolution of convergent form and function or convergent function via divergent form (i.e. many-to-one mapping) will be a fruitful area of research in the future. Such studies will allow a better understanding of how selection on the entire phenotype acts to shape locomotor systems in addition to direct selection on the locomotor apparatus and its performance.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Data S1 Wing Mounting Techniques.

Table S1 Results from phenotypic trajectory analysis, showing differences in magnitude (upper diagonal) and angle (lower diagonal) of change between the least-squares means of males and females for each species vector.

Figure S1 Plot of 14 landmarks shown in Figure 1 from stalk-eyed fly wings using three different techniques.

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