



Wing shape, wing size, and sexual dimorphism in eye-span in stalk-eyed flies (Diopsidae)

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The eyes of stalk-eyed flies (Diopsidae) are positioned at the end of rigid peduncles ('stalks') protruding laterally from the head. Eye-stalk length varies within the family and, in some species, varies between males and females. Larger eye-stalks in males result from sexual selection for longer stalks, a trait that increases male reproductive success. In the present study, we examined whether an increase in eye-stalk length results in an adjustment of wing size and shape to deal with the burden of bearing an exaggerated 'ornament'. We compared wing morphology among ten species of stalk-eyed flies that differ in eye-span and the degree of sexual dimorphism. Mass-specific wing length differed between males and females in seven out of the ten species. Nondimensional wing shape parameters differed between the species ($P < 0.001$), but mostly did not differ between males and females of the same species. Dimorphism in eye-span closely correlated with dimorphism in wing length ($r = 0.89$, $P < 0.001$) and the correlation remained significant ($r = 0.81$, $P = 0.006$) after correcting for phylogenetic relationships. Once corrected for phylogenetic relatedness, the mass-specific wing length of males (but not females) was weakly correlated with mass-specific eye-span ($r = 0.66$, $P = 0.042$). We propose that the observed proportional increase in wing length associated with increased eye-span can facilitate aerial manoeuvrability, which would otherwise be handicapped by the elevated moment of inertia imposed by the wider head. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **98**, 860–871.

ADDITIONAL KEYWORDS: aspect-ratio – insect-flight – moment-of-area – moment-of-inertia – sexual selection – wing-loading.

INTRODUCTION

The allometry of eye-span (i.e. the distance between the outer edges of the left and right eyes) on body length is a widely used measure of sexual dimorphism in diopsid stalk-eyed flies (Shillito, 1971; Burkhardt & de la Motte, 1988; Wilkinson, 1993; Baker & Wilkinson, 2001). These flies have their eyes placed on rigid peduncles protruding laterally from the head, with eye-span exceeding body length in males of some species (Shillito, 1940; de la Motte & Burkhardt, 1983; Fig. 1). In sexually-dimorphic species, despite overlapping body size distributions, male eye-span exceeds female eye-span, suggesting that sexual selection is driving the degree of dimorphism in eye-span (Wilkinson & Dobson, 1997). Studies showing

that females of sexually dimorphic species favour males with longer stalks (Burkhardt & de la Motte, 1988; Wilkinson & Reillo, 1994; Wilkinson, Kahler & Baker, 1998) and that males with longer eye-stalks tend to win male–male territorial confrontations (Panhuis & Wilkinson, 1999) confirm ongoing sexual selection in these dimorphic species.

Insects are a diverse group, and this diversity pertains also to the morphology of the flight apparatus (Dudley, 2000). Ellington (1984) showed that, although insects vary greatly in morphology, kinematics, and flight performance, some morphological properties are directly relevant to the force production during flight. Adopting the suggestion of Weis-Fogh (1973), Ellington (1984) showed how the contribution of wing shape and wing size can be incorporated by a group of parameters (i.e. the first three moments of wing area and their nondimensional radii) that are

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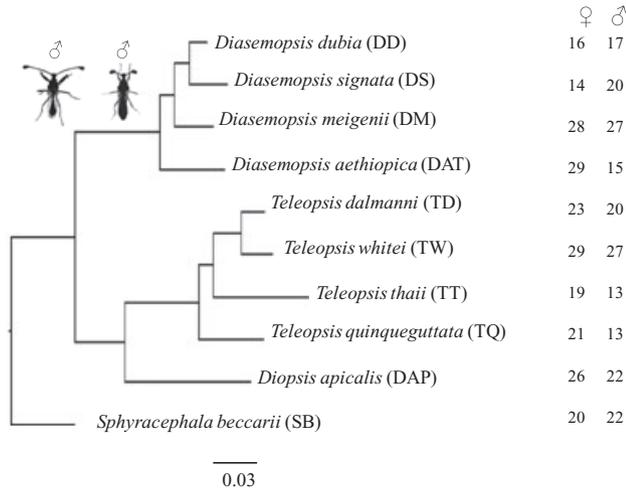


Figure 1. Phylogenetic relationships and branch lengths of the ten species of stalk-eyed flies (Diopsidae) used in the present study. Each species name is followed by an acronym code name in brackets. The numbers on the right are sample sizes for male and females of each species in this study. The images in the upper left corner show a male TW and a male TQ, side by side, illustrating the extreme inter-specific variation in relative eye-span.

proportional to lift and aerodynamic moments. The studies of Weis-Fogh (1973) and Ellington (1984) relate the average quasi-steady aerodynamic force to the cubic power of wing length and the aerodynamic moment to the quadruple power of wing length. Thus, very small changes in wing length can result in amplified aerodynamic performance, even when wing-beat kinematics remain constant.

The extent to which the cost of producing and maintaining ornaments trades-off with the fitness of the individuals bearing them, remains a fundamental question in studies of evolution by sexual selection (Darwin, 1871; Fisher, 1930; Zahavi & Zahavi, 1997). In a recent study, Ribak & Swallow (2007) measured the aerial turning (manoeuvring) performance of male and female *Cyrtodiopsis dalmanni* Widemann, 1830, a species with sexually dimorphic eye-span (Wilkinson, 1993; Wilkinson & Reillo, 1994) and found little evidence for a functional trade-off between eye-span and performance. [The genus *Cyrtodiopsis* has been synonymized with *Teleopsis* recently (Meier & Baker, 2002). We use this later (updated) nomenclature from here on.] Despite an elevated moment of inertia associated with increased eye-span that would require approximately 1.5-fold larger torques to rotate their bodies in air, males engaged in aerial manoeuvres that were equal to, or surpassed, those of females with shorter eye stalks. To explain this counterintuitive observation, the authors suggested that male *Teleopsis dalmanni* compensate for their

increased rotational inertia by having longer wings (5% mass-specific increase relative to females). This small difference in mass-specific wing length leads to a larger difference in area of the wing pair (approximately 8%) and can result in even larger differences in aerodynamic power (Ribak & Swallow, 2007).

We tested the hypothesis that wings coevolved with variation in eye-span as a mechanism to compensate for the increase in moment of inertia of the body as the large compound eyes shifted away from the midline of the body. If the wings indeed compensate for increased eye-span, as proposed for *T. dalmanni*, we would expect the increase in eye-span to correlate with changes in wing morphology within the family. We took advantage of the fact that sexual dimorphism in eye-span varies among the various species within Diopsidae (Burkhardt & de la Motte, 1985; Wilkinson & Dobson, 1997; Baker & Wilkinson, 2001; Kotrba & Balke, 2006) and compared mass-specific wing sizes and wing shape of males and females from ten species within the family. The species used differ in mean body size, eye-span, and the degree of eye-span dimorphism. If changes in the wings are related to an increase in eye stalk length, we expect to find a correlation between eye-span and wing dimorphism, as well as a correlation between mass-specific eye-span and mass-specific wing size and/or shape parameters across all species.

MATERIAL AND METHODS

SPECIES SAMPLED AND PHYLOGENETIC RELATEDNESS
The flies employed in the present study (Fig. 1, Table 1) were chosen based on availability from populations raised in captivity at the University of Maryland at College Park. The species represent four of the major genera and capture much of the range in ornament size and sexual dimorphism seen in the family. Because phylogenetic relationships between species can bias comparative statistical analyses that assume the independence of data points, we used phylogenetically independent contrasts (Felsenstein, 1985). These analyses require an independently-generated phylogenetic hypothesis. We developed a phylogenetic hypothesis for the ten species in the present study using DNA sequence data from fragments of two mitochondrial gene regions, cytochrome oxidase II and 16S ribosomal RNA. Details concerning fly sampling localities, DNA amplification, and GenBank accession numbers were taken from Baker, Wilkinson & DeSalle (2001) and Földvári *et al.* (2007), and are summarized in Table 1. The concatenated two-gene data set, which consisted of 1100 bp, was aligned and subjected to parsimony analysis. *Sphyracephala beccarii* was chosen as the outgroup and used

Table 1. Location of collection sites and references of molecular data for the ten taxa used in the present study

Species	Locality	GenBank Accession No.	
		COII	16S
<i>Diasemopsis dubia</i>	Pietermaritzburg, South Africa (S29°35', E30°21')	AF304750	AF304175
<i>Diasemopsis signata</i>	Limbe, Cameroon (N04°01', E09°13')	AF304755	AF304720
<i>Diasemopsis meigenii</i>	Pietermaritzburg, South Africa (S29°35', E30°21')	AF304752	AF304717
<i>Diasemopsis aethiopica</i>	Nelspruit, South Africa (S25°22', E30°41')	AF304771	AF304736
<i>Teleopsis dalmani</i>	Ulu Gombak, Malaysia (N03°12', E101°42')	AF305782	AF304747
<i>Teleopsis whitei</i>	Ulu Gombak, Malaysia (N03°12', E101°42')	AF304784	AF304749
<i>Teleopsis thaii</i>	Doi Suthep National Park, Thailand (N16°14', E101°10')	DQ191300	DQ191299
<i>Teleopsis quinqueguttata</i>	Bukit Ringit, Malaysia (N03°42', E102°08')	AF304783	AF304748
<i>Diopsis apicalis</i>	Pietermaritzburg, South Africa (S29°35', E30°21')	AF304777	AF304742
<i>Sphyracephala beccarii</i>	Rivulets, South Africa (S25°26', E30°45')	AF304772	AF304737

to root the tree. Two most parsimonious trees were obtained using PAUP* (Swofford, 2003). The two trees differed by the placement of *Teleopsis thaii*. We chose the tree that placed it as sister to the sexually dimorphic species rather than to the monomorphic *Teleopsis quinqueguttata* (Fig. 1), consistent with a recent phylogenetic analyses of the *dalmani* species group (Földvári *et al.*, 2007) and of the family Diopsidae (Baker *et al.*, 2001), which used larger molecular datasets. Branch lengths were estimated for the selected tree in PAUP* using maximum likelihood with an over-parameterized model (GTR + I + G). The topology and branch lengths obtained from these analyses were used to generate the independent contrasts in PDTREE.

MORPHOLOGICAL MEASUREMENTS

Large stock population cages were maintained for each species in cages (40 × 40 × 120 cm) lined with moist cotton and blotting paper in a temperature (25 °C) and humidity (> 60%) controlled environmental chamber under a 12 : 12 h light/dark cycle. Flies in the large population cages were allowed to feed and oviposit *ad libitum* on pureed corn that was changed twice weekly. For measurement, each fly was removed from the population cage, placed in a sealed vial and weighed to the nearest 0.01 mg. *Post mortem*, each fly was placed on its back under a digital camera mounted on a dissecting microscope. A single image of the ventral view of the fly, showing the entire body, was taken to measure eye-span. Then, an incision was made at the pleuron and the wing was separated from the thorax, complete with its hinge, placed on a microscope slide and covered with a microscope cover glass. We used either the left or right wing depending on which wing was successfully detached from the thorax intact. A digital image of the wing was then taken at a larger magnification for wing morphology analysis.

We limited our comparative wing morphology (size and shape) analysis to specific wing traits: wing length, wing area, wing moment of area and aspect-ratio. We focused only on these parameters rather than using a more general method like geometrical morphometrics (Gilchrist, Azevedo & Partridge, 2000; Rohlf, 2002) because, although the latter can be more sensitive to subtle differences in wing shape, the results would be less informative regarding aerodynamic performance. The morphological traits compared in the present study are directly relevant to Ellington's (1984) quasi-steady model of insect flapping flight.

Measurements were performed on the images using image analysis software (ScionImage, Scion Corp.). Eye-span (E) was measured to the nearest 0.01 mm between the outer edges of the eyes. The images of the wings were first oriented so that wing length (R , the line connecting the base of the wing with the wing tip) was horizontal in the image. The wing base was defined as the first articulation of the wing veins (Figs 2, 3). The wing tip was defined as the point furthest away from the wing base on the curved wing tip. At steps of five image pixels along the length of the wing in the image, the positions (two-dimensional coordinates) of points on the leading and trailing edges of the wing were stored into a computer file. Image magnification (> 175 pixel mm⁻¹) was adjusted between the species so that we typically obtained > 200 points on each wing edge for all wings regardless of wing size. An image analysis macro program (custom written for ScionImage), which identified wing edges in the images and measured the positions (Fig. 2), allowed some automation to the process enabling the measurement of wings from a total of 421 flies.

The wing edge position data were used to calculate wing length ($R = \text{number of measurements} \times 5 \text{ pixel} \times \text{image magnification factor}$) and area of the

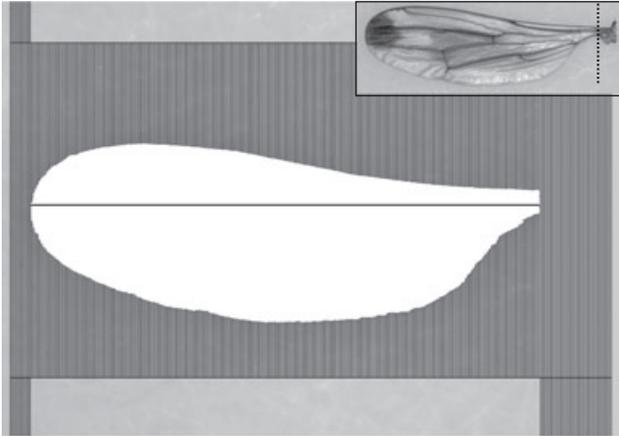


Figure 2. Wing morphology measurement. Image analysis software uses the image contrast to find the wing (white area) edges from the background and measures positions on the wing edges every five image pixels (vertical lines in the background) along the wing length (the horizontal line connecting wing base and tip). In this example, the measured wing is the one shown in the insert at the top right corner (belongs to a male *Diopsis apicalis*). Only the area distal to the first vein articulation (left of the dashed vertical line in the insert) is measured.

wing pair (S , from integrating the distances between wing edges along R and multiplying by 2 to account for both wings), and the first three moments of wing area and their nondimensional radii were calculated *sensu* Ellington (1984). The k th ($k = 1, 2, 3$) moment of wing area (S_k) is:

$$S_k = 2 \int_0^R cr^k dr \quad (1)$$

denoting the product of the local wing chord (c) with its distance to the wing base (r), where the latter is raised to the k th power. The product is integrated (dr is the variable of integration) over the entire wing length ($0 \leq r \leq R$) and multiplied by 2 to account for the wing pair. The moment radius (r_k) is a nondimensional representation of the same moments. According to Ellington (1984):

$$r_k = \sqrt[k]{\frac{S_k}{SR^k}} \quad (2)$$

Because moments 1–3 (and their radii) are highly correlated with each other (Ellington, 1984; Eqns 1–2), we focused our analysis on the third moment ($k = 3$, i.e. S_3 with units of m^5), which is the most sensitive to small variations in wing length (as seen in Eqn. 1) and relates to the aerodynamic moment generated by the wing (Weis-Fogh, 1973). The nondimensional radius of this moment, r_3 , is a function

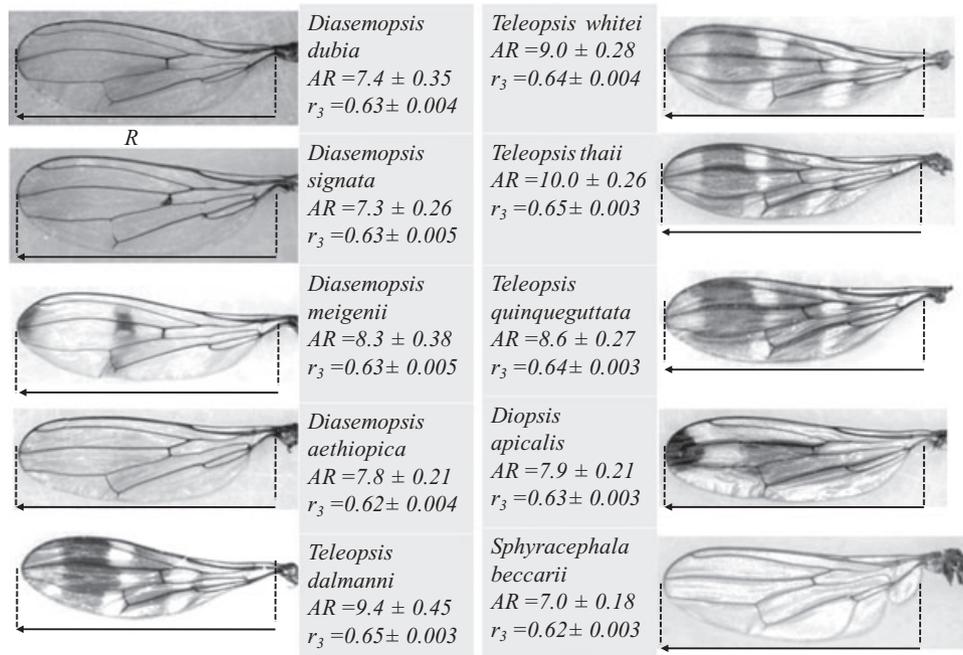


Figure 3. Wing shapes of the species analysed. All wing images are scaled to have similar lengths (R) to emphasize changes to wing shape. AR is aspect-ratio and r_3 is the nondimensional radius of the third moment of wing area. Values are the mean \pm SD. The wings shown are taken from males.

of wing shape. It is related to the distribution of wing area along the wing length. The larger the radius, the more wing area is located away from the wing base and closer to the wing tip. Wing aspect-ratio (AR) is a more general nondimensional representation of wing shape. It is the length (span) of the wings relative to width (average chord). Aspect-ratio increases for narrower (elongated) wing shapes. For the wing pair, AR was calculated from wing span ($= 2R$) and area as:

$$AR = \frac{(2R)^2}{S} \quad (3)$$

Wing-loading (ρ_w) equals the ratio of body weight and total wing area. Wing-loading represents the average pressure ($N\ m^{-2}$) exerted by the wings on the air to support body mass:

$$\rho_w = \frac{mg}{S} \quad (4)$$

where m is the measured fresh body mass of the fly and g is gravitational acceleration ($9.8\ ms^{-2}$).

We used analysis of covariance (ANCOVA) with body mass as a covariate to test for species and sex effects in the measured parameters: E , R , S , S_k and ρ_w . All variables were \log_{10} -transformed prior to analyses. We used analysis of variance (ANOVA) to test for the same effects in the nondimensional parameters r_k and AR . Using the least-squares adjusted-means from the ANCOVA for males and female of each species, we calculated a 'dimorphism index' for each parameter. For example, sexual dimorphism in eye-span (I_E) was calculated for each species as the difference in the adjusted means of eye-span of males (\bar{E}_m) and females (\bar{E}_f):

$$I_E = \bar{E}_m - \bar{E}_f \quad (5)$$

Similar differences were calculated for the remaining traits (wing length, I_R ; wing area, I_S ; wing-loading, I_p ; moment of area, I_{Sk}). The dimorphism indices allowed scoring each species based on the degree of dimor-

phism. We used eye-span dimorphism (I_E) as the independent variable and regressed the wing dimorphism indices for all ten species to test for a relationship between eye-span dimorphism and wing dimorphism. A second analysis tested for a direct relationship between wing size and eye-span within the two sexes separately (i.e. after eliminating the sex effect). For each sex, we regressed wing length and eye-span on body mass (after log transforming all variables) and calculated the residuals. The residuals of wing length were then plotted against the residuals of eye-span and tested for a correlation. All correlation tests were repeated using phylogenetically independent contrasts calculated in PDM (freeware) according to methods outlined above and *sensu* Garland, Harvey & Ives (1992) and Garland *et al.* (1993). Throughout the present study, we report correlations between variables using the Pearson coefficient (r) and the P -value of the regression analysis and P' and, in some cases, r' to distinguish the P -value and Pearson coefficient of the same test performed on the phylogenetically corrected regression on standardized contrasts. Statistical tests were performed using SYSTAT, version 10 (SYSTAT software) and STATISTICA, version 5.0 (StatSoft Inc.).

RESULTS

Figure 4 shows the means per species per sex of the measured body mass, eye-span, wing length, and wing area. The ANCOVA on eye-span using body mass as a covariate (Table 2) revealed significant effects of species ($F_{9,384} = 886$, $P < 0.001$), sex ($F_{1,384} = 1757$, $P < 0.001$), and a significant interaction between species and sex ($F_{9,384} = 92$, $P < 0.001$). Tukey post-hoc tests of the interaction showed that three of the species (TQ, DS, SB; for list of code names, see Fig. 1) had no significant differences in the mass-adjusted eye-span between the sexes ($P > 0.212$). The remaining species exhibited significant dimorphism in eye-span, with males having significantly longer eye stalks for their body mass than females ($P < 0.001$

Table 2. Results of the analysis of covariance for eye-span (E), wing length (R), wing area (S), and the third moment of wing area (S_3)

Dependent variable	Species	Sex	Species \times Sex	Body mass
E	$F_{9,384} = 886.3^{***}$	$F_{1,384} = 1757.4^{***}$	$F_{9,384} = 92.3^{***}$	$F_{1,384} = 209.6^{***}$
R	$F_{9,400} = 109.4^{***}$	$F_{1,400} = 10.6^{**}$	$F_{9,400} = 8.3^{***}$	$F_{1,400} = 207.3^{***}$
S	$F_{9,400} = 129.8^{***}$	$F_{1,400} = 5.2^*$	$F_{9,400} = 6.5^{***}$	$F_{1,400} = 232.6^{***}$
S_3	$F_{9,386} = 80.3^{***}$	$F_{1,386} = 10.3^{**}$	$F_{9,386} = 6.2^{***}$	$F_{1,386} = 227.6^{***}$

Asterisk denote statistical significance: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. The covariate is body mass and all data are log-transformed for the analysis.

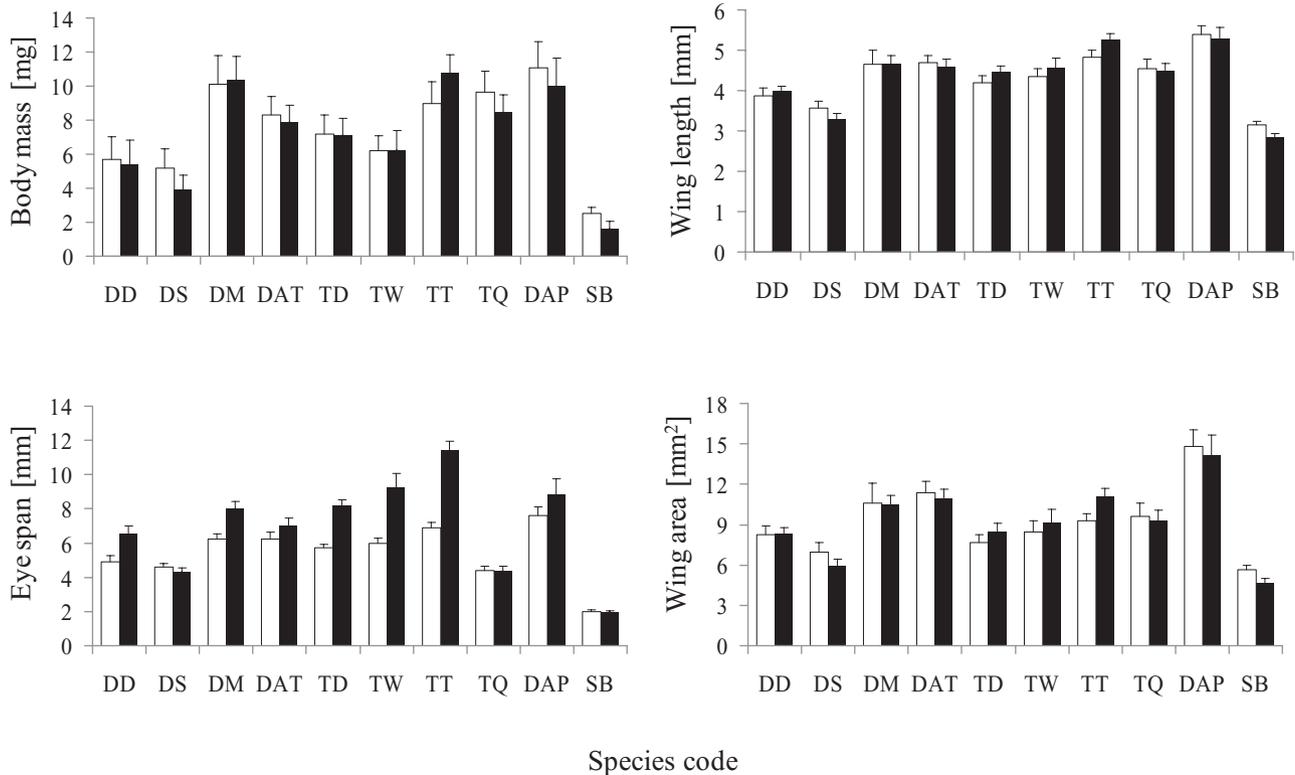


Figure 4. Mean \pm SD of the measured values per species per sex. Black bars indicate males and open bars indicate females. A key to species code names is provided in Fig. 1.

for all comparisons). The differences between males and females in the adjusted means revealed that the dimorphism index for eye-span (I_E) was greater than zero for all ten species (Fig. 5). Its value ranged between 0.032 mm in the least dimorphic species-*Diasemopsis signata* (DS) and 3.87 mm in the most dimorphic species *T. thaii* (TT). I_E was not correlated with the average body mass of each species ($r = 0.44$, $P = 0.193$, $P' = 0.428$), demonstrating that the index was independent of the variation in body mass among the various species.

VARIATION IN WING SIZE AS A FUNCTION OF EYE-SPAN

An ANCOVA on wing length using body mass as a covariate revealed significant species ($F_{9,400} = 109$, $P < 0.001$), sex (male wings > female wings, $F_{1,400} = 10.6$, $P < 0.001$) and interaction ($F_{9,400} = 8.3$, $P < 0.001$) effects. Qualitatively similar results were obtained in analyses of the remaining traits that were mathematical variations of wing size (Table 2). I_R ranged between -0.14 to 0.27 mm (Fig. 5A), reflecting the fact that dimorphism in wing length in Diopsidae can be bidirectional where females of some species can have longer wings, relative to their body mass, than males. I_R was positively correlated with I_E ($r = 0.893$, $P <$

0.001 , $P' = 0.006$; Fig. 5A). Dimorphism in wing length (I_R) and dimorphism in wing area (I_S) were closely correlated ($r = 0.97$, $P < 0.001$, $P' < 0.001$), so that a similar trend of positive correlation between I_E and I_S was observed (Fig. 5B). Species with large sexual dimorphism in eye-span had significantly lower wing-loading dimorphism ($r = -0.89$, $P < 0.001$; $P' = 0.008$; Fig. 5C) and a higher third moment of wing area dimorphism ($r = 0.91$, $P < 0.001$; $P' < 0.001$; Fig. 5D).

Wing length and eye-span both increased with body mass (Fig. 6). The residuals from the regression of wing length on body mass did not correlate significantly with the residuals from the regression of eye-span on body mass (males: $r = 0.46$, $P = 0.181$; females: $r = 0.273$, $P = 0.445$). However, after correcting for phylogeny, contrasts of residuals showed a weak association that was marginally significant in males ($r' = 0.66$, $P' = 0.042$) but not in females ($r' = 0.49$, $P' = 0.154$). A similar residual analysis with wing area revealed no significant correlation with eye-span in males or females ($P > 0.6$, $P' > 0.19$ in all tests).

VARIATION IN WING SHAPE AS A FUNCTION OF EYE-SPAN

Variation in wing shape is evident in Figure 3, where images of wings of the ten species are scaled to

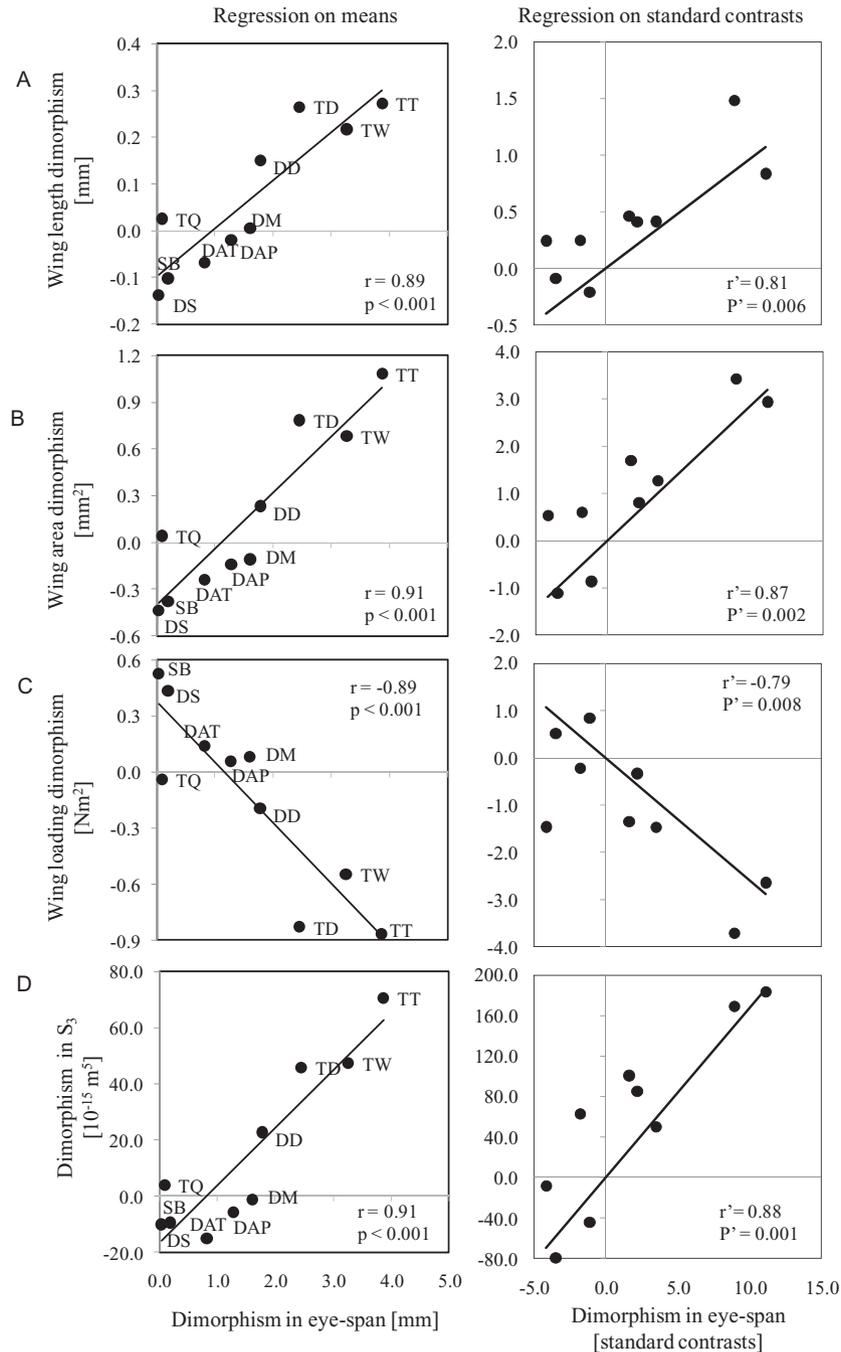


Figure 5. Sexual dimorphism in stalk-eyed flies. Sexual dimorphism is defined as the difference in adjusted means (adjusted for body mass) of males minus females (left column of plots). In each row (A–D), the plot to the right is the standard contrasts of the same data as shown on the left. r and r' are the Pearson coefficients and P and P' are the P -values of the regressions. Plotted as a function of dimorphism in eye-span are dimorphism in wing length (A), dimorphism in wing area (B), dimorphism in wing-loading (C), and dimorphism in the third moment of wing area (D). A key to species code names is provided in Fig. 1.

similar wing lengths. ANOVA showed that AR varied between species ($F_{9,401} = 407$, $P < 0.001$) and had a significant sex effect (AR of males $>$ AR of females, $F_{1,401} = 6.03$, $P = 0.014$). The interaction between

species and sex was significant ($F_{9,401} = 2.53$, $P = 0.008$) but post-hoc tests (Tukey) revealed that within species only one (DD) had sexually dimorphic AR ($P = 0.014$). The radii of the third moment of wing

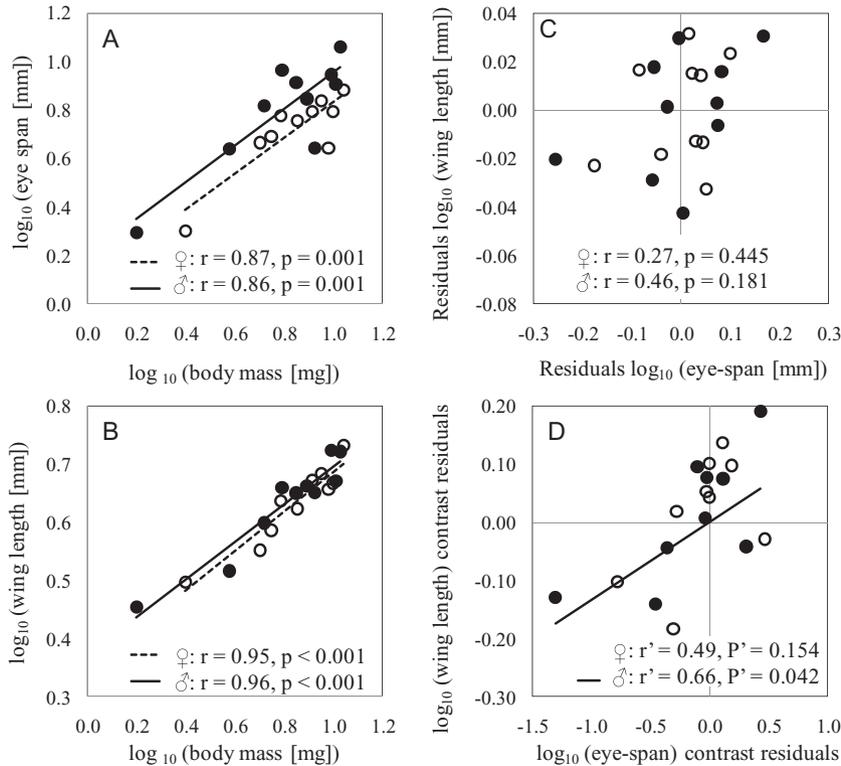


Figure 6. The relationship between mean wing length and mean eye-span in ten species of stalk-eyed flies. Males are denoted with black circles and females with open circles. A, log eye-span as a function of log body mass. B, log wing length as a function of log body mass. C, residuals from the regression in (A) plotted against residuals from the regression in (B). D, same as in (C), but this time using the residuals from an independent contrast analysis to account for phylogeny of the species used in the present study. Note that the correlation between the contrasts of residuals is marginally significant in males but not significant in females.

area (r_3) differed significantly between the species ($F_{9,387} = 335$, $P < 0.001$) but not between sexes ($F_{1,387} = 0.74$, $P = 0.389$) in all species (nonsignificant interaction, $F_{9,387} = 1.02$, $P = 0.422$). To identify whether a trend can be found in the variation of wing shape between the different species, we regressed I_E with the mean (per species) AR and r_3 . I_E was significantly correlated with AR ($r = 0.789$, $P = 0.007$) and had a weaker correlation ($r = 0.633$, $P = 0.049$) with r_3 ; Fig. 7). However, the statistical significance of these correlations vanished once the data were adjusted to account for phylogeny ($P' = 0.126$ and $P' = 0.455$ for AR and r_3 , respectively).

DISCUSSION

In holometabolous insects, resource allocation during growth can lead to the expression of a sexually-selected morphological trait at the expense of other morphological traits (Nijhout & Emlen, 1998; Emlen & Nijhout, 2000; Emlen, 2001). Thus, Kawano (1995) found that male Rhinoceros beetles with larger horns possessed relatively smaller wings than hornless

females or males with smaller horns. For the traits measured in the present study, we report the opposite scenario. In sexually dimorphic species of stalk-eyed flies, the males with larger eye stalks also grow longer wings for their body mass.

There appears to be little doubt that exaggerated morphologies come at some cost to the individual bearing them. However, identifying and quantifying these costs as an effect on locomotion can sometimes be intricate. The dimorphic tail streamers of the male barn swallow have been suggested both as a handicap on flight and as an adaptation for improved flight manoeuvrability (see Buchanan & Evans, 2000 and references therein). Artificially adding long tail feathers to short tailed hummingbirds showed that maximum flight speed was reduced by only a few percent ($< 4\%$) and the metabolic rate increased up to 11%, even when the length of the tail was artificially elongated by approximately six-fold (Clark & Dudley, 2009). In the dimorphic stalk-eyed fly *T. dalmanni*, males with longer eye-stalks were able to match and even exceed the aerial manoeuvrability of shorter eye-span females (Ribak & Swallow, 2007). A plau-

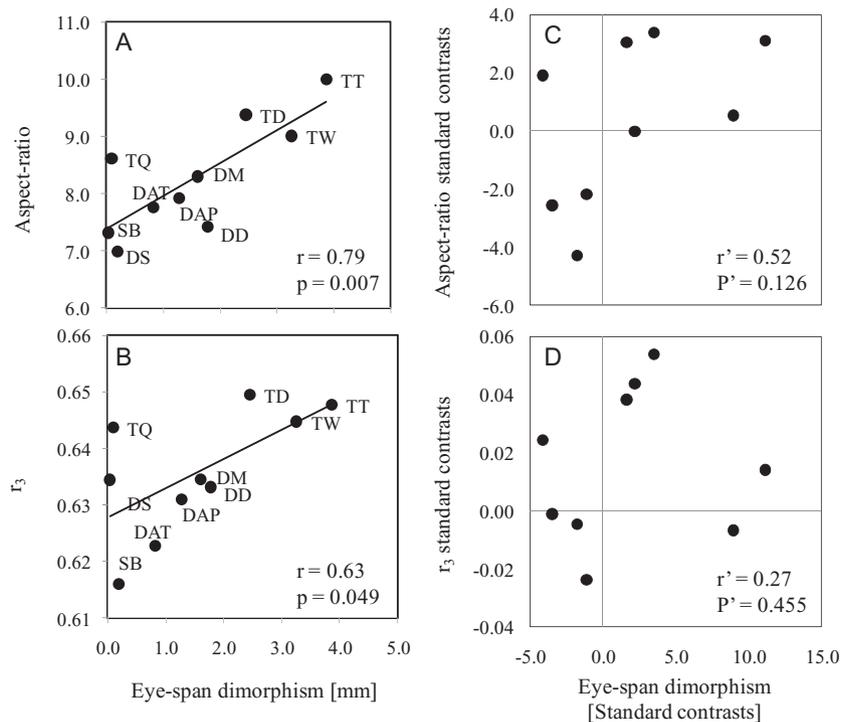


Figure 7. Relationship between wing shape and eye-span dimorphism. Shown as a function of eye-span dimorphism (I_E , see text) are the wing aspect-ratio (A) and the radius of the third moment of wing area (B). Note that the significant relations in (A) and (B) were not significant when the analyses were repeated using independent contrasts, (C) and (D), respectively. A key to species code names is provided in Fig. 1.

sible explanation for the latter finding is the ability of individuals with exaggerated ornaments to compensate for the handicap associated with these ornaments (Zahavi & Zahavi, 1997). The morphological analysis reported in the present study suggests that, in stalk-eyed flies, compensation is alleviated by dimorphism in wing size.

We hypothesized that wing morphology (size and/or shape) coevolved with increased eye-span and its associated constraints on flight manoeuvrability. Using dimorphism indices, we found that dimorphism in wing size correlated with dimorphism in eye-span in support of this hypothesis. The ANOVA analyses on wing shape parameters (AR , r_3) revealed significant differences between species and sex, but dimorphism in wing shape in only one species. Furthermore, analysis of independent contrasts indicated that dimorphism in eye-span is not significantly correlated with wing shape parameters. Therefore, it appears that the evolution of ornament sexual dimorphism in stalk-eyed flies occurs in parallel with a relative increase in male wing size compared to females, but the increase in wing size occurs without changes in wing shape. When wing shape stays the same all wing size parameters (R , S and S_3) are inter-correlated; hence, it was not unexpected to note that,

once dimorphism in eye-span correlated with dimorphism in wing length, similar relationships existed for wing area and S_3 .

The second set of analyses compared actual trait size (instead of inter-sex differences) within each sex. The hypothesis tested here was that eye-span is directly correlated with wing length once the effect of body size (mass) is removed. Because variation in eye-span is smaller among females than among males (Fig. 6), we anticipated a weaker statistical correlation between eye-span and wing traits in females. Furthermore, within both sexes, we can expect the relationships between eye-span and wing size to be obscured by inter-specific variance in morphology, life style, flight performance, and phylogeny. Indeed, we found a weak correlation between mass-specific eye-span and mass-specific wing-length but only in males, and only after correcting for the phylogenetic relationships between species. Therefore, there does not appear to be a constraint on wing size imposed by eye-span but rather a trend within each species where males with longer eye stalks, compared to females, also have larger wings.

Wing shape, particularly r_3 , appeared to vary between species depending on phylogeny. The phylogenetically independent regression revealed no

relationship between wing shape parameters and eye-span dimorphism. For example, Fig. 3 indicates that two members of the genus *Teleopsis*, TQ and TW, have very similar wing shapes (Figs 3, 7), despite the former being a monomorphic species with short eye-span and the latter a dimorphic species with long eye-span (Fig. 1). Conversely, the lack of statistical correlation may also result from the small range of variation in r_3 among species (Fig. 3) and the relatively small sample size ($N = 10$ species).

Increasing the moment of area of the wing without changing body mass can be achieved by increasing the wing area without changing wing shape (AR or r_3), in which case wing-loading is reduced. The moment of area can also be increased by deviating from geometrical similarity, changing wing shape by increasing AR and r_3 without changing wing area, thus keeping wing-loading the same. Within species, we see that the first scenario is the case because an increase in wing size in males is not associated with a change in wing shape but does result in eye-span dimorphic males having lower wing loading. One question that remains unaddressed concerns why should larger, longer wings be an advantage for a stalk-eyed fly with larger eye-span? We propose that one answer may be to increase the torque produced by the flapping wings to compensate for the elevated inertia of the body during turning in air.

In equally-sized (body mass) male and female *T. dalmanni*, the longer eye-span of males is not associated with an increase in the total mass of the head compared to females. Rather, the increased eye-span mainly affects the lateral distribution of the same head mass and this contributes to a higher moment of inertia (MOI) of the head for roll and yaw. In *T. dalmanni*, the higher MOI of the head increases the total MOI of the body for rotation about a vertical axis by approximately 50% when the body is at flight posture (Ribak & Swallow, 2007). The MOI of a body is inversely related to the responsiveness of the body to changes in rotation speed when acted upon by a force moment. Thus, a higher MOI has the potential of reducing manoeuvrability. High-speed films show that, when fruit-flies turn 90° in less than 50 ms, it takes the body approximately ten wing-beat cycles to complete the turn (Fry, Sayaman & Dickinson, 2003). The torque to rotate the body is developed in the wings over several wing-beat cycles to overcome the inertia of the body and wing (Fry *et al.*, 2003; Hedrick, Cheng & Deng, 2009); but, for the relative importance of drag, see also Hesselberg & Lehmann (2007). When hovering, if a fly is to change the direction it is facing by rotating about a vertical axis (i.e. with little or no banking), the wings must generate a net torque. The magnitude of this net torque is the difference between the force moments produced by

the left and right wings. Because the force moments generated by the wings are related to the third moment of wing area (Weis-Fogh, 1973), for the same kinematic parameters, larger wing length contributes to larger force and moments through high-order (power) functions that amplify small changes in wing length. Here, it is important to note that the small increase in R ($0.27\text{ mm} = 6\%$ inter-sex difference of the adjusted mean of TT) results in a much larger (32%) increase in S_3 , which can explain how slightly longer wings of males with longer eye-spans generate stronger moments per wing beat, potentially improving the turning performance by compensating for a higher MOI of the body.

This proposed explanation for the observed variation in wing morphology ignores the contribution of wing-beat kinematics to flight plasticity. It is impossible to link flight performance directly to variation in wing morphology *per se* because morphological relationships are further complicated by variation in wing-beat kinematics. Wing-beat frequency will tend to reduce with the increase in wing length (Sotavalta, 1947, 1952) and male stalk-eyed flies can possibly compensate for increased inertia by supplementing the variation in wing morphology with variation in wing-beat kinematics. However, the complexity of insect flight does not eliminate selection for adaptive wing morphologies. Geometrical constraints on kinematics, as well as muscle and aerodynamic efficiency, should result in selective pressures to adjust wing morphology to flight requirements (Dudley, 2002; Wootton, 2002). Lines of fruit flies provide an example for adjustment of wing morphology to flight requirements. Wing aspect-ratio and wing length have been shown to increase in *Drosophila* via morphological phenotypic plasticity, in correlation with latitude (Azevedo *et al.*, 1998). Flies reared in colder areas increase wing AR to increase the lift produced by their wings that flap at reduced frequency (Starmer & Wolf, 1989; Frazier *et al.*, 2008). Hence, to compensate for reduced flight performance, flies reared in the cold grow longer wings that have a larger aerodynamic moment arm and have the potential of producing larger forces per wingbeat.

Verifying the link between wing morphology and sexual selection for increased eye-span requires more studies on the wing-beat kinematics of stalk-eyed flies and how this varies with wing morphology and inertia of the body. The present study demonstrates that dimorphism in eye-span is correlated with dimorphism in wing size, supporting the idea that wings grow in size as a compensatory mechanism to prevent male flies from exhibiting reduced flight performance. If this is the case, then trade-offs between natural and sexual selection that regulate the evolution of eye-span growth most likely do not result from

reduced flight performance but may occur in resource allocation between other traits during larval development (Nijhout & Emlen, 1998) or in the energetic cost of locomotion for the mature flies.

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