Naturalis Repository

Habitat variation and wing coloration affect wing shape evolution in dragonflies

D. Outomuro, K.-D. B. Dijkstra, F. Johansson

Downloaded from:
http://dx.doi.org/10.1111/jeb.12203

Article 25fa Dutch Copyright Act (DCA) - End User Rights

This publication is distributed under the terms of Article 25fa of the Dutch Copyright Act (Auteurswet) with consent from the author. Dutch law entitles the maker of a short scientific work funded either wholly or partially by Dutch public funds to make that work publicly available following a reasonable period after the work was first published, provided that reference is made to the source of the first publication of the work.

This publication is distributed under the Naturalis Biodiversity Center 'Taverne implementation' programme. In this programme, research output of Naturalis researchers and collection managers that complies with the legal requirements of Article 25fa of the Dutch Copyright Act is distributed online and free of barriers in the Naturalis institutional repository. Research output is distributed six months after its first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and copyrights owner(s) of this work. Any use of the publication other than authorized under this license or copyright law is prohibited.

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the department of Collection Information know, stating your reasons. In case of a legitimate complaint, Collection Information will make the material inaccessible. Please contact us through email: collectie.informatie@naturalis.nl. We will contact you as soon as possible.

JOURNAL OF Evolutionary Biology

doi: 10.1111/jeb.12203

Habitat variation and wing coloration affect wing shape evolution in dragonflies

D. OUTOMURO*, K.-D. B. DIJKSTRA† & F. JOHANSSON*

*Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden
†Naturalis Biodiversity Center, Leiden, The Netherlands

Keywords:

geometric morphometrics;
independent contrasts;
landscape;
phenotypic correlation;
Trithemis;
water body.

Abstract

Habitats are spatially and temporally variable, and organisms must be able to track these changes. One potential mechanism for this is dispersal by flight. Therefore, we would expect flying animals to show adaptations in wing shape related to habitat variation. In this work, we explored variation in wing shape in relation to preferred water body (flowing water or standing water with tolerance for temporary conditions) and landscape (forested to open) using 32 species of dragonflies of the genus *Trithemis* (80% of the known species). We included a potential source of variation linked to sexual selection: the extent of wing coloration on hindwings. We used geometric morphometric methods for studying wing shape. We also explored the phenotypic correlation of wing shape between the sexes. We found that wing shape showed a phylogenetic structure and therefore also ran phylogenetic independent contrasts. After correcting for the phylogenetic effects, we found (i) no significant effect of water body on wing shape; (ii) male forewings and female hindwings differed with regard to landscape, being progressively broader from forested to open habitats; (iii) hindwings showed a wider base in wings with more coloration, especially in males; and (iv) evidence for phenotypic correlation of wing shape between the sexes across species. Hence, our results suggest that natural and sexual selection are acting partially independently on fore- and hindwings and with differences between the sexes, despite evidence for phenotypic correlation of wing shape between males and females.

Introduction

Habitats are temporally and spatially variable, and many organisms adapt to this variation by dispersal and/or migration (Southwood, 1962; Hedenström, 2002; Dockx, 2007). Most insects show complex life cycles, where the larval stage is usually sedentary and the adult stage disperses by flight. Adult dispersal ability is expected to vary among taxa if the temporal and spatial variation or unpredictability of their larval habitats differs. For instance, standing water habitats (lentic) are spatially and temporarily less predictable than flowing water (lotic) (Ribera & Vogler, 2000). Therefore, adults with aquatic larval development would be expected to show better dispersal ability in lentic species than in lotic species (Ribera & Vogler, 2000). Similarly, dispersal ability should be greater in temporary water species compared with permanent water species (Gascón *et al.*, 2008). Studying interspecific trait variation in different habitats may provide insight into the facultative mechanisms behind adaptation.

Wing shape is related to migration and dispersal abilities in birds (Bowlin & Wikelski, 2008; Förschler & Bairlein, 2011), bats (Norberg & Rayner, 1987), butterflies (Breuker *et al.*, 2007; Dockx, 2007), and dragonflies (Johansson *et al.*, 2009) and is therefore expected to be adaptive (Kölliker-Ott *et al.*, 2003). Thus, the study of how wing shape varies with habitat may help to understand how species can track spatial and temporal changes in their environment, not just contemporarily but at an evolutionary scale. This is of special relevance for species inhabiting freshwater ecosystems, where dispersal may be vital under the context of climate change: for example, in areas where

Correspondence: David Outomuro, Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden.
Tel.: +46 (0)18 471 2756; fax: +46 (0)18 471 6484;
e-mail: outomuro.david@gmail.com

temperature is expected to increase, the distance between suitable freshwater habitats will be larger due to the disappearance of those habitats.

The dragonfly genus *Trithemis* Brauer, 1868 (Odonata, Libellulidae) is an excellent model group to study the adaptive response of larval habitat requirements and adult dispersal abilities. Over 40 species of *Trithemis* occur mainly in the Old World tropics (Dijkstra & Lewington, 2006), and they show a high diversity of habitat requirements. The ancestral form was inferred to have preferred temporary stagnant waters and open habitats (i.e. nonforested) (Damm *et al.*, 2010). Thereafter, multiple shifts to forested habitats and flowing waters, but also reversals, have occurred during the rapid radiation of the group (Damm *et al.*, 2010). These different habitats could have caused adaptive selection on wing shape related to dispersal, although no evidence is available so far. Dragonflies show a greater development of the basal part of the hindwing in migrating species (Johansson *et al.*, 2009), probably related to their gliding ability (Corbet, 1962). Therefore, a wider hindwing base would be expected to improve spatial movements. In addition, gliding in dragonflies has been suggested to be a thermoregulatory strategy to avoid overheating (May, 1976) and would therefore be expected to be more common in species that live in open habitats. As *Trithemis* originated in open, standing waters, a wide hindwing base is expected in the ancestor of the genus. Such a hindwing could allow for optimal gliding flight in the open habitat, as well as dispersal among water bodies and better cooling. However, wing traits might have evolved in different directions due to new selection pressures or due to a lack of function in new habitats. For example, we may expect that the broad hindwing base would be kept in open habitats but not necessarily in forested ones. In addition, a broader forewing base physically interferes with the hindwing (Wakeling, 1997). Therefore, it is difficult to predict the response of forewings in the present context. However, in general, shorter and broader wings improve flight agility (Betts & Wootton, 1988) and would be expected in species preferring more forested habitats.

Selection on wing shape within this genus could also be affected by the presence of wing coloration on the hindwing base. Wing coloration is important for sexual selection in damselflies and dragonflies (Corbet, 1999; Córdoba-Aguilar & Cordero-Rivera, 2005) both for males and females (Córdoba-Aguilar *et al.*, 2003; Svensson *et al.*, 2007). Although not much investigated yet, wing coloration was shown to be related to wing shape variation in damselflies (Outomuro & Johansson, 2011; Outomuro *et al.*, 2013) and butterflies (Srygley, 1999). For example, in damselflies, the wing area where the colour was present was larger, suggesting an effect of the need for an effective display (Outomuro *et al.*, 2013). Therefore, wing shape would be expected to change due to selection on wing coloration, with a larger wing area where the colour is present. Although wing coloration may have other evolutionary roles such as interspecific discrimination (e.g. Tynkkynen *et al.*, 2004), it has been shown for damselflies that sexual selection is stronger that natural selection in population divergence (Svensson *et al.*, 2006). Therefore, sexual selection might interact with other natural selection pressures involved in wing shape evolution.

We studied wing shape evolution of males and females of 32 species of the genus *Trithemis* (around 80% of the known species) in relation to larval and adult habitat requirements, that is, preferred water body (flowing water or standing water with tolerance for temporary conditions) and landscape (forested or open), and the presence of wing coloration. Previous studies have shown that there is phylogenetic structure in wing shape (*Drosophila*: Klingenberg & Gidaszewski, 2010; damselflies: Outomuro *et al.*, 2013). Therefore, we first confirmed the presence of phylogenetic structure of wing shape evolution in our data set, and we then performed phylogenetic independent contrasts. We used geometric morphometric approaches to analyse wing shape. As we found different significant effects for fore- and hindwings between males and females, and there was a conspicuous sexual dimorphism in wing shape, we also studied whether there was phenotypic correlation of wing shape between the sexes. Males and females share most of the genes, and conflicts in the evolutionary interests of each sex can occur (reviewed in Bonduriansky & Chenoweth, 2009). A previous study on damselflies with clear sexual dimorphism in wing shape suggested that male and female wing shapes show phenotypic correlation between the sexes (Outomuro *et al.*, 2012). We predicted that (i) taxa from standing water and open habitats should show an expanded hindwing base; (ii) taxa from forested habitats should have shorter and broader wings, independently of the hindwing base; (iii) taxa with wing coloration should show a broader wing area where the pigment is present, that is, at the hindwing base; and (iv) there is phenotypic correlation of wing shape between the sexes. We found significant effects due to landscape variation and the extension of wing coloration. Those effects differed between fore- and hindwings and between males and females. We also found evidence for phenotypic correlation of wing shape between the sexes across species.

Materials and methods

Study taxa and phylogeny

We studied 32 taxa of the genus *Trithemis* (32 males and 29 females; Table 1). Specimens were photographed together with a scale using a digital camera at Naturalis Biodiversity Center (Leiden). Pictures were subsequently analysed for studying wing shape. Taxa were categorized into three main variables following Damm

Table 1 List of studied taxa and variables used for the comparative analyses: water body (1: running water, 2: standing waters with tolerance for temporary conditions), landscape (1: forested habitat, 2: open habitat) and hindwing coloration (0: absent, 1: extensive, 2: very extensive).

Taxa	Water body	Landscape	Hindwing coloration
Trithemis aconita	1	1	0
T. adelpha	2	2	1
T. aenea	1	1	0
T. aequalis	1	2	0
T. africana	1	1	2
T. annulata	2	2	1
T. arteriosa	2	2	1 (0 for female)
T. aurora	2	2	1
T. basitincta	1	1	0
T. bifida	1	1	0
T. bredoi (only male)	1	2	1
T. dejouxi (only male)	1	2	0
T. dichroa	1	1	0
T. donaldsoni (only male)	1	2	0
T. dorsalis	1	2	0
T. ellenbeckii	1	2	0
T. festiva	1	2	1
T. furva	1	2	0
T. grouti	1	1	0
T. hartwigi	2	1	1
T. hecate	2	2	0
T. imitata	2	2	2
T. kalula	2	2	1
T. kirbyi	2	2	2 (1 for female)
T. monardi	2	2	2
T. nuptialis	1	1	0
T. pluvialis	1	2	1
T. pruinata	1	1	0
T. selika	2	2	1
T. stictica	1	2	0
T. tropicana	1	1	2
T. werneri	1	2	1

et al. (2010): water body (flowing water and standing water with tolerance for temporary conditions), landscape (forested or open with regard to vegetation) and presence of wing coloration on the hindwing base (see Table 1). Wing coloration was defined as very extensive when it extended to the central vein of the anal loop (Askew, 2004), extensive when it did not reach this vein and absent when no conspicuous coloration was visible (Fig. S1).

The phylogeny was constructed using 39 taxa of the genus *Trithemis*, with *Pantala flavescens* (Fabricius, 1798) as outgroup (sequences published in Damm *et al.*, 2010) (Table S1). We used two mitochondrial genes (the NADH-dehydrogenase subunit 1 and the 16S rDNA) and the nuclear regions ITSI and ITSII with the 5.8S region in between. The sequences were downloaded from GenBank and aligned in MEGA (Tamura *et al.*, 2011) using MUSCLE (Edgar, 2004). The nuclear sequence was not available for *Trithemis africana* (Brauer, 1867). We ran two independent Bayesian phylogenetic analyses in the package BEAST version 1.7.1 (Drummond *et al.*, 2012) using a SRD06 model as the nucleotide substitution model, an uncorrelated lognormal relaxed molecular clock and a birth–death process as tree prior. We ran 10^7 generations for the MCMC sampling, logging every 1000 generations. The resulting tree was similar to that previously published by Damm *et al.* (2010) (Fig. S2) and was subsequently pruned to contain only our study taxa.

Wing shape

We used geometric morphometric methods to study wing shape. The relative position of landmark coordinates was used to analyse shape, after keeping mathematically constant the effects of nonshape variation (position, orientation and scale) (Bookstein, 1991; Rohlf & Marcus, 1993; Adams *et al.*, 2004). Landmarks were digitized on wing pictures (11 landmarks for forewings and 12 landmarks for hindwings) using tpsDig2 (Rohlf, 2010a). Ten landmarks were associated with biologically constant points in forewings, and one semi-landmark was use to capture wing curvature (Fig. 1). For hindwings, it was not possible to find biologically constant landmarks in the basal part of the wing. Therefore, we used a star with the lines separated constantly by 9 degrees. The star was fixed to the wing node and the front margin of the wing (Fig. 1). We established five landmarks where the first, fourth, seventh, ninth and tenth star radii cross the hindwing outline (Fig. 1).

Using tpsRelw (Rohlf, 2010b), landmarks were subjected to a generalized Procrustes analysis (Rohlf & Slice, 1990), for both fore- and hindwings, as well as males and females separately. The specimens were translated to the origin, scaled into unit centroid size and rotated to minimize the total sum-of-squares devia-

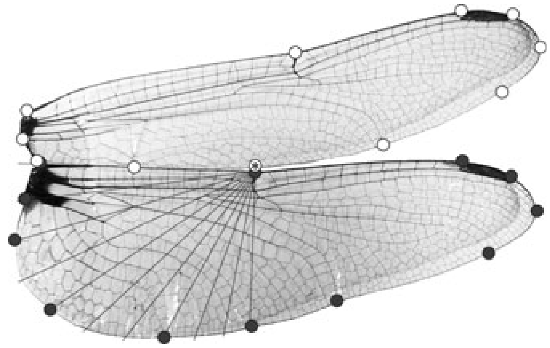

Fig. 1 Landmarks used for capturing outline wing shape for fore- (white circles) and hindwings (grey circles) on the right wings of a male of *Trithemis integra* Dijkstra, 2007. The asterisk indicates the semi-landmark on forewings. The star used for capturing landmarks on the hindwing base is shown.

tions of the coordinates from all specimens to the average configuration. The semi-landmark of the forewing was permitted to slide along its tangent direction until minimizing the Procrustes distance between specimens (Bookstein, 1991). Individual wings were then aligned using the consensus wing and the shape variables computed (uniform and nonuniform shape components). The individual centroid size was also computed for each wing using its corresponding landmark coordinates. Centroid size of wings was shown to be correlated with body size in Odonata (Outomuro & Johansson, 2011) and was used as a proxy for body size in our analyses. We also computed the shape variables using merged data sets for male and female forewings, and male and female hindwings, for their use in the analyses of phenotypic correlation (see below).

Statistical analyses

We studied how wing shape differed due to the effect of the three study variables (water body, landscape and wing coloration, see Table 1), separately for fore- and hindwings, and for males and females. All statistical analyses were performed either in Statistica 10 (StatSoft, 2011) or in R version 2.15 (R Development Core Team, 2011). No effects of multicollinearity were detected among the three studied variables. We first ran a MANCOVA on all the shape components as the dependent variables, with centroid size as the covariate, and water body, landscape and wing coloration as factors. We note that the last factor was only included for hindwings. Nonsignificant interactions were removed one by one in the model.

We also tested whether there was a phylogenetic structure in wing shape evolution (i.e. departed from Brownian motion) by analysing the presence of phylogenetic signal in its divergence (Blomberg *et al.*, 2003; Klingenberg & Gidaszewski, 2010). We applied the approach of Klingenberg & Gidaszewski (2010) which quantifies the phylogenetic signal of the shape components as the sum of squared shape changes along the branches of the phylogeny. Smaller values of the sum of squared shape changes imply greater phylogenetic signal, that is, closely related species are more similar among each other. To test the significance of sum of squared shape changes, a permutation approach was used to simulate the null hypothesis of complete absence of phylogenetic signal. The simulation is based on a random and repeated reassignment of the shape components to the tips of the phylogeny. The *P*-value is equal to the proportion of permuted data sets with a lower or equal sum of squared shape changes than the sum of squared shape changes obtained for the original data (Klingenberg & Gidaszewski, 2010).

The role of allometry on wing shape was also studied. First, we ran a multivariate phylogenetic generalized least-squares analysis (PGLS) (Rohlf, 2001) following Outomuro *et al.* (2013), separately for fore- and hindwings of males and females. The results of the PGLS were not significant for any of the data sets (see Results). Therefore, it was decided to keep the allometric component of wing shape. Second, the phylogenetic mapping analysis showed the presence of a significant phylogenetic signal (see Results), so we performed a phylogenetic MANOVA (Garland *et al.*, 1993) on wing shape testing each of the significant effects obtained in the MANCOVA's. The phylogenetic MANOVA's were run using the R-package geiger (Harmon *et al.*, 2008), which uses the test statistic from a MANOVA and compute the null distribution of this test statistic by simulating new sets of the shape components on the phylogenetic tree. The simulations are run under Brownian motion. For the significant terms in the phylogenetic MANOVA's, we visualized how wing shape changed among the different levels of the variable. We did this using tpsSplin (Rohlf, 2004) which computes thin-plate spline deformation grids among wing shapes and a reference wing shape. For instance, we compared how wing shape differed among landscapes (see Results). To obtain a finer graphical resolution of the change along that landscape gradient, we used the five original categories in the study by Damm *et al.* (2010): forested, openings in the forest, half-open, open and cooler+open (open highlands). However, we could not test the differences among these because we had too few species in each category. Therefore, forested, openings and half-open were combined into the forested landscapes, and the other two were regarded as open landscapes.

Finally, we tested for phenotypic correlation of wing shape between the sexes. We used tpsPLS (Rohlf, 2006) for computing a partial least-squares analysis of the covariation between the shape components of males and the shape components of females (obtained in the mixed data set), for fore- and hindwings separately. This method does not assume that one shape is a function of the other, as regression models usually do. The analysis results in a set of paired vectors, or covariance dimensions, accounting for the covariance between the two sets of shapes. The first two covariance dimensions explained more than 80% of variance in forewings and more than 92% in hindwings (see Results). We used the male and female scores of each dimension to run phylogenetic independent contrasts (Felsenstein, 1985). For performing these analyses, we used the package PDAP:PDTREE version 1.16 (Midford *et al.*, 2011) for Mesquite version 2.75 (Maddison & Maddison, 2011). We exponentially transformed the tree branch lengths to satisfy the requirements of the independent contrasts (Garland *et al.*, 1992). The contrasts plots were standardized for both axes, positivized for the X axis and forced through the origin (Garland *et al.*, 1992). The slopes of the regression were calculated using a major axis regression (Model II regression). We investigated whether the slope of the regression differed significantly from one, by inspecting the 95% CI.

Results

Before correcting for the phylogenetic relationships, male forewing shape only differed between landscapes (Table 2). A significant interaction term between water body and landscape was also found (Table 2). On the other hand, female forewings showed marginally nonsignificant differences between landscapes, but no effect of water body (Table 2). Male hindwing shape differed with regard to water body and wing coloration, but not to landscape (Table 2). Finally, female hindwing shape showed significant differences with regard to landscape, wing coloration and the interaction between these two terms (Table 2).

When we investigated the presence of phylogenetic structure in wing shape evolution, we found that wing shape showed a significant phylogenetic signal in its divergence for both sex and wing (male forewings: phyl. signal = 0.00912, $P = 0.001$; female forewings: phyl. signal = 0.00735, $P = 0.001$; male hindwings: phyl. signal = 0.01320, $P = 0.001$; female hindwings: phyl. signal = 0.01311, $P = 0.001$). These results imply that the evolution of wing shape significantly departed from Brownian motion and that related species tend to have similar wing shape. Centroid size did not have a significant effect on the evolution of wing shape (PGLS: male forewings: Pillai's trace = 1.091, $F_{36,28} = 0.934$, $P = 0.581$; female forewings: Pillai's trace = 0.920, $F_{36,22} = 0.520$, $P = 0.960$; male hindwings: Pillai's trace = 1.123, $F_{40,24} = 0.768$, $P = 0.775$; female hindwings: Pillai's trace = 1.016, $F_{40,18} = 0.464$, $P = 0.978$).

As we found that wing shape showed a phylogenetic structure, we investigated whether the significant effects found in the MANCOVA's were kept significant after correcting for the phylogenetic effects. The phylogenetic MANOVA showed that the effects of landscape on male forewing shape were significant after the phylogenetic relationships were taken into account (Table 3). A visualization of the male forewing shapes revealed that wings were progressively broader from forested to open habitats (Fig. 2). For female forewing shape, the phylogenetic MANOVA was nonsignificant and the possible effect of landscape was driven by the phylogenetic relationships (Table 3). For male hindwing shape, the wing coloration effect was significant, but not the water body effect, after correcting for the phylogenetic relationships (Table 3). The deformation grids provided strong support for this statistical result, because the wing base was gradually wider from non-coloured wings to wings with very extensive coloration (Fig. 3). Finally, for female hindwing shape, the phylogenetic MANOVA revealed a significant effect due to landscape (Table 3), with a trend

Table 2 Results for the MANCOVA on fore- and hindwing shapes of males and females.

	Wilks'λ	*F*	d.f. effect	d.f. error	*P*
Male forewings					
Water body	0.242	1.743	18	10	0.186
Landscape	0.098	5.120	18	10	0.006
Log Centroid size	0.354	1.015	18	10	0.511
Water body × Landscape	0.093	5.403	18	10	0.005
Female forewings					
Water body	0.194	1.845	18	8	0.190
Landscape	0.133	2.890	18	8	0.065
Log Centroid size	0.275	1.173	18	8	0.429
Male hindwings					
Water body	0.073	4.419	20	7	0.026
Landscape	0.211	1.308	20	7	0.377
Coloration	0.005	4.544	40	14	0.002
Log Centroid size	0.096	3.301	20	7	0.056
Female hindwings					
Water body	0.077	1.190	20	2	0.554
Landscape	0.003	35.960	20	2	0.027
Coloration	0.001	16.710	40	4	0.007
Log Centroid size	0.017	5.830	20	2	0.156
Landscape × Coloration	0.001	10.720	40	4	0.016

Table 3 Results for the phylogenetic MANOVA on fore- and hindwing shapes of males and females.

	Wilks'λ	*F*	d.f. effect	d.f. error	*P*	Phylogenetic *P*
Male forewings						
Landscape	0.185	3.199	1	30	0.019	0.014
Female forewings						
Landscape	0.206	2.140	1	27	0.110	0.112
Male hindwings						
Water body	0.134	3.566	1	30	0.017	0.691
Coloration	0.006	5.953	2	29	<0.001	0.037
Female hindwings						
Landscape	0.111	3.197	1	27	0.048	0.045
Coloration	0.004	5.356	2	26	<0.001	0.058

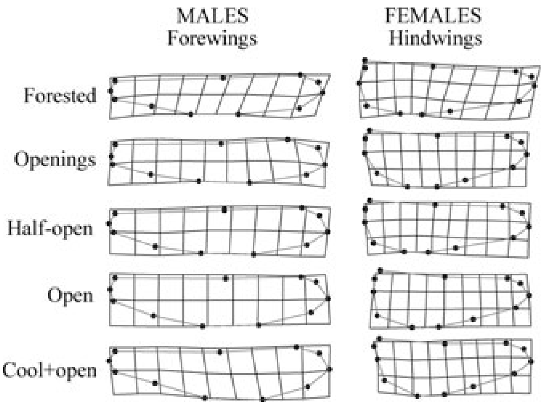

Fig. 2 Deformation grids showing the shape variation of male forewings and female hindwings among different landscapes (cool+open are open habitats in highlands). Lines between the landmarks are included to improve the interpretation of results.

from more slender wings at forested landscapes towards broader wings at open landscapes (Fig. 2). Regarding wing coloration variation, female hindwing shape showed marginally nonsignificant results after the phylogenetic relationships were considered (Table 3). Similar to males, female hindwings showed a larger wing base when wings were more coloured (Fig. 3).

The partial least-squares analyses showed that male and female shapes were highly correlated for fore- and hindwings. For forewings, the first two covariance dimensions explained 80.72% of the variance (dimension 1: 54.33% of variance, $r = 0.761$, $P = 0.02$; dimension 2: 26.39% of variance, $r = 0.804$, $P = 0.01$). For the hindwings, the first two covariance dimensions explained 92.21% of the variance (dimension 1: 77.47% of variance, $r = 0.851$, $P = 0.01$; dimension 2: 14.74% of variance, $r = 0.666$, $P = 0.03$). Those first two dimensions were used to run phylogenetic independent contrasts between female and male wing shape. For forewings, the first two dimensions showed a fairly high correlation and a slope that did not significantly differ from one, suggesting phenotypic correlation of wing shape between the sexes across species (dimension 1: $r = 0.690$, slope = 0.900, CI = 0.573–1.384, $R^2 = 0.476$, $P < 0.001$; dimension 2: $r = 0.709$, slope = 1.110, CI = 0.740–1.696, $R^2 = 0.503$, $P < 0.001$; Fig. 4). The results for hindwings were similar to forewings (dimension 1: $r = 0.727$, slope = 1.233, CI = 0.847–1.853, $R^2 = 0.529$, $P < 0.001$; dimension 2: $r = 0.546$, slope = 1.339, CI = 0.713–2.909, $R^2 = 0.298$, $P = 0.002$; Fig. 4).

Discussion

Wing shape showed a phylogenetic structure in the genus *Trithemis*. A similar result was previously shown in *Drosophila* Fallén, 1832 (Klingenberg & Gidaszewski, 2010) and damselflies (Outomuro *et al.*, 2013). Thus, the phylogenetic history of wing shape appears to constrain to a certain extent the anticipated direction outlined by selection. We found that landscape and wing coloration, but not water body explained the variation in wing shape, differently for fore- and hindwings.

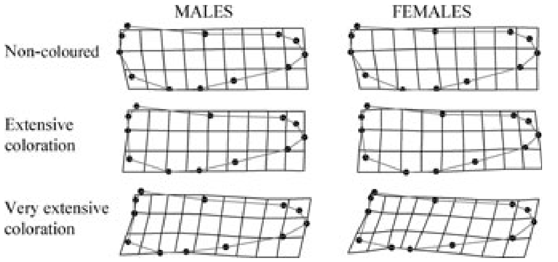

Fig. 3 Variation of wing shape among the groups of hindwing coloration, from a non-coloured wing to very extensive wing coloration. Lines linking the landmarks are shown to improve interpretation of results.

Water body, wing coloration and hindwings

In contrast to our predictions, we did not find significant variation in male or female hindwing shape in relation to water body, that is, between flowing and standing water bodies. Retention of wing traits from the ancestral stage might occur, which was inferred to live in standing water bodies (Damm *et al.*, 2010). According to this hypothesis, the ancestral hindwing shape (i.e. with a broader wing base) would not be selected against in the transition to running water habitats. Therefore, the dispersal abilities of sedentary species may remain high.

The most striking effect on hindwing shape was the extent of coloration, which was positively related to a greater development of the wing base. These results were clearer for males than for females. Therefore, our results point at a major role of sexual selection on the evolution of hindwing shape. However, we cannot exclude the possibility that the presence of wing coloration is a trait developed as a by-product of other habitat variables acting primarily on the evolution of hindwing shape. Sexual selection on coloration might have also caused an exaggeration of the trait (i.e. wider base) that would not be expected by other habitat variables alone (Andersson, 1994; Emlen & Nijhout, 2000). A number of arguments support our suggestion of a stronger role of sexual selection on hindwing evolution. First, wing coloration has a major role in intra- and intersexual selection in some damselfly families (e.g. Calopterygidae, Chlorocyphidae; Corbet, 1999), and behavioural studies suggest that this is the case for species in the family Libellulidae, which includes the genus *Trithemis* (Jacobs, 1955; Frantsevich & Mokrushov, 1984; Corbet, 1999). Second, we have recently shown that a larger wing area is found where the coloration is located in male damselflies with intra- and intersexually selected wing coloration (Outomuro *et al.*, 2013). Despite this strong association between hindwing shape and the presence of coloration, a greater development of the hindwing base is also related to gliding ability in dragonflies, that is, dispersal ability and thermoregulation (Corbet, 1962; May, 1976; Johansson *et al.*, 2009). Gliding might be also advantageous for signalling during courtships (Corbet, 1999). Therefore, the results of the present study suggest that a wider hindwing base with extensive wing coloration might be sexually selected for, at least in males.

Landscape and wing shape

We predicted that shorter and broader wings would be expected in more forested landscapes, because such wings would improve flight agility (Betts & Wootton,

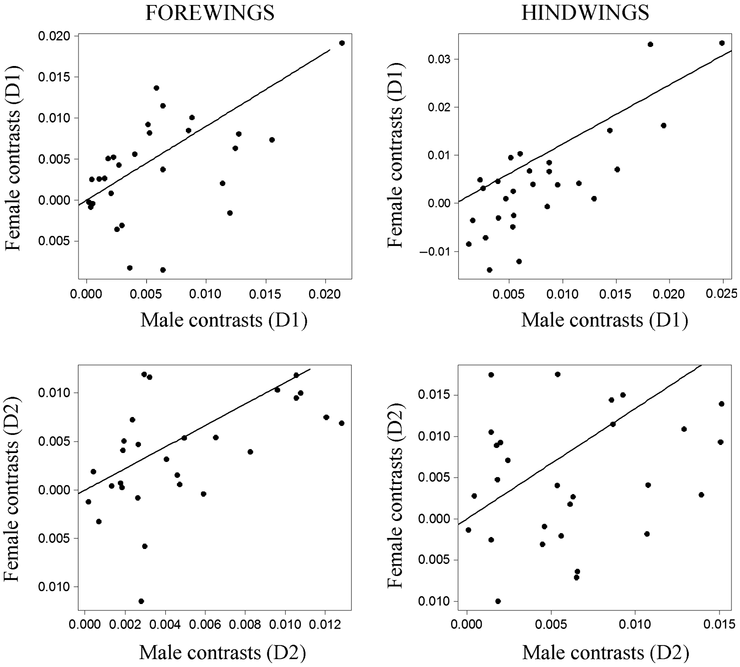

Fig. 4 Phylogenetic independent contrasts between female and male wing shape scores. The scores were obtained from the first two covariance dimensions of a partial least-squares analysis (see main text).

1988). We found significant variation in wing shape relative to landscape, but these did not agree with our predictions. Instead, we found that forewings of males were broader in the open landscape, and a similar result was observed in female hindwing shape. We note that the forewing shape change across landscapes was continuous, broadening from forested to open landscapes (Fig. 2). This successive change suggests that wing shape patterns are affected by landscape type and that broader wings can be beneficial in more open landscapes. Unlike the hindwing, the forewing base cannot become broader, because it physically interferes with the hindwings (Wakeling, 1997). Perhaps an overall broader forewing improves gliding ability, which is related to dispersal and thermoregulation (Corbet, 1962; May, 1976; Johansson *et al.*, 2009), although further research is needed to support this hypothesis. Moreover, if that is the case, a larger space between the bases of fore- and hindwings would be necessary for avoiding physical interference between both wings.

The lack of support for our predictions suggests that our current knowledge on aerodynamics in dragonflies is insufficient. Although there is a rich research background on wing aerodynamics in certain groups of animals (e.g. Weis-Fogh, 1973; Ellington, 1984; Betts & Wootton, 1988; Wakeling & Ellington, 1997a,b; Hedenström, 2002), much more functional work is needed. Moreover and most importantly, there is a general lack of knowledge on how wing shape variation between or within species may affect flight. For example, quantitative aerodynamic theory within genera showing different wing shapes is currently lacking. Therefore, it is difficult to make strong predictions relating wing shape and function. Despite this, experimental approaches in the laboratory and field are certainly promising (e.g. Combes *et al.*, 2010).

Selection on forewing versus hindwing

We found that landscape explained forewing and coloration explained hindwing variation in male wing shape. Landscape also explained some of the variation in hindwing shape of females. This suggests that different selective pressures may act on each pair of wings and that fore- and hindwings are partially evolving independently. Wings may be affected by different selective pressures within the individual, as has previously been found in coloured wings of butterflies (Oliver *et al.*, 2009; Rutowski *et al.*, 2010) and suggested for damselflies (Outomuro *et al.*, 2013). For instance, the ventral pattern of *Bicyclus* Kirby, 1871 butterflies is related to predator avoidance, whereas the dorsal pattern has a role in mate signalling (Oliver *et al.*, 2009). Our study shows that not only the presence of ornaments on wings may lead to a physical separation of wing function, but that other selective pressures, such as the preferred landscape, may also evoke different responses in the wings of the same individual.

Wing shape correlation between the sexes

In line with our predictions, we found evidence for phenotypic correlation of wing shape between the sexes, as has previously been suggested for damselflies with coloured wings (Outomuro *et al.*, 2012). We also note that this phenotypic correlation was across species. As we found that different selection pressures are probably acting on fore- and hindwings of males and females, our results may suggest that selection on wing shape in one sex might be displacing the other sex (i.e. intralocus sexual conflict, reviewed in Bonduriansky & Chenoweth, 2009). For instance, sexual selection on hindwing shape on male coloration might lead to wing shape evolution of female hindwing shape. Previous studies support that sex-specific selective pressures act on wing shape due to gender-specific life strategies (birds: Stiles *et al.*, 2005; Fernández & Lank, 2007; butterflies: Berwaerts *et al.*, 2008; DeVries *et al.*, 2010). However, estimates of selection acting on male and female wing shape as well as of intersexual genetic correlation would be necessary to infer true intralocus sexual conflict in our study system (Bonduriansky & Chenoweth, 2009).

Conclusion

In summary, we found that wing coloration and landscape were associated with a certain wing shape and that males and females differ in these patterns. We reiterate that experimental manipulation on wing shape is needed to show whether these patterns are caused by sexual and/or natural selection. We also suggest that more development on wing shape and aerodynamic theory is needed in organisms with two pairs of wings that can beat independently.

Acknowledgments

We are very grateful to L. Faulks for her help with the English correction. This study has been supported by a postdoc position to DO from the Spanish Ministry of Education. FJ was supported by The Swedish Research Council.

References

Adams, D.C., Rohlf, F.J. & Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the ‘revolution’. *Ital. J. Zool.* **71**: 5–16.

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

Askew, R.R. 2004. *The Dragonflies of Europe (Revised Edition)*. Harley Books, Colchester.

Berwaerts, K., Matthysen, E. & Van Dyck, H. 2008. Take-off flight performance in the butterfly *Pararge aegeria* relative to sex and morphology: a quantitative genetic assessment. *Evolution* **62**: 2525–2533.

Betts, C.R. & Wootton, R.J. 1988. Wing shape and flight behaviour in butterflies (Lepidoptera: Papilionoidea and Hesperioidea): a preliminary analysis. *J. Exp. Biol.* **138**: 271–288.

Blomberg, S.P., Garland, T. & Ives, A.R. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. *Evolution* **57**: 717–745.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280–288.

Bookstein, F.L. 1991. *Morphometric Tools for Landmark Data Geometry and Biology*. Cambridge University Press, Cambridge.

Bowlin, M.S. & Wikelski, M. 2008. Pointed wings, low wing-loading and calm air reduce migratory flight costs in songbirds. *PLoS ONE* **3**: e2154.

Breuker, C.J., Brakefield, P.M. & Gibbs, M. 2007. The association between wing morphology and dispersal is sex-specific in the glanville fritillary butterfly *Melitaea cinxia* (Lepidoptera: Nymphalidae). *Eur. J. Entomol.* **104**: 445–452.

Combes, S.A., Crall, J.D. & Mukherjee, S. 2010. Dynamics of animal movement in an ecological context: dragonfly wing damage reduces flight performance and predation success. *Biol. Lett.* **6**: 426–429.

Corbet, P.S. 1962. *A Biology of Dragonflies*. H. F. & G. Whiterby LTD, London.

Corbet, P.S. 1999. *Dragonflies Behaviour and Ecology of Odonata*. Cornell University Press, New York.

Córdoba-Aguilar, A. & Cordero-Rivera, A. 2005. Evolution and ecology of Calopterygidae (Zygoptera: Odonata): Status of knowledge and research perspectives. *Neotrop. Entomol.* **34**: 861–879.

Córdoba-Aguilar, A., Salamanca- Ocaña, J.C. & Lopezaraiza, M. 2003. Female reproductive decisions and parasite burden in a calopterygid damselfly (Insecta: Odonata). *Anim. Behav.* **66**: 81–87.

Damm, S., Dijkstra, K.D.B. & Hadrys, H. 2010. Red drifters and dark residents: the phylogeny and ecology of a Plio-Pleistocene dragonfly radiation reflects Africa’s changing environment (Odonata, Libellulidae, Trithemis). *Mol. Phylogenet. Evol.* **54**: 870–882.

DeVries, P.J., Penz, C.M. & Hill, R.I. 2010. Vertical distribution, flight behaviour and evolution of wing morphology in *Morpho* butterflies. *J. Anim. Ecol.* **79**: 1077–1085.

Dijkstra, K.-D.B. & Lewington, R. 2006. *Field Guide to the Dragonflies of Britain and Europe*. British Wildlife Publishing, Dorset.

Dockx, C. 2007. Directional and stabilizing selection on wing size and shape in migrant and resident monarch butterflies, *Danaus plexippus* (L.), in Cuba. *Biol. J. Linn. Soc.* **92**: 605–616.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol. Biol. Evol.* **29**: 1969–1973.

Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**: 1792–1797.

Ellington, C.P. 1984. The aerodynamics of hovering insect flight. II. Morphological parameters. *Phil. Trans. R. Soc. Lond. B* **305**: 17–40.

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

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* **125**: 1–15.

Fernández, G. & Lank, D.B. 2007. Variation in the wing morphology of western sandpipers (*Calidris mauri*) in relation to sex, age class, and annual cycle. *Auk* **124**: 1037–1046.

Förschler, M.I. & Bairlein, F. 2011. Morphological shifts of the external flight apparatus across the range of a passerine (Northern wheatear) with diverging migratory behaviour. *PLoS ONE* **6**: e18732.

Frantsevich, L.I. & Mokrushov, P.A. 1984. Visual stimuli releasing attack of a territorial male in *Sympetrum* (Anisoptera: Libellulidae). *Odonatologica* **13**: 335–350.

Garland, T. Jr, Harvey, P.H. & Ives, A.R. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. *Syst. Biol.* **41**: 18–32.

Garland, T. Jr, Dickerman, A.W., Janis, C.M. & Jones, J.A. 1993. Phylogenetic analysis of covariance by computer simulation. *Syst. Biol.* **42**: 265–292.

Gascón, S., Boix, D., Sala, J. & Quintana, X.D. 2008. Relation between macroinvertebrate life strategies and habitat traits in Mediterranean salt marsh ponds (Empordà wetlands, NE Iberian Peninsula). *Hydrobiologia* **597**: 71–83.

Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E. & Challenger, W. 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* **24**: 129–131.

Hedenström, A. 2002. Aerodynamics, evolution and ecology of avian flight. *Trends Ecol. Evol.* **17**: 415–422.

Jacobs, M.E. 1955. Studies on territorialism and sexual selection in dragonflies. *Ecology* **36**: 566–586.

Johansson, F., Söderquist, M. & Bokma, F. 2009. Insect wing shape evolution: independent effects of migratory and mate guarding flight on dragonfly wings. *Biol. J. Linn. Soc.* **97**: 362–372.

Klingenberg, C.P. & Gidaszewski, N.A. 2010. Testing and quantifying phylogenetic signals and homoplasy in morphometric data. *Syst. Biol.* **59**: 245–261.

Kölliker-Ott, U.M., Blows, M.W. & Hoffmann, A.A. 2003. Are wing size, wing shape and asymmetry related to field fitness of *Trichogramma* egg parasitoids? *Oikos* **100**: 563–573.

Maddison, W.P. & Maddison, D.R. 2011. Mesquite: A modular system for evolutionary analysis. Version 2.75. URL http://mesquiteproject.org.

May, M.L. 1976. Thermoregulation and adaptation to tempe rature in dragonflies (Odonata: Anisoptera). *Ecol. Monogr.* **46**: 1–32.

Midford, P.E., Garland, T. Jr & Maddison, W.P. 2011. PDAP Package version 1.16.

Norberg, U.M. & Rayner, J.M.V. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. *Phil. Trans. R. Soc. Lond. B* **316**: 335–427.

Oliver, J.C., Robertson, K.A. & Monteiro, A. 2009. Accommodating natural and sexual selection in butterfly wing pattern evolution. *Proc. R. Soc. B* **276**: 2369–2375.

Outomuro, D. & Johansson, F. 2011. The effects of latitude, body size, and sexual selection on wing shape in a damselfly. *Biol. J. Linn. Soc.* **102**: 263–274.

Outomuro, D., Bokma, F. & Johansson, F. 2012. Hind wing shape evolves faster than front wing shape in *Calopteryx* damselflies. *Evol. Biol.* **39**: 116–125.

Outomuro, D., Adams, D.C. & Johansson, F. 2013. The evolution of wing shape in ornamented-winged damselflies (Calopterygidae, Odonata). *Evol. Biol.* **40**: 300–309.

R Development Core Team 2011. *R: A language and environment for statistical computing*. Version 2.15.0. http://www.R-project.org.

Ribera, I. & Vogler, A.P. 2000. Habitat type as a determinant of species range sizes: the example of lotic-lentic differences in aquatic Coleoptera. *Biol. J. Linn. Soc.* **71**: 33–52.

Rohlf, F.J. 2001. Comparative methods for the analysis of continuous variables: geometric interpretations. *Evolution* **55**: 2143–2160.

Rohlf, F.J. 2004. tpsSplin. Thin-plate spline version 1.20. URL http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2010a. tpsDig version 2.16. URL http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2010b. tpsRelw. Relative warps version 1.49. URL http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. 2006. tpsPLS version 1.18. URL http://life.bio.sunysb.edu/morph/.

Rohlf, F.J. & Marcus, L.F. 1993. A revolution in morphometrics. *Trends Ecol. Evol.* **8**: 129–132.

Rohlf, F.J. & Slice, D. 1990. Extension of the Procrustes method for the optimal superimposition of landmarks. *Syst. Zool.* **39**: 40–59.

Rutowski, R.L., Nahm, A.C. & Macedonia, J.M. 2010. Iridescent hindwing patches in the Pipevine Swallowtail: differences in dorsal and ventral surfaces relate to signal function and context. *Funct. Ecol.* **24**: 767–775.

Southwood, T.R.E. 1962. Migration of terrestrial arthropods in relation to habitat. *Biol. Rev.* **37**: 171–211.

Srygley, R.B. 1999. Locomotor mimicry in *Heliconius* butterflies: contrast analyses of flight morphology and kinematics. *Phil. Trans. R. Soc. Lond. B* **354**: 203–214.

StatSoft, Inc (2011) STATISTICA (data analysis software system), version 10. URL: http://www.statsoft.com.

Stiles, F.G., Altshuler, D.L. & Dudley, R. 2005. Wing morphology and flight behaviour of some North American hummingbird species. *Auk* **122**: 872–886.

Svensson, E.I., Eroukhmanoff, F. & Friberg, M. 2006. Effects of natural and sexual selection on adaptive population divergence and premating isolation in a damselfly. *Evolution* **60**: 1242–1253.

Svensson, E.I., Karlsson, K., Friberg, M. & Eroukhmanoff, F. 2007. Gender differences in species recognition and the evolution of asymmetric sexual isolation. *Curr. Biol.* **17**: 1943–1947.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.* **28**: 2731–2739.

Tynkkynen, K., Rantala, M.J. & Suhonen, J. 2004. Interspecific aggression and character displacement in the damselfly *Calopteryx splendens*. *J. Evol. Biol.* **17**: 759–767.

Wakeling, J.M. 1997. Odonatan wing and body morphologies. *Odonatologica* **26**: 35–52.

Wakeling, J.M. & Ellington, C.P. 1997a. Dragonfly flight. I. Gliding flight and steady-state aerodynamic forces. *J. Exp. Biol.* **200**: 543–556.

Wakeling, J.M. & Ellington, C.P. 1997b. Dragonfly flight. II. Velocities, accelerations and kinematics of flapping flight. *J. Exp. Biol.* **200**: 557–582.

Weis-Fogh, T. 1973. Quick estimates of flight fitness in hovering animals, including novel mechanisms for lift production. *J. Exp. Biol.* **59**: 169–230.

Supporting information

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

Table S1 GenBank accession numbers of the taxa used for constructing the phylogeny.

Figure S1 Wing pictures showing an example of the three categories of wing coloration.

Figure S2 Phylogeny of the genus *Trithemis* used in the present study.

Data deposited at Dryad: doi: 10.5061/dryad.nh618

Received 4 March 2013; revised 21 May 2013; accepted 21 May 2013