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RESEARCH ARTICLE

The Evolution of Wing Shape in Ornamented-Winged Damselflies (Calopterygidae, Odonata)

David Outomuro · Dean C. Adams · Frank Johansson

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Abstract Flight has conferred an extraordinary advantage to some groups of animals. Wing shape is directly related to flight performance and evolves in response to multiple selective pressures. In some species, wings have ornaments such as pigmented patches that are sexually selected. Since organisms with pigmented wings need to display the ornament while flying in an optimal way, we might expect a correlative evolution between the wing ornament and wing shape. We examined males from 36 taxa of calopterygid damselflies that differ in wing pigmentation, which is used in sexual displays. We used geometric morphometrics and phylogenetic comparative approaches to analyse whether wing shape and wing pigmentation show correlated evolution. We found that wing pigmentation is associated with certain wing shapes that probably increase the quality of the signal: wings being broader where the pigmentation is located. Our results also showed correlated evolution between wing pigmentation and wing shape in hind wings, but not in front wings, probably because hind wings are more involved in signalling than front wings. The results imply that the evolution of diversity in wing pigmentations and behavioural sexual displays might be an important driver of speciation due to important pre-copulatory selective pressures.

Keywords Geometric morphometrics · Phylogeny · Sexual signaling · Wing pigmentation

Introduction

Flight is a key adaptation in animals and wing shape is an essential part of flight performance. The evolution of wing shape, while constrained by aerodynamic limitations, is also influenced by selection operating on other aspects of organismal performance, including migration, dispersal, foraging, predator avoidance, specific-gender strategies as well as sexual selection (e.g. Hedenström and Møller 1992; Wickman 1992; Marchetti et al. 1995; Srygley 1999; Breuker et al. 2007; Dockx 2007; Johansson et al. 2009; DeVries et al. 2010; Förschler and Bairlein 2011; Outomuro et al. 2012). Many of these selection pressures may result in species diversification. Thus, understanding the phylogenetic changes in wing shape is necessary for comprehending the evolution of flight.

Wings can be a fundamental structure used in sexual displays by showing secondary sexual traits. Such traits are important in species recognition and mate choice, and may have an important role in biological diversification and eventual speciation (Svensson and Gosden 2007; Derryberry et al. 2012). One example of secondary sexual traits on wings are ornaments, which typically are positively sexually selected, either by male–male interactions and/or by female choice, and are condition dependent (e.g. Andersson 1994; Contreras-Garduño et al. 2008; Legagneux et al. 2010; Rutowski et al. 2010). Wing shape may also play a crucial role in ornament sexual signaling, since

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D. Outomuro (✉) · F. Johansson
Department of Ecology and Genetics, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 752 36 Uppsala, Sweden
e-mail: outomuro.david@gmail.com

D. C. Adams
Department of Ecology, Evolution, and Organismal Biology, Iowa State University, 241 Bessey Hall, Ames, IA 50011, USA

certain wing shapes might improve both flight performance (e.g. maneuverability and ornament display) and the quality of the ornamental signal (e.g. size and shape of the ornament) (Monteiro et al. 1997, Srygley 1999). Hence, we would expect a correlative evolution between aspects of wing shape that confer optimal flight and those that enable optimal wing display. Some evidence for this pattern comes from butterflies that exhibit Müllerian mimicry (i.e. pairs of species that share wing pigmentation patterns): Srygley (1999) found that wing shape diverged among species within lineages, and converged among species within mimicry groups. However, to our knowledge there are no other available studies that have evaluated the correlative evolution of wing shape and wing ornaments.

Males of the damselfly family Calopterygidae are an excellent group to study the potential correlative evolution of wing shape and wing ornamentation for two main reasons. First, calopterygids show a wide variety of flight modes such as hunting, dispersal, territorial (threats, chasing, circling) and displaying flights (cross display, dive display, courtship arc) (e.g. Pajunen 1966; Waage 1973). Flight mode usually has a strong impact on wing shape (e.g. Betts and Wootton 1988; Berwaerts et al. 2006; DeVries et al. 2010), and therefore the wing shape differences among calopterygids (e.g. Sadeghi et al. 2009; Outomuro et al. 2012) might be related to flight to some degree. Second, the family Calopterygidae exhibit conspicuous ornaments on wings, in the form of wing pigmentation (Córdoba-Aguilar and Cordero-Rivera 2005). Interestingly, the conspicuousness of the wing pigmentation varies from one species to another, ranging from highly pigmented wings to almost complete hyaline wings (see "Materials and methods").Wing pigmentation is based on melanin, which is costly to produce, condition-dependent and has been shown to be positively selected both in male–male territorial contests and by female choice (Grether 1996a; Hooper et al. 1999; Siva-Jothy 1999, 2000; Rantala et al. 2000; Córdoba-Aguilar 2002; Contreras-Garduño et al. 2006, 2008; Svensson et al. 2007). Further, it has been shown that the expression of wing pigmentation is correlated with wing shape in one species within this group of damselflies (*Calopteryx virgo* (Linnaeus, 1758): Outomuro and Johansson 2011). Hence, we have some direct and indirect evidence that biological diversification at the species level could be driven by wing pigmentation. Moreover, behavioral studies have shown that males of some taxa within this family have very conspicuous sexual displays towards the female during which hind wings are more involved in signaling than front wings (summarized in Table 1 in Outomuro et al. 2012). We might therefore expect shape of hind wings to evolve faster than shape of front wings, and such a pattern has been found in the European calopterygids (Outomuro et al. 2012). We also note that other selective pressures can also affect wing pigmentation. For example, bird predators select negatively wing pigmentation (Svensson and Friberg 2007; Rantala et al. 2011) and interactions between conspecific species may lead to wing pigmentation displacement (e.g. Tynkkynen et al. 2004; Anderson and Grether 2010).

If improved signaling represents an important selective force on male wing shape, males of calopterygid species with different kinds of wing pigmentation may be expected

Table 1 Study taxa from the damselfly family Calopterygidae, sample size (*N* front-/hind-wings) and assigned pigmentation group (front-/hind-wings)

Taxa	*N*	Pigmentation group
Archineura incarnata	8/8	1/1
Atrocalopteryx atrata	9/10	3/3
Caliphaea confusa	6/6	6/6
Calopteryx aequabilis	10/10	4/4
Calopteryx amata	5/5	6/5
Calopteryx cornelia	10/10	2/2
Calopteryx exul	6/6	6/6
Calopteryx haemorrhoidalis	10/10	3/3
Calopteryx maculata	10/10	3/3
Calopteryx splendens splendens	10/10	4/4
C. virgo meridionalis	10/10	3/3
C. virgo virgo	10/10	3/3
Calopteryx xanthostoma	10/10	4/4
Echo modesta	10/10	6/6
Hetaerina americana	10/10	1/1
Hetaerina titia	10/10	1/1
Matrona basilaris	10/10	3/3
Matronoides cyanipennis	5/7	3/3
Mnais andersoni	10/10	2/2
Mnais costalis	10/10	2/2
Mnais mneme	10/10	2/2
Mnais pruinosa	10/10	2/2
Mnais tenuis	10/10	2/2
Neurobasis chinensis	10/10	6/3
Phaon camerunensis	10/10	6/6
Phaon iridipennis	10/10	6/6
Phaon sp. from Madagascar	6/6	6/6
Psolodesmus mandarinus dorothea	6/6	5/5
Sapho bicolor	10/10	4/4
Sapho ciliata	10/8	3/3
Sapho gloriosa	5/7	3/3
Umma longistigma	10/10	6/6
Umma saphirina	10/10	6/6
Vestalis amoena	10/10	6/6
Vestalis gracilis	10/10	5/5
Vestalis lugens	10/10	3/3

to exhibit different wing shapes. It is the relative wing pigmentation which is selected for and not the overall size of the patch itself (Grether 1996a, b; Siva-Jothy 1999; Córdoba-Aguilar 2002), since individuals with proportional larger wing pigmentation generally have a higher condition (Rantala et al. 2000; Contreras-Garduño et al. 2006). We could therefore hypothesize that the wing region with the pigmentation would be expected to be larger. For instance, species with a colored wing patch located at the wing base would be predicted to have a broader wing base.

In the present study, we tested the hypothesis that wing pigmentation is evolving in a correlated manner with wing shape. We evaluated this hypothesis using a phylogenetic comparative approach, and used males of 36 taxa of the family Calopterygidae which differ in wing shape and wing pigmentation position, extension and/or color. We also focused on the differences between front- and hind-wings, given that they have different roles in flight and displaying in this lineage (Outomuro et al. 2012). We used geometric morphometric techniques to quantify wing shape. We addressed the following questions: (1) does wing shape differ among different wing pigmentation groups; (2) does the relationship between wing shape and wing pigmentation differ between front- and hind-wings; and (3) is the region of the wing with pigmentation more developed than the rest of the wing?

Materials and Methods

Study Taxa

We studied a total of 331 males from 36 taxa of the damselfly family Calopterygidae for which there are previous data on their phylogenetic relationships based on nucleotide sequences. Apart from our own sampled taxa, we also obtained dried specimens from museums (NCB Naturalis of Leiden and The Swedish Museum of Natural History, Stockholm) or colleagues (Table 1). Wings were scanned in a flatbed scanner or photographed, always together with a scale used as a reference for size estimates. Wing pictures were used for wing shape analyses. Sample size for each taxon varied from 5 to 10 specimens (Table 1).

Wing pigmentation varies among the study taxa not only in extension, but also in position and color. We were interested in determining whether the presence of wing pigmentation was associated to a certain wing shape and thus we needed to summarize all the aforementioned variables in discrete groups to allow comparative analyses. We chose to classify the taxa into six pigmentation groups which efficiently gather the information on the position, extension and color of the wing pigmentation (Table 1; Fig. 1): (1) pigmentation on the wing base; (2) yellow pigmentation on most of the wing; (3) extensive dark wing pigmentation, covering more than 85 % of the whole wing; (4) dark pigmentation as a wing spot, covering 30–85 % of the wing surface, either located at the central part of the wing or at the wing apex; (5) dark pigmentation covering <20 % of the wing surface, restricted to the wing tip; and (6) no conspicuous pigmentation. In the case of the species of the genus *Mnais* Sélys, 1853, for which there are territorial and non-territorial morphs that differ in wing pigmentation (Tsubaki 2003), we only used the territorial morphs due to statistical limitations in the use of phylogenetic procedures. For *Hetaerina titia* (Drury, 1773), we only used morphs with reduced wing pigmentation in hind wings.

Phylogenetic Tree

To obtain a phylogenetic tree that included all our study taxa we re-analyzed previously published nucleotide sequences of the following genes: 18S, 5.8S, partial 28S rDNA, and of the spacers ITS1 and ITS2 (Weekers et al. 2001; Hayashi et al. 2004; Dumont et al. 2005; Dumont et al. 2010; Guan et al. 2012). We used a total of 70 taxa from the family Calopterygidae, with two species of non-calopterygid damselflies and one dragonfly as outgroups (Supplementary Table 1). Sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) in MEGA version 5 (Tamura et al. 2011). Aligned sequences were then subjected to a Bayesian phylogenetic analysis in the package BEAST version 1.7.1 (Drummond et al. 2012) to obtain an estimate of the phylogenetic relationships among taxa. For this we used a SRD06 model as the nucleotide substitution model, a relaxed molecular clock (uncorrelated lognormal) and a birth–death process as a tree prior. The MCMC sampling was run for 10^7 generations and logged every 1,000. The resulting consensus tree was then pruned to contain only our study taxa, and was then used in all subsequent phylogenetic comparative analyses (Fig. 1). The phylogenetic tree we obtained in this study resembled previous published trees (e.g. Dumont et al. 2005).

Wing Shape and Phylogenetic Analyses

We used geometric morphometrics techniques to study wing shape. These methods allow for quantification of shape from landmark coordinates after the effects of non-shape variation (position, orientation, and scale) have been mathematically held constant (Bookstein 1991; Rohlf and Marcus 1993; Adams et al. 2004). From each wing we digitized 12 landmarks and semilandmarks to capture wing shape using TpsDig2 (Rohlf 2010a). First, ten biologically homologous landmarks were digitized, located at the wing

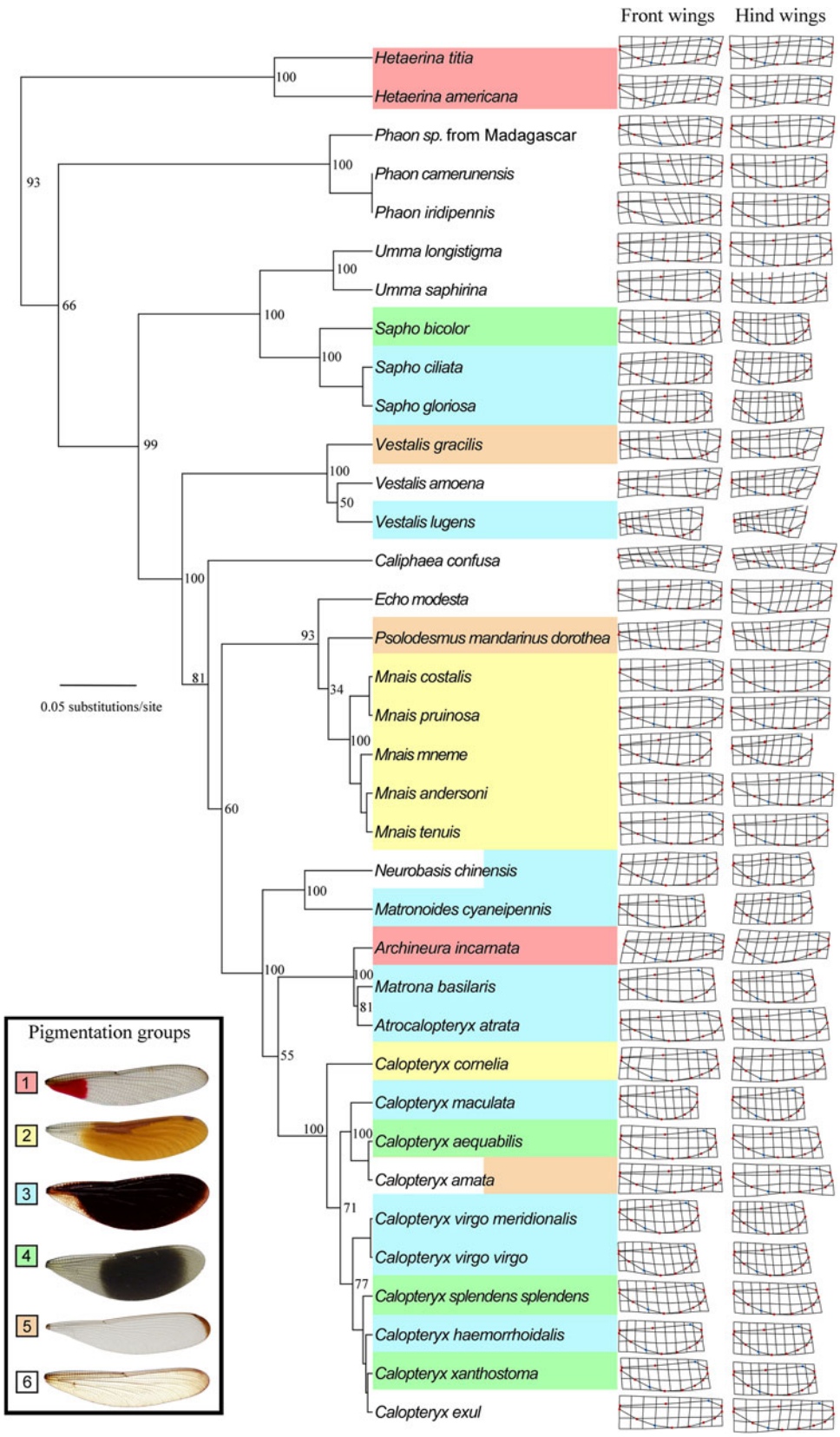


Fig. 1 Consensus Bayesian tree used in the present study. Posterior Bayesian probabilities are shown on the nodes. Deformation grids for each taxon are mapped, showing separately front- and hind-wings. Each *colour* indicates a pigmentation group. Note that for *N. chinensis* and *C. amata* front- and hind-wings differ in pigmentation (*right* and *left colours* respectively). Examples of front wing pictures for each pigmentation group are also included (*1 H. americana*, *2 M. costalis*, *3 C. haemorrhoidalis*, *4 C. splendens splendens*, *5 P. mandarinus dorothea*, *6 C. amata*

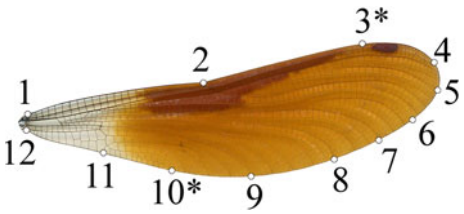


Fig. 2 Front wing of male *Mnais costalis* showing the landmarks and semilandmarks (*asterisk*) used for the study of wing shape

base and along the wing margin where it is intersected by major wing veins (Fig. 2). Additionally, two semilandmarks were used to incorporate aspects of wing curvature. The landmarks and semilandmarks were subjected to a generalized procrustes analysis (GPA) (Rohlf and Slice 1990). Here, all specimens are translated to the origin, scaled to unit centroid size, and optimally rotated to minimize the total sums-of-squares deviations of the landmark coordinates from all specimens to the average configuration. During GPA, semilandmarks were permitted to slide along their tangent directions (Bookstein 1991; Gunz et al. 2005) so as to minimize Procrustes distance between specimens. A single reference shape configuration was obtained (front- and hind-wings combined) and used for aligning all individual wing shapes, and for computing shape variables (i.e. uniform and non-uniform shape components) in tpsRelw (Rohlf 2010b). These shape variables were subsequently used for the wing shape analyses as the response variables. Centroid size was also computed for each wing, and since this was highly correlated with body size (e.g. Outomuro and Johansson 2011), it was used as a proxy for body size.

To visualize how wing shape differed among taxa and among pigmentation groups, we first computed the average wing shape for both front- and hind-wings for each taxon, as well as for each pigmentation group. We then generated thin-plate spline deformation grids for each of these (relative to the overall reference shape) using tpsSplin (Rohlf 2004).

We inspected how wing shape was related to centroid size. For this we graphically represented wing shape on centroid size by using an approach by Drake and Klingenberg (2008). This method performs multiple regressions of the Procrustes coordinates on centroid size and then computes shape scores, which are the predicted shape variables in the regression including the residual variation in the direction of the shape space. Since we were interested in the relationship across taxa, we used the mean wing shape for each taxon and we run the analysis separately for front- and hind-wings. We found that wing shape and centroid size showed a linear relationship (see Supplementary Fig. 1). To remove the allometric component of wing shape, we performed a multivariate linear regression of the wing shape variables on centroid size, separately for front- and for hind-wings. We kept the residuals from these regressions and used them subsequently as the non-allometric shape variables. The residuals were not related to centroid size.

For both front- and hind-wings we performed a nested MANOVA to determine whether wing shape differed among the six pigmentation groups. The non-allometric shape variables were used. Here we included pigmentation group and taxa nested on pigmentation group. We then performed pairwise comparisons between the different pigmentation groups by using a permutation procedure shuffling the specimens with respect to the pigmentation group (e.g. Adams and Nistri 2010). At each step of the permutation procedure, we used the least-squares means for each pigmentation group obtained from a nested MANOVA (same design model as above), and for each iteration, the Euclidean distances among group means were compared to the original values to assess significance (see Adams and Collyer 2007, 2009; Collyer and Adams 2007). We used R version 2.15 for all statistical analyses (R Development Core Team 2011).

To test whether wing shape tracked the evolutionary history of the study lineage and was not randomly distributed across taxa, i.e. significantly departed from Brownian motion, we assessed the phylogenetic signal of wing shape (Blomberg et al. 2003; Klingenberg and Gidaszewski 2010; Blankers et al. 2012). We did it in front- and hind-wing shape, both before and after removing size effects. We first computed the average front- and hind-wing shape for each species using respectively the original shape variables and the non-allometric shape variables. We then obtained estimates of phylogenetic signal using the approach by Klingenberg and Gidaszewski (2010). This method quantifies phylogenetic signal for multi-dimensional traits (such as shape) as the sum of squared shape changes along the branches of the phylogeny. Here, smaller values are found when closely related species are also similar in shape: thus, smaller values imply greater phylogenetic signal in shape. To assess the degree of phylogenetic signal we used a permutation procedure, where wing shapes are permuted across the tips of the phylogeny and an estimate of phylogenetic signal is obtained. The significance of the phylogenetic signal is calculated as the proportion of permuted shapes sets in which the sum of squares changes is lower or equal to the observed sum of squares in the original dataset (see Klingenberg and Gidaszewski 2010).

Finally, we performed several phylogenetic comparative analyses to examine correlative evolutionary patterns in wing shape. First we assessed the evolutionary relationship between wing shape (including the allometric component) and centroid size among species using multivariate

phylogenetic generalized least squares (PGLS) (e.g. Rohlf 2001). This was accomplished using a new function written by one of us in R, and is implemented under a Brownian motion model of evolution (see Supplementary Material). Additionally we used phylogenetic MANOVA (Garland et al. 1993) to evaluate whether the non-allometric component of wing shape and wing shape with the allometric component differed among pigment groups while accounting for phylogenetic relationships.

Results

There was clear variation in wing shape among species, and front wings frequently differed from hind wings in both their shape and pigmentation (Fig. 1). MANOVAs on the non-allometric component of wing shape showed that both front- and hind-wings differed in shape among the different pigmentation groups and among the taxa (Table 2). Pairwise comparisons among pigmentation groups based on the MANOVA revealed that most of the groups differed from each other in wing shape, with the exception of pigmentation groups 5–6 in hind wings (Table 3). Wings with basal pigmentation (group 1, Fig. 3) were broader at the base, especially in the hind wings. From the midpoint and onwards the wings were however more slender. Yellow-pigmented wings were close to the consensus wing both for front- and for hind wings (group 2, Fig. 3). Highly dark pigmented or less extensively dark pigmented wings (groups 3 and 4, Fig. 3) were much shorter and broader, especially for the hind wings. Wings with only apical pigmentation were broader at the wing apex and showed more slender wing base (group 5, Fig. 3). Finally, hyaline wings were longer and more slender in shape (group 6, Fig. 3).

There was significant phylogenetic signal for both the wing shape with the allometric component and for the non-allometric wing shape. This was true for both front- (with allometric component: phylo. signal = 0.0735; $P = 0.001$; non-allometric component: phyl. signal = 0.0707;

Table 2 Results from the nested MANOVA on wing shape for front- and hind-wings

	Pillai's trace	Approx. *F*	*df* num	*df* den	*P* values
Front wings					
Pigmentation	4.0544	58.958	100	1,375	<0.001
Pigmentation (taxa)	9.8653	9.410	600	5,800	<0.001
Hind wings					
Pigmentation	3.8611	47.125	100	1,390	<0.001
Pigmentation (taxa)	9.8471	9.473	600	5,860	<0.001

Table 3 Pairwise comparisons between the pigmentation groups (1–6) in front- (A) and hind-wings (B), including the Euclidean distance (above the diagonal) and the values of significance (below the diagonal) (the 0.05 level of significance after Bonferroni correction is 0.003)

	1	2	3	4	5	6
A. Front wings						
1	1	0.062	0.111	0.106	0.089	0.072
2	0.001	1	0.056	0.053	0.066	0.044
3	0.001	0.001	1	0.035	0.086	0.079
4	0.001	0.001	0.001	1	0.096	0.069
5	0.001	0.001	0.001	0.001	1	0.081
6	0.001	0.001	0.001	0.001	0.001	1
B. Hind wings						
1	1	0.055	0.112	0.106	0.070	0.050
2	0.001	1	0.070	0.073	0.053	0.034
3	0.001	0.001	1	0.045	0.101	0.086
4	0.001	0.001	0.001	1	0.113	0.091
5	0.001	0.001	0.001	0.001	1	0.036
6	0.001	0.001	0.001	0.001	0.016	1

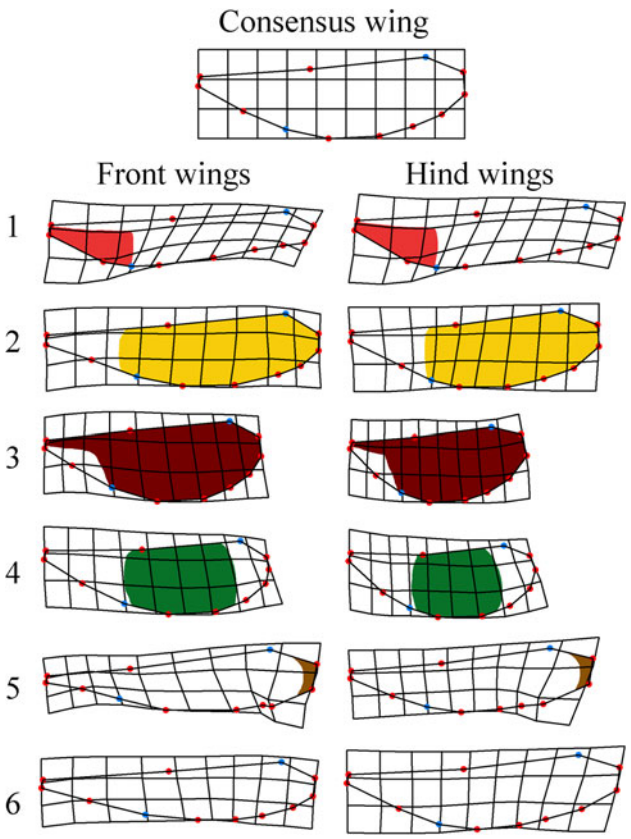


Fig. 3 Thin-plates spline deformation grids showing the differences in wing shape among the pigmentation groups for front- and hind-wings. Deformation grids are based on the average specimen for each group

Table 4 Results of the multivariate PGLS of centroid size on wing shape

PGLS	Pillai's trace	Approx. *F*	*df* num	*df* den	*P* values
Front wings	0.9972	0.7955	40	32	0.7555
Hind wings	0.75261	0.48268	40	32	0.9852

$P = 0.001$) and hind wings (with allometric component: phyl. signal = 0.0772; $P = 0.001$; non-allometric component: phyl. signal = 0.0746; $P = 0.001$). Thus, variation in wing shape displayed a phylogenetic structure that was not predicted by a Brownian motion of evolution, both before and after removing size effects in wing shape. There was no relationship between wing shape and centroid size among species when phylogeny was taken into account in the analysis (Table 4). Additionally, after accounting for phylogeny in the analysis of the wing shape with the allometric component, there were no significant differences among pigmentation groups for front wing shape, while there were marginally non-significant differences in hind wing shape among the pigmentation groups (Table 5A). When the non-allometric component of wing shape was corrected for phylogenetic effects, there were significant differences among pigmentation groups for hind wings, but not for front wings (Table 5B). Hence, wing shape differed with regard to pigmentation groups in hind wings when the allometric component was subtracted from wing shape and centroid size itself has no significant effects on wing shape when phylogeny was taken into account.

Discussion

We showed that wing shape evolution was correlated to wing ornamentation and not only to its allometric component. In fact, wing shape specifically differed between the pigmentation groups, with a higher development of the wing where the pigmentation was located. After accounting for phylogenetic non-independence, only the non-allometric component of hind wing shape showed significant differences between the pigmentation groups.

Wing Pigmentation and Wing Shape

Wing shape differed between pigmentation groups. Our results showed that a broader wing or a broader part of the wing occurs where wing pigmentation is present. Field studies showed that signal quality is related to the relative size (and probably shape) of the wing pigmentation (e.g. Grether 1996b; Siva-Jothy 1999; Córdoba-Aguilar 2002). Our findings suggest that wing shape is evolving in a correlative manner with wing pigmentation, leading to differences among pigmentation groups and probably to an optimization of the wing pigmentation display. The proposed correlative evolution between color and shape is supported by previous examples on Müllerian mimicry groups of butterflies (Srygley 1999). However, in the case of the calopterygid damselflies examined here, wing pigmentation is not related to predator avoidance, but to sexual signaling. Since our results are based on correlations, we still do not know if wing shape is evolving due to wing pigmentation or vice versa. For example, wing pigmentation evolution might be constrained by wing shape, which might have evolved due different habitat and/or predation selective pressures (e.g. Svensson and Friberg 2007).

Functional Differences Between Front- and Hind-Wings

When phylogeny was taken into account, only hind wing shape showed significant differences between pigmentation groups. This different pattern of evolution between front- and hind-wing shape is in agreement with behavioral studies on Calopterygidae which suggested that hind wings are more involved in ornament displaying than front wings (see Table 1 in Outomuro et al. 2012). Moreover, our previous study showed that hind wing shape is evolving faster than front wing shape (Outomuro et al. 2012). The present results suggest that front- and hind-wings are evolving partially independently, accommodating different selection pressures within the individual as it has been previously shown for butterflies (Oliver et al. 2009;

Table 5 Results of the phylogenetic MANOVA on wing shape testing the effect of the wing pigmentation group, before (A) and after (B) removing size effects

	Wilk's λ	Approx. *F*	*df* num	*df* den	*P* values	Phyl. *P* values
A. With allometric component						
Front wings	0.0002	2.8151	100	58.328	<0.001	0.1648
Hind wings	0.0001	2.9713	100	58.328	<0.001	0.0739
B. Non-allometric component						
Front wings	0.0002	2.6709	100	58.328	<0.001	0.2617
Hind wings	0.00008	3.4105	100	58.328	0.001	0.0249

Rutowski et al. 2010). For instance, the wing dorsal surface of *Bicyclus* butterflies is used for mate signaling and is evolving faster than the ventral surface, which is not sexually selected (Oliver et al. 2009). Under this framework, the evolution of wing pigmentation and wing shape might also lead to interspecific differences in wing ornaments and behavioral sexual displays, thus increasing phenotypic and behavioral diversity. Therefore, our results suggest that pre-copulatory selection, together with post-mating selection (Cordero Rivera et al. 2004), might be contributing to speciation events in calopterygid damselflies.

Our results showed that both front- and hind-wing shape exhibit significant phylogenetic signal, i.e. their patterns of evolution significantly departed from Brownian motion. Therefore, although closely-related taxa are more similar in wing shape, they also showed divergence in wing shape depending on the wing pigmentation they express, at least in hind wings. Since hind wing shape is evolving faster than front wing shape in some calopterygid species (Outomuro et al. 2012), it would be expected to find different strengths of phylogenetic signal between front- and hind-wings. Contrary to this expectation, the strength of the phylogenetic signal was similar between front- and hind-wings, both before and after removing size effects. We lack a satisfactory explanation for these results, although we suggest that correlative selection between front- and hind-wings might be leading to similar strengths in the phylogenetic signals.

The Allometric Component of Wing Shape

Interestingly, centroid size did not show significant effects on wing shape in a phylogenetic context and therefore no macroevolutionary pattern was evident with regard to wing size. However, body size has an allometric relationship with the shape of a body structure (Debat et al. 2003; Shingleton et al. 2007) and this relationship is expected to improve the aerodynamics requirements for a given size and sex of the individual (e.g. wings in butterflies: Berwaerts et al. 2006; DeVries et al. 2010; head of stalk-eyed flies: Worthington et al. 2012).

Conclusions

The reported differences in wing shape related to each pigmentation group may have important effects on the aerodynamics properties of the wing (Ellington 1984; Betts and Wootton 1988; Wakeling and Ellington 1997). Calopterygid wing design (broad and non-pedunculated wings) allows higher levels of agility than other damselflies, necessary for territorial and displaying behaviors (Serrano-Meneses et al. 2008). With the present results and data it is not possible to describe patterns of flight such as agility in the different pigmentation groups. However, we are currently developing a study relating wing shape to wing agility.

We showed that the evolution of wing shape is correlated with the evolution of wing pigmentation and wing size such that correlative evolution between optimal flight performance and sexual signaling may be present. Moreover, we found differences between front- and hind-wings, supporting previous hypothesis of a higher role of hind wings in sexual signaling. Wing shape evolution involves a plethora of environmental factors that lead to an optimal wing shape, but sexual selection on wing pigmentation is likely to have a key role in driving wing shape evolution and perhaps eventual speciation. Our study is correlative but we note that manipulative field experiments cited above support our findings. Nevertheless experiments focusing on mechanistic studies are needed to add more support to our results.

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