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## Strong longitudinal variation in wing aspect ratio of a damselfly, Calopteryx maculata (Odonata: Zygoptera)

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Geographical patterns in body size have been described across a wide range of species, leading to the development of a series of fundamental biological rules. However, shape variables are less well-described despite having substantial consequences for organismal performance. Wing aspect ratio (AR) has been proposed as a key shape parameter that determines function in flying animals, with high AR corresponding to longer, thinner wings that promote high manoeuvrability, low speed flight, and low AR corresponding to shorter, broader wings that promote high efficiency long distance flight. From this principle it might be predicted that populations at range edges would exhibit low AR wings. I test this hypothesis using the riverine damselfly, Calopteryx maculata, sampled from 34 sites across its range margin in North America. Nine hundred and seven male specimens were captured from across the 34 sites (mean=26.7 $\pm 2.9$ SE per site), dissected and measured to quantify the area and length of all four wings. Geometric morphometrics were employed to investigate geographical variation in wing shape. The majority of variation in wing shape involved changes in wing aspect ratio, confirmed independently by geometric morphometrics and wing measurements. There was a weak positive relationship between wing aspect ratio and temperature, in line with work on other insects. However, there was a strong longitudinal pattern in which western populations exhibited lower wing aspect ratio. This longitudinal pattern may be related to increasing variability in precipitation from east to west in North America. I discuss my findings in light of research of the functional ecology of wing shape across vertebrate and invertebrate taxa

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9 Running title: Damselfly wing morphology


#### Abstract

Geographical patterns in body size have been described across a wide range of species, leading to the development of a series of fundamental biological rules. However, shape variables are less well-described despite having substantial consequences for organism performance. Wing aspect ratio (AR) has been proposed as a key shape parameter that determines function in flying animals, with high AR corresponding to longer, thinner wings that promote high manoeuvrability, low speed flight, and low AR corresponding to shorter, broader wings that promote high efficiency long distance flight. From this principle it might be predicted that populations living in cooler areas would exhibit low AR wings to compensate for reduced muscle efficiency at lower temperatures. I test this hypothesis using the riverine damselfly, Calopteryx maculata, sampled from 34 sites across its range margin in North America. Nine hundred and seven male specimens were captured from across the 34 sites (mean $=26.7 \pm 2.9$ SE per site), dissected and measured to quantify the area and length of all four wings. Geometric morphometrics were employed to investigate geographical variation in wing shape. The majority of variation in wing shape involved changes in wing aspect ratio, confirmed independently by geometric morphometrics and wing measurements. There was a weak positive relationship between wing aspect ratio and temperature, in line with work on other insects. However, there was a much stronger longitudinal pattern in which western populations exhibited lower wing aspect ratio. This longitudinal pattern may be related to increasing variability in precipitation from east to west in North America. I discuss my findings in light of research of the functional ecology of wing shape across vertebrate and invertebrate taxa.


Keywords: wing morphology, aspect ratio, flight, dispersal, habitat stability, precipitation, damselfly, Odonata.

## INTRODUCTION

Powered flight has evolved independently in four different lineages: the pterosaurs, insects, birds, and bats, allowing animals to exploit novel niches and avoid predators. The adaptations that allowed each of these transitions to an aerial niche represent a suite of similar traits that can be broken down into a number of functional morphological components that influence inter- and intraspecific variation in flight performance. First, absolute body size is correlated with dispersal ability across a wide range of taxa (Jenkins et al. 2007). Second, the ratio of body mass to wing area - known as "wing loading" - has a strong influence on the amount of thrust generated per wingbeat (Dudley 2002). However, for the purposes of this study I am most interested in the third component of variation: that of wing shape. One of the principle measures of functional variation in wing shape is the length of the wing relative to the width, known as aspect ratio. In vertebrates, higher aspect ratio (longer, thinner wings) is predicted to give faster and more efficient flight (Norberg 1989) and has been shown to be associated with migratory species in birds (Mönkkönen 1995). However, there has been speculation that the benefits of high aspect ratio may be reduced or even reversed at the low Reynolds numbers experienced by insects (Ennos 1989; Wootton 1992). This fact, along with the difference in the nature of flight - number, structure and locomotory independence of wings - between birds and insects complicates the formation of hypotheses concerning the implications of variation in odonate morphology (Johansson et al. 2009). The literature on the functional relevance of insect wing morphology is heavily biased towards theory (Dudley 2002), laboratory studies (Marden 1995) and observations of kinematics (Rüppell 1989; Wakeling \& Ellington 1997a, b, c) rather than field observations.

Contrary to predictions for birds, a number of findings point towards lower wing aspect ratio as being beneficial for dispersal in insects. Wing aspect ratio is lower in populations of Pararge aegeria that have recently been founded (Hill et al. 1999). Populations of P. aegeria (Hughes et al. 2007; Vandewoestijne \& Van Dyck 2011), Drosophila melanogaster (Azevedo et al. 1998), and a number of damselflies (Hassall et al. 2009; Taylor \& Merriam 1995) show lower aspect ratio at higher latitudes where temperature reduces the efficiency of flight in ectotherms. Other studies have shown higher wing aspect ratio only in species of damselflies with expanding
range margins (Hassall et al. 2009), and those marginal populations exhibit wing shapes that deviate progressively away from the species average closer to the range margin (Hassall et al. 2008). Studies using common garden rearing of Drosophila from a range of latitudes have shown that individuals reared at lower temperatures have lower aspect ratio (Azevedo et al. 1998). While there is no clear relationship between aspect ratio and flight speed in butterflies (Berwaerts et al. 2008; but cf Berwaerts et al. 2002; Dudley 1990), species in which males "patrol" (i.e. exhibit prolonged flight) tend to have lower aspect ratios (Wickman 1992). Chironomid females have broader wings (characteristic of lower aspect ratio) to assist with flying for long periods between habitat patches (McLachlan 1986). While there are exceptions (increased fragmentation does not correlate with aspect ratio in Plebejus argus (Thomas et al. 1998) or Pararge aegeria (Merckx \& Van Dyck 2006)) these findings seem to suggest that lower wing aspect ratio in insects is associated with greater dispersal.

Wing morphology in Odonata may be affected by a combination of sexual selection during intrasexual, agonistic interactions, intersexual courtship displays and dispersal (Johansson et al. 2009). In the field, intrasexual territorial contests in C. maculata are determined by fat reserves (Marden \& Rollins 1994; Marden \& Waage 1990) and contests in Plathemis lydia are determined by flight muscle ratio (Marden 1989). In both cases, aspect ratio was shown not to influence the outcome of the contests. Sexual selection on courtship displays focuses on patterns of pigmentation in Calopteryx species (Siva-Jothy 1999; Waage 1973). However, wing shape has been shown to vary with landscape structure in C. maculata (Taylor \& Merriam 1995) and between some closely-related species of Calopterygidae in Europe (Sadeghi et al. 2009), although not all species exhibited distinct wing shapes. Based on these results, it seems that wing shape variation is under natural selection due to dispersal (within or between sites), rather than sexual selection.

Based on the reasoning presented above, I evaluate the hypothesis that, based on negative correlations between latitude and aspect ratio, a positive relationship should be found between temperature and aspect ratio to compensate for lower flight efficiency at lower temperatures. Uncertainties over the ecological role of morphology variation may stem from the partial sampling of geographical ranges (Hassall 2013). Limited
sampling of non-linear trends that occur over large spatial scales may produce misleading results and so I provide an analysis of wing shape variation across the entire range of the damselfly Calopteryx maculata in North America.

## METHODS

A total of 907 specimens of male C. maculata were collected from 34 sites across the range by 25 collectors (Figure 1, Table 1). Collections took place between 13 May and 7 August 2010 and mean sample size from each site varied between 4 and 84 individuals (mean=26.7 $\pm 2.9 \mathrm{SE}$, details of sample sizes and mean measurements can be found in Table 1). Wings were dissected from the body as close to the thorax as possible and mounted on adhesive tape (Scotch Matte Finish Magic Tape). Wings were scanned using the slide scanner on an Epson V500 PHOTO flatbed scanner with fixed exposure at 1200dpi. Wing length (the length from the costal end of the vein separating the arculus from the discoidal cell to the tip of the wing) and wing area were calculated for each of the four wings on each individual. All measurements were carried out in ImageJ (Rasband 1997-2007). During measurement, any damage to wings was noted and those measurements (length or area) which could not be accurately quantified were excluded. This resulted in the exclusion of 7 fore wing and 9 hind wing lengths, and 28 fore wing and 45 hind wing areas. Aspect ratio was then calculated separately for both fore and hind wings as wingspan $2 /$ wing area (see Table 1 for summary statistics and sample sizes).

It has been suggested that wing aspect ratio does not provide sufficient detail to be morphologically informative in butterflies (Betts \& Wootton 1988) or dragonflies (Johansson et al. 2009). Therefore, in addition to calculating aspect ratio, I also use geometric morphometrics to derive descriptors of the shape of the wing. A subset of up to 10 individuals from each site were selected at random and a set of 14 landmarks were digitised on 1 fore wing and 1 hind wing (Figure 2) using tpsDig2 (v.2.12, Rohlf 2008). Mean locations for each of the 14 landmarks were found for each of the 34 sites. Principal components analysis (PCA) was carried out on these landmarks after Procrustes transformation using the PAST software package (Hammer et al. 2001). Relationships between the principal components and absolute measurements were investigated using Pearson correlations. Fore and hind wings were compared using paired Hotelling's $\mathrm{t}^{2}$ tests in PAST to assess whether the two datasets could be combined.

Mean annual temperature was extracted for each site from the WORLDCLIM dataset (Haylock et al. 2008) to test the central hypothesis of the study. After initial data examination revealed a longitudinal pattern, the coefficient of variation $(\sigma / \mu)$ of precipitation, which exhibits strong variability from west to east, was calculated for each site using the WORLDCLIM dataset (bioclimatic variable 15, Haylock et al. 2008) in ArcGIS v9.2 (ESRI 2006). Aspect ratio and the informative principal components from the shape analysis were regressed against temperature and seasonality of precipitation using linear regressions weighted by the square-root of the sample size. In each case, the models were tested with a quadratic predictor term using Akaike's information criterion (AIC) to evaluate any improvement in model fit.

## RESULTS

Fore and hind wings vary significantly in shape $\left(\mathrm{t}^{2}=122500, \mathrm{p} \ll 0.001\right)$ and were completely separated along the PC1 axis which explained $80.2 \%$ of the variance in shape. As a result, fore and hind wing data are treated separately for the rest of the analysis.

PCA of both fore and hind wings resulted in three components that lay before the "elbow" of the scree plot. These explained $38.7 \%, 23.2 \%$ and $18.6 \%$ (total $80.5 \%$ ) of the variance in fore wing shape and $44.9 \%, 21.4 \%$, and $12.6 \%$ (total $78.9 \%$ ) of the variance in hind wing shape. PC1 in both cases involved a variation in the width of the wing relative to its length, such that an increase in PC1 leads to a decrease in the width of the wing relative to the length (Figure 3). PC1 was significantly positively correlated with aspect ratio (fore wings, $\mathrm{r}=0.875, \mathrm{p}<0.001$; hind wings, $\mathrm{r}=0.854, \mathrm{p}<0.001$, Figure 4). The PC 2 and PC3 involved more subtle shape changes which were still consistent between wings. PC2 appears to involve a shortening of the pre-nodal region and a blunting of the tip, while PC3 corresponds to a movement of wing area towards the wing tip.

Geographical patterns of wing aspect ratio showed strong longitudinal variation (Figure 5A), with significant and positive relationships between longitude and aspect ratio in both fore $(\mathrm{r}=0.816, \mathrm{p}<0.001)$ and hind wings $(\mathrm{r}=0.800, \mathrm{p}<0.001)$ indicating a decline in aspect ratio further west. Latitudinal patterns were significant and positive but weaker than for longitude (fore: $\mathrm{r}=0.441, \mathrm{p}=0.010$; hind: $\mathrm{r}=0.375, \mathrm{p}=0.029$ ). For regression models containing either longitude and latitude or both, models with longitude only performed best and the addition of latitude did not produce a significant improvement in model performance $(\Delta \mathrm{AIC}<2)$.

Regressions between aspect ratio and temperature showed no significant increase in explanatory power for the quadratic model in the fore (linear $\mathrm{AIC}=-50.188$; quadratic $\mathrm{AIC}=-50.947 ; \Delta \mathrm{AIC}=0.76$ ) or hind wings (linear $\mathrm{AIC}=-53.582$; quadratic $\mathrm{AIC}=-53.509 ; \Delta \mathrm{AIC}=0.073$ ) and so the quadratic term was not added. Aspect ratio was positive and significantly related to temperature for fore wings $\left(F_{1,32}=6.262, p=0.018, R^{2}=0.138\right)$ but there was no significant relationship with aspect ratio of hind wings $\left(\mathrm{F}_{1,32}=3.302, \mathrm{p}=0.079, \mathrm{R}^{2}=0.065\right)$. Regressions
between aspect ratio and the seasonality of precipitation also showed no increase in explanatory power for the quadratic model in fore (linear $\mathrm{AIC}=-63.124$; quadratic $\mathrm{AIC}=-64.723 ; \Delta \mathrm{AIC}=1.599$ ) or hind wings (linear $\mathrm{AIC}=-$ 67.687; hind: $\mathrm{AIC}=-68.840 ; \Delta \mathrm{AIC}=1.154$ ) and so the quadratic term was not added. Linear regressions between aspect ratio and the seasonality of precipitation were highly significant in both fore ( $\mathrm{F}_{1,32}=25.687, \mathrm{p}<0.001$, $\mathrm{R}^{2}=0.428$ ) and hind wings ( $\mathrm{F}_{1,32}=23.792, \mathrm{p}<0.001, \mathrm{R}^{2}=0.409$, Figure 5 B ) and explained a high proportion of the variation in the data. Taken together, models explaining aspect ratio with seasonality of precipitation had a substantially greater explanatory power than those using temperature in both fore ( $\triangle \mathrm{AIC}=13.776$ ) and hind wings ( $\triangle \mathrm{AIC}=15.331$ ). However, the geographical distribution of aspect ratio values (Figure 1) suggests that there may be a step-change in wing shape at a certain longitude, rather than a gradual trend.

## DISCUSSION

I provide the first comprehensive assessment of intraspecific variation in wing morphology across a range in a damselfly. The use of geometric morphometrics to analyse shape confirms that changes in aspect ratio (i.e. changes in the length of the wing relative to the width) constitute the major source of variation between specimens from different sites. I demonstrate a weakly significant effect of temperature in fore wing shape, with lower wing aspect ratios at cooler temperatures as is predicted by theory. However, the dominant pattern is one of increasing aspect ratio from west to east, which has not been documented in previous studies and may be related to the greater seasonality of precipitation in western populations. This finding is consistent with the habitat-stability-dispersal hypothesis if increasing seasonality of precipitation disrupts flow regimes in Calopteryx breeding sites. It is worth noting that the nature of this study is such that I cannot disentangle the effects of selection from those of phenotypic plasticity. Indeed, previous studies have demonstrated that while some flight morphological parameters are under genetic control, wing aspect ratio shows a plastic response to the environment in Drosophila (Azevedo et al. 1998).

The literature on the functional relevance of aspect ratio has produced conflicting findings. While the pattern observed in the present study is consistent with a range of studies that suggest lower aspect ratio favours more efficient or longer flight, higher aspect ratios have been associated with fragmented habitat (Taylor \& Merriam 1995) and range expansion (Hassall et al. 2009) in calopterygid damselflies in other studies. The sites involved in Taylor and Merriam's study were all north of Ottawa, Canada, where my results indicate aspect ratios are high. It may be that the increase in distance between foraging and reproductive habitat that was associated with morphological change in their study operates on a different scale to the patterns describe in the present study. I suggest that the findings of previous studies are limited by their consideration of a relatively small area and number of populations.

It is generally considered that higher aspect ratios provide a benefit for longer-distance (Mönkkönen 1995) or efficient, gliding flight (Ennos 1989), although this is equivocal in Lepidoptera (Betts \& Wootton 1988).

However, as I argue in the introduction, the opposite appears to be true for the majority of studies of insects where smaller Reynolds numbers operate. Indeed, this study provides support for a positive association between aspect ratio and temperature, which may be selected for because lower aspect ratio is more efficient at lower temperatures (Vandewoestijne \& Van Dyck 2011). There remains a gap in the literature that needs to be filled with flight laboratory experiments of the functional implications of aspect ratio variation in odonates and other insects as have been carried out in some butterflies (Berwaerts et al. 2008; Berwaerts et al. 2002; Davis et al. 2012). In particular, a test of the hypothesis that lower aspect ratios enhance flight efficiency at lower temperatures is warranted given the increasing evidence for the correlation between aspect ratio and temperature.

The findings here are consistent with selection for dispersal in habitats with a greater variability in precipitation which causes greater variability in streamflow. The range of potential oviposition sites is limited by fluctuations in rainfall which may raise or lower flow-rates, moving them outside of the narrow range of values that $C$. maculata prefer (Gibbons \& Pain 1992), such that a habitat that is suitable in one year may not remain so in the next. It is also conceivable that seasonality of precipitation increases the fragmentation of these suitable sites: sites which are only marginally suitable become unsuitable during extreme precipitation events, while a subset of sites which are resilient to flow rate variation remain suitable. Such resilience may be provided by geomorphological characteristics of the river channel (Goldstein et al. 2007) or the presence of microhabitats which are less susceptible to variations in flow rate (Brooks et al. 2005). This elimination of marginal larval habitats effectively fragments the landscape resulting in spatial variability.

Habitat selection in Odonata is a hierarchical process, with selection steps occurring at the level of the landscape, the habitat, and the oviposition site (Buchwald 1995; Wildermuth 1994). At least the first two of these steps involves visual cues which include the presence of conspecifics (Corbet 1999), linear polarised light reflected from water bodies (Wildermuth 1998) and structural features, including vegetation (Wildermuth 1992). Calopterygid damselflies also exhibit very specific habitat requirements relating to the depth and flow rate of the
rivers which form the larval habitat (Abbott 2005). Male Calopteryx splendens and Calopteryx xanthostoma land on the water to demonstrate the flow-rate of the stream in their territory, and successful courtship is associated with increased flow-rate up to $0.15 \mathrm{~m} \cdot \mathrm{~s}^{-1}$ (Gibbons \& Pain 1992). The benefits of this flow-rate to the resulting offspring are higher embryonic development rates and lower accumulation of encrusting algae (Siva-Jothy et al. 1995). Changes in flow rates may result in either (i) variation in larval mortality rates, or (ii) variation in the tendency for females to oviposit. It is well-established that variability in precipitation produces disproportionately large variations in streamflow (Dettinger \& Diaz 2000). Given the apparent sensitivity of Calopterygidae to variations in flow rates (Gibbons \& Pain 1992; Siva-Jothy et al. 1995), an assessment of regional variations in population sizes with respect to extremes of precipitation (and, hence, streamflow) might be warranted. Extremes of temperature and precipitation resulting from climate change are likely to manifest long before the gradual increase in long-term mean climate (Karl \& Trenberth 2003; Palmer \& Raisanen 2002) and may pose a particular threat to this family.

Previous studies have questioned the use of aspect ratio as a single numerical metric describing wing shape in insects, due to its inability to represent the complexity of wing morphology (Betts \& Wootton 1988; Johansson et al. 2009). However, I find that a complex method of shape analysis using geometric morphometrics yields patterns that strongly resemble variation in the simpler concept of aspect ratio. However, it is clear from the explanatory power of those principal components that correlate with aspect ratio ( $38.7 \%$ and $44.9 \%$ ) that there is a great deal of variability in addition to this dimension. It is worth noting that insects exhibit a great deal of variation in aspect ratio. Odonates have high aspect ratios compared to some other insects, for example Drosophila virilis with an aspect ratio of 2 (Vogel 1957), and Bombus terrestris with an aspect ratio of 6.4. However, butterflies show higher aspect ratios of 9.8-10.5 in Pararge aegeria (Berwaerts et al. 2008; Berwaerts et al. 2002). The data presented here show aspect ratios of hind wings between 5.61 and 7.79 and of forewings between 5.70 and 7.56. Aeshna cyanea, a large odonate, exhibits aspect ratio of 8.4 and 11.6 for hind and fore wings, respectively (Ellington 1984). What makes the odonate wing very different is the extent of the venation

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## 363 <br> TABLES

364 Table 1 - Sampling site locations, sample sizes and aspect ratios of wings of male Calopteryx maculata.
365 "Measurements" gives the sample size for the total number of measured specimens, "Geo Morph" gives the 366 sample sizes used in the geometric morphometric analysis ( $\mathrm{N}_{\text {fore }}=$ sample size for fore wings, $\mathrm{N}_{\text {hind }}=$ sample size 367 for hind wings).

|  |  |  |  |  |  |  | Measurements |  |  | Geo Morph |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Region | Site | Latitude | Longitude | Date | Fore wing aspect ratio ( $\pm$ SE) | $\begin{array}{r} \text { Hind wing } \\ \text { aspect ratio }( \pm \mathrm{SE}) \end{array}$ | $\mathrm{N}_{\text {total }}$ | $\mathrm{N}_{\text {fore }}$ | $\mathrm{N}_{\text {hind }}$ | $\mathrm{N}_{\text {fore }}$ | $\mathrm{N}_{\text {hind }}$ |
| $=$ Ontario | Blakeney Falls | 45.268 | -76.250 | 31/05/10 | $6.845( \pm 0.044)$ | $6.392( \pm 0.037)$ | 23 | 23 | 23 | 10 | 10 |
| Ontario | Dorset | 45.271 | -78.960 | 31/07/10 | $7.053( \pm 0.075)$ | $6.564( \pm 0.069)$ | 7 | 6 | 7 | 6 | 7 |
| (1) Ontario | Heber Down | 43.941 | -78.988 | 08/06/10 | $6.845( \pm 0.034)$ | $6.380( \pm 0.034)$ | 20 | 20 | 20 | 10 | 10 |
| Ontario | Lucknow | 43.954 | -81.497 | 28/07/10 | $7.018( \pm 0.041)$ | $6.578( \pm 0.040)$ | 20 | 20 | 19 | 10 | 10 |
| Ontario | North Bay | 44.947 | -79.471 | 20/06/10-21/06/10 | $6.811( \pm 0.019)$ | $6.372( \pm 0.019)$ | 84 | 84 | 84 | 10 | 10 |
| Ontario | Peterborough | 44.315 | -78.343 | 15/06/10 | $6.792( \pm 0.048)$ | $6.352( \pm 0.052)$ | 20 | 20 | 20 | 10 | 10 |
| Ontario | Ridgetown | 42.439 | -81.831 | 11/07/10 | $6.707( \pm 0.048)$ | $6.280( \pm 0.039)$ | 18 | 18 | 18 | 10 | 10 |
| Ontario | Sault Ste Marie | 46.582 | -84.300 | 24/06/10-26/06/10 | $6.651( \pm 0.025)$ | $6.231( \pm 0.023)$ | 60 | 60 | 59 | 10 | 10 |
| Ontario | Serena Gundy Park | 43.716 | -79.353 | 15/07/10 | $6.772( \pm 0.042)$ | $6.378( \pm 0.040)$ | 25 | 25 | 25 | 10 | 10 |
| Quebec | Dunany | 45.758 | -74.304 | 25/06/10 | $6.925( \pm 0.036)$ | $6.457( \pm 0.040)$ | 15 | 14 | 15 | 10 | 10 |
| Quebec | Shawinigan | 46.514 | -72.679 | 27/06/10 | $6.857( \pm 0.032)$ | $6.491( \pm 0.059)$ | 33 | 26 | 25 | 10 | 10 |
| Arkansas | Smithville | 36.235 | -91.470 | 22/05/10-07/08/10 | 6.382 ( $\pm 0.027)$ | 6.014 ( $\pm 0.028)$ | 35 | 35 | 33 | 10 | 10 |
| Florida | 8 Mile Creek | 30.483 | -87.326 | 26/06 | $6.653( \pm 0.045)$ | $6.278( \pm 0.039)$ | 20 | 19 | 19 | 10 | 10 |
| Georgia | Conyers Monastery | 33.584 | -84.073 | 04/08 | $6.755( \pm 0.049)$ | $6.331( \pm 0.045)$ | 11 | 11 | 11 | 10 | 10 |
| Georgia | Rome | 34.443 | -85.150 | 18/06/10-27/06/10 | $6.651( \pm 0.041)$ | $6.221( \pm 0.036)$ | 20 | 19 | 15 | 10 | 10 |
| Illinois | Rockford | 42.211 | -88.976 | 17/07/10 | $6.332( \pm 0.040)$ | $5.956( \pm 0.040)$ | 20 | 20 | 20 | 10 | 10 |
| Iowa | Gateway Hills Park | 42.008 | -93.647 | 24/06/10 | $6.298( \pm 0.037)$ | $5.879( \pm 0.035)$ | 20 | 20 | 20 | 10 | 10 |
| Iowa | Odebolt | 42.274 | -95.129 | 15/07/10 | $6.391( \pm 0.025)$ | $6.040( \pm 0.024)$ | 73 | 73 | 73 | 10 | 10 |
| Kentucky | Fossil Creek | 37.773 | -84.561 | 07/06/10 | $6.757( \pm 0.046)$ | $6.265( \pm 0.036)$ | 25 | 25 | 25 | 10 | 10 |
| Maryland | Folly Quarter Creek | 39.255 | -76.927 | 13/07/10 | $6.603( \pm 0.029)$ | $6.247( \pm 0.031)$ | 33 | 32 | 32 | 10 | 10 |
| Michigan | Johnson Creek | 42.399 | -83.528 | 19/06/10-26/06/10 | $6.826( \pm 0.041)$ | $6.405( \pm 0.038)$ | 24 | 23 | 21 | 10 | 10 |
| Mississippi | Starkville | 33.567 | -89.041 | 05/07/10 | $6.580( \pm 0.035)$ | 6.190 ( $\pm 0.031$ ) | 26 | 26 | 24 | 10 | 10 |
| Missouri | Eleven Point River | 36.793 | -91.331 | 05/06/10 | $6.279( \pm 0.047)$ | $5.885( \pm 0.042)$ | 12 | 12 | 12 | 10 | 10 |
| Missouri | White River | 36.654 | -92.230 | 05/06/10 | $6.273( \pm 0.028)$ | $5.903( \pm 0.028)$ | 25 | 24 | 21 | 10 | 10 |
| Nebraska | Chappell | 41.083 | -102.467 | 30/06/10 | $6.408( \pm 0.065)$ | $6.070( \pm 0.061)$ | 6 | 6 | 6 | 6 | 6 |


| Nebraska | Kimball | 41.232 | -103.843 | $01 / 07 / 10$ | $6.401( \pm 0.030)$ | $6.038( \pm 0.030)$ | 32 | 32 | 32 | 10 | 10 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| Nebraska | Leigh | 41.701 | -97.247 | $21 / 06 / 10$ | $6.359( \pm 0.034)$ | $5.963( \pm 0.034)$ | 25 | 23 | 22 | 10 | 10 |
| Ohio | Mt Vernon | 40.405 | -82.487 | $16 / 06 / 10$ | $6.748( \pm 0.023)$ | $6.300( \pm 0.025)$ | 40 | 39 | 39 | 10 | 10 |
| South Carolina | Four Holes Swamp | 33.212 | -80.348 | $14 / 07 / 10$ | $6.782( \pm 0.059)$ | $6.445( \pm 0.046)$ | 21 | 21 | 21 | 10 | 10 |
| South Carolina | Little Creek | 34.842 | -82.402 | $15 / 07 / 10$ | $6.777( \pm 0.040)$ | $6.529( \pm 0.050)$ | 29 | 28 | 28 | 10 | 10 |
| Texas | Powderly | 33.753 | -95.605 | $13 / 05 / 10$ | $6.287( \pm 0.033)$ | $5.929( \pm 0.030)$ | 22 | 19 | 18 | 10 | 10 |
| Vermont | Lamoille River | 44.681 | -73.068 | $18 / 06 / 10$ | $6.873( \pm 0.123)$ | $6.473( \pm 0.112)$ | 4 | 4 | 4 | 4 | 4 |
| Vermont | West Haven | 43.624 | -73.362 | $24 / 07 / 10$ | $6.688( \pm 0.037)$ | $6.277( \pm 0.035)$ | 17 | 11 | 10 | 10 | 10 |
| Vermont | Winooski River | 46.352 | -72.571 | $04 / 07 / 10-18 / 07 / 10$ | $6.895( \pm 0.034)$ | $6.477( \pm 0.028)$ | 42 | 42 | 41 | 10 | 10 |
| 368 |  |  |  |  |  |  |  |  |  |  |  |

FIGURE LEGENDS


Figure 1 -
Geographic distribution of Calopteryx maculata (light area) and 34 sampling locations (symbol size is proportional to aspect ratio, see legend in lower left).


Figure 2 - Locations of 14 landmarks on the wing of Calopteryx maculata.


Figure 3 - Deformation plots showing the effect of increasing the value of each principal component on the relative locations of wing landmarks. Arrows indicate the direction and extent of change. Percentages are the percentage of variation explained by each principal component for fore and hind wings, respectively.


Figure 4 - Relationship between aspect ratio and the first principal component describing variation in wing shape for fore (closed symbols, solid line) and hind wings (open symbols, dotted line). Points are mean values from each of 34 sampling sites for both variables.



Figure 5 - Relationships between (A) aspect ratio and longitude for fore (closed symbols, solid line) and hind wings (open symbols, dotted line), and (B) aspect ratio and seasonality of precipitation (only fore wing data are shown). Points are mean values from each of 34 sampling sites for both variables.

## 1

## Map of sampling sites

Figure 1 - Geographic distribution of Calopteryx maculata (light area) and 34 sampling locations (symbol size is proportional to aspect ratio, see legend in lower left).


## 2

## Geometric morphometric landmarks

Figure 2 - Locations of 14 geometric morphometric landmarks on the wing of Calopteryx maculata.


## 3

Calopteryx maculata wing deformation plots

Figure 3 - Deformation plots showing the effect of increasing the value of each principal component on the relative locations of wing landmarks. Arrows indicate the direction and extent of change. Percentages are the percentage of variation explained by each principal component for fore and hind wings, respectively.


## 4

Relationship between aspect ratio and wing shape principal component
Figure 4 - Relationship between aspect ratio and the first principal component describing variation in wing shape for fore (closed symbols, solid line) and hind wings (open symbols, dotted line). Points are mean values from each of 34 sampling sites for both variables.


## 5

Relationship between longitude, seasonality of precipitation and aspect ratio in Calopteryx maculata.

Figure 5 - Relationships between (A) aspect ratio and longitude for fore (closed symbols, solid line) and hind wings (open symbols, dotted line), and (B) aspect ratio and seasonality of precipitation (only fore wing data are shown). Points are mean values from each of 34 sampling sites for both variables.


