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

Christopher Hassall

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 ±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

2 **Strong longitudinal variation in wing aspect ratio of a damselfly, *Calopteryx***

3 ***maculata* (Odonata: Zygoptera)**

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10 **ABSTRACT**

11 Geographical patterns in body size have been described across a wide range of species, leading to the

12 development of a series of fundamental biological rules. However, shape variables are less well-described

13 despite having substantial consequences for organism performance. Wing aspect ratio (AR) has been proposed

14 as a key shape parameter that determines function in flying animals, with high AR corresponding to longer,

15 thinner wings that promote high manoeuvrability, low speed flight, and low AR corresponding to shorter,

16 broader wings that promote high efficiency long distance flight. From this principle it might be predicted that

17 populations living in cooler areas would exhibit low AR wings to compensate for reduced muscle efficiency at

18 lower temperatures. I test this hypothesis using the riverine damselfly, *Calopteryx maculata*, sampled from 34

19 sites across its range margin in North America. Nine hundred and seven male specimens were captured from

20 across the 34 sites (mean=26.7 ±2.9 SE per site), dissected and measured to quantify the area and length of all

21 four wings. Geometric morphometrics were employed to investigate geographical variation in wing shape. The

22 majority of variation in wing shape involved changes in wing aspect ratio, confirmed independently by

23 geometric morphometrics and wing measurements. There was a weak positive relationship between wing aspect

24 ratio and temperature, in line with work on other insects. However, there was a much stronger longitudinal

25 pattern in which western populations exhibited lower wing aspect ratio. This longitudinal pattern may be related

26 to increasing variability in precipitation from east to west in North America. I discuss my findings in light of

27 research of the functional ecology of wing shape across vertebrate and invertebrate taxa.

28

29 **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

56 range margins (Hassall et al. 2009), and those marginal populations exhibit wing shapes that deviate

57 progressively away from the species average closer to the range margin (Hassall et al. 2008). Studies using

58 common garden rearing of *Drosophila* from a range of latitudes have shown that individuals reared at lower

59 temperatures have lower aspect ratio (Azevedo et al. 1998). While there is no clear relationship between aspect

60 ratio and flight speed in butterflies (Berwaerts et al. 2008; but cf Berwaerts et al. 2002; Dudley 1990), species in

61 which males "patrol" (i.e. exhibit prolonged flight) tend to have lower aspect ratios (Wickman 1992).

62 Chironomid females have broader wings (characteristic of lower aspect ratio) to assist with flying for long

63 periods between habitat patches (McLachlan 1986). While there are exceptions (increased fragmentation does

64 not correlate with aspect ratio in *Plebejus argus* (Thomas et al. 1998) or *Pararge aegeria* (Merckx & Van Dyck

65 2006)) these findings seem to suggest that lower wing aspect ratio in insects is associated with greater dispersal.

66

67 Wing morphology in Odonata may be affected by a combination of sexual selection during intrasexual,

68 agonistic interactions, intersexual courtship displays and dispersal (Johansson et al. 2009). In the field,

69 intrasexual territorial contests in *C. maculata* are determined by fat reserves (Marden & Rollins 1994; Marden

70 & Waage 1990) and contests in *Plathemis lydia* are determined by flight muscle ratio (Marden 1989). In both

71 cases, aspect ratio was shown not to influence the outcome of the contests. Sexual selection on courtship

72 displays focuses on patterns of pigmentation in *Calopteryx* species (Siva-Jothy 1999; Waage 1973). However,

73 wing shape has been shown to vary with landscape structure in *C. maculata* (Taylor & Merriam 1995) and

74 between some closely-related species of Calopterygidae in Europe (Sadeghi et al. 2009), although not all

75 species exhibited distinct wing shapes. Based on these results, it seems that wing shape variation is under

76 natural selection due to dispersal (within or between sites), rather than sexual selection.

77

78 Based on the reasoning presented above, I evaluate the hypothesis that, based on negative correlations between

79 latitude and aspect ratio, a positive relationship should be found between temperature and aspect ratio to

80 compensate for lower flight efficiency at lower temperatures. Uncertainties over the ecological role of

81 morphology variation may stem from the partial sampling of geographical ranges (Hassall 2013). Limited

82   sampling of non-linear trends that occur over large spatial scales may produce misleading results and so I

83   provide an analysis of wing shape variation across the entire range of the damselfly *Calopteryx maculata* in

84   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 ±2.9 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 t^2 tests in PAST to assess whether the two datasets could be combined.

110   Mean annual temperature was extracted for each site from the WORLDCLIM dataset (Haylock et al. 2008) to

111   test the central hypothesis of the study. After initial data examination revealed a longitudinal pattern, the

112   coefficient of variation (σ/μ) of precipitation, which exhibits strong variability from west to east, was calculated

113   for each site using the WORLDCLIM dataset (bioclimatic variable 15, Haylock et al. 2008) in ArcGIS v9.2

114   (ESRI 2006). Aspect ratio and the informative principal components from the shape analysis were regressed

115   against temperature and seasonality of precipitation using linear regressions weighted by the square-root of the

116   sample size. In each case, the models were tested with a quadratic predictor term using Akaike's information

117   criterion (AIC) to evaluate any improvement in model fit.

RESULTS

Fore and hind wings vary significantly in shape (t^2=122500, p<<0.001) 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, r=0.875, p<0.001; hind wings, r=0.854, p<0.001, Figure 4). The PC2 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 (r=0.816, p<0.001) and hind wings (r=0.800, p<0.001) indicating a decline in aspect ratio further west. Latitudinal patterns were significant and positive but weaker than for longitude (fore: r=0.441, p=0.010; hind: r=0.375, 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 (ΔAIC<2).

Regressions between aspect ratio and temperature showed no significant increase in explanatory power for the quadratic model in the fore (linear AIC=-50.188; quadratic AIC=-50.947; ΔAIC=0.76) or hind wings (linear AIC=-53.582; quadratic AIC=-53.509; ΔAIC=0.073) and so the quadratic term was not added. Aspect ratio was positive and significantly related to temperature for fore wings ($F_{1,32}$=6.262, p=0.018, R^2=0.138) but there was no significant relationship with aspect ratio of hind wings ($F_{1,32}$=3.302, p=0.079, R^2=0.065). Regressions

144   between aspect ratio and the seasonality of precipitation also showed no increase in explanatory power for the

145   quadratic model in fore (linear AIC=-63.124; quadratic AIC=-64.723; ΔAIC=1.599) or hind wings (linear AIC=-

146   67.687; hind: AIC=-68.840; ΔAIC=1.154) and so the quadratic term was not added. Linear regressions between

147   aspect ratio and the seasonality of precipitation were highly significant in both fore ($F_{1,32}$=25.687, p<0.001,

148   R^2=0.428) and hind wings ($F_{1,32}$=23.792, p<0.001, R^2=0.409, Figure 5B) and explained a high proportion of the

149   variation in the data. Taken together, models explaining aspect ratio with seasonality of precipitation had a

150   substantially greater explanatory power than those using temperature in both fore (ΔAIC=13.776) and hind

151   wings (ΔAIC=15.331). However, the geographical distribution of aspect ratio values (Figure 1) suggests that

152   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).

179 However, as I argue in the introduction, the opposite appears to be true for the majority of studies of insects

180 where smaller Reynolds numbers operate. Indeed, this study provides support for a positive association between

181 aspect ratio and temperature, which may be selected for because lower aspect ratio is more efficient at lower

182 temperatures (Vandewoestijne & Van Dyck 2011). There remains a gap in the literature that needs to be filled

183 with flight laboratory experiments of the functional implications of aspect ratio variation in odonates and other

184 insects as have been carried out in some butterflies (Berwaerts et al. 2008; Berwaerts et al. 2002; Davis et al.

185 2012). In particular, a test of the hypothesis that lower aspect ratios enhance flight efficiency at lower

186 temperatures is warranted given the increasing evidence for the correlation between aspect ratio and

187 temperature.

188

189 The findings here are consistent with selection for dispersal in habitats with a greater variability in precipitation

190 which causes greater variability in streamflow. The range of potential oviposition sites is limited by fluctuations

191 in rainfall which may raise or lower flow-rates, moving them outside of the narrow range of values that *C.*

192 *maculata* prefer (Gibbons & Pain 1992), such that a habitat that is suitable in one year may not remain so in the

193 next. It is also conceivable that seasonality of precipitation increases the fragmentation of these suitable sites:

194 sites which are only marginally suitable become unsuitable during extreme precipitation events, while a subset

195 of sites which are resilient to flow rate variation remain suitable. Such resilience may be provided by

196 geomorphological characteristics of the river channel (Goldstein et al. 2007) or the presence of microhabitats

197 which are less susceptible to variations in flow rate (Brooks et al. 2005). This elimination of marginal larval

198 habitats effectively fragments the landscape resulting in spatial variability.

199

200 Habitat selection in Odonata is a hierarchical process, with selection steps occurring at the level of the

201 landscape, the habitat, and the oviposition site (Buchwald 1995; Wildermuth 1994). At least the first two of

202 these steps involves visual cues which include the presence of conspecifics (Corbet 1999), linear polarised light

203 reflected from water bodies (Wildermuth 1998) and structural features, including vegetation (Wildermuth 1992).

204 Calopterygid damselflies also exhibit very specific habitat requirements relating to the depth and flow rate of the

205 rivers which form the larval habitat (Abbott 2005). Male *Calopteryx splendens* and *Calopteryx xanthostoma* land

206 on the water to demonstrate the flow-rate of the stream in their territory, and successful courtship is associated

207 with increased flow-rate up to 0.15m.s^{-1} (Gibbons & Pain 1992). The benefits of this flow-rate to the resulting

208 offspring are higher embryonic development rates and lower accumulation of encrusting algae (Siva-Jothy et al.

209 1995). Changes in flow rates may result in either (i) variation in larval mortality rates, or (ii) variation in the

210 tendency for females to oviposit. It is well-established that variability in precipitation produces

211 disproportionately large variations in streamflow (Dettinger & Diaz 2000). Given the apparent sensitivity of

212 Calopterygidae to variations in flow rates (Gibbons & Pain 1992; Siva-Jothy et al. 1995), an assessment of

213 regional variations in population sizes with respect to extremes of precipitation (and, hence, streamflow) might

214 be warranted. Extremes of temperature and precipitation resulting from climate change are likely to manifest

215 long before the gradual increase in long-term mean climate (Karl & Trenberth 2003; Palmer & Raisanen 2002)

216 and may pose a particular threat to this family.

217

218 Previous studies have questioned the use of aspect ratio as a single numerical metric describing wing shape in

219 insects, due to its inability to represent the complexity of wing morphology (Betts & Wootton 1988; Johansson

220 et al. 2009). However, I find that a complex method of shape analysis using geometric morphometrics yields

221 patterns that strongly resemble variation in the simpler concept of aspect ratio. However, it is clear from the

222 explanatory power of those principal components that correlate with aspect ratio (38.7% and 44.9%) that there is

223 a great deal of variability in addition to this dimension. It is worth noting that insects exhibit a great deal of

224 variation in aspect ratio. Odonates have high aspect ratios compared to some other insects, for example

225 *Drosophila virilis* with an aspect ratio of 2 (Vogel 1957), and *Bombus terrestris* with an aspect ratio of 6.4.

226 However, butterflies show higher aspect ratios of 9.8-10.5 in *Pararge aegeria* (Berwaerts et al. 2008; Berwaerts

227 et al. 2002). The data presented here show aspect ratios of hind wings between 5.61 and 7.79 and of forewings

228 between 5.70 and 7.56. *Aeshna cyanea*, a large odonate, exhibits aspect ratio of 8.4 and 11.6 for hind and fore

229 wings, respectively (Ellington 1984). What makes the odonate wing very different is the extent of the venation

230 in odonate wings compared to other taxa. This venation may be associated with the pleating of the wing, which

231 enhances aerodynamic performance relative to a smooth with of the same shape (Vargas et al. 2008).

232

233 The results presented here demonstrate clear geographical variation in flight morphology in a damselfly across

234 its entire range. While the other studies investigating geographical variation in odonate morphology have

235 focused on north-south transects (Johansson 2003), there are clearly important patterns occurring along the east-

236 west axis of the range highlighting the need to consider range-wide surveys to understand macroecological and

237 macroevolutionary patterns (Hassall 2013, 2014). Furthermore, although habitat fragmentation is associated with

238 higher aspect ratios in *C. maculata* at local scales, lower aspect ratios may be associated with lower habitat

239 stability at continental scales. From the survey of studies that have included aspect ratio, it is clear that

240 laboratory studies are needed to clarify the relationship between form and function in odonate wing shape.

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TABLES

363 **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 (N_{fore} = sample size for fore wings, N_{hind} = sample size

367 for hind wings).

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

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

368

FIGURE LEGENDS

370   Figure 1 –

371   Geographic distribution of *Calopteryx maculata* (light area) and 34 sampling locations (symbol size is

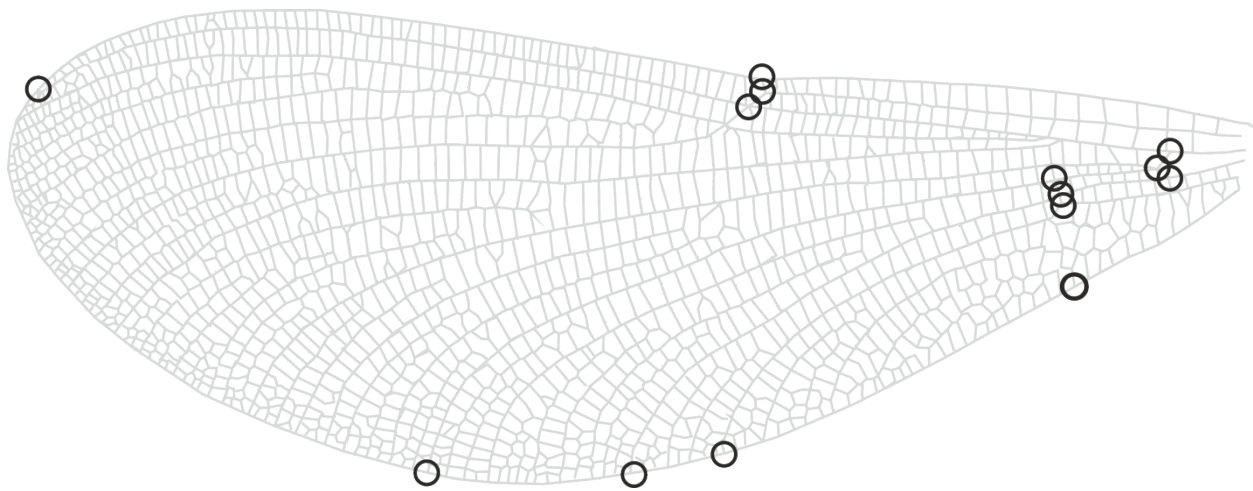372   proportional to aspect ratio, see legend in lower left).

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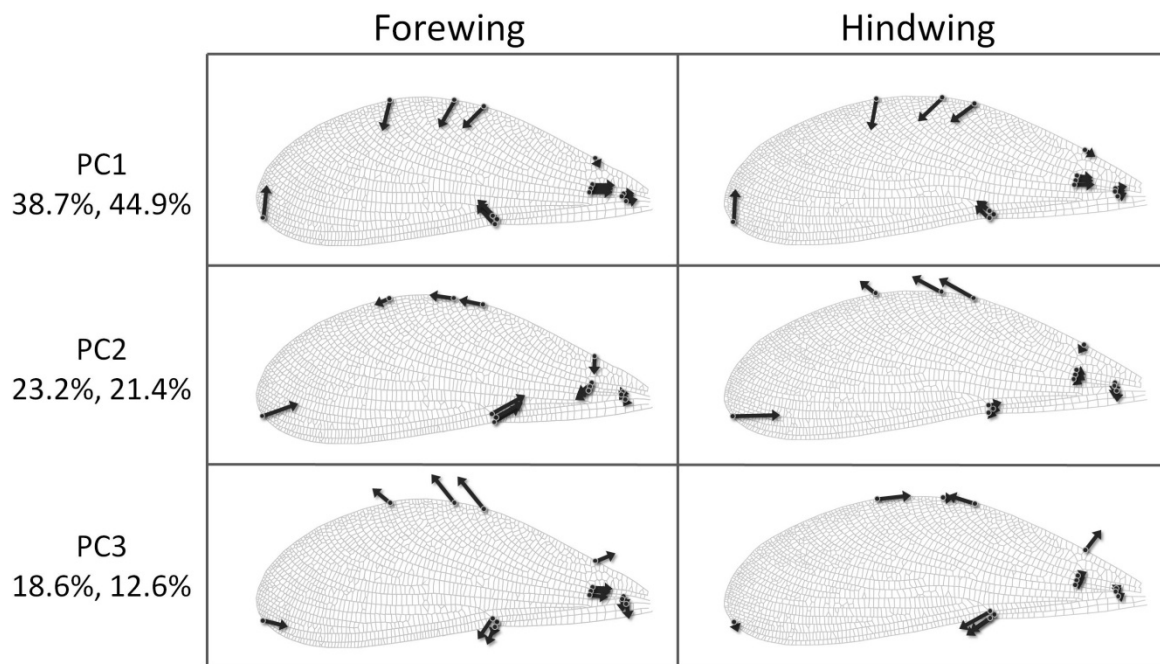375   Figure 2 – Locations of 14 landmarks on the wing of *Calopteryx maculata*.

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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.

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Figure 4 – Relationship between aspect ratio and the first principal component describing variation in wing

384 shape for fore (closed symbols, solid line) and hind wings (open symbols, dotted line). Points are mean values

385 from each of 34 sampling sites for both variables.

386

387

388 Figure 5 – Relationships between (A) aspect ratio and longitude for fore (closed symbols, solid line) and hind

389 wings (open symbols, dotted line), and (B) aspect ratio and seasonality of precipitation (only fore wing data are

390 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).
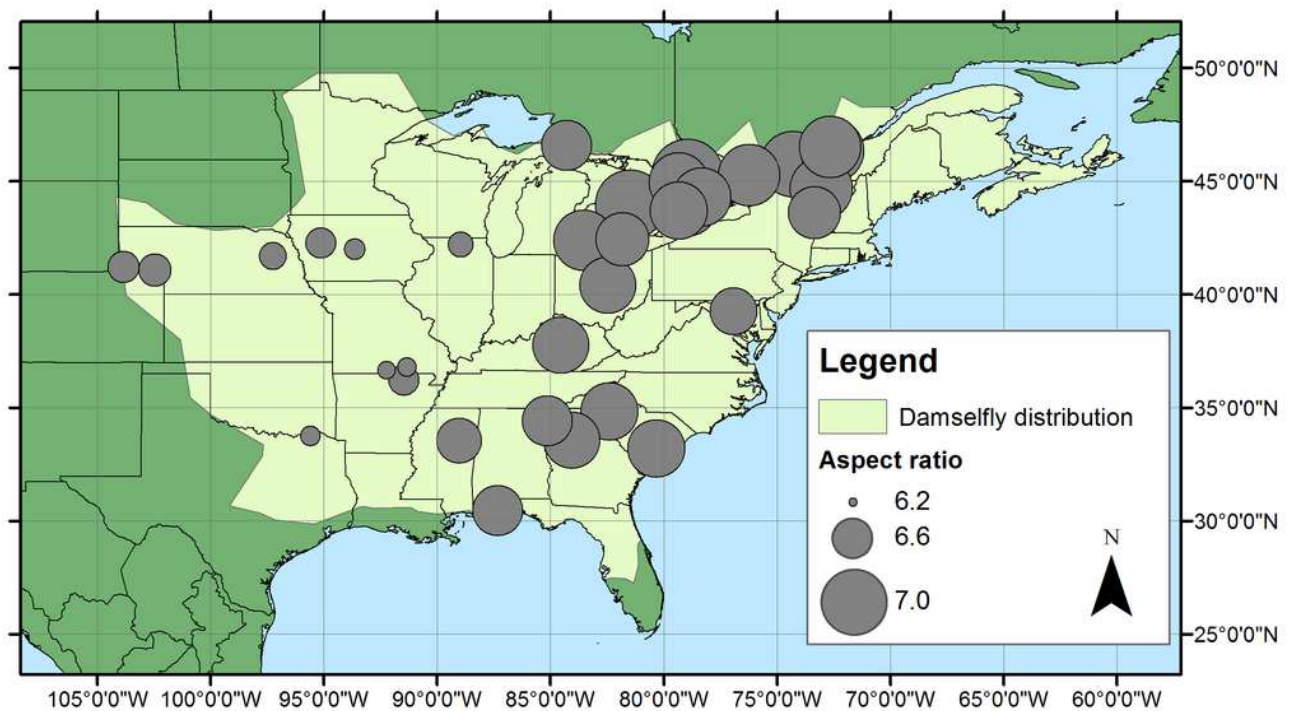
Geometric morphometric landmarks

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

3
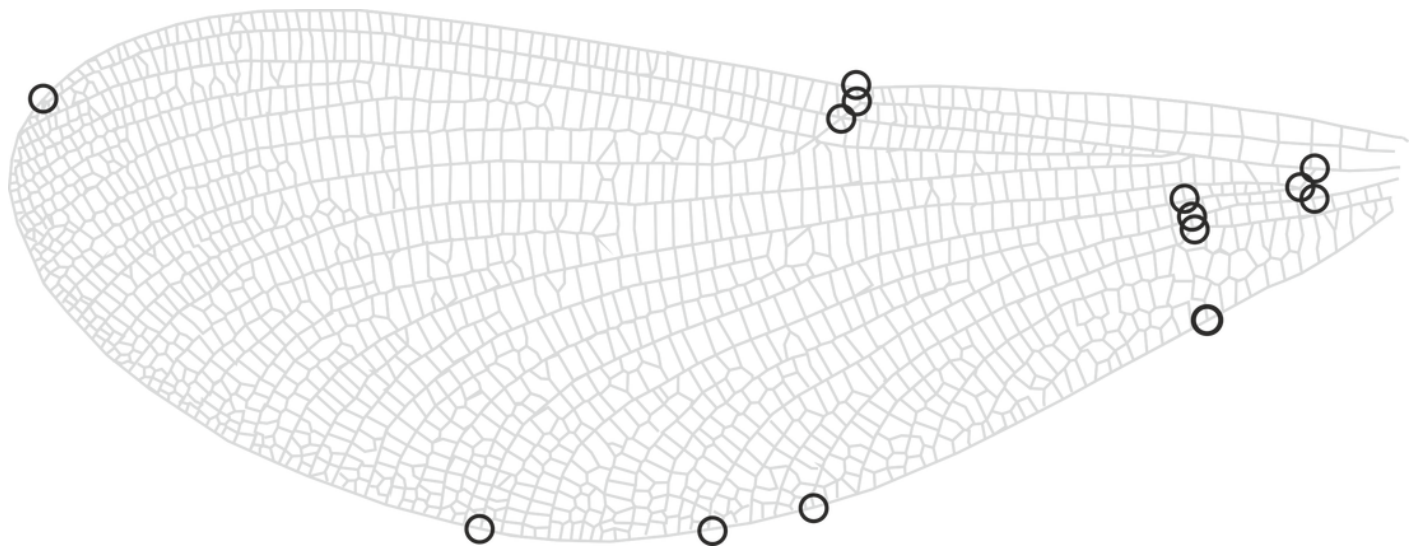
Calopteryx maculata wing deformation plots

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
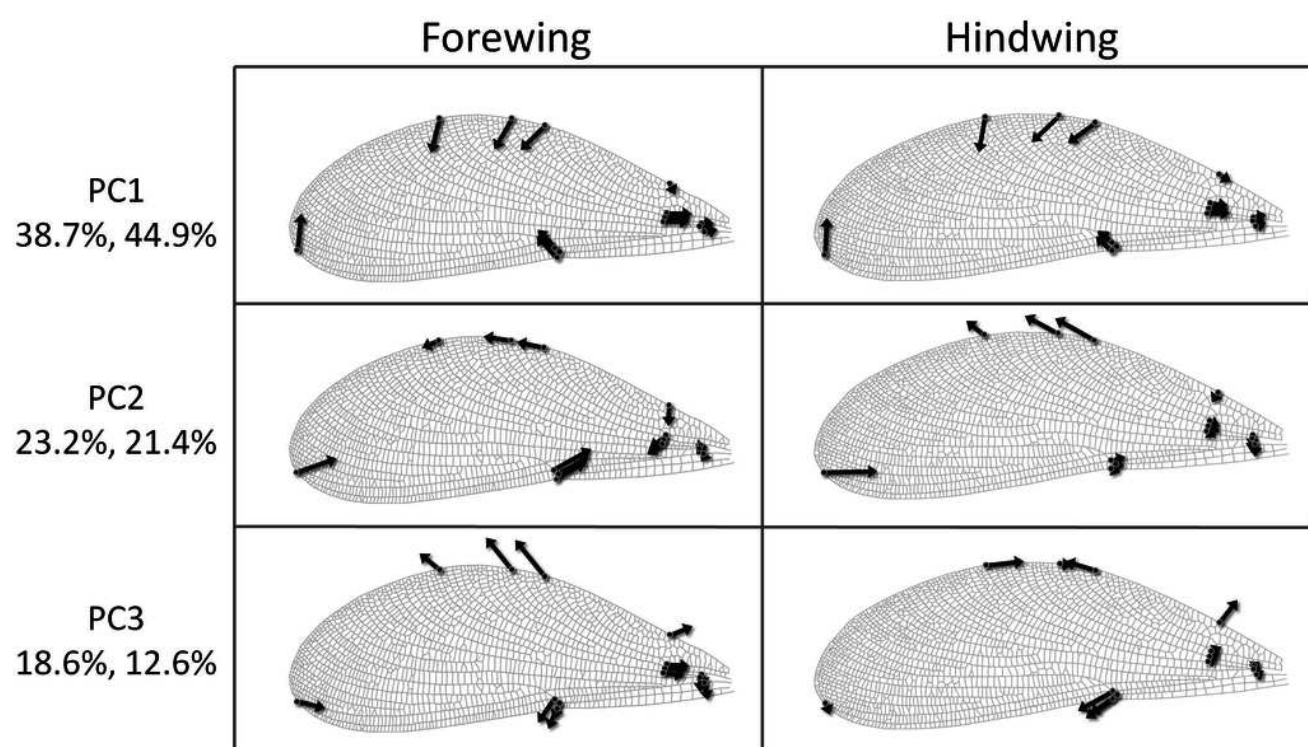
Relationship between aspect ratio and wing shape principal component

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.

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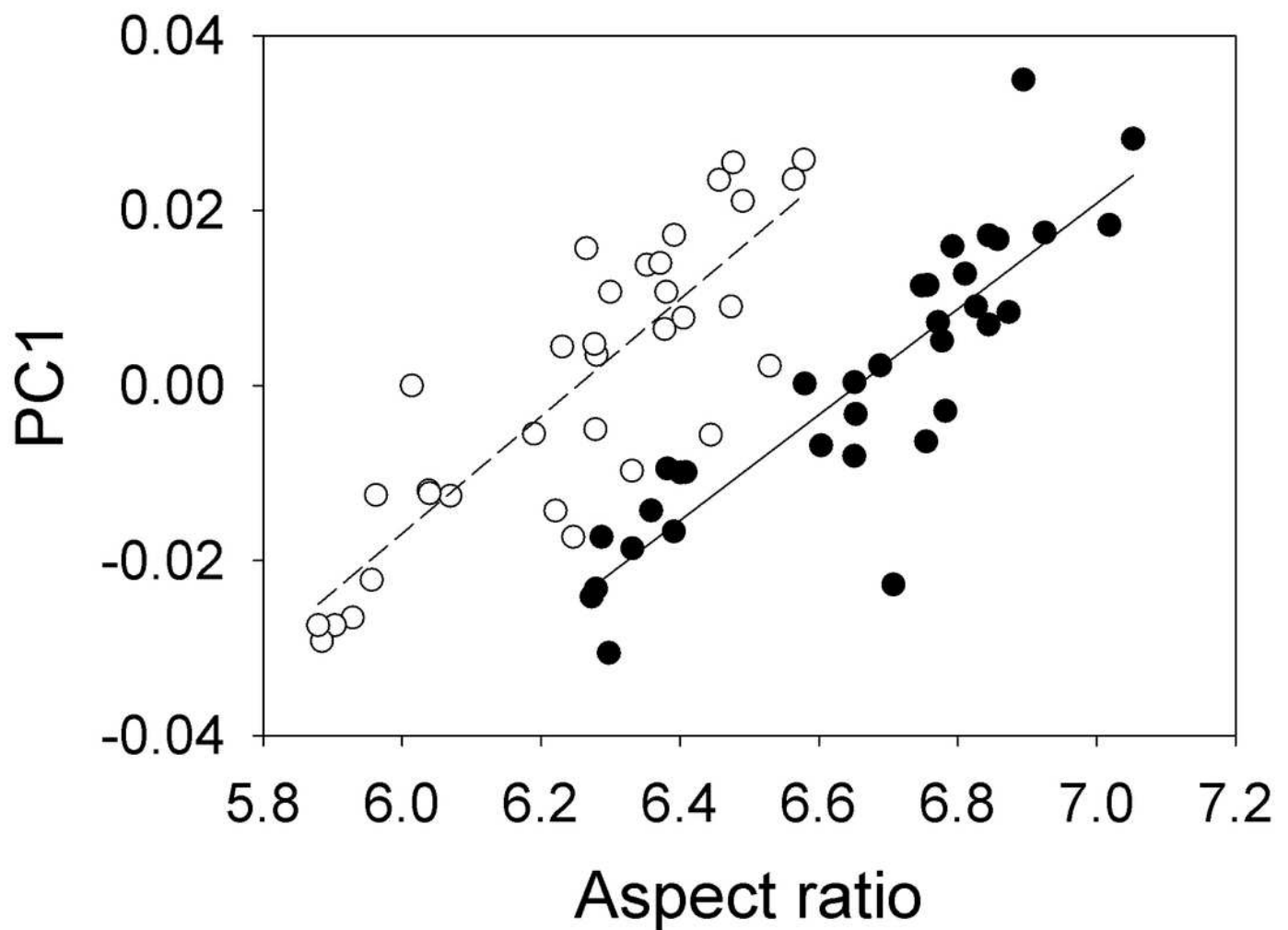
Relationship between longitude, seasonality of precipitation and aspect ratio in *Calopteryx maculata*.

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.