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**Wings of *Coenagrion puella* vary in shape at the northern range margin(Odonata: Coenagrionidae), Hassall et al (2008)
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Wings of *Coenagrion puella* vary in shape at the northern range margin(Odonata: Coenagrionidae)

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ABSTRACT

A previous study has shown that wing size in *Coenagrion puella* varied considerably along a latitudinal gradient in the UK. Using landmark data from wing images, patterns of shape variation were also determined along the same transect by geometric morphometric analysis of wing shape. Wing shape was uniform at all sites other than those closest to the range margin, which differed significantly. The potential mechanisms that might have generated such between-population variation are discussed.

INTRODUCTION

It has always been assumed that odonate wing venation varies relatively little between individuals of the same species and this assumption has formed the basis for studies of phylogeny within this taxon (Trueman, 1996). However, damselflies and dragonflies have been shown to vary in body size with latitude (for a review see Corbet, 1999 p.624) and a component of this is likely to be manifested in wing size.

During sampling of *Coenagrion puella* (Linnaeus) for a study on variation in body size with latitude, it was noted that there was substantial variation in wing length (C. Hassall unpubl. data). The aim of the present study was to ascertain which area of the wing was undergoing the size change.

Geometric morphometrics is a field which has been greatly facilitated by the increase in computing power over the past few decades (Richtsmeier et al., 2002). This technique makes use of x,y coordinates ("landmarks") to quantify shape and allows both a comparison of shape between objects and an investigation into which landmarks cause the variation. Geometric morphometrics has been used to distinguish between species and sexes of Hymenoptera (Pretorius, 2005) through the analysis of multivariate, landmark data.

MATERIALS AND METHODS

During the summer of 2007, at least 30 male adult *Coenagrion puella* were removed from each of a series of eight sites across Britain (Table 1) to investigate variation in morphometry. The sampling sites are shown in Fig. 1 in the context of the British (main) and European (inset) ranges. These individuals were scanned using a flatbed scanner and measured using the ImageJ analysis tool (Rasband, 1997-2007). To look at changes in shape of wings, the tpsDIG2 computer program (available from <http://life.bio.sunysb.edu/morph/>) was used to

digitise 14 out of roughly 220 nodes as landmarks on the right fore wing (or left when the right was missing or damaged) of all *C. puella* specimens that possessed at least one intact fore wing (Fig. 2, Table 2). Those specimens not possessing at least one intact forewing were excluded, resulting in reduced sample sizes. These landmarks were considered to represent the major dimensions of the wing.

Landmarks taken from wing scans were analysed using the PAST software package (available from <http://folk.uio.no/ohammer/past/>). First, landmarks were procrustes transformed to standardise the locations. This method of standardisation removes variation in scaling and rotation but preserves shape. A MANOVA test was then used to examine differences between sites in shape and a post-hoc pairwise comparison of sites was performed using Hotelling's T^2 test.

RESULTS

The variation in the location of transformed wing landmarks is plotted in Fig. 3. A qualitative analysis of this image suggested that there was relatively little pre-nodal variation in size or shape judging by the small clusters of points around landmarks 1-5. The greatest variation occurred in the central portion of the wing, with elongated clouds of points around landmarks 6-10. There was little variation in landmarks 11-14. From this cursory glance, it appeared that the majority of the size variation was generated by a general elongation of the post-nodal section of the wing.

This was supported by a comparison of the mean conformations of landmarks from the two sites exhibiting the greatest divergence in size (Coronation Pit as the smallest and Loch of Kinnordy as the largest; Fig. 4). Again, landmarks 1-5 and 11-14 exhibited little variation between the two sites. However, landmarks 6-8 were closer to the proximal portion of the wing in the population from Coronation Pit. This suggested that the variation in size may be due to an elongation of this region of the wing in the population from Loch of Kinnordy.

The sites were shown to vary significantly in shape using the MANOVA test in PAST (Wilk's $\lambda = 0.2195$, $F_{196,1292} = 1.644$, $p < 0.0001$). A comparison of the sites using Hotelling's T^2 test showed that specimens from site 8 (Loch of Kinnordy) were significantly different from specimens caught at all other sites (Table 2). After a Bonferroni correction for multiple tests, specimens from site 8 were still significantly different from those of the four most southern sites and specimens from site 7 were significantly different from those of sites 2 and 4. This difference in shape between sites could also be seen in the canonical variates analysis plot (Fig. 5) where sites 6, 7 and 8 became progressively more distinct from the group comprising the other five sites along the first CVA axis.

DISCUSSION

Although wing venation was shown to be highly conserved between populations in the southern sites, those individuals inhabiting sites closest to the northern range margin exhibited a progressive increase in differentiation from the shape characteristic of sites closer to the core of the range. The mechanism for the variation in shape is not clear. It is likely that wing size would be closely correlated with body mass, since wingloading (the mass of an organism per unit area of wing) is an important energetic constraint.

An explanation could be that the wing shape exhibited by marginal populations is that which carried the initial colonisers to those sites. A founder effect can be caused by highly dispersive individuals establishing new populations with subsequent progeny inheriting those dispersal characteristics. This pattern has been demonstrated for *Lepidoptera* (Hill et al., 2006, Hughes et al., 2003). However, the complexity inherent within odonate flight makes it difficult to assess whether or not the change in shape confers an advantage to dispersal. In

support of this hypothesis, however, *Coenagrion puella* had not been recorded at either Loch of Kinnordy or Tailend Moss prior to our visit (P. Batty pers. comm.). The short history of the species at these sites may have preserved the dispersal characteristics of the founders.

A final explanation may lie in the thermal stress under which the damselflies are developing. Environmental stressors have been linked to variation in wing shape in Lepidoptera (Hoffman et al., 2002) and fluctuating asymmetry in a wide range of species (Parsons, 1990) as well as Odonata (e.g. Chang et al., 2007). This low-temperature stress may be affecting the development of highly conserved genetic traits which may otherwise conform to the general species pattern. However, if this were the case, one would expect the variation to lack the direction that appears to be present in Fig. 5, so the developmental instability may be directional. This second hypothesis assumes that the wing shapes exhibited by the southern populations are “ideal” and that deviations from that shape caused by thermal stress would be detrimental. Such deviations may contribute to the inability of *C. puella* to exist further north than these sites. This runs counter to the previous hypothesis, which suggested that the northern wing shape has an advantage for individuals inhabiting those sites.

Wing venation has long been used as a character to trace odonate phylogenies. This study has shown that wing shape varies between populations, even when only a relatively small proportion of vein junctions are analysed. The results of this study represent the first to demonstrate the morphometric flexibility of this trait. Care should be taken to assess intra- and inter-population variation in shape where venation is used to discriminate between closely related species.

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Tables

Table 1. Locations of sites comprising a latitudinal gradient of the UK and sample sizes (n) of male *Coenagrion puella* with intact forewings collected from each site.

Site number	Name	Long	Lat	n
1	Queen Elizabeth Country Park	-0.978	50.961	29
2	Coronation Pit	-0.495	52.083	30
3	Felmersham Nature Reserve	-0.551	52.215	29
4	Vale Royal Locks	-2.541	53.23	25
5	Duddon Mosses	-3.199	54.258	28
6	Drumburgh Moss	-3.159	54.919	22
7	Tailend Moss	-3.591	55.893	30
8	Loch of Kinnordy	-3.047	56.676	30

Table 2. Pairwise comparison of wing shape in *Coenagrion puella* at eight sites along a latitudinal transect in the UK. Values are p-values arising from Hotelling's T^2 test. Values above the diagonal are raw p-values and those below the diagonal are corrected for multiple tests (Bonferroni's correction by multiplying p-values by the number of tests – 16). Significant p-values, indicating significant differences in the shape of wings between two sites, are shown in **bold**.

Site	1	2	3	4	5	6	7	8
1		0.643	0.632	0.519	0.384	0.154	0.004	0.002
2	17.998		0.821	0.085	0.148	0.215	0.001	0.000
3	17.687	22.981		0.354	0.422	0.236	0.012	0.001
4	14.542	2.370	9.901		0.181	0.166	0.001	0.000
5	10.745	4.140	11.812	5.056		0.237	0.012	0.012
6	4.303	6.013	6.600	4.651	6.641		0.193	0.021
7	0.101	0.019	0.332	0.024	0.337	5.412		0.047
8	0.044	0.000	0.020	0.000	0.330	0.589	1.303	

Figure legends

Figure 1: The distribution of *Coenagrion puella* in Britain (main – dark grey squares are 10km grid squares in which *C. puella* has been found) and Europe (inset – reproduced from Askew (2004), black represents distributional range). Sampling sites are marked with “x”.

Figure 2: The 14 landmarks digitised from the *Coenagrion puella* wing scans — 1: 1st junction of costa; 2: junction of analis and anal crossing; 3: nodal junction of costa; 4: sub-nodus; 5: nodus; 6: junction of cubitus and 5th post-nodal cross vein; 7: split of 2nd and 3rd branch of radius; 8: junction of costa and 5th post-nodal cross vein; 9: termination of cubitus; 10: beginning of 2nd intercalary radial vein; 11: termination of 3rd radial vein; 12: termination of 2nd radial vein; 13: distal, exterior corner of pterostigma; 14: proximal, exterior corner of pterostigma.

Figure 3: Variation in the locations of 14 wing landmarks in *Coenagrion puella* across eight sites after standardisation by procrustes transformation.

Figure 4: Superimposition of mean relative warps of *Coenagrion puella* wing shape from specimens from Kinnordy (hollow circles on solid mesh) onto those from Coronation Pit (filled circles on dotted mesh).

Figure 5: Ordination plot from a canonical variates analysis showing the variation in wing shape in *Coenagrion puella* at eight sites along a latitudinal transect. Polygons are convex hulls (encompassing all data points for each site) and ellipses are 95% confidence ellipses. The 5 southern sites are shown as solid lines (————), site 6 is shown as long dashes (———), site 7 as short dashes (-----) and site 8 as dotted lines (······). The two axes explained 42.99% (axis 1) and 18.36% (axis 2) of the variation in the data.



