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Analysis of symmetry of the fore- and hindwings of male dragonfly *Orthetrumsabina*

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Random deviations from perfect symmetry in bilateral traits results from developmental perturbations is often used as an indicator of developmental instability and fitness of an organisms. The wings of dragonflies are good examples of determining symmetry as these are found in opposite sides of the body. We analyzed a population of the dragonfly Orthetrumsabina for symmetry in wing venations using landmark-based methods. Anatomical landmarks were assigned to both left and right fore- and hindwings. Data were appended and subjected to Procustes superimposition and Principal Component Analysis using Symmetry and Asymmetry in Geometric Data (SAGE). Procrustes ANOVAindicates Fluctuating Asymmetry in the fore and hind wings of O. Sabina. Principal component analysis show the differences observed were in the triangle, pterostigma, vein distances and shapes between left and right wings.

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INTRODUCTION

Dragonflies are known for impressive flying abilities. They do this by propelling themselves upwards and downwards, backwards and forwards, side to side and hover in mid-air using both the fore and hind wings in tandem or independently. This is possible because their two sets of distinctive wings composed of a network of tubular veins that are the main structural units acting like cantilever beams (Kruez *et al* 2001; Minke at al 1978; Wooton 1991; Zhong-xue *et al* 2009). The seemingly complicated system of veins are connected by main and cross veins both transversely and longitudinally, with main veins running approximately parallel to one another. The many cross veins running orthogonal to the tubular veins and a thin membrane aids in support of the wing against aerodynamic and environmental forces (Song *et al* 2007; Minke et 1978; Wooton et 2000; Zhing-xue 2009) thus allow them to fly further, higher and faster than most insects. The membranes also contain randomly oriented microtubules that form a composite structure that also aids in stiffening the wing [Ress 1975b] but the corrugated design absorb stress and allow deformations to occur (Minke at al 1978; Wooton 1991; Wooton *et al* 2000) believed to provide good aerodynamic performance (Rees 1975a, b; Okamoto *et al* 1996; Kesel 2000; Tamai *et al* 2007; Smith 1996; Mingallon and Ramaswamy, 2011). Many other studies on the wings were numerical analyses to better understand their structural functions (Smith 1996, Kesel *et al*. 1998, Herbert *et al*. 2000, Combes and Daniel 203a, b, c and Wootton *et al*. 2003), however, only a few have been done in understanding size and shape relationships and symmetry in both left and right fore and hind wings (Tabugo *et al* 2014). With advances in the fields of imaging, geometry, biology and statistics new tools known as geometric morphometrics (GM) have helped understand quantitatively the nature of variations in biological structures (Bookstein1993). We used the tools of GM in understanding the nature of symmetry in the fore and hind-wings of a population of *O. Sabina* (Villanueva, 2009), a tolerant species to a constantly changing environment. It is for this reason that we believed that the wings of this species may have small random deviations from the ideal bilateral symmetry (Palmer *et al*., 1993 and Valen, 1962). This is known as fluctuating asymmetry (FA) and has been hypothesized to increase in response to both genetic and environmental stress experienced by a population (Trokovic *et al*., 2012 and Bonn *et al*., 1962) this species of dragonfly included. It is hypothesized that phenotypic variation in a population increases when introduced to environmental stress which affects the development of an individual and that FA arises when an organism is unable to buffer against disruptive factors during development (Palmer, 1996) such as those that may originate from various exogenous and endogenous stresses such as pollutants, natural environmental features such as temperature, humidity, density, and shifts in

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resource use induced by global warming (Castrezana, 1997), habitat degradation (Sarre, 1996), predation and competition [Badyaev *et al*., 2000). The resultswill be developmental instability, a suite of processes that tend to disrupt the precise development of bilateral traits (Palmer, 1994) along a developmental trajectory in an environment (Nijhout, 2003). Increased FA is a reflection of poor developmental homeostasis (Carter *et al*., 2009). Lower values of FA may reflect development that proceeds more precisely (Parsons, 1990) whereas elevated FA values may be due to problems during development that reflect increased developmental instability (Hermita, 2013). It is therefore the main aim of this study to know if the wings of the species are perfectly bilaterally symmetrical or if there are any variations in symmetry. To be able to determine if developmental instability is occurring in both the fore- and hind- wings of *O. Sabina*, the geometric morphometric data were subjected to symmetry analysis using Symmetry and Asymmetry in Geometric Data (SAGE) (Marquezand Knowles 2007).This software uses landmark based methods which is then decomposes variance into symmetric and asymmetric components and latter is further decomposed to directional and fluctuating asymmetry.

Methodology:

The specimens were randomly collected from the field in Tigbao, Zamboangadel Sur by either handpicking or the use of a sweep net. Collected samples were placed in an envelope to avoid wing breakage. Digital images of the wings were taken and subjected to landmark geometric morphometric analysis.A total of 29 landmark points were assigned to theforewing and 35 for the hind wing (Fig. 1.). Landmark descriptions were based on an earlier publication (Tabugo, 2014) (Tables 1 and 2). Image were digitized using TpsDig2 software (Rohlf2004). Tps data file for the left and the right fore and hind wings were appended using TpsUtil (Rohlf2013) and were subjected to SAGE (Marquezand Knowles 2007). In SAGE, ProcrustesAnova which assessed relative amounts of asymmetry was performed and supported by Principal Component Analysis (PCA), a tool which displays variation within a sample and which characterizes the main features of shape variation.

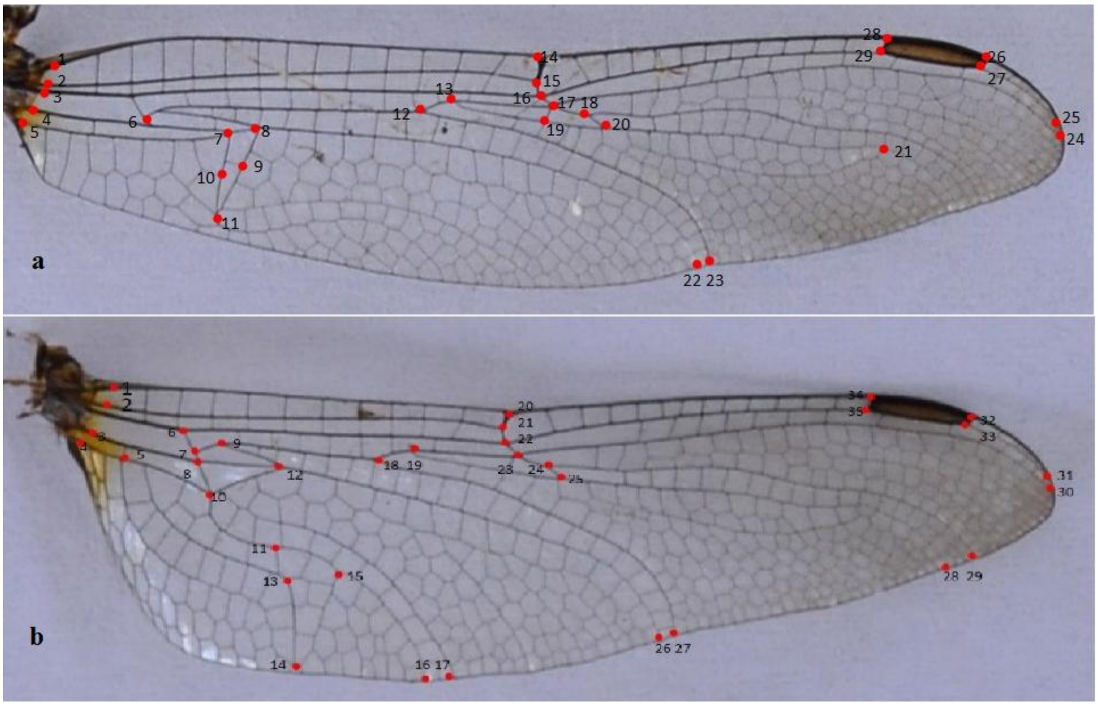

Fig. 1: Designated landmarks of the right (a) forewing and (b) hindwing of *O. sabina*.

Table 1: Description of assigned landmark in *O. sabina* left and right forewings (based on Tabugo *et al* 2014).

Landmark #	Descriptive location	Landmark #	Descriptive location
1	Proximal End of the Costa (C)	16	Distal End of the Radius (R)
2	Proximal End of the Subcosta (Sc)	17	Origin of the Radial Branches (R2 and R3)
3	Proximal End of the Radius + Media (R+M)	18	Anterior End of the 2nd Crossvein between Radial Branches (R2 and R3)
4	Proximal End of the Cubitus (Cu)	19	Posterior End of the 2nd Crossvein between Radial Branches (R2 and R3); Origin of Radial Supplement (Rspl)
5	Proximal End of the 1st Anal Vein (A/IA)	20	Proximal End of Radial Supplement (Rspl)
6	Basal End of the Arculus (Arc)	21	Distal End of Radial Supplement

7	Proximal End of the Anterior Margin of the Triangle (T)	22	Distal End of Anterior Media (MA)
8	Distal End of the Anterior Margin of the Triangle (T)	23	Distal End of Radial Branch (R4)
9	Midpoint of the Triangle (T)	24	Distal End of Intercalary Radial Vein (IR2)
10	Midpoint of the Triangle (T)	25	Distal End of Radial Branch (R2)
11	Posterior End of the Triangle (T)	26	Antero-lateral and Distal End of the Pterostigma
12	Origin of Radial Branches (R2 and R4)	27	Postero-lateral and Distal End of the Pterostigma
13	Origin of Intercalary Vein (IR3)	28	Antero-lateral and Proximal End of the Pterostigma
14	Nodus (N)	29	Postero-lateral and Proximal End of the Pterostigma
15	Distal End of the Subcosta (Sc)		

Table 2: Description of assigned landmark in *O. sabina*left and right hindwings.

Landmark #	Descriptive location	Landmark #	Descriptive location
1	Proximal End of the Costa (C)	19	Origin of the Intercalary Radial Vein (IR3)
2	Proximal End of the Subcosta (Sc)	20	Nodus (n)
3	Proximal End of the Media (m)	21	Distal End of the Subcosta (Sc)
4	Proximal End of the Cubitus (Cu)	22	Distal End of the Radius (R)
5	Posterior End of the Anal Crossing (Ac)	23	Origin of the Radial Branches (R2 and R3)
6	Basal End of the Arculus (Arc)	24	Distal End of Radial Supplement
7	Posterior and Proximal Vertex of the Hypertrigone (ht)	25	Posterior End of the 2nd Crossvein between Radial Branches (R2 and R3); Origin of Radial Supplement (Rspl)
8	Anterior and Proximal Vertex of the Subtrigone (t)	26	Distal End of the Anterior Media (AM)
9	Anterior and Proximal Vertex of the Hypertrigone (ht)	27	Distal End of the Radial Branch (R4)
10	Posterior and Proximal Vertex of the Subtrigone (t)	28	Distal End of the Intercalary Radial Vein (IR3)
11	(Cu2 + A2)	29	Distal End of the Radial Branch (R3)
12	Distal Vertex of the Subtrigone (t)	30	Distal End of Intercalary Radial Vein (IR2)
13	Anal Supplement (Aspl)	31	Distal End of Radial Branch (R2)
14	Basal end of the Anal Vein (A3)	32	Antero-lateral and Distal end of the Pterostigma
15	Second Branch of Cubital Vein (Cu2)	33	Postero- lateral and Distal end of the Pterostigma
16	e	34	Antero-lateral and Proximal end of the Pterostigma
17	Distal End of the Posterior Cubital Vein	35	Postero-lateral and Proximal End of the Pterostigma
18	Origin of the Radial Branch (R4)		

RESULTS AND DISCUSSION

Procrustesanova showed a significant level of asymmetryin individuals of both fore- and hindwings of *O. Sabina* (Table 3). The measurement error recorded a low mean square value in sides compared to data recorded in individual x side interactionshow the asymmetry in the wings is FA. To further support the first analysis, PCA analysis is performed (Table 4) to visualize shape variation in the wings (Figure 2)and also used to investigate patterns of covariation in the positions of landmarks (Dryden, 1998). In Fig 2a and 2b, there were observed differences in the shape in almost of the landmarks described between the left and the right fore- and hind wings of the dragonfly. While the triangle provides strong wing framework and adapts the wings for rapid sculling forward motion (Needham, 1899), a noticeable deformation of the triangle is seen in the wings. Most veins also deviates from its original shape and length indicating that the asymmetry observed may alter the frequency and the amplitude of wing-flapping (Balmford, 1993 and McLachlan, 1997).

Table 3: Procrustes ANOVA results of the fore- and hindwings of *O.sabina*.

Effect	SS	dF	MS	F	Remarks
Appended Forewings					
Individual (shape/symmetry variation)	0.13174	1566	8.4123e-005	1.4393	significant
Sides (Directional Asymmetry)	0.010773	54	0.00019949	3.4133	Significant
Individual x Sides (Fluctuating Asymmetry)	0.091527	1566	$5.8466x10^{-005}$	5.6378	Significant

Measurement error	0.069964	6480	$1.0797x10^{-005}$	--	
Appended Hindwings					
Individual (shape/symmetry variation)	0.11107	1914	5.8032e-005	1.2142	signficant
Sides (Directional Asymmetry)	0.0019196	66	$2.9084x10^{-005}$	0.60852	Significant
Individual x Sides (Fluctuating Asymmetry)	0.09148	1914	$4.7795x10^{-005}$	6.6582	Significant
Measurement error	0.056853	7920	$7.1785x10^{-005}$	--	

Table 4: First two principal component showing variance in *O. sabina* appended fore and hind wings.

	PC 1 (%)	PC 2 (%)	Overall (%)
Appended Forewings	58.73	14.63	73.36
Appended Hindwings	63.79	7.88	71.67

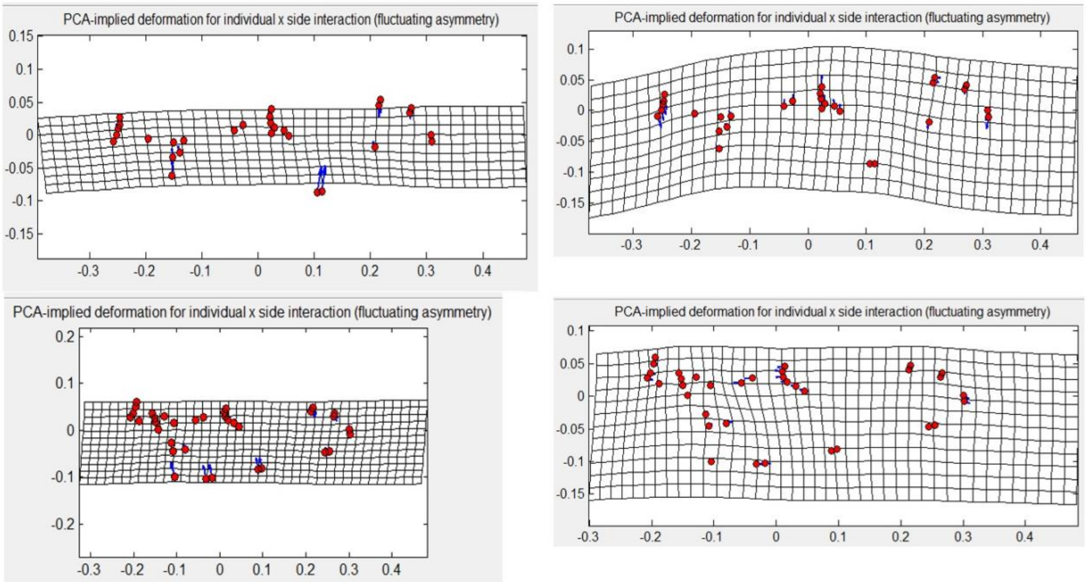

Fig. 2: PCA implied deformation of individual x side interaction (FA) in *O. sabina*appended fore- and hindwings.

There are many possible explanations to the results of this study that show FA is the type of asymmetry in the wings of male *O. Sabina*. This may indicate the species is affected by developmental noise and may likely experiencing developmental instability as reflected by high level of fluctuation in the data recorded. It is argued that increased FA is a reflection of poor developmental homeostasis at the molecular, chromosomal and epigenetic levels (Carter *et al*., 2009; Polak and Taylor, 2007; Hermita, 2013). The high FA values observed could also have resulted from the interplay of developmental "noise" and stabilizing processes associated by conditions of environment where the species is found (Graham *et al*. 1993; Badyaev (2000). The observed wing asymmetry in the male *O. Sabina* can be also be argued to influence their success in territorial defense and mating (Beáta, 2005; Moller, 1996) since*O. sabina* are territorial species (Klingenberg, 1992).

Conclusion:

Deviations from bilaterally symmetrical structures such as the fore- and hind wings of the dragonfly can be examined byProcustes superimposition and principal component analysis using Symmetry and Asymmetry in Geometric Data (SAGE). In this study, Procrustes ANOVA showed a significant high level of fluctuating asymmetry in the appended fore- and hind wings of *O. Sabina* and the differences in the shape in most of the trait was clearly described using PCA.

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