

## Geometric Perspectives of Sexual Dimorphism in the Wing Shape of Lepidoptera: the Case of *Synneuria* sp. (Lepidoptera: Geometridae)

Hugo A. BENÍTEZ<sup>1,2</sup> Luis E. PARRA<sup>2</sup> Einer SEPULVEDA<sup>2</sup> Maria J. SANZANA<sup>2</sup>

<sup>1</sup>Departamento de Zoología, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, Concepción, Casilla: 160-C - 4070386, CHILE e-mails: luparra@udec.cl; einersepulveda@udec.cl; mariajosanzana@udec.cl

<sup>2</sup> Instituto de Alta Investigación, Universidad de Tarapacá, Casilla 7-D Arica CHILE  
e-mail: hugobenitez@udec.cl (Corresponding author)

### ABSTRACT

We investigated the sexual dimorphism in wing shape in *Synneuria* sp., using the tools of geometric morphometrics. This species, which has taxonomic problems, has a limited geographic distribution. In the spring and summer of 2006-2007 we collected 63 males and 58 females in farms named "El Guindo (36°50'12"W - 73°01'25"S) and Coyanmahuida" (36°49'28.66"S - 72°44'1.34"W) in the province of Concepción, Biobío Region of Chile. We photographed the right wing of each individual and constructed a photographic matrix in which we digitalized 18 morphological landmarks based on the shape and vein pattern of the wing. A multivariate factorial analysis of wing shape showed significant differences between sexes ( $F = 4.237$ ;  $p = < 0.05$ ) but not among localities ( $F = 0.169$ ;  $p = > 0.05$ ), in which the sectorized landmarks indicated large contributions to wing shape in the matrix  $R^2$ : #7: 0.49945 and #8: 0.46818. The geometric variation demonstrated that the points in the bases of the radial veins were key characters to differentiate between sexes.

**Key words:** Geometric morphometric, wing shape, moths, phenotype, sexual shape dimorphism, interpopulation.

### INTRODUCTION

One of the most interesting sources of phenotypic variation in animals and plants has been sexual dimorphism, whose study continues to be an important area of study in evolutionary biology. Sexual differences in morphological characters are a common phenomenon in many animal taxa, whose most conspicuous aspect is body size (Gannon & Rácz, 2006). The direction of these differences are, whether, males or females are larger or differ in distinctive groups (Wainwright 1994; Koehl, 1996). Most of the morphological variations in moth and butterflies are due to the effects associated with the environment, whether phenotypic responses (plasticity) or particularly those which act during ontogenetic development (Mutanen & Kaitala, 2006; Meyer-Rochow & Lau 2008; Sihvonen, 2008). Females are generally larger than males, which gives them adaptive advantages such as greater fecundity and better parental care (Forrest, 1987; Andersson, 1994; Moller & Zamora-Muñoz, 1997;

Reeve & Farbain, 1999). However, in some species males are longer but have less relative mass (e.g. Cepeda-Pizarro *et al.* 1996), which implies that the determination of sexual dimorphism requires more complex measurement techniques related, for example, the shape (Benítez *et al.* 2010a). Sexual dimorphism is of interest in entomological studies since frequently the differences between sexes are not obvious or the individuals are very small; thus finding discriminating characters allows easy determination of the sexes. The main object of this study is to describe in a new, more simplified manner to determine the sexual dimorphism in moths based on morphological techniques which permit a better understanding of the shape.

## MATERIALS AND METHODS

### Geographical area

The study area was the farms named “El Guindo (36°50'12”W- 73°01'25”S) and Coyanmahuida” (36°49'28.66”S - 72°44'1.34”W) in the province of Concepción, Biobío Region of Chile, which are relict native forests with *Nothofagus obliqua* and *Peumus boldus*, among others separated 20 km from one another.

### Sampling

In order to determine intra- and interpopulation variation by means of geometric morphometrics we used imagoes of *Synneuria* sp. (Lepidoptera, Geometridae). We collected the moths with phototropic UV traps using an 800 watt electric generator; the light sources were placed over a white sheet to increase the luminosity. These traps were installed for a period of 4 hours in different sampling points in the two farms. Collected individuals were processed and mounted.

### Digitalization of images and morphological analysis

The geometrical analysis, which considered variations attributed exclusively to shape, was pershapeed using a photographic register of 63 males and 58 females of *Synneuria* sp., whose wings were each mounted in a fixed mould. The right wing of each was photographed with a Sony 10 DSC-H7 camera with directed fiber optics lighting, with which we constructed photographic matrixes using the TpsUtil 1.40 program (Rohlf, 2008b). We digitalized 18 morphological landmarks based upon the shape and vein pattern of the wing (Fig. 1) for all individuals. The selection of the morphological landmarks was based on the external anatomy (Table 1) using landmark type 1 (anatomic) and landmark type 3 (Pseudolandmarks) (#2 and #3) (Zelditch *et al.* 2004), and digitalized using the TpsDig 2.12 program (Rohlf, 2008a). The X-Y coordinates of the biologically homologous landmarks were aligned and superimposed with the method of minimum least squares based on the Generalized Procrustes Analysis, GPA (Rohlf & Slice, 1990), in order to remove variation not related to shape (i. e., rotation, translation and mathematical scale).

To obtain the shape variables of the aligned specimens we used the Tps Relw 1.45 program (Rohlf, 2007), which makes an interpolation that projects the data in a Euclidean plane. With this last program we pershapeed a relative warp analysis.

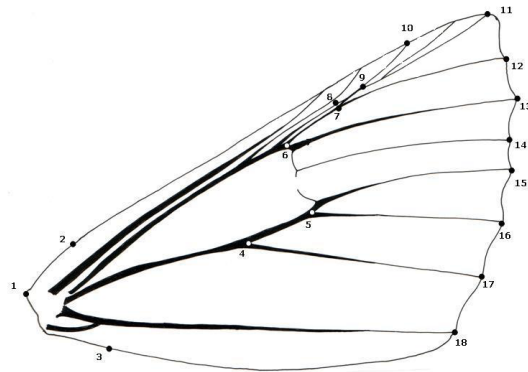


Fig. 1. Representation of 18 morphological landmarks of the right wing of *Synneuria* sp.

Table 1. Anatomical description of 18 external landmarks in the right wing of *Synneuria* sp.

Landmark	Anatomical structure	Landmark	Anatomical structure
#1	Humeral Apex	#10	Radial Vein Vertex 3
#2	Superior Vertex of the basal spot	#11	Radial Vein Vertex 4
#3	Inferior Vertex of the basal spot	#12	Radial Vein Vertex 5
#4	Cubital Trunk 2	#13	Medial Vein Vertex 1
#5	Cubital Trunk 1	#14	Medial Vein Vertex 2
#6	Radial Trunk	#15	Medial Vein Vertex 3
#7	Radial Base Point 5	#16	Cubital Vein Vertex 1
#8	Radial Base Point 4	#17	Caudal Vein Vertex 2
#9	Radial Point 3 + Radial 4	#18	Anal Vein Vertex

### Measurement error

To diminish the measurement errors and avoid bias in taking photographs and digitalizing landmarks, we selected a random sub-sample of 80 individuals, took new photographs and made new digitalizations of the morphological landmarks. The proportional measurement error was calculated with the following shapeula, using the results of an analysis of variance (Dryden & Mardia, 1998):

$$\%ME = S^2A / (S^2w + S^2A) \times 100$$

where  $S^2w = MS_{within}$

and  $S^2A = (MS_{among} - MS_{within}) / n$  ( $n^o$  repeated measures)

in which the components (between and within) are from the mean squares of a one-way ANOVA considering individuals as a source of variation.

### RESULTS

The multivariate factorial analysis of wing shape found significant differences between sexes (Fig. 2.) ( $F=4.237$ ;  $p < 0.05$ ) but not among localities ( $F=0.169$ ;  $p > 0.05$ ),

(Table 2) in which the digitalized landmarks indicated large contributions to wing shape in the matrix R` #7: 0.49945 and #8: 0.46818 (Table 3).

The morphological variation among moths was determined by fusing the matrixes for both sexes and populations in TpsUtil; in the Generalized Procrustes Analysis the first three relative warps explained 91.74% of the variation in shape (Rohlf & Slice, 1990). In order to visualize the variation in wing shape graphically we took the means of the first three relative warps. We found different morphotypes for the males and females of *Coyanmahuida* and *Guindo* (Fig. 3).

The proportional measurement error compared to the real data had relative contributions of 2.33% (Rw1) and 0.5% (Rw2). The percentage of error for both shapeations and all sites was less than 4%.

Table 2. Two-way ANOVA for the differences in form of *Synneuria* sp using the first relative warp as dependent variable.

Effect	SS	df	MS	F	p
Sex	<b>0.001526</b>	<b>1</b>	<b>0.001526</b>	<b>4.237204</b>	<b>0.018961</b>
Locality	0.000061	1	0.000061	0.169012	0.684121
Sex x Locality	0.000490	1	0.000490	1.360991	0.253206
Error	0.010086	28	0.000360		

Table 3. Relative contributions (RC) of each landmark (LM#) to *Synneuria* sp. wing shape.

LM#	RC	LM#	RC
1	0.00112	10	0.00031
2	0.00058	11	0.00167
3	0.00047	12	0.0065
4	0.00052	13	0.00629
5	0.00298	14	0.00503
6	0.0007	15	0.00316
7	<b>0.49945</b>	16	0.00174
8	<b>0.46818</b>	17	0.00084
9	0.0003	18	0.00015

## DISCUSSION

The variation of wing shape in *Synneuria* sp. was clearly shown by the techniques of geometric morphometrics. Although we found differences in shape between the sexes, these were not so notable between the two sampling areas.

A number of hypotheses have been proposed to explain sexual dimorphism in insects (Wilkund & Forsberg, 1991; Fairbairn, 1997; Walker & Rypstra, 2001; Esperk *et al.* 2007; Benítez *et al.* 2010b). The hypotheses indicating the connection between sexual selection *versus* natural selection and environmental variation are those most commonly used to determine sexual dimorphism (Anderson, 1994; Moller & Zamora-Muñoz, 1997).

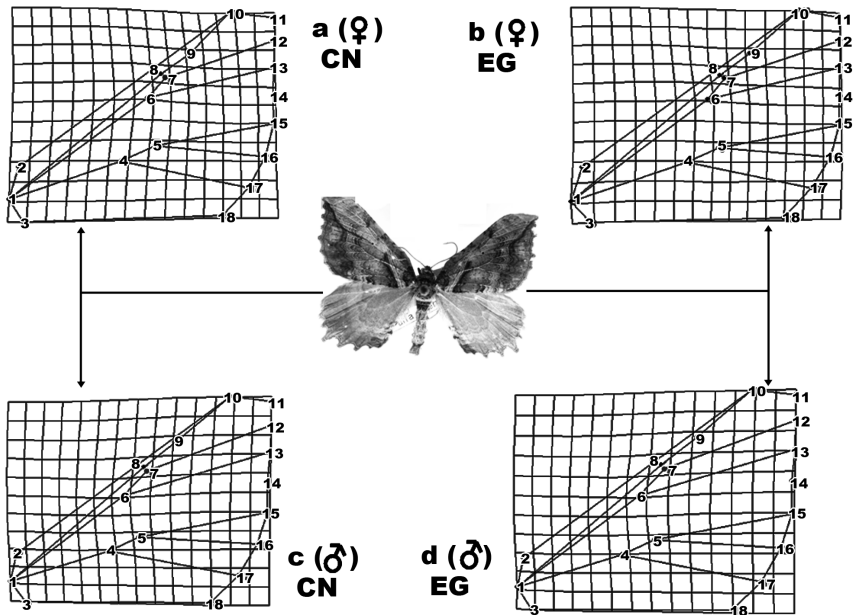


Fig. 2. Morphological deshapeation grids which show the distributions of shape for males and females of *Synneuria* sp. in the different localities, CN: Coyamahuida and EG: El Guindo.

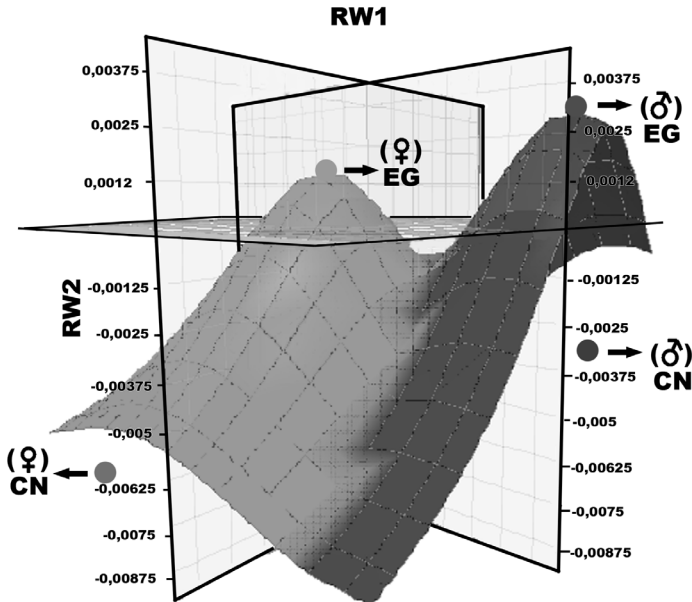


Fig. 3. 3-D dispersion graph of the shape variables by sex and locality in moths of the genus *Synneuria*. The points in the dark area indicate populations of males from El Guindo and Coyamahuida, respectively, and in the clear area females from El Guindo and Coyamahuida. \*\* Each point within the volumetric sector indicates a variable with distinct shape.

Our results indicate that the wings in *Synneuria* sp. have sexual dimorphism in shape (Fig. 2.). The evaluation and sectorization of wing structure using landmarks was the key to determining that it is submitted to a high degree of pressure, both environmental and life history. The differences among the individual configurations of each sex were captured using mathematical functions which vary depending upon the position of each landmark in the wing image. These differences were located in landmarks 5, 6, 7 and 8, respectively. The geometric variation detected showed that the landmarks located in the base of the radial veins were key characters to distinguish different wing morphotypes among populations and sexes. The crucial attributes for the group would benefit the dispersion, migration and sexual selection; in males for the nuptial flight, territoriality and sexual selection, and in females primarily as a characteristic flight behavior in the search for host plants (e.g. Ellington, 1984; Benson *et al.* 1989; Srygley, 1994; Dudley, 2000; Breuker *et al.* 2007; Dockx, 2007; Johansson *et al.* 2009). Therefore, selection would act on wing shape to optimize flight characteristics (DeVries *et al.* 2010).

For a number of authors, the variation in wing shape does not contribute sufficient evidence to conclude this is only a product of sexual dimorphism. It is frequently argued that individual variation in shape may be strongly dependent on environmental conditions (Adams & Funk, 1997; Tatsuta *et al.* 2001). However, our study showed significant different in wing shape between sexes, both with and between localities; thus we conclude that the differences found here are analytical for the species and areas studied.

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