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Chapter 3

Sexual Dimorphism Using Geometric Morphometric Approach

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1. Introduction

Comparison of anatomical characters between organisms has been a core element in comparative biology for centuries. Historically, taxonomic classification and understanding of biological diversity have been based mainly on morphological descriptions [1]. In the early twentieth century, comparative biology entered a transition from the description field and quantitative science, where morphological analysis had a similar revolution of quantification [2]. Based on this quantitative mathematical revolution, the study of morphology has had an important emphasis by developing statistical shape analysis. This made possible the combination of multivariate statistical methods and new ways to visualize a structure [3,4].

In geometric morphometrics (GM), the shape is defined as "any geometric information that remains when the effects of translation, scaling and rotation are removed from an object"[5]. According to [6,7] two techniques have been described: Landmark and Outline methods. Landmark geometric morphometrics is currently the most used tool in sexual dimorphism studies, where equivalent and homologous specific points are fixed in the biological structure being studied. Whereas outliner GM reduces contour shape in a structure by means of points built and located in its boundaries [8-10]. These tools allow studying organism shape and also size, providing sound graphic analyses to quantify and visualize morphometric variation within and between organism samples.

One of the most interesting sources of phenotypic variation in animals and plants has been sexual dimorphism, the study of which continues to be an important area of research in evolutionary biology. Sexual differences in morphological characters are a common phenomenon in many animal taxa, and their most conspicuous aspect is body size [11]. The direction of these differences, that is whether males or females are larger, varies from one group to another [12]. Most of the morphological variations of insects are due to effects...
Sexual Dimorphism

associated with the environment, either phenotypic responses (plasticity) or particularly those which act during ontogenetic development [13]. Females are generally larger than males, and this gives them adaptive advantages such as greater fecundity and better parental care [14,15]. However, in some species males are longer but have less relative mass e.g. [16], which implies that the determination of sexual dimorphism requires more complex measurement techniques related, for example, with geometric shape [17]. 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 sexes.

Studies of *Ceroglossus chilensis* shape have discussed that sexual dimorphism is usually concentrated in two sections of body shape: in the abdominal section, where this dimorphism variation is associated with an adaptative character due to the presence of female eggs; and changes in the pronotal section associated with male-male competition due to variation in sexual ratio in populations [17,18]. Other studies have used geometric variation of wing shape in insects as the dimorphism character, where the integrated geometric variation of veins is differentiated between male and female [19].

The following chapter is a brief description of sexual dimorphism of shape in insects and its evaluation by using new morphological tools that provide a visualization of the geometric shape, besides a description in 2 insect orders about the way in which sexual dimorphism variations that are not easily observed may be distinguished in different populations.

2. Geometric morphometrics methodology

Morphometrics is the study of shape variation and its covariation with other variables. The development of its new properties, capable of capturing shape, renders this new morphology to be considered geometric, being its introduction received as a “revolution” for the morphological analysis realm [20]. Shape is mathematically defined as all the geometric features of an object except its size, position and orientation [4]. In other words, changes in size, position and orientation do not change the shape of an object. Most of the research efforts in geometric morphometrics have concentrated on landmark data. Morphological landmarks are points that can be located precisely on each specimen under study with a clear correspondence in a one-to-one manner from specimen to specimen [7,21]. There are several methods for the analysis of curves and outlines. Outlines can be analyzed using semi-landmarks, which are the points that fall at defined intervals along a curve between two landmarks [22]. Semilandmarks can be analyzed with Procrustes superimposition like ordinary landmarks. Another outline method is perhaps the oldest type of geometric morphometrics – Fourier analysis [23]. Fourier methods use sine and cosine harmonic functions to describe the positions of outline coordinates. Fourier analysis can be applied to 2D outlines [23,24] or 3D closed surfaces [25,26]. Eigenshape is a third method for the analysis of outlines or curves [27,28]. In eigenshape, the coordinate points of an outline or curve are converted to a phi function, which is a list of the angles from one point to the next one in the series. Outline methods have been criticized because their
individual coordinate points are not biologically homologous to each other [29], but this issue is important only in cases where a one-to-one mapping between individual variables and biological homology is required.

The principal and most important analysis of geometric morphometrics is called Procrustes superimposition, where only the shape information is extracted and the other components of variation in size, position and orientation can be removed, while taking care not to alter shape in any step of the procedure [4,9,30]. The extra components of variation can be removed by rescaling the configurations to a standard size, shifting them to a standard position, and rotating them to a standard orientation (Figure 1). Moreover, since none of the steps has changed the shape of the configurations, the variation after the procedure is the complete shape variation.

![Figure 1. Summary of Procrustes superimposition. Components of variation other than shape are eliminated by scaling to the same size, translating to the same location of centroids, and rotating to an overall best fit of corresponding landmarks. (Figure Idea by C.P Klingenberg)](image)

3. Sexual shape dimorphism

Insects in many species vary greatly in the expression of sexual traits [14]. In some species variation in the expression of such traits is discontinuous, resulting in the co-occurrence of two or more discrete phenotypes within one sex. The discrete expression of sexual traits or secondary sexual traits has attracted particular attention, as it is thought to reflect alternative adaptations to heterogeneous social conditions [31]. Sexual size dimorphism
(SSD) in body size is considered to be one of the major determinants of mating success in many species [32-35]. Because larger males are generally more aggressive and more competitive than smaller males, larger males often attain greater reproductive success through intrasexual selection [14]. In contrast, sexual shape dimorphism (SShD) has been much less investigated [17-19,36]. From those studies that considered SShD, most have discussed it as a diagnostic trait for diverse purposes, such as sex identification or the analysis of ontogeny [37-40]. Nevertheless, some other authors have considered sexual dimorphism evolution covering only some aspects of a limited number of taxa, such as: the evolution of cranium in primates [41-44]; the proportions and dimensions in lizard bodies [45,46]; newts [47]; or in flies [48]; and variation of shape in insect heads [49]; and variation of sexual dimorphism in *Drosophila* wings [36].

4. Sexual shape dimorphism examples in insect body and wing shape

4.1. Case 1 Body shape

In coleopteran of the genus *Ceroglossus* (Carabidae) a phenomenon occurs which is completely opposed to that described above. *Ceroglossus* Solier is a genus endemic to *Nothofagus* forests of the extreme south of South America.

Studies of body shape in *Ceroglossus chilensis* have demonstrated that the similarity of males and females is directly associated with the sex ratio of this species [50]. Morphological sex dimorphism is much reduced and only visible under a microscope. However, in terms of geometric morphometrics the differences are visible in two body regions; the abdomen of females, whose variation has been reported to have an adaptive value due to the presence of eggs, and changes in the pronotum of the thorax in males, which has been attributed to intrasexual competition in this species[17,18,50].

4.1.1. Methodology

For the morphometric analyses a total of 116 specimens of *C. chilensis* were used from 2 populations (53 males and 63 females) of Santa Juana area in the Coast Range (37º10’S, 72º57’W) and near San Fabián de Alico in the Andes Foothills (36º37’ S, 71º50’W), both localities in the Región del Bío-Bío. The geometric analysis considered exclusively variation in shape, and it was performed using a photograph in ventral view of males and females with an Olympus X-715 digital camera; using the methodology of [51], we digitized 17 landmarks (LMs, anatomical homologous points) on every picture, by TpsDig 2.10 (Figure 2). All analyses were then run using MorphoJ software version 1.05a [53].

Once the Cartesian x-y coordinates were obtained for all landmarks, the shape information was extracted with a full Procrustes fit [4,9], taking into account the object symmetry of the structure. Procrustes superimposition is a procedure that removes the information of size, position and orientation to standardize each specimen according to centroid size. Due to the high difficulty to check the differences in sexual dimorphism in this group, the only way to differentiate was based on the presence of antennal careens located from the fifth to ninth
Figure 2. Location of the 17 landmarks in ventral view of *Ceroglossus chilensis*

Figure 3. Canonical Variate Analysis (CVA) for the sexual shape dimorphism population of *Ceroglossus chilensis*. Each point represents a shape variable for female and male individuals in ventral view. The figure shows the first two CV components’ axes with shape deformation images associated, and their antennal structure that is differentiation characteristic based on optic microscopy (careens presence in males).
Sexual Dimorphism

segment [54], present "only in males" and observable under a dissecting microscope (Figure 3). Because of the symmetry of the structure, reflection is removed by including the original and mirror image of all configurations in the analysis and simultaneously superimposing all of them [55]. To examine the amount of symmetric variation and sexual shape dimorphism we used Procrustes ANOVA to assess studies on object symmetry. Differences between locations and sex were assessed using canonical variate analysis (CVA), a multivariate statistical method used to find the shape characters that best distinguish multiple groups of specimens.

4.1.2. Results

The PCA plot for the symmetric component (individual variation) shows some differences between the populations analyzed. The first two PCs account for 53.643% (PC1 + PC2 + PC3 = 27.619% + 14.88% + 11.14) of the total shape variation and provide a reasonable approximation of the total amount of variation, with the other PC components that account each no more than 9.5% of the variation. The canonic analysis showed a clear differentiation of sexual shape dimorphism in both populations (Figure 3).

The Procrustes ANOVA for size does not show significant differences between populations and sex. Instead, Procrustes ANOVA for shape shows differences between populations ($F = 3.79, P<0.0001$) and high differences between sex ($F = 11.76, P<0.0001$). Besides, MANOVA tests, for both symmetric and asymmetric components, confirm these results ($\text{Pillay} = 0.64, P<0.0001$; $\text{Pillay} = 0.31, P<0.0001$ respectively).

4.2. Case 2 Wing shape

Within species, sexual dimorphism is a source of variation in life history (e.g., sexual size dimorphism and protandry), morphology (e.g., wing shape and colour pattern), and behaviour (e.g., chemical and visual signaling). Sexual selection and mating systems have been considered the primary forces driving the evolution of sexual dimorphism in insects especially in lepidoptera, and alternative hypotheses have been neglected [56]. Recent analyses demonstrate that many lepidopteran species exhibit female-biased sexual size dimorphism 73% of 48 species in Reference [57]. Size and shape differences are established during the larval period [58,59] by developmental and physiological mechanisms (e.g., number of larval instars and hormonal regulation). Because females of many species are capital breeders (i.e., they allocate larval resources for reproduction), and large size is related directly to fecundity [60-62], selection for large female body size appears to be driven by natural selection for increased fecundity [63]. Most of the morphological variations between males and females 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 [64-66]. Females are generally larger than males; this gives them adaptive advantages such as greater fecundity and better parental care [14,15,63,67].

In this section, sexual dimorphism was determined to be present in the wing shape of moths of the Synneuria genus, suggesting that the wing shape may be selected as a character to determine sex between lepidopteran species.
4.2.1. Methodology

Sampling: The study area was the farms named “El Guindo (36º50'12"W- 73º01'25"S) and Coyanmahuida” (36º49'28.66"S - 72º44'1.34"W) separated 20 km from one another in the province of Concepción, Biobío Region of Chile, where there are relict native forests with *Nothofagus obliqua* and *Peumus boldus*, among others. In order to determine intra- and interpopulation variation by means of geometric morphometrics, we used adults of *Synneuria* sp. (Lepidoptera, Geometridae). The individuals were collected by 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. Finally we collected individuals which were processed, males and females separated, wings cut, and mounted.

The geometrical analysis, which considered variations attributed exclusively to shape, was performed 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 fibre optics lighting, with which we constructed photographic matrices using the TpsUtil 1.40 program [68]. We digitalized 18 morphological landmarks based upon the shape and vein pattern of the wing (Figure 4) for all individuals using the TpsDig 2.12 program (52). To determine if there are significant differences between male and female populations of *Synneuria*, a factorial variance analysis (ANOVA) was calculated base on the matrix of covariance between sexes generated by means of the Procustes analysis.

**Figure 4.** Location of 18 morphological landmarks in the right wing of *Synneuria* sp. (Benitez, Neotropical Entomology unpublished data)
4.2.2. Results

The morphological variation among moths determined by Procrustes ANOVA indicates that variation in shape between sexes is highly significant (Table 1).

<table>
<thead>
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<th>Effect</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
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</tr>
<tr>
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<td>0.010086</td>
<td>28</td>
<td>0.000360</td>
<td></td>
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</tr>
</tbody>
</table>

Table 1. Two-way ANOVA for differences in shape of *Synneuria* sp using the first relative warp as dependent variable.

The relative warp plot shows some differences between sexes within each population analyzed (Figure 5). The first three Rw components account for 91.74% of the total shape variation and provide a reasonable approximation of the total amount of variation, and the other Rw components account for no more than 5% of variation. In order to visualize the variation in wing shape graphically we took the mean of the first three relative warps. We found different morphotypes for males and females of Coyanmahuida and Guindo (Figure 6).

![Figure 5. Morphological deformation grids showing distributions of shape for males and females of *Synneuria* sp. in the different localities, CN: Coyanmahuida and EG: El Guindo.](image)
Figure 6. 3-D dispersion graph of 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 different shape.
4.3. Discussion Case 1 and 2

The adoption of new techniques to determine variation in shape of both animals and plants is currently a widely discussed issue [69,70]. Geometric morphometrics can unify methodologies to quantify and visualize shape in all the ways that are possible.

For case 1, geometric morphometric was capable of detecting variation between species that are not clearly visible on plain sight, but rather at a sexual selection level between species. However, small variations on body shape could mark the difference in both populations, and these were proven according to Procustes distances and also by means of variance analyses. It is worth noting that the populations studied were subject to climate differences from which individuals were collected.

Although these differences are not obvious, individuals of the Coast Range had less thickened bodies than those of the Andes foothills. It has been reported that a climate with high relative humidity and constant temperatures promotes a thinner, subelytral cavity; this result was therefore expected for the Coast Range. The individuals of the Andes foothills had more visible morphological variations, which may be a consequence of the instability of the environment in this area (more variation in temperature, leading to thicker subelytral cavities). However, in spite of the climatic differences between populations there were not large morphological differences in the sexual dimorphism between populations. We may infer from this that gene flow has not been interrupted between them [17].

Regarding the case of Lepidoptera wing, it was very similar to the findings in beetles, but in this case differences were determined by means of small variations in wing shape associated with venation and flying styles of males and females [18].

For a number of authors, the variation in wing shape does not provide sufficient evidence to conclude that this is only a product of sexual dimorphism. It is frequently argued that individual variation in shape may be strongly dependent on environmental conditions [3,71]. However, our study showed a significant difference in wing shape between sexes, both within and between localities. Therefore, we conclude that the differences found here are analytical for the species and areas studied.

The differences among the individual configurations of each sex were captured using mathematical functions varying according to 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 on 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 behaviour in the search for host plants e.g. [72-75]. Therefore, selection would act on wing shape to optimize flight characteristics [76].
5. Conclusions

This revision is intended to provide a wide view of GM use in some of the diverse study areas of sexual dimorphism in insects, confirming that by using the new tools that define shape as a differentiation characteristic it is possible to determine variations at minimum scales, which can be explained by means of sexual selection. Furthermore, by using geometric morphometric, besides identifying variations regarding sex, the researcher may determine relations between anatomic points of shape, in order to identify asymmetry patterns and generate hypotheses about the group development stability, [55,77]. Therefore, it is worth noting that in recent years research efforts have increased exponentially, and GM gains attention every day as a useful tool for quantitative integration in morphology study due to its easy, inexpensive and fast application. Consequently, scientists are taking steps to combine these advanced techniques of morphometry study, to unify methodologies with molecular and genetic studies, in order to get results with total evidence within the analysis itself.

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Sexual Dimorphism Using Geometric Morphometric Approach


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