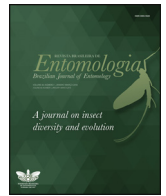




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Systematics, Morphology and Biogeography

Sexual dimorphism and population differentiation in the Chilean Neotropical moth *Macaria mirthae* (Lepidoptera, Geometridae): a wing geometric morphometric example



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ARTICLE INFO

Article history:

Received 16 April 2017

Accepted 20 June 2017

Available online 5 July 2017

Associate Editor: Livia Pinheiro

Keywords:

Wing shape

Sexual dimorphism

Geometridae

Azapa valley

Chaca valley

ABSTRACT

Sexual shape dimorphism is the differentiation of male and female organisms based on their shape variation; this definition was proposed for the use of geometric morphometrics analysis where the geometric features of the shape are analyzed without the influence of the size. *Macaria mirthae* (Lepidoptera: Geometridae) is a moth that inhabits different valleys in the north of Chile principally associated to *Acacia macracantha* and lately *Leucaena leucocephala* both trees of the Fabaceae family. The Sexual dimorphism was analyzed in this species in order to corroborate studies on the use of wing as a sexual differentiation trait, and specific influence of localities was also evaluated. A clear shape variation was found where the male wings are more contracted compared to female wings. A climate influence is also suggested that could differentiate the wing shape from the individuals that inhabit two different valleys in the neotropical region of the north of Chile. This research supports previous studies identifying a clear Sexual shape dimorphism in the wing, as a selected trait, suggesting that oviposition and male competition of this group of moths is reflected in their wings. These differences raise the question whether Sexual shape dimorphism can be modulated by natural selection.

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Introduction

In the last centuries, comparative biologists have been focused in studies of morphological variation where the most used method is the comparison of phenotypic variation. One important source of variation is the sexual dimorphism with its corresponding classification and understanding (Andersson, 1994).

Sexual differences in morphological characters are a common phenomenon in many animal taxa, and their most conspicuous aspect is body size (Andersson, 1994; Fairbairn, 1997; Teder and Tammaru, 2005; Stillwell and Davidowitz, 2010). Nevertheless, size variation is not enough to identify or characterize the sexual dimorphism (sexual size dimorphism (SSD)) (Benítez et al., 2013).

In Lepidoptera, Benítez et al. (2011) identified through geometric morphometrics approach a pattern of sexual dimorphism in external morphology using the shape of the organism (sexual shape dimorphism, SShD). The above-mentioned authors found that this tool was useful to differentiate males and females of a geometrid

moth (*Synneuria* sp.) using only the wings (this analysis uses only the shape, excluding the size).

Additionally, another factor that contributes to the morphological variation in organisms is the variation on the climatic condition, where same species modify their morphology for a better adaptation to the environment (adaptation of the phenotype to the environment).

The Atacama Desert is the most arid desert of the world (Clarke, 2006). In the northernmost part of Chile, it is crossed by several narrow, sub-parallel, transverse valleys extending from the western slopes of the Andes Mountain Range to the Pacific Ocean. These valleys are recognized to be among the most important places for the biodiversity of the Atacama Desert (Luebert and Plissock, 2006; Estades et al., 2007). However, contiguous valleys are separated by a wider area of absolute desert mainly devoid of vegetation, which could work as effective barriers for low dispersal of organisms inhabiting different valleys. It is expected that, depending on the dispersal capacities, this effect could be particularly serious for some specialized phytophagous insects associated with plants restricted to the bottom of the valleys.

Studies on morphological variation due to climatic condition are many; nevertheless, there are few that combine Lepidoptera

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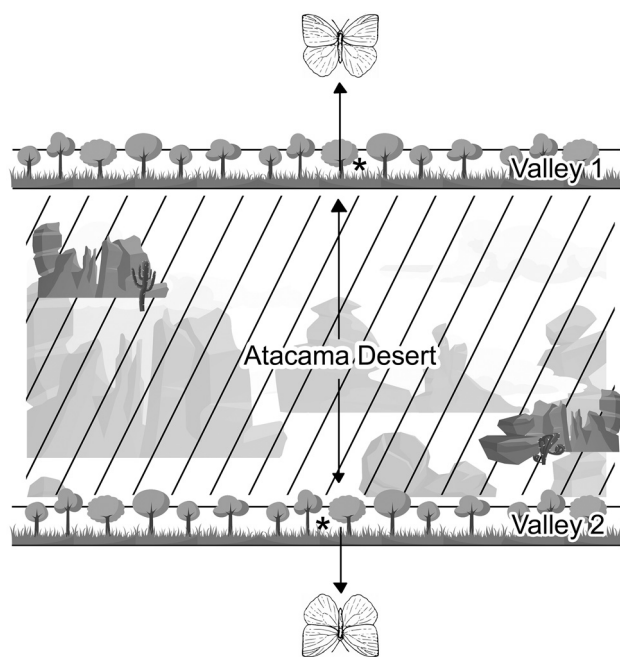


Fig. 1. Graphical scheme of the location of the two Valleys in Atacama Desert in the north of Chile.

and geometric morphometrics. Sanzana et al. (2013) use the wing shape of *Auca coctei* (Lepidoptera: Nymphalidae) to identify the wing shape adaptation in their distributional cline, suggesting the presence of ecological rules regarding the morphology (Converse Bergman Rule). Benítez et al. (2013) studied the isolated effect of island on the morphology of tenebrionid beetles and they found that individuals of the same species can vary their geometric body shape responding to different climatic conditions.

The aim of this study was to evaluate the wing sexual shape dimorphism and the wing morphological variation on two contiguous coastal valleys of the Atacama Desert in the Neotropical moth *Macaria mirthae*.

Materials and methods

Study sites

The study was undertaken in two coastal valleys of the Atacama Desert in northern Chile: the Azapa Valley (18°31'12"S, 70°10'41"W) and the Chaca Valley (18°48'56"S, 70°09'07"W), both belonging to the Arica Province (Fig. 1). This area is characterized by a tropical hyper desertic bioclimate with mean annual precipitation about 0 mm (Luebert and Plissock, 2006). As a result of the summer rains that occur in the high plateau and the western slopes of the Andes, the two valleys harbour semi perennial streams which enable the presence of native vegetation.

Sampling and rearing

Larvae (only last instar) of *M. mirthae* were collected in January 2013 on *A. macracantha* in the Azapa and Chaca valleys. As *M. mirthae* is able to use additional Fabaceae host plants (Vargas and Mundaca, 2014) in the two study sites, and since wing morphology can be affected by different larval host in this moth (Benítez et al., 2015), surveys for larvae were restricted to isolated trees of *A. macracantha* collected by canning trees. The collected larvae were placed into individual plastic vials with towel paper on the bottom. The vials were kept in the laboratory at room temperature and were cleaned daily. Fresh leaves of *A. macracantha* were added daily until

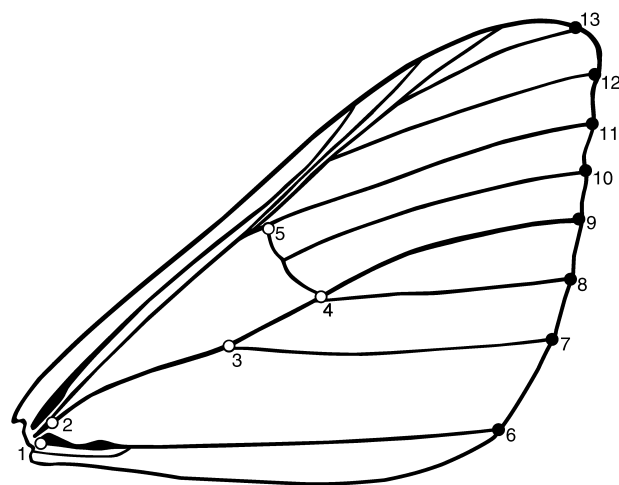


Fig. 2. Benítez et al. (2015), representation of the 13 morphological landmarks identified in the forewings of *Macaria mirthae*.

the larvae finished feeding. Pupation occurred between the towel paper pieces at the bottom of the vial. The adults obtained from the pupae were killed with ethyl acetate 24 hours after emergence. Wings were removed from the body of the adults and then compressed between two microscope slides for the subsequent image capture.

Geometric morphometrics analysis

Following the procedure of Benítez et al. (2015), from 230 individuals right and left forewings were photographed with a digital camera Micropublisher 3.3 RTV-QImaging (Q-imaging, Canada) attached to a stereoscopic microscope Olympus SZ61. Thirteen landmarks were digitized, using TpsDig 2.17 software (Rohlf, 2013). On all wings (both left and right) upper wing side landmarks were identified according to external anatomy and wings vein pattern (Fig. 2) (Benítez et al., 2015).

The shape information was extracted using a Procrustes fit (Rohlf and Slice, 1990; Dryden and Mardia, 1998) and the exploratory shape variation between sexes was analyzed using principal components analysis (PCA). In order to evaluate the location effect on the morphology, a discriminant analysis was performed using the covariance matrix of shape. A PCA of the pool by sex covariance matrix was performed to evaluate if localities effect was not confused with the analyzed sexual dimorphism. Finally, a multivariate regression of size on shape was performed in order to analyze if the size has an influence on the sexual dimorphism (Monteiro, 1999). All the morphometrics analysis were performed using the software MorphoJ 1.06d (Klingenberg, 2011).

Results

The geometrical attributes of the wing shape were visible using the PCA, the accumulated variation of shape in the first 3 principal components reached 61.4% (PC1 = 34.12%; PC2 = 16.20%; PC3 = 11.08%). The scatterplot of variation (simulation of the morphospace in a PCA) shows a clear differentiation between males (black dots) and females (grey dots) (Fig. 3). There is a noticeable SShD by the contraction of the tip and radial veins of the wing in males were the mean vector of movement on the landmarks is to the left direction. On the contrary, female wing shape expands the tip section of the wing but contracts the landmarks 1, 2, 3, 5 of the wings. The PCA to identify variation between localities was not able to find differences from the pool by sex covariance matrix; however, the discriminant analysis shows a noticeable differentiation

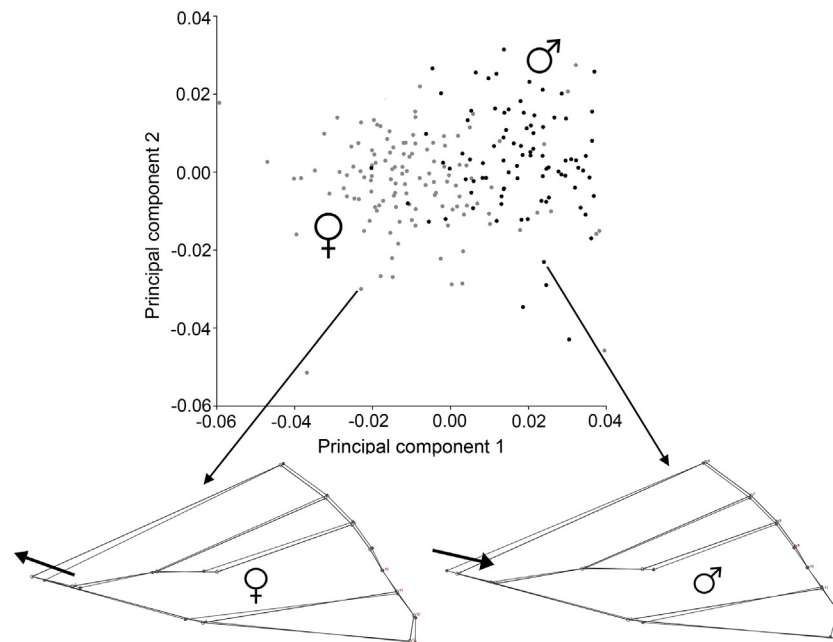


Fig. 3. PCA analysis of the sexual shape dimorphism of *Macaria mirthae*: the figure shows the first two orthogonal PC components' axes that represent the shape space dimensions, also a decomposition of shape variation between sexes. *Each point represents a different shape.

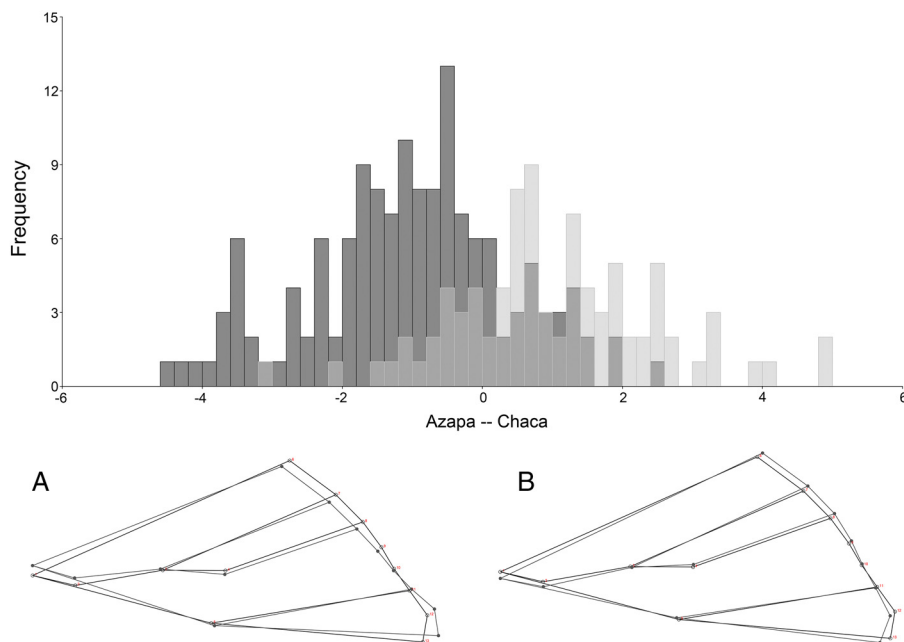


Fig. 4. Discriminant analysis of the wing shape between the two Azapa (grey bars) and Chaca valley (white bars) at the Atacama Desert.

between the wing shape of Azapa valley moths and the wing of the individuals from Chaca valley moths (Fig. 4). The wing shape of Azapa moths shows a more variable shape with more disorganized movement of the vector at the different sections of the wing, in contrast to the wings from the individuals of Chaca. These shape differences were statistically significant (p -values for permutation tests (1000 permutation runs): <0.0001). Finally, the multivariate regression of the shape on centroid size (Fig. 5) shows that the size has a significant but small influence (8.4%) on the shape differentiation.

Discussion

Analysis of geometric morphometrics can confirm a clear difference in both: sexual shape dimorphism and localities. The tool

of geometric morphometrics is a good method to analyze the sexual differences in the wings of *M. mirthae*. Wing shape was well distinguished in the two valleys in the extreme north region of Chile. The Scatterplot of the PCA (Fig. 3) shows that the PC1 differentiates the group of males and females. The landmark differences detected at the SShD were principally located in the base of the radial veins, key traits to distinguish different wing morphotypes between sexes. Benítez et al. (2011) explain that this morphological change on the veins could be due to dispersion, migration and sexual selection. Additionally, in males it could be the result of the nuptial flight, territoriality and sexual selection, and in females, primarily due to their flight behaviour while searching for host plants to lay eggs (Dockx, 2007; Johansson et al., 2009).

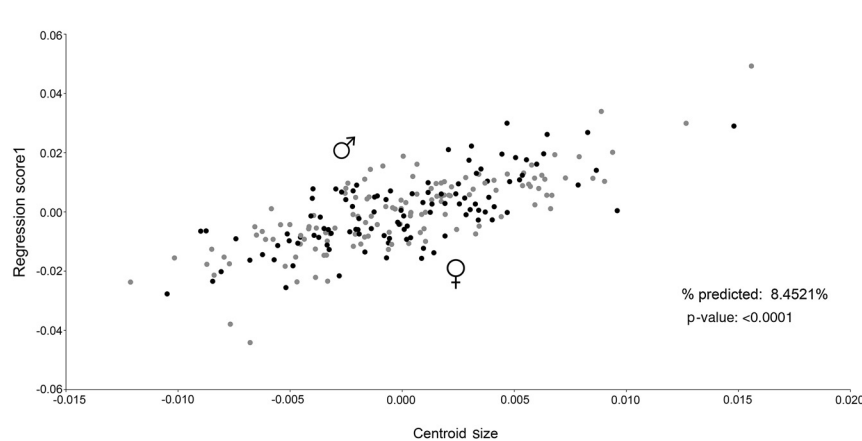


Fig. 5. Multivariate regression of the wing shape on the wing centroid size of *Macaria mirthae*. Grey points represent female wings and black points represent male wings.

This hypothesis could work for insects in case their male sexual behaviour was more pronounced than in females, condition that could force sexual selection (Thornhill, 1976; Savalli and Fox, 1998; Fairbairn et al., 2007). Nevertheless, more studies are needed on this topic for *M. mirthae*.

The observed differences in the two valley populations could be caused by a possible isolation of the species (30 km of desert) from the populations (Fig. 4). Nevertheless, more studies are needed in order to clarify if the modifications between valleys have a genetic impact or due to local adaptation (Cepeda-Pizarro et al., 2005; Valdivia et al., 2011; Benítez et al., 2014). Benítez et al. (2014) found similar shape adaptation in non-winged beetle (*Praocis spinolai*). Also, morphological plasticity could be observed in insects at multiple traits, caused by the influence of different hosts (Mozaffarian et al., 2007; Soto et al., 2008) or clinal latitudinal and altitudinal effects (Outomuro and Johansson, 2011; Sanzana et al., 2013).

The multivariate regression of the shape and centroid size (Fig. 5) was used to test the influence of centroid size on the shape. It is well known that in insect the size is one of the most important attributes in sexual dimorphism (Andersson, 1994; Fairbairn, 1997; Teder and Tammaru, 2005; Stillwell and Davidowitz, 2010). However, for specific traits this rule is no longer effective, since the results shown that centroid size is not a significant trait to differentiate males from females.

For *M. mirthae* morphological variation has been found at the wings due to host influence, where the individuals from the north (Azapa) have been forced to change their diet behaviour from a unique host plant (*Acacia macracantha*) to include in their diet a more abundant plant (*Leucaena leucocephala*). This change has affected the symmetric pattern of the wings in populations by developmental instability as a consequence of stochastic processes and environmental stress (Benítez et al., 2015).

Finally, this research corroborates the studies of Benítez et al. (2011) identifying a clear SShD, suggesting that the oviposition behaviour and male competition of this moth is reflected in their wings. This study also confirms the efficiency of the geometric morphometrics tool and raises the question of whether SShD may be modulated by natural selection. In addition, GM tools enabled us to detect subtle morphological differentiation of isolated populations of a specialized phytophagous. Accordingly, geometric morphometric tools can be helpful to study the patterns of sexual dimorphism and morphological differentiation of organisms inhabiting the extreme environments of the Atacama Desert.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors would like to thanks to Ms Maria Raquel Lazo de la Vega, for the proof reading of the article and to the Dr. Wilson Huanca to provide the facilities on the micro-lab pictures. Financial support was obtained from project DGI-9719-17, from Universidad de Tarapacá.

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