

Latitudinal gradient effect on the wing geometry of *Auca coctei* (Guérin) (Lepidoptera, Nymphalidae)

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ABSTRACT. Latitudinal gradient effect on the wing geometry of *Auca coctei* (Guérin) (Lepidoptera, Nymphalidae). When the environmental conditions change locally, the organisms and populations may also change in response to the selection pressure, so that the development of individuals may become affected in different degrees. There have been only a few studies in which the patterns of wing morphology variation have been looked into along a latitudinal gradient by means of geometric morphometrics. The aim of this work was to assess the morphologic differentiation of wing among butterfly populations of the species *Auca coctei*. For this purpose, 9 sampling locations were used which are representative of the distribution range of the butterfly and cover a wide latitudinal range in Chile. The wing morphology was studied in a total of 202 specimens of *A. coctei* (150 males and 52 females), based on digitization of 17 morphologic landmarks. The results show variation of wing shape in both sexes; however, for the centroid size there was significant variation only in females. Females show smaller centroid size at higher latitudes, therefore in this study the Bergmann reverse rule is confirmed for females of *A. coctei*. Our study extends morphologic projections with latitude, suggesting that wing variation is an environmental response from diverse origins and may influence different characteristics of the life history of a butterfly.

KEYWORDS. Centroid size; geometric morphometrics; Insecta; inverse Bergman rule.

Study of variations in the biological characteristics of organisms as a response to a geographic gradient is being deeply studied under the approach of macroecological patterns. The most studied are those of Bergmann, Allen, Gloger and Jordan, among others. Bergmann's rule (Bergmann 1847; Mayr 1956) states that body size of species increases with latitude. Originally this rule was limited to endothermic vertebrates, being those of larger size found at higher latitudes (Blackburn *et al.* 1999; Ashton *et al.* 2000). Ray (1960) was one of the pioneers in suggesting that ectothermic invertebrates such as insects also fulfill Bergmann's rule. However, the inverse Bergmann rule has also been suggested for insects (Van Voorhies 1997; García-Barros 2000), stating that for diverse taxa body size decreases from the tropics toward the poles, that is, from warmer to colder climates (Brennan & Fairbairn 1995; Mousseau 1997).

Wing morphology has been used as an indicator of changes in environmental conditions by measuring changes in developmental instability on the shape using fluctuating asymmetry (Woods *et al.* 1999; Hoffmann *et al.* 2002; 2005; Takahashi *et al.* 2011). Other factors that affect the stability are the decrease in temperature, atmospheric pressure, oxygen availability and increase in solar radiation leading to morphological changes in insects, *e.g.*, compromising insect thermoregulation at high altitude and reducing convective heat loss of insects (Hodkinson 2005; Dillon *et al.* 2006).

Many studies have related environmental temperature to wing morphology, mainly in the Diptera (Gilchrist *et al.* 2004). However, studies of wing morphology of butterflies related to variation in climatic conditions are scarce (Altizer & Davis 2010; Gibbs *et al.* 2010). This study evaluates morphological variation among populations of a day-flying nymphalid butterfly. The butterfly *Auca coctei* (Guérin, 1838) belongs to the family most widely distributed in Chile and the second most diverse (45 species) (Peña & Ugarte 2006). This species is widely distributed in Chile and Argentina and is very common in the entire temperate zone. It inhabits the high Andes, the Altiplano, the coastal area, the Patagonian steppes, the forests of southern South America and shrubby zones of the foothills (Peña & Ugarte 2006). It is abundant and flies in sunlight and shade, including dense shade; it prefers to visit yellow flowers of species of the genus *Senecio*, but it can also be found associated with grasses, developing its entire life cycle in the habitats where it lives (Shapiro 2009). Given the great variety of habitats that this species inhabits, and the fact that it does not move far from its host plants where it deposits its eggs and undergoes the rest of its life cycle (Fernández & Jordano 2009), these behavior patterns could explain the morphological variation that exists among populations in its wide distribution range. In this study we have used geometric morphometrics as a powerful morphological tool to examine the hypothesis that these butter-

flies respond with morphological variation of the wing to different latitudinal gradients. To do this, we explore the wing morphology of nine populations of *A. coctei* from different latitudes of Chile, expecting to find changes associated with the shape and size of the wing among populations related to their latitudinal distribution, due to a possible association with one of the macroecological patterns mentioned above.

MATERIAL AND METHODS

Study area and sampling. The populations of *A. coctei* were collected from 9 localities for male and 8 for female from the Region de Coquimbo to the Region de Los Lagos in Chile (Fig. 1). Although the populations of this butterfly are found along the latitudinal gradient, this sampling design was followed to include the latitudinal extremes of the range, 29° in the north and 40° in the south. This allowed sampling populations which live in contrasting environmental situations.

Individuals were captured between November, 2010 and February, 2011 using aerial entomological nets. We explored the lower and medium strata of forests, as well as forest borders, non-disturbed and disturbed interiors, on sunny days in the hours of greatest sunlight when the temperature was above 15° C. A total of 202 butterflies were collected, with a minimum of 5 at each sampling site (Table I). The fore wings of each butterfly were separated at the base of the thorax and mounted on slides; the ventral side was photographed using an Olympus x 715 camera with directional optical fiber lamps. The genitalia of each individual were used to confirm the identity of the species, comparing them with the type material deposited in the Zoology Museum of the *Universidad de Concepción* (UCCC-MZUC).

Table I. Study sites for *Auca coctei* in Chile, with indication of geographic coordinates (latitude, longitude), Chilean regions and sample size (n).

Locality	Region	Latitude	n
La Serena	IV	29°55'S 71°14'W	39
Valle Elqui	IV	29°56'S 71°09'W	38
Valparaíso	V	32°58'S 71°00'W	21
Dichato	VIII	36°32'S 72°55'W	29
Tumbes	VIII	36°42'S 73°08'W	32
Concepción	VIII	36°49'S 73°01'W	8
Collipulli	IX	38°43'S 72°36'W	13
Loncoche	IX	39°21'S 72°37'W	5
Los Lagos	X	40°13'S 72°19'W	17

Wing landmark acquisition and measurement error.

The photographic matrixes were constructed, using TpsUtil 2.12 program (Rohlf 2008a). Based on the methodology proposed by Benítez *et al.* (2011a), 17 landmarks were digitized on the ventral side of the right wing (Fig. 2), using the software program tpsDIG V2.16 (Rohlf 2008b). The measurement error (ME) is of critical importance when analysing data in GM (*e.g.* Klingenberg & McIntyre 1998). To assess the significance of ME, the wings of 50 individual of *A. coctei*

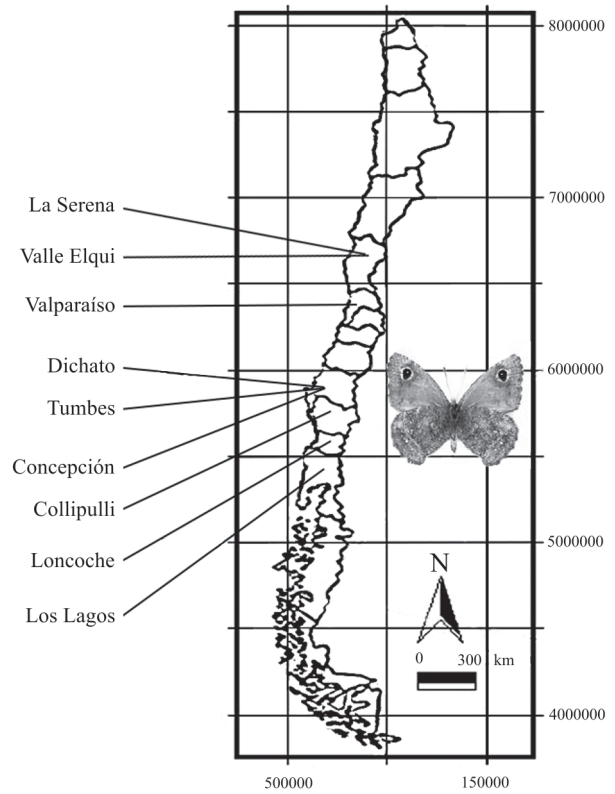


Fig. 1. Map of the study area and the sampling sites for the study of latitudinal gradient effect in *Auca coctei* in Chile. In terms of latitude the northernmost site is located in La Serena (29°55'S, 71°14'W), while the southernmost one is in Los Lagos (40°13'S, 72°19'W).

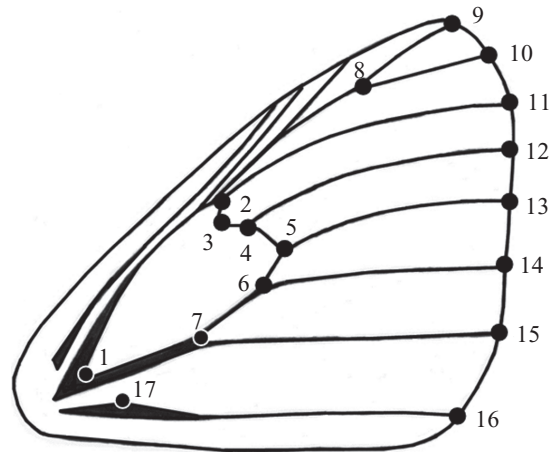


Fig. 2. Positions of the 17 landmarks digitized on the right wing of *Auca coctei* to study the latitudinal gradient effect in Chile.

were digitized twice. We then applied a conventional analysis of variance (ANOVA) on centroid size for size and a Procrustes ANOVA for shape (Rohlf & Slice 1990).

Morphometric and statistical analysis. Once the Cartesian x-y coordinates were obtained for all landmarks, the shape information was extracted with a generalized Procrustes

analysis (GPA) (Rohlf & Slice 1990; Dryden & Mardia 1998). The geometric size among populations was estimated using the centroid size as an estimator of size (Alibert *et al.* 2001; Benítez *et al.* 2011b).

The amount of individual variation (dimensions) and the shape variation in the entire dataset was analysed using principal component analysis (PCA) based on the covariance matrix of the wing shape variation. The inter-location differences were assessed using Procrustes distances which were the product of a canonical variate analysis (CVA). The results were reported as Procrustes distances and the respective P values for these distances, after permutation tests (10,000 runs), were reported. Additionally to assess shape and size variation between location and sexes, we performed Procrustes ANOVA; results are reported as sums of squares (SS) and mean squares (MS) that are dimensionless (Klingenberg & McIntyre 1998; Klingenberg *et al.* 2002). All morphometric and statistical analyses were performed using MorphoJ (Klingenberg 2011).

RESULTS

The first three PC's of the PCA distribution of the shape for the nine study sites explained 57.27% (PC1 + PC2 + PC3: 33.29% + 13.92% + 10.05%) of the total shape variation and provide an approximation of the total amount of variation, with the other PC components accounting each no more than 7.5% of the variation.

Differentiation of shape among sites and sexes. The Procrustes ANOVA revealed a significant difference in shape between males and females (Table II). According with PCA, CVA analysis shows significant differences in both sexes and localities examined and after permutation test ($p < 0.005$; 10,000 permutation runs) showing a particular significant distance to each other (Tables III, IV). The CVA analysis shows that the sexes are clearly separated in their shape (Fig. 3). The deformation grids that express the range of variation of shape indicated that the sexual dimorphism is in the same part of the wing for all sites. Specifically, the differences were accentuated in points 3 and for (the discal cell) which generated a narrowing of the wing in the males, while points 8 (radial point) and 17 (vertex of the anal wing) generated a widening of the wing in females. Fig. 3 illustrates this widening of the wing in females and the narrowing of the wing in males of *A. coctei*. Also, in all sites the marginal wing border was straight in males and concave in females.

Size variation. The ANOVA revealed a significant variation in centroid size for females (Table II). The correlation

Table III. Paired comparisons using analysis of canonical components among eight sites for females of *Auca coctei*. Results are reported in significance values estimated with a permutation test (10,000 replicates).

P value	H	E	I	K	G	B	A	C
H	0.0199							
E	0.0039	0.0057						
I	0.0269	0.1022	0.0179					
K	0.1460	0.1667	0.1044	0.2506				
G	0.0004	0.0004	<0.0001	0.0036	0.0116			
B	0.0006	0.0015	0.0003	0.0061	0.0807	<0.0001		
A	0.0028	0.0077	0.0014	0.0035	0.1274	<0.0001	0.0002	
C	0.0058	0.0233	0.0168	0.0047	0.0012	0.0893	0.0022	<0.0001

Table IV. Paired comparisons using analysis of canonical components among nine sites for males of *Auca coctei*. Results are reported in significance values estimated with a permutation test (10,000 replicates).

P Value	H	E	I	K	J	G	B	A	C
H	<0.0001								
E	<0.0001	<0.0001							
I	<0.0001	0.0002	<0.0001						
K	<0.0001	0.0002	<0.0001	<0.0001					
J	0.0047	0.0415	0.0026	0.0128	0.0019				
G	<0.0001	<0.0001	<0.0001	0.001	<0.0001	0.0011			
B	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0014	<0.0001		
A	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	
C	<0.0001	<0.0001	<0.0001	<0.0001	0.0013	<0.0001	<0.0001	<0.0001	<0.0001

analysis indicated a significant negative relation between mean centroid size of females and latitude ($t = 0$; $P < 0.05$; $r = -0.198$). There were size differences among populations, which were more pronounced in females. Fig. 4 shows for females, that La Serena and Tumbes have larger individuals than Dichato and Concepción, with the smallest ones. Despite these outgoing points, the relationship between latitude and centroid size becomes significant, resulting in smaller sizes at higher latitudes.

DISCUSSION

The variation in wing shape of *Auca coctei* is clearly observed using the techniques of geometric morphometrics. Each of the nine populations showed a significantly different wing shape. This variation was most important in landmarks 3, 4 and 8, which refer to the point situated in the base of the radial vein, a key anatomical character to distinguish different wing morphotypes among the populations (Benítez *et al.* 2011a). Although they have intersecting areas, each of them had a significantly different wing shape.

Table II. Procrustes ANOVA significance test for geometric shape (SH) and centroid size (CS) as dependent variables and sex as the independent variable for *Auca coctei*. Sums of squares (SS) and mean squares (MS).

Sex	Trait	SS	MS	Df	F	P	Pillai tr.	P (param.)
Females	CS	0.000008000	0.000001000	7	3.89	0.0034	–	–
	SH	0.000188436	0.000188436	210	4.54	<0.0001	5.69	0.0369
Males	CS	0.000003000	0.000001000	8	1.34	0.2261	–	–
	SH	0.103002870	0.000429100	240	9.00	<0.0001	3.59	<0.0001

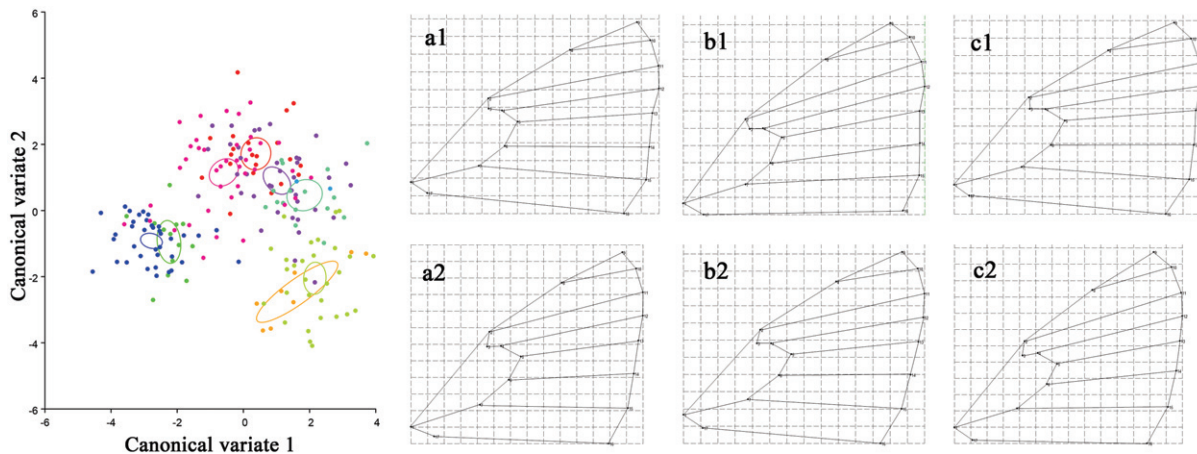


Fig. 3. Canonical variate analysis of the differentiation among sampling sites for *Auca coctei*. Color represents a shape variable which groups all individuals from the same site. Dark red points: La Serena (a); dark green: Collipulli; dark blue: Tumbes (c); lilac: Valle Elqui; light green: Dichato; orange: Concepción; red: Valparaíso; calypso: Los Lagos (b); fluorescent green: Loncoche. a1, b1, c1: female; a2, b2, c2: male.

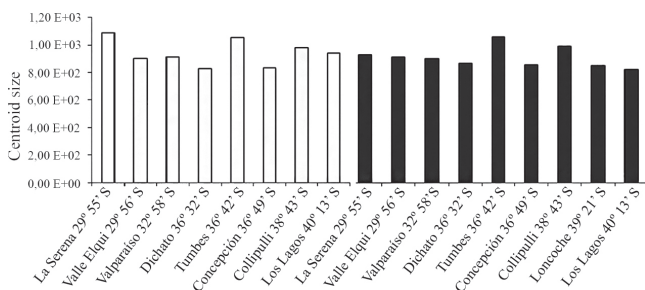


Fig. 4. Centroid size representation of all the sampled localities with their respective latitude for males and females of *Auca coctei* in Chile. White bars represent females; black bars represent males.

There are many processes which could explain the fact that all males had a straight wing marginal border while females had a concave border (Dudley 2000; Breuker *et al.* 2007a). These variations may be explained by the flight characteristics of lepidopterans. Dudley (2000) indicated that the type of flight is important to explain wing shape, which may vary depending on the type and velocity of flight. The distinct variants of wing shape give the nymphalid butterflies characteristics such as better power of orientation, protection against predators, identification of their food plants, migration, dispersion, etc. (Dudley 2000; Breuker *et al.* 2007b; Dockx 2007). The shape of the wing appears to be optimized to improve flight characteristics (DeVries *et al.* 2010; Benítez *et al.* 2011a).

The latitudinal gradient in wing size of *A. coctei* shows an inverse Bergmann law for females. This is the first study on variability of wing size of a Chilean lepidopteran. The plasticity response could explain this pattern, where growth and available energy (temperature) or other vital elements (water) in combination with the time available to complete development produce a decrease in development at low temperatures in high altitude sites (Loh *et al.* 2008).

One of the adaptations relating to the fact that butterflies have to regulate their body temperature is wing size varia-

tion. A larger wing size will enable the butterfly to receive more energy and transfer more heat to the body (Fernández & Baz 2006). This data is consistent with the works performed on dipterans (Gilchrist & Huey 2004; Dillon & Frazier 2006; Frazier *et al.* 2008; Hernández *et al.* 2010), where they suggest that due to aerodynamic reasons larger wings are found at regions with higher temperatures and smaller wings at regions with lower temperatures. Based on our results, females in higher latitudes showed smaller wing size, compared to those in lower latitudes. This could be explained by the abiotic similarities of these sampling sites: little light availability and much wind. Considering these conditions, they would not be adopting this regulatory mechanism. There may exist other factors having an influence in wing size reduction, as for example, presence of a natural enemy or predator. On the other hand, when the temperature is low, as in these sites, the butterfly with a smaller wing size needs a higher wing flapping frequency when flying in order to increase its body temperature (Brodsky 1994). Exceptionally, the specimens captured in Tumbes show a considerable increase in wing size. Based on the environmental characteristics of the site, mainly the stronger winds, a larger size would benefit these individuals with a stronger power and aerodynamic capacity (Berwaerts *et al.* 2002; Frazier *et al.* 2008), reducing the amount of energy for flying. It has been documented that there is a larger proportional development of the mesothorax carrying the first wing pair, in order to face the adverse wind conditions (Brodsky 1994).

However, wing morphology may respond to different types of environmental signals, mainly during the period of development, in which the genes that should be activated for wing development are altered by environmental factors (Hoffmann *et al.* 2005). According to many authors, size variation depends strongly on environmental factors (Adams & Funk 1997; Tatsuta *et al.* 2001), such as seasonality and the length of time which is favorable for growth (Nylín & Svárd 1991). Nevertheless, the plastic response may be me-

diated by physiological mechanisms (Blanckenhorn & Demont 2004), without intervention of adaptation. For example, specific wing cells of butterflies are activated when exposed to high temperatures (Ramos *et al.* 2006), generating an increase in cell size and thus a larger wing. Another physiological explanation for the pattern is that an increase in temperature produces high oxygen diffusion in the cells. This may possibly induce an increase in size and better functioning of cells at high temperatures (Hernández *et al.* 2010).

ACKNOWLEDGEMENTS

The authors thank Project DIUC 210.113.079-1.0 of the *Dirección de Investigación* of the *Universidad de Concepción* for financial support. We also thank Christian Muñoz, Jaime Pizarro and Fermín Alfaro for their collaboration with the field work, and the *Museo de Zoología* of the *Universidad de Concepción* (UCCC-MZUC).

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