Neotrop Entomol (2013) 42:164–169
DOI 10.1007/s13744-012-0107-z

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Assessment of Patterns of Fluctuating Asymmetry and Sexual Dimorphism in Carabid Body Shape

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Keywords

Asymmetry, *Ceroglossus chilensis*, forest plantation, geometric morphometrics

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Edited by Roberto A Zucchi – ESALQ/USP

Received 8 September 2012 and accepted 17 December 2012
Published online 17 January 2013

Abstract

The measurement tool most used to estimate developmental stability (DS) is fluctuating asymmetry (FA), which is a measure of the small random deviations that occur between the left and right sides of bilaterally symmetrical traits. In the Biobío Region of Chile, forest plantations are a widely extended phenomenon, which affect 27% of the surface area of the region and which are dominated by the monoculture of *Pinus radiata*. This study evaluated the presence of FA in the body shape of two populations of *Ceroglossus chilensis* (Eschscholtz) in two 13-year-old forest plantations (commercial thinning) using insects collected with interception traps. Since the biotic and abiotic components of forest plantations are subject to continual anthropic modifications that affect almost all ecological processes, including population dynamics, community composition, and material and energy flows, these characteristics are reflected in the DS of individuals. The results showed that there was greater precision using geometric morphometrics to detect the presence of asymmetry in plantations due to shape analysis, as proposed by studies in antennal morphology using traditional measures. It should be noted that the populations were exposed to different environments; the population in the Coast Range is more humid, while the Andes Foothills population is in a drier area with drier soils. In spite of this, there was minimum phenotypic variation detected at the population level, which reflected the different environments and may be associated with patterns of environmental phenotypic plasticity.

Introduction

Developmental stability (DS) is defined as the capacity of an organism to produce a phenotype predetermined by an adaptive body design under a group of genetic conditions and specific environments (Waddington 1942). It, therefore, refers to the intrinsic capacity of an organism to withstand accidents and perturbations during its growth and development (Clarke 1998). The capacity of an organism to produce an "ideal" phenotype in spite of the perturbations encountered during development reflects the causal mechanism of DS. This capacity is used to evaluate a variety of stress types and the genetic capacity to correct them (Auffray *et al* 1999). The tendency of a development system to produce morphological changes as responses to random perturbations is often called developmental instability or developmental noise (Klingenberg 2003, Nijhout & Davidowitz 2003, van Dongen 2006).

The measurement tool most used to estimate DS is fluctuating asymmetry (FA) (Palmer & Strobeck 1986, Palmer 1994, Clarke 1998, Pither & Taylor 2000). FA is a measure of the small random deviations which occur between the left and right (L and R) sides of bilaterally symmetrical traits (van Valen 1962). The main purpose of using FA to measure DS is that the L and R sides of an organism may be viewed as two independent replicates of the same development, in which the sides of the body of an individual share the same genotype and a homogeneous environment (Klingenberg 2003). In other words, the determining factors of the development of the L and R sides are identical, thus in ideal conditions, perfect symmetry is expected (Auffray *et al* 1999, Klingenberg 2003, van Dongen 2006). However, in the natural process of organism development, there are almost always small perturbations at the cellular level (developmental noise) (Polak 2003). Since these perturbations are produced in a small part of the organism, it is expected that their effects will accumulate independently on the L and R sides (Klingenberg 2003). This means that the visible and quantifiable asymmetry of the L and R sides of morphological structures will, therefore, be the expression of the perturbations which have accumulated during development.

The biotic and abiotic components of forest plantations are subject to continual anthropic modifications which affect almost all ecological processes, including population dynamics, community composition, and material and energy flows (Covarrubias 1993, Albrecht 2003, Briones & Jerez 2007). In the Biobío Region of Chile, forest plantations are a widely extended phenomenon, which affect 27% of the surface area of the region and are dominated by the monoculture of *Pinus radiata* (Briones & Jerez 2004).

There is evidence that temperature, adverse nutritional stress, presence of chemicals, population density, and many other factors causing stress during development can lead to increased FA in Coleoptera (Garnier *et al* 2006, Benítez *et al* 2008, 2011, Henríquez *et al* 2009). Therefore, it is expected that, when environmental conditions change, organisms and populations should adapt to the new conditions (Clarke 1993). As a consequence, the generation of symmetrical phenotypes is conditioned by the dampening of the phenotype in response to the disturbances that occur during morphogenesis (Labrie *et al* 2003, Leamy & Klingenberg 2005).

Ceroglossus chilensis (Eschscholtz) (Coleoptera: Carabidae) is distributed from the Maule region to the extreme south of the Aysén region. It is also present in Argentina, and it is the southernmost species of the genus and the one with the widest distribution in Chile. It prefers more xeric habitats and is more tolerant to arid conditions than its congeners. It is not known if its size, which is relatively large for a carabid, is related to its ability to resist the aridity of the environment (Jiroux 2006). There is evidence indicating that the developmental and environmental instability of *C. chilensis* with highly dense activity is affected by the modified environment rather than the natural environment (Briones & Jerez 2007, Benítez *et al* 2008, Henríquez *et al* 2009).

FA has become a focus of multiple studies in developmental biology as it is easy to manage and results are rapidly obtained. Studies using traditional morphometrics and FA in *C. chilensis* in pine plantations showed that the effect of the resin on the developmental stages was one of the reasons of the developmental instability of antenna development (Benítez *et al* 2008). The increasing extension of pine plantations in Chile is causing a growing socioeconomic, physical, and biological impact. However, the effects of these plantations on the faunal community and especially on the insects have received little attention (Schlatter & Otero 1995).

The objective of this study was to investigate the FA in *C. chilensis* from two plantations of *P. radiata* to confirm the suggestions of Benítez *et al* (2008)) and to increase the precision of traditional measurement tools by using morphological tools based on the study of the geometrical shape of the body.

Material and Methods

Data acquisition

For the morphometric analyses, a total of 116 specimens (53 males and 63 females) of *C. chilensis* were used from 2 populations, one from the Santa Juana area in the Coast Range (37°10′ S, 72°57′ W) and the other from an area near San Fabián de Alico in the Andes Foothills (36°37′ S, 71°50′ W), both localities in the Biobío Region. In order to confirm the results of the study by Benítez *et al* (2008)), in each area, collection sites in 13-year-old forest plantations, which had undergone commercial thinning, were selected.

Sampling was performed between December 2004 and January 2005. In each plantation, 10 interception traps were left in place for 15 days following Briones & Jerez (2007). The traps were placed in a line 5 m away from trees and 20 m away from the border of the plantation to avoid edge effects.

Statistical and shape analysis

The geometric analysis exclusively considered variation in shape, and it was performed using a photograph in ventral view of males and females with an Olympus X-715 digital camera. Twenty-two landmarks (LMs; anatomical homologous points) were digitized following Alibert *et al* (2001) in every photograph using the TpsDig 2.10 software (Rohlf 2008) (Fig 1, Table 1). All analyses were repeated with new photographs to avoid measurement errors, and all analyses were run using MorphoJ software version 1.05a (Klingenberg 2011).

Once the Cartesian *x*–*y* coordinates were obtained for all LMs, the shape information was extracted with a full Procrustes fit (GPA; Rohlf & Slice 1990, Dryden & Mardia 1998), taking into account the object symmetry of the structure. Procrustes superimposition is a procedure that

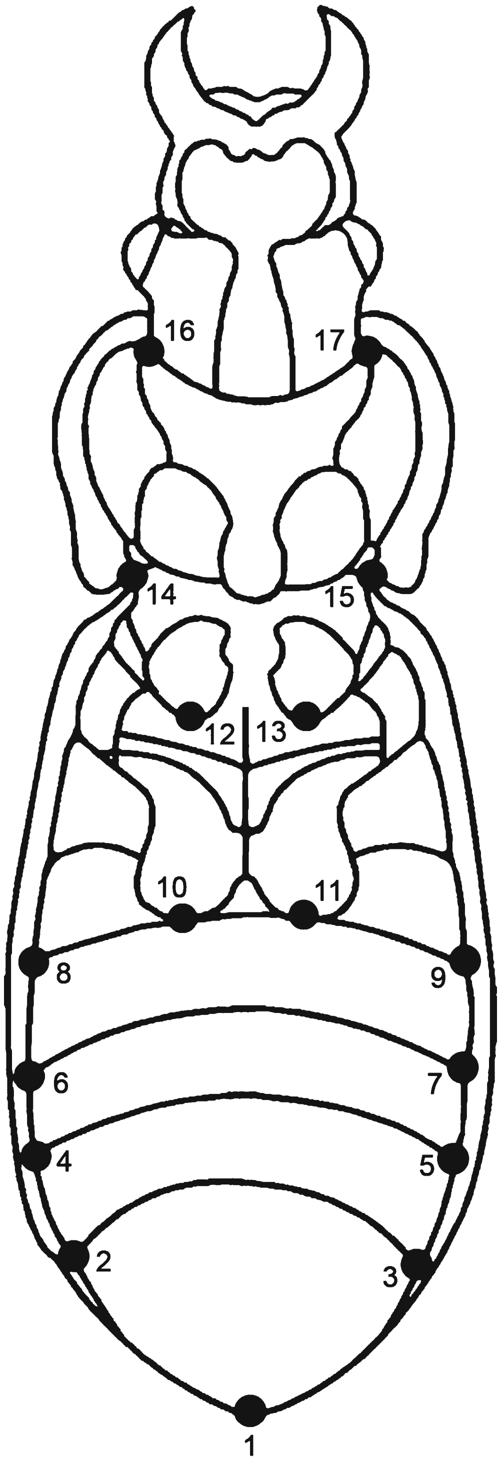

Fig 1 Location of the 17 LMs in ventral view of *Ceroglossus chilensis*.

removes the information of size, position, and orientation to standardize each specimen according to the size of the centroid. Due to the great difficulty in checking the differences in sexual dimorphism in this group, the only way to differentiate the sexes was based on the presence of antennal careens located from the fifth to ninth segments (Jiroux 2006), which are present "only in males" and observable under a dissecting microscope. Because of the symmetry of the structure, reflection is removed by including the original image and the mirror image of all configurations in the analysis and by their simultaneous superimposition (Klingenberg *et al* 2002). To examine the amount of symmetric variation and sexual shape dimorphism, I used the Procrustes analysis of variance (ANOVA) as assessed for 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 characteristics that best distinguish among multiple groups of specimens.

Table 1 Anatomical description of 17 external LMs in *Ceroglossus chilensis* body shape.

Landmark	Anatomical structure
#1	Pygidium
#2	Left lateral vertex of 6th abdominal segment
#3	Right lateral vertex of 6th abdominal segment
#4	Left lateral vertex of 5th abdominal segment
#5	Right lateral vertex of 5th abdominal segment
#6	Left lateral vertex of 4th abdominal segment
#7	Right lateral vertex of 4th abdominal segment
#8	Left lateral vertex of 3rd abdominal segment
#9	Right lateral vertex of 3rd abdominal segment
#10	Mean point of 3rd abdominal left segment
#11	Mean point of 3rd abdominal right segment
#12	Lower left mean point of mesosternum
#13	Lower right mean point of mesosternum
#14	Left vertex of pronotal carina
#15	Right vertex of pronotal carina
#16	Left vertex of pronotal epimere
#17	Right vertex of pronotal epimere

Results

The Procustes ANOVA confirmed the pattern of FA on the significant differences for shape but not for size and confirmed the pattern for directional asymmetry of the body shape in both populations (Table 2). At the same time, Procrustes ANOVA for shape showed differences between populations and large differences between sexes.

In addition, a multivariate analysis of variance test was used to calculate the non-isotropic variation of each LM and confirmed the presence of highly significant FA in the samples for symmetric (Pillay=0.64, $P<0.000$) and asymmetric components (Pillay=0.31, $P<0.0001$). The principal component analysis plot for the symmetric component

Table 2 Procrustes ANOVA for both CS and SH of *Ceroglossus chilensis*.

		SS	MS	*df*	*F*	*P*
CS	SD	0.000001	0.000001	1	2.41	0.1234
	VP	0.000001	0.000001	1	0	0.9501
	FA	0.000025	0.00001	114		
SH	SD	0.013744	0.00091626	15	11.76	<0.0001
	VP	0.0044254	0.00029502	15	3.79	<0.0001
	FA	0.1332845	0.00007794	1,710	2.6	<0.0001
	DA	0.0114243	0.00076162	15	25.41	<0.0001

Units of Procrustes distances: dimensionless.

CS centroid size, *SH* shape, *SS* sum of squares, *MS* mean squares, *VP* variation between populations (Coast Range and Andes Foothills), *FA* fluctuating asymmetry, *DA* directional asymmetry, *SD* sexual dimorphism.

(individual variation) showed differences between the two populations analyzed associated with a thicker abdominal section and a perceptible elongation of the pronotal segment in males. The first three principal components (PCs) accounted for 53.64% (PC1+PC2+PC3=27.62%+14.88%+11.14%) of the total shape variation and provided a reasonable approximation of the total amount of variation, with the other PCs each accounting for no more than 9.5% of the variation. The canonical analysis showed a clear differentiation of sexual shape dimorphism in both populations (Fig 2).

Discussion

The aggressive conduct of a pioneer species, such as *P. radiata*, is probably adequate for the colonization and habilitation of recent or eroded soils; however, in other soils, its effects must be neutralized with silvicultural measures and improvement of the soil to maintain medium and long range fertility to avoid possible effects on the entomofauna which coexists in the secondary growth of native

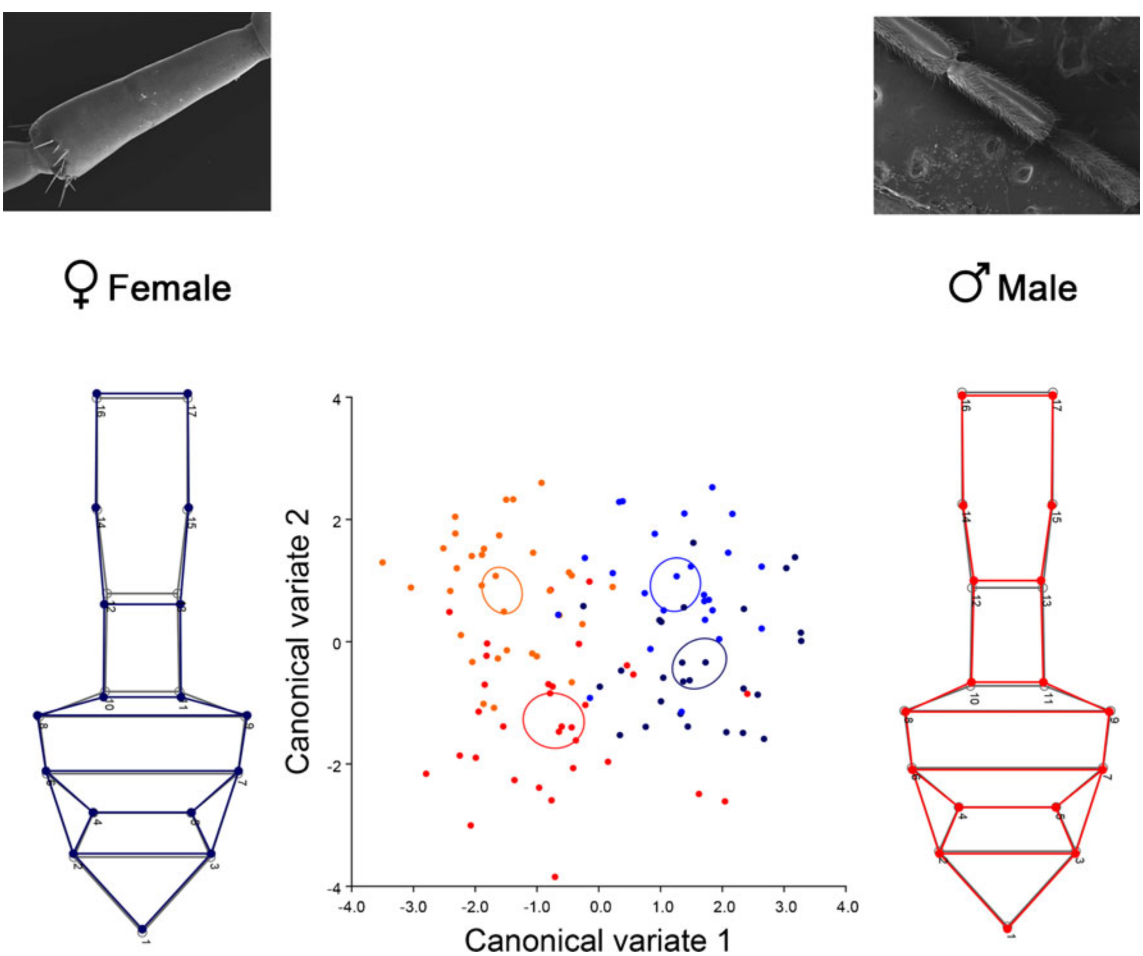

Fig 2 CVA for the sexual shape dimorphism population of *Ceroglossus chilensis* (each point represents a shape variable for female and male individuals in ventral view). The figure shows the first two PCs' axes with shape deformation images associated and their antennal structure that is a distinguishing characteristic (careens present in males).

forests present in these plantations (Schlatter & Otero 1995).

Following the recommendation of Benítez *et al* (2008) for a more exhaustive evaluation of characters related to the fitness of the species in pine plantations, the results of this study show that the populations of *C. chilensis* analyzed present evidence of FA in body shape, thus confirming the asymmetric character of the populations which live under the canopy of *P. radiata* plantations. The advantage of these new analyses was due to the incorporation of tools of geometric morphometrics which decrease the measurement errors in the samples evaluated (Klingenberg & McIntyre 1998).

Polak (2003) gave a number of examples which related fitness to instability during embryonic development (understood as the result of a set of small stochastic accidents that tend to alter the precision of development in a given environment). Among these, the form of the body and also the asymmetrical antennae would negatively affect the capture of prey and the finding of a mate, probably reflecting the effect that the type of management of the plantation has on the ontogeny of individuals (more acidified soils have less presence of microfauna and macrofauna). This would generate an active migration of species; large numbers of *C. chilensis* were found near to and somewhat distant from these plantations (they may migrate more than 10 km) in the search for less altered undergrowth (Briones & Jerez 2007).

The slight sexual dimorphism of the species is known for the group. This study verified that, in both populations from different ranges submitted to different environmental pressures, the results for sexual dimorphism are consistent with those already published (Benítez *et al* 2010) (Fig 2). *C. chilensis* appear not to modify their reproductive style in which the chance of a male to have a sexual partner is almost 1. It is not necessary for males to generate showy sexual attributes, thus avoiding a greater energetic cost. Another important character in this genus is that *C. chilensis* are apterous, implying limited variability, which in turn would restrict the search for a sexual partner (Lailvaux & Irschick 2006). Thus, the geometric morphometrics analysis confirmed the patterns of FA associated with the stress produced by thinning of the plantations of *P. radiata* on the body shape of *C. chilensis* in both populations. It must be emphasized that the populations inhabit different environments: a more humid environment in the Coast Range and a drier environment with drier soils in the Andes Foothills. However, these environmental differences reflected minimum phenotypic variation, which was detected at the level of population variation and which may be associated with patterns of phenotypic plasticity.

Acknowledgments The author thanks Dr. Viviane Jerez and Dr. Raul Briones for their crucial collaboration in sampling and obtaining individuals for this study. I also thank Dr. Lafayette Eaton for translating the manuscript and Mrs. Fiorella Vallebuona for providing good advice in the statistics and format of the manuscript.

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