Patterns of asymmetry in body traits and genitalia in two distant populations of a Neotropical scorpion

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The ability of a genotype to control the stable development of a phenotype under different environmental conditions may explain the level of developmental instability. An indicator to developmental instability is Fluctuating Asymmetry. Traits under pressures of sexual selection exhibit greater values of asymmetry than others. These traits have more physiological requirements during morphogenesis and a more complex development. The aims of this work are to analyze asymmetry patterns of genitalic and somatic traits in the scorpion *Bothriurus bonariensis* (Bothriuridae) and to compare these values between central and peripheral populations of its distribution. We observed that types of asymmetry in genitalic traits differed in both populations. Also, traits of the hemispermaphores (two halves that constitute the spermatophore) showed the highest levels of fluctuating asymmetry, particularly in those characteristics with important role for insemination success. This work offers fundamental baseline knowledge for further works focused on asymmetry of genitalia and mating success in scorpions.

Keywords: arachnids; *Bothriurus bonariensis*; developmental instability; selective pressure; South America

Introduction

Stress can be characterized as a force shaping adaptation and evolution in changing environments, and it is a property of both the stressor and the stressed (Bijlsma and Loeschke 2005). Stressful conditions of the environment on the physiology of a species may limit the distribution range of the species. In many species, peripheral populations suffer stressful environmental conditions, and in this context, high metabolic costs are enough to limit expansions of their living range (McNab 1973; Root 1988a, 1988b; Alerstam 1990; Dennis 1993). Environmental conditions restrict species distribution range not only physiologically but also genetically (Møller and Swaddle 1997; Kark et al. 2004). The ability of a genotype to control the stable development of a phenotype under a range of environmental conditions may explain the level of developmental stability of a particular trait (Møller et al. 1995). A wide variety of environmental and genetic factors may result in a reduction of the developmental stability that makes individuals less efficient in terms of growth, survival and reproduction (Graham et al. 2000; Santos et al. 2006; Vishalakshi and Singh 2008a, 2008b).

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A measure of developmental instability is fluctuating asymmetry, which is defined as small random differences between left and right sides of a bilateral trait. Indeed, this is a result of the inability of individuals to undergo identical development of both sides of a bilaterally symmetrical trait (Palmer and Strobeck 1992; Møller and Pomiankowski 1993; Swaddle et al. 1994; Møller 1997; Peretti et al. 2001; Leung et al. 2003; Helle et al. 2011). When an individual faces a sub-optimal set of environmental conditions, the developmental process becomes unstable and the levels of fluctuating asymmetry grow (Vishalakshi 2011). Other forms of asymmetry that can occur are Directional Asymmetry and Antisymmetry. The former occurs when there is a tendency for one side of an organism to develop more than the other (Van Valen 1962). In contrast, Antisymmetry shows one side of a character larger than the other but not handed-bias as to which side of the character will be bigger (Timofeeff-Ressovsky 1934; Graham et al. 1993, 2000; Palmer 2004, 2005). Fluctuating asymmetry is widely used to assess developmental instability; great differences appear in some fluctuating asymmetry values of different populations of a species (Picton et al. 1990; Møller 1993), as well as in the periphery of a distribution range (Parsons 1993; Kark 2001). In addition, traits influenced by sexual selection may exhibit greater values of fluctuating asymmetry in comparison with other traits in which such type of selection does not occur (Møller and Eriksson 1994; Peretti et al. 2001; Santos 2001; Uetz et al. 2009). An explanation for this trend is that many traits under sexual selection have more physiological requirements during morphogenesis and a more complex development (Pomiankowski et al. 1991).

Developmental instability has been explored in a wide variety of taxa (Lens and Van-Dongen 2000; Hendrickx et al. 2003; Vishalakshi and Singh 2008a, 2008b, 2008c; Băncilă et al. 2010; Helle et al. 2011). In arthropods, Huber et al. (2007) make an interesting review on genitalic evolution in spiders and insects, focusing on conspicuous asymmetry of sexual traits rather than minor random changes like fluctuating asymmetry. Among insects, fluctuating asymmetry patterns are not uniform. For example, in crickets and beetles habitat fragmentation is unrelated to fluctuating asymmetry (Berggren 2005; Henríquez et al. 2009). On the other hand, in *Drosophila* fluctuating asymmetry increases because of the influence of environmental stressors (Graham et al. 2000; Santos et al. 2006; Vishalakshi and Singh 2008a, 2008b).

Among arachnids, scorpions are a good model to study fluctuating asymmetry, in particular in genitalic traits. They show indirect sperm transfer by a sclerotized spermatophore. The spermatophore consists of two halves (hemispermatothores) produced by the male's paraxial organs. These two halves join to exit through the gonopore to form the spermatophore, immediately prior to sperm transfer (Hjelle 1990). So, if the two halves are developed differently, the spermatophore will show more asymmetry. This spermatophore is relatively complex and, from previous studies, it is known that certain structures may vary in size and shape (Peretti 1993). Peretti et al. (2001) examined some patterns of asymmetry in sexual and non-sexual traits in a population of *Bothriurus bonariensis* (Bothriuridae). This species has a wide distribution in South America, ranging from southeast Brazil to central Argentina, with a core population in Uruguay (Mattoni and Acosta 2005; Ojanguren Affilastro 2005). In Argentina, *B. bonariensis* inhabits the provinces of Entre Ríos, Corrientes, Buenos Aires, Santa Fe, La Pampa, Córdoba and San Luis. This area belongs to 'the Pampa' and 'the Espinal' phytogeographic provinces (Ojanguren Affilastro 2005) (Figure 1), both showing different environments depending on vegetation, soil and

Figure 1. Geographic distribution of *Bothriurus bonariensis*. Black circles indicate the total distribution of the species known to date; grey grid squares show the populations used in this study.

climate (Cabrera and Willink 1973), factors that could affect development in this population on different ways (Kark et al. 2004; Olivero et al. 2012). The habitat of the Mendiola population (peripheral population in Córdoba Province) belongs to the phytogeographical province of the Espinal, while the Piedras de Afilar population (core population in Uruguay) corresponds to the phytogeographical province of Pampa. In comparison, the climate is wetter in the latter with a greater level of rains and lower temperatures (Cabrera and Willink 1973; Argentina's national meteorological service -online www.smn.gov.ar; Uruguay's National direction of meteorology -online www.meteorologia.com.uy) (Figure 2).

Along its distribution, populations of this species show differences in colouring patterns and a wide range of sizes in morphological and sexual traits (Ojanguren Affilastro 2005; Olivero et al. 2012). This variation could be related to environmental characteristics that change throughout the species' range. Scorpion species belonging to wet zones show a greater amount of pigment than species inhabiting drier zones (Lourenço and Cloudsley-Thompson 1996; Mattoni 2002).

The aims of this work are to detect and analyze asymmetry patterns of genitalic and somatic traits in the scorpion *Bothriurus bonariensis* (Bothriuridae) and to compare these values between two different populations (central and peripheral) of its distribution. Our study focuses on two principal lines: (1) Given that the peripheral population may suffer more stressful conditions compared with core populations (Moller 1995; Auffray et al. 1999), we would expect to observe higher levels of asymmetry in the peripheral population; (2) Since traits under sexual selection tend to show higher levels of fluctuating asymmetry due to a higher sensitivity and physiological requirements for development (Pomiankowski et al. 1991), we would expect to find higher levels of asymmetry in spermatophore and sexually selected traits in comparison with other body traits.

Figure 2. Mean temperature and annual rainfall in Mendiolaza (Argentina) and Piedras de Afilas (Uruguay) during 2010–2011.

Materials and methods

Study material

We analyzed specimens of *B. bonariensis* from two distant populations selected in order to represent the core and periphery of their distribution (Figure 1). We selected populations from which specimens were abundant (allowing statistical comparisons) and well preserved (poor preservation alters the hemispermatozoon form) (Olivero

et al. 2012). Selected populations were the following. Core population: Piedras de Afilar (Canelones province, Uruguay – 34°43'0,44" S, 55°31'59,79" W, 40 m.a.s.l), 16 males, 10 females, 16 pair of hemispermatothores; Peripheral population: Mendiola (Córdoba Province, Argentina – 31°15' 52,59" S, 64°18'10" W, 540 m. a.s.l), 20 males, 11 females, 20 pairs of hemispermatothores (Figure 1). The Mendiola population was considered the peripheral population because of its location and also by the climatic conditions of the area, which has an arid climate with high temperatures and low humidity. By contrast, the Piedras de Afilar population lives in a wetter and cooler climate with more favourable conditions (Figure 2), therefore was considered the core population, although this is not exactly located in the centre of the total distribution of the species (Cabrera and Willink 1973; Argentina's national meteorological service -online www.smn.gov.ar; Uruguay's National direction of meteorology -online www.meteorologia.com.uy).

Measurements of individuals

To compare different populations of *B. bonariensis*, we followed the biological species concept (Mayr 1942). Although it is known that individuals of these two populations have significant morphological differences (Mattoni 2003; Ojanguren Affilastro 2005; Olivero et al. 2012) and probably could be considered as different species, this separation is not yet confirmed. For this reason, we consider these populations as one species. All study specimens were preserved in 80% ethanol. Both hemispermatothores from each male specimen were removed, cleaned by hand and preserved following Sissom et al. (1990). Morphological traits were measured in males and females and genitalic traits corresponded to both hemispermatothores (Peretti et al. 2001). The female has an ovoid and flexible genital atrium and two spermathecae which increase in size with successive inseminations (Peretti 2003). These traits were not included in this work because their elasticity makes confident measurements difficult. Sixteen traits were analyzed in males (four morphological and 12 from hemispermatothores) and four traits in females (Figure 3). Some measurements

Figure 3. Body and genitalic traits measured in males and females of *Bothirurus bonariensis*.

were also used in a systematic study of Bothriuridae and for the description of spermatophores and hemispermaphores (Acosta 1989; Peretti 1992, 1993, 1995). Moreover, they are characters that have relevance in the context of sexual biology in general (Peretti 1992; Peretti et al. 2001; Peretti 2003). Each trait was measured twice by only one person, in order to compensate for measuring errors. Fluctuating asymmetry can be confused by measurement error, therefore repeat measurement should be undertaken to ensure that fluctuating asymmetry is detectable (Palmer and Strobeck 2003). Specimens were analyzed using a stereoscopic dissecting microscope (Nikon SMZ1500), and pictures were taken using a digital camera (Nikon Digital Sight DS-Fi1). Digital pictures were analyzed with the Image Tool 3.0 measuring software (© UTHSCSA 1996–2002).

Statistical analyses and types of asymmetry

Statistical analyses were performed with the PC program NCSS 2007 (© Hintze 2007). To control for measurement errors, a repeated measures ANOVA was used. All measurements of the variables were significantly repeatable (Table 1 in Appendix). For analyses and evaluation of the magnitude of asymmetry of each trait, the absolute level of asymmetry was estimated as the unsigned left-minus-right character value. Relative asymmetry was estimated as absolute asymmetry divided by the mean left and right character value. Results are expressed as percentage of relative asymmetry (relative asymmetry value was multiplied by 100). To test if the signed left-minus-right character value fit a normal distribution, Shapiro–Wilks and Kolmogorov–Smirnov one-sample tests were used (Siegel and Castellan 1988). One-sample *t*-test was employed to establish the presence of directional asymmetry to data with normal distribution. Data with non-normal distribution were considered antisymmetry. To control the relationship between the absolute asymmetry and the mean size of sides (left and right) a linear regression analysis was performed. Three linear regressions were performed for individuals of each of the populations considering the traits showing higher levels of asymmetry: width of capsular lobe, length of lateral edge and length of crest. In each regression, independent variable was the size of the feature, and dependent variable was the absolute value of absolute asymmetry in the trait.

Multivariate analyses

To compare relative asymmetry of selected traits between the core and peripheral populations, we performed a multivariate discriminant analysis (Fisher 1936; Fukunaga 1990; Duda et al. 2001). Discriminant analysis results in a set of prediction equations (linear functions) based on independent variables that are used to classify individuals into groups. As an estimating method, the discriminant lineal function was used without setting *a priori* probabilities and without selecting variables. Canonical Correlation Coefficient and Wilks' Lambda Statistical were calculated, which allowed us to choose the most appropriate model for all models that can be constructed from the variables previously selected. Both ratios measure the differences between the groups due to discriminant functions (El Ouardighi et al. 2007). This technique allowed us to analyze if there was a separation among populations

considering the studied variables and to determine the influence of each variable on the population discrimination level (Brown and Wicker 2000; Vignoli et al. 2005).

Results

Types and levels of asymmetry

Core population

In the Piedras de Afilar population, both males and females showed fluctuating asymmetry in most of the analyzed traits. In traits with directional asymmetry, this was always to the left side (Tables 1 and 2). Three of the four somatic traits measured in males showed directional asymmetry (hand length, hand height and movable finger length). In females, three of the four somatic traits showed fluctuating asymmetry (hand length, hand width, and movable finger length) (Table 1). Genitalic traits had higher values of asymmetry than somatic traits (mean of genitalic traits = 6.7%, mean of somatic traits of males = 1.6%, mean of somatic traits of females = 2.7%). The genital trait that showed highest levels of asymmetry was the distance of dorsal fold–lateral edge (24%). Then, length of lateral edge showed a level of asymmetry of 10%, length of crest showed 9% and width of capsular lobe showed 12% (Table 2, Figure 4).

Peripheral population

In the Mendiolaza population the results were similar. Tables 1 and 2 show that most of the analyzed traits showed fluctuating asymmetry. Somatic traits in males had fluctuating asymmetry in three of the four measured traits (hand height, hand width and movable finger length), while one had antisymmetry (hand length). Regarding somatic traits of females, two of the four measured traits showed antisymmetry (hand length and hand height), one showed fluctuating asymmetry (movable finger length) and the other showed left directional asymmetry (hand width). Genitalic traits also had higher values of fluctuating asymmetry than somatic traits in this population (mean of genitalic traits = 6.9%, mean of somatic traits of males = 3%, mean of somatic traits of females = 2.6%). Distance of dorsal fold–lateral edge was the feature with highest level of asymmetry (16%). Length of lateral edge showed a level of asymmetry of 12%, length of crest showed 12% and width of capsular lobe showed 9% (Table 2, Figure 4).

Comparison between populations

In both studied populations, most of the analyzed traits showed fluctuating asymmetry or antisymmetry. Traits of hemispermaphores presented the highest values of fluctuating asymmetry, whereas values of somatic traits were lower. The genitalic character ‘distance of dorsal fold–lateral edge’ showed the greatest percentage of fluctuating asymmetry. Both populations showed values of asymmetry higher than 16% in this character (Table 2). Other important genitalic traits that showed high values in the level of asymmetry in both populations were length of lateral edge, length of crest and width of capsular lobe (Table 2, Figure 4).

Directional asymmetry in genital traits occurs in different traits of both populations. In peripheral population, length and width of the lamina showed directional asymmetry, while in the core population these traits had fluctuating asymmetry (median width of the

Table 1. Type, level of relative asymmetry (percentage) and standard deviation for morphological traits measured in males and females in the core and peripheral populations of the *Bothriurus bonariensis*.

Populations		Mendiolaza			Piedras de Afilar	
Type of asymmetry		AS	DA	FA	DA	FA
Males	Hand length	2.326 ± 1.9			(L) 1.644 ± 1.3 <i>p</i> < 0.001	
	Hand height			1.795 ± 1.7 <i>p</i> = 0.098	(L) 4.678 ± 3.6 <i>p</i> < 0.001	
	Hand width			1.709 ± 1.0 <i>p</i> = 0.062		1.821 ± 1.6 <i>p</i> = 0.343
	Movable finger length			6.202 ± 4.9 <i>p</i> = 0.474	(L) 6.021 ± 4.2 <i>p</i> < 0.001	
Females	Hand length	2.076 ± 1.5				1.906 ± 1.2 <i>p</i> = 0.196
	Hand height	2.173 ± 1.9			(L) 2.696 ± 1.4 <i>p</i> < 0.001	
	Hand width		(L) 1.119 ± 0.9 <i>p</i> = 0.037			1.603 ± 1.3 <i>p</i> = 0.104
	Movable finger length			5.164 ± 2.8 <i>p</i> = 0.149		4.618 ± 2.3 <i>p</i> = 0.412

Abbreviations: AS: antisymmetry (non-normal distribution). DA: directional asymmetry. FA: fluctuating asymmetry. R: directional asymmetry biased to right side. L: directional asymmetry biased toward left side.

Table 2. Type, level of relative asymmetry (percentage) and standard deviation for male's genital traits measured in the core and peripheral populations of *Bothriurus bonariensis*.

Populations		Mendiolaza			Piedras de Afilar		
Type of asymmetry	AS	DA	FA	AS	DA	FA	
Lamina length		(R) 3.513 ± 3.3 <i>p</i> = 0.019		2.184 ± 1.4			
Lamina width (distal)		(R) 3.44 ± 3.1 <i>p</i> = 0.027		2.521 ± 2.5			
Lamina width (median)		(L) 1.938 ± 2.1 <i>p</i> = 0.019				1.936 ± 1.8 <i>p</i> = 0.268	
Lamina width (basal)			6.555 ± 4.5 <i>p</i> = 0.051			5.138 ± 4.0 <i>p</i> = 0.488	
Trunk length			4.574 ± 4.5 <i>p</i> = 0.338			1.811 ± 2.6 <i>p</i> = 0.210	
Capsular lobe length			3.566 ± 2.7 <i>p</i> = 0.158			3.385 ± 3.4 <i>p</i> = 0.424	
Capsular lobe width	8.597 ± 7.6					11.814 ± 7.5 <i>p</i> = 0.106	
Dorsal fold length			5.493 ± 4.8 <i>p</i> = 0.130	3.988 ± 4.0			
Dorsal fold width	5.709 ± 4.9				(L) 4.645 ± 3.4 <i>p</i> = 0.008		
Distance of dorsal fold–lateral edge			16.412 ± 13.7 <i>p</i> = 0.255		(L) 23.79 ± 22.1 <i>p</i> = 0.049		
Lateral edge length			12.011 ± 10.1 <i>p</i> = 0.118			10.248 ± 8.2 <i>p</i> = 0.470	
Crest length	11.85 ± 10.3					9.034 ± 6.6 <i>p</i> = 0.348	

Abbreviations: AS: antisymmetry (non-normal distribution). DA: directional asymmetry. FA: fluctuating asymmetry. R: directional asymmetry biased to right side. L: directional asymmetry biased toward left side.

Figure 4. Level of relative asymmetry in genital traits of males of *Bothriurus bonariensis* in studied populations. White boxes show the values of the Mendiolaza population (peripheral), while grey boxes show the values of the Piedras de Afilar population (core).

lamina) and antisymmetry (length and distal width of the lamina). On the other hand, the core population showed directional asymmetry in traits such as dorsal fold width and distance of dorsal fold–lateral edge, whereas that in the Mendiolaza population had antisymmetry and fluctuating asymmetry, respectively (Table 2).

Relationship between asymmetry and trait size

Of three linear regressions that relate the asymmetry with trait size in peripheral population, no significant results were obtained (Table 3). However, in the core population, the length of crest showed a positive relationship between antisymmetry and trait size: antisymmetry is greater in longer hemispermatothores crests (Table 3).

Table 3. Linear regressions between size and asymmetry of spermatophore variables of *Bothriurus bonariensis*.

	Traits	R ²	Slope	Est.	F	p
Mendiolaza	Capsular lobe width	0.022	0.209	0.005	0.365	0.554
	Lateral edge length	0.028	−0.089	0.005	0.494	0.492
	Crest length	0.630	1.078	0.017	25.501	0.0001
Piedras de Afilar	Capsular lobe width	0.089	0.323	0.001	1.369	0.261
	Lateral edge length	<0.001	−0.007	0.015	0.001	0.969
	Crest length	0.002	0.028	0.006	0.032	0.860

Multivariate analysis

The discriminant analysis showed significant differences between the core and peripheral populations only for relative asymmetry of the somatic traits of males ($F = 4.7$, $p = 0.004$, Wilks lambda = 0.623; Figure 5). The most influential trait in this separation was the length of movable finger (Table 2 in Appendix). Instead, we found no significant differences between populations in genitalic traits of males ($F = 1.1$, $p = 0.448$, Wilks lambda = 0.573) or somatic traits of females ($F = 0.5$, $p = 0.714$, Wilks lambda = 0.883). However, genital variables of males that most

Figure 5. Spatial separation of populations of *Bothriurus bonariensis* according somatic traits of males as Canonical Discriminant Analysis (Score 1: $F = 4.7$, $p = 0.004$, Wilk's lambda = 0.6228).

influenced a separation among populations were the dorsal fold length, the distance of dorsal fold–lateral edge, the length of lateral edge and the width of capsular lobe (Table 3 in Appendix). The last three also had higher values of relative asymmetry (see Table 2).

Discussion

Types and levels of asymmetry

Asymmetry within populations

Peretti et al. (2001) demonstrated that male genitalia of *Bothriurus bonariensis* could vary between some populations; in Argentina, males from Córdoba (peripheral distributions of the species) showed greater values of fluctuating asymmetry compared with specimens from southern Buenos Aires. In addition, in the Córdoba population, hemispermatophores are shorter as well as capsular lobes and dorsal folds, compared with population in the core of the geographic distribution (Uruguay population) of the species (Olivero et al. 2012). In this study, although we did not find differences in levels of asymmetry, we observed that types of asymmetry in genitalic traits differed in both populations. A strong developmental instability not only produces changes in the levels of fluctuating asymmetry but also translates into a transition from fluctuating asymmetry to other forms of asymmetry (Lens and Van Dongen 2000; Schneider et al. 2003; Leamy and Klingenberg 2005). Data concerning other groups of animals (Møller and Swaddle 1997) show that there could be evolutionary transitions between different types of asymmetries in response to intense directional selection. In this sense, asymmetry in hemispermatophores may reflect developmental instability, possibly due to a higher physiological sensitivity to environmental stress on the paraxial organs that produce the hemispermatophores (Peretti et al. 2001).

On the other hand, according to the discriminant analysis, somatic traits of males show a significant separation in the two populations studied, and the most influential variable in this separation is the length of movable finger. These differences could be caused by different environmental pressures in the two populations. Somatic traits analyzed in this study involved measurements of the pedipalp hands. The pedipalps are instrumental in prey capture, and they are the platform for one of the primary sensory systems in scorpions (Hjelle 1990). But also, pedipalps are sexually dimorphic and are involved in courtship, where the male leads the female in a classical *promenade à deux* until a suitable location is found for depositing the spermatophore and transferring sperm (Polis and Sissom 1990). For this reason, pedipalp hands also could be under the influence of sexual selection.

Asymmetry of genitalic and somatic traits

Another aspect associated with the possibility of evolutionary transitions between types of asymmetry is the following: the sister species of *B. bonariensis*, *B. chacoensis* Maury and Acosta (1993) presents a spermatophore rather similar to that of *B. bonariensis*, except that it possesses a long distal filament in the right capsular lobe. This is a case of directional asymmetry, as observed in the left lobe and other capsular structures of *Brachistosternus* species (Maury 1975; Acosta 1989; Peretti 1993). Some

B. bonariensis males have rudiments of different lengths of a filament similar to that in *B. chacoensis*, perhaps representing different evolutionary stages (Peretti et al. 2001). This could indicate that genitalic traits in this species may be particularly sensitive to developmental instability, either by sexual selection or by stressful environmental conditions. In the present study, male genitalia shows highest levels of fluctuating asymmetry in both populations, especially in traits with an important role for sperm transfer success such as the dorsal fold, lateral edge and crest (Peretti 1992; Peretti et al. 2001; Peretti 2003). Although no significant differences were detected in fluctuating asymmetry of core and peripheral populations, the dorsal fold, lateral edge and capsular lobe are traits that may have greater influence on the separation of populations, as discriminant analysis showed. Indeed, the inner face of female operculum fits against the dorsal fold of the lamina. Along with the fit of the female operculum with the lamina, the external part of the capsule (which is represented in this stage by two capsular lobes) enters the genital atrium. This initial intromission ensures that capsular eversion takes place inside the atrium. The medial region of lamina always fits in the intercoxal space of the female's legs, and the crest helps to fit the distal part of lamina (Peretti 2003). The developmental stability of these traits could be altered as a result of sexual selection. Indeed, sexual selection can promote a greater directional selective pressure, and by this means may increase the level of developmental instability (Clarke 1997). Secondary sexual traits will show higher values of asymmetry than morphological traits under stabilizing selection (Møller 1993; Møller and Eriksson 1994; Hunt and Simmons 1998; Peretti et al. 2001; Rasmuson 2002; Vishalakshi and Singh 2006), due to the effects of genomic stress on developmental stability that are imposed by their different mode of selection (Møller and Pomiankowski 1993). Sexual selection can relax the internal mechanisms of control in the morphogenetic development of a character, leading to increased phenotypic variation and increased susceptibility to environmental stress (Møller and Hoglund 1991; Møller 1993).

On the other hand, in scorpions the hemispermatophores are formed in paired secretory sacs called 'paraxial organs' (Farley 2001). These paraxial organs, as part of the reproductive system, are present only in adult males (Hjelle 1990; Farley 2001). For this reason, the development of the hemispermatophores can be indirectly affected by environmental stresses experienced by sub-adults. Indeed, environmental pressures leading to fluctuating asymmetry may be affected by stresses at the time of sexual maturation, when the paraxial organs develop (sub-adult stage) and/or the shorter time between matings during which the each set of hemispermatophores develop (adult stage). In contrast, the pedipalp hands are present in all the stages of development and therefore the environmental pressures affect these structures throughout development. However, they are more likely to be reflective of stresses shortly before and during the moulting stages, since these are the actual stages of development of the new cuticle. Therefore, although all structures are subjected to environmental pressures, each is affected differently according to their development time.

The study of the symmetry of hemispermatophores represents an interesting area, since these structures offer an opportunity to investigate variations in the patterns of asymmetry on the adult male, where local environmental stresses may show short-term fluctuations. For example, further studies could examine the asymmetry patterns between sets of hemispermatophores produced during the male's reproductive life.

Relationship between asymmetry and trait size

Fluctuating asymmetry has been found to be a good predictor of mating success and sexual attractiveness in fish, birds and insects (Tomkins and Simmons 2003; Gross et al. 2007). The fluctuating asymmetry may reflect the genome's ability to resist noise development, and thus indicates genetic quality. Therefore, individuals with larger and more symmetrical traits would have higher reproductive success (Andersson 1994), and females could use those traits to assess male quality (Møller 1993; Møller and Pomiankowski 1993; Andersson and Simmons 2006). Some studies have demonstrated that the production of larger traits is more expensive compared with that for smaller traits, and only bigger specimens with a better genetic value could develop symmetrical traits (Møller 1993; Møller and Swaddle 1997). However, the associations between the size and symmetry traits, especially sexual traits, have been considerably debated (Evans and Hatchwell 1993; Tomkins and Simmons 1995; Clarke 1998; Bjorksten et al. 2000).

In this study, only one of linear regressions performed had significant results, showing a positive relationship between the length of crest hemispermatophore and antisymmetry, and it has been observed that a morphological trait's size is constant, with a non-significant relation with body size (Olivero unpub. data). Therefore, it is possible that smaller specimens could produce large traits but with greater levels of asymmetry. Other studies have found no relationship between size of traits and fluctuating asymmetry (Tomkins and Simmons 1995; Hosken 2001). Another alternative could be that these levels of asymmetry may be the result of mechanical function selecting against asymmetry, and the ability of each individual to maintain symmetry during development (David et al. 1998; Hunt and Simmons 1998). Further experimental studies are needed in *Bothriurus bonariensis* to analyze the relationship between the asymmetry of the spermatophore with mating success, since it represents an interesting subject that is poorly understood in scorpion biology. The present work offered fundamental baseline knowledge for that purpose.

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APPENDIX

Table 1. Repeated Measures ANOVA in males and females of *Bothriurus bonariensis* in the core and peripheral populations.

Populations	Mendiolaza				Piedras de Afilar			
	Right Side		Left Side		Right Side		Left Side	
	F	p	F	p	F	p	F	p
Males								
Hand length	1.26	0.276	0.276	0.276	1.22	0.287	0.287	0.287
Hand height	2.57	0.125	0.125	0.125	0.48	0.497	0.497	0.497
Hand width	0.76	0.393	0.393	0.393	3.29	0.089	0.089	0.089
Movable finger length	3.27	0.086	0.086	0.086	1.52	0.236	0.236	0.236
Lamina length	0.01	0.920	0.920	0.920	1.75	0.207	0.207	0.207
Lamina width (distal)	2.28	0.148	0.148	0.148	2.30	0.149	0.149	0.149
Lamina width (median)	0.01	0.916	0.916	0.916	0.10	0.750	0.750	0.750
Lamina width (basal)	0.42	0.525	0.525	0.525	0.03	0.864	0.864	0.864
Trunk length	0.66	0.427	0.427	0.427	0.00	1.000	1.000	1.000
Capsular lobe length	0.85	0.369	0.369	0.369	1.18	0.295	0.295	0.295
Capsular lobe width	0.05	0.832	0.832	0.832	0.18	0.676	0.676	0.676
Dorsal fold length	0.01	0.942	0.942	0.942	2.00	0.177	0.177	0.177
Dorsal fold width	1.67	0.212	0.212	0.212	0.00	1.000	1.000	1.000
Distance of Dorsal fold-Lateral edge	4.12	0.057	0.057	0.057	3.00	0.103	0.103	0.103
Lateral edge length	0.38	0.545	0.545	0.545	0.01	0.907	0.907	0.907
Crest length	1.50	0.238	0.238	0.238	1.52	0.236	0.236	0.236
Females								
Hand length	0.88	0.370	0.370	0.370	1.45	0.259	0.259	0.259
Hand height	1.93	0.195	0.195	0.195	0.71	0.422	0.422	0.422
Hand width	0.00	1.000	1.000	1.000	1.82	0.209	0.209	0.209
Movable finger length	0.49	0.501	0.501	0.501	1.22	0.298	0.298	0.298

Table 2. Influence of somatic traits analyzed in the Canonical Discriminant Analysis in males of the studied populations of *Bothriurus bonariensis*.

Variable Influence Section		
Variable	F	p
Movable finger length	14.57	0.0005
Hand heigth	12.41	0.0012
Hand length	4.89	0.0338
Hand width	0.35	0.5567

Table 3. Influence of genital traits considered in the Canonical Discriminant Analysis for group separation in males of the studied populations of *Bothriurus bonariensis*.

Variable Influence Section		
Variable	F	p
Dorsal fold length	2.27	0.143
Distance of dorsal fold-lateral edge	1.91	0.178
Lateral edge length	1.5	0.230
Capsular lobe width	1.31	0.262
Lamina length	1.15	0.292
Lamina width (distal)	1.06	0.313
Lamina width (basal)	0.94	0.341
Lamina width (medial)	0.84	0.366
Trunk length	0.68	0.415
Crest length	0.37	0.549
Dorsal fold width	0.24	0.630
Capsular lobe length	0.17	0.686