



Biology, Ecology and Diversity

## Constant fluctuating asymmetry but not directional asymmetry along the geographic distribution of *Drosophila antonietae* (Diptera, Drosophilidae)



Marcelo Costa<sup>a,\*</sup>, Rogério P. Mateus<sup>a</sup>, Mauricio O. Moura<sup>b</sup>

<sup>a</sup> Departamento de Ciências Biológicas, Universidade Estadual do Centro Oeste, Guarapuava, PR, Brazil

<sup>b</sup> Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, PR, Brazil

### ARTICLE INFO

#### Article history:

Received 27 February 2015

Accepted 9 September 2015

Available online 9 October 2015

Associate Editor: Márcio R. Pie

#### Keywords:

Developmental instability

Generalized Procrustes Analysis

Geographical variation

Geometric morphometrics

Shape

### ABSTRACT

The population dynamics of a species tends to change from the core to the periphery of its distribution. Therefore, one could expect peripheral populations to be subject to a higher level of stress than more central populations (the center–periphery hypothesis) and consequently should present a higher level of fluctuating asymmetry. To test these predictions we study asymmetry in wing shape of five populations of *Drosophila antonietae* collected throughout the distribution of the species using fluctuating asymmetry as a proxy for developmental instability. More specifically, we addressed the following questions: (1) what types of asymmetry occur in populations of *D. antonietae*? (2) Does the level of fluctuating asymmetry vary among populations? (3) Does peripheral populations have a higher fluctuating asymmetry level than central populations? We used 12 anatomical landmarks to quantify patterns of asymmetry in wing shape in five populations of *D. antonietae* within the framework of geometric morphometrics. Net asymmetry – a composite measure of directional asymmetry + fluctuating asymmetry – varied significantly among populations. However, once net asymmetry of each population is decomposed into directional asymmetry and fluctuating asymmetry, most of the variation in asymmetry was explained by directional asymmetry alone, suggesting that populations of *D. antonietae* have the same magnitude of fluctuating asymmetry throughout the geographical distribution of the species. We hypothesize that larval development in rotting cladodes might play an important role in explaining our results. In addition, our study underscores the importance of understanding the interplay between the biology of a species and its geographical patterns of asymmetry.

© 2015 Sociedade Brasileira de Entomologia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### Introduction

Any species is faced with a variety of climates and environments throughout its geographical distribution, resulting in different levels of stress and selective pressures (Brown et al., 1996; Bridle and Vines, 2007). These factors can affect genetic and phenotypic traits, thus generating clines or discontinuities throughout the distribution (Hoffmann and Shirriffs, 2002).

Models of morphological development have emphasized the interaction of a variety of components in an organism to generate a functional structure under a set of conditions (Klingenberg et al., 1998). However, stressing factors such as variation in nutrition, temperature, population density, pollutants, and habitat

fragmentation can lead to developmental instability (DI) (Moller and Swaddle, 1997; Lens et al., 1999; Hosken et al., 2000). Given that such perturbations will be visible at the level of the phenotype, the presence of phenotypic changes can indicate the extent to which an organism is responding to its stressors (Hosken et al., 2000). In this context, the pattern of symmetry of bilateral structures has been widely used as a marker for developmental instability (Moller and Swaddle, 1997). Given that both sides of an organism are under the control of the same genetic pathways during development, one might expect that any deviation from symmetry might be the product of local disturbances that would break developmental homeostasis (Hosken et al., 2000; Breuker et al., 2006).

The asymmetry can be described by the frequency distribution of the difference between the left and the right sides of the individuals of a population (Palmer and Strobeck, 1986). In general, there are three main types of bilateral asymmetry: fluctuating asymmetry (FA), directional asymmetry (DA) and antisymmetry (AS) (Palmer and Strobeck, 1986, 1992, 2003; Palmer, 1994).

\* Corresponding author.

E-mail: [marcelokosta@yahoo.com.br](mailto:marcelokosta@yahoo.com.br) (M. Costa).

FA is a model of variation in which deviations from symmetry are distributed close to a mean of zero, and are random and non-directional. Alternatively, deviations can be distributed preferentially in one direction, thus generating DA, in which there is a tendency for the excessive development of one specific side in relation to the other, leading to a distribution with the average deviates being significantly different from zero (Palmer, 1994). Finally, AS is found whenever one side is usually greater than the other, yet the position of the larger side varies randomly in a population, leading to a bimodal distribution of the differences between the left and right sides of the body (Palmer and Strobeck, 1992; Palmer, 1994).

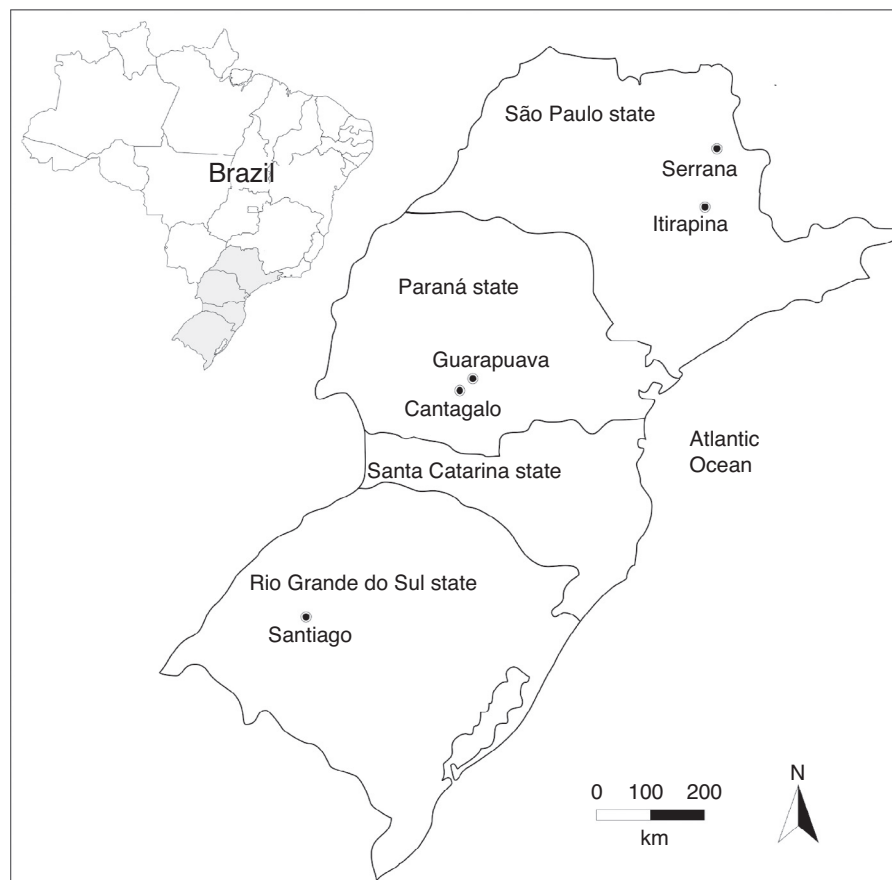
Of all three types of asymmetry, FA has been considered as a measure of DI, given that it reflects the inability of an organism to cope with stressing factors and the resulting perturbations during development (Palmer, 1994; Klingenberg and McIntyre, 1998). Contrary to FA, other types of asymmetry are caused in part by either genetic or environmental factors and are therefore harder to associate with DI, nor to be used as a proxy to measure it (Palmer and Strobeck, 2003). However, the three types of asymmetry are related, with a continuum between them (Graham et al., 1998; Kark, 2001). Therefore, the transition from FA to DA or AS can indicate severe instability during development (Graham et al., 1998), yet these relationships have not been fully understood. Although the relationship between FA and the mechanisms causing stress is also unclear, with some tests providing conflicting results (Hoffmann et al., 2005; Vangestel and Lens, 2011), the FA has been considered a good indicator of DI and thus, act as a biomarker to environmental stress (Beasley et al., 2013; Lazić et al., 2013; Lezcano et al., 2015).

The population dynamics of a species tends to change from the core to the periphery of its distribution (Brown et al., 1996; Lens

et al., 1999). Therefore, one could expect peripheral populations to be subject to a higher level of stress than more central populations (the center–periphery hypothesis) and consequently should present a higher level of FA (Kark, 2001). One spatial pattern of asymmetry was detected in the partridge *Alectoris chukar* (Gray, 1830), which showed an increase in the proportion of asymmetric individuals in peripheral populations, as well as higher levels of DA and AS (Kark, 2001). However, in the same geographical region, two species of *Euchloe* butterflies did not differ with respect to the level of asymmetry between populations in the center and the periphery of their ranges (Kark et al., 2004), suggesting that the response of asymmetry in relation to geographical variation could vary depending on the studied organism and its habitat.

There is strong evidence supporting latitudinal variation in several morphological traits in *Drosophila*, including body size and wing size and shape (Hoffmann and Shirriffs, 2002; Griffiths et al., 2005). However, most of these studies have been carried out using cosmopolitan species, whereas little is known about species with ranges restricted to specific types of habitat (Griffiths et al., 2005). One particularly model system in this respect is the cactophilic species *Drosophila antonietae* Tidon-Sklorz and Sene, 2001. *D. antonietae* is endemic to South America found from South and Southeast Brazil to the eastern edge of the Argentinean Chaco (Manfrin and Sene, 2006). Its distribution is associated with fragments of xerophytic vegetation that include the cactus *Cereus hildmannianus* K. Schum (Mateus and Sene, 2003; Manfrin and Sene, 2006). The larvae of *D. antonietae* develop within rotting cladodes, feeding on the yeast present in this environment (Pereira et al., 1983; Manfrin and Sene, 2006).

Despite the considerable geographical distance between these populations and the limited capacity for dispersal in *D. antonietae*,



**Fig. 1.** Locations of the sampled populations of *Drosophila antonietae*. Serrana (21°14' S, 47°34' W), Itirapina (22°16' S, 47°48' W), Guarapuava (25°17' S, 51°53' W), Cantagalo (25°25' S, 52°04' W), Santiago (29°23' S, 54°44' W).

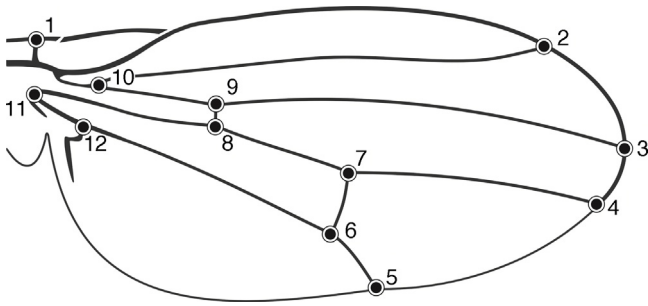


Fig. 2. Representation of the wing of *Drosophila antonietae* indicating the position of the 12 anatomical landmarks.

there is still uncertainty regarding their level of isolation (Manfrin and Sene, 2006; Mateus and Sene, 2007). Local populations display high genetic variability and moderate genetic diversity, leading to hypotheses suggesting either a moderate level of gene flow or short periods of differentiation followed by the maintenance of ancestral polymorphism (Mateus and Sene, 2007). In general, populations of *D. antonietae* are fragmented and have low additive genetic variance (Mateus and Sene, 2007). Given that the fragmentation could produce DI (Lens et al., 1999), it is possible that the same scenario applies to the fragmented populations of *D. antonietae*. Also, as fragmentation occurs along the geographical distribution of *D. antonietae* it is also possible that the level of DI varies spatially as predicted by the center–periphery hypothesis. If this scenario holds, it would be expected to find FA in all populations sampled and, also, a higher level of FA in peripheral than in central populations. To test these predictions we study asymmetry in wing shape of five populations of *D. antonietae* collected throughout the distribution of the species using FA as a proxy for DI. More specifically, we addressed the following questions: (1) what types of asymmetry occur in populations of *D. antonietae*? (2) Does the level of FA vary among populations? (3) Does peripheral populations have a higher FA level than central populations?

## Material and methods

### Specimen collection

We collected a total of 201 males from five populations of *D. antonietae*, namely Serrana ( $n=22$ ) and Itirapina ( $n=40$ ) located in the state of São Paulo (the northernmost limit of the geographical distribution of the species), Guarapuava ( $n=23$ ) and Cantagalo ( $n=66$ ) located in the state of Paraná (the geographical center of the species distribution), and Santiago ( $n=50$ ) located in the state of Rio Grande do Sul (the southernmost limit of the geographical distribution of the species) (Fig. 1). The drosophilid specimens were captured by closed traps (Penariol et al., 2008) containing banana and orange baits fermented by yeast (*Saccharomyces cerevisiae*).

### Data acquisition and geometric morphometrics

We used the right and left wings of males of *D. antonietae* as our morphometric marker due to the ease of identification of homologous landmarks formed by the intersection of veins, and by the extensive understanding of the mechanisms underlying wing development in *Drosophila* (Hoffmann and Shirriffs, 2002; Griffiths et al., 2005). Wings were removed, mounted on semi-permanent slides, and digitally photographed under a microscope. Twelve type 1 anatomical landmarks were located on the dorsal surface of the wings (Fig. 2) using the software TPSDig 2.16 (Rohlf, 2010). Type 1 landmarks are characterized by the intersection of three structures and are formed by the juxtaposition

of tissues (Monteiro and Reis, 1999), which in our case corresponds to the intersection of wing veins of *D. antonietae*. Each landmark was digitalized three times in each wing by the same person on different days to allow for estimating measurement error.

Landmark configurations were superimposed using Generalized Procrustes Analysis (GPA) (Klingenberg and McIntyre, 1998; Monteiro and Reis, 1999). GPA begins by reflecting landmark configurations from one of the sides and superimposing them by their centroid (midpoint of a configuration of anatomical landmarks). The size of the centroid, the square-root of the sum of the squared distances from a set of landmark to their centroid (Monteiro and Reis, 1999), is then scaled to one. Finally, each landmark configuration is rotated such that the squared distances between homologous landmarks are minimized. As a result of all of these calculations, the distances between the superimposed configurations of left and right structures correspond to the extent to which they differ in shape, given that they are an approximation to Procrustes distances (Klingenberg and McIntyre, 1998).

### Statistical analyses

Shape asymmetry was analyzed using configurations superimposed as dependent variables in a Procrustes ANOVA (Klingenberg and McIntyre, 1998), such that the specimen identity considered as a random effect and side of the body was used as a fixed effect. In particular, the among-species main effect stands for individual shape variation, the effect of the side of the body corresponded to directional asymmetry (DA), the interaction between the side of the body and the specimen identity corresponded to fluctuating asymmetry (FA) and the residual term corresponded to the measurement error in the model (Palmer, 1994; Klingenberg and McIntyre, 1998; Palmer and Strobeck, 2003). In the Procrustes ANOVA, the degrees of freedom are calculated by multiplying the number of degrees of freedom of each factor by the total number of dimensions in shape space (Klingenberg and McIntyre, 1998; Monteiro and Reis, 1999). Antisymmetry (AS) in wing shape was analyzed using scatterplots of the differences between the left and right side for each landmark. The formation of clusters of points in this distribution would correspond to a bimodal distribution in the differences between the left and the right sides and consequently to the presence of AS (Klingenberg and McIntyre, 1998; Palmer and Strobeck, 2003).

In each individual, shape asymmetry can be measured as the deviation from the perfect superimposition of left and right configurations (Klingenberg and McIntyre, 1998). Therefore, the individual net asymmetry (NAi) was estimated by the Procrustes distance between the left and right configurations of each individual (Marchand et al., 2003). On the other hand, the population net asymmetry (NA) was estimated by averaging individual net asymmetries (NAi). Given that NA is composed of both FA and DA, NA can be partitioned into those two types of asymmetry (Graham et al., 1998; Marchand et al., 2003). The population estimate of DA was obtained by calculating the Procrustes distance between the average of the left and right configurations of each population (Schneider et al., 2003), whereas the difference between NA and DA was considered as a population-level estimate of FA (Marchand et al., 2003). Therefore, the values of individual fluctuating asymmetry (FAi) were obtained by calculating the difference between NAi and DA of each specimen.

The differences in the levels of NA and FA between populations were tested using ANOVAs with NAi and FAi as response variables, whereas population of origin and replicates as predictor variables. Replicates were added to the ANOVAs to estimate the effect of measurement error on the level of FA.

All analyses were carried out using the softwares Morpho J 1.05c (Klingenberg, 2011) and R 2.15.1 (R Development Core Team, 2012).

**Table 1**  
Analysis of shape asymmetry. Result of Procrustes ANOVA of populations of *Drosophila antonietae*.

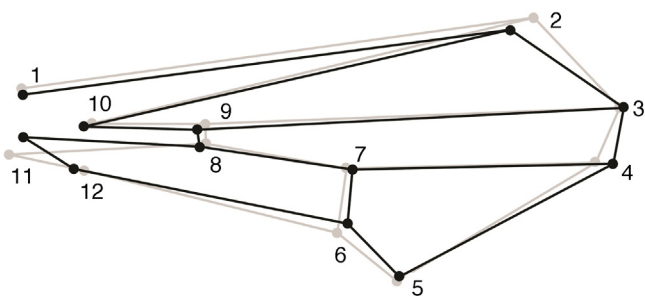
Effect	DF	MS	F
Population	80	0.0004897	4.45 <sup>a</sup>
Individual (I)	3920	0.0001101	6.79 <sup>a</sup>
Side (S)	20	0.0005663	34.94 <sup>a</sup>
I * S	4000	0.0000162	4.83 <sup>a</sup>
Error	16,080	0.0000034	

<sup>a</sup>  $p < 0.001$ .

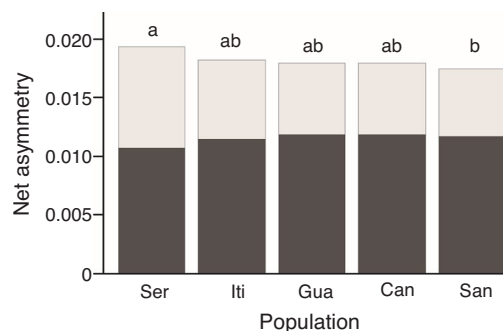
## Results

The analyzed populations of *D. antonietae* showed both directional asymmetry (DA) and fluctuating asymmetry (FA) in wing shape (Table 1). The partitioning of variance obtained from the Procrustes ANOVA indicated that the effect of the side of the body and the interaction between the side and specimen identity were significant, implying not only that *D. antonietae* has both DA and FA in wing shape, but also that the level of FA is higher than the error term. However, asymmetry is not homogeneous with respect to the effect of specimen identity and source population. In addition, the analysis of a scatterplot of differences between left and right sides demonstrated that the analyzed populations do not display AS. The DA is mainly related to a dislocation in the anterior–posterior wing axis, produced by landmarks 11 (wing base), 06 (posterior wing margin) and 02 (anterior wing margin), and in the base–apex axis from movement of the landmark 04 (Fig. 3).

The level of net asymmetry (NA) – composed of the sum of FA and DA, differed only between populations of Serrana and Santiago ( $F_{4,596} = 27.85$ ;  $p < 0.05$ ), which display the highest and the lowest values of NA ( $0.0193 \pm 0.0037$  and  $0.0174 \pm 0.0044$ , respectively), whereas the remaining populations had intermediate values (Fig. 4). On the other hand, partitioning NA into its FA and DA components allows for determining which of those types of asymmetry accounts for variation in NA between populations. Curiously, there was no difference in FA between populations ( $F_{4,596} = 0.82$ ;  $p > 0.05$ ), suggesting that populations of *D. antonietae* have the same magnitude of FA throughout the geographical distribution of the species (Fig. 4). Therefore, the difference in NA between the populations of Serrana and Santiago is due to DA, not FA (Fig. 4). Again, populations of Serrana and Santiago showed the highest and the lowest levels of DA (0.0087 and 0.0057), whereas the remaining populations had intermediate values (Fig. 4). No significant effect of measurement error was detected in the NA analysis ( $F_{2,596} = 16.72$ ;  $p > 0.05$ ), as well as in the FA analysis ( $F_{2,596} = 16.72$ ;  $p > 0.05$ ), suggesting that measurement error in this study is random and does not affect the outcome of asymmetry analyses.



**Fig. 3.** Diagram of the difference between the shape averages of the left wing (gray lines) and right wing (black lines), corresponding to directional asymmetry in *Drosophila antonietae*. Deformations magnified 10 times.



**Fig. 4.** Net asymmetry level (DA + FA) of populations of *Drosophila antonietae*. DA, directional asymmetry (light gray); FA, fluctuating asymmetry (dark gray); Ser, Serrana; Iti, Itirapina; Gua, Guarapuava; Can, Cantagalo; San, Santiago. Lowercase letters denotes homogeneous groups ( $p < 0.05$ ) according to a Tukey test.

## Discussion

Our results indicated that, although populations of *D. antonietae* showed both fluctuating and directional asymmetry, net asymmetry (NA = DA + AF) varied significantly among populations. However, most of the variation is explained by DA alone, suggesting that the level of FA is not structured geographically in *D. antonietae*. We hypothesize that larval development in rotting cladodes might play an important role in explaining our results.

Fluctuating asymmetry has been associated with geographical distribution as an indicator of developmental instability (DI), possibly reflecting environmental changes along the distribution of a species (Kark, 2001). However, the relationship between asymmetry (based on measurements of linear traits) and the geographical distribution of species has been controversial (Jenkins and Hoffmann, 2000; Kark, 2001; Kark et al., 2004) because some species do not show this expected spatial pattern of asymmetry (Jenkins and Hoffmann, 2000; Kark et al., 2004), suggesting that asymmetry varies depending on the studied organism and its habitat. Jenkins and Hoffmann (2000) found that the populations of *Drosophila serrata* Malloch (1927) along eastern Australia also did not show variation in the level of FA in central and peripheral populations. Likewise, our results indicate that, although *D. antonietae* did show FA, the differences in NA between populations of Serrana and Santiago can be accounted for by changes in DA rather than FA. This suggests that the level of FA is not structured geographically in *D. antonietae*, thus indicating that other factors related to the biology of *D. antonietae* might act in opposition to environmental variation to buffer the level of asymmetry found in populations.

Three hypotheses can be formulated to explain the patterns of FA recorded for *D. antonietae*, which will be addressed in turn. First, environmental variation along the geographical distribution of *D. antonietae* might not be sufficient to generate different levels of FA among populations. It is widely recognized that environmental pressures, particularly those related to temperature and relative humidity, might affect the development of an organism and thus generate FA (Hosken et al., 2000; Hoffmann et al., 2005). Although the position of the populations of *D. antonietae* in the space formed by the principal component axes indicates that there are recognizable differences in climatic conditions between populations, there was no correspondent response in the levels of asymmetry in response to this environmental variation. In this context, Hoffmann et al. (2005) compared several studies of DI and demonstrated that variation in wing shape is more sensitive to stressing factors than FA and proposed that FA is not a good indicator of DI. Therefore, either FA is not sensitive to the environmental differences found throughout the distribution of the species, or there are other nonclimatic factors influencing FA in *D. antonietae*. Second, *D. antonietae*

might have “escaped” the environmental variation due to the life history of the species as being associated with a host plant, the cactus *C. hildmannianus*. The larvae of *D. antonietae* develop within decomposing tissues inside cladodes, feeding on yeast (Manfrin and Sene, 2006). These cladodes might provide a microenvironment (Soto et al., 2007) where environmental variation is buffered, thus decreasing stress due to environmental factors. A similar scenario has been suggested to explain the absence of asymmetry along the geographical distribution of *Euchloe* butterflies (Kark et al., 2004). On the other hand, Gibbs et al. (2003) investigated directly the microclimate in decomposing cladodes in the Sonoran desert and demonstrated that, although cladodes might buffer to some extent environmental changes, they might nevertheless experience marked variation in relative humidity. Moreover, the temperature within cladode might actually exceed the external temperature, which could suggest that these structures are likely not an efficient thermic refugium for *Drosophila*. However, the conditions in deserts (with exposed cladode) might not be representative of the conditions on cacti found in gallery forests along the Paraná, Paraguay, and Uruguay river basins (Manfrin and Sene, 2006; Mateus and Sene, 2007). In this case, rotting cladodes are “protected” by a vegetation cover, which might facilitate environmental buffering and possibly act as a climatic refugium for *D. antonietae*. Finally, it is possible that another stressing factor might be homogeneously affecting populations such that they express a similar level of asymmetry. *D. antonietae* is more viable and has a lower development time when reared in culture media with exudates from the cactus *Pilosocereus machrisii* Y. Dawson in comparison with media from its own host plant, *C. hildmannianus* (Soto et al., 2007). On the other hand, the level of FA in the wing of *D. antonietae* is higher when reared in culture media based on *C. hildmannianus* than on *P. machrisii* (Soto et al., 2010). These combined observations might indicate that *C. hildmannianus* itself could be considered as a stressing factor for the development of *D. antonietae*. As a consequence, all populations of *D. antonietae* would be under a similar level of stress, regardless of geographical distribution, thus leading to similar levels of response in terms of asymmetry. This demonstrates that the interaction with *C. hildmannianus* can play an important role in the pattern of asymmetry observed between populations of *D. antonietae*.

Our results are also consistent with the presence of DA in populations of *D. antonietae*. Directional asymmetry is not a rare effect in Diptera (Klingenberg and McIntyre, 1998; Klingenberg et al., 1998; Pélabon and Hansen, 2008; Soto et al., 2010), as well in other invertebrate (Graham et al., 1998; Schneider et al., 2003) and vertebrate groups (Kark, 2001; Marchand et al., 2003; Loehr et al., 2013). In Diptera, Klingenberg et al. (1998) suggested that the genetic basis of DA has been conserved over evolutionary time due to the existence of a left-right axis determining the position of imaginal disks (developmental precursors of wings) on each side of the body. In the case of size DA, Pélabon and Hansen (2008) carried out a review of studies on DA in insect wings and showed that the direction of asymmetry is consistent within species, but is not conserved at higher taxonomic levels (including Diptera). It has been suggested that both DA and AS can be generated in response to a high level of stress, thus providing a continuum between these types of asymmetry (Graham et al., 1998, but see Palmer and Strobeck, 1992). The association between different types of asymmetry has been found in the partridge *A. chucker* (Kark, 2001), where the pattern of size asymmetry changes geographically (from the center to the periphery), such that peripheral populations have higher levels of asymmetry (FA, DA, and AS) than central ones. The relationship between DA and DI has also been suggested for *Drosophila* by Soto et al. (2010) when investigating the development of *D. antonietae* and *Drosophila gouveai* Tidon-Sklorz and Sene, 2001 in culture media based on different host cacti (*C. hildmannianus* and

*P. machrisii*). The presence of DA in wing shape was detected when either species was reared on *C. hildmannianus* media, but not on *P. machrisii* (Soto et al., 2010). The presence of DA in populations of *D. antonietae* is likely a product of an evolutionarily conserved pattern in Diptera and the variation in DA found in the populations of Serrana and Santiago are possibly related to local (historical or ecological) differences between these populations.

Mapping the effects of perturbations on phenotypic development is a complex issue. In general, our results suggested that fluctuating asymmetry in *D. antonietae* has similar levels in all populations, which suggests a homogeneous perturbation. Given that DA varies on the edge of the distribution of the species, we hypothesize that local evolutionary effects on demography might have generated this difference. Given that the cactus is the stage where development takes place, there is a potential for stress to be generated from this interaction. Thus, understanding how asymmetry patterns are generated in this system would entail a better comprehension of how the plant/herbivore interaction occurs and varies between populations.

### Conflicts of interest

The authors declare no conflicts of interest.

### Acknowledgments

MC was supported by a graduate scholarship from the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). This study was partially funded by grants to MOM from Conselho de Desenvolvimento Científico e Tecnológico (CNPq – processes 475461/2007 e 312357/2006) and to RPM from SETI/Fundação Araucária (processes 103/2005, 231/2007 and 415/2009) and from FINEP (processes 01.05.0420.00/2003 and 1663/2005).

### References

- Beasley, D.A.E., Bonisoli-Alquati, A., Mousseau, T.A., 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. *Ecol. Indic.* 30, 218–226.
- Breuker, C.J., Patterson, J.S., Klingenberg, C.P., 2006. A single basis for developmental buffering of *Drosophila* wing shape. *PLoS one* 1, e7.
- Bridle, J.R., Vines, T.H., 2007. Limits to evolution at range margins: when and why does adaptation fail? *Trends Ecol. Evol.* 22, 140–147.
- Brown, J.H., Stevens, G.C., Kaufman, D.M., 1996. The geographic range: size, shape, boundaries, and internal structure. *Annu. Rev. Ecol. Syst.* 27, 597–623.
- Gibbs, A.G., Perkins, M.C., Markow, T.A., 2003. No place to hide: microclimates of Sonoran Desert *Drosophila*. *J. Thermal Biol.* 28, 353–362.
- Graham, J.H., Emlen, J.M., Freeman, D.C., Leamy, L.J., Kieser, J.A., 1998. Directional asymmetry and the measurement of developmental instability. *Biol. J. Linn. Soc.* 64, 1–16.
- Griffiths, J.A., Schiffer, M., Hoffmann, A.A., 2005. Clinal variation and laboratory adaptation in the rainforest species *Drosophila birchii* for stress resistance, wing size, wing shape and development time. *J. Evol. Biol.* 18, 13–222.
- Hoffmann, A.A., Sherriffs, J., 2002. Geographic variation for wing shape in *Drosophila serrata*. *Evolution* 50, 1068–1073.
- Hoffmann, A.A., Woods, R.E., Collins, E., Wallin, K., White, A., McKenzie, J.A., 2005. Wing shape versus asymmetry as an indicator of changing environmental conditions in insects. *Aust. J. Entomol.* 44, 233–243.
- Hosken, D.J., Blanckenhorn, W.U., Ward, P.I., 2000. Developmental stability in yellow dung flies (*Scathophaga stercoraria*): fluctuating asymmetry, heterozygosity and environmental stress. *J. Evol. Biol.* 13, 919–926.
- Jenkins, N.L., Hoffmann, A.A., 2000. Variation in morphological traits and trait asymmetry in field *Drosophila serrata* from marginal populations. *J. Evol. Biol.* 13, 113–130.
- Kark, S., 2001. Shifts in bilateral asymmetry within a distribution range: the case of the chucar partridge. *Evolution* 55, 2088–2096.
- Kark, S., Lens, L., Van Dongen, S., Schmidt, E., 2004. Asymmetry patterns across the distribution range: does the species matter? *Biol. J. Linn. Soc.* 81, 313–324.
- Klingenberg, C.P., 2011. Morpho: an integrated software package for geometric morphometrics. *Mol. Ecol. Resour.* 11, 353–357.
- Klingenberg, C.P., McIntyre, G.S., 1998. Geometric morphometric of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution* 52, 1363–1375.
- Klingenberg, C.P., McIntyre, G.S., Zaklan, S.D., 1998. Left-right asymmetry of fly wings and the evolution of body axes. *Proc. R. Soc. Ser. B* 265, 1255–1259.

- Lazić, M.M., Kaliontzopoulou, A., Carretero, M.A., Crnobrnja-Isailović, J., 2013. Lizards from urban areas are more asymmetric: using fluctuating asymmetry to evaluate environmental disturbance. *PLoS one* 8, e84190.
- Lens, L., Dongen, S., Wilder, C.M., Brooks, T.M., Matthysen, E., 1999. Fluctuating asymmetry increases with habitat disturbance in seven bird species of a fragmented afro-tropical forest. *Proc. R. Soc. Ser. B* 266, 1241–1246.
- Lezcano, A.H., Quiroga, M.L.R., Liberoff, A.L., Van der Molen, S., 2015. Marine pollution effects on the southern surf crab *Ovalipes trimaculatus* (Crustacea: Brachyura: Polybiidae) in Patagonia Argentina. *Mar. Pollut. Bull.* 91, 524–529.
- Loehr, J., Herczeg, G., Leinonen, T., Gonda, A., Van Dongen, S., Merilä, J., 2013. Asymmetry in threespine stickleback lateral plates. *J. Zool.* 289, 279–284.
- Manfrin, M.H., Sene, F.M., 2006. Cactophilic *Drosophila* in South America: a model for evolutionary studies. *Genetica* 126, 57–75.
- Marchand, H., Paillat, G., Montuire, S., Butet, A., 2003. Fluctuating asymmetry in bank vole populations (Rodentia Arvicolinae) reflects stress caused by landscape fragmentation in the Mont-Saint-Michel Bay. *Biol. J. Linn. Soc.* 80, 37–44.
- Mateus, R.P., Sene, F.M., 2003. Temporal and spatial allozyme variation in the South American cactophilic *Drosophila antonietae* (Diptera Drosophilidae). *Biochem. Genet.* 41, 219–233.
- Mateus, R.P., Sene, F.M., 2007. Population genetic study of allozyme variation in natural populations of *Drosophila antonietae* (Insecta, Diptera). *J. Zool. Syst. Evol. Res.* 45, 136–143.
- Moller, A.P., Swaddle, J.P., 1997. *Asymmetry, Developmental Stability and Evolution*. Oxford University Press, Oxford.
- Monteiro, L.R., Reis, S.F., 1999. *Princípios de morfometria geométrica*. Holos Editora, Ribeirão Preto.
- Palmer, A.R., 1994. Fluctuating asymmetry analyses: a primer. In: Markow, T.A. (Ed.), *Developmental Instability: Its Origins and Evolutionary Implications*. Kluwer, Academic Publishers, Dordrecht, pp. 335–364.
- Palmer, A.R., Strobeck, C., 1986. Fluctuating asymmetry: measurement, analysis, patterns. *Annu. Rev. Ecol. System.* 17, 391–421.
- Palmer, A.R., Strobeck, C., 1992. Fluctuating asymmetry as a measure of developmental stability: Implications of non-normal distributions and power of statistical tests. *Acta Zool. Fenn.* 191, 55–70.
- Palmer, A.R., Strobeck, C., 2003. Fluctuating asymmetry analysis revisited. In: Polak, M. (Ed.), *Developmental Instability (DI): Causes and Consequences*. Oxford University Press, Oxford, pp. 279–319.
- Pélabon, C., Hansen, T.F., 2008. On the adaptive accuracy of directional asymmetry in insect wing size. *Evolution* 62, 2855–2867.
- Penariol, L., Bicudo, H.E.M.D.C., Madi-Ravazzi, L., 2008. On the use of open or closed traps in the capture of drosophilids. *Biota Neotropica* 8, 47–51.
- Pereira, M.A.Q.R., Vilela, C.R., Sene, F.M., 1983. Notes on breeding and feeding sites of some species of the *repleta* group of the Genus *Drosophila* (Diptera Drosophilidae). *Ciênc. Cult.* 35, 1313–1319.
- R Development Core Team, 2012. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. Available at <http://www.R-project.org/>.
- Rohlf, F.J., 2010. TPSDig, Digitize Landmarks and Outlines, Version 2.16. State University of New York at Stony Brook, Stony Brook, Available at <http://life.bio.sunysb.edu/morph/>.
- Schneider, S.S., Leamy, L.J., Lewis, L.A., DeGrandi-Hoffman, G., 2003. The influence of hybridization between African and European honeybees, *Apis mellifera*, on asymmetries in wing size and shape. *Evolution* 57, 2350–2364.
- Soto, I.M., Manfrin, M.H., Sene, F.M., Hasson, E., 2007. Viability and developmental time in the cactophilic *Drosophila gouveai* and *Drosophila antonietae* (Diptera: Drosophilidae) are dependent of the cactus host. *Ann. Entomol. Soc. Am.* 4, 490–496.
- Soto, I.M., Carreira, V.P., Corio, C., Soto, E.M., Hasson, E., 2010. Host use and developmental instability in the cactophilic sibling species *Drosophila gouveai* and *D. antonietae*. *Entomol. Exp. Appl.* 137, 165–175.
- Vangestel, C., Lens, L., 2011. Does fluctuating asymmetry constitute a sensitive biomarker of nutritional stress in house sparrows (*Passer domesticus*)? *Ecol. Indic.* 11, 389–394.