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ORIGINAL PAPER

Left–right asymmetry and morphological consequences of a host shift in the oligophagous Neotropical moth *Macaria mirthae* (Lepidoptera: Geometridae)

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Abstract Phytophagous insects are excellent model organisms to study the genetic and ecological components of adaptation and morphological divergence, because their host plants are one of the main environmental factors influencing their early life stages. Although many lepidopterans are highly specialized in their host use, shifts to exotic plants have been reported for some species. *Macaria mirthae* is a native moth from Northern Chile that feeds preferentially on the Fabacea species *Acacia macracantha*, however due to habitat loss a host shift has recently been observed to the introduced fabacean *Leucaena leucocephala*. We studied the impact that different host plants have on the developmental instability levels in the moth's wing morphology evaluating both fluctuating asymmetry (FA) and directional asymmetry (DA). FA measures the small random deviations existing between the left and right sides of bilaterally symmetrical traits and it widely used as a biomonitor of environmental quality. DA refers to the tendency for a trait to be consistently developed in a different manner on the right and left sides of the body. It has been recently shown that subtle DA patterns seem to be a ubiquitous phenomenon among bilaterian animals. Our results confirmed the presence of FA in *M. mirthae* forewings by applying geometric morphometric techniques. Furthermore, it was found that the individuals feeding on the endemic tree (*A. macracantha*) showed marked DA levels, while the specimens inhabiting the exotic plant (*L. leucocephala*) did not. The absence of DA in the individuals occupying the exotic plant is striking, because it has been established that this asymmetry pattern is widespread among insect wings. This phenomenon could be related to the influence of *L. leucocephala* on normal wing development. Despite the reduced quality of *L. leucocephala* as host plant, its wider presence in the Azapa valley (Chile) could explain the host shift made by *M. mirthae*.

Keywords Geometric morphometrics · Fluctuating asymmetry · Directional asymmetry · Geometridae · Wing shape

Introduction

Phytophagous insects are excellent model organisms to study the genetic and ecological components of adaptation and morphological divergence, because their host plants are one of the main environmental factors affecting the early stages of their life cycle (Jorge et al. 2011; Matsubayashi et al. 2010, 2011; Ohshima 2008). Shifts to new host plants involve the exploitation of new food sources, facing chemically diverse environments (including potentially toxic substances), new mating locations, and the possible infection by parasitoids, bacteria and fungi (Fogleman and Abril 1990; Kircher 1982; Via 1990). In turn, morphological changes associated with host plant shifts are well documented in insects, for example in beetles, fruit flies, aphids or moths (Dambroski et al. 2005; Hawthorne and Via 2001; Jones 1998, 2004; Marohasy 1996; Matsubayashi et al. 2011; Soto et al. 2008).

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The adaptation to a new host plant may also result—either as a by-product or direct consequence—in the evolution of sexual isolation, thus emphasizing the evolutionary role of host plant shifts in cladogenesis (e.g. Coyne and Orr 2004; Etges et al. 2006). On the other hand, these new plant hosts can impose physiological stresses on the herbivore which may be expressed in disruption of morphology, possibly generating developmental destabilization at certain development stages. Even though many lepidopterans are highly specialized in their host use, shifts to exotic plants have been reported for some species (Graves and Shapiro 2003; Shapiro 2002, 2006; Vargas 2013). The capacity to shift from one host to another has been associated in moths with the native host range of each species (Fraser and Lawton 1994), which means that those species that have wider native host ranges usually adapt better to new introduced hosts. Meanwhile in butterflies it may be predicted by the geographic range and the native diet breadth (Jahner et al. 2011). Furthermore, the new hosts may be extremely important for the preservation of native lepidopteran populations in some human-modified environments, because the new introduced hosts can be a vital resource for the subsistence of species facing habitat loss (Graves and Shapiro 2003; Shapiro 2002; Vargas 2013).

These new host associations may affect a number of different life history traits of phytophagous insects (Vanbergen et al. 2003). From an ecological and evolutionary perspective, it is important to determine if host shifts are associated with modifications in insect morphological attributes that could have some effect on their life history traits, such as survival, longevity and reproductive capacity. Since most moths are flying insects at the adult stage, any modification in shape of their wings could be important in processes such as migration or mating behaviour. It is known that wing phenotypic variation is host-dependent in some species of the order Lepidoptera (Jorge et al. 2011; Mozaffarian et al. 2007).

Macaria mirthae Vargas, Parra and Hausmann (Lepidoptera, Geometridae), is a geometrid moth native of the Atacama Desert in northern Chile that feeds on three species to native Fabaceae (*Acacia macracantha*, *Geoffroea decorticans*, and *Prosopis tamarugo*) (Vargas et al. 2005). Two of these three native Fabaceae have been described as the primary host plants for *M. mirthae* in the coastal valleys of this hyper-arid desert: *A. macracanhta* and *G. decorticans* (Vargas et al. 2005). Nonetheless, the main native host in these localities is *A. macracantha*, while *G. decorticans* is just an occasional host. Even though *A. macracantha* is the main native host of *M. mirthae*, this species has recently been found feeding on an introduced fabacean, *Leucaena leucocephala*. This exotic and invasive Fabaceae tree has been recently recorded as host of *M. mirthae* based on morphology and DNA barcodes (Vargas and Mundaca 2014; Rivera-Cabello et al. 2015). Even though it is not clear when *L. leucocephala* was first introduced to Chile, one author has pointed out that it might have arrived at the end of the 1800s as forage plant (Reiche 1906). Nonetheless, it has only recently replaced *A. macracantha* in several areas of northern Chile, most probably during the last four decades (Rivera-Cabello et al. 2015). Due to the great importance of host plants in the phenotypic variation of phytophagous insects (Carreira et al. 2006; Marohasy 1996; Soto et al. 2008) it presents an opportunity to explore the developmental impact of adapting to a new host from a morphological perspective.

Developmental stability is defined as the capacity of an organism to produce a phenotype that is predetermined by an adaptive body design modelled by genetic conditions and specific environments (Auffray et al. 1999; Klingenberg 2003; Nijhout and Davidowitz 2003; Van Dongen 2006; Waddington 1942). This means that the capacity of an organism to produce an “ideal” phenotype in spite of the perturbations faced during development reflects the causal mechanism of DS. This capacity has been used to assess different types of stress, as well as the genetic aptitude to correct them (Auffray et al. 1999). The propensity of a development system to generate morphological changes as a response to random perturbations is often called developmental instability (DI) or developmental noise (DN) (Polak 2003). One of the most suitable tools to analyse the influence of DI on a certain morphological trait is fluctuating asymmetry (FA) (Klingenberg 2003; Nijhout and Davidowitz 2003; Van Dongen 2006). FA measures the small random deviations existing between the left and right sides of bilaterally symmetrical traits (Van Valen 1962). FA is particularly interesting as a result of its potential as a biomonitor of environmental quality (Benítez and Parra 2011; Floate and Fox 2000; Graham et al. 1993, 2010; Parsons 1992). The primary principle underlying the application of FA as measure of developmental stability is that the left (L) and right (R) sides of an organism may be considered as two independent replicates of the same developmental process, where the sides of the body of an individual share the same genotype and a relatively homogeneous environment (Klingenberg 2003). Nonetheless, during the natural developmental process of an organism, there are almost always small perturbations at the cellular level (i.e. developmental noise) (Polak 2003). Since these perturbations occur in restricted parts of the organism, it is expected that their effects will accumulate independently on the left and right sides (Klingenberg 2003, 2004). This means that the observed asymmetry of the left and right sides of morphological structures is a result of the expression of these subtle perturbations that have been accumulated throughout development.

On the other hand, directional asymmetry (DA) refers to the tendency for a trait to be consistently developed in a different manner on the right and left sides of the body and it is usually quantified by the difference between right and left averages (Graham et al. 1998, 2010; Klingenberg et al. 1998). This difference can be small or large, nonetheless the average left–right difference differs from zero (Palmer and Strobeck 1986). DA has been recognized for a long time as a common phenomenon among bilaterian animals, especially in internal organs (Boorman and Shimeld 2002; Ligoxygakis et al. 2001; Palmer 2004; Toga and Thompson 2003). However, it was only with the advent of geometric morphometric techniques that subtle but statistically significant DA has been found in virtually every early study of external shape asymmetry (Auffray et al. 1996; Klingenberg et al. 1998; Smith et al. 1997). External asymmetries seem to be therefore conspicuous, evolving in many lineages including fish, birds and mice in different traits (e.g. observed in mandibles and skulls) (Palmer 2004). In fact DA in wing size, usually with a left–right biased asymmetry, is common in insects and has been reported in 47 of 49 species from seven different orders (Pélabon and Hansen 2008). As previously mentioned, geometric morphometric methods have routinely revealed subtle DA levels, therefore it has been established that it seems to be a ubiquitous phenomenon among bilateral organisms (e.g. Auffray et al. 1996; Debat et al. 2000; Klingenberg et al. 1998; Smith et al. 1997). The study of wing morphology in moths is an important initial approach to assess the effect of new host associations in the morphological development of these phytophagous insects. In addition, here we present preliminary results indicating different levels of developmental instability in the wing morphology of the *M. mirthae* developing on different host plants. We addressed the following question using fluctuating asymmetry and directional asymmetry as proxies: Is the development of wing size and shape of *M. mirthae* affected by the larval host plant?

Materials and methods

Data collection and background information

Seventy-two larval specimens were collected in January 2013 in the Azapa valley (18°31′S; 70°10′W), Arica Province, northern Atacama Desert, Chile. We collected last instar larvae from two different host plants: the native *Acacia macracantha* and the invasive *Leucaena leucocephala*. *A. macracantha* is a broadly distributed Neotropical species that has its southern native range along the northern Pacific coast of Chile (Aronson 1991). This tree was traditionally found in the northernmost valleys of Chile, where it is an important host plant for many native Lepidoptera (Vargas and Parra 2009). On the other hand, *L. leucocephala* is native to Central America and was introduced in many tropical and subtropical areas of the world because it is used as forage plant, but it is currently included among the 100 most invasive alien species in the world (Lowe et al. 2000).

The sampled individuals were recorded feeding from the same host during previous instars, therefore both potential larval choice differences and female oviposition preferences were controlled for. The collected larvae were brought to the laboratory and placed into individual plastic vials at room temperature, where leaves of the respective host plants were changed and frass was removed daily until last instar larvae finished their feeding period. Then towel paper was added to assist in pupation. Vials were inspected daily to detect adult emergence and when emerged they were killed with ethyl acetate and mounted. Right and left wings were removed from the body and then compressed between two microscope slides for the subsequent image capture (see below).

Landmark acquisition and shape analysis

One hundred and forty-four right and left forewings (72 specimens; 42 from *A. macracantha* and 30 from *L. leucocephala*) of each moth were photographed with a digital camera Micropublisher 3.3 RTV-QImaging (Q-imaging, Canada) attached to a stereoscopic microscope Olympus SZ61. We digitized 13 morphological landmarks (LMs) using TpsDig 2.17 software (Rohlf 2013), both in the left and right wings of all the specimens on the upper side, according to external anatomy and the vein pattern of the wings (Fig. 1). All the landmarks were type I, which means that these are mathematical points whose stated homology is supported by the strongest available evidence, such as a local pattern of juxtaposition of tissue types, which in this case corresponded to vein intersections (Bookstein 1991). The LMs were then aligned applying a Procrustes superimposition, thus generating shape variables which are the Cartesian coordinates of the LMs after removing the differences due to scale, translation and rotation (Dryden and Mardia 1998; Rohlf and Slice 1990). Due to the fact that wings exhibit matching symmetry (i.e. two separate copies that are located on the left and right body sides as mirror images of each other), the dataset comprised two separate landmark configurations (i.e. one for each side). The shape analysis included the reflection of all configurations from one body side to its mirror image (for details see Klingenberg 2002; Klingenberg and McIntyre 1998). After the Procrustes superimposition, the shape coordinates were used to generate a covariance matrix pooled by sex in order to avoid sex influence in the data. The shape variation of the whole dataset was then analysed by performing a Principal Component Analysis (PCA) using the covariance matrix of the symmetric component (Klingenberg and Monteiro 2005). Scatterplots of scores along the first two principal components were used to visualize the variation in shape of the specimens inhabiting the two different host plants.

Measurement error (ME) has a critical importance when analysing symmetries (e.g. Palmer 1994). Therefore to assess the significance of FA and DA relative to ME, the left and right wings of the individual moths were digitized twice (Klingenberg and McIntyre 1998).

Procrustes ANOVA is a well-known geometric morphometric tool commonly applied to estimate asymmetry patterns, thus the elements of the ANOVA (i.e. mean squares MS and sum of squares SS, which are dimensionless) are essential to assess the intensity of the observed asymmetry (Klingenberg 2002; Klingenberg and McIntyre 1998). Due to its suitability, a Procrustes ANOVA was then carried out to assess asymmetry on shape and size and variation between sexes and hosts. As previously mentioned the results were reported as SS and MS, which are the ANOVA elements used to quantify either the presence or absence of asymmetry, as well as the intensity of the results (Klingenberg 2002; Klingenberg and McIntyre 1998). Both a F-test and a Pillai trace test were used to assess significance levels, due to the fact they are routinely used in morphometrics and because they are implemented in MorphoJ.

As previously indicated, FA is defined as those random differences that occur between the left and right sides in a bilaterally symmetric organism (Benítez and Parra 2011; Graham et al. 2010; Palmer and Strobeck 1986; Van Dongen 2006; Van Valen 1962). It is the variation of the individual asymmetry vectors around the means of all the configurations from each side. In the Procrustes ANOVA the MS related to the individual effect were used as an estimator of an individual's variation, while the MS related to the interaction individual * side was used as an estimator of FA. The existence of DA was statistically tested through the main effect of "side" (for further details about both the theory and implementation of Procrustes ANOVA see Klingenberg and McIntyre 1998, pp. 1366–1367).

Finally, a discriminant analysis was performed to assess whether there were significant differences in the wing shape due to the use of different plant hosts (native vs. exotic). A Hotelling's T^2 was used as a significance test for this analysis. All the aforementioned morphometric and statistical analyses were performed using MorphoJ (Klingenberg 2011).

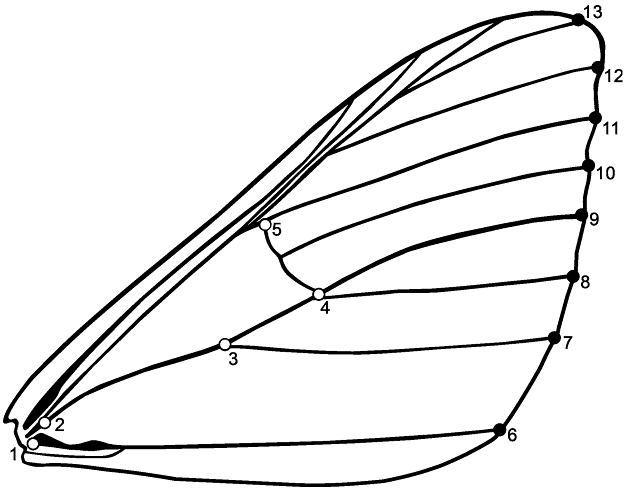

Fig. 1 Location of the 13 landmarks on the forewings of *M. mirthae*

Results

The Procrustes ANOVA applied to assess the measurement error showed that MS individual variation due to FA exceeded the measurement error (Table 1), therefore this latter factor was negligible. FA was noticeable in the forewings of the specimens inhabiting different host plants. Interestingly, this level was higher in those individuals that fed from the invasive host plant *L. leucocephala* than in those that consumed the native *A. macracantha.* The Procrustes ANOVA also confirmed the presence of directional asymmetry with significant differences in the side factor detected in the shape of individuals inhabiting *A. macracantha* (Table 2), whereas the specimens occupying *L. leucocephala* did not show significant DA levels for the values of the Pillai's trace in shape (Table 3). There were no significant DA levels for size in either of the two groups (Tables 2, 3).

The first three principal components accounted for 61.4 % (PC1 = 34.12 %; PC2 = 16.20 %; PC3 = 11.08 %) of the total symmetric shape variation and explained more than half of the total amount of wing shape variation (Fig. 2a–c).

The PCA showed a slight overlap between individuals occupying different hosts; however the Procrustes ANOVA exhibited significant differences due to this latter factor. Shape changes associated with the first 2 PCs are illustrated in Fig. 3.

The Hotelling's T^2 test showed that the mean wing shape of the individuals inhabiting the two different host plants (*A. macracantha* vs. *L. leucocephala)* was significantly different (T^2 105.6195; p value <0.0001). Furthermore, the cross-validated discriminant analysis was able to correctly classify the 80.95 % of the individuals inhabiting *A. macracantha* and the 80 % of the specimens occupying *L. leucocephala* (Fig. 2d). The Procrustes

Table 1 Procrustes ANOVA performed to assess measurement error for both centroid size and shape of *M. mirthae*

Effect	SS	MS	*df*	F	*P*	Pillai tr.	*P* (param)
Centroid size							
Individual	0.000163	0.00001	16	0.89	0.593		
Side	0.000098	0.000098	1	8.53	0.01		
Ind * side	0.000184	0.000012	16	0	1		
Error 1	0.085112	0.002503	34				
Shape							
Individual	0.0455309	0.000129349	352	2.41	<0.0001		
Side	0.00127137	0.000057789	22	1.08	0.369		
Ind * side	0.01887335	0.00005362	352	1.35	0.0004	9.43	<0.0001
Error 1	0.0297658	0.000003979	748				

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The size value that is present in the table refers to centroid size which is the size estimator generated after a Procrustes superimposition

Table 2 Procrustes ANOVA performed to assess fluctuating and directional asymmetry for both centroid size and shape of the *M. mirthae* individuals that inhabit the endemic host *A. macracantha*

Effect	SS	MS	*df*	F	*P*	Pillai tr.	*P* (param)
Centroid size							
Individual	0.002275	0.000078	29	3.98	0.0002		
Side	0[a]	0[a]	1	0[a]	0.9749		
Ind * side	0.000572	0.00002	29	8.16	<0.0001		
Error 1	0.000145	0.000002	60				
Shape							
Individual	0.09199874	0.0001442	638	3.33	<0.0001	13.35	<0.0001
Side	0.00164652	0.00007484	22	1.73	0.0207	0.81	0.2592
Ind * side	0.02761457	0.00004328	638	3.29	<0.0001	10.91	<0.0001
Error 1	0.01735788	0.00001315	1320				

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The size value that is present in the table refers to centroid size which is the size estimator generated after a Procrustes superimposition

[a] Values close to 0 are not shown because their exponential factor is over 7 and MorphoJ cannot display them

Table 3 Procrustes ANOVA performed to assess fluctuating and directional asymmetry for both centroid size and shape of the *M. mirthae* individuals that inhabit the exotic host *L. leucocephala*

Effect	SS	MS	*df*	F	*P*	Pillai tr.	*P* (param)
Centroid size							
Individual	0.002272	0.000057	40	4.7	<0.0001		
Side	0[a]	0[a]	1	0[a]	0.9648		
Ind * side	0.000484	0.000012	40	3.97	<0.0001		
Error 1	0.000261	0.000003	84				
Shape							
Individual	0.1167956	0.000132722	880	3.3	<0.0001	12.95	<0.0001
Side	0.0015829	0.0000719485	22	1.79	0.0142	0.81	0.0026
Ind * side	0.0353539	0.0000401749	880	2.97	<0.0001	11.63	<0.0001
Error 1	0.0244107	0.0000135314	1804				

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The size value that is present in the table refers to centroid size which is the size estimator generated after a Procrustes superimposition

[a] Values close to 0 are not shown because their exponential factor is over 7 and MorphoJ cannot display them

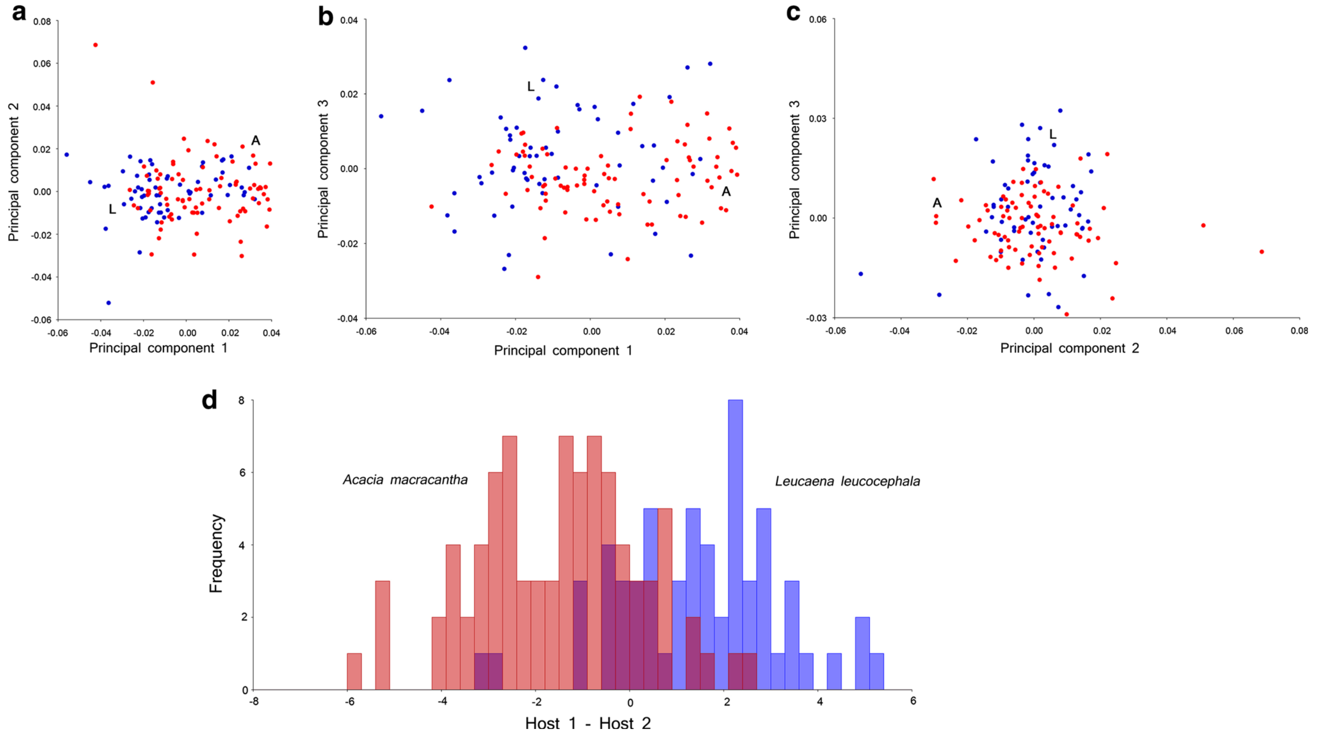

Fig. 2 *Top* PCA *scatterplot* of *M. mirthae* wing shape variables color-coded according to their plant host: *A. macracantha* (*red*), *L. leucocephala* (*blue*). The figure shows the first three components that represent the 61.4 % of the wing shape variation (**a–c**). *Bottom* the classification results obtained from the cross-validated discriminant analysis are shown (**d**). The figure shows a relatively clear distinction of the wing shape depending on the larval host plant. (Color figure online)

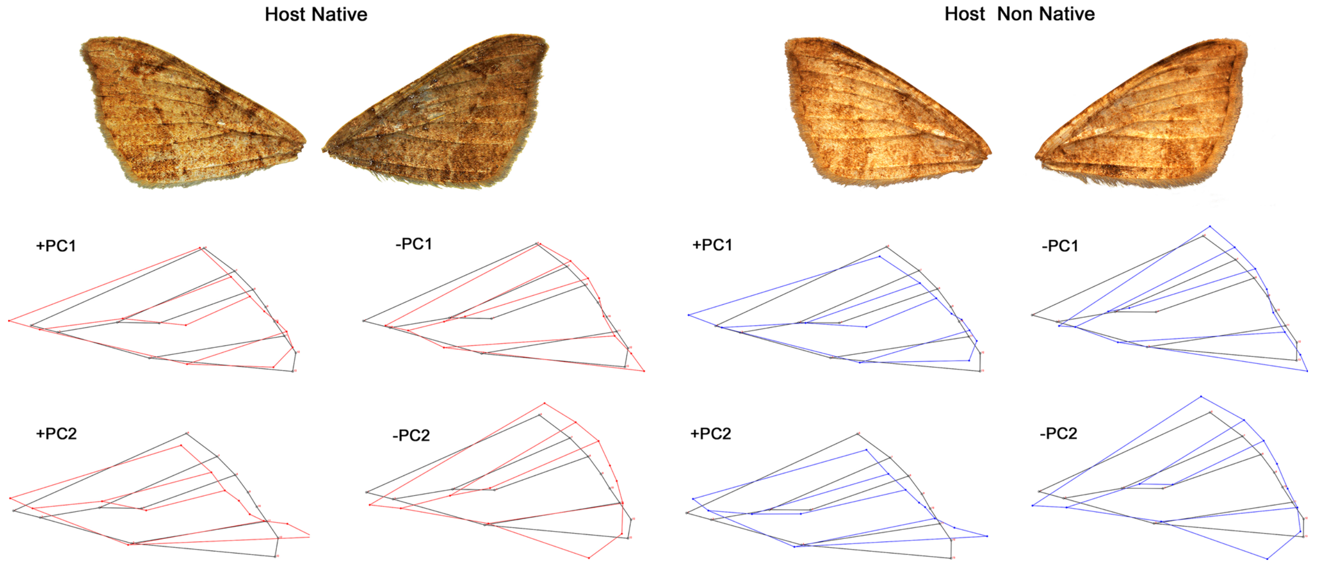

Fig. 3 Wireframe representation of the wing shape variation of *M. mirthae*. The first two principal components (PCs) for each host are shown **a** *A. macracantha* and **b** *L. leucocephala*. For each PC, the diagrams show both the shape that corresponds to the average shape and the observed extremes in the positive and negative directions of the PC axes. In order to achieve a better visualization, the scaling factor was increased 0.18 times

ANOVA performed to test for differences due to host and sex indicated that the shape variation due to these factors is highly significant (sex = F 20.17; $P < 0.0001$; host = F 4.38; P 0.0021). On the other hand, the centroid size differences were only significant for the sex factor (sex = F 25.37; $P < 0.0001$; host = F 0.18; P 0.6714) (Table 4).

Discussion

Geometric morphometric tools were used here for the first time to assess the morphological shape effects caused by a host shift to an alien invasive plant in a moth of the Geometridae family.

Our results confirmed the presence of FA in *M. mirthae* forewings, showing that they were dependent on the larval host plant. Interestingly, the level of FA was higher in the moths that fed from the exotic host plant *L. leucocephala* during their larval stage than in the specimens that consumed the native *A. macracantha*. It is known that FA is a measure of developmental instability (Klingenberg 2003; Leamy and Klingenberg 2005; Van Dongen 2006), so broadly speaking, more symmetrical individuals would have greater survival possibilities than those with lower levels of symmetry (Benítez et al. 2008; Lens et al. 2002; Møller 1997; Møller and Swaddle 1997). Hence, the higher FA level in the moth forewings occupying the exotic plant suggests a lower quality of *L. leucocephala* as a host for the larval development of *M. mirthae*, regardless of the absence of differences in centroid size. Polak (2003) provides several examples that relate fitness to instability (i.e. the result of many subtle stochastic accidents that tend to alter the accuracy of development in a given environment) during larval development. For instance, changes in levels of bilateral asymmetry could negatively affect the search for an adequate oviposition place, mate choice, etc.

Possible causes underlying the pattern observed in this research could be related to different aspects of plant physiology such as the presence of toxic substances in *L. leucocephala* (Hammond 1995), the C/N ratio, leaf toughness, or other factors. However, the effects from these on *M. mirthae* are unknown, further studies are required to positively identify which features could be involved in the distinct responses to the hosts (e.g. L. *leucocephala* leaf composition; associated microorganisms).

Additionally, it was observed that the individuals occupying the native host species showed a consistent directional asymmetry level, while the moths that occupied the invasive plant during their larval stage did not exhibit this asymmetry pattern (De Coster et al. 2013; Kark et al. 2004; Lens et al. 2002). DA in wing size, usually with a left–right biased asymmetry, is common in insects and has been reported in 47 of 49 species from seven different orders (Pélabon and Hansen 2008). However, efforts to estimate the genetic variation underlying DA have repeatedly failed (Coyne 1987; Monedero et al. 1997; Smith and Sondhi 1960; Tuinstra et al. 1990), casting doubts on the evolutionary potential of DA (Lewontin 2000; Smith et al. 1985).

This possible absence of genetic variation could perhaps lead to evolutionary stasis in DA. Hence, DA could correspond to a fitness optimum resulting from some selective pressures operating on asymmetry (Pélabon and Hansen 2008). Therefore, the absence of this widespread asymmetry pattern in the wings of the individuals that consumed the exotic plant could indicate that these specimens were subjected to high developmental pressures that impact their adult phenotype by inhibiting the expression of the normal DA pattern. The absence of DA and the higher FA levels in these individuals represent cumulative evidence showing that the host shift experienced by some populations of *M. mirthae* has affected their normal development. Future studies should try to determine if this forced host shift due to habitat loss is leading to long-term adaptive change in this phytophagous moth. Interestingly, centroid size was not different between the forewings of *M. mirthae* moths inhabiting the two different host plants. This result differs with previous records for butterflies (Jorge et al. 2011) and moths (Mozaffarian et al. 2007) that have documented striking differences in wing size due to host shifts. These studies have shown that individuals inhabiting non-preferred host plants, develop smaller wings.

In spite of this absence of size differences, the present study confirmed the effect of the two different host plants on the phenotypic variation of *M. mirthae* forewings. The forewing shape was significantly different depending on the host plant consumed by the larvae. The discriminant analysis showed that the wing shape between the

Table 4 Procrustes ANOVA applied to assess host and sex differences for both centroid size and shape of *M. mirthae*

Effect	SS	MS	*df*	F	*P*	Pillai tr.	*P* (param)
Centroid size							
Sex	0.000655	0.000655	1	25.37	<0.0001		
Host	0.000006	0.000006	1	0.18	0.6714		
Shape							
Sex	0.03164292	0.001438314	22	20.17	<0.0001	0.55	<0.0001
Host	0.00763011	0.000346823	22	4.38	<0.0001	0.37	0.0021

Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). The size value that is present in the table refers to centroid size which is the size estimator generated after a Procrustes superimposition specimens inhabiting different host plants were significantly different.

This ecomorphological response observed in *M. mirthae* shape variation is consistent with other studies that have detected this kind of host-related plastic variation in insects (Jorge et al. 2011; Mozaffarian et al. 2007; Soto et al. 2008). Nevertheless, there is currently no evidence that the observed shape variation is disadvantageous at the adult stage for those individuals occupying the exotic host (Jorge et al. 2011). The larval host-plant could be regarded as a relevant component of the adult environment only if the butterfly or moth species has a specific and restricted host (e.g. if food source changes are lethal) (Jorge et al. 2011; Nylin et al. 2009). Future research could try to measure if this host shift has a negative impact on the fitness of this moth (quantitative genetic experiments), in order to quantify the possible selective effect of *L. leucocephala.*

The contrasting FA levels observed between the individuals feeding on different host plants, pose several questions regarding the conservation status of the *M. mirthae* populations inhabiting areas where *A. macracantha* has been almost totally replaced with *L. leucocephala.* The specimens that fed on the exotic plant showed higher FA levels, as well as the absence of DA, thus suggesting an impact on the development of the moths that could affect their fitness. In general terms, much of the area originally covered by native vegetation in the coastal valleys of northern Chile has been drastically modified, mostly transformed into agricultural crops (Luebert and Pliscoff 2006). This anthropic process has severely affected the distribution of *A. macracantha* that now is scarcely present in the coastal valleys, while *L. leucocephala* is evidently more abundant (Rivera-Cabello et al. 2015). This phenomenon obviously affects all the species that ecologically depend on this plant, such as *M. mirthae* because it implies that the gravid females of this species have more possibilities of finding *L. leucocephala* trees instead of *A. macracantha* specimens when looking for oviposition sites. This means that they have fewer chances to deposit their eggs on their most suitable host plant. Thus, the decreasing density of *A. macracantha* could imply that in the relatively near future there will be a higher proportion of morphologically sub-optimal *M. mirthae* adults looking for conspecifics to copulate with, as well as for oviposition sites that would be primarily sub-optimal due to the decreasing density of *A. macracantha.* This situation could logically have negative consequences for the conservation of this endemic moth in the coastal valleys of northern Chile. Preliminary surveys have shown that the larval densities of *M. mirthae* are higher in *A. macracantha* when compared to *L. leucocephala* (HAV; unpublished data), which could indicate a higher oviposition preference by the female moths and/or a better larval performance in the native host. Therefore, it would be highly interesting to compare between the two host plants both in larval performance as well as in female oviposition preference. Furthermore, it has been observed that *M. mirthae* is an important prey of a potter wasp *Hypodynerus andeus* (Packard) (Hymenoptera, Vespidae, Eumeninae) native of the Azapa valley (Méndez-Abarca et al. 2012; Vargas et al. 2014); hence it would be relevant to additionally study how the decreasing number of *A. macracantha* affects this trophic interaction.

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