

Host-plant dependent wing phenotypic variation in the neotropical butterfly *Heliconius erato*

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Most phytophagous insects feed on a single plant during development, and this may influence not only performance-linked traits, but also more subtle morphological differences. Insect–plant interactions are thus valuable for studying environmental influences on phenotypes. By using geometric morphometrics, we investigated the variation in forewing size and shape in the butterfly *Heliconius erato phyllis* reared on six species of passion vines (*Passiflora* spp.). We detected wing shape sexual dimorphism, for which the adaptive significance deserves further investigation. There was size as well as wing shape variation among individuals fed on different hosts. These subtle differences in shape were interpreted as environmental effects on development, which should be under weak natural selection for these traits, and therefore not strongly canalized. This result reinforces the role of plasticity on host-plant use, as well as the corresponding consequences on developmental variability among phytophagous insects. We propose that this variation can be an important factor in resource specialization and partner recognition, possibly triggering reproductive isolation and sympatric speciation in phytophagous insects. This interaction also shows itself as a good model for studying the role of environmental and interaction diversity in evolution. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **102**, 765–774.

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INTRODUCTION

Variation in size and shape has been considered a key factor in evolutionary studies ever since the onset of the field (Darwin, 1859). When considering this kind of variation at the intraspecific level, the influence of genetic and environmental factors is a major concern.

Although relatively neglected until the 1980s, the framework of phenotypic plasticity and reaction norms is essential for the understanding of the development and maintenance of size and shape variation (Pigliucci, 2005). Recently, it was suggested that plasticity may have a primary role in the origin of evolutionary novelties and diversification (West-Eberhard, 2003; Pfennig *et al.*, 2010) and, specifically in the case of herbivorous insects, plasticity in host-plant use has

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been proposed as a promoter of speciation (Nylin & Janz, 2009). In this context, the interaction between herbivorous insects and their host-plants is a very adequate model for studying the influence of the biotic environment on an organism's phenotype and its evolutionary consequences.

In most herbivorous insects, the host-plant is the only food resource during larval development, having potential variation in terms of nutritional quality and the presence of diverse chemical compounds influencing development. In addition, the host-plant is the main factor modulating the interactions with other species, such as predators, parasites, and pathogens (Nylin & Janz, 2009). Given these primary roles, it is expected that plants have a direct influence on different dimensions of an insect's phenotype, from life-history traits directly related to performance (Thompson, 1988), such as mortality, body size, and development time, to more subtle morphological variation that is not necessarily associated with fitness. The former traits are expected to be optimized in primary hosts by means of adaptation, as a part of the process of host-plant colonization (Nylin & Gotthard, 1998). Inadequate development and therefore higher variation in these traits are expected only for secondary hosts or in the case of generalist species. By contrast, subtle morphological variation can be considered as a reliable surrogate of the effects that different environments have on the developmental process because they are less related to fitness, and therefore subject to weaker selection. This makes most herbivore–plant interactions valuable systems for studying phenotypic plasticity and canalization (i.e. the reduction of phenotypic variation by developmental mechanisms; Stearns, Kaiser & Kawecki, 1995).

Butterflies represent the major model for the study of the interaction between herbivorous insects and their host-plants (Thompson & Pellmyr, 1991). Many studies on preference and performance, the evolution of host range, and co-evolution have been carried out using this group (Ehrlich & Raven, 1964; Thompson & Pellmyr, 1991; Janz, Nylin & Wahlberg, 2006). Within butterflies, the tribe Heliconiini and its host-plants, the passion vines (Passifloraceae), are amongst the most studied organisms in this regard (Benson, Brown & Gilbert, 1975; Brown, 1981; Brower, 1997). Besides the large amount of data on interactions with host-plants, the genus *Heliconius* has been largely used as a model in evolutionary research, mainly in relation to wing colour patterns (Brower, 1996; Gilbert, 2003), speciation (Jiggins, 2008), and evo-devo (McMillan, Monteiro & Kaplan, 2002; Joron *et al.*, 2006; Parchem, Perry & Patel, 2007). However, studies on wing shape, especially in the context of host-plant use, have not yet been performed, and could integrate these fields of research,

contributing both to the comprehension of the developmental aspects of insect–hosts interaction, and to a better understanding of the influence of different environments on development and evolution.

Heliconius erato, one of the best studied species in the genus, as well as the most widespread, occurs from the south of Mexico to southern South America (Brown, 1979, 1981) and presents several geographical subspecies, all involved in local mimicry rings with other butterfly species (McMillan *et al.*, 2002). The extra-amazonian *Heliconius erato phyllis*, is the most widespread, occurring in several different biomes and, although each individual uses a single food source during development, different individuals can use a wide range of host-plants of the genus *Passiflora* (Brown & Mielke, 1972). Previous studies have shown that *H. erato phyllis* is highly selective with respect to oviposition sites, concerning plant parts and species, nitrogen content and presence of conspecifics (Williams & Gilbert, 1981; Mugrabi-Oliveira & Moreira, 1996; Rodrigues & Moreira, 1999, 2002; Kerpel, Soprano & Moreira, 2006). These ecological characteristics and the current knowledge on *H. erato phyllis* make it an appropriate model for studying the influence of the host-plant on the phenotype.

In the present study, we aimed to investigate host-plant influence on the development of *H. erato phyllis*, examining the variation in wing size and shape, decomposed by using geometric morphometric tools. Specifically, we investigated whether shape and size differences among individuals arise on different host-plants. The expectation was that the host-plants represent environments that are sufficiently different to exert an influence on development, causing significant variations in the form of individuals.

MATERIAL AND METHODS

STUDY SITES AND BUTTERFLY REARING

We reared individuals from eggs laid by females collected in two populations located in two different regions, where host-plant use and performance had been previously investigated in detail: the first population was from 'Morro do Santana' in Porto Alegre municipality, Rio Grande do Sul State, southern Brazil; a subtropical region in the southern limit of *H. erato* distribution (hereafter termed the RS population). The second population was from 'Morro do Voturuá', in São Vicente municipality, São Paulo State, southeastern Brazil; in a tropical region (hereafter termed the SP population). Both are coastal sites with high levels of rainfall. Detailed descriptions of each site are provided in Rodrigues & Moreira (2002) and Ramos & Freitas (1999), respectively.

All individuals used in the present study were kindly provided by R. R. Ramos and A. Elpino-

Campos (butterflies from SP and RS populations, respectively), who have reared them for performance and behavioural studies (Ramos, Rodrigues & Freitas, unpubl. data; A. Elpino-Campos, unpubl. data). Even though rearing was held in each of the localities, it was made under the same controlled conditions. Eggs were collected daily from wild caught females kept in insectaries and taken to a climate room at 25 °C under fluorescent light. They were kept in Petri dishes lined with moistened paper until larval hatching. First instars were then randomly transferred individually to shoot tip cuttings of potted plants cultivated specially for this purpose under semi-natural conditions. Individuals of each population were fed *ad libitum* until pupation in plants that occur naturally in each site: *Passiflora capsularis* ($N = 13$ individuals), *Passiflora edulis* ($N = 16$) and *Passiflora jileki* ($N = 13$) in SP; and *Passiflora misera* ($N = 17$), *Passiflora suberosa* ($N = 17$), *Passiflora caerulea* ($N = 17$), and *P. edulis* ($N = 20$) in RS. For *P. edulis*, plants were obtained from seeds of the most common cultivar from Brazil, such that they are nutritionally similar in both sites. After emergence, all the individuals were killed by freezing, then dried and kept in entomological envelopes until the image capture procedure.

DATA ACQUISITION AND MORPHOMETRICS

We removed the forewings of all individuals (42 from SP and 71 from RS; 61 males and 52 females) and captured images in 1200 dpi resolution of their ventral side, using a flatbed scanner (HP Scanjet 3800; Hewlett-Packard). The ventral side was chosen because the veins are more pronounced compared to the dorsal surface. A total of 19 landmarks, vein intersections or vein distal tips, were scored in each wing (Fig. 1A) using TPSDig, version 2.1 (Rohlf, 2006). The right forewing of each individual was scored twice to account for measurement error. These coordinates were averaged before the superimposition procedure.

We decomposed the form of all the landmark configurations into shape and size by means of geometric morphometrics (Bookstein, 1991, 1996; Dryden & Mardia, 1998). Size was measured as centroid size (CS), the square root of the sum of the squared distances of each landmark from the centroid, or gravity centre, of the landmark configuration. To measure shape, all configurations were scaled to unit CS, and superimposed by a generalized least squares (GLS) Procrustes procedure. A mean shape was calculated and the differences between its landmarks and the ones of each individual were the residuals of the GLS procedure. We used the relative warps as shape variables [the axes of a principal components analysis (PCA) on the covariance matrix of the GLS residuals.

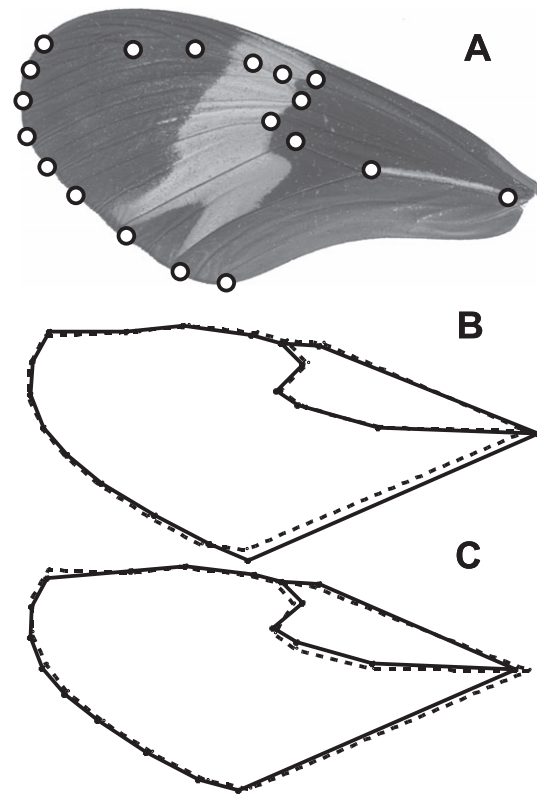


Figure 1. Landmarks used in this study, sexual dimorphism and allometry in the shape of forewings of *Heliconius erato phyllis*. A, ventral view of the right wing of a *H. erato* butterfly. Open circles indicate the landmarks used in the present study. B, female (dashed line) and male (solid line) wing shape, obtained by multiple regression. C, small (dashed line) and large (solid line) individuals, also by multiple regression.

The four last axes are null, given the dimensionality lost in the procrustes superimposition].

STATISTICAL ANALYSIS

We carried out some preliminary analyses to test for the existence of differences in shape and size between sexes to validate subsequent analysis. Otherwise, sexual dimorphism could mask the variation among individuals reared on the host-plants described above. We carried out a one-way analysis of variance (ANOVA) for CS and a multivariate analysis of variance (MANOVA) for the 34 PCA axes, both using sex as the factor, to test for size and shape differences, respectively.

To test for host-plant dependent variation in size, we performed a one-way ANOVA for CS, with host-plant as the factor, with data for individuals reared in *P. edulis* from both localities kept separated. We made this separation in all tests in which host-plant varia-

Table 1. Multivariate analysis of variance of the shape variables (non null principal components) testing for sexual and host-plant based differences in shape among *Heliconius erato phyllis* individuals. *P* values in bold emphasize significant effects

	d.f.	Pillai	approximate <i>F</i>	Numerator d.f.	Denominator d.f.	<i>P</i>
Sex	1	0.8419	9.0861	34	58	< 0.0001
Host-plant	6	3.2987	2.2627	204	378	< 0.0001
Size	1	0.5902	2.4566	34	58	0.0013
Sex × Host-plant	6	2.0501	0.9618	204	378	0.620
Sex × Size	1	0.3157	0.787	34	58	0.772
Host-plant × Size	6	2.1926	1.0671	204	378	0.294
Residuals	91					

tion was considered. For shape variation, because there was sexual dimorphism (see Results) and the possibility of allometrical shape variation, we carried out a three-way MANOVA for the 34 PCA axes with sex, size, and host-plant as factors.

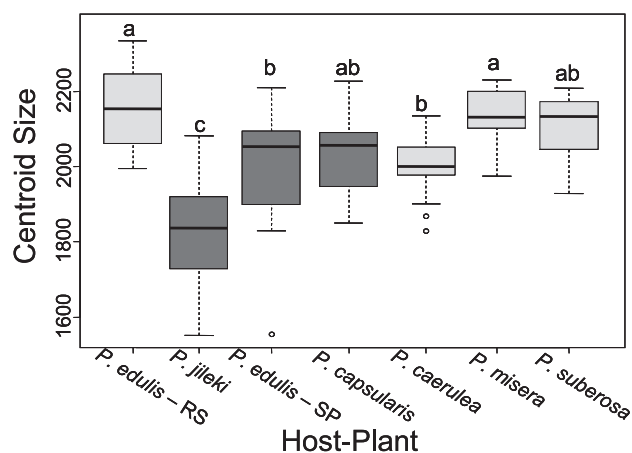
To test for the capacity to discriminate *H. erato phyllis* individuals reared on different host-plants based only on forewing size and shape, we calculated an optimal linear discriminant function between the seven host-plants (again separating *P. edulis* from SP and RS). Accordingly, we performed a linear discriminant analysis (LDA) on the 34 PCs, in combination with the CS. We used a leave-one-out, cross-validation procedure that allows an unbiased estimate of classification percentages (Baylac, Villemant & Simbolotti, 2003). In the leave-one-out cross-validation, all the data except one individual are used to calculate the discriminant function. The individual not used is then classified according to this function. The procedure is repeated to compute a mean classification error and a probability of group membership for each individual. All morphometric and statistical analyses were performed with R software (R Development Core Team, 2008), with morphometric procedures using the Rmorph library for R software (Baylac, 2007).

RESULTS

SHAPE AND SIZE VARIATION

We found no sexual dimorphism in size ($F_{1,111} = 0.017$, $P = 0.90$) but a significant difference in shape between sexes (Pillai = 0.799, $F_{1,112} = 9.14$, $P < 0.0001$; Fig. 1B). Given this difference, we used sex as a factor in the MANOVA testing for shape variation among individuals reared on different hosts but not for the ANOVA testing for size variation.

There was significant size ($F_{6,106} = 14.11$, $P < 0.0001$) and shape (Table 1) variation among individuals reared on the different host-plant species, and this effect did not interact with either size or sex (Table 1).

**Figure 2.** Wing size variation among *Heliconius erato phyllis* reared on different host-plants from different localities. Light grey boxes are from individuals from 'Morro do Santana', Rio Grande do Sul state (RS) and dark grey individuals are from 'Morro do Voturuá', São Paulo state (SP). The host-plants are ordered increasingly by larval survival. Different letters above boxes represent significant differences among groups (Tukey's multiple comparison tests). Dots represent outliers for each host-plant.

The absence of interaction between sex and host-plant for shape allowed discriminant analysis to be carried out not considering sex. Regarding centroid size variation, the smallest individuals were those reared on *P. jileki*, and the largest ones were fed with *P. edulis*, *P. misera*, and *P. suberosa* (Fig. 2). To show the main axes of shape variation, we plotted the mean value of the individuals grown in each of the host-plants for the first two axes of the PCA for the shape variables (Fig. 3). These axes accounted for more than 44% of the total shape variation. By showing the deformations along each axis of the PCA, Figure 3 allows a visualization of the general wing deformations caused by feeding on different plants.

The LDA among individuals reared on each host-plant provided a considerable rate of discrimination, with 51% of the individuals being assigned to the host-plant on which they were reared. There was some variation in the correct classification rate, with a success as high as 75% in *P. edulis*-RS, whereas

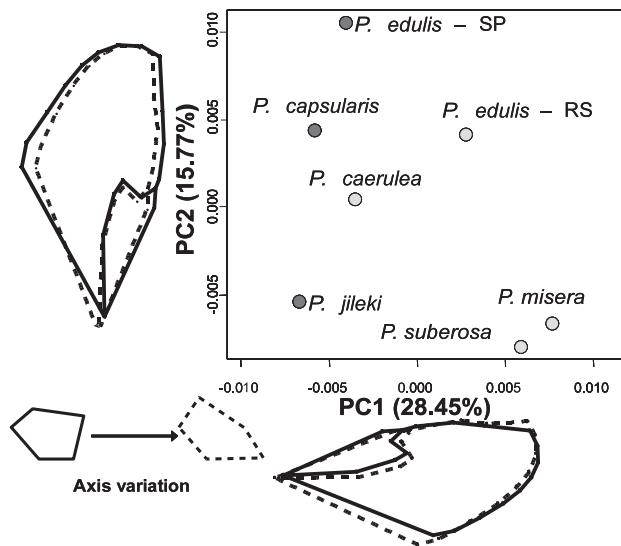


Figure 3. Mean values on the two first axes of the principal components analysis on shape residuals (percentage of shape variation described) of *Heliconius erato phyllis* individuals reared on different host-plant species. The shape variation along each axis is indicated next to each axis, where the dashed line represents the shape at minimum negative values in the axis and the solid line represents the shape at maximum values. Dark grey dots represent passion vines from 'Morro do Voturuá', São Paulo state (SP) and light grey dots from 'Morro do Santana', Rio Grande do Sul state (RS).

others were much lower; for example, *P. edulis*-SP, with 37.5% (Table 2). However, even the host-plants with the least success had a higher correct classification than expected by chance. The LDA also allowed us to build a dendrogram (Fig. 4) from the Mahalanobis distances among the individuals reared on each host, showing a strong similarity between individuals fed on *P. misera* and *P. suberosa* and an absence of a geographical pattern in shape variation.

DISCUSSION

The present study shows that wing shape is consistently influenced by the larval food source in *H. erato*

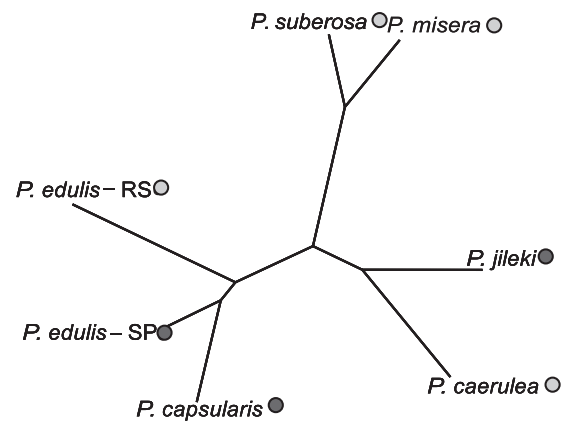


Figure 4. Unrooted Neighbour-joining dendrogram of the Mahalanobis' distances of forewing shape variables between *Heliconius erato phyllis* individuals reared on different host-plants. Open circles represent passion vines from 'Morro do Voturuá', São Paulo state (SP) and filled circles represent passion vines from 'Morro do Santana', Rio Grande do Sul state (RS).

Table 2. Percentage of correct classification of the host-plant on which *Heliconius erato phyllis* larvae were reared based on shape and size by means of a linear discriminant analysis followed by leave-one-out, cross-validation procedure

	<i>Passiflora edulis</i> -RS	<i>Passiflora jileki</i>	<i>Passiflora edulis</i> -SP	<i>Passiflora capsularis</i>	<i>Passiflora carulea</i>	<i>Passiflora misera</i>	<i>Passiflora suberosa</i>
<i>Passiflora edulis</i> -RS	75	5	10	0	5	0	5
<i>Passiflora jileki</i>	0	30.77	7.69	23.08	23.08	7.69	7.69
<i>Passiflora edulis</i> -SP	12.5	12.5	37.5	6.25	12.5	18.75	0
<i>Passiflora capsularis</i>	0	0	7.69	53.85	7.69	7.69	23.08
<i>Passiflora carulea</i>	17.65	11.76	0	17.65	41.18	5.88	5.88
<i>Passiflora misera</i>	0	0	0	0	5.88	58.82	35.29
<i>Passiflora suberosa</i>	0	11.76	5.88	11.76	0	17.65	52.94

Each line shows the individuals reared in one of the host-plants, and the percentage of individuals classified in each host-plant are shown in the columns. RS, first population from 'Morro do Santana' in Porto Alegre municipality, Rio Grande do Sul State, southern Brazil; a subtropical region in the southern limit of *H. erato* distribution; SP, second population from 'Morro do Voturuá', in São Vicente municipality, São Paulo State, southeastern Brazil; in a tropical region. The diagonal (in bold) represents the correct classification rates.

Table 3. Field use, survivorship, and centroid size (CS) of the forewings of *Heliconius erato phyllis* on the host-plants used in this study

Host-plant	Locality	Survival	Size (CS) $\pm$ SE	Use
<i>Passiflora edulis</i> -RS	RS	0.13	2156 $\pm$ 106.8	None
<i>Passiflora jileki</i>	SP	0.32	1831 $\pm$ 145.3	Low
<i>Passiflora edulis</i> -SP	SP	0.52	2000 $\pm$ 162.0	Medium
<i>Passiflora capsularis</i>	SP	0.55	2036 $\pm$ 110.8	High
<i>Passiflora caerulea</i>	RS	0.60	1996 $\pm$ 82.9	Low
<i>Passiflora misera</i>	RS	0.75	2125 $\pm$ 76.0	High
<i>Passiflora suberosa</i>	RS	0.88	2099 $\pm$ 92.1	Medium

For the RS populations, data are from Menna-Barreto & Araújo (1985) and Rodrigues & Moreira (2002). For the SP populations, data are from Ramos *et al.* (2011). Size data is from the present study. RS, first population from 'Morro do Santana' in Porto Alegre municipality, Rio Grande do Sul State, southern Brazil; a subtropical region in the southern limit of *H. erato* distribution; SP, second population from 'Morro do Voturuá', in São Vicente municipality, São Paulo State, southeastern Brazil; in a tropical region.

phyllis. To our knowledge, this is the first demonstration that larval diet influences the shape of a given body structure in a butterfly. The results obtained are in agreement with other studies that have detected this kind of host-related phenotypic plasticity in the carob moth and in cactophilic *Drosophila* (Mozaffarian, Sarafrazi & Ganbalani, 2007; Soto, Hasson & Manfrin, 2008). The shape variation provided a considerable level of discrimination among individuals reared on different host-plants. Below, we discuss the main implications of these results for the ecology of *Heliconius* and for the study of phenotypic plasticity as a whole.

SEXUAL DIMORPHISM

As observed in previous studies (Ramos & Freitas, 1999), we did not detect sexual size dimorphism in *H. erato phyllis*. On the other hand, we detected a clear sexual dimorphism in the wing shape that was already noticed in the field (A. V. L. Freitas, pers. observ.) but not properly documented previously. Although well defined, the evolutionary mechanisms at the origin of this kind of dimorphism cannot be pinpointed with the present design. There are two main problems hindering the interpretation of this kind of wing shape variation in butterflies. First, the relationship between subtle variations in wing shape and aerodynamics or flight performance is poorly understood (Strauss, 1990). In studies addressing this problem, shape is treated from a different framework, being considered as an univariate variable, generally in terms of aspect ratio or centroid position (Srygley, 1994, 1999). The second problem is the intrinsically multifunctional biological role of the wings in butterflies. Besides acting in flight, they may also function as cues for predators in the case of aposematic or

cryptic species, and are generally involved in species recognition by sexual partners, as well as in thermoregulation (Srygley, 1994; Estrada & Jiggins, 2008). Given that all of these life-history aspects can vary according to sex, it is even more difficult to determine the relationship between wing shape differences to a specific wing function. Mendoza-Cuenca & Marcías-Ordoñez (2005) studied these flight related traits considering sexual dimorphism in a *Heliconius* species. Although their study should provide a good comparison with the present study, the existence of two male phenotypes with greater differences than the observed between sexes and the existence of sexual size dimorphism in their study complicates comparisons of wing shape dimorphism. In any case, the influence of wing shape in the colour patterns used in species recognition by females and androchonia scale cell formation should be further investigated further because the first hypothesis that needs to be tested in cases of sexual dimorphism is its role in sexual selection.

SIZE VARIATION

Even though size variation was not the main focus of the present study, as a result of these aspects having been discussed extensively elsewhere (Rodrigues & Moreira, 2002, 2004), the results obtained in the present study allow comparison with these studies. Using a measure of size independent of shape (CS), we observed a pattern similar to the previously observed (Table 3), with a general relationship of this size measure with host-plant use and the survivorship of the individuals, as measured in these studies. The only exception was the individuals reared on *P. edulis* in RS. In this region, there are no reports of *H. erato phyllis* using *P. edulis* in the field; in the populations where

host-plant use has been assessed, this host-plant is absent (Rodrigues & Moreira, 2002). The absence of an effective use of this host could explain the observed pattern, with very low survival besides the large size. By rearing the larvae in a host not used in nature by the southern population, the results probably do not reflect the adaptive process by itself, but rather the physiological results of this host with respect to the ontogeny of the individuals.

SHAPE VARIATION AND EVOLUTIONARY CONSEQUENCES

The wing shape variation among *H. erato phyllis* individuals reared on different host-plants strongly suggests that this is an example of morphological phenotypic plasticity. Although it is unlikely to be adaptive in a strict sense, it accounts for a large proportion of the underlying variation observed among individuals. These results raise several questions about the origin and maintenance of such phenotypic plasticity.

As can be seen through the absence of shape differences among individuals reared in *P. edulis* from both localities, and the absence of separation among localities in the dendrogram of Mahalanobis distances, *H. erato phyllis* wing shape does not vary between localities. This gives support for the existence of a strong role of phenotypic plasticity in this trait. These results differ from those observed for wing size, which shows a significant difference between individuals reared on *P. edulis* from RS and SP, as well as a general tendency to find larger individuals in RS. These individuals were reared under the same temperatures; thus, plastic responses to climatic variation between the localities can be ruled out as an explanation for wing size variation. However, it should be considered that survival is also different in plants from different localities, and these factors could interact. These results are difficult to explain for the present scenario, and further experiments in this direction should be performed to clarify the role of geographical and plastic variation in shape and size.

The next point to be addressed is the origin of the phenotypic plasticity in *H. erato phyllis* wing shape. Although size variation in herbivorous species related to host-plants can be directly attributed to nutritional quality (Nylin & Gotthard, 1998), this correlation is not straightforward for shape variation. Unlike size, there is no evidence that the shape of a given structure is influenced by the quantity of nutrients provided, unless this shape variation is involved with static allometry (*sensu* Gould, 1966). In that case, shape differences could be attributed to size differences resulting from starvation. However, this is not an issue in the present study because there is no significant

effect of the host-plants versus size interaction factor of in the MANOVA carried to test for shape variation (Table 1) and the size related shape variation is very subtle (Fig. 1C). Besides, there are no knowledge that the shape variation observed is advantageous *per se* at the adult stage, given that, in general, individuals reared on different host-plants available in the same environment will face the same selective pressures in the adult life (unless the host-plant does influence *a priori* the adult life history). Larval host-plant cannot be considered a relevant part of the adult environment for most butterfly species because a different food source is used, especially in the case of *Heliconius*, which has a very long lifespan of several months as adult (Brown, 1981), where the Hopkins effect (i.e. adult host-plant preference induced by the host used during larval development) was not detected (Kerpel & Moreira, 2005). These characteristics preclude a long-term association between an individual and a certain host-plant species.

If wing shape phenotypic plasticity is not adaptive at the adult stage and is not directly influenced by the amount of nutrients available on each host-plant, these differences are probably the result of qualitative nutritional differences and their effects on *H. erato phyllis* development. This variation can be explained as developmental accommodation to different environments (i.e. host-plants) (West-Eberhard, 2003; Nylin & Janz, 2009; Pfennig *et al.*, 2010). This type of effect is expected to be widespread, mainly among species in which each individual uses a single food source during the development, although different individuals can use very different hosts. Although such variation is probably selectively neutral in most systems for most of the time, it represents a pool of morphological variation that cannot be neglected. Such reaction norms can be modulated by selective agents in contexts where the host-plant used by the larva is ecologically relevant to adult life (Pfennig *et al.*, 2010). The occurrence of this kind of situation is feasible in nature and could trigger genetic accommodation in situations involving host range change, and subsequent diversification, as proposed by Nylin & Janz (2009), based on the general ideas of genetic assimilation reported by Waddington (1953). In this sense, the neutral variation observed in the present study could work as a preliminary stage, which is able to lead to the kind of resource related polyphenism noted by Pfennig *et al.* (2010) as one of the most prominent roles of phenotypic plasticity on diversification.

Another possible mechanism through which this kind of variation could lead to diversification is by partner recognition and subsequent assortative mating. In *H. erato*, this possibility is assumed to be precluded by the fact that this species belongs to the pupal mating clade (whose males mate with emerging

females) and does not have a Hopkins effect. However, this path to diversification has not been considered in discussions related to host-related speciation, and could be widespread in herbivorous species that choose partners by morphological details and that, by means of low dispersal or the Hopkins effect, tend to oviposit in the same plant used as larvae. The differences observed would lead to reproductive isolation of individuals from different host-plants and the formation of sympatric host races, as well as subsequent speciation.

PERSPECTIVES AND CONCLUSIONS

The results obtained in the present study on shape variation open several possibilities for future studies on the morphological variation among individuals and its relationship with food source. First, the proximate causation of such plasticity could be investigated by identifying key chemical compounds acting on the determination of the shape variation present in the different host-plants. A second approach would be to use this type of data on shape variation in individuals reared under laboratory conditions to assign field collected ones to different host-plants. Two important issues that need to be addressed before the application of such an approach are the requirement for a sufficiently high rate of correct classification and the need to assume that the main source of shape variation is the host-plant. Subsequently, it would be necessary to validate this technique by assigning populations whose host-plant range is already known. A third approach would be to compare the morphological variation within species with different patterns of host-plant use aiming to better understand the role of different factors in the morphological determination.

In conclusion, the present study shows how host-plant can exert influences on the phenotype in *Heliconius* butterflies, from characteristics directly related to fitness to the most subtle morphological wing shape traits. We emphasize the prominent role of the host-plants as a paramount environmental factor in the ecology of herbivorous insects, and show that a large proportion of total morphological variation has an environmental origin. We also propose that this variation can be an important factor in resource specialization and partner recognition, possibly triggering reproductive isolation and sympatric speciation in phytophagous insects.

Future studies on the patterns and dynamics of host-plant use should be integrated with studies on morphological variation, given that phenotypic variation could be better understood if held in the framework of patterns of interaction structure. The separation between adaptive and intrinsic effects of different hosts could also be understood more clearly if other kinds of traits are considered in addition to

performance parameters. This integrated approach would allow a better understanding and also contribute to the solution of current problems both in ecology and evolutionary biology, such as the role of the environmental variation in diversification and speciation, as well as the role of the interactions in diverse aspects of the life history of organisms.

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