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Wing shape variations in an invasive moth are related to sexual dimorphism and altitude

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Abstract

Wing morphology has great importance in a wide variety of aspects of an insect's life. Here, we use a geometric morphometric approach to test the hypothesis that variation, in insect wing morphology patterns, occurs between sexes and along altitudinal gradients for invasive species, despite their recent association to this environment. We explored the variation in wing morphology between 12 invasive populations of the invasive potato pest, *Tecia solanivora*, at low and high altitude in the central highlands of Ecuador. After characterizing sexual dimorphism in wing shape, we investigated if moths at higher elevations differ in wing morphology from populations at lower altitudes. Results indicate wing shape and size differences between sexes and between altitudinal ranges. Females showed larger, wider wings than males, while high altitude moths showed larger, narrow-shaped wings by comparison to low-altitude moths. GLM analyses confirmed altitude was the only significant determinant of this gradient. Our study confirms a sexual dimorphism in size and wing shape for the potato moth. It also confirms and extends predictions of morphological changes with altitude to an invasive species, suggesting that wing morphology variation is an adapted response contributing to invasion success of the potato moth in mountainous landscapes. Ours is one of the first studies on the morphology of invasive insects and represents a valuable contribution to the study of insect invasions because it both offers empirical support to previous genetic studies on *T. solanivora* as well as proving broader insight into the mechanisms behind morphological evolution of a recently introduced pest.

Keywords: altitude, geometric morphometrics, potato tuber moth, sexual variation, *Tecia solanivora*, wing shape

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Introduction

Wing morphology has been used largely in taxonomic, ecological and evolutionary studies in insects. (Moraes *et al.*, 2004; Carreira *et al.*, 2006; Soto *et al.*, 2008). It is an especially attractive study trait because of its importance in a wide variety of aspects of an insect's life, such as sexual and territorial display, foraging, defense mechanisms, thermal

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regulation, and the aerodynamics and the energetic costs of flight (Betts & Wootton, 1988; Wootton, 1992; Berwaerts *et al.*, 2002, 2006). Wing configurations relate to the varying ecological roles and physiological constrains of flight that are particular to each sex. In general, female wing design would confer superior load bearing, by being larger and/or broader than that of males (e.g. Willmott & Ellington, 1997). Wing morphology, shape in particular, can further be used as an indicator of changing – and often stressful – environmental conditions (Hoffmann *et al.*, 2002, 2005). Decreasing temperature, atmospheric pressure and oxygen availability, and increased solar radiation related to high-altitude environments can lead to morphological changes in insects (Hodkinson, 2005; Dillon & Frazier, 2006). Previous studies have related the effect of temperature in wing morphology, observing larger wings and reduced wing loadings (body mass/wing area) at lower temperatures/higher altitudes (e.g. Miller, 1991a; Norry *et al.*, 2001; Altshuler & Dudley, 2002; Gilchrist & Huey, 2004).

Surprisingly, wing morphology studies of invasive insects have been rare (e.g. Loh *et al.*, 2008); however, these species are interesting models to study environmental adaptations. The environmental factors to which they are exposed act on the invasive population for relatively few generations and provide an opportunity to test for the speed of the response to new environmental gradients. In addition, new environments are often considered stressful, which may affect wing developmental canalization and, thereby, patterns of variations (Hoffmann *et al.*, 2005). Finally, invasive insects often have undergone rapid genetic change due to bottlenecks during introduction to and/or selection within the new environments. Wing developmental regulation may be affected by these genetic changes. For all these reasons, invasive insects are of great interest because not only does their study offer the prospect to understand the effects of environmental and genetics stress on wing shape patterns, but it also allows testing predictions of such patterns within relative short periods of time.

An interesting case of well-documented invasion is that of the potato tuber moth, *Tecia solanivora* Povolny (1973) (Lepidoptera: Gelechiidae), which has spread from Guatemala into Central America, northern South America and the Canary Islands during the past 30 years, attacking *Solanum tuberosum* L. tubers in the field and in storage and becoming one of the most damaging crop pests in these regions (Niño, 2004; Puillandre *et al.*, 2007). As with other invasive species, *T. solanivora* has suffered a significant genetic bottleneck during the invasion process (Puillandre *et al.*, 2007) but has established successful populations in a wide variety of mountainous landscapes and a wide altitudinal range (from 200 m to >3500 m a.s.l.). In Ecuador, the potato moth is found from 2300 m to almost 3800 m a.s.l., its lowest elevation coinciding with some of its highest ranges in Central America. Another interesting characteristic of this invasive pest is that the rapid upward expansion of the agricultural frontier in the Ecuadorian highlands represents a constant opportunity for new populations of the potato moth to become established. It also involves climatic conditions that become more extreme the higher this frontier goes, thus posing new ecological, physiological and behavioral challenges to the moths.

Here, we use a geometric morphometric approach to test the hypothesis that insect wing morphology patterns among sexes and along altitudinal gradients are observed for invasive species despite their recent association to a new environment. To achieve this goal, we explored the variation in wing morphology between 12 populations of T. *solanivora*, at low and high altitude in the central highlands of Ecuador. With traditional morphometrics, biological measures are limited to linear distances, ratios or angles, thus failing to capture the geometrical relations between the anatomical points analyzed (Rohlf, 1990). Geometric morphometrics, on the other hand, offer a more comprehensive approach to the study of shape through the multivariate statistical analysis of anatomical landmarks of biological homology (Bookstein, 1991; Rohlf & Marcus, 1993; Adams *et al.*, 2004). It preserves the information about the relative spatial arrangement of the data throughout the analysis (Zelditch *et al.*, 2004), making it possible to find and analyze shape variations in the organisms within and between populations (Walker, 2000). Moreover, geometric morphometric tools present the advantage of laying results that not only have high statistical power but also have easily visualized results, helping with their interpretation and communication (Rohlf & Marcus, 1993; Adams *et al.*, 2004; Zelditch *et al.*, 2004). In this study, we first characterized sexual dimorphism in wing shape and then investigated whether the populations of the invasive moth at higher altitudes show changes in the wing morphology that can differentiate them from the populations at lower altitudes. We expect to find that previously observed sexual dimorphism in coloration and shape extends to variations of the wing shape between the males and females of *T. solanivora*. We also expect that if there is variation of the wing shape between altitudinal ranges, there would be a relation with environmental factors characteristic of each altitude.

Materials and methods

Study area and background

Tecia solanivora attacks potato tubers both in the field and in store, burrowing deep feeding tunnels into the tuber (Pumisacho & Sherwood, 2002). Adults show sexual dimorphism in both size and coloration, with generally bigger, light-brown colored females versus smaller, deep-brown colored males (EPPO/OEPP, 2005; see Pumisacho & Sherwood, 2002; Barragán, 2005 for further details on *T. solanivora*'s biology). In Ecuador, potatoes are cultivated in the highlands, where climatic conditions are rather stable across seasons and allow year round growth of this crop (Dangles *et al.*, 2008). Mean temperature has little seasonal variation: in the upper elevations, the warmest month averages 18°C and the coolest month averages 12°C (Cáceres *et al.*, 1998). Instead, daily temperature variation is much higher: air temperature can vary daily between 0° and 30°C. Under such favorable climatic conditions and extended food resources available, populations of the potato tuber moth are active throughout the year (Pollet *et al.*, 2003; Pruna 2004; Dangles *et al.*, 2008).

Sampling

Populations of *T. solanivora* were sampled in 12 sites located on the western slope in the central Ecuadorian highlands (table 1). Although moth population occurs continuously along the elevation gradient, moth sampling was designed in order to explore two altitudinal extremes:

Table 1. Data on localities where *Tecia solanivora* specimens were collected for this study (localities considered for sex-related variation analysis are marked with*).

Code	Altitude (m a.s.l.)	Locality	Province	Coordinates	Mean Temperature (min/max) °C	Natural abundance (ind/week)	Individuals analyzed	
							Females	Males
	Low-altitude populations							
A	2400	San Pablo*	Bolivar	01°48′09″S 79°03′28″W	15.6(5–25)	36.9	34	24
B	2586	Pumamaqui	Bolivar	01°47′16″S 79°04′16″W	17.6(5–29)	39.8	–	27
C	2645	San Simón*	Bolivar	01°38′25″S 78°59′18″W	17.5(5–28)	42.3	29	25
D	2667	Salache	Cotopaxi	01°01′51″S 78°36′35″W	15.7 (0–30)	25.6	–	17
E	2700	San Miguelito	Bolivar	01°33′15″S 79°00′08″W	14.1 (5–29)	49.2	–	22
F	2711	Santa Ana	Cotopaxi	01°01′23″S 78°33′30″W	16.1 (3–28)	58.7	–	29
G	2713	La Hoya	Cotopaxi	01°00′24″S 78°34′19″W	14.5 (0–33)	46.8	–	30
H	2727	Anchilibí	Cotopaxi	01°03′00″S 78°33′53″W	16.0 (3–29)	29.8	–	19
	High-altitude populations							
I	3043	San Francisco	Cotopaxi	01°00′01″S 78°32′32″W	14.9 (4–27)	11.1	–	31
J	3152	Palama Bajo	Cotopaxi	01°00′36″S 78°31′52″W	13 (3–24)	11.5	–	29
K	3226	Carbón Chimipamba	Bolivar	01°33′16″S 79°00′085′W	12.7(2–24)	10.9	–	8
L	3280	Palama Medio	Cotopaxi	01°00′14″S 78°31′52″W	11.4 (2–22)	9.72	–	21

Detail of number of individuals used for each locality and natural abundance (calculated based on the number of male catch in pheromone traps per week during the period November 2006–March 2007, see Dangles *et al.*, 2008 for more details).

sites below 2750 m and sites above 3000 m (see fig. 1 and fitted bimodal distribution). This allowed us sampling populations living under contrasting environmental (especially thermal) conditions. Intermediate altitude sites were discarded as they could potentially be influenced by both high and low altitude climates indistinctively. This design implies that populations were treated as nested samples within altitudinal classes as low or high altitude, allowing the use of discriminant analyses with *a priori* established groups. Sampling took place between October of 2006 and January of 2007 using dome traps baited with species-specific pheromones (Pherobank, Wageningen, The Netherlands) placed at about 1 m height in potato fields. Pheromone traps for *T. solanivora* make adult male sampling comparatively easier to female sampling. For this reason, only male individuals were used to test for wing shape variation related to altitude. However, during January of 2006, we also collected adult moths from potato sacks in the localities of San Pablo and San Simón (table 1) that we used to study sexual variation in wing morphology. Preliminary exploration showed that there was no bias induced by pheromone trapping in the samples. Sampled specimens were kept in individual vials with 90% ethanol.

Specimen's preparation and data collection

In all cases, forewings of adult moths were separated from the body at the base and the scales removed by gently sweeping them away with a fine brush in a Petri dish

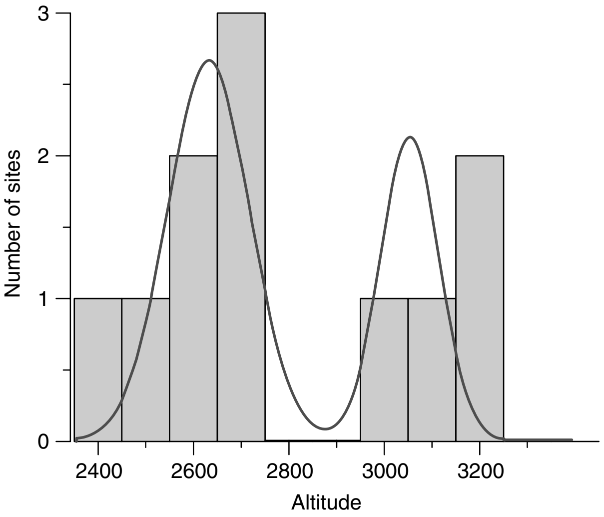


Fig. 1. Methodology used to group the study sites into two altitude classes. Frequency distributions of altitudes were decomposed into Gaussian distributions using a combination of a Newton-type method and expectation maximization algorithms. The mean (μ) and variance (σ) of the altitude distribution for both groups of sites were calculated using the 'mixture distribution' package of R software (R Development Core Team, 2008). For low altitude sites, $\mu = 2548$ and $\sigma = 108$, and for high altitude sites, $\mu = 3075$ and $\sigma = 77$.

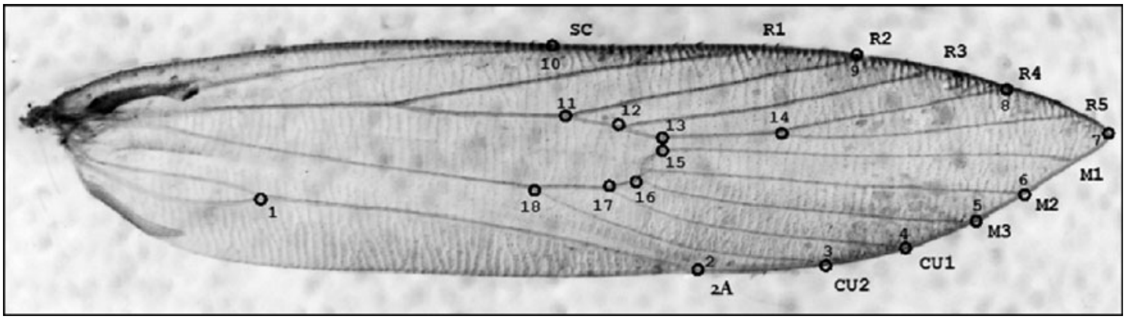


Fig. 2. Right wing of a male specimen of *Tecia solanivora* depicting the position of the 18 landmarks used for the analyses. Vein nomenclature from Borror *et al.* (1981).

containing 90% ethanol. In some cases, the base of the wings was damaged during extraction, due to fragility caused by being retained in the pheromone traps for long durations. This also affected the quality of the whole body, making it impossible to obtain other morphometrics related to body measures. Photographs of each wing were taken using a digital camera (Powershot S40, Canon, Tokyo, Japan) attached to a stereo microscope (Leica M275, Bannockburn, IL, USA) with a black background and a millimeter grid for size reference.

Following Zelditch's *et al* (2004) criteria of homology and comprehensive coverage, a total of 18 landmarks were identified on each wing and their coordinates obtained using tpsDig 2.10 (Rohlf, 2006). Of these, 17 correspond to type I landmarks (whose claimed homology from case to case is supported by morphological evidence, e.g. vein-vein and vein-edge intersections) and one to type II landmarks (whose claimed homology from case to case is supported only by geometric evidence, e.g. structure tip), according to Bookstein's (1991) classification (fig. 2). Landmarks at the base of the wing were not considered because wing veins in this area are quite thick, and positioning of landmarks was not easily repeatable; moreover, due to sampling and preparation constrains, wing base of some specimens was not in suitable condition. All landmark coordinates were superimposed with a Generalized Procrustes Analysis (GPA) algorithm using tpsSmall 1.20 (Rohlf, 2003) to remove the effect of scale, position and orientation from the coordinates (Rohlf, 1999; Zelditch *et al.*, 2004). The centroid size (CS), calculated as the square root of the sum of squared distances from the landmarks to their centroid (Bookstein, 1991), was obtained as an estimator of size. The coordinates were also divided by CS to obtain size-free, though not allometry-free, data (Baylac *et al.*, 2003). Adjusted coordinates and CS were averaged between the right and left wings of each individual when both wings were available, in order to reduce measurement error (Chris Klingenberg, personal communication). These repeated measurements also provided an estimate of the digitizing error of >1 mm (see Arnqvist & Martensson, 1998).

Morphometric and statistical analyses

Projection of data from Kendall's shape space into a linear tangent space is necessary for using standard multivariate analyses (Rohlf, 1999). We confirmed that data variation due to this projection was small enough for data set to correctly represent the original distribution in the shape space. A regression of the Procrustes distance (Pd) from the consensus configuration in the shape space on the PD calculated on the linear tangent space was performed using tpsSmall.

Sex related variation

To evaluate wing shape dimorphism, shape variables (also called partial warps: Rohlf, 1993) were used for a principal components analysis called relative warps analysis (RWA), performed with tpsRelw 1.45 (Rohlf, 2007). Using the total shape variables (both uniform and non-uniform components) the variation between male and female wings was assessed with a multivariate analysis of variance (MANOVA) and a canonical variate analysis (CVA) (following Cardini & O'Higgins, 2004). Statistical significance was evaluated with a two-group multivariate permutation analysis ($N = 10{,}000$). All statistical analyses were performed using PAST 1.6 (Hammer *et al.*, 2001) and groups were established *a priori* to maximize the differences (Rohlf *et al.*, 1996; Cavalcanti *et al.*, 1999). To visualize the existing variation, a thin-plate spline analysis was performed using tpsSpline (Rohlf, 2004) with the consensus configurations of each sex on a deformation grid based on the reference configuration calculated from the total sample ($n = 112$).

Altitude related variation

A RWA was also used for evaluating wing shape variation among high and low altitude groups with the overall sample from the 12 localities. A second RWA was performed using the consensus configuration of each locality to give an equal weight to each one of the localities instead of to each specimen (Rohlf *et al.*, 1996). A combined MANOVA/CVA along with a multivariate permutation analysis ($N = 10{,}000$) were run to test the significance of the separation between the two groups. Visual analysis of the variation was performed between the consensus configurations of each locality on a deformation grid based on the reference configuration calculated from the total sample ($n = 281$) using tpsSpline. Although we were aware that cross-validation would have improve our discriminant analyses results and made them more robust (e.g. Klimov *et al.*, 2006), limitation of the number of available specimens prevented us from doing it. However, random removal of 1–5 individuals did not change the significance of our results.

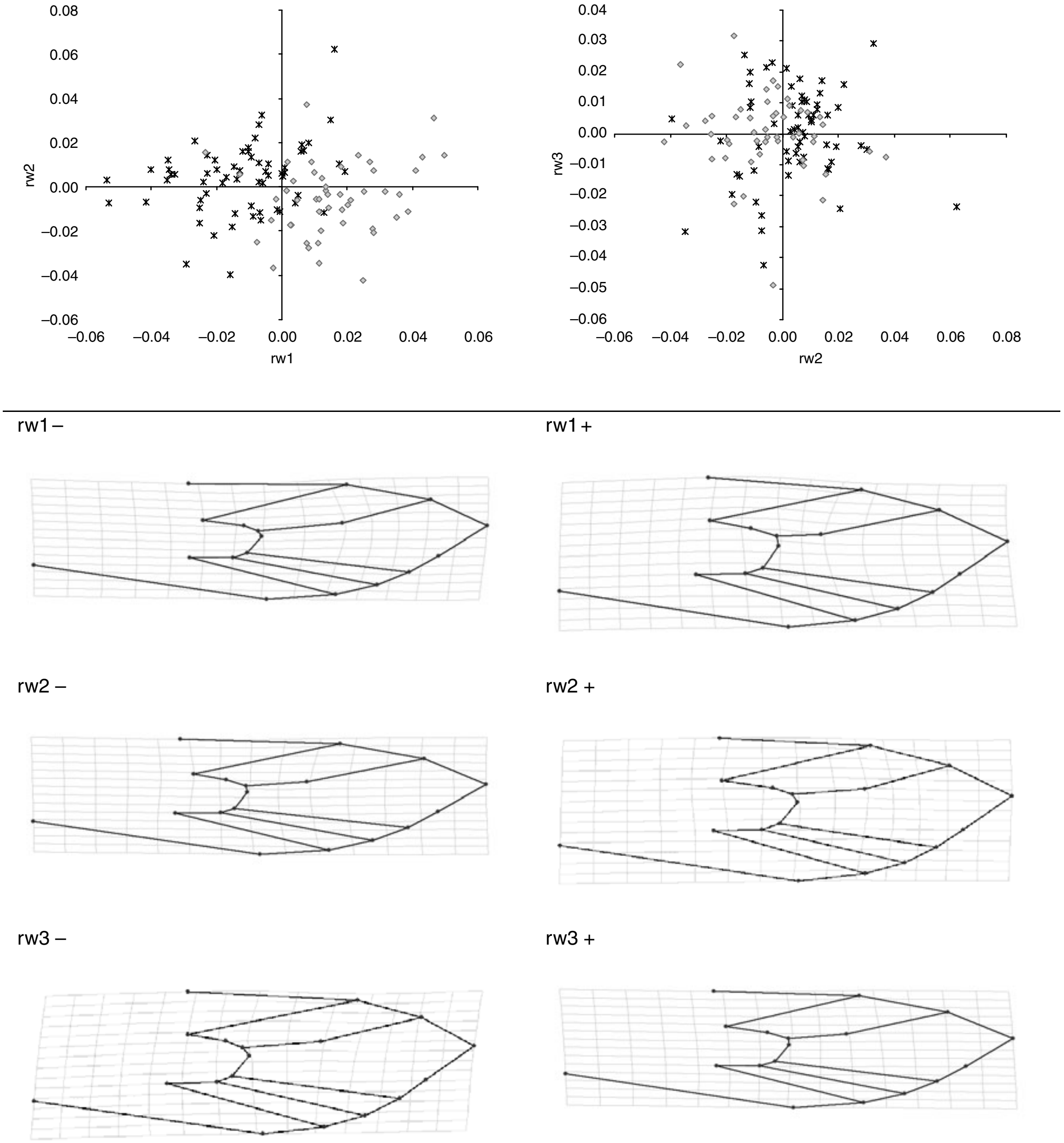


Fig. 3. Upper part: Scatter plot for the RWA on male and female wings. Distribution of specimens for (a) *x*: rw1, *y*: rw2 and (b) *x*: rw2, *y*: rw3 (males: $n = 49$, in grey; females: $n = 63$, in black). Lower part: Deformation grids for both negative and positive extremes of the three relative warps: rw1, rw2 and rw3.

Allometry and allometric effect

Forewing morphometry can be taken as a reliable body size index in Lepidoptera (Miller, 1991b). Consequently, to test for overall body size differences between sexes and between specimens from different sites, we performed a one-way ANOVA using the isometric estimator centroid size (CS). This was obtained from the coordinates' data with tpsSmall and averaged across left and right wings of individuals (see Gomez & Monteiro, 2008). We did not use thorax mass for size related exploration, as recent findings show that it decreases with age in some butterflies (Stjernholm *et al.*, 2005), and our sampling method did not allow for determining age in the captured individuals. Additionally, we ran two analyses using tpsRegr 1.31 (Rohlf, 2005) to examine the influence of allometry on any variation found between the studied groups. First, a multivariate regression of the total shape variables, using the CS logarithm (CS loge) as the independent variable. Second, we ran a multivariate analysis of covariance (MANCOVA), using the total shape variables as dependent variables and the CS loge as the independent variable.

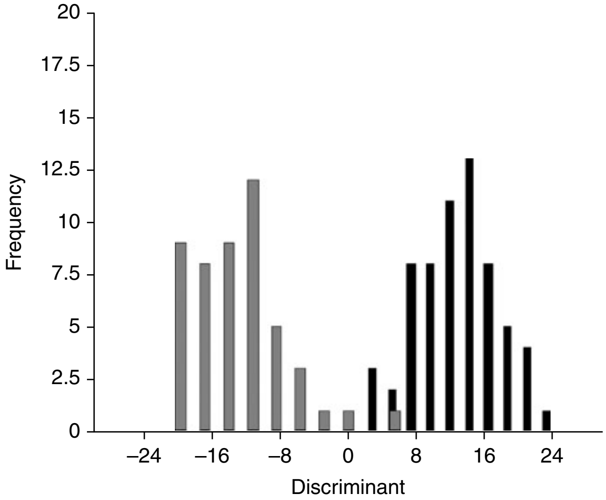


Fig. 4. Histogram of the specimens grouped by sex (males: $n=49$, in grey; females: $n=63$, in black) along the Discriminant axis. Percent of individuals correctly assigned to their original groups: 99.11%.

Environmental impact on wing morphology

Generalized Linear Model analysis (GLM) were used to test the effect of environmental variables on mean values of two wing morphometric variables (size: CS, and shape: CV1 scores) for *T. solanivora* populations at each sampling point. The Poisson log-linear model included the following ($\text{Log}_{10}+1$) transformed variables: region, altitude, mean temperature, min temperature, max temperature, moth density and relative humidity. These explanatory variables were chosen based on their potential use as surrogate measures of processes and factors that might have a direct effect on wing morphology along the gradient. The analysis was run using mean, minimum and maximum temperatures, but the most significant results were always obtained with mean temperature. We also included a 'site' variable to allow within-site comparisons while controlling for variation resulting from unmeasured site-specific parameters. Change in wing morphometrics due to each factor was modeled considering each factor independently and in combination with other factors, including biologically reasonable two-way interactions and squared variables. The more parsimonious model was identified using the Akaike's Information Criterion (AIC: see Venables & Ripley, 2002) in likelihood ratio tests to find the difference between the initial model (including all terms) and the reduced model (in which one effect term was removed). All analyses were performed using the mass library for R (R Development Core Team, 2008).

Results

Wing shape variation and sexual dimorphism

The first three relative warps, which are the principal components of the distribution of wing shapes, explain 50.92% of the total wing shape variation between the 63 female and 49 male adult moths studied (rw1 = 24.62%, rw2 = 14.94%, rw3 = 11.35%). A plot of rw1 and rw2 showed a tendency of females to be grouped at the negative side of the first relative warp and males at the positive side; deformation grids for both extremes showed slender wings on the positive end and wider wings at the negative end (fig. 3). Sexual dimorphism in wing shape was highly significant (MANOVA/CVA of shape variables, sex, Wilks' lambda = 0.129, $F=16.62$, df = 32, $P<0.001$). Both sexes were effectively separated along the discriminant axis (fig. 4), with 99.11% of correctly classified specimens, as did too the two-group multivariate permutation analysis (Mahalanobis distance = 0.4944, $P<0.0001$). The grid deformations of the consensus configuration of each sex from the reference configuration revealed that male and female wings were mostly differentiated by their width, and not so much by their longitude (fig. 5). This result is consistent with what was observed with the RWA.

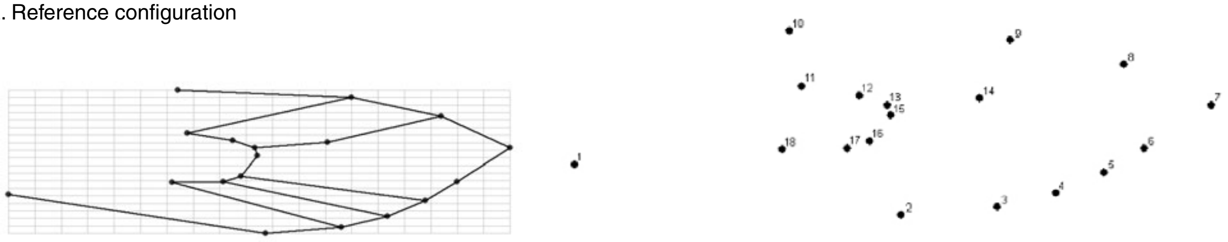
Wing shape differentiation among low and high altitude populations

A total of 281 adult males were collected from our pheromone sampling: 192 corresponding to localities underneath 2800 m a.s.l. (low altitude group) and 89 to localities above 3000 m a.s.l. (high altitude group) (table 1). The RWA for all the individuals of the 12 localities showed that 45.33% of the overall variation in wing shape was explained by the first three relative warps: rw1 = 21.03%, rw2 = 13.10%, rw3 = 11.20% (fig. 6). As expected, a RWA for the consensus configurations of each site showed a better differentiation of the groups, with a 75.51% of the overall variation explained by the first three relative warps (data not shown). Deformation grids for rw1 showed that wing shapes narrow at the positive extreme, and widen at the negative extreme. High altitude specimens tended to group at the positive end of the first relative warp, while low altitude specimens grouped around the center and negative end. The effect of altitude on the wing shape variation was highly significant (MANOVA/CVA of shape variables, altitude, Wilks' Lambda = 0.565, $F=5.958$, df = 32, $P<0.001$). Low and high altitude groups were effectively separated along the discriminant axis (fig. 7), with 83.99% correctly classified specimens. Similar results were obtained with the multivariate permutation (Mahalanobis distance = 0.1124 and $P<0.0001$). TpsSpline deformation grids and displacement vectors from the consensus configurations show that, for the high altitude group, wing shape is narrower than wing shape of the low altitude group, especially at the middle part of the wing (fig. 8).

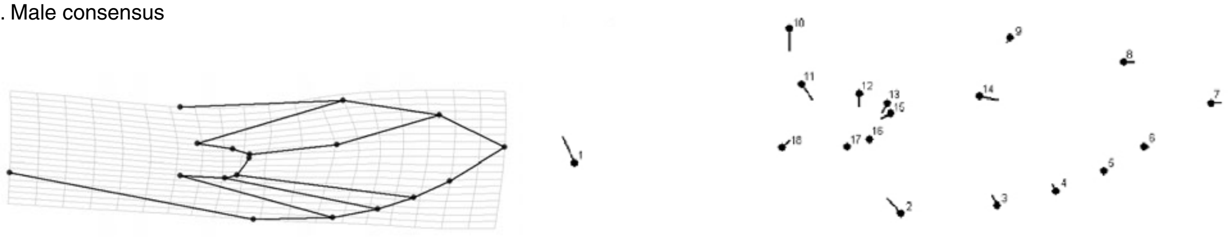
Allometry and allometric effect

One-way ANOVA was highly significant for centroid size variation (i.e. variation of wing size as calculated through the isometric estimator of size, centroid size) between sexes ($F=19.24$, df = 1, $P<0.001$) and between altitudinal sites ($F=9.979$, df = 1, $P=0.0017$). Female wings were generally larger than male wings (fig. 9), and high altitude wings tended to present higher values for centroid size (i.e. larger wings; fig. 10). Regression of shape variables on size for male and female wings showed significant allometric relationship (Wilks' Lambda = 0.318, $F=5.288$, df = 32, $P<0.001$), but MANCOVA indicated allometric slopes were not significantly different (Wilks' Lambda = 0.732, $F=0.401$, df = 64, $P=1.00$) and, when size was held constant, shape variables

a. Reference configuration



b. Male consensus



c. Female consensus

Fig. 5. Deformation grids for the consensus configurations and displacement vectors of a. males and b. females, from the reference configuration calculated on the overall sample (n = 112, top). Grids and vectors obtained using tpsSpline, exaggerated by a factor of 5 for better visualization of changes.

proved to differ significantly (Wilks' lambda = 0.151, $F = 13.697$, df = 32, $P < 0.001$). When looking for allometric effect on the altitude group's variation, similar outcomes were obtained. The regression of shape variables on size showed highly significant values (Wilks' Lambda = 0.618, $F = 4.797$, df = 32, $P < 0.001$) and again no difference was observed between allometric slopes with MANCOVA (Wilks' Lambda = 0.857, $F = 0.614$, df = 63, $P = 0.992$). The difference observed on wing shape between low and high altitude groups remained significant once size was held constant (Wilks' lambda = 0.151, $F = 13.697$, df = 32, $P < 0.001$).

Environmental impact on wing morphology

Of the eight factors included in the GLM analysis, only altitude significantly affected the two wing morphometric variables (CS: $\Delta AIC = 3.1$, $P = 0.035$, CV1 scores: $\Delta AIC = 2.5$, $P = 0.045$). The following most important predictors of wing morphometrics were 'mean temperatures' and 'moth abundance' but these were not significant ($P > 0.12$). We found neither significant effect of 'site' nor 'region' on wing morphometrics ($P > 0.66$).

Discussion

Sexual dimorphism in wing shape

Our results not only confirm a female-biased sexual dimorphism in size for *T. solanivora* but also show dimorphism in the shape of the wings. Several studies have shown that sexual dimorphism in life-history traits of insects, such as size and shape, may be adaptive (reviewed in Nylin & Gotthard, 1998). The larger size of females may be favored by natural selection for increased fecundity (Gilchrist, 1990; Calvo & Molina, 2005) and suggests a higher fitness importance of size advantages for female's fecundity compared to those offered to males (Nylin & Gotthard, 1998). Small size of males can be the result of sexual selection for rapid development that leads to early eclosion (Gilchrist, 1990). Although populations with high overlapping of generations (as is the case of populations studied here) were not usually expected to present protandry (Singer, 1982; Nylin *et al.*, 1993), more recent findings show that aseasonal moths can present it (Muralimohan & Srinivasa, 2008). Elongated wings observed in males can be related to the longer flying periods they require for finding sexually active females (Douwes, 1976; Kingsolver, 1983; Shreeve, 1984; Gilchrist, 1990). The observed tendency of larger wing size of

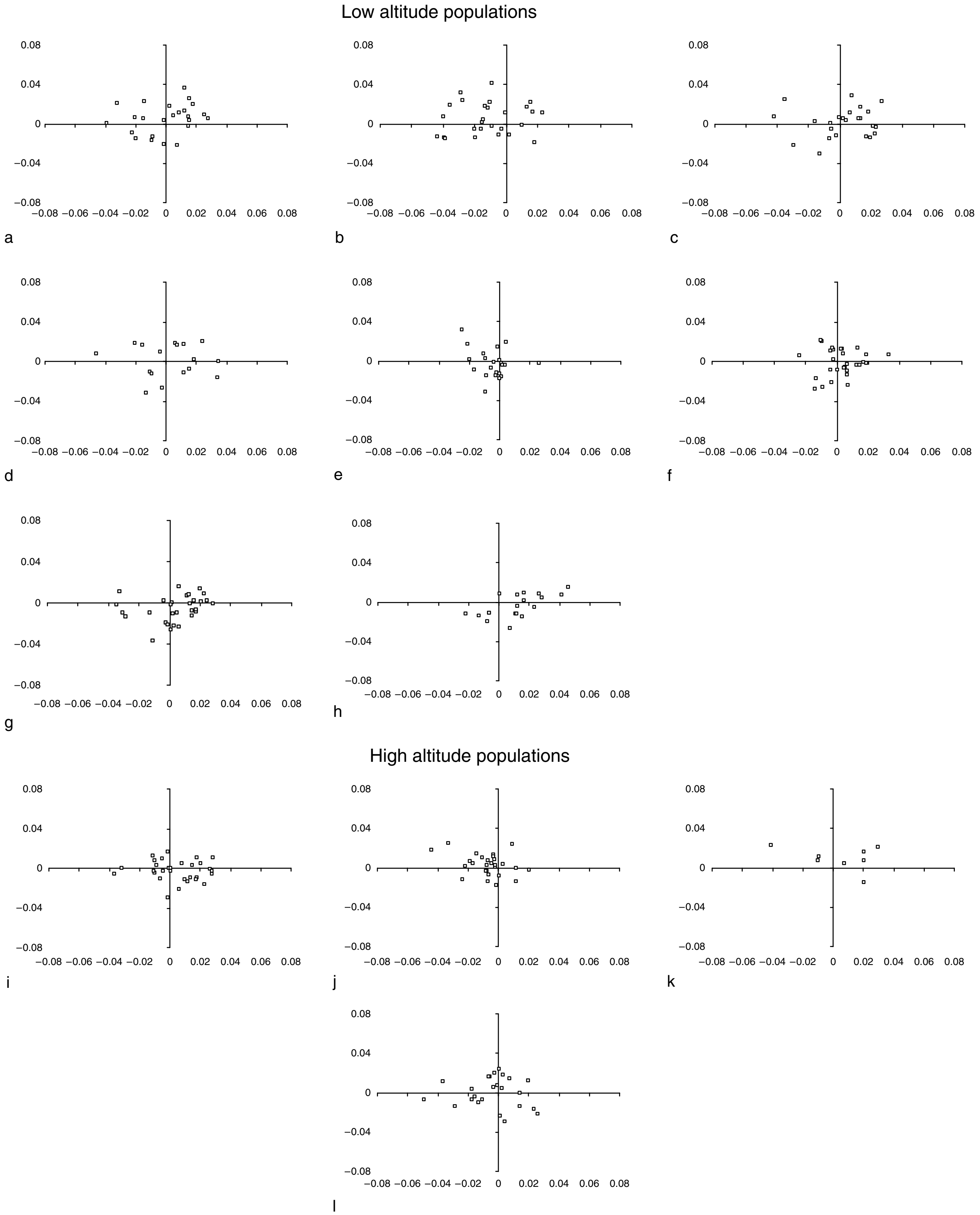


Fig. 6. Scatter plot for the RWA on males from the 12 localities studied. Breakdown of the distribution of specimens for each locality on *x*: rw1, *y*: rw2. (a) San Pablo, (b) Pumamaqui, (c) San Simón, (d) Salache, (e) San Miguelito, (f) Salache, (g) La Hoya, (h) Anchilibí, (i) San Francisco, (j) Palama Bajo, (k) Carbón Chimipamba, (l) Palama Medio. The figure shows a rw1 and rw2 plot breakdown of each site for better visualization of the distribution.

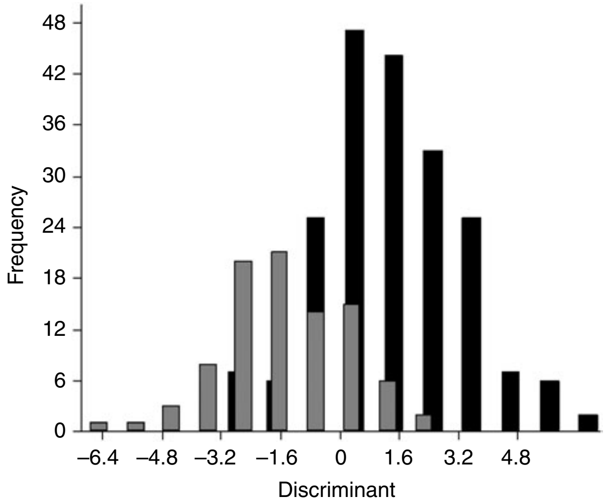


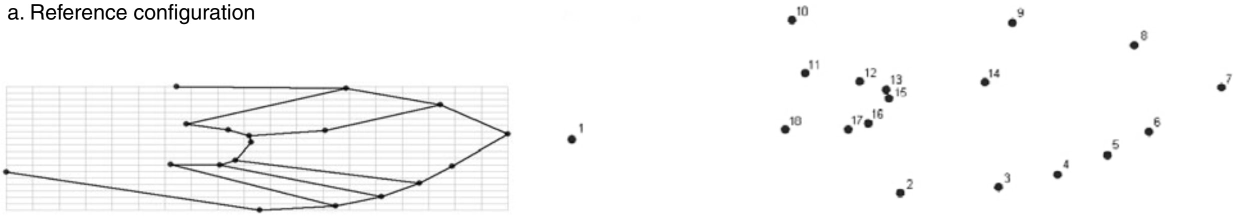
Fig. 7. Histogram of the specimens grouped by altitude (high altitude: grey, low altitude: black) along the discriminant axis. Percent of individuals correctly assigned to their original groups: 83.99%.

female wings than that of males is probably a consequence of the wider wing configuration in females; similar wing configurations were observed in other Lepidoptera, the speckled wood butterfly, *Pararge aegeria* (Berwaerts *et al.*, 2002).

Wing shape variation with altitude

Our study confirms and extends predictions of morphological changes with altitude to an invasive species. We observed significant differences in size and wing morphology between populations from localities under 2750 m and above 3050 m a.s.l., with larger moths at higher altitudes and most of the variation in shape occurring on the width of the middle region of the wings; higher altitude populations tend to have slender wings, while the lower altitude populations show broader wings by comparison. One of the hypotheses behind our research was that the differences in temperature we observed in the different sampled sites might somehow affect the wing morphology of the moths. This hypothesis was sustained by previous studies on *D. melanogaster* (Dillon & Frazier, 2006; Frazier *et al.*, 2008) and *D. suboscura* (Gilchrist & Huey, 2004), which suggest aerodynamic reasons for larger (and proportionally longer) wings at lower temperatures and lower atmospheric pressure. Slender wings might compensate for the environmental constraints on flight at higher altitudes, since they can help in reducing the energetic requirement for flight as well as

a. Reference configuration



b. High altitude consensus

c. Low altitude consensus

Fig. 8. Deformation grids for the consensus configurations and displacement vectors of specimens from low and high altitude sites, from the reference configuration calculated on the overall sample ($n = 281$, top). Grids and vectors obtained using tpsSpline, exaggerated by a factor of ten for better visualization of changes.

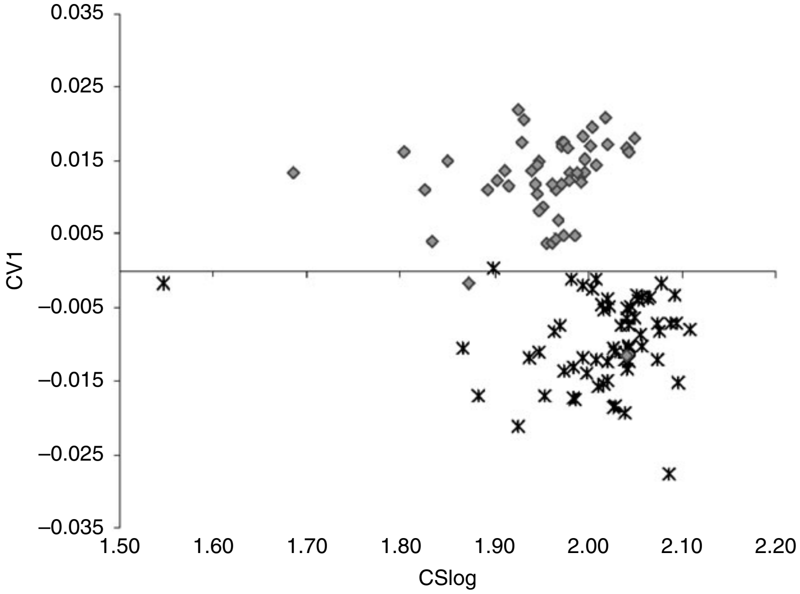


Fig. 9. Regression of the natural logarithm of centroid size (CS $\log_e$) on the first canonical axis (CV1) of the shape variables of male ($n = 49$, in grey) and female ($n = 63$, in black) wings.

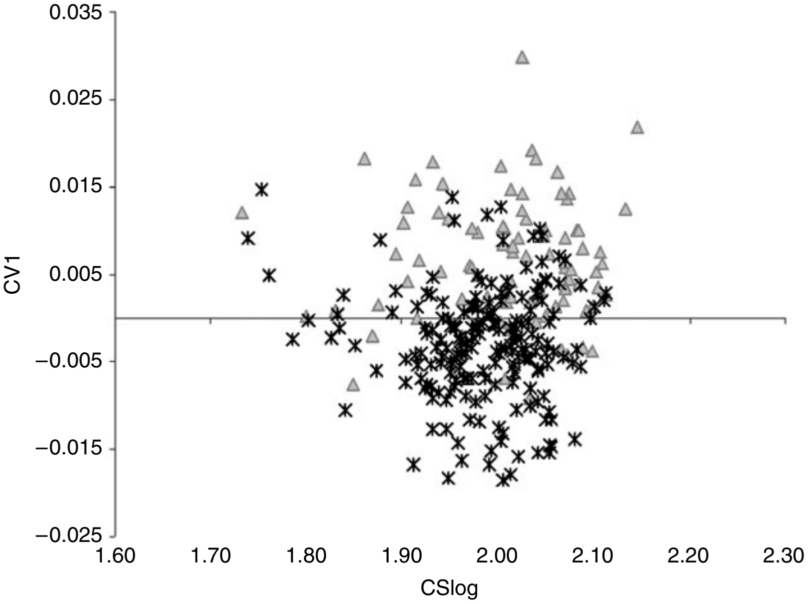


Fig. 10. Regression of the natural logarithm of centroid size (CS $\log_e$) on the first canonical axis (CV1) of the shape variables of high altitude ($n = 89$, in grey) and low altitude ($n = 192$, in black) wings.

dragging forces while yielding greater aerodynamic forces (Wootton, 1992; Berwaerts *et al.*, 2002; Frazier *et al.*, 2008 and references therein). We also expected moths with lager body sizes at lower temperatures – and, therefore, higher altitudes – as is predicted by Bergmann's rule (see Miller, 1991a).

Despite the fact that our observations fit the predictions for body size and wing morphology variations at different altitudes, we found no evidence of a strong relationship between such changes and the decreasing temperature at higher elevations. The GLM analysis showed that the only significant factor behind the observed variation was altitude; and, although the second-most important predictor was mean temperature, it was not significant. However, altitude is a combination of temperature, atmospheric pressure, humidity and solar radiation variables, each of them potentially exerting separate selective pressures on wing shape and size.

Wing shape of an invasive species

The invasive potato moth developed altitudinal differences in wing morphology consistent with patterns observed for non-invasive species. Our results show that such patterns are able to develop in a short period of time. To our knowledge, the *T. solanivora*'s wing size altitudinal cline in Ecuador is the only described example of an invasive insect rapidly developing altitudinal Bergmann clines in their invasive range (*D. subobscura* and other invasive *Drosophila* clines are latitudinal). Below, we discuss three hypotheses that may explain such a process.

The first hypothesis is a rapid genetic adaptation. In case of genetic adaptation, larger and broader thoraxes, smaller abdomens and higher wing aspect ratios may reflect increased dispersal ability of high-altitude potato moth populations – which are also those that are the most recently established (see Hughes *et al*., 2007; Breuker *et al*., 2007). However, the rapidity of the establishment of the cline (ten years for *T. solanivora* versus 20 years for *D. subobscura*) and the arguments detailed below question the genetic nature of this cline. The second, and most likely, hypothesis is that these patterns are a purely plastic response, and the differences between altitudes are not genetically based as observed for another invasive insect species (Loh *et al*., 2008). Potato moths show a significant decrease in development time at higher temperatures (Notz, 1996; Dangles *et al*., 2008) and a reduced number of generations at high altitudes (Dangles *et al*., 2008), which can directly relate to size variation between low and high altitudes. Wing morphology can also respond to environmental cues, in particular during the developmental period, when genes that need to be activated during wing development can be altered by environmental conditions (Hoffmann *et al*., 2005 and references therein). Another argument against genetically-based variation is the absence of non-altitudinal differentiation; we indeed ruled out geographic distance as a variability predictor because sampled localities did not differ significantly between provinces of origin (i.e. the largest geographical distance; data not shown). This lack of significance was also reinforced by the results of GLM showing that province and site were not influencing factors in morphological variation. The third hypothesis is that, in addition to being plastic, the response may be non-adaptive (i.e. it does not provide any advantage to the insect to have long wings at high altitude) but due to purely developmental and physiological mechanisms (see Blanckenhorn & Demont, 2004 for a review). For example, a differential ratio between cell division and cell growth increment with temperature lead to smaller adults at higher temperature (with no adaptive value of this reaction norm). Another physiological explanation for these clines involves slower oxygen diffusion at increasing temperatures in large cells compared with their oxygen consumption, possibly inducing malfunction of such cells at higher temperatures.

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