

# Evidence of selection on phenotypic plasticity and cost of plasticity in response to host-feeding sources in the major Chagas disease vector *Triatoma infestans*



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## ABSTRACT

Phenotypic plasticity is the ability of a genotype to display alternative phenotypes in different environments. Understanding how plasticity evolves and the factors that favor and constrain its evolution have attracted great interest. We investigated whether selection on phenotypic plasticity and costs of plasticity affect head and wing morphology in response to host-feeding sources in the major Chagas disease vector *Triatoma infestans*. Full-sib families were assigned to blood-feeding on either live pigeons or guinea pigs throughout their lives. We measured diet-induced phenotypic plasticity on wing and head size and shape; characterized selection on phenotypic plasticity for female and male fecundity rates, and evaluated costs of plasticity. Wing size and shape variables exhibited significant differences in phenotypic plasticity associated with host-feeding source in female and male bugs. Evidence of selection on phenotypic plasticity was detected in head size and shape for guinea pig-fed females. A lower female fecundity rate was detected in more plastic families for traits that showed selection on plasticity. These results provide insights into the morphological phenotypic plasticity of *T. infestans*, documenting fitness advantages of head size and shape for females fed on guinea pigs. This vector species showed measurable benefits of responding plastically to environmental variation rather than adopting a fixed development plan. The presence of cost of plasticity suggests constraints on the evolution of plasticity. Our study indicates that females fed on guinea pigs (and perhaps on other suitable mammalian hosts) have greater chances of evolving under selection on phenotypic plasticity subject to some constraints.

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## 1. Introduction

Phenotypic plasticity is the ability of an organism or a single genotype to exhibit distinct phenotypes when exposed to different environments throughout its ontogeny (Pigliucci, 2005). The evolution of phenotypic plasticity is thought to provide a mechanism for adaptation to spatially or temporally variable environments

(Dudley and Schmitt, 1996). Understanding how plasticity evolves and the factors that favor and constrain its evolution has attracted a great interest (Pigliucci, 2005; van Kleunen and Fischer, 2005). However, few studies have experimentally tested the hypothesis that the phenotype evoked by a specific environment results in higher relative fitness than the alternative phenotype, *i.e.*, selection on phenotypic plasticity (*e.g.*, Baythavong and Stanton, 2010; Caruso *et al.*, 2006).

The evolution of adaptive traits in variable habitats depends on the contributions of plasticity and inheritance of the phenotypic expression (Via *et al.*, 1995). However, selection on phenotypic plasticity only tests the specific plastic responses to a fitness component (Pigliucci, 2005; van Kleunen and Fischer, 2005) and does not imply heritability measures. Constraints on the evolution of adaptive plasticity were evidenced by cost for traits under selection on phenotypic plasticity (Mooney and Agrawal, 2008). The cost of phenotypic plasticity is related to the lower fitness that more plastic

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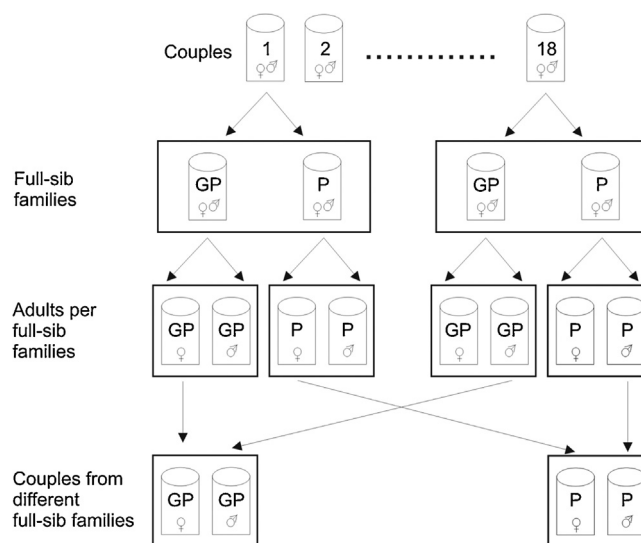
families display when compared with less plastic families in a given environment (Stinchcombe et al., 2004; van Kleunen and Fischer, 2005). Evidence for the cost of plasticity was reported in *Drosophila melanogaster* (Krebs and Feder, 1997); in larval wood frogs, *Rana sylvatica*, in the presence of aquatic predators (Relyea, 2002); in gypsy moth larvae related to digestive enzymes (Mrdaković et al., 2014), whereas scant evidence of costs of plasticity in response to predators was recorded in the crustacean *Daphnia pulex* (Scheiner and Berrigan, 1998).

*Triatoma infestans* is the most important vector of *Trypanosoma cruzi*, the etiological agent of Chagas disease, in the southern cone of South America (Gürtler et al., 2007). This vector has successfully adapted to human dwellings, and presents high levels of natural infection with *T. cruzi* (Zeledón and Rabinovich, 1981). Likewise other Triatominae, the main bloodmeal hosts of *T. infestans* are birds and mammals (Rabinovich et al., 2011). Mammalian and avian blood differs in several respects mainly related to the hemostatic mechanism (i.e., thrombocytes vs. platelets), hematocrit, viscosity, and water content (Lehane, 2005; Lewis, 1996). Avian blood has lower nutritional quality than mammalian blood (Lehane, 2005). Host-feeding sources affect fitness traits: cohorts of *T. infestans* fed on guinea pigs throughout their lives exhibited higher fecundity and fertility, and needed less blood to produce an egg compared with bugs that fed on pigeons alone (Nattero et al., 2011).

Host-feeding sources represent environments that are different enough as to influence development (e.g., Jorge et al., 2011; Laparie et al., 2010; Nattero et al., 2013). Functional arguments for the value of diet-induced selection on phenotypic plasticity in the morphological traits of *T. infestans* are based on experimental evidence showing variations in head capsule size along the ontogenetic trajectory (Nattero et al., 2013): adult bugs fed on guinea pigs exhibited larger head capsules and higher shape variation than those fed on pigeons. This pattern may be related to differences in blood viscosity between avian and mammalian hosts during the process of blood ingestion in which the cibarial pump (i.e., a complex of muscles that nearly fill the head capsule) is involved.

The expression of plasticity in head capsules and wings are not expected to vary in the same way since the relation with host-feeding sources is not necessarily the same. For example, head capsule is related directly to the substantial differences involved in blood-feeding on mammalian and avian hosts or to their blood characteristics, whereas wings and the probability of flight initiation are related to other variables such as temperature, density, weight-length ratio and sex. The probability of flight initiation of *T. infestans* males exceeded that of females in the field (Gürtler et al., 2014; Vázquez-Prokopec et al., 2002) but not in experimental settings (Gurevitz et al., 2006 and references therein). The relation between wing morphology and host-feeding source is plausible in view of the reported differences in wing size and shape and flight initiation probabilities across habitats and sexes (Gürtler et al., 2014; Schachter-Broide et al., 2004).

For a particular trait to be selected by adaptive phenotypic plasticity and become fixed in the population as an adaptive novelty, this trait should exhibit low costs of plasticity, among other factors (Relyea, 2002). This would imply that phenotypic plasticity advantages and low costs of plasticity might favor the fixation of a particular characteristic of that trait in a given population. Under such conditions, these characters could be useful for vector control purposes as phenotypic markers of a given environment or habitat. Our study is based on the following hypotheses: (1) evidence of wing phenotypic plasticity will be detected between host-feeding sources, based on differences in blood composition between mammalian and avian hosts; (2) both wings and head capsules will be targets of selection on phenotypic plasticity in guinea pig-fed bugs based on evidence showing that bugs fed on mammalian hosts exhibited higher fecundity and fertility than bugs fed on avian



**Fig. 1.** Experimental setup for adaptive phenotypic plasticity in *T. infestans*. Couples from newly-emerged adults were constituted and the entire offspring was considered a full sib-family. Nymphs from the full-sib families were assigned to feed on guinea pig (GP) and pigeon (P). Females per full-sib family were matched with males from different full-sib families to establish experimental couples whose reproductive rates were registered.

hosts (Nattero et al., 2011); (3) evidence of selection on phenotypic plasticity in two fitness components (female and male fecundity rates) would differ between females and males, because total adult blood intake directly affects female and male reproductive output (Nattero et al., 2011); and (4) more plastic individuals will exhibit costs, derived from expressing plastic development in wing or head size and shape rather than adopting a fixed development plan.

This study may be the first attempt to determine diet-induced selection on phenotypic plasticity and cost of plasticity in an insect species. To evaluate this, we experimentally measured diet-induced patterns of phenotypic plasticity in wing size and shape of *T. infestans*. We also characterized selection on phenotypic plasticity, measured as selection on phenotypic plasticity in two fitness components, and estimated the cost of plasticity for wing and head capsule size and shape.

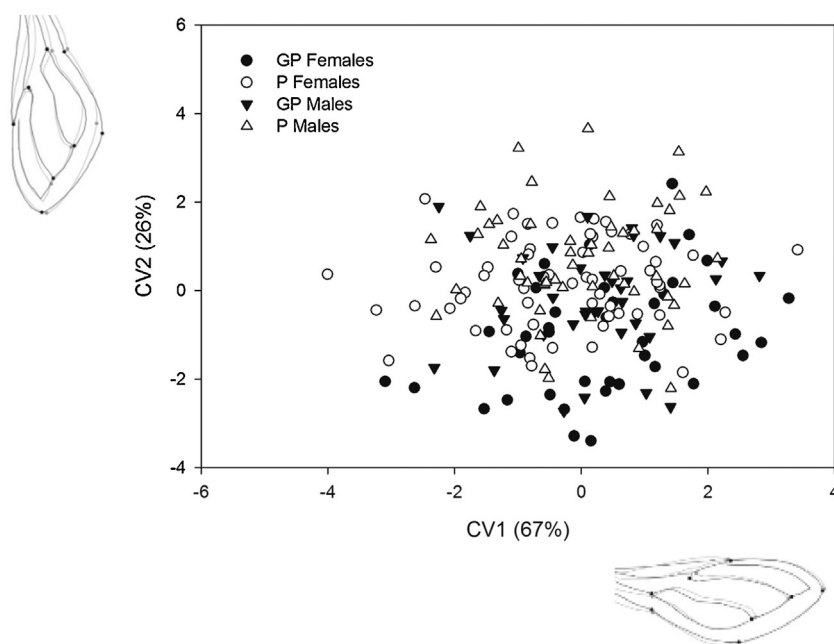
## 2. Materials and methods

### 2.1. Experimental design

*T. infestans* fifth-instar nymphs were collected from chicken coops and at a mud oven in two neighboring rural communities in Belgrano, San Luis province (Argentina) in April 2010. Houses were made of bricks walls, and the main peridomestic structures were chicken coops. Chickens were the most common host-feeding source of *T. infestans* (about 80%), and dogs contributed to the remaining 20%. Annual mean rainfall was about 500 mm; the mean annual air temperature ranged from a minimum of 10.7 °C and a maximum of 24.4 °C.

The collected nymphs were held in cylindrical vials in groups of 10 individuals and kept separately by habitat, maintained in the laboratory and fed regularly on pigeons (*Columba livia*) until molting to the adult stage. The first laboratory generation was used for the experiments. Insects were maintained in the laboratory at 26 ± 2 °C, 60–70% relative humidity and a photoperiod of 12:12 h (light:dark) throughout the experiments.

The experimental setup is described graphically in Fig. 1. One male and one female from the newly-emerged adults were held in a cylindrical glass vial. In order to obtain different genotypes,



**Fig. 2.** Canonical variate analysis on the whole wing shape dataset. The shape changes associated with two canonical axes (CV) are visualized as configurations corresponding to extreme positions on them. Grey—configurations for negative PC scores; black—configurations for positive scores. Shape changes correspond to an arbitrary value of 2.5 standard deviations. The percentages described on each axis correspond to the explained variance. GP—guinea pig, P—pigeon.

couples were formed with individuals from different habitats; the entire offspring of each couple was considered a full-sib family. All emerged first-instar nymphs from 18 full-sib families were randomly assigned to one of the two host species used: guinea pigs (*Cavia porcellus*) and pigeons (*C. livia*). Since the main bloodmeal hosts of triatomines are birds and mammals (Rabinovich et al., 2011), we choose pigeons and guinea pigs as host-feeding sources because they are appropriate experimental hosts. Pigeons, guinea pigs and mice have traditionally been used for experimental feeding of triatomine bugs (e.g., Aldana et al., 2009; Guarneri et al., 2000a,b; Nattero et al., 2011). All insects were fed every 15 days on the same host-feeding source during the entire life cycle. Seven pigeons and 6 guinea pigs were used throughout the assays. All hosts used were naive, in good health, and were periodically replaced during the experiment. Experiments were done following the ethics guidelines for biomedical research (Resolution No. 1047, 2005) of the National Council of Scientific and Technical Research (CONICET) of Argentina. For feeding bugs, pigeons were immobilized by folding their wings. Bugs were placed inside a plastic vial with tulle covering the open side of a cylindrical glass vial which was exposed to the pigeon beneath the wings. Guinea pigs were placed in small plastic cases (20 × 10 cm) containing a piece of brick, and bugs were introduced into the case and used the brick as a refuge. Each assay included up to 5 bugs per host, which were allowed to feed *ad lib* until the bug removed its proboscis and ceased to probe again.

Four or five female and male bugs per full-sib family were matched with individuals from different full-sib families that fed on the same host-feeding source to establish the experimental couples (Fig. 1). All insects were weighed on the day of imaginal molt. Only one meal was offered to each bug during the first 10 days in the adult stage; all bugs were weighed individually before and immediately after each feeding event with a Mettler balance (precision, 0.001 mg) to estimate the amount of blood (mg) consumed. The number of eggs and spermatophores produced by each couple were counted twice a week until no oviposition or mating was registered during 20 consecutive days. Female fecundity rate was estimated as numbers of eggs laid per female-day; for this calculation, we divided the total number of eggs laid per female by

the number of reproductive days (days between the onset and the end of oviposition) for each female. Male fecundity rate was estimated as numbers of spermatophores produced per male-week; we divided the total number of spermatophores produced by the number of reproductive weeks of each male.

## 2.2. Wing and head geometric morphometry

Wing and head shape descriptors using landmark-based methodology were recorded on 4–5 female and male adults per each full-sib family. For wings, eight coplanar landmarks were located along the outline and the venation intersections of the right wing (Fig. 2). For heads, 6 coplanar landmarks located along the outline were defined and collected only from the right side. Landmark digitalizations were done using TPSdig2 (version 2.09; available at [www.life.bio.sunysb/morpho](http://www.life.bio.sunysb/morpho)).

Generalized least squares Procrustes superimposition was used to extract shape variation from the landmark data using the MOP module from the CLIC package (available at [mome-clic.com](http://mome-clic.com)). To avoid problems related to the loss of dimensions due to the superimposition, we calculated relative warps (RWs) (Bookstein, 1991), i.e., a principal component analysis (PCA) of shape variables (partial warp and uniform components) both for wings and head capsules. The RWs of each individual were used to investigate allometric and phenotypic plasticity on wing and head shape. For analysis we used the RWs that accounted for 90% of the variability in all cases.

To identify the wing shape features that best differentiated between females and males fed on each host-feeding source, we performed a Canonical Variate Analysis (CVA) using MorphoJ version 1.05f (available at [www.flywings.org.uk/morphoj\\_page.htm](http://www.flywings.org.uk/morphoj_page.htm)).

To investigate the occurrence of allometry in females and males fed on each of the hosts, we followed procedures proposed by Debat et al. (2003): we performed a multivariate analysis of covariance (MANCOVA) of the RWs considering host source as a categorical predictor and centroid size as a covariate. This procedure allowed us to test simultaneously for the effects of wing size on shape (allometric effect); of host source on shape variation independent of wing size (i.e., the nonallometric component), and the consistency of the

allometric effect between host sources (size-host source interaction).

### 2.3. Wing phenotypic plasticity

Phenotypic plasticity represents measurable morphometric variation, and is often expressed and analyzed with analysis of variance (Pigliucci, 2001). To test phenotypic plasticity on wing shape variables independent of size (the allometric effect was evaluated *a priori*), RWs were used as dependent variables in a multivariate analysis of variance (MANOVA) to investigate the relative importance of (1) host-feeding source, (2) full-sib family, and (3) the host source-family interaction term, as main sources of variation of plasticity among families.

The centroid size (CS) of each individual bug was used to conduct the phenotypic plasticity analysis of wing size. CS is a single variable of size that integrates different axes of growth and is measured as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein, 1991). The assumption of normality for CS was tested using the Shapiro–Wilk's test.

For wing size variation, a mixed-model ANOVA was conducted to investigate the relative importance of full-sib family, host-feeding source, and host source-family interaction. The full-sib family term and its interactions were taken as random effects.

The results of phenotypic plasticity on head size and shape during the ontogenetic trajectory for the individuals included in this experiment were reported elsewhere (Nattero et al., 2013). The methodology used for these analyses was similar to the ones described above for the assessment of wing phenotypic plasticity.

### 2.4. Selection on phenotypic plasticity

To test for the hypotheses of direct selection on head and/or wing size or shape differences between host sources, we carried out phenotypic selection analyses following procedures described by Lande and Arnold (1983). We regressed each individual's relativized fitness on its standardized morphological trait for each group (*i.e.*, females fed on pigeons, females fed on guinea pigs, males fed on pigeons, and males fed on guinea pigs). We distinguished two categories of traits depending on the module (head and wing) to which they belonged: (a) head capsule-related traits, which included head capsule size (head CS) and head capsule shape (*i.e.*, the first and second RWs of the head capsule shape descriptor, respectively), and (b) wing-related traits, which included wing size (wing CS) and wing shape (*i.e.*, the first and second RWs of the wing shape descriptor, respectively). RWs were obtained separately for each group. Only the first two RWs of wing and head capsule shape were used because they included at least 60% of the total variation in all cases.

Directional standardized selection gradients were obtained by multiple regression analysis of each individual relativized fitness on the standardized traits (within-environment phenotypic selection analysis for plasticity; reviewed by van Kleunen and Fischer, 2005). All traits were standardized to zero mean and unit variance (*Z*-score transformation). This transformation is equivalent to expressing the original individual trait in units of standard deviation to permit valid comparisons of the strength of selection among groups. We ran separate regression models for each combination of host-feeding source and trait. This selection analysis assessed the relationship between head capsule and wing size and shape with fitness measures between two different host sources. As fitness measures, the first selection analysis included female fecundity rate and the second one included male fecundity rate. In all analyses the dependent variables were head capsule size, head capsule RW1, head capsule RW2, wing CS, wing RW1 and wing RW2.

We performed a second group of selection analyses at the family level. Family means were estimated for each trait and fitness measure. Family mean selection analyses were calculated for each selection analysis as above (Dudley and Schmitt, 1996). Results tended to be similar, although less significant because of the smaller sample size (18 families vs. approximately 140 individuals for each host source), and are therefore not shown.

The form of the selection surface acting upon the characters was examined by using the univariate cubic spline routine available at [www.zoology.ubc.ca/~schluter/wordpress/software/](http://www.zoology.ubc.ca/~schluter/wordpress/software/).

The cubic spline procedure is a nonparametric fitting function that provides a quantitative prediction of fitness across a range of trait values. The standard error for the predicted regression surface was estimated by bootstrapping the dataset 10,000 times.

### 2.5. Costs of phenotypic plasticity

We followed the technique proposed by Stinchcombe et al. (2004) for estimating the costs of plasticity within individual host-feeding sources. The cost of plasticity was tested by regressing family mean fitness within an individual environment on the family means of the trait within that environment and a measure of plasticity. The measure of plasticity was estimated as the difference of each trait between host sources for each full-sib family. For head capsule and wing, differences in shape were estimated as the Mahalanobis distance for each family between host sources. This distance quantifies shape variation and measures differences between groups (full-sib families) relative to the within-group variation, and therefore accounts for the group-specific direction of shape variation (Klingenberg and Monteiro, 2005). For CS variation, the mean CS per full-sib family for each host source was used. We ran separate regression models for each combination of treatment and trait for females and males. The fitness measures were relativized and each independent trait was standardized. A significant and negative regression coefficient for plasticity indicates that more plastic full-sib families have lower fitness for the host-feeding source under consideration, *i.e.*, plasticity is costly.

## 3. Results

### 3.1. Wings variation

Bugs fed on pigeons had larger wing CS (mean  $\pm$  SD: females, 516.60  $\pm$  36 mm; males, 307.77  $\pm$  52.09 mm) than bugs fed on guinea pigs (females, 498  $\pm$  25.13 mm; males, 498  $\pm$  25.13 mm). Wing CS of bugs fed on guinea pigs and pigeons showed significant differences both in females ( $F_{143,1} = 85.39$ ,  $p < 0.0001$ ) and males ( $F_{147,1} = 17.76$ ,  $p < 0.0001$ ).

Results from the CVA showed overall significant differences in wing shape of females and males from both host-feeding sources (Wilk's  $\lambda = 0.612$ ;  $p < 0.05$ ). Pairwise comparisons showed also significant differences ( $p < 0.05$ ), except for the comparison between females and males fed on pigeons (Fig. 2).

The MANCOVAs showed that there were no allometric effects of wing size on shape variation both in females ( $F_{10,136} = 0.26$ ,  $P = 0.608$ ) and males ( $F_{10,140} = 0.17$ ,  $P = 0.119$ ). Nevertheless, host-feeding source effects were statistically significant in females ( $F_{10,136} = 2.97$ ,  $p < 0.01$ ) and males ( $F_{10,140} = 3.05$ ,  $p < 0.01$ ), suggesting an effect on the nonallometric component of shape. The interaction term (wing size–host source) was nonsignificant both in females and males, suggesting that the allometric effect remained relatively consistent between host sources.



**Table 1**

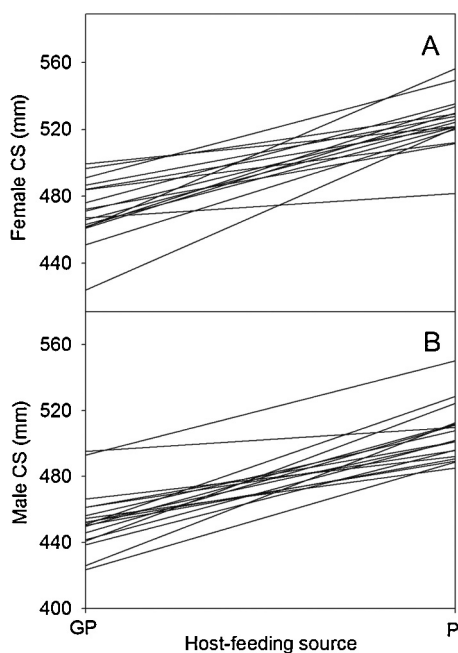
Multivariate analysis of variance (MANOVA) tests for wing shape differences in female and male *T. infestans* fed on guinea pigs and pigeons. Significant ( $p < 0.05$ ) coefficients are in bold;  $n$  indicates sample size.

Source of variation	Females				Males			
	$n$	Wilk's value	$F$	$p$ -Value	$n$	Wilk's value	$F$	$p$ -Value
<b>Wings</b>	144	0.489			148	0.51		
Whole model								
Full-sib family			4.103	<b>0.000</b>			2.354	<b>0.021</b>
Host source			3.1	<b>0.026</b>			2.412	<b>0.018</b>
Host source $\times$ family			1.16	0.330			1.924	0.061

**Table 2**

ANOVA for wing size (centroid size) for female and male *T. infestans* fed on guinea pigs and pigeons. The full-sib family term and its interactions are taken as random effects. Significant ( $p < 0.05$ ) coefficients are in bold;  $n$  indicates sample size.

Source of variation	Females				Males			
	$n$	MS	$F$	$p$ -Value	$n$	MS	$F$	$p$ -Value
<b>Wings</b>	144				148			
Full-sib family		1891.08	8.41	<b>0.000</b>		1877.96	6.53	<b>0.000</b>
Host source		62769.7	279.09	<b>0.000</b>		48584.43	169.06	<b>0.000</b>
Host source $\times$ family		7119.34	2.43	<b>0.008</b>		472.18	1.64	0.104



**Fig. 3.** Variation in wing centroid size (CS) between host-feeding sources. (A) Mean wing size reaction norm for females of the 18 families. (B) Mean wing size reaction norm for males of the 18 families. GP—guinea pig, P—pigeon.

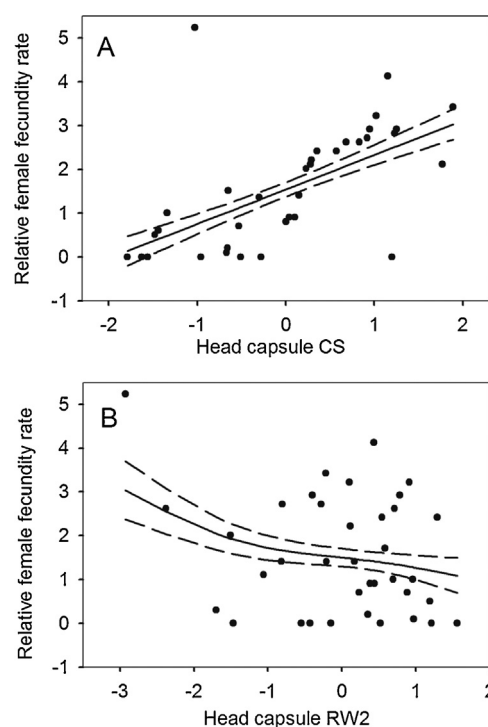
### 3.2. Phenotypic plasticity

Results from the MANOVA performed for wing shape variables showed that both females and males exhibited significant differences in wing shape associated with host source variation (Table 1). The full-sib family also showed significant effects on wing shape in both sexes.

The ANOVA model showed significant differences in wing size expression for both host sources and at the family level (Table 2). No significant host source-family interaction was detected (Table 2, Fig. 3).

### 3.3. Relations between fecundity rate and head and wing size and shape

The ANOVA test showed that females fed on guinea pigs exhibited significantly higher fecundity rate than those fed on pigeons



**Fig. 4.** Cubic spline estimates for head capsule centroid size, CS (A) and head capsule shape, RW2 (B) for females of *T. infestans* fed on guinea pigs ( $\lambda = 4$  for head capsule CS and  $\lambda = 1$  for head capsule RW2). Female fecundity rate was measure as eggs laid per female-day.

( $F_{143,1} = 16.74, p < 0.001$ ), whereas for male fecundity rate, no significant differences were detected ( $F_{147,1} = 0.69, P = 0.407$ ).

Phenotypic selection analyses detected a significant association between head capsule size and shape with female fecundity rate in bugs fed on guinea pig (Table 3). Female head size showed a positive and significant selection gradient (Fig. 4A). For head shape, the RW2 showed also a significant directional coefficient gradient (Fig. 4B). For male fecundity rate, there were no significant selection gradients for bugs fed on guinea pigs or on pigeons (Table 3).

Analysis of costs of plasticity within each host-feeding source revealed that there were significant costs for head capsule size and shape in females fed on guinea pigs, and in wing shape for females fed on guinea pigs or pigeons (Table 4). This implies that

**Table 3**  
Within-environment analysis of whether plasticity in morphological traits of individuals of *T. infestans* in response to host-feeding source is adaptive. Fitness measures were fecundity rates (eggs per female-day, spermatophores per male-week). Significant ( $p < 0.05$ ) coefficients are in bold. Sample sizes for females: 71 for guinea pigs, 73 for pigeons; males: 72 for guinea pigs, 76 for pigeons.

Fecundity		Guinea pigs			Pigeons		
		$\beta$	SE	<i>p</i> -Value	$\beta$	SE	<i>p</i> -Value
Female	Head capsule CS	0.77	0.17	<b>0.000</b>	0.23	0.12	0.070
	Head capsule RW1	0.20	0.18	0.265	0.15	0.10	0.141
	Head capsule RW2	-0.51	0.17	<b>0.005</b>	-0.02	0.11	0.884
	Wing CS	0.15	0.18	0.394	0.12	0.12	0.310
	Wing RW1	-0.35	0.18	0.066	-0.20	0.11	0.065
	Wing RW2	-0.05	0.17	0.767	-0.06	0.11	0.561
Male	Head capsule CS	-0.15	0.14	0.278	-0.03	0.16	0.830
	Head capsule RW1	-0.08	0.18	0.658	0.16	0.19	0.391
	Head capsule RW2	-0.16	0.15	0.296	0.05	0.14	0.707
	Wing CS	0.15	0.17	0.365	-0.03	0.16	0.859
	Wing RW1	0.00	0.11	0.999	0.28	0.18	0.112
	Wing RW2	0.15	0.17	0.365	0.28	0.18	0.112

**Table 4**  
Analysis of costs of plasticity in wing and head morphological traits of *T. infestans* in response to host-feeding source. Significant ( $p < 0.05$ ) coefficients are in bold. Sample size was 18 full-sib families.

Fecundity		Guinea pigs			Pigeons		
		$\beta$	SE	<i>p</i> -Value	$\beta$	SE	<i>p</i> -Value
Female	Head capsule size	-1.57	0.57	<b>0.017</b>	0.10	0.65	0.878
	Head capsule shape	-1.51	0.58	<b>0.022</b>	-0.49	0.68	0.485
	Wing size	-0.48	0.91	0.603	-0.05	0.44	0.913
	Wing shape	-1.13	0.60	<b>0.047</b>	-1.08	0.53	<b>0.050</b>
Male	Head capsule size	0.21	0.65	0.902	-0.05	0.44	0.913
	Head capsule shape	-0.64	0.62	0.318	0.08	0.53	0.878
	Wing size	-0.65	0.62	0.313	0.27	0.64	0.684
	Wing shape	0.88	0.49	0.099	-0.93	0.76	0.243

those females that fed on guinea pig and exhibited more plastic phenotypes had significantly lower fecundity rate.

#### 4. Discussion

Our results document phenotypic plasticity for wing size and shape in *T. infestans* that fed on guinea pigs and pigeons throughout their life cycle, and selection on plasticity in head capsule size and shape in females that fed on guinea pigs. Conversely, we found no consistent pattern of selection on phenotypic plasticity between female and male fitness components. Although adaptive plasticity has become widely recognized as an important component of phenotypic response to variations in environmental factors (e.g., Baythavong and Stanton, 2010; Mrdaković et al., 2014; Via et al., 1995), this appears to be the first study that may demonstrate possible selection on phenotypic plasticity and costs of plasticity in an insect species in response to variations in host-feeding sources.

##### 4.1. Phenotypic plasticity

Our results showed that bugs fed on pigeons showed bigger wing CS than those fed on guinea pigs. The direction of the phenotypic plasticity response was consistent with an allometric effect: the greater the wing size variation detected in bugs fed on pigeons, the greater the body length (Nattero et al., 2013). Other experimental studies showed that bugs fed on pigeons ingested larger quantities of blood than those fed on rodent hosts (Guarneri et al., 2000a,b; Nattero et al., 2011). This pattern is consistent with field results showing that *T. infestans* bugs from habitats with chickens associated (where bugs mostly or only fed on chickens) had greater blood-feeding rates and engorgement levels than bugs from pig corrals and goat corrals in the dry Argentine Chaco (Gürtler

et al., 2014). Similarly, *T. infestans* males and females collected from chicken coops had significantly larger wing CS than those collected from pig or goat corrals and wood piles in a different study area (Schachter-Broide et al., 2004). Blood proteins are expected to influence wing plasticity since they are involved directly or indirectly in metabolism and flight. Blood protein composition varied between mammalian and avian host sources (Lehane, 2005). In some moths, aphids, flies and butterflies, food items ingested during larval development included diverse chemical compounds that influenced wing plasticity and other morphological traits. For example, in the carob moth *Ectomyelois ceratoniae*, wing size and shape were related to different host plants that provided different stored nutritional reserves during the larval stage (Mozaffarian et al., 2007). In the aphid *Brevicoryne brassicae*, the nutritional quality of different host plants was considered as a source of phenotypic plasticity (Leal-Aguilar et al., 2008). In cactophilic species of *Drosophila*, wing size significant differed between and within fly species depending on the cactus host species on which they had developed (Soto et al., 2009). In the butterfly *Heliconius erato phyllis*, wing size and shape variations occurred among individuals fed on different species of *Passiflora* vines (Jorge et al., 2011).

For head capsule size, diet-induced phenotypic plasticity showed bigger heads when bugs fed on guinea pigs rather than on pigeons in both sexes (Nattero et al., 2013). The negative direction of this response suggested a nonallometric effect of body size on head size and a re-allocation of resources to the head capsule in guinea pig-fed bugs. Evidence of a plastic response in heads of bugs fed on guinea pigs could be related to the fact that these experiments were performed with the first-generation offspring of bugs originally collected from two habitats in which they most likely fed on chickens during their entire development; these bugs later fed on pigeons until the experiment started. Maternal effects (i.e., the

influence of the mother's genotype or phenotype on her offspring, Wolf and Wade, 2009) produced by the previous and subsequent use of avian host-feeding sources (chicken or pigeon) may operate on morphological traits such as wings.

For wing size, there were no significant interaction effects between full-sib family and host source, implying that there is no genetic variation for phenotypic plasticity. These results are consistent with the lack of phenotypic selection on wing size plasticity since the absence of an interaction between full-sib family and host-feeding source may limit evolutionary changes (West-Eberhard, 2003). In contrast, evidence of a full-sib family and host-source interaction effect on head capsule size in both sexes (Nattero et al., 2013) implies that this trait could be a target for evolutionary changes.

#### 4.2. Selection on phenotypic plasticity

Our results showed that wing size or shape plasticity did not translate into selection on phenotypic plasticity on female and male fecundity rate. For females fed on guinea pigs, head capsule size and shape gave evidence of phenotypic plasticity, thus supporting hypothesis 2 partially. The fact that bigger head capsules for females fed on guinea pigs (rather than on pigeons) were positively associated with a higher number of eggs per female-day (fecundity rate) suggests higher allocation of resources to the feeding process than to other metabolic processes such as flight dispersal. The efficiency of exploiting different host sources may significantly influence at least one component of individual fitness since phenotypic selection for plasticity was only evident for females fed on guinea pigs. These characteristics may favor the enhanced development of the cibarial pump muscles in insects fed on guinea pigs. The above-mentioned attributes may explain the differences found in phenotypic plasticity between adult bugs that had fed on guinea pigs vs. pigeons.

When considering male fecundity rate in the analysis of phenotypic selection, we obtained no evidence of selection on phenotypic plasticity in head capsule and wing morphology. This disagreement may be related to the male fitness metric chosen. The fecundity of *T. infestans* females significantly increased with an increasing number of copulas (Nattero et al., 2011). These findings suggest that females are not able to maintain adequate amounts of spermatozooids in the spermathecae after mating only once, or alternatively, male seminal secretions may stimulate the female hormones directly involved in egg-laying behavior (Stoka et al., 1987). A better measure of male fitness would perhaps be the number of spermatozooids that a male delivers in its first copula. In other insects, the male fitness measures considered included the number and duration of copulas (Sakaluk and Muller, 2008) and the number of spermatheca (Schäfer et al., 2013).

Evidence of selection on phenotypic plasticity in morphological traits of other insect species are scarce. In the aphid *Myzus persicae*, strong selection for larger body size occurred among individuals reared on an unfavorable host plant (Peppe and Lomônaco, 2003), whereas in the gypsy moth *Lymantria dispar* phenotypic selection analyses revealed that specific activities of digestive enzymes were adaptive (Mrdaković et al., 2014).

Our study has some limitations related to the selection on phenotypic plasticity analysis used. We did not estimate a global fitness metric that would allow for possible trade-offs between fecundity- and survival-related traits, and we only present results of selection on phenotypic plasticity for two fitness components which suggest that plasticity is plausible adaptive. Another limitation of this study is that all experiments were conducted using insects fed *ad lib*, with no resource limitation. Under natural conditions resources are limited, and trade-offs usually are not expressed in *ad lib* contexts.

#### 4.3. Costs of plasticity

Bugs that fed on guinea pigs displayed maximum fecundity and fertility and showed traits (head capsule size and shape) that were under selection on phenotypic plasticity, at the expense of a higher cost of plasticity associated. These results might imply that even though there is evidence of selection on phenotypic plasticity for head capsule size and shape, the evolution of plasticity in a trait may be constrained by its costs. Though these results, selection on phenotypic plasticity and fecundity might change independently by another uncontrolled phenomenon and interpretation of results on costs of plasticity may be considered carefully.

In reference to the arguments put forth by van Kleunen and Fischer (2005) related to the nature of the costs of plasticity, our data illustrate at least three potential costs associated with head capsule size and shape and wing shape in females fed on guinea pig. First, a maintenance cost may be incurred if facultative development requires the maintenance of regulatory development of head capsule size and shape. Second, the process of sampling the environment can also have energetic costs associated with, for example, temporary reductions in foraging or mating efficiency. Third, considering that head capsules from females fed on guinea pigs were bigger and showed more deformations than those fed on pigeons (Nattero et al., 2013), a production cost of structures may be operating through plastic development. Our results showed that in females, plasticity had a fitness cost when guinea pigs were the blood meal source. This result might suggest that the ability to respond to a host is costly, and that these costs might be offset by the benefits of plasticity that ensue when only one host species is available.

An investigation on adaptive plasticity should determine the costs of plasticity, *i.e.*, whether the ability to change phenotypes is itself adaptive (*e.g.*, Caruso et al., 2006; Relyea, 2002). Our findings provide evidence for the rarely tested assumption that evolution of phenotypic plasticity is constrained by costs of plasticity, and new insights into the phenotypic plasticity of *T. infestans*, evidencing the fitness benefits of head capsule size and shape in female bugs fed on guinea pigs. This suggests that females fed on guinea pigs (and perhaps on other suitable mammalian hosts) have greater chances of evolving under selection on phenotypic plasticity subject to some constraints.

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