

## Developmental thermal plasticity among *Drosophila melanogaster* populations

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### Abstract

Many biotic and abiotic variables influence the dispersal and distribution of organisms. Temperature has a major role in determining these patterns because it changes daily, seasonally and spatially, and these fluctuations have a significant impact on an organism's behaviour and fitness. Most ecologically relevant phenotypes that are adaptive are also complex and thus they are influenced by many underlying loci that interact with the environment. In this study, we quantified the degree of thermal phenotypic plasticity within and among populations by measuring chill-coma recovery times of lines reared from egg to adult at two different environmental temperatures. We used sixty genotypes from six natural populations of *Drosophila melanogaster* sampled along a latitudinal gradient in South America. We found significant variation in thermal plasticity both within and among populations. All populations exhibit a cold acclimation response, with flies reared at lower temperatures having increased resistance to cold. We tested a series of environmental parameters against the variation in population mean thermal plasticity and discovered the mean thermal plasticity was significantly correlated with altitude of origin of the population. Pairing our data with previous experiments on viability fitness assays in the same populations in fixed and variable environments suggests an adaptive role of this thermal plasticity in variable laboratory environments. Altogether, these data demonstrate abundant variation in adaptive thermal plasticity within and among populations.

### Introduction

Environmental variation in temperature is a critical parameter that influences many components of fitness (Umina *et al.*, 2005; Rashkovetsky *et al.*, 2006; Reusch & Wood, 2007), drives patterns of local adaptation (Hoffmann *et al.*, 2003b) and affects species distributions (Clarke 1996) in nature. Variation in temperature occurs on a daily, spatial and seasonal scale (Gibbs *et al.* 2003) and thus for a population to persist in the long term, it must harbour sufficient genetic variation to adapt across generations, the capacity of individuals to respond plastically within a generation, or some combination of both genetic and plastic responses (Hoffmann

& Parsons, 1991; Ayrinhac *et al.*, 2004; Hoffmann *et al.*, 2005; Hoffmann & Willi, 2008).

There is abundant evidence that the thermal response phenotypes that mediate the adaptive or plastic responses are in fact under both genetic (Hoffmann *et al.*, 2003a; Morgan & Mackay, 2006; Zhen & Ungerer, 2008; Fallis *et al.*, 2012) and environmental control (Gibert & Huey, 2001; Gibert *et al.*, 2001). Many studies have documented significant gene-by-thermal environment effects on thermal response phenotypes *within* populations (Ayrinhac *et al.*, 2004; Deere *et al.*, 2006; Swindell *et al.*, 2007; Winterhalter & Mousseau, 2007; Levine *et al.*, 2011; Bublly *et al.*, 2012); however, few studies have examined genetic variation in thermal plasticity across broad geographic ranges (Trotta *et al.*, 2006; Winterhalter & Mousseau, 2007). Studies of genetic variation in thermal plasticity from species with broad geographic ranges allow fundamental questions

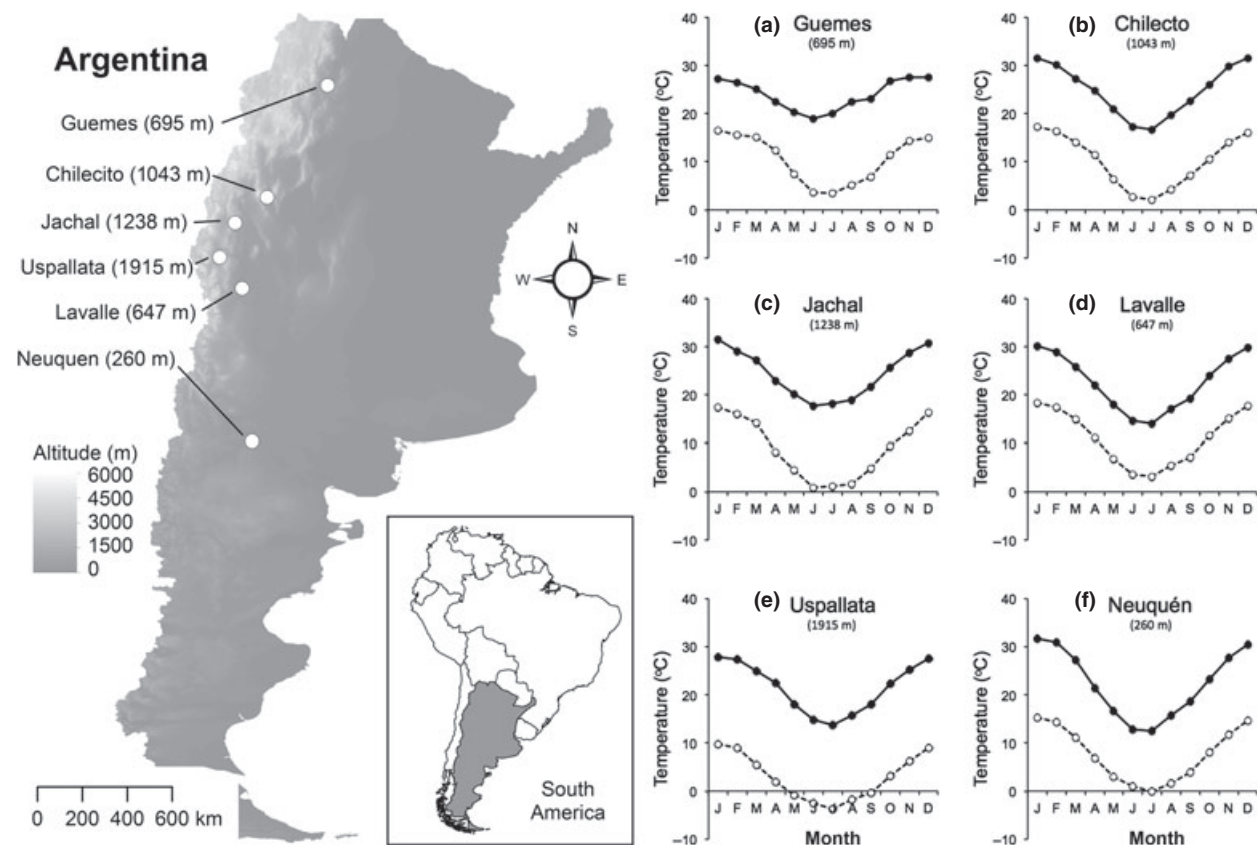
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to be addressed including is there genetic variation in plastic traits among populations with distinct environments? And what is the evolutionary significance of this variation in plasticity across these diverse natural environments?

Genetic variation in plasticity, within or among populations, confirms plasticity has a genetic basis and can evolve as a complex trait (Scheiner, 1993). Natural selection should favour plasticity when environmental change is frequent and environmental cues for such changes are reliable (Mitchell-Olds & Rutledge, 1986; Schlichting & Smith, 2002). The corresponding reaction norm should maintain plasticity across environments (i.e. reaction norm slopes  $\neq 0$ ). Conversely, natural selection should limit plasticity when environmental fluctuations are rare or when cues for change are not predictable (DeWitt *et al.*, 1998). For example, in such environments, fluctuations may be faster than the organismal response time, making a single phenotype the most fit in all environments. A single phenotype may also be favoured when an organism can actively select the most suitable habitat (Hoffmann & Parsons, 1991, 1997; Schlichting & Smith, 2002). Thus, the

degree of plasticity for such populations should be low (i.e. reaction norm slopes = 0) and genotypes should be robust across environments.

*Drosophila melanogaster* is a broadly distributed species that has been extremely successful in adapting to a wide range of thermal environments (David & Capy, 1988; Ayriñac *et al.*, 2004), and harbours ample amounts of genetic and phenotypic variation in thermotolerance phenotypes (David & Capy, 1988; Ayriñac *et al.*, 2004). Many studies have documented robust thermal responses on cold and/or heat survival/tolerance phenotypes across multiple populations (Lee *et al.*, 1987; Overgaard *et al.*, 2008; Sgro *et al.*, 2010; Fallis *et al.*, 2012); however, few have measured genetic variation in thermal plasticity across multiple populations (Trotta *et al.*, 2006; Winterhalter & Mousseau, 2007; Austin & Moehring, 2013). Here, we quantify the amount of thermal phenotypic plasticity variation within and among six natural populations of *D. melanogaster* from a latitudinal gradient in South America (Fig. 1). The six collection sites are diverse in many geographic, climate and environmental parameters, including yearly thermal profiles and seasonal thermal



**Fig. 1** Population sites. Geographic locations of the populations used in this study: (a) = Guemes, (b) = Chilecito, (c) = Jachal, (d) = Uspallata, (e) = Lavalle and (f) = Neuquén. Insets show mean highest and lowest monthly temperatures (filled and open circles, respectively) from collection locations. Meteorological data from <http://www.smn.gov.ar/>.

**Table 1** Collection sites and selected climatological data for the six populations of *Drosophila melanogaster* in Argentina.

Population	Latitude	Altitude (m)	Temperature (°C)					Isofemale lines ( <i>n</i> )
			Mean annual	Max. monthly high mean	Min. monthly low mean	Mean rainfall (mm)	Mean humidity (%)	
A. Guemes	24°41'S	695	16.58	27.5	3.4	69.73	73.83	10
B. Chilecito	29°10'S	1043	17.25	31.6	2.1	15.75	59.66	10
C. Jachal	30°12'S	1238	16.45	31.6	0.9	11.84	54.25	10
D. Lavalle	32°50'S	647	15.93	30.2	3.2	22.53	58.75	10
E. Uspallata	32°35'S	1915	11.61	27.9	-3.7	12.75	51.45	10
F. Neuquén	38°57'S	260	14.74	31.7	-0.1	15.23	52.08	10

variation (Table 1; Fig. 1). We quantitatively measured plasticity in cold tolerance using a chill-coma recovery time assay (Morgan & Mackay, 2006) on ten genotypes from each population after rearing individuals from egg to adult at two different temperatures (18 or 25 °C). We found significant variation in thermal plasticity within populations and adaptive variation in mean thermal plasticity among populations. Among population variation in mean thermal plasticity was strongly associated with the altitude of origin of each population. Finally, we paired our data with work from Folguera *et al.* (2008) we are able to conclude that this variation in thermal plasticity is likely beneficial (i.e. increases fitness) in variable laboratory environments.

## Materials and methods

### *Drosophila* stocks

Gravid females were collected from six populations in central Argentina, described previously (Lavagnino *et al.*, 2008). Flies were collected by net sweeping over fermented banana baits at six locations along a north to south latitudinal gradient ranging from approximately 24–38° south latitude in Argentina (Fig. 1). Populations were named for the nearby city or provinces where sampling took place (i.e. Guemes, Jachal, Chilecito, Lavalle, Uspallata and Neuquén). Collection locations, latitude, longitude, altitude and climatological data (<http://www.smn.gov.ar/>) for each population are presented in Table 1. Ten isofemale lines were created from single wild-caught females from each population and inbred via full-sib mating for 10 generations. Following the 10 generations of full-sib mating, the isofemale lines have been maintained in mass cultures since February 2004 on standard cornmeal-agar-molasses (10 : 1 : 2) medium sprinkled with live yeast to stimulate oviposition. Flies were maintained from egg to adult at either 25 or 18 °C and on a light/dark cycle of 12 h. All phenotypic assays were conducted in 2009 and used 5- to 7-day old flies, separated by sex to account for sex-specific differences in phenotype. Separation of individual flies was performed via CO<sub>2</sub>

anaesthesia, and flies were allowed at least 24 h to recover before being used in experiments.

### Phenotypic assays

To measure thermal plasticity, we measured chill-coma recovery time on flies reared from egg to adult at 18 and 25 °C. Chill-coma recovery time was measured as in Morgan & Mackay (2006). Briefly, assays were conducted by transferring 25 same-sex individuals, without the use of anaesthesia, to empty shell vials immediately before cold stress. Each line was subjected to a 0 °C cold stress for a 3-h period. Upon removal from the cold, flies were placed at room temperature and allowed to recover from chill coma (i.e. able to stand on their legs) for up to 30 min. Chill-coma recovery times were quantified as time (in minutes) required for a fly to recover within the 30-min period. Individuals that did not recover during the observational period were given a score of 30 min. There was no mortality during the assay. We performed three replicates containing 25 individuals per line, sex and developmental temperature (18 or 25°).

### Statistical analysis

We tested for the presence of variation in reaction norm slope among genotypes within each population by assessing the degree of genotype-by-environment interaction using the following mixed model:  $y = \mu + G + S + E + G \times S + G \times E + S \times E + S \times E + R(G \times S \times E) + \epsilon$ , where  $y$  is the sex-, line- and environment-specific chill-coma recovery times.  $G$ ,  $S$  and  $E$  are the fixed effects of genotype, sex and developmental environment (18 or 25 °C).  $R(G \times S \times E)$  is the random effect of replicate vial nested within genotype, sex and developmental environments.  $G \times S$ ,  $G \times E$ ,  $S \times E$  and  $G \times S \times E$  are the interaction effects between genotype and sex, genotype and environment, sex and environment, and genotype and sex and environment, respectively, and  $\epsilon$  is the residual error. The terms of primary interest in the within population analysis are  $G$  and  $G \times E$  as they represent significant

genetic variation and genotype-by-environment interaction within populations.

Variation in mean plasticity among populations was calculated by first quantifying the line-specific reaction norm slope. The line-specific regression coefficient was estimated from a simple linear regression between chill-coma recovery time and developmental environment. Specifically, for each line, a simple linear regression was made using the following model:  $y = \beta_0 + \beta_1 E + \epsilon$ , where  $y$  is again the sex-, line- and environment-specific chill-coma recovery time and  $E$  is the developmental environment (18 or 25 °C). The slopes of the regression coefficients (i.e. the  $\beta_1$ s) were retained as they represent the line-specific reaction norm slope. We tested for variation in thermal plasticity (i.e. the reaction norm slopes) by performing a two-way analysis of variance with fixed effects of population and sex.

To test whether variation in environmental or geographic factors associated with variation in chill-coma recovery, we used a stepwise forward-backward selection model implemented in PROC REG in SAS 9.2 (SAS Institute 2009). This approach tests for associations between the line-specific mean plasticity and the geographic and/or climatological data, by evaluating the significance of each geographic or climatological factor (Table 1) on thermal plasticity.

## Results

The developmental environment (18 vs. 25 °C) had a significant effect on chill-coma recovery time (Fig. 2). The majority of the thermal reaction norms had positive slopes, because flies reared at 18 °C generally have more rapid chill-coma recovery times [ $\bar{x}_{18} = 12 \text{ min } 40 \text{ s } (\pm 3 \text{ s})$ ] than flies reared at 25 °C [ $\bar{x}_{25} = 15 \text{ min } 5 \text{ s } (\pm 3 \text{ s})$ ]. The effect of developmental environment was highly significant in five of six populations (Table 2). Although there is a general pattern that decreased developmental temperature results in more rapid chill-coma recovery, there is significant variation among the genotypes within each population (Fig. 2).

There was significant within-population genetic variation in the chill-coma recovery times in all six populations (Table 2; Fig. 2). All six of the populations had highly significant variation among the ten genotypes within each population, while three of the six populations (Uspallata, Lavalle and Jachal) had significant genotype-by-environment interaction (Table 2; Fig. 2). To compare the population-specific thermal plasticity among the six populations, we analysed the variation among populations in the reaction norm shapes (Fig. 2). The thick dashed lines, superimposed on each population's set of reaction norms, represents the population mean thermal plasticity (Fig. 2). There was significant variation in thermal plasticity among the six populations [(Fig. 3A; Table 3) ( $F_{5,108} = 3.13$ ;  $P = 0.0113$ )]. The Lavalle population had the lowest thermal plasticity  $\bar{\beta}_{LAV} = 0.079 \pm 0.099$  and thus the smallest shift in the chill-coma recovery time between 18 and 25 °C, while the greatest thermal plasticity occurred in the populations from Chilecito  $\bar{\beta}_{CHI} = 0.472 \pm 0.039$  and Uspallata  $\bar{\beta}_{USP} = 0.453 \pm 0.131$ .

The single environmental factor that was positively associated with variation in thermal plasticity among the six populations was population altitude (Fig. 3B;  $\beta_1 = 0.00015 \pm 0.00006$ ,  $P = 0.0229$ ). The populations from low altitude (Guemes, Neuquén and Lavalle) had the lowest mean thermal plasticity, while populations from high altitude (Chilecito and Uspallata) generally had increased mean thermal plasticity (Fig. 3B). The population from Jachal is a high altitude population (1238 m), but exhibits a mean thermal plasticity  $\bar{\beta}_{JACH} = 0.225 \pm 0.073$  that is similar to low altitude populations (Fig. 3A).

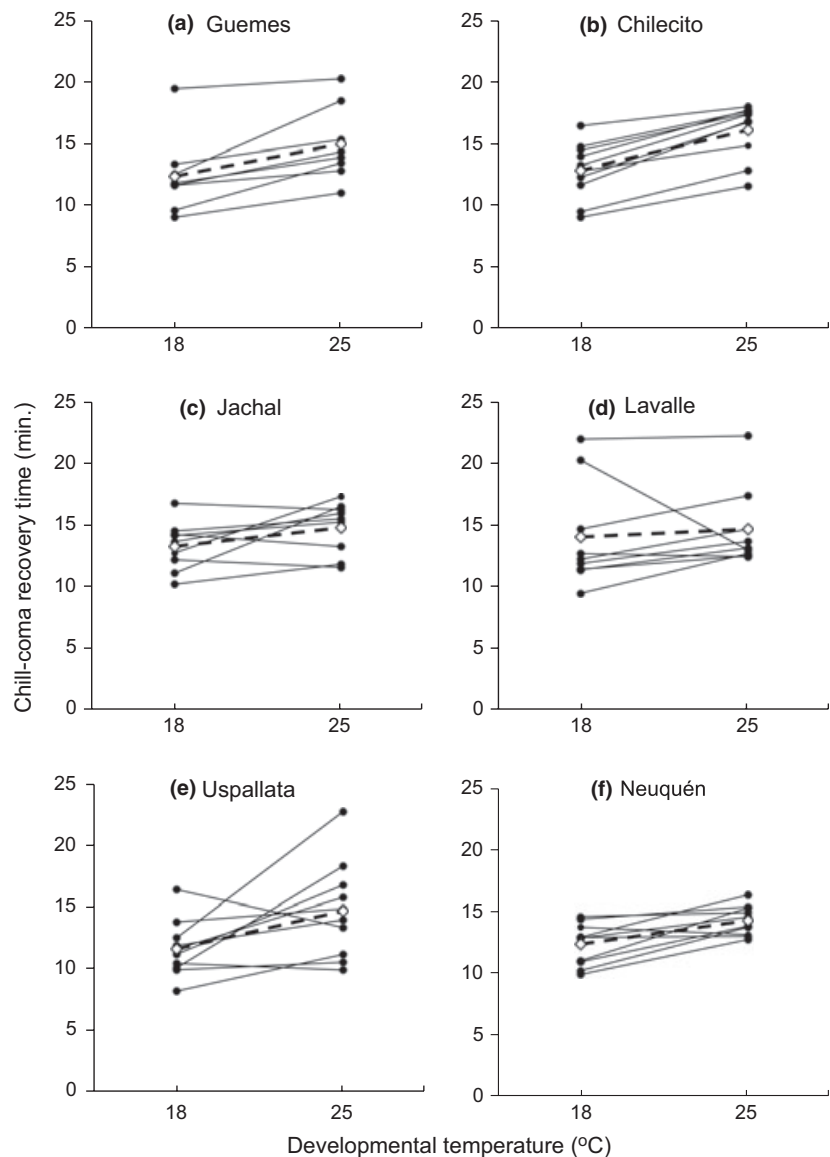
## Discussion

The role of phenotypic plasticity in adaptation has been controversial, with some studies suggesting plasticity aids in creating new phenotypes on which evolution can act (Robinson & Dukas, 1999; Pigliucci & Murren, 2003; Price *et al.*, 2003), while others suggest plasticity inhibits evolution because genotypes may

	Population					
	Guemes	Chilecito	Jachal	Lavalle	Uspallata	Neuquén
Environment (E)	18.28****	50.65****	11.10**	1.95	73.39****	19.38****
Genotype (G)	10.93****	9.23****	6.42****	26.54****	19.12****	4.24****
G × E	0.96 <sup>NS</sup>	0.51 <sup>NS</sup>	2.11*	4.92****	13.19****	1.52 <sup>NS</sup>
Sex (S)	0.54 <sup>NS</sup>	3.75 <sup>NS</sup>	4.35*	0.28 <sup>NS</sup>	6.45*	7.28**
E × S	1.04 <sup>NS</sup>	0.05 <sup>NS</sup>	0.12 <sup>NS</sup>	0.02 <sup>NS</sup>	1.52 <sup>NS</sup>	0.22 <sup>NS</sup>
G × S	1.04 <sup>NS</sup>	0.54 <sup>NS</sup>	1.05 <sup>NS</sup>	0.91 <sup>NS</sup>	1.19 <sup>NS</sup>	0.37 <sup>NS</sup>
G × E × S	0.40 <sup>NS</sup>	0.26 <sup>NS</sup>	0.05 <sup>NS</sup>	0.56 <sup>NS</sup>	0.73 <sup>NS</sup>	0.3 <sup>NS</sup>
R(G × E × S)	12.78****	7.57****	8.54****	6.54****	5.66****	10.93****

<sup>NS</sup> $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ .

**Table 2** Genetic and genotype-by-environment variation within each of the six populations.  $F$  values and significance are presented for each population.

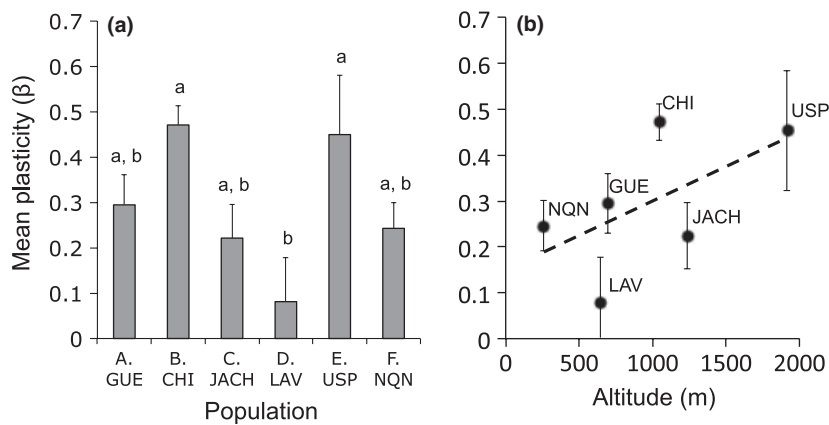


**Fig. 2** Thermal reaction norms. Line-specific chill-coma recovery reaction norms are presented for the each line grouped within the six populations (a–f; See Fig. 1). The reaction norm with the open diamond and bold dashed line is the mean population chill-coma recovery reaction norm. The *x*-axis is the developmental temperature, 18 or 25 °C, while the *y*-axis is the chill-coma recovery time in minutes.

become hidden from natural selection (Grant, 1977; Levin, 1988; Ghalambor *et al.*, 2007). To link the pervasive nature of phenotypic plasticity with long-standing questions about its role in adaptation, it is essential to analyse many populations spanning climatically variable regions, where different degrees of phenotypic plasticity may vary in response to different evolutionary processes. Here, we examined the level of phenotypic plasticity in chill-coma recovery time, an adaptive cold response phenotype (Gibert *et al.*, 2001), within and among six *D. melanogaster* populations collected along a latitudinal and altitudinal transect in Argentina. We found significant levels of genetic variation within all populations (Table 2, Fig. 2). We found that the thermal plasticity significantly varied among populations (Fig. 3A) and that

mean thermal plasticity was best explained by altitude of each population (Fig. 3B). Populations from higher altitudes exhibited a higher level of plasticity than populations at low altitudes.

Pairing our results with the results of Folguera *et al.* (2008) demonstrates the potential adaptive significance of among population variation in thermal plasticity in these South American populations. Briefly, in Folguera *et al.* (2008), fitness (i.e. larval to adult viability) was measured in stable and fluctuating thermal environments on two of the populations used here, Uspallata (which has high mean thermal plasticity and occurs at high altitude) and Lavalle (which has low mean thermal plasticity and occurs at low altitude) (Fig. 3). The study consisted of two fixed temperature treatments (constant 17 or 25 °C) and three variable temperature



**Fig. 3** Among population variation in thermal reaction norms. (a) Variation in population mean plasticity among the six populations. The  $y$ -axis is the population mean plasticity, while the  $x$ -axis is the population of origin (ordered by latitude from north to south). Lower case letters denote the Duncan's *post hoc* means groupings. (b) Relationship between population mean plasticity and altitude of the population. Error bars denote plus or minus one standard error.

**Table 3** Variation in thermal plasticity (reaction norm slope) among populations.

Source	d.f.	MS	$F$	$P$
Population	5	0.4404	3.13	0.0113
Sex	1	0.0030	0.02	0.8836
Population x Sex	5	0.0434	0.31	0.9068
Error	108			

treatments [day temperature : night temperature (25 : 17 °C, 30 : 9 °C and 25 : 9 °C)]. Folguera *et al.* (2008) found no significant differences between the two populations under the fixed temperature treatments; however, under two of the variable temperature treatments (25 : 17 °C, and 25 : 9 °C), the high plasticity (high altitude) population, Uspallata, had higher viability than the low plasticity (low altitude) population, Lavalle. This combination of results suggests that populations from high altitude exhibit higher levels of thermal phenotypic plasticity and this thermal plasticity is associated with increased fitness in variable thermal environments (Folguera *et al.*, 2008).

The overall pattern of thermal plasticity observed in each of these six populations is consistent with previous studies (Gibert & Huey, 2001; Ayrinhac *et al.*, 2004), which have shown chill-coma recovery time to be significantly decreased when flies are developmentally acclimated in low temperature rearing environments. Across all our populations, this trend is confirmed by the population mean reaction norms (Fig. 2), where flies reared at 18 °C recover more rapidly on average than flies reared at 25 °C. Although the mean reaction norms are consistent with expectations, the significant variability in reaction norm slope and position is different from previous studies of chill-coma recovery time. Both Ayrinhac *et al.* (2004) and Gibert & Huey (2001) have previously shown that both genetic variation and developmental temperature have strong effect on chill-coma recovery time, but their effects are largely independent. In our study, we find

effects that are consistent with previous studies for three populations, Guemes, Chilecito and Neuquén; however, we identified significant variation in the degree of thermal plasticity (i.e. genotype-by-environment interaction) within the Jachal, Lavelle and Uspallata populations (Table 2; Fig. 2). This variation in the degree of thermal plasticity represents genetically based differences in how genotypes within a population respond to thermal rearing environment. The finding that there was a significant effect of genetic, environmental and genotype-by-environment interactions on the expression of within population variation in chill-coma recovery time is not unexpected given the complex genetic architecture (Norry *et al.*, 2004, 2008; Morgan & Mackay, 2006) that has been shown to underlie chill-coma recovery time and other thermal phenotypes.

An extremely interesting finding from the current study was the significant differences in the response to thermal rearing environment among geographically distinct populations. This is the first study to our knowledge that has quantified the among population differences in mean thermal plasticity, based on the analysis of multiple genotypes and not overall population samples. Our finding that there is significant variation in mean thermal plasticity among populations, suggests that the evolutionary history of each population has shaped the patterns of variation in thermal plasticity among populations. Because this variation in mean plasticity is also associated with the altitude of the population of origin, it is likely that these among-population changes in thermal plasticity were driven by biotic or abiotic differences among the sites. Our findings that high altitude populations have increased thermal plasticity relative to low altitude populations is largely consistent with previous studies that have documented increased phenotypic plasticity in populations from variable environments, relative to robust phenotypes in stable environments (Ishihara, 1999; Trussell, 2000; Winterhalter & Mousseau, 2007; Cheviron *et al.*, 2008; Karl *et al.*, 2009; Crispo & Chapman, 2010). Thus,

our data are consistent with the prediction that variable abiotic environments should favour the maintenance of plasticity (Mitchell-Olds & Rutledge, 1986; Schlichting & Smith, 2002). Altogether, our results demonstrate there is abundant variation in thermal plasticity within and among populations and the significant among population variation in thermal plasticity was likely shaped by local adaptation to local environment heterogeneity.

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