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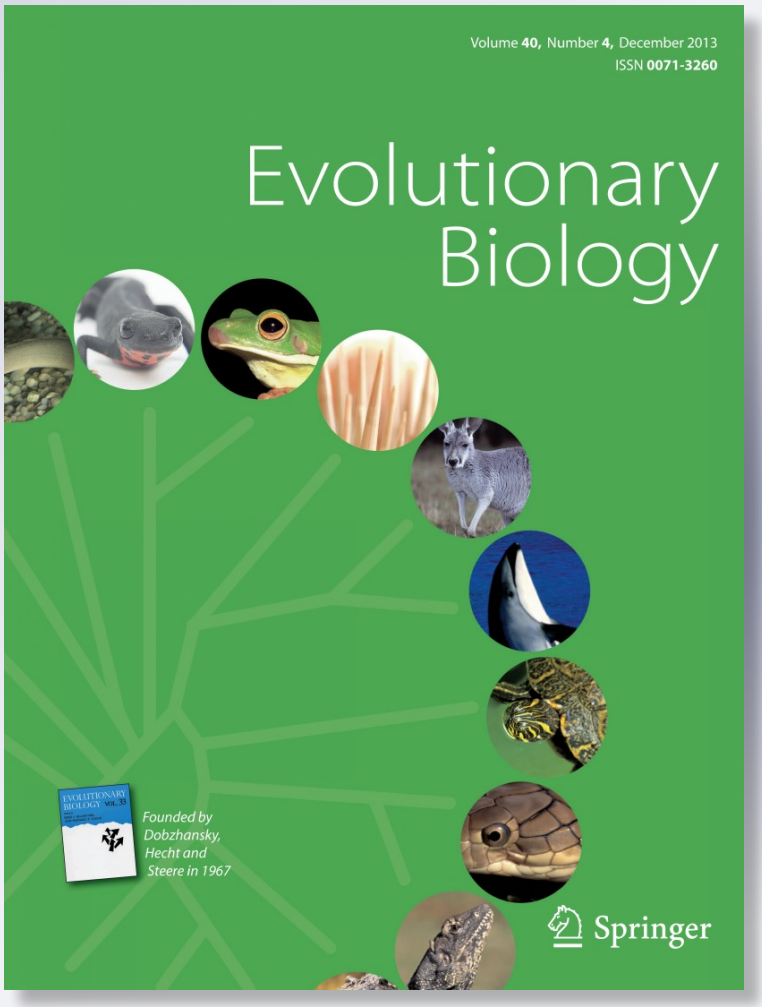

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RESEARCH ARTICLE

Latitudinal Variation in Starvation Resistance is Explained by Lipid Content in Natural Populations of *Drosophila melanogaster*

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Abstract One of the most common environmental stressors is a shortage or suboptimal quality of food, thus all animals deal with periods of starvation. In the present study we examine variation in starvation resistance, longevity and body lipid content and the correlations between traits along an environmental gradient using isofemale lines recently derived from natural populations of *Drosophila melanogaster* from South America. The use of isofemale lines and controlled rearing laboratory conditions allows us to investigate within and among population components of genetic variation and the potential associations among starvation resistance, longevity and body lipid content. All these traits were analyzed separately in females and males, improving our understanding of sexual dimorphism. Our results revealed significant differences among populations in starvation resistance and longevity. Actually, the opposing latitudinal cline detected for starvation resistance suggests that natural selection played an essential role in shaping the pattern of geographic variation in this trait. Moreover, we also detected a positive relationship between starvation resistance and body lipid content in both sexes, providing evidence for a physiological and/or evolutionary association between these traits. Conversely, starvation resistance was not correlated with longevity indicating that these traits might be enabled to evolve independently. Finally, our study reveals that there is abundant within population genetic variation for all traits that may be maintained by sex-specific effects.

Keywords Genetic correlation · Latitudinal cline · Genetic variation · Genotype × sex interaction · Starvation resistance · *Drosophila melanogaster*

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Introduction

Organisms are often exposed to environmental conditions that change continuously along a spatial–temporal scale which could potentially affect fitness. Such environmental conditions are often recognized as stress factors (Rion and Kawecki 2007) and as strong selective agents driving the evolution of traits that help to mitigate the consequences of environmental stress (Hoffmann and Parsons 1991; Randall et al. 1997; Rion and Kawecki 2007). One of the most powerful and common stress factors is the shortage of food sources which force the evolution of strategies to survive periods of malnutrition or starvation. The ability to survive periods of food shortage is an adult fitness component which has special importance during adverse periods (e.g., winters in temperate zones), which are often accompanied by severe reductions in food availability (Izquierdo 1991; Mitrovski and Hoffmann 2001; Boulétreau-Merle and Fouillet 2002). In *Drosophila*, starvation resistance is an adaptive trait orchestrated by many genes and affected by external and sexual environments (Harbison et al. 2004, 2005; Hoffmann et al. 2005a, b; Goenaga et al. 2010). Several studies have reported that the tolerance to starvation is correlated with life-history traits (Rion and Kawecki

2007), revealing an intricate genetic architecture in which pleiotropy could play an essential role. Nevertheless, unveiling the underlying genetic architecture of a trait also requires the identification of the genes responsible of natural trait variation and the characterization of patterns of genetic variation within and among natural populations. The study of these patterns may help to understand how genetic variation is maintained in the face of continuing erosion by natural selection.

Studies of patterns of geographic variation in starvation resistance using natural populations have reported latitudinal clines (Karan and Parkash 1998; Karan et al. 1998; Parkash and Munjal 2000; Arthur et al. 2008; Sisodia and Singh 2010; Goenaga et al. 2010) that were interpreted as the result of varying intensities of natural selection. However the substantial amounts of within-population genetic variation suggest weak selective pressures or the involvement of processes that offset the loss of genetic variation (Schmidt et al. 2005a; Goenaga et al. 2010).

Geographical patterns of variation in starvation resistance (SR) may also arise as an indirect response to natural selection acting on other(s) trait(s) genetically correlated with SR. Actually, the study of correlations among traits is essential to understand the degree of genetic independence between traits and to predict the selective responses, since the evolutionary trajectories of correlated traits are linked. Most studies of genetic associations of SR with other traits mainly focused on longevity. However, the results of such studies are controversial. Some studies reported a positive relationship between SR and longevity (Service et al. 1985; Rose et al. 1992; Zwaan et al. 1995; Chippindale et al. 1996; Archer et al. 2003; Phelan et al. 2003; Magwire et al. 2010) while others did not find a relationship between these two complex traits (Force et al. 1995; Harshman et al. 1999b; Schmidt et al. 2005b; Baldal et al. 2006). Interestingly, most of these investigations were carried out by means of experiments based on phenotypic manipulations or have studied the effect of particular mutants on both traits, and just a small fraction used flies derived from natural populations (Schmidt et al. 2005b; Ballard et al. 2008).

Artificial selection experiments revealed that tolerance to starvation is positively correlated with the amount of lipid reserves (Chippindale et al. 1996; Harshman et al. 1999b; Hoffmann et al. 2005b; Baldal et al. 2006; Aguila et al. 2007; Slack et al. 2010; Schwasinger-Schmidt et al. 2012; Hansen et al. 2013). Moreover, this association is often proposed as one of the physiological pathways through which this stress resistance trait can evolve. Actually, lipid reserves in insects provide essential energy supply during development (Ziegler and Van Antwerpen 2006), diapause (Hahn and Denlinger 2007), to fuel prolonged periods of flight and to survive during periods of food shortage (Arrese and Soulages 2010). However, very few studies using flies derived from natural populations did not detect the association between SR and body lipid content, suggesting that this relationship is not widespread in nature (Hoffmann et al. 2001; Schmidt et al. 2005b; Jumbo-Lucioni et al. 2010; Parkash and Aggarwal 2012).

In addition, studies in insects have shown that mating may have beneficial effects on female starvation tolerance probably through the transfer of nutritive nuptial gifts and/or stimulation of food intake that increase lipid reserves (Boucher and Huignard 1987; Butlin et al. 1987; Ivy et al. 1999; Edvardsson 2007; Carvalho et al. 2006; Rush et al. 2007; Goenaga et al. 2012). Actually, the accumulation of lipid reserves has been shown to be one of the main causes for adaptation to starvation, and energy conservation has also been postulated as a likely mechanism (Arrese and Soulages 2010; Parkash and Aggarwal 2012; Schwasinger-Schmidt et al. 2012). However, it is essential to investigate whether these mechanisms have also evolved in natural populations since most of these conclusions rely on studies of experimental evolution.

In the present study we investigated (1) patterns of geographic variation in SR, longevity and body lipid content using isofemale lines derived from five natural populations of *D. melanogaster* sampled along a latitudinal/altitudinal gradient, (2) the associations between traits, and (3) patterns of variation in the sexual dimorphism for the three traits.

Materials and Methods

Drosophila melanogaster Stocks

Gravid females were collected in 5 localities along a North–South latitudinal gradient in Western Argentina (Table 1). Flies were collected by net-sweeping on fermented banana baits, sorted by sex upon arrival to the laboratory. Singly inseminated females were used to establish isofemale lines by rearing their offspring. All isofemale lines were maintained in high numbers ($N > 300$) under the same laboratory conditions for ten generations on a cornmeal-molasses-agar medium treated with an antifungal agent and with antibiotics to eliminate bacterial infection. About 15 bottles per isofemale line were always maintained in order to reduce common environment effects (see below). Sets of isofemale lines were randomly chosen from pools of more than 50 lines established for each sampling locality for the analyses of starvation resistance, longevity and body lipid content (Table 1).

Table 1 Geographical coordinates, altitude of the five populations of *D. melanogaster* sampled for this study and number of isofemale lines analysed for each population and trait

Populations	Latitude (south)	Longitude (west)	Altitude (m)	Number of isofemale lines tested		
				SR	L	BLC
Güemes	24°41′	65°03′	695	10	15	14
Cachi	25°07′	69°09′	2,280	10	10	6
Lavalle	32°50′	68°28′	647	15	10	7
Uspallata	32°50′	68°15′	1,900	13	12	12
Neuquén	38°58′	68°08′	260	15	14	9

Phenotypic Assays

Experimental flies used in phenotypic assays were reared under optimal conditions to minimize the influence of environmental variation in the measurement of SR, longevity (L) and body lipid content (BLC). For each line 4 egg-collecting chambers (plastic containers of 15 × 10 × 4 cm) were prepared by releasing 200 sexually mature flies of both sexes in plastic containers. In each chamber a Petri dish containing egg-laying medium (2 % agar in distilled water and baker's yeast) was used for egg collection. Petri dishes were removed after 12 h and incubated until egg hatching at 25 °C ± 1 (approximately 24 h). First-instar larvae were transferred to 10 vials (30 individuals per vial) containing lab medium. Vials were incubated at the same conditions described above. To obtain virgin flies newly emerged adults were recovered from the vials every 5 h, separated by sex under light CO_2 anesthesia and maintained in groups of 20 individuals of the same sex in vials with fresh food before the assays. Groups of females and males were stored at −80° for the future quantification of lipid. The number of lines analyzed varied across localities between 10 and 15 for SR and L and between 6 and 14 for the quantification of BLC (Table 1).

The stocks maintenance and all phenotypic assays were conducted at constant temperature (25 °C ± 1), humidity (60–70 %) and 12 h light/dark cycle and populations and the flies for different replicates came from different culture vials. Each trait investigated was analyzed simultaneously for all lines.

Starvation Resistance

Survival time under starvation condition was measured in groups of 5 virgin flies (4–6 day-old) per vial and assessed as the time elapsed (in hours) since flies were exposed to the starvation diet until the death of all flies. Seven to ten vials (replicates) were set up for each combination of line and sex, making a total of 5,646 flies scored for SR. Starvation diet consisted of 5 ml of 1.7 % agar in distilled water, which provided moisture but not food to the flies. The same methodology with minor modification has been previously used in several studies (e.g. Ayroles et al. 2009; Magwire et al. 2010). Survival was scored daily at 8.00, 14.00 and 20.00 h, until the death of all flies. Scores of SR for each individual fly were used to estimate mean survival time per vial (N = 5), which was the variable considered in statistical analysis.

Longevity

L was measured in groups of 5 virgin flies (4–6 day-old) per vial and estimated as the time elapsed (in days) since the day that the groups of flies were assembled until death of all flies. Seven to ten vials (replicates) were set up for each combination of line and sex, making a total of 4,705 flies scored for longevity. Flies were placed in vials containing 5 ml of cornmeal-molasses-agar medium without live yeast on the surface. We set up seven to ten replicated vials for each combination of line and sex. The same protocol has been used in Pasyukova et al. (2004), Ayroles et al. (2009) and Magwire et al. (2010) except that in our study we transferred flies to new vials every 3 days and recorded survivorship every 24 h. L scores for each individual fly were used to estimate mean survival time per vial which was the variable considered in statistical analysis.

Body Lipid Content

We quantified BLC in groups of 10 virgin flies (4–6 day old) per vial using the methods described in Robinson et al. (2000) and Hoffmann et al. (2005b) with minor modifications. Five to eight vials (replicates) were set up for each combination of line and sex, making a total of 3,750 flies scored for lipid content. This procedure integrates all lipid classes and does not distinguish between lipids sequestered in eggs and somatic tissues. Ten flies per vial were dried for 24 h at 60 °C and the dry weight determined on a Sartorius microbalance with an accuracy of 0.001 mg. Flies were then transferred to tubes containing 1.5 ml of diethyl ether that were gently agitated at room temperature for 24 h. Afterwards, the remaining ether was removed and flies were dried for 24 h at 60 °C. Flies were then re-weighted to determine fat-free dry weight per group. BLC per fly was then calculated as (dry weight − fat-free dry weight)/10, which was the variable considered in statistical analyses.

Statistical Analyses

Correlations Among Traits

We estimated the correlation between pairs of traits using Pearson's correlation coefficient (r) which measures the degree of co-variation between traits (Lynch and Walsh 1998; Quinn and Keough 2002). Standardized mean trait values for each line were the variables considered in correlation analyses. We only analyzed lines in which both traits were measured in females and males. We considered the deviations from the overall mean within each sex rather than the raw values in statistical tests. In addition, we recomputed correlations using the covariance among standardized line means between traits calculated in a bivariate model.

It is important to mention that the estimation of correlation coefficients using isofemale line means provides a reliable approximation of genetic correlations, though it is possible that uncertain environmental effects could also contribute to variance among line means. However, it is worth mentioning that all along the study we tried to reduce the impact of common culture conditions by distributing flies emerged in the bottles of each line at random in the vials in which traits were measured.

Statistical analyses were performed using CORR procedure in STATISTICA 8.0 software package (StatSoft Inc. 2007).

Phenotypic Variation Analysis

The full data set of each trait was analyzed using a mixed model analysis of variance (ANOVA) to determine the sources of variation according to the model:

$$y = \mu + P + L(P) + S + P \times S + L(P) \times S + \varepsilon,$$

where μ is the overall mean, P and S are the fixed effects of population and sex, respectively, L(P) stands for the random effect of Line nested in Population and ε is the error or the among replicate variance. The population effect tests for phenotypic differentiation among populations and the variance components associated with Line and Line by Sex interaction factors, giving an estimation of within-population genetic variation. Similar analysis has been conducted in early studies using isofemale lines from natural populations (Griffiths et al. 2005; Arthur et al. 2008; Goenaga et al. 2010; Fallis et al. 2011).

We also performed two-way ANOVAs for each population separately to investigate if there are genetic differences within each population for SR, L and BLC and also the sexual dimorphism (SD) based on the model:

$$y = \mu + L + S + L \times S + \varepsilon$$

In these ANOVAs, a significant L effect may be interpreted as the presence of genetic components of phenotypic variance. Variance component for line is an indication of the genetic variation within population (Hoffmann et al. 2001; Griffiths et al. 2005; Arthur et al. 2008; Goenaga et al. 2010). Genetic variation among isofemale lines includes additive components but non-additive effects (i.e. epitasis and dominance) may also contribute to differences across lines.

A significant Line by Sex interaction (L × S) may be interpreted as indicative of sex-specific effects of Lines on trait expression or as evidence of genetic variation in the degree of the sexual dimorphism. Considering that further evolution of the SD for a trait may occur if the correlation between sexes is less than one, we also calculated the cross-sex correlation for each trait using Robertson (1959) equation:

$$r_{fm} = Cov_{fm}/\sigma_m\sigma_f,$$

where Cov_{fm} is the covariance among line means between males and females, calculated by means of the bivariate ANOVA and σ_m and σ_f are the square roots of the among lines variance components from one-way ANOVAs performed for males and females, respectively. This correlation was used to estimate to what extent the sexes have the potential to evolve independently (Falconer 1952; Muir et al. 1992).

Statistical analyses were carried out using GLM procedure and the variance components of random factors were computed by a restricted maximum-likelihood (REML) approach using the VARCOMP procedure (Lynch and Walsh 1998) both in STATISTICA 8.0 (StatSoft Inc. 2007). BLC was arcsine square root transformed before the ANOVAs.

Regression Analyses

We performed multiple linear regression analyses of each trait on latitude and altitude to examine whether variation for each trait investigated is associated with geographic variables. These analyses were performed using a Multiple Regression procedure in STATISTICA 8.0 (StatSoft Inc. 2007) wherein the variable was the mean trait value of each line pooled across sexes. Each line was treated as an independent data point for any particular latitude/altitude because they were founded from a different wild caught female (Hoffmann et al. 2002).

Results

Geographic Variation

The general ANOVAs revealed that populations were significantly differentiated for both SR and L but not for

BLC (Fig. 1; Table 2). However, patterns of variation for SR and L were markedly different as indicated by post hoc comparisons (Fig. 1). On one hand, mean values of SR for flies derived from Southern populations (Lavalle, Uspallata and Neuquén) differed significantly from Northern populations (Güemes and Cachi) while for L only Lavalle was significantly differentiated from the rest (Fig. 1a, b). The populations were not significantly differed for BLC.

To further investigate the underlying causes of differentiation among population, we tested the hypothesis of clinal variation by means of multiple regression analysis of SR and L on latitude and altitude separately. The regression for SR revealed a significant and negative association with latitude ($F_{2,58} = 5.28$; $p = 0.007$, Table 3) that accounted for 15 % of overall among-population variation (Fig. 2). In contrast, the regression analysis for L was not significant (Table 3).

In the ANOVAs for SR, L and BLC the sex term was significant, indicating that these traits are sexually dimorphic (Table 3). Females were, on average, significantly more resistant to starvation (45.7 ± 0.2 h) than males (43 ± 0.3 h), outlived males by approximately 4 days (mean time to death was 40 days for females and 36 days for males) and stored almost twice amount of lipids than males (0.062 ± 0.001 μg vs. 0.036 ± 0.001 μg) (Fig. 1; Table 2). The pattern of variation of the SD was consistent across populations for the three traits as suggested by the non-significant Population by Sex interaction (Table 2). The general ANOVAs also revealed that these populations harbor substantial amounts of genetic variation for these traits as indicated by the significant Line and Line by Sex interaction terms (Table 2). Overall, these factors, which may be construed as a combined estimation of the genetic component of trait variation, jointly accounted for 57.5, 32.8 and 18.3 % of total phenotypic variation for SR, L and BLC, respectively (Table 2). The Line by Sex interaction was also significant in the three ANOVAs indicating that there could be genetic factors with sex-specific effects affecting all traits analyzed or, in other words, that there may be genetic variation for the SD.

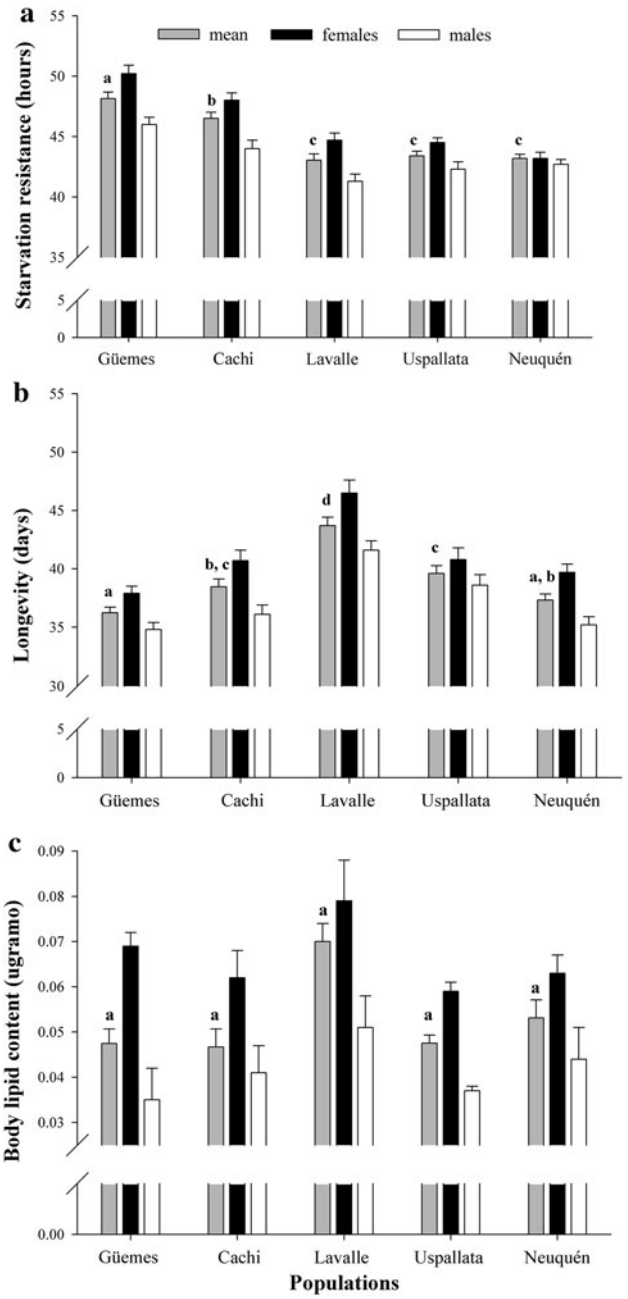

Fig. 1 Mean values and standard errors for (**a**) starvation resistance (h), (**b**) longevity (days) and (**c**) body lipid content (μg) in females and males from five natural populations of *D. melanogaster* located along a latitudinal gradient

Analysis of Correlations Among Traits

To evaluate if SR, L and BLC are genetically correlated, we calculated Pearson's product-moment coefficients (r) in females and males separately. These analysis showed that SR and BLC are positively and significantly correlated in females ($r = 0.357 \pm 0.165$, $p = 0.038$, $n = 34$) and males ($r = 0.383 \pm 0.166$, $p = 0.029$, $n = 33$) (Fig. 3). Moreover, the values of the determination coefficients (r^2) indicated that 12 and 14 % of total variation in SR may be explained by differences in BLC in females and males, respectively. In contrast, the correlation coefficients between SR and L were not significant neither in females ($r = 0.154 \pm 0.148$, $p = 0.305$, $n = 46$) nor in males ($r = 0.238 \pm 0.144$, $p = 0.107$, $n = 47$), suggesting a potential independence of these traits. The same results were obtained using bivariate models for the calculation of covariation between traits (data not shown).

Table 2 Results of the nested ANOVAs for SR, L and BLC examining differences among populations, among lines and between sexes

Sources of variation	SR			L			BLC		
	df	*F*	σ^2	*df*	*F*	σ^2	*df*	*F*	σ^2
Population	4	3.0*	–	4	4.5**	–	4	1.2	–
Sex	1	11.0**	–	1	16.4***	–	1	51.6***	–
Population × sex	4	0.5	–	4	0.5	–	4	0.7	–
Line (population)	56	17.2***	16.9	55	6.7***	12.7	23	2.9***	6.4
Line (population) × sex	56	9.8***	40.6	55	3.5***	20.1	23	1.9**	11.9
Error	1,020		42.5	930		67.2	319		81.7

The percentage of total phenotypic variance explained by each random factor is also given (σ^2)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 3 Linear regression analyses for starvation resistance (SR), longevity (L) and body lipid content (BLC) on latitude and altitude based on the isofemale line mean pooled sexes. The intercept, the slope and the 95 % confidence intervals for each parameter of the regressions are shown in parenthesis. Also shown the coefficient of determination (R^2)

Traits	Intercept ± SE	β1 ± SE (latitude)	β2 ± SE (altitude)	R^2
SR	58.4 ± 4.6***	−0.44 ± 0.1**	−0.14 ± 0.13	0.15
	(50.2–67.1)	(−0.71 to −0.16)	(−0.42 to 0.13)	
L	32.24 ± 4.61	0.18 ± 0.14	0.12 ± 0.11	0.03
	(22.93–41.48)	(−0.09 to 0.46)	(−0.15 to 0.40)	
BLC	0.03 ± 0.01	0.21 ± 0.14	−0.15 ± 0.13	0.09
	(0.007–0.06)	(−0.05 to 0.48)	(−0.43 to 0.11)	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

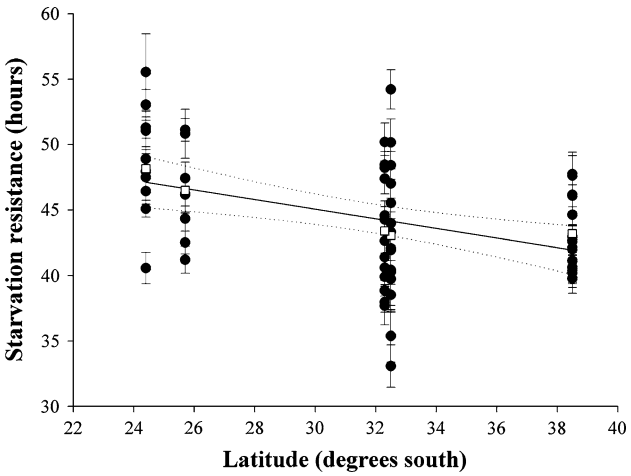

Fig. 2 Linear regression between latitude and mean starvation resistance for each isofemale lines originating from natural populations along a latitudinal gradient. Mean starvation resistance of each isofemale line overall by sexes (*circle*) and standard errors (*bars*); mean starvation resistance of each population (*square*); regression line (*solid line*) and the 95 % of confidence interval (*dotted line*)

Phenotypic Variation, Sexual Dimorphism and Cross Sex Correlations

The general ANOVAs revealed that the Line and the Line by Sex interaction terms were highly significant for SR, L and BLC (Table 2). We also carried out two-way ANOVAs for each population and trait separately to further examine patterns of genetic variation within populations. These ANOVAs showed that either the Line and/or the Line by Sex interaction factors were significant for SR, L and BLC. These results indicate that substantial amounts of natural genetic variation underlie the three traits in all populations examined (Table 4). However, the contribution of Line and Line by Sex interaction terms to total phenotypic variation varied among traits and populations.

For SR the sign of the SD was consistent across all sampling localities (Fig. 1a; Table 4). Moreover, highly significant differences among lines that explained 5–40 % of total phenotypic variance were detected in all populations. The Line by Sex interaction was also significant in all populations, suggesting that variation in SD has a genetic basis. Variance components associated with the interaction term accounted from 18.7 to 62.4 % of overall phenotypic variance (Table 4).

Two-way ANOVAs for L revealed that females outlived males in all populations, though differences between sexes were only significant in three out of five populations analyzed. Furthermore, a significant Line effect was detected in all populations, except Lavalle, accounting for 9.1–29.9 % of total phenotypic variance. The Line by Sex interaction was significant in all populations and explained from 10.7 to 24.9 % of total trait variation (Table 4).

BLC differences between sexes were significant in all populations, females stored significantly greater amounts of lipids than males, a pattern that was consistent across all populations. The Line effect, which accounted for 4 to 30 % of total phenotypic variance, was significant in Cachi, Uspallata y Neuquén (Table 4). The Line by Sex interaction term was only significant in Güemes and Cachi, accounting for 43 and 15 % of total phenotypic variance, respectively.

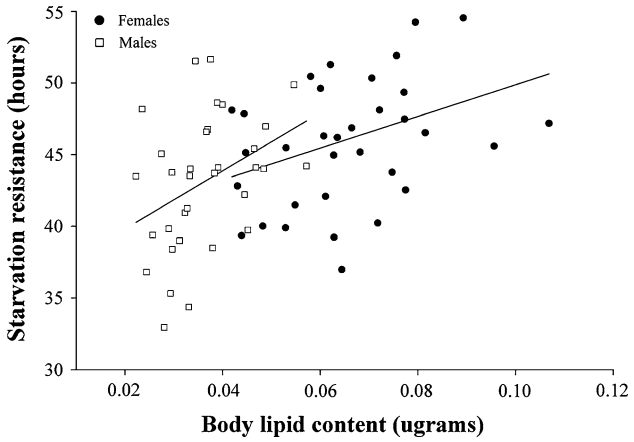

Fig. 3 Starvation resistance increases significantly with body lipid content in females and males. *Each point* represents an isofemale lines tested

We also calculated the cross-sex correlation (r_{fm}) for each trait pooling all isofemale lines irrespective of the populations. These analyses showed a significant and positive correlation for SR (r = 0.336; $p = 0.007$) and L (r = 0.445; $p < 0.001$) but not for BLC (r = 0.292; $p = 0.06$). These analyses revealed that only 10 and 19 % of variation among females in SR and L, respectively, may be explained by genetic factors shared with males. We also estimated the cross-sex correlations (r_{fm}) for each population and trait (Table 4). For SR the r_{fm} differed significantly from 0 and 1 in Güemes and for L the r_{fm} differed significantly from 0 and 1 in Güemes and Uspallata. Finally, r_{fm} was different from 1 but not from 0 in the rest of populations and traits.

Discussion

Our survey extends the comparative study of patterns of geographical variation across continents, an essential point in the assessment of the role of natural selection shaping variation in life history, stress resistance and physiological traits. To our knowledge, the present is the first survey investigating variation for three complex quantitative traits

Table 4 *F* and percent of total phenotypic variance (in brackets) accounted for by differences among lines and the interaction line × sex in the two-way ANOVAs performed for each population and trait

	Güemes	Cachi	Uspallata	Lavalle	Neuquén
Starvation resistance					
Sex	7.64*	6.17*	2.19	2.22	0.56
Line	22.01***	10.68***	19.28***	27.64***	6.17***
	(40.3)	(23.6)	(27.8)	(8.9)	(5)
Line × sex	5.27***	3.74***	8.19***	20.44***	6.58***
	(19.4)	(18.7)	(28.1)	(62.4)	(36.8)
r^{a}_{fm}	0.61	0.42	0. 5	0.2	−0.12
	(0.3)	(0.4)	(0.3)	(0.3)	(0.3)
Longevity					
Sex	5.39*	7.63*	0.52	0.94	6.72*
Line	4.94	6.82	16.07	4.49	5.52
	(13.9)***	(18.2)***	(29.9)***		(9.1)***
Line × sex	2.25**	2.42*	5.51***	3.9***	3.31***
	(10.7)	(11.7)	(24.9)	(24)	(19)
r^{a}_{fm}	0.37	0.39	0.52	−0.03	0.26
	(0.2)	(0.3)	(0.2)	(0.4)	(0.2)
Body lipid content					
Sex	12.19*	11.43*	37.83***	1.17	44.16*
Line	2.41	3.05*	2.44*	2.54	5.09**
		(12.4)	(4.4)	(4.7)	(31.1)
Line × sex	5.32***	2.69*	1.58	2.04	0.83
	(43.4)	(14.8)	(5.9)	(20)	
r^{a}_{fm}	−0.40	0.15	0.31	0.15	1.08
	(0.32)	(0.5)	(0.67)	(0.4)	(UD)

UD undefined, *NS* not significant

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

[a] Cross-sex correlation coefficient between line means (standard error)

in natural populations of *D. melanogaster* from South America. We detected substantial amounts of genetic variation within populations and slight differentiation among populations for SR, L and BLC. However, the patterns of variation differed among traits and populations, suggesting different evolutionary trajectories for each trait.

Patterns of Geographic Variation

Differentiation among populations accounted for a small though significant proportion of total phenotypic variation in SR. Such pattern of variation may be accounted for geographic variation of weak selection intensities probably related to variation in food availability along a latitudinal gradient, as indicated by the opposite latitudinal cline. However, it is important to point out that the cline found in this study is in the opposite direction to our a priori expectations of increased resistance to starvation in flies that come from high latitude localities, since southern populations face temperate climates and thus, more marked degree of seasonality in food availability.

The evidence of latitudinal clines in SR is still a controversial issue. Studies in natural populations have reported opposite clines in *D. melanogaster*, *D. ananassae* and *D. kikkawai* from India (Karan and Parkash 1998; Karan et al. 1998; Parkash and Munjal 2000; Sisodia and Singh 2010) and *D. simulans* from Australia (Arthur et al. 2008), a positive cline in *D. birchii* from Australia (Griffiths et al. 2005) and also no clinal variation in *D. melanogaster* from Chile (Robinson et al. 2000), Australia (Hoffmann et al. 2001) (though in these cases there is an obvious opposite trend) and North America (Schmidt et al. 2005a) and *D. serrata* from Australia (Hallas et al. 2002). Overall, these studies indicate that the patterns of variation of this ecologically relevant trait vary greatly across continents, hindering the understanding of the potential evolutionary mechanisms underlying natural variation.

Nevertheless, it is important to notice that fruit flies are saprophytophagous insects that feed upon decaying fruit tissues and the microbial community (bacteria molds and yeasts) associated with this process (Markow and O'Grady 2008). Food sources should decay more slowly in higher latitudes as a result of lower mean temperatures, thus, flies living at higher latitudes (i.e. cooler temperatures) are expected to be exposed to shorter periods of food scarcity, reducing their ability to survive starvation conditions, providing a plausible explanation for the opposing cline detected for SR.

Demographic and historical factors may also account for the latitudinal cline in SR, although, clines resulting from non-adaptive factors should vanish as a result of gene flow populations. Furthermore, historical and demographic factors should have similar effects on the pattern of variation across different traits, diluting the outcome of selection acting upon individual traits. Interestingly, the different patterns of variation documented here for the other traits (L and BLC) provide additional and indirect evidence supporting the hypothesis of the adaptive nature of the opposing cline in SR. In particular, we reported that the relative contribution of the population factor to total phenotypic variation in L was close (8 %) to the value estimated for SR (7.2 %) and there were not differences among population in BLC. Furthermore, we did not detect a relationship of L and BLC with latitude and/or altitude. In contrast, Schmidt et al. (2005a) and Schmidt and Paaby (2008) examined patterns of variation for SR, L and lipid content, and found that significant differentiation among population for lipid content and L but not for SR. In addition, surveys conducted in the same set of natural populations studied here revealed differences in the patterns of among population variation for adult and larval olfactory behavior (Lavagnino et al. 2008), thermal-stress tolerance (Fallis et al. 2011) as well as latitudinal clines for developmental time and viability (Folguera et al. 2008; Mensch et al. 2010). Overall, these findings are in line with the idea that specific population processes drive the evolutionary trajectories of each trait and lend support to the adaptive nature of the SR cline.

Another possible explanation for the latitudinal cline for SR is that it may have arisen as a correlated response to natural selection acting on trait(s) genetically correlated with SR and that also covary with latitude. A potential candidate trait is cold resistance because not only covaries with latitude (Hoffmann et al. 2002; Schmidt and Paaby 2008) but also is negatively correlated with SR as has been shown by artificial selection experiments and studies in natural populations (Chippindale et al. 1996; Harshman et al. 1999b; Hoffmann et al. 2005b; Schmidt et al. 2005b; Kenny et al. 2008; Ayroles et al. 2009). Another trait exhibiting latitudinal clinal variation is the incidence of diapause (the frequency of genotypes expressing reproductive diapause). Moreover, it has been shown that genotypes expressing reproductive diapause are more resistant to starvation (Schmidt et al. 2005a, b). However, on the basis of their results the authors predict a potential positive cline for SR, which is in conflict with the opposing cline detected herein. More recently, a consistent relationship between body melanization and SR, mediated by the metabolism of lipid reserves, has been shown in natural populations (Parkash and Aggarwal 2012). These results suggest that the opposing cline in SR may be explained in terms of its correlation with other traits and might interpreted as adaptive responses to environmental heterogeneity among habitats, however, the selective mechanisms responsible for these geographical patterns are complex and still elusive.

Correlations Between Traits

The study of correlations between traits is essential to elucidate and understand potential constraints affecting the evolutionary trajectory of traits. In this study we did not find a significant correlation between SR and L, which is in agreement with earlier studies (Force et al. 1995; Harshman et al. 1999b; Baldal et al. 2006; Schmidt et al. 2005b) but in conflict with the results of artificial selection experiments on SR that showed a correlated response in L (Service et al. 1985; Rose et al. 1992; Zwaan et al. 1995; Chippindale et al. 1996; Archer et al. 2003; Phelan et al. 2003). This discrepancy among isofemale lines and artificial selection experiments may be due to genetic differences among population sets, differences in laboratory conditions and/or in the methodologies used (natural populations vs. artificial selection experiments) (Harshman and Hoffmann 2000). It may be argued that the correlations between traits based on the study of isofemale lines are not strictly genetic and that differences among lines may be also due to non-genetic, common environmental factors. However, common environmental factors can be ruled out since flies of the same isofemale line emerge in the bottles were distributed at random across the experiment. In conclusion, our results suggest that independent sets of genes may underlie variation in SR and L.

Regarding studies of the genetic architecture of the studied traits, QTL mapping studies have identified genomic regions that affect both SR and L, suggesting a common genetic basis (Vieira et al. 2000; Wang et al. 2004). Nonetheless, these results might not be considered as indicative that the same genes are affecting both traits since QTL mapping has a low resolution for the identification of individual genes. Actually, Vieira et al. (2000) supports the hypothesis of the genetic independence between SR and L. Similarly, studies using flies in which the signaling pathway target of rapamycin (TOR) has been inhibited by mutation in the TORC1 complex produced long-lived flies without any effect on SR, while, blocking TORC2 complex affected SR but not L (Kapahi et al. 2004; Bjedov et al. 2010). In addition, the inactivation of the apokinetic hormone, main responsible for energy mobilization and feeding behavior, produces an increase in SR independently of L (Lee and Park 2004). All these evidence suggests that SR and L are largely genetically decoupled and therefore, might be evolving independently.

Our study also shows that SR and BLC are correlated in both sexes, suggesting that the ability to survive famine increases with the amount of reserves stored as lipids. This finding is consistent with studies conducting in natural populations (Aguila et al. 2007; Ballard et al. 2008; Sisodia and Singh 2010; Parkash and Aggarwal 2012) and artificial selection experiments (Chippindale et al. 1996; Harshman and Schmid 1998; Harshman et al. 1999a; Hoffmann et al. 2005b; Baldal et al. 2006; Schwasinger-Schmidt et al. 2012; Hansen et al. 2013) and it suggests that abundance of lipid reserves is one of the physiological mechanisms to resist periods of food shortage. However, additional evidence for a common genetic basis of SR and BLC in *Drosophila* comes from QTL mapping studies showing that a region in the third chromosome has effects in both traits (Wang et al. 2004, 2005). On the other hand, Harbison et al. (2004) has reported that mutations in the gene *spalt major* (*salm*) affected SR through limiting required lipids or access to them. Further evidence in favour of a link between these traits has revealed that lines selected for starvation tolerance accumulated more lipids (Schwasinger-Schmidt et al. 2012). Finally, it has been shown that starvation induces morphology alterations in the adiposites which include the aggregation of lipid droplets and energy mobilization (Zhang et al. 2000; Britton et al. 2002; Colombani et al. 2003), causing a noticeable reduction in adiposites size (Butterworth et al. 1965).

Sexual Dimorphism Variation

Differences in life history strategies between males and females are the most accepted explanation for the evolution of sexually dimorphic traits. Specifically, the SD may be an outcome of differences in sex-specific selection on shared traits favoring sex-specific optima (Rice and Chippindale 2002; Fairbairn et al. 2007). This study shows that SR, L and BLC are sexually dimorphic traits, in agreement with previous work in *Drosophila* (Vieira et al. 2000; Robinson et al. 2000; Schmidt et at. 2005a, b; Schmidt and Paaby 2008; Ballard et al. 2008; Magwire et al. 2010; Harbison et al. 2005; De Luca et al. 2003; Goenaga et al. 2010). The SD in starvation tolerance may be explained by sex-specific expression of reproductive traits that are known to be correlated with SR as egg production (Salmon et al. 2001), ovariole number (Wayne et al. 2006) and the ability to enter in reproductive diapause (Schmidt et al. 2005a).

Our study also reveals a significant Genotype by Sex interaction in all populations or, in other words, that genotypes differ in the magnitude of the SD, suggesting the presence of segregating genetic variation for the SD of the traits studied in this work. The analysis of the cross-sex correlation (r_{mf}) indicates a certain degree of genetic independence between sexes that may allow further evolution of the SD.

Studies with *P*-element insertion lines allowed to identify candidate genes involved in the expression of SR, L and BLC which exhibited a clear sex-specific effect on phenotype (Harbison et al. 2004; Wang et al. 2005; Magwire et al. 2010). Interestingly, we detected that more than 50 % of the genotypes tested here showed clear sex-

specific effects on the expression of SR, L and BLC. Consequently, the significant Genotype by Sex interaction may be considered as evidence that natural population harbor genotypes with sex-specific effects on these complex traits. Thus, an important conclusion of our study is that these effects could play an important role in the maintenance of the standing amounts of genetic variation within populations for the traits examined despite the continued erosion by the action of natural selection.

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References

Aguila, J. R., Suszko, J., Gibbs, A. G., & Hoshizaki, D. K. (2007). The role of larval fat cells in adult *Drosophila melanogaster*. *Journal of Experimental Biology, 210*(6), 956–963.

Archer, M. A., Phelan, J. P., Beckman, K. A., & Rose, M. R. (2003). Breakdown in correlations during laboratory evolution. II. Selection on stress resistance in *Drosophila* populations. *Evolution, 57*(3), 536–543.

Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: Energy metabolism and regulation. *Annual Review of Entomology, 55*, 207–225.

Arthur, A. L., Weeks, A. R., & Sgrò, C. M. (2008). Investigating latitudinal clines for life history and stress resistance traits in *Drosophila simulans* from eastern Australia. *Journal of Evolutionary Biology, 21*(6), 1470–1479.

Ayroles, J. F., Carbone, M. A., Stone, E. A., Jordan, K. W., Lyman, R. F., et al. (2009). Systems genetics of complex traits in *Drosophila melanogaster*. *Nature Genetics, 41*(3), 299–307.

Baldal, E. G., Bbrakefield, P. M., & Zwaan, B. J. (2006). Multitrait evolution in lines of *Drosophila melanogaster* selected for increased starvation resistance: The role of metabolic rate and implications for the evolution of longevity. *Evolution, 60*(7), 1435–1444.

Ballard, W. O., Melvin, R. G., & Simpson, S. J. (2008). Starvation resistance is positively correlated with body lipid proportion in five wild caught *Drosophila simulans* populations. *Journal of Insect Physiology, 54*(9), 1371–1376.

Bjedov, I., Toivonen, J. M., Kerr, F., Slack, C., Jacobson, J., Foley, A., et al. (2010). Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*. *Cell Metabolism, 11*(1), 35–46.

Boucher, L., & Huignard, J. (1987). Transfer of male secretions from the spermatophore to the female insect *Caryedon serratus* (OI.): Analysis of the possible trophic role of these secretions. *Journal of Insect Physiology, 33*(12), 949–957.

Boulétreau-Merle, J., & Fouillet, P. (2002). How to overwinter and be a founder: Egg-retention phenotypes and mating status in *Drosophila melanogaster*. *Evolutionary Ecology, 16*(4), 309–332.

Britton, J. S., Lockwood, W. K., Li, L., Cohen, S. M., & Edgar, B. A. (2002). Drosophila's insulin/pi3-kinase pathway coordinates cellular metabolism with nutritional conditions. *Developmental Cell, 2*(2), 239–249.

Butlin, R. K., Woodhatch, C. W., & Hewitt, G. M. (1987). Male spermatophore investment increases female fecundity in a grasshopper. *Evolution, 41*(1), 221–225.

Butterworth, F. M., Bodenstein, D., & King, R. C. (1965). Adipose tissue of *Drosophila melanogaster*. I. An experimental study of larval fat body. *Journal of Experimental Zoology, 158*(2), 141–153.

Carvalho, G. B., Kapahi, P., Anderson, D. J., & Benzer, S. (2006). Allocrine modulation of feeding behavior by the sex peptide of *Drosophila*. *Current Biology, 16*(7), 692–696.

Chippindale, A. K., Chu, T. J. F., & Rose, M. R. (1996). Complex trade-offs and the evolution of starvation resistance in *Drosophila melanogaster*. *Evolution, 50*(2), 753–766.

Colombani, J., Raisin, S., Pantalacci, S., Radimerski, T., Montagne, J., & Leopold, P. (2003). A nutrient sensor mechanism controls *Drosophila* growth. *Cell, 114*(6), 739–749.

De Luca, M., Roshina, N. V., Geiger-Thornsberry, G. L., Lyman, R. F., Pasyukova, E. G., & Mackay, T. F. C. (2003). Dopa decarboxylase (*Ddc*) affects variation in *Drosophila* longevity. *Nature Genetics, 34*(4), 429–433.

Edvardsson, M. (2007). Female *Callosobruchus maculatus* mate when they are thirsty: Resource-rich ejaculates as mating effort in a beetle. *Animal Behaviuor, 74*(2), 183–188.

Fairbairn, D. J., Blanckenhorn, W. U., & Székely, T. (2007). *Sex size and gender roles: Evolutionary studies of sexual dimorphism*. Oxford: Oxford University Press.

Falconer, D. S. (1952). The problem of environment and selection. *American Naturalist, 86*(830), 293–298.

Fallis, L. C., Fanara, J. J., & Morgan, T. J. (2011). Genetic variation in heat-stress tolerance among South American *Drosophil*a populations. *Genetica, 139*(10), 1331–1337.

Folguera, G., Ceballos, S., Spezzi, L., Fanara, J. J., & Hasson, E. (2008). Clinal variation in developmental time and viability and the response to thermal treatments in two species of *Drosophila*. *Biological Journal of the Linnean Society, 95*(2), 233–245.

Force, A. G., Staples, T., Soliman, S., & Arking, R. (1995). Comparative biochemical and stress analysis of genetically selected *Drosophila* strains with different longevities. *Developmental Genetic, 17*(4), 340–351.

Goenaga, J., Fanara, J. J., & Hasson, E. (2010). A quantitative genetic study of starvation resistance at different geographic scales in natural populations of *Drosophila melanogaster*. *Genetic Research, 92*(4), 253–259.

Goenaga, J., Fanara, J. J., & Hasson, E. (2012). The effect of mating on starvation resistance in natural populations of *Drosophila melanogaster*. *Evolutionary Ecology, 26*(4), 813–823.

Griffiths, J. A., Schiffer, M., & Hoffmann, A. A. (2005). Clinal variation and laboratory adaptation in the rainforest species *Drosophila birchii* for stress resistance wing size wing shape and development time. *Journal of Evolutionary Biology, 18*(1), 213–222.

Hahn, D. A., & Denlinger, D. L. (2007). Meeting the energetic demands of insect diapause: Nutrient storage and utilization. *Journal of Insect Physiology, 53*(8), 760–773.

Hallas, R., Schiffer, M., & Hoffmann, A. A. (2002). Clinal variation in *Drosophila serrata* for stress resistance and body size. *Genetics Research, 79*(2), 141–148.

Hansen, M., Flatt, T., & Aguilaniu, H. (2013). Reproduction, fat metabolism, and life span: What is the connection? *Cell Metabomis, 17*(8), 10–19.

Harbison, S. T., Chang, S., Kamdar, K. P., & Mackay, T. F. C. (2005). Quantitative genomics of starvation stress resistance in *Drosophila*. *Genome Biology, 6*, R36. doi:10.1186/gb-2005-6-4-r36.

Harbison, S. T., Yamamoto, A. H., Fanara, J. J., Norga, K. K., & Mackay, T. F. C. (2004). Quantitative trait loci affecting starvation resistance in *Drosophila melanogaster*. *Genetics, 166*(4), 1807–1823.

Harshman, L. G., & Hoffmann, A. A. (2000). Laboratory selection experiments on life history and stress-related traits in

Drosophila: What do they really tell us? *Trends in Ecology & Evolution, 15*(1), 32–36.

Harshman, L. G., Hoffmann, A. A., & Clark, A. G. (1999a). Selection for starvation resistance in *Drosophila melanogaster*: Physiological correlates enzyme activities and multiple stress responses. *Journal of Evolutionary Biology, 12*(2), 370–379.

Harshman, L. G., Moore, K. M., Sty, M. A., & Magwire, M. M. (1999b). Stress resistance and longevity in selected lines of *Drosophila melanogaster*. *Neurobiology Aging, 20*(5), 521–529.

Harshman, L. G., & Schmid, J. L. (1998). Evolution of starvation resistance in *Drosophila melanogaster*: Aspects of metabolism and counter-impact selection. *Evolution, 52*(6), 1679–1685.

Hoffmann, A. A., Anderson, A., & Hallas, R. (2002). Opposing clines for high and low temperature resistance in *Drosophila melanogaster*. *Ecoogy Letters, 5*(5), 614–618.

Hoffmann, A. A., Hallas, R., Anderson, A. R., & Telonis-Scott, M. (2005a). Evidence for a robust sex-specific trade-off between cold resistance and starvation resistance in *Drosophila melanogaster*. *Journal of Evolutionary Biology, 18*(4), 804–810.

Hoffmann, A. A., Hallas, R., Sinclair, C., & Mitrovski, P. (2001). Levels of variation in stress resistance in *Drosophila* among strains local populations and geographic regions: Patterns for desiccation starvation cold resistance and associated traits. *Evolution, 55*(8), 1621–1630.

Hoffmann, A. A., & Parsons, P. A. (1991). *Evolutionary genetics and environmental stress*. New York: Oxford University Press.

Hoffmann, A. A., Shirriffs, J., & Scott, M. (2005b). Relative importance of plastic vs genetic factors in adaptive differentiation: Geographical variation for stress resistance in *Drosophila melanogaster* from eastern Australia. *Functional Ecology, 19*(2), 222–227.

Ivy, T. M., Johnson, J. C., & Sakaluk, S. K. (1999). Hydration benefits to courtship feeding in crickets. *Proceedings of the Royal Society of London. Series B, 266*(1428), 1523–1527.

Izquierdo, J. I. (1991). How does *Drosophila melanogaster* overwinter? *Entomologia Experimentalis et Applicata, 59*(1), 51–58.

Jumbo-Lucioni, P., Ayroles, J. F., Chambers, M. M., Jordan, K. W., Leips, J., Mackay, T. F. C., et al. (2010). Systems genetics analysis of body weight and energy metabolism traits in *Drosophila melanogaster*. *BMC Genomics, 11*, 297–310.

Kapahi, P., Zid, B. M., Harper, T., Koslover, D., Sapin, V., & Benzer, S. (2004). Regulation of lifespan in *Drosophila* by modulation of genes in the TOR signaling pathway. *Current Biology, 14*(10), 885–890.

Karan, D., Dahiya, N., Munjal, A. K., Gibert, P., Moreteau, B., Parkash, R., et al. (1998). Desiccation and starvation tolerance of adult *Drosophila*: Opposite latitudinal clines in natural populations of three different species. *Evolution, 52*(3), 825–831.

Karan, D., & Parkash, R. (1998). Desiccation tolerance and starvation resistance exhibit opposite latitudinal clines in Indian geographical populations of *Drosophila kikkawai*. *Ecological Entomology, 23*(4), 391–396.

Kenny, M., Wilton, A., & Ballard, W. O. (2008). Seasonal trade-off between starvationresistance and cold resistance in temperate wild-caught *Drosophila simulans*. *Australian Journal of Entomology, 47*(1), 20–23.

Lavagnino, N. J., Anholt, R. R., & Fanara, J. J. (2008). Variation in genetic architecture of olfactory behaviour among wild-derived populations of *Drosophila melanogaster*. *Journal of Evolutionary Biology, 21*(4), 988–996.

Lee, G. H., & Park, J. H. (2004). Hemolymph sugar homeostasis and starvation-induced hyperactivity affected by genetic manipulations of the adipokinetic hormone encoding gene in *Drosophila melanogaster*. *Genetics, 167*(1), 311–323.

Lynch, M., & Walsh, B. (1998). *Genetics and analysis of quantitative traits*. Sunderland: Sinauer.

Magwire, M. M., Yamamoto, A., Carbone, M. A., Roshina, N. V., Symonenko, A. V., Pasyukova, E. G., et al. (2010). Quantitative and molecular genetic analyses of mutations increasing *Drosophila* life span. *PLoS Genetics, 6*(7), e1001037.

Markow, T. A., & O'Grady, P. M. (2008). Reproductive ecology of *Drosophila*. *Functional Ecology, 22*(5), 747–759.

Mensch, J., Carreira, V., Lavagnino, N., Goenaga, J., Folguera, G., Hasson, E., et al. (2010). Stage-specific effects of Candidate heterochronic genes on variation in developmental time along an altitudinal cline of *Drosophila melanogaster*. *PLoS ONE, 5*(6), e11229.

Mitrovski, P., & Hoffmann, A. A. (2001). Postponed reproduction as an adaptation to winter conditions in *Drosophila melanogaster*: Evidence for clinal variation under semi-natural conditions. *Proceedings of the Royal Society. B, 268*(1481), 2163–2168.

Muir, W. M., Nyquist, Y., & Xu, S. (1992). Alternative partitioning of the genotype by environment interaction. *Theoretical and Applied Genetics, 84*, 193–200.

Parkash, R., & Aggarwal, D. D. (2012). Trade-off of energy metabolites as well as body color phenotypes for starvation and desiccation resistance in montane populations of *Drosophila melanogaster*. *Comparative Biochemistry and Physiology Part A, 161*(2), 102–113.

Parkash, R., & Munjal, A. K. (2000). Evidence of independent climatic selection for desiccation and starvation tolerance in Indian tropical populations of *Drosophila melanogaster*. *Evolutionary Ecology Research, 2*(5), 685–699.

Pasyukova, E. G., Roshina, N. V., & Mackay, T. F. C. (2004). Shuttle craft: A candidate quantitative trait gene for *Drosophila* lifespan. *Aging Cell, 3*(5), 297–307.

Phelan, J. P., Archer, M. A., Beckman, K. A., Chippindale, A. K., Nusbaum, T. J., & Rose, M. R. (2003). Breakdown in correlations during laboratory evolution. I. Comparative analyses of *Drosophila* populations. *Evolution, 57*(3), 527–535.

Quinn, G. P., & Keough, M. J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge: Cambridge University Press.

Randall, D., Burggren, W., & French, K. (1997). *Eckert animal physiology: Mechanisms and adaptations* (4th ed.). New York: W.H. Freeman Company.

Rice, W. R., & Chippindale, A. K. (2002). The evolution of hybrid infertility: Perpetual coevolution between gender-specific and sexual antagonistic genes. *Genetica, 116*(2–3), 179–188.

Rion, S., & Kawecki, T. J. (2007). Evolutionary biology of starvation resistance: What we have learned from *Drosophila*. *Journal of Evolutionary Biology, 20*(5), 1655–1664.

Robertson, A. (1959). The sampling variance of the genetic correlation coefficient. *Biometric, 15*(3), 469–485.

Robinson, S. J. W., Zwaan, B., & Partridge, L. (2000). Starvation resistance and adult body composition in a latitudinal cline of *Drosophila melanogaster*. *Evolution, 54*(5), 1819–1824.

Rose, M. R., Vu, L. N., Park, S. U., & Graves, J. L. (1992). Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Experimental Gerontology, 27*(2), 241–250.

Rush, B., Sandver, S., Bruer, J., Roche, R., Wells, M., & Giebultowicz, J. (2007). Mating increases starvation resistance and decreases oxidative stress resistance in *Drosophila melanogaster* females. *Aging Cell, 6*(5), 723–726.

Salmon, A. B., Marx, D. B., & Harshman, L. G. (2001). A cost of reproduction in *Drosophila* melanogaster: Stress susceptibility. *Evolution, 55*(8), 1600–1608.

Schmidt, P. S., Matzkin, L., Ippolito, M., & Eanes, W. F. (2005a). Geographic variation in diapause incidence life-history traits and climatic adaptation in *Drosophila melanogaster*. *Evolution, 59*(8), 1721–1732.

Schmidt, P. S., & Paaby, A. B. (2008). Reproductive diapause and life-history clines in North American populations of *Drosophila melanogaster*. *Evolution, 62*(5), 1204–1215.

Schmidt, P. S., Paaby, A. B., & Heschel, M. S. (2005b). Genetic variance for diapauses expression and associated life histories in *Drosophila melanogaster*. *Evolution, 59*(12), 2616–2625.

Schwasinger-Schmidt, T. E., Kachman, S. D., & Harshman, L. G. (2012). Evolution of starvation resistance in *Drosophila melanogaster*: Measurement of direct and correlated responses to artificial selection. *Journal of Evolutionary Biology, 25*(2), 378–387.

Service, P. M., Hutchinson, E. W., Mackinley, M. D., & Rose, M. R. (1985). Resistance to environmental stress in *Drosophila melanogaster* selected for postponed senescence. *Physiological Zoology, 58*(4), 380–389.

Sisodia, S., & Singh, B. N. (2010). Resistance to environmental stress in *Drosophila ananassae*: Latitudinal variation and adaptation among populations. *Journal of Evolutionary Biology, 23*(9), 1979–1988.

Slack, C., Werz, C., Wieser, D., Alic, N., Foley, A., Stocke, H., et al. (2010). Regulation of lifespan metabolism and stress responses by the *Drosophila* SH2B protein Lnk. *PLoS Genetics*. doi:10.1371/journal.pgen.1000881.

StatSoft (2007). Methods and applications. Version 8.0 StatSoft Tulsa.

Vieira, C., Pasyukova, E. G., Zeng, A., Hackett, J. B., Lyman, R. F., & Mackay, T. F. C. (2000). Genotype-environment interaction for quantitative trait loci affecting life span in *Drosophila melanogaster*. *Genetics, 154*(1), 213–227.

Wang, M., Harshman, L. G., & Nuzhdin, S. V. (2005). Quantitative trait loci for lipid content in Drosophila melanogaster. *Obesity Research, 13*(11), 1891–1897.

Wang, M., Lazebny, O., Harshman, L. G., & Nuzhdin, S. V. (2004). Environment-dependent survival of *Drosophila melanogaster*: A quantitative genetic analysis. *Aging Cell, 3*(4), 133–140.

Wayne, M., Soundararajan, U., & Harshman, L. (2006). Environmental stress and reproduction in *Drosophila melanogaster*: Starvation resistance ovariole numbers and early age egg production. *BMC Evolutionary Biology*. doi:10.1186/1471-2148-6-57.

Zhang, H., Stallock, J. P., Ng, J. C., Reinhard, C., & Neufeld, T. P. (2000). Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. *Genes & Development, 14*(21), 2712–2724.

Ziegler, R., & Van Antwerpen, R. (2006). Lipid uptake by insect oocytes. *Insect Biochemistry and Molecular Biology, 36*(4), 264–272.

Zwaan, B., Bijlsma, R., & Hoekstra, R. F. (1995). Direct selection on life span in *Drosophila melanogaster*. *Evolution, 49*(4), 649–659.