

# A quantitative genetic study of starvation resistance at different geographic scales in natural populations of *Drosophila melanogaster*

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## Summary

Food shortage is a stress factor that commonly affects organisms in nature. Resistance to food shortage or starvation resistance (SR) is a complex quantitative trait with direct implications on fitness. However, surveys of natural genetic variation in SR at different geographic scales are scarce. Here, we have measured variation in SR in sets of lines derived from nine natural populations of *Drosophila melanogaster* collected in western Argentina. Our study shows that within population variation explained a larger proportion of overall phenotypic variance (80%) than among populations (7.2%). We also noticed that an important fraction of variation was sex-specific. Overall females were more resistant to starvation than males; however, the magnitude of the sexual dimorphism (SD) in SR varied among lines and explained a significant fraction of phenotypic variance in all populations. Estimates of cross-sex genetic correlations suggest that the genetic architecture of SR is only partially shared between sexes in the populations examined, thus, facilitating further evolution of the SD.

## 1. Introduction

In nature, organisms are often exposed to a wide range of fluctuating environmental conditions that may reduce their fitness. Such conditions are often called *stress factors* (Rion & Kawecki, 2007). Organisms deal with stressful environmental conditions by changing their survival strategies, a process that may drive local adaptation (Hoffmann & Parsons, 1991; Randall *et al.*, 1997). The study of changes in fitness-related traits across habitats is essential to our understanding of adaptation and biogeographic patterns of organisms exposed to marked environmental seasonal variation. Hence, it is crucial to explore phenotypic variation in adaptive traits and their underlying genetic basis in order to predict potential phenotypic shifts triggered by local adaptation.

Usually, species that inhabit temperate areas are exposed to seasonal food shortage. Consequently, the

ability to survive periods of food shortage is one of the most important stress traits. In *Drosophila*, starvation resistance (SR) is an adult fitness component that acquires special relevance during adverse periods, like winters in temperate regions (Izquierdo, 1991; Mitrovski & Hoffmann, 2001; Boulétreau-Merle & Fouillet, 2002). SR is governed by many genes and influenced by environmental factors (Service & Rose, 1985; Da Lage *et al.*, 1990; Karan *et al.*, 1998; Harbison *et al.*, 2004; Hoffmann *et al.*, 2005) and, a number of studies have shown that it is correlated with several life-history traits such as fecundity (Service & Rose, 1985; Leroi *et al.*, 1994a, 1994b), lifespan (Service *et al.*, 1985; Force *et al.*, 1995; Lin *et al.*, 1998; Archer *et al.*, 2003), developmental time (Chippindale *et al.*, 1996; Harshman *et al.*, 1999) and viability (Chippindale *et al.*, 1996), suggesting a complex genetic architecture in which pleiotropy plays a pervasive role. Furthermore, the fact that SR is a sexually dimorphic trait, adds an additional level of complexity in the genotype–phenotype map (Hansen, 2006; Rion & Kawecki, 2007).

A screening of *P*-element insertion lines revealed that a large number of genes affect SR and, also,

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Table 1. Mean SR  $\pm$  SE (in hours) in females and males in nine populations of *D. melanogaster* from Argentina along with information of geographical coordinates

Populations	Latitude (South)	Longitude (West)	Altitude (m)	N	Sex	SR (h)	n
Güemes	24°41''	65°03'	695	13	Females	61.48 $\pm$ 1.35	104
					Males	48.04 $\pm$ 0.81	94
Cachi	25°07'	69°09'	2280	8	Females	61.54 $\pm$ 1.59	68
					Males	45.27 $\pm$ 1.02	58
San Blas	28°25'	67°06'	1061	13	Females	62.62 $\pm$ 1.35	118
					Males	51.70 $\pm$ 0.82	109
Chilecito	29°10'	67°28'	1043	16	Females	55.71 $\pm$ 1.36	151
					Males	43.17 $\pm$ 0.71	143
Jáchal	30°12'	68°45'	1238	13	Females	65.75 $\pm$ 0.56	110
					Males	53.67 $\pm$ 0.60	101
San Juan	31°27'	68°31'	671	12	Females	65.12 $\pm$ 1.14	114
					Males	50.21 $\pm$ 0.82	107
Barreal	31°32'	69°27'	1910	15	Females	58.58 $\pm$ 0.96	136
					Males	49.42 $\pm$ 0.54	136
Lavalle	32°50'	68°28'	647	11	Females	54.08 $\pm$ 1.65	98
					Males	44.28 $\pm$ 1.02	89
San Rafael	34°35'	69°	800	15	Females	56.66 $\pm$ 0.93	142
					Males	40.72 $\pm$ 0.56	130

N: number of lines analysed.

n: number of vials analysed for each population and sex.

that several of these genes have sex-specific effects (Harbison *et al.*, 2004). These results suggest both a different genetic basis for SR in females and males, and that variation in the sexual dimorphism (SD) has a genetic basis, highlighting the fundamental role that genotype by sex interactions may play in the evolution of natural genetic variation in SR.

Most surveys of variation in SR in natural populations of *D. melanogaster* aimed at unveiling geographical trends and, in some cases, assessed correlations with other traits such as lipid content (Robinson *et al.*, 2000; Hoffmann *et al.*, 2001). However, studies addressing the relative contribution of within and among populations variation to overall variance and/or providing estimates of genetic components of variance in experimental flies raised in common garden conditions are scarce (but see Hoffmann *et al.*, 2001).

One of the objectives of the present paper is to investigate variation in SR in nine natural populations of *D. melanogaster* sampled in western Argentina, and the apportionment of total phenotypic variation at two different geographic scales, within and among populations. We show that natural populations harbour substantial amount of genetic variation and that a significant fraction of SR variation may be accounted for by differences among populations. In addition, we also show that the genotype by sex interaction constitutes a significant component of natural genetic variation indicating that this factor may be involved in the maintenance of SR variation in nature. We also obtain estimates of cross-sex

genetic correlations in order to evaluate the evolutionary potential of the SD in SR.

## 2. Materials and methods

### (i) *Drosophila melanogaster* stocks

Flies were collected by means of net sweeping on fermented banana baits in nine localities along a north–south transect in Argentina (Table 1). Upon arrival to the laboratory, inseminated females collected in the field were isolated in individual vials to establish lines by rearing their respective progenies. All lines were maintained for ten generations in glass vials containing cornmeal–molasses–agar medium under a regime of constant temperature (25 °C), humidity (70%) and 12 h light/dark cycle before the onset of the experiments described below. Eight to sixteen lines from each locality were randomly chosen from the sets of lines for the analysis of SR (Table 1).

### (ii) SR assays

We measured SR as the time elapsed (in hours) from the moment in which flies were exposed to a starvation diet until death. Starvation diet consisted of 5 ml of 1.7% agar in water that provided moisture but not food, in standard culture vials. For each line, 200 sexually mature flies of both sexes were released in egg-collecting chambers. In each chamber, a Petri dish containing an 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 at 25 °C until egg hatching (approximately 24 h). First-instar larvae were transferred to 10 vials (30 individuals per vial) containing cornmeal–molasses–agar medium. Adult flies were recovered from the vials and separated by sex under CO<sub>2</sub> anaesthesia and then transferred to vials before the SR assays. Groups of ten 2–7 day-old non-virgin flies were transferred to the vials in which SR was assessed (see also Harbison *et al.*, 2004). Seven to ten replicated vials were set up per line and sex. All replicates were incubated at a constant temperature of 25 °C under a 12 h light/dark cycle. Survival was scored daily at 8.00 h, 14.00 h 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 replicate which was the variable considered in all statistical analysis.

### (iii) Statistical analyses

The full data set was initially explored using a mixed three-way analysis of variance (ANOVA), to investigate the contribution of population, lines (nested in populations) and sex to total phenotypic variance, an approach that is commonly used in quantitative genetic studies (see, for instance, Lavagnino *et al.*, 2008; Ballard *et al.*, 2008; Kenny *et al.*, 2008), according to the model:

$$y = u + P + L(P) + S + P \times S + L(P) \times S + E,$$

where  $u$  is the overall mean,  $P$  and  $L(P)$  are the random effects of population and line (nested in population), respectively,  $S$  is the fixed effect of sex, and  $E$  is the error, which according to our experimental design corresponds to the among replicate variance.

Additional ANOVAs were performed for each population separately according to the model:

$$y = u + L + S + L \times S + E.$$

In these ANOVAs, a significant  $L$  effect may be interpreted as an estimate of the genetic component of phenotypic variance, since lines (families) may be considered as different genotypes (David *et al.*, 2005). Likewise, the line by sex interaction ( $L \times S$ ) may be considered as an estimate of the genotype by sex interaction ( $G \times S$ ). A significant  $G \times S$  interaction may be interpreted as variation in the SD across genotypes and detected as deviations from the perfect genetic correlation between sexes (Muir *et al.*, 1992).

To estimate the cross-sex genetic correlation in each population we used the equation:

$$r_{mf} = \text{Cov}_{mf} / \sigma_m \sigma_f,$$

where  $\text{Cov}_{mf}$  represents the covariance between males and females, calculated by means of the bivariate

ANOVAs and  $\sigma_m$  and  $\sigma_f$  are the square roots of the among lines variance components obtained from univariate ANOVAs performed for males and females, respectively.

### (iv) Estimation of quantitative genetics parameters

We estimated quantitative genetic parameters for each natural population and for females and males, separately. Under our experimental design, the contribution of the variance component among lines ( $\sigma_L^2$ ) may be considered as an estimate of genetic variance ( $V_G$ ) (Lynch & Walsh, 1998; Morgan & Mackay, 2006). Environmental variance ( $V_E$ ) was estimated from the error term. Finally, total phenotypic variance ( $V_P$ ) was calculated as  $V_G + V_E$  (Falconer & Mackay, 1996; Lynch & Walsh, 1998).

To facilitate comparisons between sexes and among populations, we calculated the genetic coefficients of variation using the equation:

$$\text{CV}_G = 100(V_G)^{1/2} / \hat{u},$$

where  $\hat{u}$  is the SR population mean. This procedure is based on the notion that trait means, rather than variances, are more appropriate for standardizing genetic variance when the objective is to compare among traits and/or populations (Houle, 1992).

Statistical analyses and estimates of variance components of random effects were performed using GLM and VARCOMP procedure, respectively, implemented in the STATISTICA 8.0 software package (StatSoft, Inc., 2007).

## 3. Results

The mean values of survival in males and females under starvation are shown in Table 1. The general ANOVA revealed that differences among populations, between sexes and among lines (within populations), as well as the line by sex interaction were significant. Females (60.4 h) were, on average, significantly more resistant than males (47.5 h) ( $F_{1,108} = 181.8$ ,  $P < 0.00001$ ), a pattern that was homogeneous across populations as indicated by the non-significant population by sex interaction ( $F_{8,108} = 0.94$ ,  $P = 0.48$ ). The population effect was significant and accounted for 7.2% of total phenotypic variance, suggesting that the genetic factors that orchestrate variation in SR vary among sampling localities ( $F_{8,108} = 2.34$ ,  $P = 0.023$ ).

We also tested for clinal variation by means of multiple regression analyses of SR on geographic (latitude and altitude) and climatic variables (mean annual temperature; mean annual maximum and minimum temperature). This analysis revealed that among populations differentiation did not show any significant trend ( $F_{2,7} = 0.41$ ,  $P = 0.67$ ).

Table 2. Relative contribution of each source of variation to total phenotypic variation for SR in each population

	Güemes	Cachi	San Blas	Chilecito	Jáchal	San Juan	Barreal	Lavalle	San Rafael
Sex	***	***	**	***	***	***	**	*	***
Line	33.4	67.5**	54**	61.3**	6.2	62.1**	45.2*	42.0	58.3**
Line by sex	44.6***	24.0***	30.5***	31.3***	36***	25.7***	40.8***	48.6***	25.1***
Error	220	8.5	15.5	7.4	57.8	12.2	14	9.4	16.6
$r_{mf} \dagger$	0.48 (0.25)	0.78 (0.2)	0.70 (0.2)	0.78 (0.12)	-0.04 (0.29)	0.70 (0.21)	0.73 (0.22)	0.54 (0.26)	0.78 (0.16)

† Cross-sex genetic correlation coefficient (SE).

Significance level: \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$ .

In addition, the general ANOVA showed that the line (47.8%) and the line by sex interaction (32.1%) effects were significant and accounted, jointly, for 80% of overall phenotypic variance ( $F_{108, 1776} = 85.14$ ,  $P < 0.00001$ ;  $F_{108, 1776} = 22.15$ ,  $P < 0.00001$ , respectively).

We studied the relative contribution of differences among lines (genetic variance) and the line by sex interaction (genotype by sex interaction) to total phenotypic variation in each individual population to investigate whether the trends detected by the general ANOVA are homogeneous across populations. The results of the ANOVAs revealed that sex, line and the line by sex interaction were significant in most cases (Table 2). Differences among lines were highly significant in six out of nine populations (Table 2) suggesting that *D. melanogaster* populations harbour substantial amount of genetic variation in SR. The line by sex interaction term was significant in all populations (Table 2) indicating sex-specific variation in SR among lines, or, in other words that variation in the SD has a genetic basis.

A significant line by sex interaction term may be due to deviations from the perfect genetic correlation across sexes (in other words, changes in the ranking order of lines across sexes) and/or when variance among lines differs across sexes (Robertson, 1959). Further analyses of the line by sex interaction showed that the above mentioned components contributed almost equally to the interaction in most populations analysed (data not shown).

We also calculated the SD in SR using a female/male ratio for each population using the mean values given in Table 1. SD ranged from 1.18 to 1.39, meaning that females survived, on average 27% longer than males. We further analysed the SD data by means of an ANOVA with population and line (within population) as main sources of variation. The ANOVA revealed that differences among populations were not significant, whereas differences among lines were highly significant and accounted for 66% of total variance (data not shown).

The cross-sex genetic correlation,  $r_{mf}$ , may also be used to determine to what extent the same genes affect

a certain trait in females and males. An  $r_{mf}$  equal to one indicates that the same genes are involved in trait variation in both sexes, while an  $r_{mf}$  close to zero indicates that sets of genes affecting the trait in males and females are completely different (Falconer, 1952).  $r_{mf}$  varied among populations and in most cases differed significantly from zero and from unity. The lowest value was detected in Jáchal and the highest in Cachi and San Rafael. However,  $r_{mf}$  differed from one but not from zero in Jáchal, Güemes and Lavalle. We can use the results of the ANOVAs given in Table 2 to examine this issue, since the cross-sex genetic correlation was significantly different from unity when the line by sex interaction was significant, whereas it was significantly different from zero when the line term was significant.

Estimates of quantitative genetic parameters provided a supplementary view of patterns of variation in SR in each natural population and in the SD (Table 3). As expected, estimates of  $V_G$  were large (Table 3) and varied widely among populations, with Chilecito and Jáchal showing the highest and lowest values, respectively. Moreover, estimates of  $V_G$  and  $V_P$  were larger in females than in males confirming the trends described above. However, estimates of  $V_E$  were invariably lower than  $V_G$  and quite similar between sexes (Table 3). These results are certainly a consequence of our experimental design, since flies were reared under controlled conditions of density, temperature, humidity and photoperiod to minimize environmental variation. Another plausible explanation for the low  $V_E$  is that we considered the vial as the experimental unit, implying that the input data consisted of the average of 10 observations per vial.

A similar pattern was detected for the genetic coefficient of variation ( $CV_G$ ), which ranged from 5.4% to 30.5% in females and from 8% to 19.2% in males (Table 3).

#### 4. Discussion

Our study of SR variation in nine natural populations of *D. melanogaster* from Argentina revealed that populations harbour a substantial amount of genetic



Table 3. Estimates of quantitative genetic parameters of SR for each population in females and males

	Güemes	Cachi	San Blas	Chilecito	Jáchal	San Juan	Barreal	Lavalle	San Rafael	Average
Females										
$V_P$	210.94	234.47	250.37	307.94	37.69	159.38	131.69	283.82	128.59	193.86
$V_G$	188.55	222.48	224.51	288.96	12.59	142.63	119.89	269.17	110.37	175.45
$V_E$	22.39	11.99	25.86	18.98	25.10	16.75	11.80	14.65	18.22	18.41
$CV_G$	22.33	24.24	23.93	30.51	5.40	18.34	18.69	30.34	18.54	21.36
Males										
$V_P$	68.59	76.37	76.75	77.62	34.78	77.41	41.79	89.33	43.32	65.1
$V_G$	28.31	61.77	53.39	68.99	18.68	65.33	29.15	66.64	32.96	47.24
$V_E$	40.28	14.60	23.36	8.63	16.10	12.08	12.64	22.69	10.36	17.86
$CV_G$	11.08	17.36	14.13	19.24	8.05	16.10	10.92	18.43	14.09	14.38

$V_P$ : phenotypic variance;  $V_G$ : genetic variance;  $V_E$ : environmental variance;  $CV_G$ : genetic coefficient of variation.

variation and that populations are slightly (though significantly) differentiated in their ability to survive to food shortage. Differentiation among populations in SR may be an adaptive response to environmental heterogeneity and/or the result of demographic processes. Unfortunately, we do not have any direct evidence to rule out demographic factors. However, it is noteworthy that most populations studied here were also assessed for larval and adult olfactory behaviour and developmental time showing extensive among population differentiation (Lavagnino *et al.*, 2008; Folguera *et al.*, 2008; Mensch *et al.*, 2010). Taking into consideration that non-adaptive processes (demographic and historical) are expected to equally affect all traits, the dissimilar patterns of geographic variation observed across traits studied so far suggest that non-adaptive processes cannot be responsible for the among populations variation in SR.

Moreover, differentiation among populations did not correlate with geographic (latitude nor altitude) nor climatic variables, suggesting that the ability to survive during periods of food shortage does not correlate with ecologically relevant variables that covary with latitude or altitude or climatic variables. One possible explanation for the absence of clinal trends is that the availability of feeding resources (rotting fruits in orchards and vineyards) does not covary with latitude or altitude. We should also consider the possibility that limitations of the sampling strategy may account for the failure to detect clines. However, populations were sampled over an area encompassing an ample range of latitude (12°) and altitude (1600 m) uncovering wide spectrum of habitats involving different types of fruits (see Lavagnino *et al.*, 2008 for details). Our results are concordant with previous reports that also failed to find evidences of latitudinal clines in SR in other areas of *D. melanogaster* vast distribution (Robinson *et al.*, 2000; Hoffmann *et al.*, 2005). Nevertheless, negative latitudinal clines for SR were reported for *D. melanogaster* and other *Drosophila* species in India, as well as

for *D. simulans* in Australia (Karan & Parkash, 1998; Karan *et al.*, 1998; Arthur *et al.*, 2008). Our results suggest that trends of geographic variation in SR vary greatly across continents.

The study of the apportionment of genetic variation across different geographic scales may help to understand how natural genetic variation for quantitative traits is organized and the evolutionary processes involved. Our study shows that within population variation explains a greater proportion of SR variation than among populations, a pattern that is consistent with observations in Australian *D. melanogaster* and other species as *D. simulans* and *D. birchii* from the same area (Hoffmann *et al.*, 2001; Griffiths *et al.*, 2005; Arthur *et al.*, 2008). Taking into consideration all these evidences, we suggest that the large amount of genetic variation within populations may be a common feature of stress resistance traits.

We also detected a clear SD in SR; females outlived males under starving conditions, a pattern that was consistent across all populations surveyed. SD is presumed to be the result of natural selection favouring different optimal character states in the two sexes (Fairbairn *et al.*, 2007). Thus, the SD in SR may be explained by sex-specific expression in reproductive traits correlated with SR. In *Drosophila*, SR has been shown to be correlated with egg production (Chippindale *et al.*, 1993; Salmon *et al.*, 2001), ovariole number (Wayne *et al.*, 2006) and the ability to enter in reproductive diapause (Schmidt *et al.*, 2005). In contrast, several male reproductive aspects such as the production of accessory gland fluids and sperm and courtship are not correlated with SR (Cordts & Partridge, 1996). However, it is known that the expression of some male accessory gland proteins is affected by the absence of food, leading to a decrease in male reproductive success (Harbison *et al.*, 2005; Fricke *et al.*, 2008). Alternatively, the SD in SR may result from differences in basic physiological processes that determine the allocation of limiting energy

reserves between survival and reproduction (Service *et al.*, 1985; van Noordwijk & de Jong, 1986; Chippindale *et al.*, 1993; Zwaan *et al.*, 1995; Magwere *et al.*, 2004; Harbison *et al.*, 2005).

Interestingly, estimates of quantitative genetic parameters also differed between sexes. Again females showed larger values than males in all populations. Such dissimilarity may denote differential constraints imposed by genetic correlations with other complex traits, indicating differences in the genetic architecture of SR across sexes. In our work, measures of SR were obtained in non-virgin females, and, thus, differences in the reproductive status among females could be a plausible explanation for the sex-specificity observed.

Our study detected noticeable changes in the magnitude of the SD among genotypes (lines) in all populations, as shown by the significant contribution of the  $G \times S$  interaction to total phenotypic variation. A previous study using *P*-element insertion lines identified a large number of genes that affected the expression of SR and revealed that many of these genes exhibited a clear sex-specific effect (Harbison *et al.*, 2004). In this sense, the significant  $G \times S$  interaction detected in our study provides evidence that the sex-specific effect detected in *P*-element insertion lines (Harbison *et al.*, 2004) is also present in natural populations. Therefore, it might be argued that even though natural selection may act upon genetic factors involved in the  $G \times S$  interaction, still would explain a high percentage of variation in nature. Indeed,  $G \times S$  may be a key factor in the maintenance of genetic variation in SR in nature.

Homologous traits in females and males usually covary (Poissant *et al.*, 2009), because they are typically influenced by the same genes (Roff, 1997). Therefore, sex-specific selection may preclude that males and females reach an optimum phenotype (Lande, 1980). In this sense, the cross-sex genetic correlation,  $r_{mf}$ , is a quantitative genetic parameter that may be used to predict the potential for independent evolution of sexes (Bonduriansky & Chenoweth, 2009). Additionally,  $r_{mf}$  may indicate to what extent the same traits are affected by the same set of genes in each sex. In our study, cross-sex genetic correlations were positive but significantly lower than unity, suggesting a certain degree of genetic independence between sexes. Such independence may allow further evolution of the SD in SR.

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