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Thermal-specific patterns of longevity and fecundity in a set of heat-sensitive and heat-resistant genotypes of *Drosophila melanogaster*

L. Stazione, F.M. Norry & P. Sambucetti*

Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires – IEGEBA (CONICET-UBA), (C-1428-EHA) Buenos Aires, Argentina

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Abstract

Fitness-related traits are often affected by temperature. Heat-resistant genotypes could influence the dependence of fitness traits on temperature, which should be important in adaptation to directional changes in temperature including global warming. Here, we tested temperature-dependent variation in longevity and fecundity between *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) genotypes that differ in heat-resistance QTL. Longevity and fecundity were affected by heat-resistance genotypes at constant moderate and high temperature. However, these differences between heat-resistant and heat-sensitive genotypes disappeared in a cyclic thermal regime. Analysis with the logistic mortality function indicated that mortality patterns are dependent on temperature and genotype. The results suggest that genotype*temperature interactions are substantial for senescence-related traits. In particular, fluctuating temperatures can drastically reduce any differences in life-history traits between heat-resistance genotypes, even if such genotypes differentially affect the traits at constant temperatures.

Introduction

Heat resistance is an important determinant of *Drosophila* species distributions in contemporaneous terrestrial environments (Kellermann et al., 2012). Some fitness traits could be affected by heat-resistance genotypes and the identification of such traits will aid in predicting potential evolutionary responses to climate change (e.g., Hoffmann et al., 2003; Kellermann et al., 2009). However, few studies have tested directly for links between fitness-related traits and heat-resistance genotypes at elevated temperatures.

Several QTL mapping studies revealed some potentially pleiotropic regions with effects on both stress resistance and longevity (Vieira et al., 2000; Wang et al., 2004; Defays et al., 2011; Rodriguez et al., 2012; Sambucetti et al., 2015), suggesting an association of the genetic variation for stress resistance with longevity. Previous studies in *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) have found a major QTL affecting heat resistance in the middle of chromosome 2 (Norry et al., 2004, 2007a, 2008; Morgan & Mackay, 2006; Arias et al., 2012). Another large-effect QTL was identified for heat resistance in band 10 of the X chromosome (Norry et al., 2004, 2007b, 2008; Rand et al., 2010; Arias et al., 2012). Some candidate genes included within these QTLs are associated with stress resistance in *Drosophila* (e.g., *trap1*, *catsup*, *ddc*, *hsp60*, *hsc70*; Norry et al., 2009). Some temperature-specific QTL for longevity co-localize with heat-resistance QTL (Sambucetti et al., 2015). For example, the X-linked QTL mentioned above has an allele with positive effects on both KRHT (knockdown resistance to high temperature) and longevity at high temperature, indicating that the QTL conferring heat resistance also increases longevity at high temperature (Sambucetti et al., 2015). In spite of these possible links between heat resistance and longevity, the possible effects of heat-resistance genotypes remains to be explored on other fitness traits, such as fecundity in *Drosophila*.

The association between longevity and reproduction is frequently studied in model organisms like *Drosophila*. Extended longevity has been shown to have a cost in reproduction at benign temperature (reviewed in Rose, 1999; Partridge et al., 2005; Le Bourg, 2007; Paaby &

*Correspondence: E-mail: pablosambucetti@ege.fcen.uba.ar

Schmidt, 2009; Flatt, 2011). Studies of experimental evolution showed that selection for increased life span results in an increase in late-age reproduction and that this increase is correlated with a reduced reproduction early in life at benign temperature (Rose & Charlesworth, 1980; Luckinbill et al., 1984; Rose, 1984; Zwaan et al., 1995; Scannapieco et al., 2009). However, some studies suggest that longevity and early reproduction can sometimes be uncoupled (Kengeri et al., 2013; Khazaeli & Curtsinger, 2013; Wit et al., 2013; Tarin et al., 2014). Given the well-known association between longevity and fecundity, it is interesting to test these two classes of traits for their possible associations to heat-resistance genotypes at elevated temperature (e.g., Loeschcke et al., 2011). QTL mapping in recombinant inbred lines (RIL) from crosses between heat-selected populations provided information about genomic regions affecting heat resistance (Norry et al., 2004, 2007a, 2008; Morgan & Mackay, 2006; Arias et al., 2012; Sambucetti et al., 2013), as well as longevity and fecundity (Defays et al., 2011; Sambucetti et al., 2015; Highfill et al., 2016). This information can be used to establish fly stocks with alternative genotypes for heat resistance, which can be used to test fitness-related traits of individuals carrying heat-sensitive genotypes in comparison to individuals carrying heat-resistant genotypes (e.g., Loeschcke et al., 2011).

Here, we used flies carrying contrasting genotypes of heat resistance, obtained from a subset of RIL segregating extensive variation in knockdown resistance to heat stress (Norry et al., 2008). Longevity and fecundity patterns were tested at high (30 °C) and moderate (25 °C) temperature, and in a cyclic temperature regime. The cyclic temperature regime allowed us to test trait associations under an ecologically more realistic condition by exposing flies to daily temperature fluctuations. This is important because it was shown that fluctuating temperatures can have an impact on a variety of traits (Bozinovic et al., 2011; Vanin et al., 2012; Klepsatel et al., 2013). Fecundity patterns should be important for adaptation to directional changes in environmental temperature such as global warming, especially if fecundity depends on both genotype and temperature. We addressed two main aims. First, we examined whether heat-resistant genotypes exhibit a higher longevity than heat-sensitive genotypes in each thermal regime and test temperature. Second, we tested possible effects of the studied heat-resistance genotypes on both total and early fecundity. Evaluating both longevity and early fecundity at both moderate and high temperature is of additional interest because an association between these traits is sometimes temperature dependent (Sgrò & Hoffmann, 2004).

Materials and methods

Fly stocks

Fly stocks in this study were constructed from crosses between a subset of RIL described in Norry et al. (2008). Briefly, parental stocks were two nearly homozygous lines derived from Denmark and Australia, denoted D48 and SH2 lines, respectively. Parental line D48 was selected for low KRHT, whereas parental line SH2 was selected for high KRHT in adult flies. F1 females (progeny of D48*SH2) were backcrossed separately to males from the D48 and SH2 parental lines to set up two panels of RIL, one from the D48 backcross (RIL-D48) and another from the SH2 backcross (RIL-SH2). Both RIL panels were obtained by full-sib mating for 15 generations. Thirty-six microsatellite loci spread throughout all three major chromosomes were used as markers to perform QTL mapping for KRHT in Norry et al. (2008). Microsatellite loci DROSEV (bands 10A1-10A2) and AC004759 (bands 38E1-38E9) are markers closely linked to two heat resistance QTLs identified on the X chromosome (10A-12D) and chromosome 2 (34C-42F), respectively (Norry et al., 2008). Subsets of RIL were chosen to allow free recombination of the genome except for fixed alleles in the above-mentioned QTL regions.

For QTL on chromosome 2, four RIL-SH2 (lines 38, 99, 122, and 300) and five RIL-D48 (lines 1, 4, 49, 78, and 106) were crossed to set up a heat-sensitive stock denoted as ACO− (Figure 1). Similarly, four RIL-SH2 (lines 12, 32, 44, and 98) and four RIL-D48 (lines 31, 35, 39, and 83) were crossed to set up a heat-resistant stock denoted ACO+ [Figure 1; see Loeschcke et al. (2011) for further information about ACO lines]. The ACO− stock was fixed for the AC004759 marker allele from the low KRHT parental D48 (QTL allele conferring low heat resistance), whereas the ACO+ stock was fixed for the AC004759 marker allele from the high KRHT parental SH2 (QTL allele conferring high heat resistance), with the rest of the genome being both polymorphic and recombinant between the parental D48 and SH2 chromosomes (Figure 1).

For QTL on the X chromosome, five RIL-SH2 (lines 49, 148, 38, 68, and 53) and five RIL-D48 (lines 8, 32, 50, 57, and 89) were crossed to set up a heat-sensitive stock denoted as DRO− (Figure 1). In addition, four RIL-SH2 (lines 82, 32, 81, and 16) and four RIL-D48 (lines 31, 35, 72, and 98) were crossed to set up a heat-resistant stock denoted as DRO+ (Figure 1). As result of these crosses, DRO− stock was fixed for the DROSEV marker allele from the low KRHT line D48 (QTL allele conferring low heat resistance), whereas DRO+ stock was fixed for the DROSEV marker allele from the high KRHT line SH2 (QTL allele conferring high heat resistance), with the rest

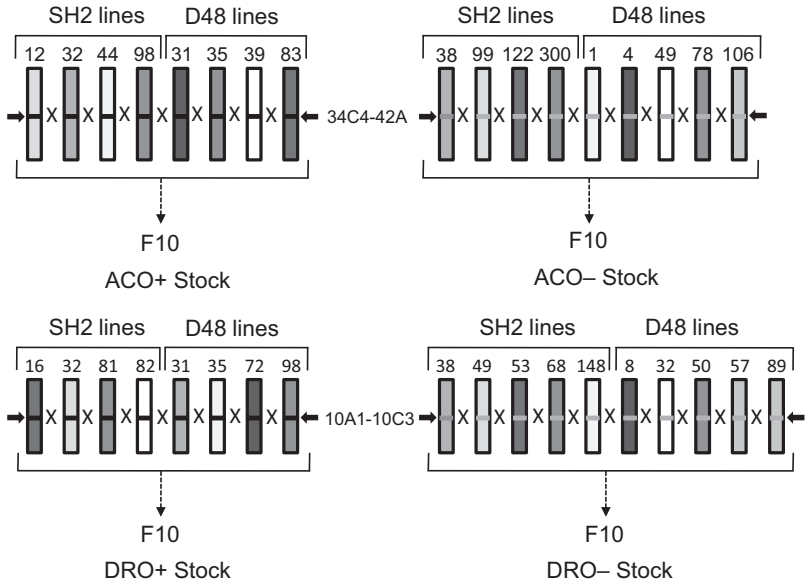

Figure 1 Schematic representation of four subsets of recombinant inbred lines (RIL) that were crossed to obtain four *Drosophila melanogaster* fly stocks on which all traits were measured in the F10 generation in this study. Chromosomes 2 (upper panels) and X (lower panels) show the localization of each microsatellite locus (arrow) used as marker of QTL alleles within two heat-resistance QTL previously identified in Norry et al. (2008). Numbered RIL selected to set up each fly stock share a single QTL allele within each fly stock but differ for the rest of the recombinant genome (represented by different shades of grey for each chromosome; detailed information about the genetic map of each RIL is given for chromosome 2 in Loeschcke et al., 2011). F10 was obtained by mass mating within each type of stock, resulting in highly polymorphic fly stocks except for each QTL allele (see text for further details).

of the genome being both polymorphic and recombinant between the parental D48 and SH2 chromosomes (Figure 1).

Each DRO and ACO stock was initially set up with 10 males plus 10 females from each one of the above mentioned RIL, in two 125-ml bottles containing 40 ml of a culture medium prepared with instant mashed potatoes plus water and nipagin (hereafter referred to as standard bottles). Stocks were maintained in five replicated standard bottles for 10 consecutive generations of random mating at 25 ± 1 °C in a L12:D12 photocycle. Flies from each line were mixed among replicated cultures every generation to allow free recombination of the whole genome except for the fixed QTL alleles as described above.

Traits measured

Longevity in the four stocks (DRO+, DRO−, ACO+, and ACO−) was measured at 25 and 30 °C as well as in a cyclic regimen of 16 h at 25 °C and 8 h at 30 °C under a L12:D12 photocycle. The 8-hour treatment at 30 °C in the cyclic regimen started at 10:00 hours and finished at 18:00 hours every day throughout the experiment. Experimental individuals were obtained by placing flies from each stock in 2-3 standard bottles per stock with 25 males plus 25 females per bottle. Flies were allowed to lay eggs for 4 days and after that were removed from the bottles. To measure longevity, 10 females and 10 males of 1 day old, all of them emerged from the above-mentioned culture bottles, were placed in 95 × 20-mm vials containing 6 ml of culture medium (hereafter referred to as standard vials). Thus, a total of 20 flies were used in each replicate assay vial. For each stock, 10 replicated vials were set up at each of the experimental temperatures. The flies were transferred to fresh vials every 2 days. Vials were examined for dead flies at each transfer until the last flies had died.

Variation in longevity at each temperature was tested using analysis of deviance with a Gamma distribution (best fitted distribution of the data) and inverse link function in a generalized linear model (GLM), with line panel (DRO+ vs. DRO− and ACO+ vs. ACO− separately) and sex (males vs. females) as fixed factors. The fitted model contained all possible interactions. Analyses were performed with InfoStat software (Di Rienzo et al., 2014). This software implements an interface of the R platform v.3.2.3 (R Development Core Team, 2015) to estimate generalized linear models through GLM and GLMER procedures from the stats and lme4 libraries (Bates et al., 2013).

Fecundity was measured at the same experimental temperatures as longevity (i.e., at constant 25 and 30 °C, and

at 16 h at 25 °C and 8 h at 30 °C under a L12:D12 photocycle) in each line. The experimental individuals were obtained in the same way as described above for longevity assays. Vials containing a small spoon with an oviposition surface were set up for each line, with one female plus two males of 1 day old, and ca. 30 vials per line. The oviposition surface consisted of a solution of agar (1.5 g) and water (140 ml) plus 0.2 ml of food coloring and yeast paste. Food coloring (Fleibor Laboratory, La Tablada, Buenos Aires, Argentina) was added to facilitate the observation of all eggs and egg shells (Sambucetti et al., 2015). The eggs were counted on the spoons using a stereo microscope every 2 days, when flies were transferred to new vials with fresh spoons. This procedure was repeated until the death of the females. Males that occasionally died were replaced by new ones of the same age from the same line. As the age of death was different among flies, total fecundity was estimated as the total number of eggs laid during the lifetime of a female relative to its age (i.e., total number of eggs/death age of the fly, in days). Early fecundity was estimated as the absolute number of eggs laid by a female during the first 5 days of its lifetime (Huey et al., 1995; Sambucetti et al., 2005).

Variation in total fecundity at each temperature was tested using analysis of deviance with a Poisson distribution (best fitted distribution of the data) and logLik link function in a generalized linear model (GLM), using line panel (DRO+ vs. DRO− and ACO+ vs. ACO− separately) as fixed factor. The same analysis was performed for early fecundity but using a normal distribution and identity link function as the best fitted model. Analyses were performed with the InfoStat software.

Mortality analysis

Mortality function was chosen by fitting mortality models [e.g., Gompertz, Gompertz-Makeham, Logistic, and Logistic-Makeham; see Pletcher (1999) for details of the mortality functions] that adequately describes the data. We tested for differences between lines for b and a parameters of the fitted model. The b parameter is usually interpreted as the demographic rate of aging (rate parameter), whereas the a parameter is often referred to as the initial mortality parameter (or intercept parameter). All estimates were obtained via maximum likelihood procedures from WinModest software (Pletcher, 1999). P-values were corrected for multiple comparisons using the sequential Bonferroni approach (Rice, 1989).

Results

Heat resistance (knockdown resistance to high temperature, measured as in Norry et al., 2008) differed between heat-resistant and heat-sensitive stocks (ANOVA, ACO stocks: line, $F_{1,290} = 5.74$, $P<0.05$; sex, $F_{1,140} = 7.49$, $P<0.01$; line*sex, $F_{1,290} = 0.41$; DRO stocks: line, $F_{1,290} = 26.84$, $P<0.0001$; sex, $F_{1,140} = 2.44$; line*sex, $F_{1,290} = 3.52$; Figure 2). At 25 °C, mean longevity was affected by a two-way interaction between stock and sex in both ACO and DRO flies (Figures 3 and 4, Table 1). GLM for each sex separately indicated differences in mean longevity in males but not in females for ACO stocks, with males from the heat-resistant line living longer than heat-sensitive males (GLM with ACO+ vs. ACO− as fixed factor, males: $\chi^2 = 2.02$, $P<0.001$; females: $\chi^2 = 0.22$, $P>0.05$, both d.f. = 1; Figures 3 and 4). The opposite pattern was observed for DRO flies: differences in mean longevity at 25 °C were significant in females but not in males, with females from the heat-sensitive stock DRO− living longer than females from the heat-resistant stock DRO+ (GLM with DRO+ vs. DRO− as fixed factor, males: $\chi^2 = 0.1$, $P>0.05$; females: $\chi^2 = 3.14$, $P<0.001$, both d.f. = 1; Figures 3 and 4).

At 30 °C, mean longevity was higher for the heat-sensitive than for the heat-resistant stock for both ACO and DRO flies (Figures 3 and 4). There was also a significant line*sex interaction at this temperature for both lines (Figures 3 and 4, Table 1). In the ACO lines differences were significant in females, and almost significant in males (GLM with ACO+ vs. ACO− as fixed factor, males: $\chi^2 = 0.33$, $P = 0.057$; females: $\chi^2 = 7.62$, $P<0.001$, both

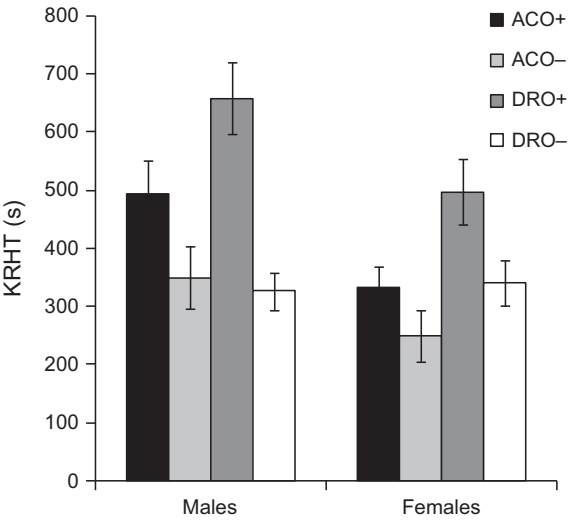

Figure 2 Mean (± SE) knockdown resistance to high temperature (KRHT) of males and females of *Drosophila melanogaster* at 37 °C for both heat-resistant (ACO+ and DRO+) and heat-sensitive (ACO− and DRO−) stocks. Significant differences are indicated in the text.

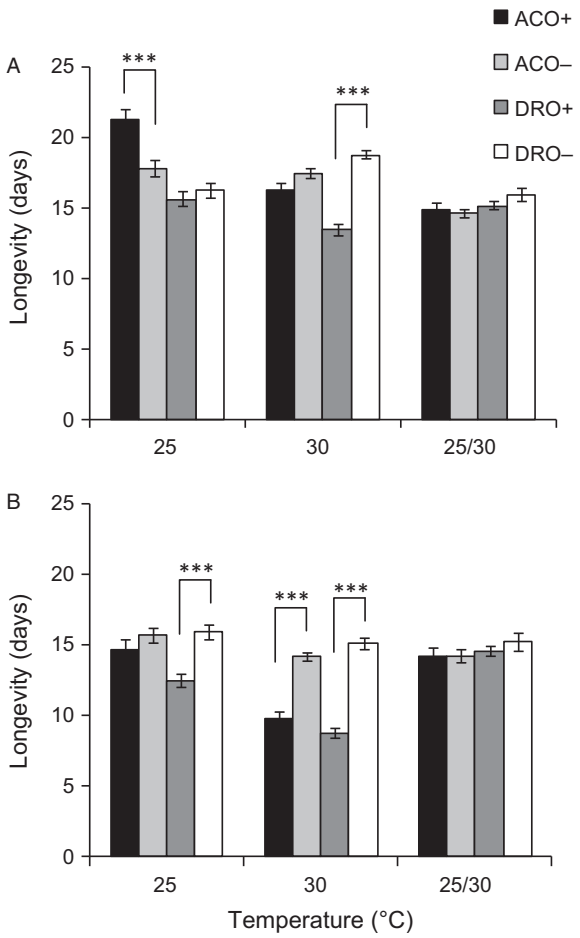

Figure 3 Mean (± SE) longevity (days) of (A) males and (B) females of *Drosophila melanogaster* at constant 25 and 30 °C and in a cyclic thermal regimen (see M & M for regimen details) for both heat-resistant (ACO+ and DRO+) and heat-sensitive stocks (ACO− and DRO−). Asterisks indicate significant differences (GLM: P<0.001).

d.f. = 1). In the DRO lines differences were significant for both sexes (GLM with DRO+ vs. DRO− as fixed factor, males: $\chi^2 = 7.91$; females: $\chi^2 = 17.01$, both d.f. = 1, P<0.001; Figures 3 and 4). At the cyclic thermal regimen, the mean longevity did not differ between ACO lines, whereas the heat-sensitive DRO line tended to live longer than the heat-resistant DRO line (almost significant; Figures 3 and 4, Table 1).

Model fitting analysis indicated that logistic function was the best fit function in most cases (Table 2). At 25 °C, ACO− males had a higher mortality rate (b) but a lower initial mortality parameter (a) than ACO+ males. The same pattern was observed in ACO lines at 30 °C for males (Table 2). ACO females differed in mortality rate at 25 °C but not at 30 °C, although this difference was not significant after correcting for multiple comparisons. Parameter a displayed the same pattern as in ACO males, with a lower value for ACO− than for ACO+ line at both 25 and 30 °C. There were no differences between DRO+ and DRO− lines in the mortality parameters at 25 °C, in both males and females. At 30 °C, DRO lines did not differ in parameter b, neither in males nor in females, but intercept parameter a was higher in DRO+ than in DRO− in both sexes, although differences in males were not significant after correcting for multiple comparisons. In the cyclic thermal regimen, parameters a and b did not differ for ACO lines, whereas for DRO lines only females showed differences in the mortality parameters, although not significant after correcting for multiple comparisons (Table 2).

There were no differences in total fecundity between heat-sensitive and heat-resistant females, neither at 25 °C nor at the cyclic regimen in ACO and DRO stocks (Figure 5A, Table 3). At 30 °C, total fecundity was higher in DRO+ than in DRO− females. The opposite pattern was observed for ACO at 30 °C, with a higher total fecundity in ACO− than in ACO+ females. There were no

Table 1 Generalized linear model to test for differences in longevity in males and females of heat-sensitive (ACO− and DRO−) vs. heat-resistant (ACO+ and DRO+) *Drosophila melanogaster* fly stocks at constant 25 and 30 °C, and in a cyclic thermal regimen (see M & M for details). χ^2 values (d.f. = 1) are shown for each fixed factor and their interaction

		Factors		
Regimen	Stock comparison	Stock	Sex	Stock*sex
25 °C	ACO+ vs. ACO−	1.12**	5.41***	1.37**
	DRO+ vs. DRO−	1.99***	1.76***	1.34**
30 °C	ACO+ vs. ACO−	2.31***	13.03***	4.08***
	DRO+ vs. DRO−	22.69***	10.87***	3.51***
25/30 °C	ACO+ vs. ACO−	0.0045	0.12	0.01
	DRO+ vs. DRO−	0.31^{+}	0.23	6×10^{-5}

**0.001<P<0.01, ***P<0.001; $^{+}$P = 0.052.

Table 2 Estimated intercept (a) and rate parameter (b) for the logistic mortality function for heat-resistant (ACO+ and DRO+) and heat-sensitive (ACO− and DRO−) *Drosophila melanogaster* fly stocks at constant 25 and 30 °C, and in a cyclic thermal regimen (see M & M for regimen details). χ^2 values (d.f. = 1) are shown for comparisons between heat-resistant and heat-sensitive stocks

	Temperature (°C)	Parameter	ACO+	ACO−	χ^2	DRO+	DRO−	χ^2
Males	25	a	0.0013	2.9×10^{-8}	10.87***	0.00057	0.0073	2.49
		b	0.20	0.89	14.94***	0.34	0.19	2.02
	30	a	0.0018	3×10^{-5}	13.01***	0.0043	0.00066	4.72*
		b	0.32	0.61	9.79**	0.34	0.35	0.0097
	25/30	a	0.0013	0.0012	0.0075	0.0011	0.0011	0.00088
		b	0.38	0.38	0.00044	0.34	0.32	0.27
Females	25	a	0.013	4.2×10^{-7}	15.62***	0.0079	0.0016	3.29
		b	0.14	0.80	6.75**	0.28	0.36	0.46
	30	a	0.031	0.0011	17.94***	0.014	0.0026	16.55***
		b	0.15	0.38	0.54	0.30	0.27	0.56
	25/30	a	0.011	0.0069	0.96	0.0031	0.0099	4.19*
		b	0.16	0.22	1.7	0.28	0.16	3.90*

*0.01<P<0.05, **0.001<P<0.01, ***P<0.001.

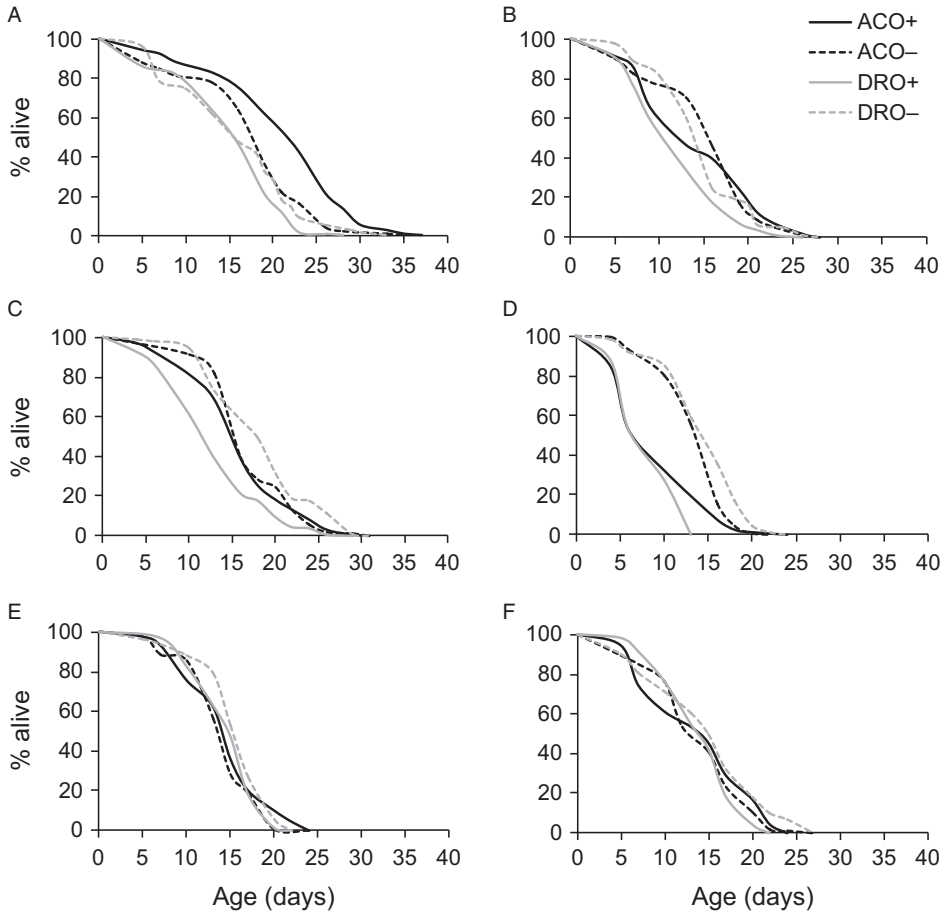

Figure 4 Survival curves of males (left panels) and females (right panels) of *Drosophila melanogaster* (A, B) at 25 °C, (C, D) at 30 °C, and (E, F) in a cyclic thermal regimen (E, F; see M & M for regimen details) for both heat-resistant (ACO+ and DRO+) and heat-sensitive stocks (ACO− and DRO−).

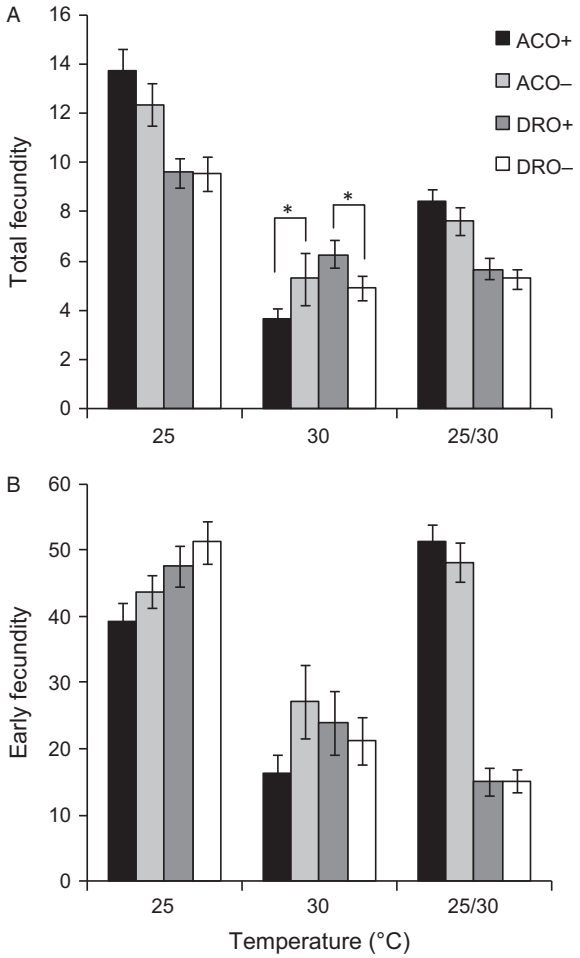

Figure 5 Mean (± SE) (A) total and (B) early fecundity (number of eggs laid in the first 5 days of adult life) of female *Drosophila melanogaster* flies at 25 and 30 °C and in a cyclic thermal regimen (see M & M for regimen details) for both heat-resistant (ACO+ and DRO+) and heat-sensitive (ACO− and DRO−) stocks. Significant differences are indicated by an asterisk (GLM: $P<0.05$).

differences in early fecundity at any of the thermal regimes (Figure 5B, Table 3) although early fecundity tended to be higher in ACO− than in ACO+ (almost significant; Figure 5B).

Discussion

Longevity and fecundity of two heat-resistance genotypes of *D. melanogaster* were compared among thermal regimens. At 25 °C, heat-resistant males from the ACO stock (ACO+) lived longer than heat-sensitive (ACO−) males, whereas their longevity was the same at 30 °C. Heat-resistant (ACO+) females lived shorter than heat-sensitive

Table 3 Generalized linear model to test for differences in fecundity in males and females of heat-sensitive (ACO− and DRO−) vs. heat-resistant (ACO+ and DRO+) *Drosophila melanogaster* fly stocks at constant 25 and 30 °C, and in a cyclic thermal regimen (see M & M for details). χ^2 values (d.f. = 1) are shown for the fixed factor 'line'

Regimen	Stock comparison	Total fecundity	Early fecundity
25 °C	ACO+ vs. ACO−	3.02	419.1
	DRO+ vs. DRO−	0.02	244.6
30 °C	ACO+ vs. ACO−	5.94*	1178.1^{+}
	DRO+ vs. DRO−	5.20*	113.1
25/30 °C	ACO+ vs. ACO−	0.58	226.4
	DRO+ vs. DRO−	1.97	0.73

*$P<0.05$; $^{+}P = 0.051$.

(ACO−) females at 30 °C. Males from the heat-sensitive DRO stock (DRO−) lived longer than heat-resistant (DRO+) males at 30 °C. DRO− females lived longer than DRO+ females at both 25 and 30 °C. Thus, on average, at constant high temperature both sexes lived longer in heat-sensitive (ACO−, DRO−) than in heat-resistant (ACO+, DRO+) lines. In sharp contrast, between-stock differences in mean longevity disappeared at fluctuating temperatures: in the cyclic thermal regimen no significant differences were observed for ACO+ vs. ACO− and DRO+ vs. DRO−. Between-stock differences in fecundity were found at 30 °C only. At this temperature, total fecundity was higher in ACO− than in ACO+ females, whereas it was higher in DRO+ than in DRO− females. These differences disappeared in the cyclic thermal regime.

Mortality analysis indicated that, at both constant temperatures, differences in longevity in ACO males are best explained by the b parameter, with a higher mortality rate in the heat-sensitive and shorter-lived flies. It is consistent with the hypothesis that longevity differences with temperature are the result of changes in the rate of aging. The lack of a significant difference in male longevity between ACO+ and ACO− at 30 °C could be explained by the a and s parameter in the logistic model (s describes the amount of deceleration in mortality rates at advanced ages; Pletcher, 1999). Both parameters are higher in the heat-resistant than in the heat-sensitive stock (data not shown for s), resulting in a possible compensation with the mortality rate and a consequent absence of differences in longevity. A general mortality pattern observed was a higher initial mortality in heat-resistant than in heat-sensitive stocks both at 25 and 30 °C.

It is interesting that at the cyclic thermal regimen, there were no significant differences between heat-resistant and heat-sensitive genotypes in both mean longevity and fecundity. Perhaps, heat-sensitive flies at constant high

temperature may attain some level of heat acclimation to that temperature whereas under fluctuating thermal conditions such an acclimation might not be achieved. In fact, previous studies showed that flies carrying heat-sensitive and short-lived genotypes were much more responsive to heat-hardening, heat-acclimation, and heat-induced hormesis treatments than flies carrying heat-resistant genotypes (Norry & Loeschcke, 2003; Gomez et al., 2009; Defays et al., 2011).

Fluctuating thermal regimes are suggested to have a greater ecological relevance than constant regimes as they are more representative of natural environments (Klepsatel et al., 2013; Manenti et al., 2016). Fluctuating temperatures can yield different results for a variety of traits when compared to constant temperatures (Bozinovic et al., 2011; Vanin et al., 2012; Manenti et al., 2016). Our results showed that longevity differences between heat-resistant and heat-sensitive stocks at constant temperatures disappear at fluctuating temperatures. Changes in environmental conditions can change trait interactions differently (Sgrò & Hoffmann, 2004), and temperature has been shown to affect trait correlations (Norry & Loeschcke, 2002; Klepsatel et al., 2013; Manenti et al., 2016). Furthermore, fluctuating temperatures are expected to demand higher energetic costs (Hoffmann et al., 2003; Bowler & Terblanche, 2008), which may result in negative correlations between stress resistance and life-history traits in flies exposed to fluctuating thermal regimes (Manenti et al., 2016). In this regard, our results show that the expected genetic correlation between heat resistance and longevity depends on the thermal conditions in which longevity is assayed.

Early fecundity did not differ between our heat-sensitive and heat-resistant stocks in any thermal regimens. However, total fecundity differed between '+' and '−' stocks at high temperature for both ACO and DRO. At 30 °C, fecundity was higher in the heat-resistant than in the heat-sensitive DRO stock, whereas it was lower in the heat-resistant than in the heat-sensitive ACO stock. Negative correlations between fecundity and longevity are usually considered consistent with an antagonistic pleiotropy model (Williams, 1966). The lines used in this study derived from parental flies that were divergent for heat resistance and fecundity at high temperature, but did not differ in fecundity at benign temperature (Sambucetti et al., 2015). Therefore, we expect a negative association between longevity and fecundity only at high temperature, as fecundity did not differ between the parental lines originally crossed for the set-up of RIL (Sambucetti et al., 2015). We found this expected association only in DRO flies, where females of the heat-sensitive stock lived longer and were less fecund. This pattern is also consistent with our mortality analysis where initial mortality was higher for the heat-resistant stock. Thus, genotype*environmental interactions are apparent, as female longevity differences in DRO stocks are also observed at 25 °C, with no related differences in fecundity at this temperature, further supporting the idea that association between survival and reproduction can be uncoupled under some conditions (Flatt, 2011). Recently, a set of RIL (RIL-SH2) was found to be longer lived and less fecund than its reciprocal RIL-D48 set at 30 °C (Sambucetti et al., 2015). On the contrary, our present results at 30 °C show that DRO+ females, which carry the SH2 allele from the heat-resistant parental line, were shorter lived and more fecund than DRO− females, which carry a D48 allele from the heat-sensitive parental line. Probably, trait associations between longevity and fecundity are affected by the genetic background, as our ACO and DRO stocks are strongly polymorphic except for the fixed QTL region, whereas the recent RIL stocks are nearly homozygous.

Identification of fitness traits influenced by genotype*temperature interactions involving heat-resistance genotypes will aid in predicting evolutionary responses to climate change in a global warming scenario (Hoffmann et al., 2003; Kellermann et al., 2009). Reproductive fitness components are also of interest to test for adaptive responses to thermal stress (Sambucetti & Norry, 2015). In this study, we analyzed heat-resistance genotypes for longevity and fecundity performances at high and moderate temperatures as well as in a cyclic thermal condition. It is apparent that both female fecundity and longevity are affected by genotype*temperature interactions at the two heat-resistance QTLs tested in this study.

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