Biological Journal of the Linnean Society, 2009, **97**, 738–748. With 4 figures

Direct and correlated responses to selection for longevity in *Drosophila buzzatii*

ALEJANDRA C. SCANNAPIECO, PABLO SAMBUCETTI and FABIAN M. NORRY*

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

Received 7 January 2009; accepted for publication 13 January 2009

The possible associations between longevity, early fecundity, and stress-resistance traits were explored using artificial selection on longevity in a laboratory population of *Drosophila buzzatii*. Three replicated lines were selected for increased lifespan (L lines) and compared with the respective unselected controls (C lines) after the 14th generation of selection. Mean longevity exhibited a significant response to selection. The baseline mortality tended to decrease in the L lines and a negative correlated response to longevity selection was found for early fecundity. Egg-to-adult developmental time increased in L lines. Longevity selection increased stress resistance for both high and low temperatures, as measured by heat knockdown resistance and chill-coma recovery. Starvation resistance also tended to be higher in L than in C lines. The results obtained are consistent with the hypothesis of trade-offs between longevity and early fecundity, and also suggest a trade-off association between adult longevity and developmental time. Correlated selection responses were generally consistent with correlations among the traits previously inferred from altitudinal clines for longevity and stress-resistance phenotypes. © 2009 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2009, **97**, 738–748.

ADDITIONAL KEYWORDS: longevity selection – chill-coma recovery – cline – early fecundity – heat knockdown resistance – senescence – starvation – trade-off.

INTRODUCTION

Longevity phenotypes are interesting not only because of their medical implications, but also because of their dramatic quantitative variation at all taxonomic levels (Promislow, 1991; Rose, 1991). Artificial selection in model organisms such as *Drosophila* comprises a quantitative genetic approach for identifying heritable patterns of phenotypic (co-)variation in fitness-related traits, including longevity (Rose, 1991, 1999; Harshman & Hoffmann, 2000; Kirkwood & Austad, 2000; Prasad & Joshi, 2003). This approach has been extensively applied in *Drosophila melanogaster* to test for genetic correlations between longevity and putatively-related traits, such as fecundity and/or stress resistance (Rose, 1984; Partridge & Fowler, 1992; Leroi, Chippindale & Rose, 1994; Zwaan, Bijlsma & Hoekstra, 1995a; Luckinbill, 1998; Harshman & Haberer, 2000; Stearns *et al.*, 2000; Clancy *et al.*, 2002; Norry & Loeschcke, 2003; Bubliy & Loeschcke, 2005; Baldal, Brakefield & Zwaan, 2006; Yadav & Singh, 2007). Artificial selection can provide a way to test whether or not the correlated responses to selection on longevity are consistent with clinal patterns of variation in fecundity and stress-resistance traits, as expected from evolutionary theories of senescence (Rose, 1991; Partridge & Mangel, 1999; Kirkwood & Austad, 2000).

According to evolutionary theories of senescence, ageing is a by-product of natural selection because mortality in wild individuals is mainly the result of extrinsic causes such as extreme temperatures, starvation, predation, and/or desiccation. In other words, the intensity of selection will decline with age if wild individuals do not live long enough to become very

*Corresponding author. Current address: Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, C-1428-EHA Buenas Aires, Argentina.
E-mail: fnorry@ege.fcen.uba.ar

aged (Rose, 1984; Finch, 1990; Partridge & Mangel, 1999; Kirkwood & Austad, 2000). This almost absent selection in late ages will result in: (1) an accumulation of late-acting deleterious mutations (i.e. the mutation accumulation theory; Medawar, 1952) and (2) an increase in the frequency of alleles with beneficial effects early in life, even if they have deleterious effects in advanced ages (i.e. the antagonistic pleiotropy theory; Williams, 1957). In addition to the antagonistic pleiotropy model, the disposable soma theory proposes that senescence has evolved in such a way that the amount of energy invested in maintaining and repairing the soma is sufficient to keep an organism alive long enough to reproduce, but less than that required to keep it alive for longer (Kirkwood & Austad, 2000).

Most of the data on age-related artificial selection in dipteran insects have been limited to *D. melanogaster* (for other dipteran models, see Miyatake, 1997). In *D. melanogaster*, early fecundity was often found to negatively correlate with longevity, as predicted from evolutionary theories of ageing. In *Drosophila buzzatii*, early fecundity and longevity at 25 °C clinally co-vary in opposite directions with the altitude of population of origin (Norry *et al.*, 2006). This correlational pattern suggested a trade-off between early fecundity and longevity (Norry *et al.*, 2006). Furthermore, long-lived populations from low altitudes were more heat-stress resistant than short-lived populations from high elevations (Sorensen *et al.*, 2005). Taken together, these correlation patterns appear to be consistent with the clinal patterns expected from evolutionary theories of ageing. Additionally, a trade-off association between developmental time and longevity may also be consistent with antagonistic pleiotropy theory (Promislow & Bugbee, 2000), but such an association was not apparent along the cline in *D. buzzatii* (Sambucetti, Loeschcke & Norry, 2006). Although considerable information is now available from age-related selection in laboratory populations of *D. melanogaster* (Rose & Charlesworth, 1980, 1981; Partridge & Fowler, 1992; Zwaan *et al.*, 1995a; Luckinbill, 1998; Partridge & Mangel, 1999; Harshman & Haberer, 2000; Stearns *et al.*, 2000; Clancy *et al.*, 2002; Baldal *et al.*, 2006), the correlated responses to selection on longevity-related traits remain to be tested in species such as *D. buzzatii* for which suggestive clines were apparent in longevity, early fecundity, and stress resistance. Correlated responses to artificial selection on longevity-related traits can provide information for comparison with provisionally inferred associations between traits across clines. Therefore, there is a need to evaluate correlated responses to longevity selection for species such as *D. buzzatii*, in which suggestive clines were found for longevity, early fecundity, and stress resistance (Sorensen *et al.*, 2005; Norry *et al.*, 2006; Sarup *et al.*, 2006).

In the present study, we explored the experimental evolution of early fecundity, thermal-stress resistance, starvation resistance, developmental time, and body size as correlated responses to truncation selection on life span in a laboratory-reared population of the cactophilic *D. buzzatii*. The base population in the study was derived from a central population of the above-mentioned altitudinal cline in Argentina. Three replicated lines were selected for 14 consecutive generations of selection on a longevity-related trait. The three main questions addressed were: (1) Are some of the previously found associations in *D. melanogaster*, involving longevity, early fecundity, developmental time, and stress resistance, as mentioned above, also seen in *D. buzzatii*? (2) Are the correlated selection responses consistent with evolutionary theories of senescence? (3) Are the laboratory selection responses consistent with the recently found clinal patterns of variation in the studied traits in *D. buzzatii*?

MATERIAL AND METHODS

Base population

The lines used in the present study were selected from a laboratory-adapted stock that was set up from a single wild population sampled in mid-April 2003 at Chumbicha, Argentina (28.52°S, 66.15°W), as described previously (Norry *et al.*, 2006). Briefly, wild flies were collected using banana baits. Twenty-two isofemale lines were derived from the wild flies and inter-crossed to set up the laboratory stock used in the present study. The above-mentioned population at Chumbicha comprises a central, large population within the altitudinal cline, described as a population at 401 m a.s.l. elsewhere (Sorensen *et al.*, 2005; Norry *et al.*, 2006). To avoid any possible confounding effects of laboratory adaptation when selecting for longevity (Sgrò & Partridge, 2000; Baldal *et al.*, 2006), the stock was maintained in the laboratory at 25 °C for 30 generations before the start of the longevity selection experiment, with five standard bottles and approximately 70 flies per bottle in each generation. The standard bottles used were 125-mL bottles containing 40 mL of culture medium. Instant *Drosophila* medium was used as culture medium throughout the experiment.

Longevity selection

Six lines were set up from the base stock. These lines were either selected for longevity (denoted L1, L2, and L3) or not selected (control lines, denoted C1, C2, and C3). All replicated lines were reared under standardized conditions at 25 °C (five bottles with 50–70

flies on 40 mL of standard laboratory medium (instant medium) under a 12 : 12 h light/dark cycle). Control lines were maintained as routine cultures in every generation. Selection in L lines was performed by placing 150–300 flies (1 day after eclosion) per replicate line in plastic cages ($35 \times 27 \times 13$ cm; one cage per replicated line) using a small aperture from which the flies were released. No anaesthetic treatment was used during this procedure. Five food dishes (2×3 cm) were placed within each cage. Food dishes contained 10 mL of standard laboratory medium plus 5 g of *Opuntia* tissues on the medium surface. Food dishes were replaced every 2 days. For each replicated L line, flies were maintained in the cage until 50% of mortality was observed. All survivors were collected from the cage and distributed at random into three standard bottles with approximately 25–50 flies per bottle. The offspring of these cultures were used for the next round of selection. This protocol was repeated for each generation of longevity selection. All experimental cages and cultures were maintained at 25 ± 1 °C. Control lines were maintained using all flies that emerged within the first 2 weeks as parents of the next generation, and were reared in otherwise similar conditions as for L lines. After 14 consecutive generations (G14) of longevity selection, all selection (L) and control (C) lines were measured for all traits in G15 (one generation after the last generation of selection) to test for direct and correlated responses to selection.

Traits studied

Longevity

To obtain experimental individuals, 2-day-old flies (25 males plus 25 females) were placed in standard bottles with standard laboratory medium. Females were allowed to lay eggs for 2 days. All cultures were maintained at 25 ± 1 °C under a 12 : 12 h light/dark cycle. Flies that emerged from these cultures were collected to set up 10–13 standard vials containing 1-day-old flies (ten males plus ten females) for all C and L replicated lines. Flies were transferred to new vials with fresh medium every 2 days when vials were examined for dead flies. The number of vials was gradually reduced as deaths occurred, with surviving adults being kept at a density as close to 20 per vial as possible. For analysis, longevity data (days) were ln-transformed because this transformation both improved normality and removed dependence of variance on mean. Residual diagnostics (Shapiro–Wilk test) revealed mostly normal error distributions.

Early fecundity

Fecundity was scored on a cactus-based medium at 25 °C under a 12 : 12 h light/dark cycle, because *Opuntia* tissues appeared to be the preferred oviposition substrates for *D. buzzatii* (Fanara & Hasson, 2001). Cactus-based medium was based on an autoclaved blend of fresh tissues of *Opuntia vulgaris* (100 mL), plus agar (2 g), yeast (5 g), and H_2O (400 mL). For each L and C line in G15, ten vials, each containing a small spoon with cactus-based medium, were set up with 1-day-old flies (one virgin female plus two males). Spoons were replaced every day. For each experimental female, the total number of eggs was scored every day for the first 7 days of adult life. Males that occasionally were dead or escaped were replaced by new males of similar age from the same replicate line. Early fecundity (EF) was estimated as the mean number of eggs laid within the first 7 days of adult life (Huey *et al.*, 1995; Sambucetti *et al.*, 2005). This range of age for males and females was shown to adequately predict EF at 25 °C not only in *D. melanogaster* (Huey *et al.*, 1995), but also in laboratory-reared *D. buzzatii* derived from the same geographical region as our origin population (Sambucetti *et al.*, 2005; Norry *et al.*, 2006). In addition, pilot studies performed by us indicated that females are inseminated between 1 and 2 days of age under the above-described experimental conditions.

Mortality analysis

We estimated age-specific mortality rate (μ_x) as the continuous form of age-specific mortality, where $\mu_x = -\ln(1 - q_x)$, $q_x = d_x/N_x$, d_x is the number of flies dying in the interval x to $x + 1$, and N_x is the number alive at day x (Elandt-Johnson & Johnson, 1980; Promislow *et al.*, 1996). WINMODEST (Pletcher, 1999) was used to fit age at death date to four different mortality models (Gompertz, Gompertz-Makeham, Logistic and Logistic-Makeham) and to determine which of them best predicts mortality trajectories in each line. Two parameters (a and b) are estimated in Gompertz model: a is the baseline mortality rate and b is the demographic rate of ageing (Pletcher, 1999). However, if mortality rates level off in older individuals, the Logistic model is suggested because it includes a third s-parameter for such a possible mortality deceleration (Pletcher, 1999). Differences in mortality parameters were tested by pairing L(i) and C(i) replicates according to the line numbers that were assigned arbitrarily to them at the start of the experiment. Gompertz was the model showing the best fit for comparisons between L and C lines. Additionally, mortality analysis was also used to test for differences in mortality parameters between laboratory-reared populations that were recently derived from the altitudinal cline described by Norry *et al.* (2006) and our L and C lines. All mortality parameters were estimated via maximum likelihood using WINMODEST (Pletcher, 1999).

Developmental time and body size

To measure egg-to-adult developmental time (DT), three shell vials containing standard laboratory medium were set up for each replicate line in G15 with 40 larvae per vial and maintained at 25 ± 1 °C. DT was determined for each replicate line by scoring the number of eclosed individuals at regular intervals of time (three times a day at 08.00 h, 14.00 h and 20.00 h), until all flies had emerged. In addition, a random sample of 15 females plus 15 males that were measured for DT was used to measure thorax length (TL, an index of body size) in each replicate line. TL was measured as the distance from the anterior margin of the thorax to the posterior tip of the scutellum from the dorsal view using an ocular micrometer at ×40 magnification.

Resistance to extreme temperatures and starvation

To measure chill-coma recovery (CCR; David *et al.*, 1998), 4-day-old experimental flies were sexed after a cold treatment of 10 min at 0 °C (flies remained temporally incapacitated by this cold shock), immediately transferred to empty vials, and placed for 20 h inside a cold chamber containing melting ice (0 °C) within a cold room at 4 °C. After 20 h, individuals were returned to 25 °C. CCR time (s) was measured by scoring the time until an individual was able to stand on its legs (Norry *et al.*, 2008). Heat-stress resistance was measured as knockdown time at high temperature (KRHT; Huey *et al.*, 1992) using a 10 × 67-cm knockdown tube in G15. Four-day-old experimental flies were released within the knockdown tube at 37 ± 0.5 °C. KRHT was scored every 30 s using a collecting vial which was replaced every 30 s until the last fly was knocked down by heat. Sample size was in the range 40–60 flies per replicate line for both KRHT and CCR. Starvation resistance (SR) was measured for 1-day-old adult flies in G15. Four shell vials each containing 3 mL of agar as the only medium were set up at 25 °C, with ten males plus ten females per vial and replicate line. SR was scored every day, three times a day (at 08.00 h, 14.00 h and 20.00 h), as the number of dead individuals, until the last fly died.

Statistical analysis

To robustly test for both direct and correlated responses to longevity selection (Norry & Loeschcke, 2002a), nested analysis of variance (ANOVA) was performed for each trait using selection regime (L versus C) and sex (except for fecundity) as fixed factors and replicate within selection regime as a random factor.

RESULTS

The response to selection for increased longevity was significant in both sexes because L flies lived generally longer than C flies for all replicated lines (Figs 1, 2, Tables 1, 2). On average, mean longevity in the pooled L line was 15.4% and 10.2% higher than in pooled C line for males and females, respectively. Males lived longer than females in both L and C lines (Tables 1, 2). By contrast to mean longevity, mortality parameters were replicate-specific (Table 3). Nevertheless, a trend was apparent for baseline mortality (*a*-parameter), with L males exhibiting a reduced rate of baseline mortality relative to C males (Table 3).

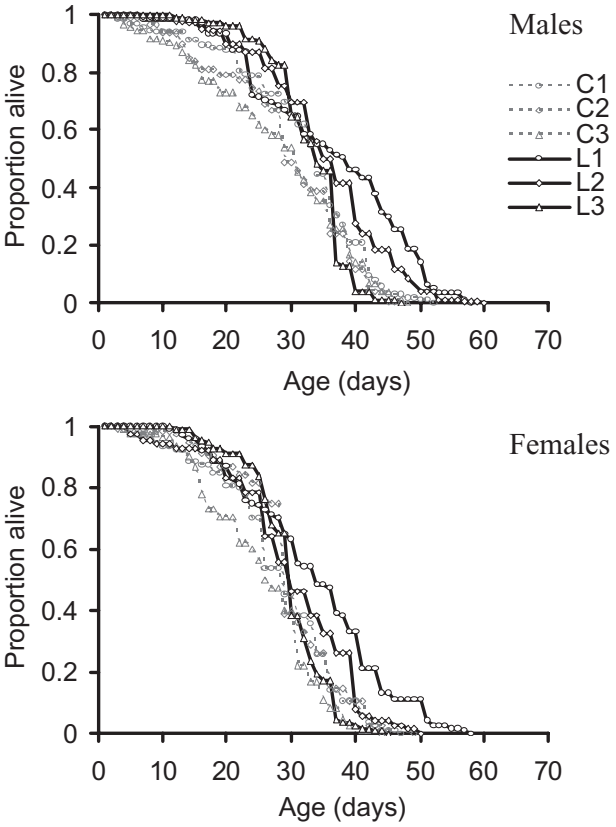

Figure 1. Survival curves for males and females are shown for control (C, grey dashed lines) and longevity-selected (L, black solid lines) replicate lines.

The results further suggest significant differentiation in the demographic rate of senescence when comparing the longevity-selected (L) line versus other populations along an altitudinal cline, including the laboratory-reared base population at G7 (Fig. 2, Table 4). Specifically, the senescence rate (*b*-parameter) was significantly lower for the L line at G15 than for other populations in G7 (Fig. 2; see comparisons in Table 4). In addition, senescence rate was either unchanged (males) or even increased rather than reduced (females) in the C line (Table 4).

EF decreased by our longevity selection regime. As expected from the antagonistic pleiotropy model, EF

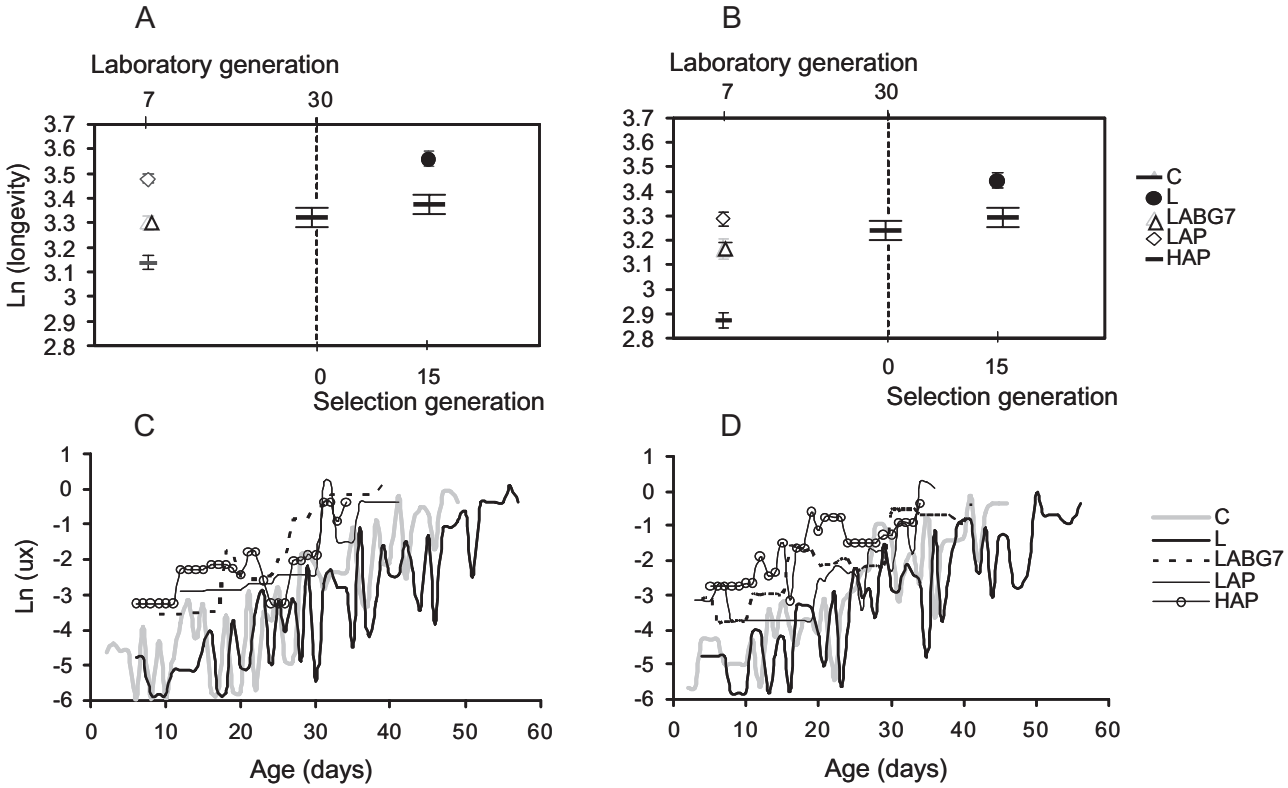

Figure 2. Mean ± SE longevity and age-specific mortality (u_x) are shown for putative comparisons between populations: LABG7 is the laboratory-reared base population at G7 (i.e. 23 generations before the start of the selection program); C is the control line averaged over replicates, L is the longevity-selected line averaged over replicates; LAP (low altitude population) is the population showing the highest longevity in an altitudinal cline from the same geographic region from where the base population in the present study was derived (Norry *et al.*, 2006); HAP (high altitude population) is the population showing the lowest longevity in the above mentioned altitudinal cline (Norry *et al.*, 2006). Longevity data are given in the same (ln-) scale as used for analyses. Error bars correspond to the SEM. A, C, males; B, D, females.

Table 1. Ln (mean ± SE longevity) is shown for males and females in control (C) and longevity-selected (L) lines after 14 generations of longevity selection

Number of replicate line	Males		Females	
	C	L	C	L
1	3.37 (0.05)	3.52 (0.04)	3.26 (0.05)	3.45 (0.03)
2	3.29 (0.04)	3.52 (0.03)	3.33 (0.03)	3.34 (0.04)
3	3.21 (0.05)	3.48 (0.02)	3.17 (0.05)	3.37 (0.02)
	3.40 (0.05)	**3.56 (0.03)**	**3.33 (0.03)**	**3.44 (0.04)**

Bold values represent the mean values for lines pooled over replicates.

was substantially lower in the longer-lived (L) than in the shorter-lived (C) lines (Fig. 3A). On average, longevity selection decreased EF from 51.3 to 35.5 per capita eggs produced per female during the first week of adult life (a 30.8% of reduction in EF of L relative to C lines).

DT increased by longevity selection because DT in the L selected line was 14.5 h and 17.3 h longer than in C line, for males and females, respectively (Fig. 3B, Table 2). In addition, DT was positively correlated with mean longevity in both sexes (Spearman rank correlation between mean traits across all L and C lines: $r_s = 0.88$, $P = 0.018$; Fig. 3B). Females showed shorter DT than males in both L and C lines (Fig. 3B). Thorax length did not differ between L and C lines (Fig. 3C; ANOVA not shown).

Table 2. Analyses of variance on ln (longevity), developmental time, early fecundity, knockdown resistance to high temperature, chill-coma recovery and starvation resistance performed to test for effects of (1) longevity-selection treatment (i.e. L versus C lines) as a fixed factor; (2) replicate within (1) as a random factor; (3) sex as a fixed factor; and all (1) × (3) and (2) × (3) respective interactions

Source of variation	d.f.	MS	*F*	d.f.	MS	*F*	d.f.	MS	*F*
	Longevity			Developmental time			Early fecundity		
(1) Selection treatment	1	10.7	14.6*	1	290069	28.2**	1	3585	9.1*
(2) Replicate within (1)	4	0.7	4.0**	4	10279	6.1***	4	394	4.2**
(3) Sex	1	2.0	7.2*	1	4455	8.2*			
(1) × (3)	1	0.7	2.4	1	1602	2.9			
(2) × (3)	4	0.3	1.5	4	544	0.3			
Within	1424	0.2		703	1675		54	93	
	Knockdown resistance			Chill-coma recovery			Starvation resistance		
(1) Selection treatment	1	57511796	7.8*	1	150346880	7.3*	1	74692	1.3
(2) Replicate within (1)	4	7328847	1.8	4	20469368	6.1***	4	59143	116.0***
(3) Sex	1	155009120	39.6**	1	302732	0.1	1	18452	32.0**
(1) × (3)	1	45814824	11.7*	1	2177417	0.5	1	531	0.9
(2) × (3)	4	3916677	0.9	4	5453611	1.6	4	576	1.1
Within	678	4145918		504	3322206		413	510	

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
d.f., degrees of freedom.

Table 3. Mortality parameters as estimated from the Gompertz model are shown for males and females in control (C) and longevity-selected (L) lines

Mortality parameters; number of replicate line	Males			Females		
	C	L	χ^2	C	L	χ^2
a-parameter						
1	0.00162	0.00206		0.00256	0.00278	
2	0.00238	0.00158		0.00158	0.00224	
3	0.00432	0.00015	44.2***	0.00338	0.00053	13.0**
	0.00271	**0.00177**	**3.5**†	**0.00243**	**0.00281**	
b-parameter						
1	0.11794	0.09035	5.0*	0.11874	0.09019	5.1*
2	0.11465	0.10502		0.13246	0.11380	
3	0.09330	0.19999	46.0***	0.12201	0.17837	10.2**
	0.10687	**0.10215**		**0.12368**	**0.10108**	**9.0****

†$P = 0.06$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
Bold values were estimated from data pooled across replicated lines. All estimates were obtained via maximum likelihood. χ^2 values (d.f. = 1) are shown only for significant comparisons between selection regime (L versus C) within each sex and replicate as based on a likelihood ratio test (each χ^2 value is twice the difference in the log-likelihood).

Thermotolerance traits exhibited positive correlated responses to longevity selection. L lines were more resistant to cold stress than C lines because CCR was shorter in L than in C flies (Table 2). Besides, CCR time was negatively correlated with longevity in males (Spearman rank correlation between mean traits across all L and C replicated lines: $r_s = -0.83$, $P = 0.041$; Fig. 4A). Regarding KRHT, a significant increase in heat-stress resistance was also found as a correlated response to longevity selection but only in females (Fig. 4B, Table 2). Longevity selection resulted in an apparent sexual dimorphism for KRHT because females were more heat resistant than males in L but not in C lines [simple-effect analysis using the appropriate MS of error from the ANOVA of Table 2: $F_{1,4} = 52.9$ ($P < 0.01$) for L;

Table 4. Mortality parameters as estimated from the Gompertz model are shown for males and females in our experimental populations

Sex and mortality parameters	Comparison 1			Comparison 2			Comparison 3		
	LABG7	C	χ^2	HAP	L	χ^2	LAP	L	χ^2
Males									
a-parameter	0.00486	0.00271		0.00240	0.00177		0.00380	0.00177	
b-parameter	0.09914	0.10687		0.16102	0.10215	10.16**	0.17555	0.10215	6.48*
Females									
a-parameter	0.00999	0.00243	13.84**	0.01284	0.00281	16.83**	0.00172	0.00281	
b-parameter	0.07827	0.12368	11.70**	0.11001	0.10108		0.14458	0.10108	5.52*

*$P < 0.05$; **$P < 0.01$.

Population abbreviations are as in Fig. 2. Comparison 1 refers to changes in mortality parameters within a single population. Comparisons 2 and 3 refer to differences between the longevity-selected L line and the two extreme populations from an implicated cline in longevity mentioned in Fig. 2 (Norry *et al.*, 2006). Comparisons between C and L are given in Table 3. All estimates were obtained via maximum likelihood. χ^2 values (d.f. = 1) are shown only for significant comparisons within each sex as based on a likelihood ratio test (χ^2 value is twice the difference in the log-likelihood).

$F_{1,4} = 2.6$ for C; Fig. 4B]. Finally, L individuals appeared to be more tolerant to starvation stress than C flies (Fig. 4C), but the difference was nonsignificant (Table 2). Females showed higher starvation resistance than males (Fig. 4C, Table 2).

DISCUSSION

Longevity is a genetically variable trait in *D. buzzatii* because artificial selection on longevity was successful in producing a significant response in the present study. On average, L females and males lived 3 and 5 days longer than C females and males, respectively (Table 1). Longevity selection tended to decrease the baseline mortality (*a*-parameter) but the senescence rate (*b*-parameter) did not consistently change by selection. This result for mortality parameters is consistent with observations in *D. melanogaster* because the increased life span of L-selected lines resulted, at least partially, from a reduction in baseline mortality rate (Pletcher, Khazaeli & Curtsinger, 2000).

Interestingly, age-specific mortality was compared between this and previous studies for populations from the same geographical region. Longevity was measured not only at the end of the experiment but also before the set up of L and C lines (Norry *et al.*, 2006). We verified that longevity, when assessed in terms of senescence rate, increased by selection in L lines not only when compared with C lines (Table 1), but also when compared with other populations along the already mentioned, altitudinal cline previously studied (Fig. 2, Table 4). These results strongly suggests that the selection program followed in the present study was successful in increasing longevity in L lines rather than reducing life span in C lines. In addition, the senescence rate was either unchanged (males) or even increased rather than reduced (females) in the C line (Table 4).

Several studies in *D. melanogaster* have shown that exposure to males (in terms of egg production and mating) has a cost in longevity for females (Partridge *et al.*, 1986; Fowler & Partridge, 1989; Chapman *et al.*, 1995). In the present study, males lived longer than females both in L and C lines, as is also often observed in other *Drosophila* species in mixed-sex environment at benign temperature (Khazaeli, Xiu & Curtsinger, 1995; Zwaan *et al.*, 1995a; Promislow *et al.*, 1996; Curtsinger & Khazaeli, 2002; Norry & Loeschcke, 2002a, b). This sex dimorphism appeared to be 5% higher in our L lines than in C lines, although this variation was not significant at the 0.05 level (Kruskal–Wallis test, $P = 0.09$).

Longevity was found to be antagonistically related to early fecundity in studies on artificial selection in *D. melanogaster* (Rose, 1984; Service & Rose, 1985; Service, Hutchinson & Rose, 1988; Roper, Pignatelly & Partridge, 1993; Leroi *et al.*, 1994; Promislow, 1995). In *D. buzzatii*, a trade-off association between longevity and fecundity was found from inter-specific patterns of variation, when comparing several sympatric populations of *D. buzzatii* and its sibling species *Drosophila koepferae* (Sambucetti *et al.*, 2005). In the present study with *D. buzzatii*, we also found that early fecundity decreased as a consequence of selection for increased longevity, with L lines showing lower fecundity at early age (Fig. 3A). This change in early fecundity was significant between L and C lines, with pilot studies suggesting that there was no sub-

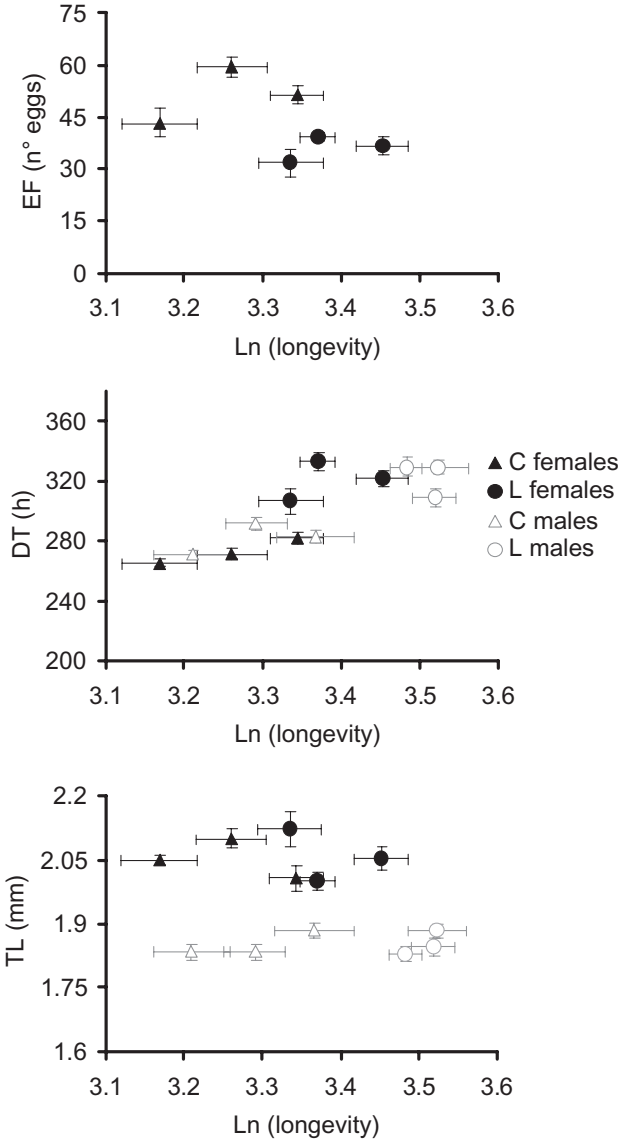

Figure 3. Mean values for three life-history traits are plotted against ln (mean longevity) for males (grey symbols) and females (black symbols) from each control (triangles) and longevity-selected (circles) line. A, early fecundity (EF, in number of eggs); B, developmental time (DT, in h); C, thorax length (TL, in mm). Data for longevity are the same as in Table 1. Error bars correspond to the SEM.

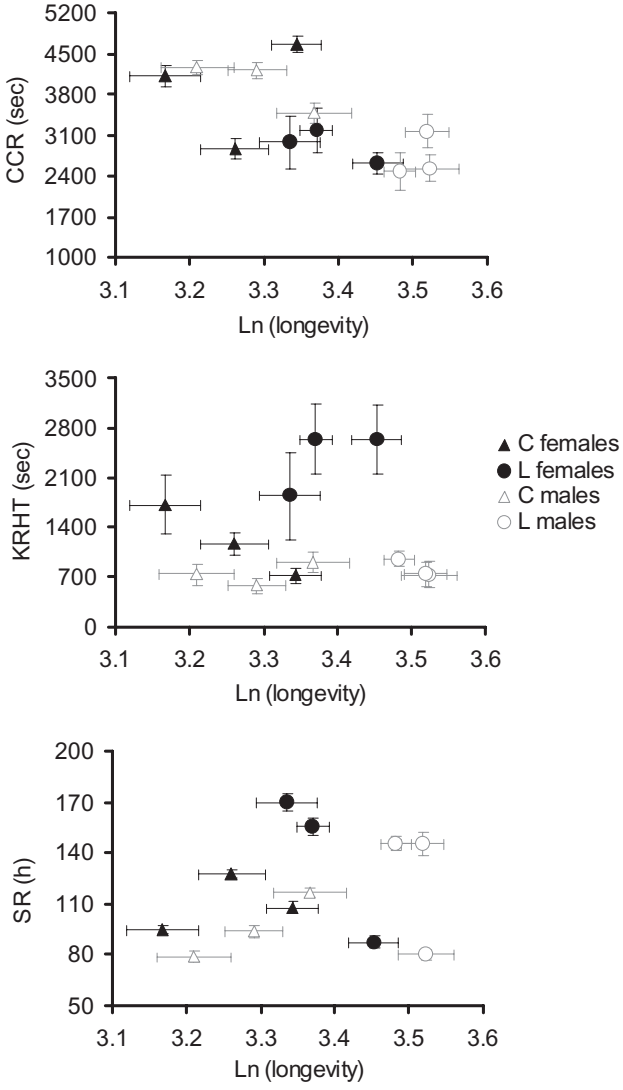

Figure 4. Mean values for three stress-resistance traits are plotted against ln (mean longevity) for males (grey symbols) and females (black symbols) from each control (triangles) and longevity-selected (circles) line. A, chill-coma recovery (CCR; s); B, knockdown resistance to high temperature (KRHT; s); (C) starvation resistance (SR; h). Error bars correspond to the SEM.

stantial change in male virility (maturity) (for related issues, see Barker & Fredline, 1985). Importantly, this pattern of experimental evolution in *D. buzzatii* is largely consistent with trade-off associations that were previously apparent between longevity and early fecundity for *D. buzzatii* along an altitudinal gradient in Argentina (Norry *et al.*, 2006). Highland populations of *D. buzzatii* partly evolved for both reduced longevity and increased early fecundity at benign temperature (Norry *et al.*, 2006). Therefore, early fecundity and longevity at benign temperature appear to be negatively correlated in *D. buzzatii* with respect to both experimental and natural evolutionary patterns (present study; Norry *et al.*, 2006).

DT is another fitness-related trait that dramatically diverged by longevity selection because DT was approximately 14–17 h longer in L than in C lines. Positive correlations between longevity and developmental time were also found in *D. melanogaster*

under diverse regimes of artificial selection (Stearns *et al.*, 2000; Vermeulen & Bijlsma, 2006), although some populations showed no correlation between the traits (Zwaan *et al.*, 1995b). The present results are consistent with the hypothesis that longevity can often be antagonistically related not only to fecundity but also to DT (Promislow & Bugbee, 2000; Stearns *et al.*, 2000; Soto *et al.*, 2006). Although DT responded to longevity selection, body size did not change as a correlated response to longevity selection in the present study. Indeed, longevity is not always positively correlated with body size in *Drosophila* (Norry & Loeschcke, 2002b; Bochdanovits & De Jong, 2003; Yadav & Singh, 2007). The correlated selection response of developmental time rather than body size itself is also consistent with observations from artificial selection on longevity-related traits in *D. melanogaster* (Hillesheim & Steams, 1992; Partridge & Mangel, 1999). Furthermore, Sambucetti *et al.* (2006) found no trade-off association between DT and body size in *D. buzzatii* along an altitudinal cline in DT for the same geographical region from where the population used in the present study originates; see also Sarup *et al.* (2006). Although DT is antagonistically related to body size in *D. buzzatii* (Cortese *et al.*, 2002), the results obtained in the present study indicate that these traits do not always show a trade-off association in studies of experimental evolution.

Another phenotype that generally correlated with longevity in previous studies in *D. melanogaster* was stress resistance (Luckinbill, 1998; Partridge & Mangel, 1999; Rose, 1999; Stearns *et al.*, 2000; Clancy *et al.*, 2002; Norry & Loeschcke, 2003; Bubliy & Loeschcke, 2005; Baldal *et al.*, 2006; Nobuhito & Kimura, 2008). Similar correlations were also significant in *D. buzzatii* (Scannapieco *et al.*, 2007; Gomez *et al.*, 2009). In the present study, L females were more tolerant to heat stress than C females, and longevity selection increased not only heat resistance, but also tolerance to cold stress (Fig. 4A, B). However, we found no consistent response to longevity selection for starvation resistance. Therefore, given that not all stress traits (e.g. starvation versus thermotolerance) were evenly affected by longevity selection in the present study, the results support the hypothesis that there is no generalized mechanism underlying increased stress resistance in long-lived individuals (Rose *et al.*, 1992; Bubliy & Loeschcke, 2005; Jørgensen, Sørensen & Bundgaard, 2006).

The present study confirms that genetic variation in longevity is segregating in *D. buzzatii*, as suggested by earlier work in this species (Norry *et al.*, 1995; Rodriguez, Fanara & Hasson, 1999). Correlations between traits, as inferred from correlated selection responses, were not different from previously reported clinal patterns of trait associations and were generally consistent not only with predictions from evolutionary theories of senescence, but also with previous findings in other species.

ACKNOWLEDGEMENTS

We wish to thank Volver Loeschcke for helpful comments on an earlier version of the manuscript. This research was supported by grants from the, Agencia Nacional de Promociòn Cientìfica y Tècnica, CONICET-Argentina, University of Buenos Aires and Fundación Antorchas to F.M.N.

REFERENCES

Baldal AE, Brakefield PM, Zwaan BJ. 2006. Multitrait evolution in lines of *Drosophila melanogaster* selected for starvation resistance: the role of metabolic rates and implications for the evolution of longevity. *Evolution* **60:** 1435–1444.

Barker JSF, Fredline DK. 1985. Reproductive biology of *Drosophila huzzatii*. *Drosophila Information Service* **61:** 28–32.

Bochdanovits Z, De Jong G. 2003. Temperature dependence of fitness components in geographical populations of *Drosophila melanogaster*: changing the association between size and fitness. *Biological Journal of the Linnean Society* **80:** 717–725.

Bubliy OA, Loeschcke V. 2005. Correlated responses to selection for stress resistance and longevity in a laboratory population of *Drosophila melanogaster*. *Journal of Evolutionary Biology* **18:** 789–803.

Chapman T, Liddle LF, Kalb JM, Wolfner MF, Partridge L. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373:** 241–244.

Clancy DJ, Gems D, Hafen E, Leevers SJ, Partridge L. 2002. Dietary restriction in long-lived dwarf flies. *Science* **296:** 319–319.

Cortese MD, Norry FM, Piccinali R, Hasson E. 2002. Direct and correlated responses to artificial selection on developmental time and wing length in *Drosophila buzzatii*. *Evolution* **56:** 2541–2547.

Curtsinger JW, Khazaeli AA. 2002. Lifespan, QTLs, age-specificity, and pleiotropy in *Drosophila*. *Mechanisms of Ageing and Development* **123:** 81–93.

David RJ, Gibert P, Pla E, Petavy G, Karan D, Moreteau B. 1998. Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *Journal of Thermal Biology* **23:** 291–299.

Elandt-Johnson R, Johnson NL. 1980. *Survival models and data analysis*. New York, NY: Wiley.

Fanara JJ, Hasson E. 2001. Oviposition acceptance and fecundity schedule in the cactophilic sibling species *Drosophila buzzatii* and *D. koepferae* on their natural hosts. *Evolution* **55:** 2615–2619.

Finch CE. 1990. *Longevity, senescence and the genome*. Chicago, IL: University of Chicago Press.

Fowler K, Partridge L. 1989. A cost of mating in females fruitflies. *Nature* **338:** 760–761.

Gomez FH, Bertoli CI, Sambucetti P, Scannapieco AC, Norry FM. 2009. Heat-induced hormesis in longevity as correlated response to thermal-stress selection in *Drosophila buzzatii*. *Journal of Thermal Biology* **34:** 17–22.

Harshman LG, Haberer BA. 2000. Oxidative stress resistance: a robust correlated response to selection in extended longevity lines of *Drosophila melanogaster*? *Journal of Gerontology: Biological Sciences and Medical Sciences* **55:** B415–417.

Harshman LG, Hoffmann AA. 2000. Laboratory selection experiments using *Drosophila*: what do they really tell us? *Trends in Ecology and Evolution* **15:** 32–36.

Hillesheim E, Steams SC. 1992. Correlated responses in life-history traits to artificial selection for body weight in *Drosophila melanogaster*. *Evolution* **46:** 745–752.

Huey RB, Crill WD, Kingsolver JG, Weber KE. 1992. A method for rapid measurement of heat or cold resistance of small insects. *Functional Ecology* **6:** 489–494.

Huey RB, Wakefield T, Crill WD, Gilchrist GW. 1995. Within-generation and between effects of temperature on early fecundity of *Drosophila melanogaster*. *Heredity* **74:** 216–223.

Jørgensen KT, Sørensen JG, Bundgaard J. 2006. Heat tolerance and the effect of mild heat stress on reproductive characters in *Drosophila melanogaster*. *Journal of Thermal Biology* **31:** 280–286.

Khazaeli AA, Xiu L, Curtsinger JW. 1995. Effect of adult cohort density on age-specific mortality in *Drosophila melanogaster*. *Journal of Gerontology: Biological Sciences and Medical Sciences* **50A:** B262–269.

Kirkwood TB, Austad SN. 2000. Why do we age? *Nature* **408:** 233–238.

Leroi AM, Chippindale AK, Rose MR. 1994. Long-term laboratory evolution of a genetic life-history trade-off in *Drosophila melanogaster*. 1. The role of genotype-by-environment interaction. *Evolution* **48:** 1244–1257.

Luckinbill LS. 1998. Selection for longevity confers resistance to low-temperature stress in *Drosophila melanogaster*. *Journal of Gerontology: Biological Sciences and Medical Sciences* **53A:** B147–153.

Medawar PB. 1952. An unsolved problem of biology. In: Medawar PB, eds. *The uniqueness of the individual*. New York, NY: Dover, 28–55.

Miyatake T. 1997. Genetic trade-off between early fecundity and longevity in *Bactrocera cucurbitae* (Diptera: Tephritidae). *Heredity* **78:** 93–100.

Nobuhito M, Kimura MT. 2008. Selection for rapid and slow recovery from chill- and heat-coma in *Drosophila melanogaster*. *Biological Journal of the Linnean Society* **95:** 72–80.

Norry FM, Loeschcke V. 2002a. Longevity and resistance to cold stress in cold-stress selected lines and their controls in *Drosophila melanogaster*. *Journal of Evolutionary Biology* **15:** 775–783.

Norry FM, Loeschcke V. 2002b. Temperature-induced shifts in associations of longevity with body size in *Drosophila melanogaster*. *Evolution* **56:** 299–306.

Norry FM, Loeschcke V. 2003. Heat-induced expression of a molecular chaperone decreases by selecting for long-lived individuals. *Experimental Gerontology* **38:** 673–681.

Norry FM, Sambucetti P, Scannapieco AC, Loeschcke V. 2006. Altitudinal patterns for longevity, fecundity and senescence in *Drosophila buzzatii*. *Genetica* **128:** 81–93.

Norry FM, Scannapieco AC, Sambucetti P, Bertoli CI, Loeschcke V. 2008. QTL for the thermotolerance effect of heat hardening, knockdown resistance to heat and chill-coma recovery in an intercontinental set of recombinant inbred lines of *Drosophila melanogaster*. *Molecular Ecology* **17:** 4570–4581.

Norry FM, Vilardi JC, Fanara JJ, Hasson E, Rodriguez C. 1995. An adaptive chromosomal polymorphism affecting size-related traits, and longevity selection in a natural population of *Drosophila buzzatii*. *Genetica* **96:** 285–291.

Partridge L, Fowler K. 1992. Direct and correlated responses to selection on age at reproduction in *Drosophila melanogaster*. *Evolution* **47:** 213–226.

Partridge L, Fowler K, Trevit S, Sharp W. 1986. An examination of the effects of males on the survival and egg-production rates of females *Drosophila melanogaster*. *Journal of Insect Physiology* **32:** 925–929.

Partridge L, Mangel M. 1999. Messages from mortality: the evolution of death rates in the old. *Trends in Ecology and Evolution* **14:** 438–442.

Pletcher SD. 1999. Model fitting and hypothesis testing for age–specific mortality data. *Journal of Evolutionary Biology* **12:** 430–439.

Pletcher SD, Khazaeli AA, Curtsinger JW. 2000. Why do life spans differ? Partitioning mean longevity differences in terms of age-specific mortality parameters. *Journal of Gerontology: Biological Sciences and Medical Sciences* **55A:** B381–389.

Prasad NG, Joshi A. 2003. What have two decades of laboratory life-history evolution studies on *Drosophila melanogaster* taught us? *Journal of Genetics* **82:** 45–76.

Promislow DEL. 1991. Senescence in natural populations of mammals: a comparative study. *Evolution* **45:** 1869–1887.

Promislow DEL. 1995. New perspectives on comparative tests of antagonistic pleiotropy using *Drosophila*. *Evolution* **49:** 394–397.

Promislow DEL, Bugbee M. 2000. Direct and correlated responses to selection on age at physiological maturity in *Drosophila simulans*. *Journal of Evoutionary Biology* **13:** 955–966.

Promislow DEL, Tatar M, Khazaeli AA, Curtsinger JW. 1996. Age-specific patterns genetic variation in *Drosophila melanogaster*. I. Mortality. *Genetics* **143:** 839–848.

Rodriguez C, Fanara JJ, Hasson E. 1999. Inversion polymorphism, longevity, and body size in natural population of *Drosophila buzzatii*. *Evolution* **53:** 612–620.

Roper C, Pignatelly P, Partridge L. 1993. Evolutionary effects of selection on age at reproduction in larval and adult *Drosophila melanogaster*. *Evolution* **47:** 445–455.

Rose MR. 1984. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. *Evolution* **38:** 1004–1010.

Rose MR. 1991. *Evolutionary biology of ageing*. Oxford: Oxford University Press.

Rose MR. 1999. Genetics of aging in *Drosophila*. *Experimental Gerontology* **34:** 577–585.

Rose MR, Charlesworth B. 1980. A test of evolutionary theories of senescence. *Nature* **287:** 141–142.

Rose MR, Charlesworth B. 1981. Genetics of life-history evolution in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics* **97:** 187–196.

Rose MR, Vu LN, Park SU, Graves JL. 1992. Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Experimental Gerontology* **27:** 241–250.

Sambucetti P, Loeschcke V, Norry FM. 2006. Developmental time and size-related traits in *Drosophila buzzatii* along an altitudinal gradient from Argentina. *Hereditas* **143:** 77–83.

Sambucetti P, Sørensen JG, Loeschcke V, Norry FM. 2005. Variation in senescence and associated traits between sympatric cactophilic sibling species of *Drosophila*. *Evolutionary Ecology Research* **7:** 915–930.

Sarup P, Sorensen JG, Dimitrov K, Barker JSF, Loeschcke V. 2006. Climatic adaptation of *Drosophila buzzatii* populations in southeast Australia. *Heredity* **96:** 479–486.

Scannapieco AC, Sørensen JG, Loeschcke V, Norry FM. 2007. Heat-induced hormesis in longevity of two sibling *Drosophila* species. *Biogerontology* **8:** 315–325.

Service PM, Hutchinson EW, Rose MR. 1988. Multiple genetic mechanisms for the evolution of senescence in *Drosophila melanogaster*. *Evolution* **42:** 708–716.

Service PM, Rose MR. 1985. Genetic covariation among life-history components – the effect of novel environments. *Evolution* **39:** 943–945.

Sgrò CM, Partridge L. 2000. Evolutionary responses of life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. *The American Naturalist* **156:** 341–353.

Sorensen JG, Norry FM, Scannapieco AC, Loeschcke V. 2005. Altitudinal variation for stress resistance traits and thermal adaptation in adult *Drosophila buzzatii* from the New World. *Journal of Evolutionary Biology* **18:** 829–837.

Soto I, Cortese M, Carreira V, Folguera G, Hasson E. 2006. Longevity differences among lines artificially selected for developmental time and wing length in *Drosophila buzzatii*. *Genetica* **127:** 199–206.

Stearns SC, Ackermann M, Doebeli M, Kaiser M. 2000. Experimental evolution of aging, growth, and reproduction in fruitflies. *Proceedings of the National Academy of Sciences of the United States of America* **97:** 3309–3313.

Vermeulen CJ, Bijlsma R. 2006. Changes in the genetic architecture during relaxation in *Drosophila melanogaster* on divergent virgin life span. *Journal of Evolutionary Biology* **19:** 216–227.

Williams GC. 1957. Pleiotropy, natural selection and the evolution of senescence. *Evolution* **11:** 398–411.

Yadav JP, Singh BN. 2007. Evolutionary genetics of *Drosophila ananassae*: evidence for trade-offs among several fitness traits. *Biological Journal of the Linnean Society* **90:** 669–685.

Zwaan B, Bijlsma R, Hoekstra RG. 1995a. Direct selection on life-span in *Drosophila melanogaster*. *Evolution* **49:** 649–659.

Zwaan B, Bijlsma R, Hoekstra RF. 1995b. Artificial selection for developmental time in *Drosophila melanogaster* in relation to the evolution of aging – direct and correlated responses. *Evolution* **49:** 635–648.