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Variation in senescence and associated traits between sympatric cactophilic sibling species of *Drosophila*

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ABSTRACT

Main hypothesis: Longevity and early fecundity are negatively correlated both within and between sympatric but ecologically divergent sibling species that differ in life span, as predicted by evolutionary theories of ageing.

Organism: *Drosophila koepferae*, which mainly breeds on the patchily distributed *Trychocereus* cacti, and *D. buzzatii*, which mainly occurs on the more abundant and uniformly distributed *Opuntia* cacti.

Methods: Flies were reared under standardized conditions from three sympatric populations. Longevity and mortality rate were studied both at 25°C and 29.5°C. Age-specific fecundity was estimated at 25°C. Other plausibly associated traits were also studied, including heat-shock resistance, Hsp70 expression and body size.

Conclusions: Senescence rate was much faster in *D. koepferae* than in *D. buzzatii* in all populations at 25°C but not at 29.5°C. The interspecific difference in longevity at 25°C shifted in sign in one of the populations at 29.5°C. Neither the resistance to a heat shock nor the heat-induced Hsp70 expression showed significant interspecific variation. Body size was larger in *D. koepferae* than in *D. buzzatii*. *Drosophila koepferae* showed a massive reproductive output at early ages compared with *D. buzzatii*. Longevity and early fecundity at the non-stressful temperature (25°C) were negatively correlated both within and between species. Genotype × temperature interactions can in turn affect interspecific variation in evolutionary trajectories of both mean longevity and demographic rate of senescence.

Keywords: age-specific senescence rate, body size, *Drosophila*, early fecundity, genotype × temperature interaction, heat-stress resistance, Hsp70 expression.

INTRODUCTION

Senescence is the progressive increase in probability of death as well as the progressive decline in reproductive fitness with advancing age. For most wild species, this process is

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unlikely to contribute strongly to mortality because mortality in the wild may be mainly due to extrinsic causes, such as cold, heat, starvation, predation and/or infection at rather young ages. As a result of such causes of extrinsic mortality, with adult ageing there is a decline in the force of natural selection acting on survival itself. In particular, senescence can evolve as a result of (1) an accumulation of late-acting deleterious mutations [the mutation accumulation theory (Medawar, 1952)] and/or (2) an adaptive increase in frequency of alleles with late deleterious but early beneficial effects [the antagonistic pleiotropy theory (Williams, 1957)].

Empirical support for evolutionary ageing theories has been documented in experimental populations of model organisms including *Drosophila* (for reviews, see Finch, 1990; Rose, 1991, 1999; Partridge and Mangel, 1999; Kirkwood and Austad, 2000). An important conclusion of these studies is that all evolutionary theories of senescence can be valid at the same time, in the same species (Partridge and Mangel, 1999; Rose, 1999). In *Drosophila*, laboratory-selected populations have been used to test for changes in longevity and its correlates in fecundity after restricting reproduction to later ages [i.e. after the intensity of selection on the maximal life span was increased (e.g. Rose, 1984; Partridge and Fowler, 1992; Roper *et al.*, 1993; Zwaan *et al.*, 1995; Partridge *et al.*, 1999)]. A general result of these studies was that the age-selected lines consistently increased in mean longevity relative to their controls, and a general correlate of this selection response has been reduced fecundity in the long-lived flies (Partridge and Mangel, 1999; Rose, 1999). These general findings from experimental evolution in *Drosophila* are consistent with the pleiotropy and disposable soma theories of senescence (Kirkwood and Austad, 2000). Comparative biology offers an alternative to artificial selection to test evolutionary hypotheses on ageing (reviewed in Rose, 1991), especially in the *Drosophila* model since cultural artifacts can potentially be responsible for any trade-offs between longevity and fecundity in experimental evolution studies (Linnen *et al.*, 2001). Specifically, closely related species or populations that differ in their ecology in a way that is likely to affect their ageing can be used to determine whether interspecific differences in senescence patterns are consistent with evolutionary theories of ageing (e.g. Schnebel and Grossfield, 1983, 1988; Promislow, 1995; Tatar *et al.*, 1997a; Promislow and Haselkorn, 2002; Dudycha, 2003; Fox *et al.*, 2003; Reznick *et al.*, 2004). For instance, when a harsh environment persists for a given species, evolutionary theory predicts that it should lead to decreased longevity and higher reproductive effort early in life relative to other closely related species that experience more benign environments.

Drosophila buzzatii and *D. koepferae* are two cactophilic sibling species whose phytogeographical distribution partially overlaps in north-western and western Argentina. These two closely related species provide an interesting model to explore interspecific patterns of evolutionary differentiation in longevity and fecundity. The two species can utilize the decaying tissues of both *Opuntia* and columnar cacti hosts (hereafter rots). *Drosophila buzzatii* occurs in several phytogeographical areas from low to high altitudes of Argentina and Brazil, whereas *D. koepferae* shows a much more restricted distribution at moderate to high elevations of Argentina as well as the Bolivian Altiplano (e.g. Fontdevila *et al.*, 1988; Hasson *et al.*, 1992; Fanara *et al.*, 1999; Norry *et al.*, 2000). In spite of some niche overlap, *D. buzzatii* is largely associated with rots of the genus *Opuntia* (subfamily Opuntioideae), whereas *D. koepferae* breeds primarily on the rotting stems of columnar cacti of the genera *Cereus* and *Trichocereus*, subfamily Ceroideae (Hasson *et al.*, 1992; Fanara *et al.*, 1999). As a rule, columnar cacti rots are much more patchily distributed and less ephemeral than the more abundant *Opuntia* rots in many wild populations (Heed and Mangan, 1986; Hasson *et al.*, 1992; Etges, 1993). In contrast to *D. buzzatii*, *D. koepferae* evolved to exhibit a massive reproductive output upon localization

of its breeding site at early ages (Fanara *et al.*, 1999). Therefore, a faster senescence rate in *D. koepferae* than in *D. buzzatii* could be predicted from evolutionary theories of ageing.

The main aim of the present study was to compare the cactophilic sibling species *D. buzzatii* and *D. koepferae* to establish whether their interspecific variation in longevity and fecundity is consistent with evolutionary theories of senescence. Additionally, we analysed interspecific variation in other plausibly associated traits such as heat-stress resistance, Hsp70 expression and body size. In our interspecific comparisons, we used laboratory-reared flies recently derived from three sympatric populations from Argentina where significant climatic differences persist. Several hypotheses were addressed. First, the main hypothesis was that longevity is negatively correlated with early fecundity both within and between the two closely related species studied at non-stressful temperatures, as expected from evolutionary theories of senescence. Second, interspecific comparisons of longevity were also performed at high temperature to test the hypothesis that interspecific variation in life span depends on genotype × temperature interactions (Vieira *et al.*, 2000; Norry and Loeschcke, 2002a). Third, we tested the null hypothesis that heat-shock resistance and longevity have evolved in the same direction across species, as both traits are positively and genetically correlated in artificial selection populations of *Drosophila* (Norry and Loeschcke, 2003). To address this hypothesis, we additionally explored interspecific variation in heat-induced Hsp70 expression, as this molecular chaperone can influence not only heat-stress resistance but also longevity and/or fecundity (e.g. Krebs and Loeschcke, 1994a,b; Tatar *et al.*, 1997b; Tatar, 1999; Silbermann and Tatar, 2000; Hercus *et al.*, 2003; for reviews, see Hoffmann *et al.*, 2003; Sørensen *et al.*, 2003). Finally, we tested for interspecific variation in body size as soma size could be correlated with longevity.

MATERIALS AND METHODS

Experimental stocks

Adult flies were collected over banana baits at three localities in north-western Argentina in mid-April 2003 (Table 1). Mass cultures were set up for each population using 40–100 wild-caught flies from each locality. Inseminated females of the laboratory G2 generation were transferred from mass cultures to individual vials to establish isofemale lines. *Drosophila buzzatii* and *D. koepferae* were identified by examining the male genitalia (Vilela, 1983). Twenty isofemale lines of each species were identified for each of the three sympatric populations, and used to set up a mass population per species and locality of origin. After

Table 1. Sites where the sibling *D. buzzatii* and *D. koepferae* species were sampled in sympatry in Argentina

Population and altitude (m a.s.l.)	Latitude (°)	Longitude (°)	T_{min} (°C)	T_{max} (°C)	Cactus
San Luis (709 m)	33.25	66.25	4	33	*O.p.*, *T.c.*
Cafayate (1654 m)	26.06	65.58	1	26	*O.s.*, *T.t.*
Quilmes (1855 m)	26.27	66.02	0	25	*O.s.*, *T.t.*

Note: Estimated minimum and maximum mean temperatures (T_{min} and T_{max}, see text for details) and cactus species are shown for each collection site.
Key to cactus species: *O.p.*, *Opuntia panpeana*; *O.s.*, *O. sulphurea*; *T.c.*, *T. candicans*; *T.t.*, *Trichocereus terschekii*.

seven laboratory generations (when all lines were checked for species), all isofemale lines of *D. buzzatii* and *D. koepferae* (about 20 per population) were *inter-se* crossed within each population to obtain an F2 mass population (i.e. the laboratory G9 generation) on which the study was performed for each species and locality sampled. All stocks were maintained at 25°C at a 12 h light/12 h dark cycle, with three bottles per population and 50–100 flies per bottle on instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA). Pilot studies showed that this medium is optimal for both cactophilic species studied as compared with other laboratory media, and adult life span in these species did not differ significantly between instant *Drosophila* medium and cactus-based media in a pilot assay at 25°C (results not shown).

Measurements and analyses of longevity, mortality and fecundity

First-instar larvae were collected from each mass population using small spoons with agar and yeast paste. Larvae were placed at a density of 35 in 95×20 mm shell vials containing 6 ml of culture medium (hereafter referred to as 'standard' vials). All vials were kept in a walk-in incubator at 25 ± 1°C. The eclosing adults were collected as virgins, sexed under light CO_2 anaesthesia, and used for measurement of longevity. Separately for each population, five vials each containing 10 males and 10 females were set up at each of two experimental temperatures, 25°C and 29.5°C at a 12 h light/12 h dark cycle (i.e. our initial cohort size was 50 per sex, population, species and temperature). The flies were transferred to fresh vials every day (experiment at 29.5°C) or 2 days (experiment at 25°C) 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 as possible to 20 per vial.

For analysis, longevity data (days) were $\log_e$-transformed to remove dependence of variances on means. Differences in mean longevity between *D. buzzatii* and *D. koepferae* were tested with analyses of variance using species, sex and temperature as fixed factors and population as a random factor nested within species.

Age-specific mortality rate (μ_x) was estimated as $\mu_x = -\ln(1 - q_x)$, $q_x = d_x/N_x$, where d_x is the number of flies dying in the interval x to $x + 1$ and N_x is the number of flies alive at day x (Elandt-Johnson and Johnson, 1980; Tatar *et al.*, 1997a). We tested for interspecific variation in the rate parameter (b, which is usually interpreted as the demographic rate of ageing) of the Gompertz equation only, as cohort sizes larger than ours may be necessary to adequately estimate the intercept parameter (Pletcher, 1999). All estimates of b were obtained via maximum likelihood using the WinModest software (Pletcher, 1999). Maximum likelihood theory provided straightforward significance tests for comparative analysis of each mortality parameter, which was performed with WinModest (Pletcher, 1999: http://www.hcoa.org/scott/softw-winmodest.asp).

Interspecific variation in mortality was further tested with the semi-parametric Cox's proportional hazards regression (CPHR) model (Cox, 1972). Survival data were arranged as a life-table with a censoring variable to run CPHR using the Statistica package (StatSoft, 1999). In CPHR, a baseline survival curve (i.e. the survival curve of a hypothetical 'completely average' individual) is systematically flexed up or down by each independent variable, and the method computes a coefficient (B) for each of them as well as a total chi-square statistic. To adjust for within-species variation among populations, the differences in mortality rate between species were evaluated statistically with a stratified CPHR analysis by using population within species as a classification stratum.

Fecundity was studied at 25°C with a 12 h light/12 h dark cycle. Shell vials containing a small spoon with agar and yeast paste were set up for each population and species, with one female plus two males of 1 day of age per vial and 15 vials per species and population. Number of eggs was scored from spoons every 2 days when flies were transferred to new vials with fresh spoons. This procedure was performed until the death of each female. Absolute early fecundity was estimated for each female (our observation unit) as the number of eggs laid within the first 6 days of adult age (Huey *et al.*, 1995). Additionally, relative early fecundity (Charlesworth, 1980; Promislow, 1995) was also estimated for each female as the ratio of the number of eggs laid within the first 6 days of adult age to the total number of eggs laid during her lifetime. Finally, we estimated overall fecundity for each female as the total number of eggs laid during her lifetime. For analysis, each measure of fecundity was tested with one-way analysis of variance (ANOVA), using species as a fixed factor and population within species as a random factor.

Hsp70 expression

Flies were heat-exposed in empty food vials. To prevent desiccation, the stoppers were moistened with tap water. All vials were evenly spaced in racks in preheated water baths with the water level exceeding the lower end of the stoppers, with 10 flies per vial and three replicated vials per sex and population.

Induction treatment was 38°C for 1 h followed by 1 h at 25°C for recovery before freezing at −80°C. Subsequently, flies were homogenized and the level of Hsp70 expression was assayed with an enzyme-linked immunosorbent assay (ELISA) using the monoclonal antibody 7.FB (Velazquez *et al.*, 1980, 1983), which is specific for the inducible Hsp70 in *D. buzzatii* (Sørensen *et al.*, 1999). Each replicate was run on a 96 microwell ELISA plate and consisted of a sample from each population, sex and species. All samples were measured in triplicate. The grand mean of each replicate plate was standardized to the grand mean of replicate one. The ELISA procedure is described in detail in Sørensen *et al.* (1999).

Survival against heat stress

Experimental individuals were reared under standardized conditions as above for longevity measurement and aged to 2 days at 25°C. Manipulation was carried out without anaesthesia. Tests were performed using shell vials with stoppers moistened with tap water. The vials were placed in a rack within a water bath with the water level exceeding the lower end of the stoppers. We used 20 flies per vial and six vials per population and species. Flies were hardened at 37°C for 40 min (no mortality was observed), followed by 1 h at 25°C to allow the flies to recover before being heat-shocked for 1 h at 41.5°C. After this heat stress, flies were transferred to vials with fresh food and allowed to recover at 25°C. The number of survivors to stress was scored (at 25°C) as the number of flies of each sex that could walk 24 h after the heat stress. For each sex, our observation unit was the proportion of survivors per vial.

Body size

Larvae were collected from small spoons with agar and yeast paste as described above. For each population and species, 80 larvae 1–3 h old were transferred to each of three bottles

containing 40 ml of instant *Drosophila* medium (Carolina Biological Supply, Burlington, NC, USA). Eighteen males and females were randomly chosen from the total of emerging flies for measurement of thorax length as an index of body size. Thorax length was measured as the distance between the anterior margin of the thorax and the posterior tip of the scutellum, using a Wild microscope fitted with an ocular micrometer.

RESULTS

Males lived longer than females under our experimental conditions at both non-stressful and high temperatures (Fig. 1; Table 2). Mean longevity at the non-stressful temperature (25°C) was, on average, 1.7 times longer in *D. buzzatii* than in its sibling *D. koepferae* (Fig. 1). Mean longevity showed a significant interaction between species and temperature as well as between populations (or altitude of origin of population) within species and temperature [ANOVA for ln-transformed longevity (days) with effects of (1) species, (2) population within species, (3) sex and (4) adult test temperature: (1) $F_{1,4} = 9.29$*, (2) $F_{4,957} = 5.37$***, (3) $F_{1,4} = 10.47$*, (4) $F_{1,4}$ 30.93**, (1) × (3) $F_{1,4} = 0.67$, (2) × (3) $F_{4,957} = 2.06$, (1) × (4) $F_{1,4} = 14.64$, (2) × (4) $F_{1,957} = 3.87$, (3) × (4) $F_{1,4} = 0.10$, (1) × (3) × (4) $F_{1,4} = 1.58$, (2) × (3) × (4) $F_{4,957} = 1.23$

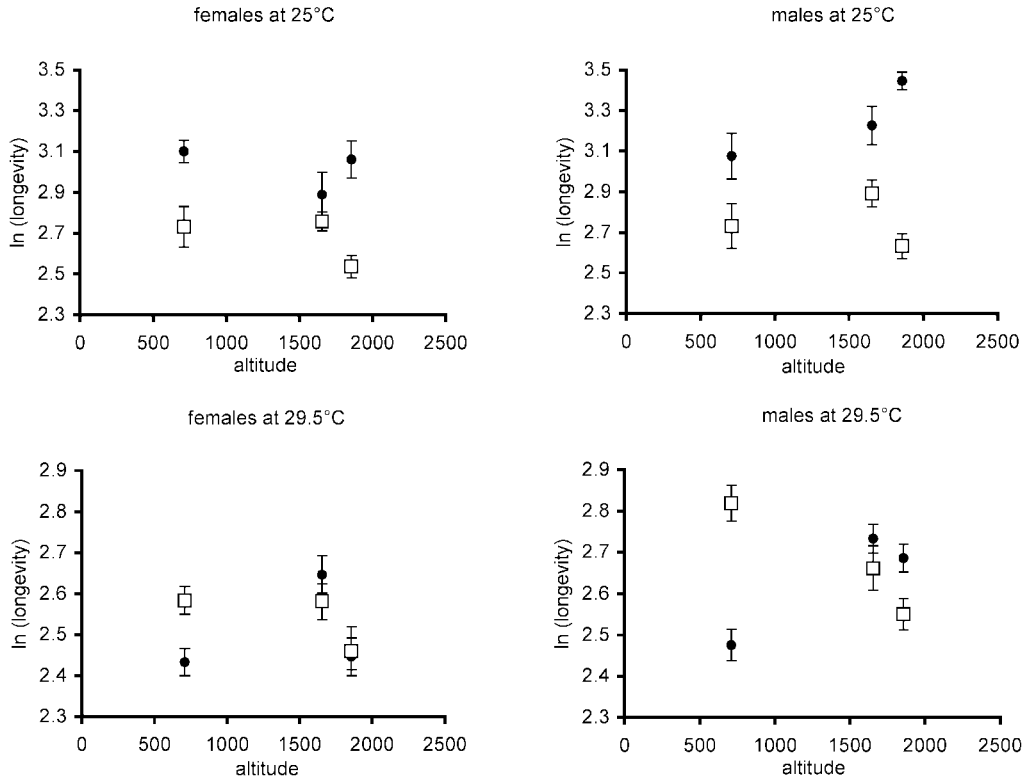

Fig. 1. Mean longevity of males and females of the sibling species *D. buzzatii* (●) and *D. koepferae* (□) at moderate (25°C) and high (29.5°C) temperatures. Data are shown for three laboratory populations each derived from sympatric wild populations originating from different altitudes as described in Table 1. Error bars indicate standard error of the mean.

Table 2. Estimated Gompertz senescence parameter *b* of *D. buzzatii* (*D.b.*) and *D. koepferae* (*D.k.*) at 25°C and 29.5°C

Population	Temp. (°C)	Females			Males		
		D.b.	*D.k.*	χ^2	*D.b.*	*D.k.*	χ^2
San Luis	25.0	0.08	0.26	18.31***	0.14	0.30	21.45***
Cafayate	25.0	0.12	0.23	16.14**	0.08	0.18	12.42*
Quilmes	25.0	0.10	0.27	17.53**	0.10	0.29	16.26**
San Luis	29.5	0.43	0.34	0.72	0.63	0.48	1.94
Cafayate	29.5	0.26	0.45	14.22*	0.21	0.57	16.23**
Quilmes	29.5	0.29	0.45	6.24	0.20	0.26	2.57

Note: All estimates were obtained via maximum likelihood. χ^2-values are shown for comparisons between species within each sex as based on a likelihood ratio test (each χ^2-value is twice the difference in the log-likelihood). *P*-values were corrected for multiple comparisons using the sequential Bonferroni technique (Rice, 1989).
$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$.

($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.005$)]. Therefore, we also performed an ANOVA for each temperature. At 25°C, the between-species difference in life span was significant [ANOVA for ln-transformed longevity at 25°C, using (1) species and (2) sex as fixed factors and (3) population as a random factor nested within species: (1) $F_{1,4} = 16.58^*$, (2) $F_{1,4} = 6.53$, (3) $F_{4,588} = 4.54^{***}$, (1) × (2) $F_{1,4} = 1.73$, (2) × (3) $F_{4,588} = 1.51$ ($^*P < 0.05$; $^{***}P < 0.005$)]. In contrast, mean longevity at 29.5°C was not significantly different between *D. buzzatii* and *D. koepferae*, while the effect of population within species was still highly significant [same ANOVA design as above, for ln-transformed longevity at 29.5°C: (1) $F_{1,4} = 0.13$, (2) $F_{1,4} = 11.00$, (3) $F_{4,588} = 12.44^{***}$, (1) × (2) $F_{1,4} = 0.03$, (2) × (3) $F_{4,450} = 1.64$ ($^{***}P < 0.005$)].

Drosophila koepferae showed a much higher mortality rate than *D. buzzatii* in all three sympatric populations at 25°C but not at 29.5°C (Fig. 2). Age-dependent mortality rate (*b*) was significantly higher in *D. koepferae* than in *D. buzzatii* in all three populations at 25°C but not at 29.5°C (Table 2). Estimates of *b* tended to be much higher at 29.5°C than at 25°C (Table 2), which is consistent with the fact that flies live longer at 25°C than at 29.5°C (Figs. 1 and 2). Consistent with the pattern of interspecific variation observed for the demographic senescence parameter (*b*), CPHR analysis showed that *D. koepferae* had a higher mortality than its sibling *D. buzzatii* at 25°C ($B = 0.59^{***}$, $\chi^2_1 = 41.21$ for females; $B = 0.37^{***}$, $\chi^2_1 = 33.53$ for males; $^{***}P < 0.005$). The same CPHR analysis indicated that mortality was not significantly different between species at 29.5°C ($B = -0.07$, $\chi^2_1 = 1.71$ for females at 29.5°C; $B = -0.09$, $\chi^2_1 = 1.64$ for males at 29.5°C).

It is clear from Fig. 3 that age-specific fecundity showed very different patterns between the species studied. Overall fecundity was substantially higher in *D. buzzatii* than in *D. koepferae* [ANOVA using (1) species as a fixed factor and (2) population within species as a random factor: (1) $F_{1,4} = 9.32^*$, (2) $F_{4,84} = 4.18^{***}$ ($^*P < 0.05$; $^{***}P < 0.005$)], whereas early fecundity was clearly higher in *D. koepferae* than in *D. buzzatii* (Fig. 3). One-way ANOVA indicated that this difference was significant for absolute early fecundity [same ANOVA model as for overall fecundity: (1) $F_{1,4} = 18.53^*$, (2) $F_{4,84} = 2.51^*$ ($^*P < 0.05$)]. Similar conclusions were achieved for relative early fecundity [mean values not shown; same ANOVA model as for overall fecundity: (1) $F_{1,4} = 82.20^{***}$, (2) $F_{4,84} = 2.49^*$ ($^*P < 0.05$;

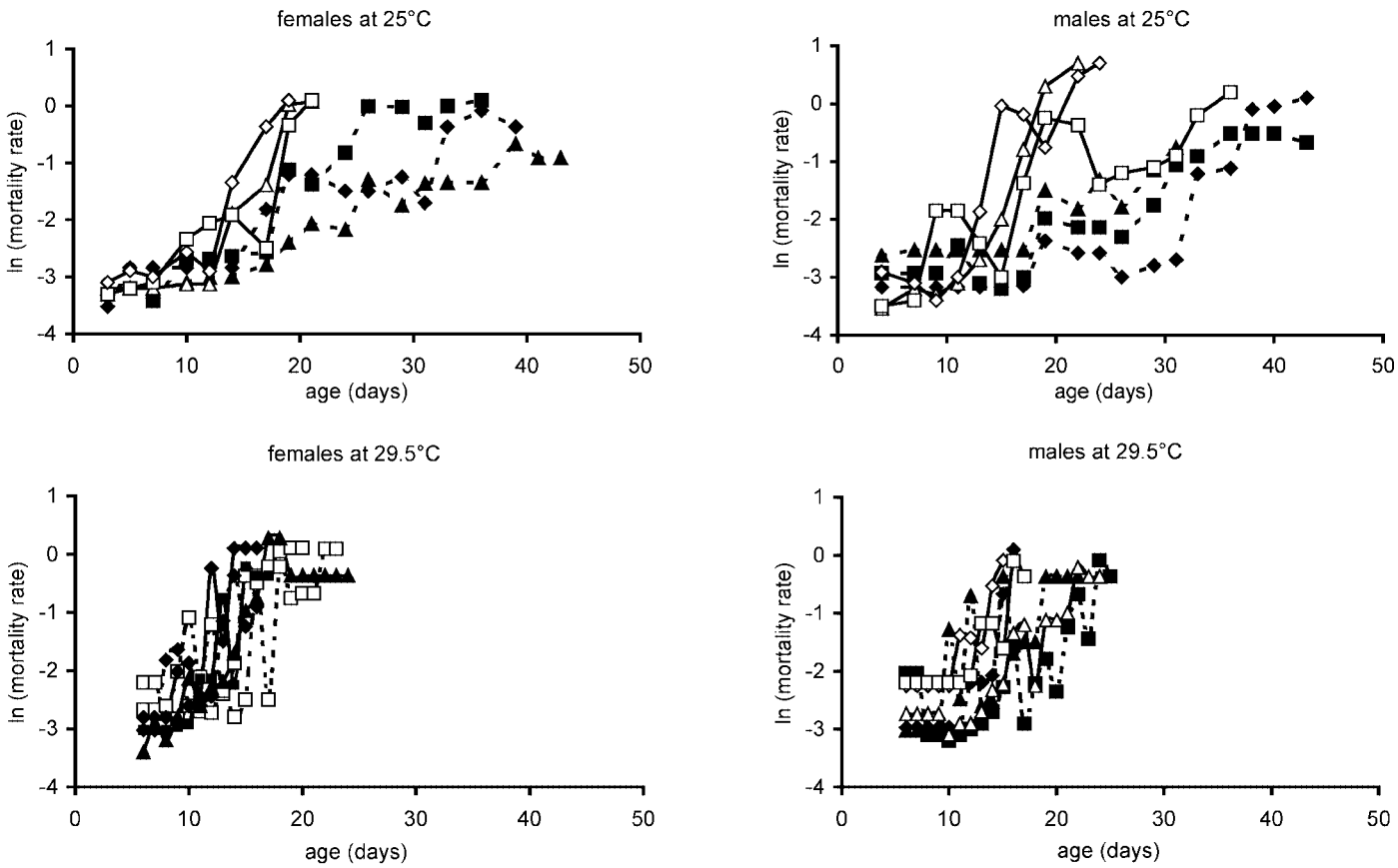

Fig. 2. Age-specific mortality rate in both *D. buzzatii* and *D. koepferae* is shown for three laboratory populations derived from sympatric wild populations originating from different altitudes as described in Table 1. *Drosophila buzzatti* from Quilmes (◆), *D. buzzatti* from San Luis (▲), *D. buzzatti* from Cafayate (■), *D. koepferae* from Quilmes (◊), *D. koepferae* from San Luis (△) and *D. koepferae* from Cafayate (□).

$^{***}P < 0.005$)]. In addition, absolute early fecundity was also negatively associated with longevity within species (Fig. 4; Pearson correlation between mean traits across populations: $r = -0.999$, $P = 0.028$ for *D. buzzatii*; $r = -0.956$, $P = 0.189$ for *D. koepferae*). Furthermore, absolute early fecundity was compared within each species between populations that not only showed the highest difference in this trait but also in both mean longevity and mortality rate of females at 25°C, namely San Luis vs. Cafayate for *D. buzzatii* and Cafayate vs. Quilmes for *D. koepferae* (see Figs. 1–4, Table 2). As expected from the antagonistic pleiotropy theory, early fecundity was significantly higher for the shortest-lived population (Cafayate for *D. buzzatii* and Quilmes for *D. koepferae*; Fig. 1, Table 2) than for the longest-lived population of each species (San Luis for *D. buzzatii* and Cafayate for *D. koepferae*; Fig. 1, Table 2), the differences being highly significant (two-tailed *t*-test, $t_{28} = 2.32^{*}$ for *D. buzzatii*, $t_{28} = 3.43^{***}$ for *D. koepferae*; $P < 0.005$). Similar conclusions were reached for relative early fecundity (results not shown).

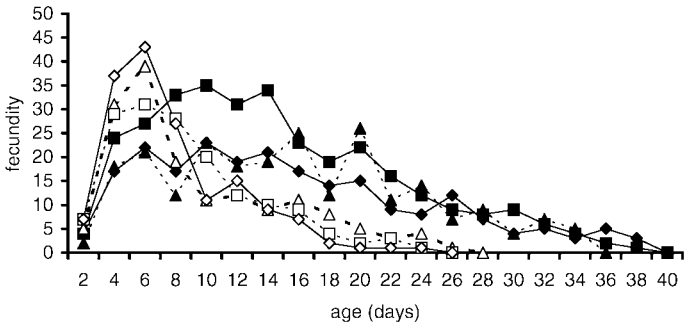

Fig. 3. Age-specific fecundity in the sibling species *D. buzzatii* and *D. koepferae* at 25°C. Estimates for fecundity are based on mean number of eggs laid every 2 days based on scores for 15 females per species and population. *Drosophila buzzatti* from Quilmes (◆), *D. buzzatti* from San Luis (▲), *D. buzzatti* from Cafayate (■), *D. koepferae* from Quilmes (◊), *D. koepferae* from San Luis (△) and *D. koepferae* from Cafayate (□).

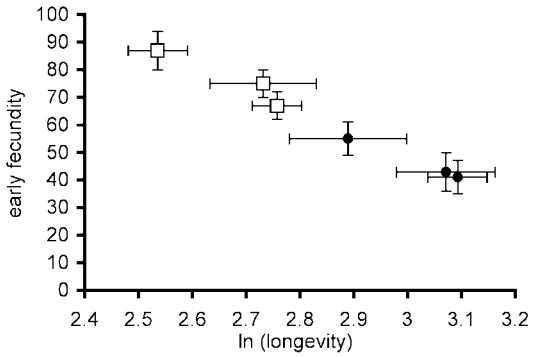

Fig. 4. Absolute early fecundity plotted against ln(mean longevity) in the sibling species *D. buzzatii* (●) and *D. koepferae* (□) at 25°C. Data for longevity are the same as in Fig. 1. Estimates for early fecundity are based on 15 females per species and population. Absolute early fecundity is the number of eggs laid during the first 6 days of adult life. References for species are given in Fig. 2. Error bars indicate standard error of the mean.

Heat-induced Hsp70 expression showed no substantial divergence between the species studied [Table 3; ANOVA using (1) species and (2) sex as fixed factors and (3) population as a random factor nested within species: (1) $F_{1,4} = 0.03$, (2) $F_{1,4} = 1.47$, (3) $F_{4,24} = 7.98$***, (1) × (2) $F_{1,4} = 0.43$, (2) × (3) $F_{4,24} = 8.17$*** (***$P < 0.005$)]. In addition, survival against heat shock showed no significant difference between species, but females were more heat-resistant than males [Table 3; ANOVA design as above: (1) $F_{1,4} = 0.12$, (2) $F_{1,4} = 17.83$*, (3) $F_{4,60} = 1.63$, (1) × (2) $F_{1,4} = 6.66$, (2) × (3) $F_{4,60} = 0.21$ for survival to heat shock (*$P < 0.05$)].

Body size was consistently larger in *D. koepferae* than in *D. buzzatii* [Table 4; ANOVA using (1) species and (2) sex as fixed factors and (3) population as a random factor nested within species: (1) $F_{1,4} = 8.25$*, (2) $F_{1,4} = 124.32$***, (3) $F_{4,204} = 28.79$***, (1) × (2) $F_{1,4} = 0.89$, (2) × (3) $F_{4,204} = 0.42$ (*$P < 0.05$; ***$P < 0.005$)].

Table 3. Mean (standard deviation) values of 38°C-induced Hsp70 expression and survival to a heat-shock stress (1 h at 41.5°C) in *D. buzzatii* (*D.b.*) and *D. koeferae* (*D.k.*)

Sex and population	Hsp70 expression		Survival against heat shock	
	D.b.	*D.k.*	*D.b.*	*D.k.*
Females				
San Luis	0.28 (0.07)	0.09 (0.06)	0.62 (0.06)	0.71 (0.08)
Cafayate	0.28 (0.08)	0.51 (0.09)	0.61 (0.07)	0.62 (0.08)
Quilmes	0.40 (0.08)	0.46 (0.07)	0.63 (0.06)	0.64 (0.06)
Males				
San Luis	0.46 (0.07)	0.41 (0.06)	0.61 (0.09)	0.64 (0.11)
Cafayate	0.41 (0.06)	0.44 (0.09)	0.63 (0.09)	0.56 (0.09)
Quilmes	0.49 (0.07)	0.33 (0.09)	0.58 (0.13)	0.57 (0.10)

Note: *N* was 12 for each Hsp70 measurement, and ranged between 54 and 78 flies per sex, population and species for heat-shock survival measurement.

Table 4. Mean (standard deviation) values of thorax length in 25°C laboratory-reared flies from three sympatric populations of *D. buzzatii* (*D.b.*) and *D. koepferae* (*D.k.*)

Sex and population	*D.b.*	*D.k.*
Females		
San Luis	1.008 (0.02)	1.063 (0.02)
Cafayate	1.033 (0.03)	1.105 (0.03)
Quilmes	1.061 (0.03)	1.116 (0.02)
Males		
San Luis	0.983 (0.02)	1.041 (0.02)
Cafayate	1.014 (0.03)	1.072 (0.04)
Quilmes	1.031 (0.02)	1.083 (0.02)

DISCUSSION

Drosophila koepferae showed a much faster senescence rate and higher early fecundity than its sibling *D. buzzatii* at a non-stressful temperature (25°C) in all three sympatric populations examined. This result suggests that the pattern of interspecific divergence in senescence rate is consistent with the fundamental trade-off between longevity and fecundity (Rose, 1991). The negative association between these traits is also consistent among populations within each species. Males lived longer than females in both species at both non-stressful and high temperatures. In contrast to the longevity–fecundity pattern, other traits such as heat-shock resistance showed no significant interspecific differentiation.

Previous studies have suggested that *D. buzzatii* lives longer than *D. koepferae* in the wild as well. For instance, for purposes other than comparing adult survival, Fanara *et al.* (1999) reported the number of bait-trapped males (BTM) as well as the number of newly emerged males (NEM) for both *D. buzzatii* and *D. koepferae* from Quilmes. Bait-trapped males were free-flying males sampled over traps of *Trichocereus* rotting patches, the rots preferred by *D. koepferae*, and newly emerged males were males that emerged from *Trichocereus* rots collected at the same time as bait-trapped males (Fanara *et al.*, 1999). If we note that bait-trapped males represents a mix of cohorts of different ages, whereas newly emerged males represent only young flies (i.e. before any possible adult survival selection), the proportion of *D. koepferae* relative to *D. buzzatii* before (NEM) and 'after' (BTM) adult survival 'selection' in the wild can be conservatively estimated (Norry, 1995). The respective proportions of *D. koepferae* relative to *D. buzzatii* were substantially different between newly emerged and bait-trapped males in the study of Fanara *et al.* (1999): 93.44% and 68.38% in NEM and BTM samples, respectively [$N_{total} = 772$ males, $\chi^2_1 = 67.24$*** (***$P < 0.005$)]. Data for flies emerging from *Opuntia* rots cannot be compared because *D. koepferae* shows highly reduced pre-adult viability in such substrates. A similar trend of differential survival in the wild could be inferred for these species from other sources of data based on banana traps (Norry, 1995; F.M. Norry, unpublished results). Such observations should not be interpreted as evidence that *D. buzzatti* shows a slower senescence than *D. koepferae* in the wild because mortality in free-flying wild *Drosophila* is due mainly to extrinsic rather than intrinsic causes. However, the very strong interspecific variation found for longevity in the present study should reflect genetic variation in intrinsic mortality rate (and thus senescence) because extrinsic causes of mortality were experimentally removed, and because all experimental individuals were reared in a common environment.

According to the senescence evolutionary theories, the fecundity patterns observed in this study appear consistent with the observation that adult survival is higher in *D. buzzatii* than in *D. koepferae* in the wild as well as in the laboratory. In addition, the dramatic divergence we found in longevity at 25°C between species cannot be easily explained as a side-effect of soma size because longevity in *Drosophila* usually increases with body size at non-stressful temperatures (Norry and Loeschcke, 2002b), whereas the short-lived *D. koepferae* was consistently larger in size than the long-lived *D. buzzatii* (Table 4). There was no apparent negative and consistent correlation between body size and longevity across populations within each species studied.

The comparative biology of ageing can be used to provisionally test whether natural selection has acted in ways that are reflected at the level of interspecific comparisons (Rose, 1991), especially if they offer an alternative approach to artificial selection experiments in

Drosophila (Linnen *et al.*, 2001). We have tested several traits for consistent patterns of divergence in senescence between *D. buzzatii* and *D. koepferae*. We found that the two species show similar resistance to heat-shock stress but differ dramatically in longevity and early fecundity. Moreover, *D. koepferae* is more resistant to knockdown at high temperatures than *D. buzzatii* (F.M. Norry, unpublished results). Females, but not males, from both species tended to express more Hsp70 if derived from the highland population of Quilmes than the lowland population of San Luis (Table 3, $P < 0.05$, *t*-test in females for each species), which is consistent with previous observations that Hsp70 expression tends to increase with altitude in *D. buzzatii* (Sørensen *et al.*, 2005). However, there was no obvious interspecific differentiation in heat-induced Hsp70 expression (Table 3). In a previous study, longevity selection decreased heat-induced Hsp70 expression while heat-shock selection increased substantially longevity in *D. melanogaster* (Norry and Loeschcke, 2003). An important conclusion in the present study is that heat-stress resistance and longevity do not always evolve in the same direction over evolutionary trajectories across species, even though both traits are often positively and genetically correlated in *Drosophila*. The evolution of heat-stress resistance can be more constrained if Hsp70 is involved in the trade-off between longevity and fecundity (Silbermann and Tatar, 2000).

Genotype × environment interactions can shift trait associations, which can influence evolutionary trajectories in populations (Gupta and Lewontin, 1982; recently reviewed in Sgrò and Hoffmann, 2004). For longevity at high versus moderate temperatures (Fig. 1), the only sympatric population that showed a significant shift in the pattern of interspecific divergence was San Luis ($P < 0.01$, *t*-test for each sex; see ANOVA results in the text for interactions, though the *b* parameter was non-significantly different between species from this population at high temperature; see Table 2). At this locality, maximal mean temperature is higher than our upper test temperature of 29.5°C (Table 1). In addition, the population that showed the highest differentiation in life span at both test temperatures was Quilmes (Fig. 1; though not for the demographic senescence parameter; see Table 2), where the maximal mean temperature is only 25°C (Table 1). These results suggest that genotype × temperature interactions can influence interspecific differentiation in evolutionary trajectories of both mean longevity and demographic rate of senescence.

Temperature almost certainly influences longevity in *Drosophila*, as noted above. However, the consistent pattern of interspecific variation observed for longevity at 25°C (a benign temperature for both species studied) cannot merely be explained by adaptation of longevity to temperature, because at high temperature (29.5°C) the 'high-altitude species' *D. koepferae* (which could be cold-adapted because of low temperature at high elevations) was longer-lived than *D. buzzatii* in the population from San Luis (Fig. 1; the *b* parameter being non-significantly different between species at 29.5°C; see Table 2). Moreover, if these two species have diverged in terms of longevity because of thermal adaptation, interspecific variation in longevity would be expected to be larger at high than at moderate temperature, but this was not the case (Fig. 1; Table 2).

The evolution of the senescence rate should be influenced by a number of extrinsic factors including, but not limited to, the distribution, abundance and stability of available resources. For the species under study, the phytogeographical distribution results in notably less abundant feeding and breeding patches (columnar cacti rots instead of *Opuntia* rots) for the short-lived *D. koepferae* than for *D. buzzatii*. As a rule, columnar cacti rots are typically bigger, much more patchily distributed (i.e. presumably less predictable for adult flies looking for suitable rots) and may offer a less ephemeral habitat for larvae than the more

abundant *Opuntia* rots (Heed and Mangan, 1986; Hasson *et al.*, 1992; Etges, 1993). In addition, host plant density and size are often negatively correlated, which may lead to the evolution of different oviposition patterns (Thompson and Pellmyr, 1991), in line with the interspecific difference in early fecundity found in the present study. In fact, utilization of a patchily distributed host plant resource like columnar cacti rots was suggested to have influenced the life-history evolution in *D. koepferae* towards a massive reproductive output upon localization of a given breeding site at early ages (Fanara *et al.*, 1999). If so, this reproductive pattern will result in an accelerated rate of senescence in *D. koepferae* due to accumulation of mutations with late-acting deleterious effects and/or selection in favour of pleiotropic alleles that increase reproduction early in life but decrease survival at advanced ages. The consistent pattern of a negative correlation between early fecundity and longevity across populations in both species might argue in favour of the antagonistic pleiotropy hypothesis. Suitable rots for each species (mainly *Trichocereus* rots for *D. koepferae* and *Opuntia* rots for *D. buzzatii*) appeared to be more abundant in the population of each *Drosophila* species that showed greater longevity of females at 25°C in this study – Cafayate for *D. koepferae* and San Luis for *D. buzzatii* (field observations at the time of sampling for this study). Other factors such as temperature almost certainly also influence longevity and fecundity within each of these species. In addition, studies in wild populations suggest that chromosomal inversions can be involved in a trade-off between fecundity and adult survival in *D. buzzatii* (Ruiz *et al.*, 1986; Hasson *et al.*, 1991). Overall, we conclude that the comparative analysis reported here under laboratory conditions is consistent with evolutionary theories of senescence in that early fecundity is negatively associated with longevity, but as discussed above our study will be best interpreted in an exploratory way to suggest plausible hypotheses to test in future work on evolution of senescence using the cactophilic *Drosophila* model. One of the hypotheses advanced in the present study is that specificity in the utilization of resources for feeding and breeding may perhaps be a crucial factor affecting the evolution of senescence. If this is the case, it does not imply that other environmental factors such as temperature and predation are not relevant for the evolution of ageing in either species. Moreover, it should be noted that all our interspecific comparisons are arbitrary with respect to whether *D. koepferae* or *D. buzzatii* is the 'ancestral' species, an issue that is unresolved (e.g. Manfbin *et al.*, 2001). Consequently, it is unknown whether evolution has led to an increase in the rate of senescence in *D. koepferae* or to a postponed senescence in *D. buzzatii*, but perhaps a combination of both evolutionary trends could also take place in the evolutionary divergence between these species.

Drosophila buzzatii is a widespread species in South America that colonized the Old World in historical times (Ruiz *et al.*, 1986), in contrast to the geographically very restricted *D. koepferae*. If colonizing success is related to interspecific patterns of senescence and fecundity, then the combination of a short life with high early fecundity did not support a successful colonization of new habitats by *D. koepferae* in contrast to its relatively long-lived sibling species, *D. buzzatii*.

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