RESEARCH ARTICLE

Effects of dietary composition on life span of *Drosophila buzzatii* and its short-lived sibling species *D. koepferae*

Federico H. Gomez · Pablo Sambucetti ·
Fabian M. Norry

Abstract Two sibling *Drosophila* species dramatically divergent in longevity, *Drosophila buzzatii* and *D. koepferae*, were examined for possible effects of both developmental culture medium and dietary composition (DC) on longevity. Longevity was greatly increased in the longer lived *D. buzzatii* when flies were reared and fed on a rich-in-nutrient and cactus-based culture (R-CBC) as compared to longevity in a poor nutrient culture (PNC). In *D. buzzatii*, life span was further increased by exposing flies to short periods of a poor-in-nutrient and cactus-based culture (P-CBC). In contrast, variation in the here used nutrient composition did not change life span in the shorter lived *D. koepferae*, as longevity in this species did not differ among R-CBC, P-CBC and PNC cultures. Hormesis is a plausible explanation for the beneficial biological effects against aging arising from brief exposure to a lowed calorie food source in *D. buzzatii*. This study shows that genetic variation between closely related species is substantial for dietary effects on longevity.

Keywords Dietary restriction · Life span extension · Environmental stress · Hormesis

F. H. Gomez (✉) · P. Sambucetti · F. M. Norry
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
e-mail: fedegz@ege.fcen.uba.ar; fedehgz@hotmail.com

Introduction

Senescence is usually defined as a progressive physiological deterioration of the organism, a process characterized by decreasing fertility and increasing mortality with age (Charlesworth 1994). The considerable amount of between-species variation in senescence rates shows that longevity can evolve (Rose 1991). In the wild, animals do not live long enough to reach advanced ages as a result of extrinsic mortality. Starvation, desiccation, predation, infection and extreme temperatures are common extrinsic mortality factors (Rose 1991; Hoffmann and Parsons 1991). As most individuals do not live long enough to reach advanced ages in the wild, the strength of natural selection declines with age (reviewed by Rose 1991; Kirkwood and Austad 2000). Mutations with deleterious effects are predicted to accumulate at advanced ages (Medawar 1952). Likewise, senescence may also evolve by selection favoring pleiotropic alleles with beneficial early-acting effects on early reproduction but deleterious late-acting effects on longevity (Williams 1957; Rose 1991; Zwaan 1999). This trade-off theory was further extended by the disposable soma theory to include the allocation of limited energetic resources to soma maintenance, reparation and reproduction (Kirkwood 1981; Kirkwood and Austad 2000).

Hormesis is a beneficial effect of mild stress involving the induction of mechanisms that protect against stress itself. Brief exposure to moderate levels of a stressor can be beneficial, for example, increasing

life span (Lithgow et al. 1995; Khazaeli et al. 1997; Calabrese et al. 1999; Hercus et al. 2003; Gems and Partridge 2008; Le Bourg 2011). Dietary restriction (DR), and low doses of other forms of stress such as high temperature, radiation, oxidative stress and hypergravity may all of them induce hormesis (e.g., Minois 2000, Rattan 2008, Le Bourg and Rattan 2008, Moskalev et al. 2011).

Dietary treatments including dietary restriction (DR) are important environmental factors in longevity determination when nutrient resources are scarce or difficult to access. The physiological response to DR appears to be relatively conserved across the animal kingdom and the evolution of life history traits occurs in environments where food supply is not optimal (Tatar 2011, Chung et al. 2013). However, it is not sufficiently clear how DR affects life span. In *Drosophila* and other fly species some studies have shown that DR increases longevity while the opposite result has also been reported (Le Bourg and Minois 2005).

Masoro (2006) showed that the effect of dietary composition and DR on the mortality rate differs among species, strains and even among different populations of the same species. Sarup and Loeschcke (2011) found that genetic background influences the position of the hormetic zone in *Drosophila melanogaster* selection lines, both between selection regimes and among the lines within selection regime, suggesting that in genetically variable populations a life enhancing treatment in one individual could have no effect or even be life shortening in other individuals. Scannapieco et al. (2007) detected heat-induced hormesis in *D. buzzatii* and its short-lived sibling species *D. koepferae*, as evidenced by either an increased mean longevity or a slower mortality rate in heat-stressed as compared to control (non-treated) flies. Heat-inducible hormesis was positively correlated with mean longevity and negatively correlated with thermal stress resistance (Scannapieco et al. 2007). At the intra-specific level in *D. buzzatii*, the hormetic impact of heat-induced hormesis on longevity was dependent on the population specific thermotolerance level, with mild-heat-stress treatment strongly inducing hormesis in heat-sensitive flies (Gomez et al. 2009). The correlation pattern in Scannapieco et al. (2007) showed that a heat-shock treatment extended longevity in long lived *D. buzzatii* but not in short lived *D. koepferae*. *Drosophila buzzatii* is less heat resistant than *D. koepferae*

(Scannapieco et al. 2007). These results suggest that correlations between heat-inducible hormesis and resistance to thermal stress appear to be negative both within and between *D. buzzatii* and *D. koepferae*. Whether similar patterns of correlation are apparent for other forms of stress-induced hormesis remains to be tested in sibling species like *D. buzzatii* and *D. koepferae*.

The main aim of this study was to investigate the possible effects of dietary composition and treatments on longevity in the sibling *D. buzzatii* and *D. koepferae*. In addition, the possible effect of culture developmental medium was also explored. These species feed and breed on rotting tissues of cactus in the wild, dramatically differing in both their mean longevity and early fecundity (Sambucetti et al. 2005). Here we considered the natural substrates of food and breed in these species to investigate the possible effects of dietary treatments on longevity in each species.

Materials and methods

Laboratory stocks

Drosophila buzzatii and *D. koepferae* stocks were derived from Quilmes (26°28′S, 65°52′W), Province of Tucuman, Argentina, where these species are sympatric (Sambucetti et al. 2005). Flies were maintained in population bottles on a 12:12 h light:dark cycle at 25 °C. In the wild, *D. buzzatii* feed and lay eggs on rotting tissues of *Opuntia* cactus species, whereas *D. koepferae* feed and oviposit on *Trichocereus* cactus species, preferentially. Our laboratory *D. buzzatii* population was reared in vials containing a culture medium prepared with *Opuntia* spp. extract, agar, dried yeast and nipagin. Similarly, our laboratory *D. koepferae* population was reared in vials containing a culture medium based on *Trichocereus* spp. extract, agar, dried yeast and nipagin. Cactus-based medium of either *Opuntia* spp or *Trichocereus* spp. extracts plus yeast is, as described above, a rich-in-nutrient culture medium, hereafter referred to as R-CBC. In addition, flies from both species were also reared in a potato-based and poor-in-nutrient culture (hereafter PNC) containing dried potato, nipagin and water, for the purpose of comparing their life span with that of R-CBC reared flies. Flies reared on R-CBC and PNC were the experimental individuals used for life span

determination. Parents of experimental flies were aged to 3–4 days, to control for the effects of parental age on offspring fitness. Culture bottles contained 10 males and 10 females and larval density was controlled by restricting oviposition to 48 h.

Longevity assays in R-CBC and PNC-reared flies

Longevity assays were carried out in R-CBC cultures. Experimental individuals from both the R-CBC-reared and the PNC-reared flies were aged to 1 day and transferred to 25 mL cylindrical vials containing 8 mL of R-CBC (i.e., *Opuntia*-based R-CBC for *D. buzzatii* and *Trichocereus*-based R-CBC for *D. koepferae*) for longevity measurement. The initial number of individuals per vial was 10 (5 females plus 5 males). Longevity data was scored at a constant temperature of 25 ± 1 °C. All vials were examined on a daily basis to check for deaths. Surviving individuals were transferred to culture vials with fresh R-CBC every 3 days. All measurements were performed in a mixed-sex environment. The sex of dead flies was determined under microscope.

Mortality assays and dietary restriction (DR) treatment

To test for any possible dietary induction of hormesis in longevity, a dietary treatment was implemented for either 1 or 2 days (see below) by transferring a sub-set of the R-CBC-reared flies to new vials containing 8 mL of a cactus-based but poor in nutrient culture, hereafter P-CBC. This P-CBC was prepared with no added yeast and smaller quantities of autoclaved cactus extract, in contrast to PNC which was based not on cactus but on potato. For the P-CBC culture, only the 25 % of the R-CBC cactus extract content was used in the preparation. The rationale for this approach is that DR is achieved in *Drosophila* by reducing the quality of the food given to the flies (Chapman and Partridge 1996), with the quantity maintained in excess of that which they consume. Experimental 2 days old flies were transferred from R-CBC vials to P-CBC vials for a period of 48 h in the case of *D. buzzatii* and 24 h in the case of the short-lived *D. koepferae*, after which they were returned to R-CBC vials for longevity measurement. This dietary treatment was compared for longevity to flies that lived on R-CBC permanently. All longevity measurements were performed in a mixed-sex environment, and flies were transferred to new vials with fresh food every 3 days. Dead flies were removed from the vials on a daily basis and sexed under microscope.

For analysis, longevity data were $\log_{(e)}$-transformed to improve normality and removing dependence of variance on means, though non-transformed raw data were already nearly normally distributed. This data transformation was successful in correcting for dependence of variances on means and normality deviations. ANOVA on transformed data yielded identical conclusions as ANOVA on non-transformed raw data, and we report ANOVA results for $\log_{(e)}$-transformed longevity. Mean longevity was first tested by a three-way ANOVA with [1] dietary-food treatment (P-CBC *vs* R-CBC *vs* PNC), [2] sex (female *vs* male), and [3] species (B [*D. buzzatii*] *vs* K [*D. koepferae*]) as fixed factors. We also performed a two-way ANOVA for each species separately by using dietary treatment and sex as fixed factors. The demographic rate of senescence *b* and instant mortality rate *a* parameters were estimated applying maximum likelihood theory (Pletcher 1999). Senescence is defined as the progressive increase of mortality with age. The age-specific mortality rate μ(x) was estimated 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 deaths in the interval x to x + 1 and N_x is the number living individuals at day x (Tatar et al. 1997). WinModest software (Pletcher 1999) was used to estimate the survivorship function that best fitted our experimental data and to perform maximum likelihood tests. The parametric Gompertz mortality model was generally better than other models to describe mortality in our data set. The model is of the form $\mu(x) = ae^{bx}$, where μ(x) is the age-specific hazard, the parameter *a* is the initial mortality parameter and *b* is the senescence rate parameter.

Results

Mean longevity is shown in Table I for each species. In all dietary treatments, *D. buzzatii* lived longer than *D. koepferae* (Fig. 1; three-way ANOVA with [1] dietary treatment, [2] sex, and [3] species as fixed factors: [1] $F_{2, 679} = 35.94^{***}$ {$^{***}P < 0.001$, $^* P < 0.05$}, [2] $F_{1, 679} = 11.09^{***}$, [3] $F_{1, 679} = 978.72^{***}$, [1] × [2] $F_{2, 679} = 1.07$, [1] × [3] $F_{2, 679} = 50.16^{***}$,

Table I Mean longevity (days ± SE) and Gompertz mortality parameters at 25 °C are shown for *D. buzzatii* and *D. koepferae*. *SE* standard error of the mean, *F* females, *M* males, *PNC* flies developed in poor nutrient culture medium, *R-CBC* flies developed in rich-in-nutrient and cactus-based culture, *P-CBC* flies exposed to poor-in-nutrient culture (individuals were exposed to a period in cactus-based poor nutrient medium without yeast and then returned to R-CBC)

	Sex	Culture	N	Mean longevity	Gompertz a	Gompertz b
D. buzzatii	F	PNC	88	13.5 (0.60)	17.6×10^{-3}	0.137
	F	R-CBC	43	18.1 (1.01)	7.2×10^{-3}	0.145
	F	P-CBC	53	23.4 (0.78)	0.5×10^{-3}	0.239
	M	PNC	58	10.2 (0.61)	30.9×10^{-3}	0.145
	M	R-CBC	33	16.9 (0.97)	10.4×10^{-3}	0.135
	M	P-CBC	40	19.7 (1.03)	5.6×10^{-3}	0.144
D. koepferae	F	PNC	47	6.23 (0.32)	23.3×10^{-3}	0.401
	F	R-CBC	52	6.25 (0.28)	21.1×10^{-3}	0.423
	F	P-CBC	83	6.18 (0.28)	37.8×10^{-3}	0.303
	M	PNC	56	6.28 (0.35)	40.4×10^{-3}	0.281
	M	R-CBC	58	5.80 (0.19)	6.2×10^{-3}	0.739
	M	P-CBC	80	5.75 (0.29)	70.8×10^{-3}	0.201

[2] × [3] $F_{1, 679} = 4.05*$, [1] × [2] × [3] $F_{2, 679} = 1.39$).

Effect of developmental culture medium in *D. buzzatii*

Survival curves and longevity parameters are shown for *D. buzzatii* in Fig 1; Table I, respectively. Mean life span was significantly different between PNC and R-CBC, with R-CBC-reared flies exhibiting higher longevity than PNC-reared individuals. In addition, females were significantly longer lived than males (Table I; two-way ANOVA with [1] dietary treatment [P-CBC *vs* R-CBC *vs* PNC] and [2] sex as fixed factors: [1] $F_{2,309} = 74.3***$, [2] $F_{1,309} = 11.4***$, [1] × [2] $F_{2,309} = 1.89$; $***P < 0.001$. Post-hoc Tukey contrasts revealed significant differences between R-CBC and PNC flies: $P < 0.001$). In males, a significant decrease in instant mortality a was found in R-CBC flies as compared to PNC flies. (Table I. χ^2-values for maximum likelihood estimate comparisons were $5.54*$ for a and 0.09 for b; $*P < 0.05$). There was no difference in the senescence rate b between R-CBC and PNC in males. Mortality parameters were not significantly different between R-CBC-reared and PNC-reared females. However, the same tendency as in males was observed in females, with a decrease in instant mortality in R-CBC (Table I. χ^2-value for a comparison between PNC and HNC females was 3.817; $P = 0.0507$).

Dietary-induced hormesis in *D. buzzatii*

Dietary induced hormesis in longevity was apparent in *D. buzzatii* when compared to R-CBC flies (Fig 1). Mean longevity increased in both P-CBC males and females. Females were longer lived than males (Table I; two-way ANOVA with (1) dietary treatment (R-CBC *vs* P-CBC *vs* PNC) and (2) sex as fixed factors: (1) $F_{2, 309} = 74.3***$, (2) $F_{1, 309} = 11.4***$, (1) × (2) $F_{2, 309} = 1.89$; $***P < 0.001$. Post-hoc Tukey contrasts showed significant differences between R-CBC and P-CBC flies: $P < 0.001$). Both instant mortality a and senescence rate b showed significant alterations in females. Instant mortality was greatly reduced in P-CBC-reared females when compared to R-CBC-reared females. In contrast, once deaths started to occur more often, senescence rate was higher in P-CBC females (Fig 1; Table I. Associated χ^2-values for maximum likelihood estimate comparisons were $13.03***$ for a and $7.70*$ for b; $*P < 0.05$; $***P < 0.001$). In males, hormesis was found in the form of life span extension only, as mortality curve parameters were not significantly different between R-CBC-reared and P-CBC-reared flies (Fig 1; Table I).

Effect of developmental culture medium in *D. koepferae*

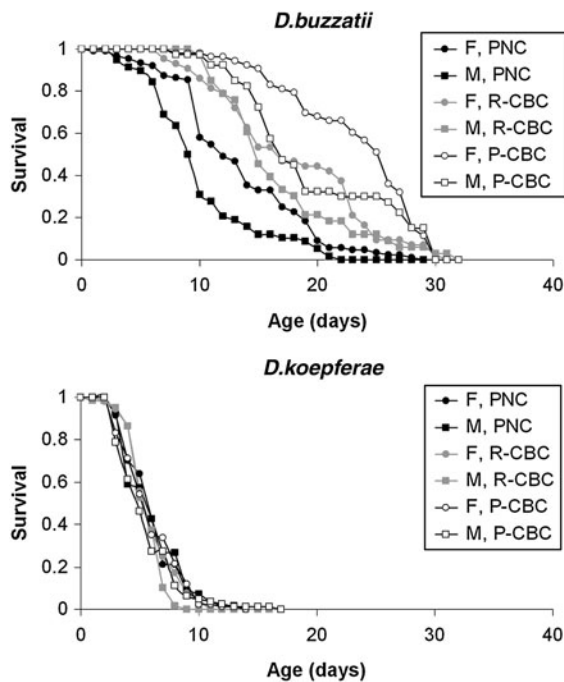Survival curves and longevity parameters are shown for *D. koepferae* in Fig 1; Table I, respectively.

Fig. 1 Survival curves are shown for males and females of *D. buzzatii* and *D. koepferae*. *F* females, *M* males, *PNC* flies developed in poor nutrient culture medium, *R-CBC* flies developed in rich-in-nutrient and cactus-based culture, *P-CBC* flies exposed to poor-in-nutrient culture (individuals were exposed to a period in cactus-based poor nutrient medium without yeast and then returned to R-CBC)

In contrast to *D. buzzatii*, mean longevity of *D. koepferae* was not significantly different neither between R-CBC-reared and PNC-reared flies nor between the sexes (Table I; two-way ANOVA with [1] dietary treatment [R-CBC *vs* P-CBC *vs* PNC] and [2] sex as fixed factors: [1] $F_{2,\ 370} = 1.29$, [2] $F_{1,\ 370} = 1.09$, [1] × [2] $F_{2,\ 370} = 0.29$). Mortality parameters differed significantly between R-CBC-reared and PNC reared males, showing both lower instant mortality and higher senescence rate in R-CBC-reared males (Fig 1; Table I). Associated χ^2-values for maximum likelihood estimate comparisons were 14.9*** for *a* and 33.5*** for *b*; ***$P < 0.001$. Mortality parameters did not differ significantly between R-CBC-reared and PNC-reared females.

Dietary treatment did not induce hormesis
in D. koepferae

In contrast to *D. buzzatii*, our dietary treatment did not change mean life span in *D. koepferae*, and mean

longevity did not significantly differ between the sexes in this species (Fig. 1; Table I; see ANOVA summarized above for this species). In males, dietary treatment affected both *a* and *b* mortality curve parameters, with P-CBC males showing increased baseline mortality but reduced senescence rate in *D. koepferae* (Table I; Fig 1. χ^2-values for maximum likelihood estimate comparisons were 28.86*** for *a* and 49.50*** for *b*; ***$P < 0.001$). Although not significantly so, the same tendency as in males was observed in females (Table I; Fig 1).

Discussion

Aging is a biological process associated with the increase and accumulation over time of levels of molecular damage, which contribute to increasing mortality at an organismal level. Research into the mechanisms underlying aging and its rate often makes use of treatments that increase life span in model organisms (e.g., *S. cerevisiae*, *C. elegans*, *D. melanogaster* and *M. musculus*), such as reduction of food intake without malnutrition through dietary restriction, DR (Kenyon 2005). In this study, two cactophilic and sibling *Drosophila* species, *D. buzzatii* and *D. koepferae*, were used to assay longevity determination of diet in a cactus-based culture. Although observed effects of dietary composition were not tested in a single-sex environment, males and females do not optimize fitness in the same way and may respond differently to resource availability. We tested whether food quality impacts on longevity under the natural condition of a mixed-sex environment. Such an experiment could also be replicated with virgin flies to further test for any possible effects of mating in the different culture media. Nevertheless, *D. buzzatii* and *D. koepferae* dramatically differed in their mean life span, consistently with previous studies (Sambucetti et al. 2005), making them especially valuable for analysis of inter-specific variation in stress-induced hormesis in longevity (Scannapieco et al. 2007). *D. buzzatii* was much longer lived than *D. koepferae*, with a lower demographic rate of senescence (Table I; Fig 1). Since extrinsic mortality sources were experimentally removed and individuals were reared in a common environment, the results indicate the presence of genetic variation in demographic senescence patterns between species. Remarkably, *D. buzzatii*

showed a substantial increase in longevity after our poor-in-nutrient treatment of DR on a cactus-based medium whereas no such effect was apparent in its short-lived sibling *D. koepferae*.

Previous studies showed that quality of nourishment rather than caloric content had a greater impact on life span extension in *Drosophila* (Mair et al. 2005). Kristensen et al. (2011) observed both plastic and evolutionary consequences on life-history traits in *D. melanogaster* exposed to nutritional environments with different protein composition, with the former being generally greater than the latter. Here, life span was greatly increased in *D. buzzatii* when the nutrient composition of the food given to parents was changed from mainly carbohydrates (PNC) to a nourishment source with proteins and lipids (R-CBC). The increase in life span, relative to PNC flies, was 34 % in R-CBC *D. buzzatii* females and 65 % in R-CBC *D. buzzatii* males, suggesting that culture protein and lipid levels had a major effect on mean survival in this species. In contrast, nutrient composition did not appear to be as relevant in determining life span in *D. koepferae* (Table I; Fig. 1). This is not the first time that stress-induced hormesis in longevity was not apparent in the shorter lived *D. koepferae*. Scannapieco et al. (2007) found that heat stress extended mean longevity in *D. buzzatii* but not in *D. koepferae*. However, heat-shock did alter mortality parameters in the latter, suggesting significant hormetic effects on the demographic rate of senescence *b* that were canceled out by an increase in baseline mortality *a* (Scannapieco et al. 2007). Coincidentally, mean life span did not increase due to P-CBC exposure in this study in *D. koepferae*, and a lower demographic rate of senescence and a higher baseline mortality were significant in males (Table I; Fig 1), suggesting that mean life span may not have changed because of the balancing of these opposing effects. Mean lifespan of *D. koepferae* was longer in Scannapieco et al. (2007) than in the present study. Thus, there is a possible difference between studies in the longevity of *D. koepferae*, such that we can not discard the possibility to induce hormesis in this species under other experimental conditions. This difference could be attributable to the rearing conditions of experimental flies and culture larval density, as well as to the different culture medium employed in each study. Nevertheless, the present results with *D. buzzatii* and *D. koepferae* add to previous evidence supporting that environmental stress, a feeding source

with low nutrient concentration in this case, can affect parameters of mortality differentially between very closely related species.

Although the magnitude of the response to P-CBC in male *D. buzzatii* is less than that in females, males do live longer when exposed to brief periods on P-CBC, and they do show the same tendency in mortality parameter changes as females. A likely hypothesis as to why there is greater life span extension induced by P-CBC in *D. buzzatii* females, is that the increased mortality in R-CBC females could partially represent a cost in reproduction, which may be lower in P-CBC than in R-CBC conditions as the level of nutrient intake and egg production are positively correlated in *Drosophila* (Chippindale et al. 1993; Chapman and Partridge 1996).

Mean life span enhancement, relative to R-CBC flies, was 16 % in P-CBC-treated *D. buzzatii* males and 29 % in P-CBC-treated *D. buzzatii* females. We detected no increase in maximum life span in *D. buzzatii*, suggesting the results here observed are consistent with a hormetic effect. Since mated flies were used in all experiments, and mated females fed *ad libitum* exhibit a high cost in reproduction (Chapman et al. 1993, Tatar and Promislow 1997), we do not rule out that life span increases in P-CBC *D. buzzatii* females may be partly due to decreased fecundity compared to mated females fed ad libitum in R-CBC. It is possible that P-CBC females invest less energy in egg production and more in soma maintenance under such conditions. Virgin females can be used to further investigate the hypothesis of a trade-off between fecundity and longevity contributing to life span enhancement. However, DR by brief periods of scarce food availability may also induce maintenance functions that protect against stress and enhance life span in the cactophilic *D. buzzatii*. The present results suggest that the hormesis hypothesis is a plausible explanation for the beneficial biological effects of brief exposure to a low calorie food source in *D. buzzatii* but not necessarily so for its short-lived sibling species, *D. koepferae*. Genetic variation between closely related species is substantial for dietary effects on longevity.

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