Experimental Gerontology 48 (2013) 539–548

Contents lists available at SciVerse ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

The lifespan-reproduction trade-off under dietary restriction is sex-specific and context-dependent

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ARTICLE INFO

Article history:
Received 4 January 2013
Received in revised form 20 February 2013
Accepted 19 March 2013
Available online 29 March 2013

Section Editor: Andrzej Bartke

Keywords:
Dietary restriction
Lifespan extension
Aging
Sex differences
Social environment
Developmental diet

ABSTRACT

Adult dietary restriction (DR) extends lifespan, but the mechanisms that underlie this effect are not well understood. Many DR studies have demonstrated that lifespan extension tends to be accompanied by a reduction in female fecundity — a correlation widely interpreted as evidence that DR triggers an adaptive re – allocation of resources from reproduction to somatic maintenance. Yet, recent evidence suggests that survival and fecundity need not always trade off under DR, calling the re-allocation hypothesis into question. Because the effects of DR on both survival and reproduction have rarely been tested in both sexes, or under a range of ecologically-relevant environments, the generality of this trade-off remains unclear. We examined the effects of DR on survival and reproduction in both sexes and across a range of environments (larval diet quality and adult sex ratio) in the neriid fly *Telostylinus angusticollis*. We found that the lifespan–reproduction trade-off is both context- and sex-dependent. Although DR extended lifespan in both sexes by 65% and rendered females completely infertile, costs of DR on male fecundity were subtle and evident only in particular environmental combinations. Our findings suggest that a re-allocation of resources may not underlie the lifespan extension response to DR. Instead, full feeding may be associated with increased costs in comparison to DR, such that lifespan extension may be achieved without an increased resource investment to the soma.

1. Introduction

While it is well known that dietary restriction during the adult stage (henceforth "DR") tends to extend lifespan and reduce or eliminate female reproduction (Masoro, 2005; Merry, 1995; but see e.g. Boggs and Ross, 1993), it remains an open question whether a reduction in reproduction is necessary to achieve lifespan extension (Carey et al., 2008; Grandison et al., 2009). The combined effects of increased lifespan and reduced fecundity that typically result from DR have long been thought to reflect a re-allocation of resources from reproductive effort to somatic maintenance, perhaps as an adaptive strategy that maximizes individuals' chances of surviving periods of resource shortage (Shanley and Kirkwood, 2000). However, the lifespan–reproduction trade-off under DR has rarely been demonstrated in males, and recent evidence (reviewed below) has called the presumed resource re-allocation into question. This question is of fundamental importance for understanding how life-history strategies evolve and, in particular, whether life-history optimization is subject to strong constraints reflecting trade-offs between key components of fitness. This question also has clear implications for the potential utility of DR as a strategy to alleviate the detrimental effects of aging in humans, in that the associated costs of DR must be taken into account. To establish whether lifespan extension through DR must be accompanied by a reduction in fecundity, it is necessary to examine DR's effects across a range of biologically relevant environments, to examine performance in a range of fitness-related traits in both sexes, and to test these effects in a variety of species (Partridge and Gems, 2007). Here, we ask how DR affects male and female survival and reproduction under contrasting developmental (larval) diets and adult social/mating environments in a neriid fly.

The proximate mechanisms mediating the DR effect remain poorly understood (Piper and Partridge, 2007), and it has been suggested that the lifespan extension effect of DR could reflect toxicity of certain nutrients or nutrient imbalance in the standard "fully-fed" laboratory diet (Raubenheimer et al., 2005; Simpson and Raubenheimer, 2007). A few recent studies lend support to the possibility that DR may not in fact trigger a re-allocation of resources, suggesting that lifespan extension may be due to other factors, or could even be an experimental or laboratory artifact — perhaps better phrased as lifespan reduction under full feeding. Grandison et al. (2009) found that *Drosophila melanogaster* females under DR supplemented with the amino acid methionine suffered no reduction in fecundity, but experienced the same lifespan extension effect as flies under regular DR, suggesting that certain other amino acids may reduce lifespan without enhancing fecundity. Mair et al. (2004) found that DR still extends life in *D. melanogaster* females even if reproduction is prevented by removal of the ovaries or a mutation that blocks vitellogenisis, suggesting that

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http://dx.doi.org/10.1016/j.exger.2013.03.007

reproduction itself is not necessary for full feeding to reduce lifespan. Fanson et al. (2012) obtained similar results in the Queensland fruit fly *Bactrocera tryoni*, detecting diet-induced reductions in lifespan for both males and females, even when the flies were kept as virgins or sterilized, leading the authors to suggest a toxic effect of high protein:carbohydrate ratios. By decoupling reproduction and nutrition, these results suggest that lifespan extension under DR is unlikely to be explained by resource re-allocation. Furthermore, insights from other nutritional geometry studies suggest that the protein: carbohydrate ratio that maximizes reproduction is not the same one that minimizes lifespan, and vice versa (Carey et al., 2008; Lee et al., 2008; Maklakov et al., 2008), supporting the possibility that these two traits need not trade off.

The DR literature is dominated by examples of the extended lifespan/reduced fecundity correlation in females. Female gametes require a greater resource investment than male gametes, so it may be expected that the fecundity costs of DR will be more apparent in females than in males. However, spermatogenesis and ejaculate production may also be very costly (Dewsbury, 1982; Wedell et al., 2002). Moreover, male accessory gland secretions, which play an important role in upregulating ovulation and oviposition in females (Arnqvist and Nilsson, 2000; Chapman, 2001; Wolfner, 2002), are likely to be costly for males to produce and limited by the resources available to them (Baker et al., 2003; Blay and Yuval, 1997; Fricke et al., 2008). Thus, if DR extends lifespan through a re-allocation of resources, it might be predicted that male fecundity should be affected in the same direction as female fecundity. However, given that males tend to experience a less pronounced lifespan extension on DR, as shown by a recent meta-analysis (Nakagawa et al., 2012), effects of DR on male fecundity may be less pronounced as well. Direct effects of DR on male fecundity have rarely been measured, but a few studies have detected effects of DR on traits related to male mating success. For example, DR resulted in reduced calling rate and increased longevity in black field crickets, *Teleogryllus commodus* (Hunt et al., 2004), and altered cuticular hydrocarbon composition in *Drosophila serrata* males (Gosden and Chenoweth, 2011). A number of other studies have also found that DR constrains some aspects of male fecundity, but the results are often inconsistent with a simple resource re-allocation scenario (Fricke et al., 2008). Surprisingly, Gosden and Chenoweth (2011) found that DR males had higher competitive mating success than fully-fed males, but DR males did not live longer.

A key assumption underlying the hypothesis that DR triggers an adaptive re-allocation of resources from reproduction to survival is that, when resources are scarce, investment in reproduction will not pay off. However, the resources drawn upon for crucial functions such as reproduction and somatic maintenance are not sourced entirely from the adult diet. Many organisms store nutrients from development for use as adults (Boggs, 2009), and the diet of the developing organism may play an important role in determining adult phenotypes and fitness, particularly for species with determinate growth. Holometabolous insects, for example, have a fixed size and shape upon eclosion, so resources acquired by the developing larva determine the adult morphology, and thereby constrain the options available to the adult. Nutrient acquisition and allocation are distinct for each life stage of a holometabolous insect, but the resources acquired in the larval stage ("developmental diet" or "larval diet") may bear strongly on the allocation, and potentially acquisition decisions and associated trade-offs, in the adult stage (Boggs, 2009). Generally, studies in holometabolous insects that have manipulated the larval diet by limiting nutrients, report detrimental fitness effects such as reduced adult size (Boggs and Freeman, 2005; Bonduriansky, 2007; Tu and Tatar, 2003; Zwaan et al., 1991), delayed eclosion (Bonduriansky, 2007; Nylin, 1988; Tu and Tatar, 2003; Zwaan et al., 1991), as well as a reduction in female fecundity (Tu and Tatar, 2003) and various aspects of male fecundity (Engels and Sauer, 2007; Gage and Cook, 1994). Effects of larval diet on adult lifespan have tended to be insignificant or inconsistent (Dmitriew and Rowe, 2011; Zajitschek et al., 2009; Zwaan et al., 1991).

The developmental/larval diet may influence trade-offs between survival and reproduction under adult DR, but few studies have tested for such interactions. Three distinct predictions are possible (Fig. 1). First, larval diet may be a cue to the adult resource environment, and phenotypes may be optimized for that environment, such that organisms reared on a nutrient-restricted ("Poor") larval diet will do best under adult DR, and those with access to a plentiful ("Rich") larval diet will do best under adult full-feeding (Fig. 1a). Second, adult resources may compensate for developmental inadequacies, such that organisms on a poor larval diet will benefit relatively more from full feeding as adults (Fig. 1b). "Catching up" tends to entail important life-history costs (Metcalfe and Monaghan, 2001), and post-eclosion growth is not an option for holometabolous insects, but they may be able to replenish otherwise depleted stores that can be used for reproduction or somatic maintenance in adulthood. Third, organisms on a poor larval diet may not be able to overcome their poor start, and may perform worse than those on a rich larval diet in both absolute and relative terms (Fig. 1c).

Two recent studies in holometabolous insects investigated the potential for interactions of larval and adult diets (Bauerfeind and Fischer, 2005; Dmitriew and Rowe, 2011), in females of the butterfly *Bicyclus anynana* and the ladybird beetle *Harmonia axyridis*, respectively. Both studies report reduced body size and a reduction in female fecundity resulting from reduced larval nutrients, as well as a reduction in fecundity resulting from adult DR. While no interaction was found between larval and adult diets on female fecundity in ladybird beetles (Dmitriew and Rowe, 2011), in butterflies, adult DR reduced fecundity in the fully-fed larval treatment, but not in the

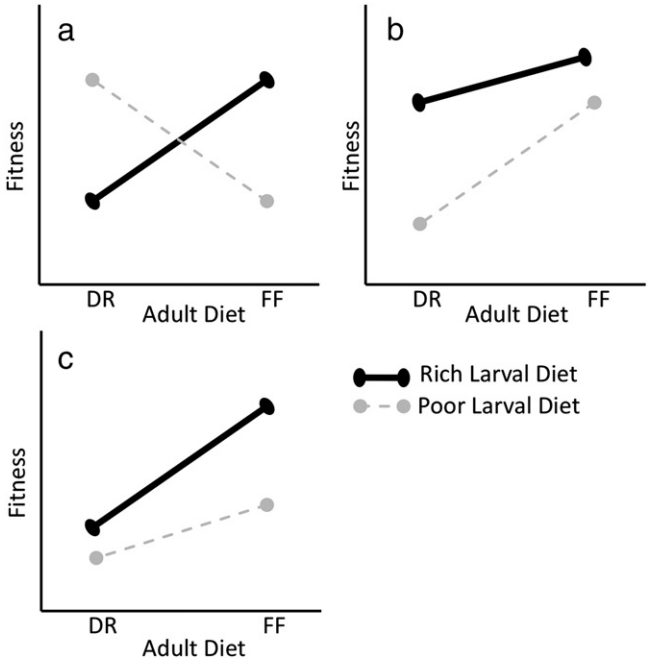

Fig. 1. Three possible response scenarios to the interaction of larval and adult diet. The three scenarios represented here are mutually exclusive, but different fitness measures may reveal different patterns of response. (a) Organisms deprived of larval nutrients will perform better under adult DR, either because they are optimized for a nutrient-limited environment – the "environment matching" hypothesis (reviewed in Dmitriew and Rowe, 2011; Monaghan et al., 2008) – or because they have a less costly phenotype, and so need fewer resources in adulthood. (b) Organisms fed a poor larval diet may benefit relatively more from full feeding in adulthood than those fed a rich larval diet, since they may have reduced larval stores and thus more to gain from adult resource abundance. (c) Organisms on a poor larval diet may be constrained in relation to those on a rich larval diet, regardless of adult resource availability. (DR: Dietary Restriction; FF: Full Feeding.)

nutrient-restricted larval treatment, suggesting that butterflies deprived of larval resources could not take full advantage of ad libitum resources in adulthood (Bauerfeind and Fischer, 2005). These studies suggest that organisms deprived of larval resources display reduced fitness as adults, regardless of the adult environment (as in Fig. 1c).

Life-history responses to DR are also likely to depend on the social/mating environment (i.e., the number and proportion of conspecific individuals of the opposite and same sex encountered within the local habitat), which may influence resource allocation to various aspects of reproduction. The social environment may vary temporally due to factors such as sex differences in maturation times, mortality rates, survival or immigration/emigration rates; seasonal changes in the operational sex ratio; and variance in arrival times at breeding sites (reviewed in Kasumovic and Brooks, 2011). The social environment of an individual can also vary spatially, particularly for a male, if females are associated with a valuable habitat or food resource, and males vie for dominance over and access to females. As male fitness tends to be limited primarily by access to females (Bateman, 1948), males that have many mating opportunities may pursue a very different strategy from those that have few (e.g. Gwynne and Simmons, 1990; Kvarnemo and Simmons, 1999). The adult social/mating environment may therefore influence the trade-off between survival and reproduction, in combination with both developmental and adult diet. A recent study by Zajitschek et al. (2012) found that lifespan effects of diet were dependent on the social environment, but only for males, and the authors suggested a need to include males as well as social environment variation in future studies.

Telostylinus angusticollis (Diptera, Neriidae) is a sexually dimorphic fly that exhibits strong, sex-specific life-history responses to larval diet (Bonduriansky, 2007) and adult social environment (Adler and Bonduriansky, 2011). Adults of both sexes reared on full larval nutrients appear to achieve higher reproductive success than those reared under restricted larval nutrients (Bonduriansky and Head, 2007). In the wild, *T. angusticollis* adults feed, mate and oviposit on the trunks of trees, and larvae develop in rotting tree bark (Adler and Bonduriansky, 2011). Females tend to aggregate at oviposition sites, and one or a few dominant males guard these territories. Dominance is established through intense male–male combat, and subordinate males tend to aggregate at separate sites, such that sex ratios in the wild tend to be highly variable, both spatially and temporally (Adler and Bonduriansky, 2012). Here, we manipulated larval diet, adult diet and adult social/mating environment in a full-factorial design, using dichotomous manipulations to allow for every possible combination of the three independent treatments. We examined responses in adult longevity and several aspects of reproductive capacity in both sexes. DR was applied by greatly reducing the protein:carbohydrate ratio in the adult diet (1:20 for DR vs. approximately 1:1 for fully-fed) because DR effects in insects appear to depend largely on the availability of protein or specific amino acids (Carey et al., 2008; Grandison et al., 2009; Lee et al., 2008; Mair et al., 2005; Maklakov et al., 2008; Zimmerman et al., 2003). While DR is typically applied by moderately reducing protein in the adult diet, we chose to restrict protein to a level that would be on the extreme end of the nutritional geometry scale (e.g. see Lee et al., 2008) in order to test in the most conservative way the possibility that lifespan extension can occur without associated reproductive costs. We crossed the adult diet manipulation with a larval diet manipulation involving a 3-fold dilution of total nutrients (sugars and protein) — a manipulation shown previously to have marked effects on larval development and adult morphology (Bonduriansky, 2007). Although both larval and adult diets varied in concentrations of nutrients, the diets were somewhat different because of the dissimilar nutritional ecologies and requirements of larvae and adults of this species. Flies subjected to each combination of larval and adult diets were maintained in same-sex (no mating) or mixed-sex (mating) social environments. In separate experiments, we also asked how larval diet affects adult lifespan under an even more extreme form of adult diet manipulation: starvation, applied at eclosion or late in life. Examining the response to adult starvation allowed us to consider in isolation the effects of stored larval reserves on lifespan. This is the first study to examine the effects of DR on lifespan and reproduction in response to variation in both the developmental (larval) and social/mating environments, in both sexes.

2. Materials and methods

This study comprises four separate experiments. In the "main experiment," we manipulated larval diet, adult diet, and social/mating environment, in a full-factorial design, and measured effects on lifespan of both sexes as well as female fecundity and male reproductive performance. We also performed separate experiments to examine effects of larval diet on starvation resistance early in life, starvation resistance late in life, and the rate of egg development in females.

2.1. Source and rearing of flies

The lab stock used in the experiments was derived from >100 individuals of *T. angusticollis* collected from aggregations on the trunks of *Acacia longifolia* trees in Fred Hollows Reserve in Sydney, Australia, and maintained in the lab as a large, outbred population for about 25 generations, supplemented annually with new wild-collected individuals. Oviposition medium consisted of a thoroughly homogenized mixture of 30 mL sugarcane molasses, 30 mL liquid barley malt and 32 g soy protein powder per liter of dry cocopeat hydrated with 800 mL of water (see Bonduriansky, 2007 for product details), frozen at −20 °C until the day of use. As the medium deteriorates rapidly over time and oviposition in stock cages occurs over a period of several days after the medium is introduced, stock larvae experience a range of nutrient availabilities depending on the age of the medium at the time of oviposition, and the density of larvae, and this results in phenotypic variation comparable to that seen in the wild source population.

2.2. Setup of main experiment

To obtain flies for the main experiment, eggs were collected from cages containing approximately 30 males and 30 females, all approximately 15 days old and kept as virgins with ad libitum food and water until the time of mating. Eggs were transferred in alternating sequence into 250-mL containers of fresh "Rich" or "Poor" larval medium provided ad libitum (i.e., >4 ml per egg). Rich larval medium was prepared in the same way as the rich oviposition medium, but all eggs were transferred to this medium when it was fresh. Poor medium contained the same ingredients at 1/3 the concentrations of rich medium.

Within each larval treatment, adult flies (which attain sexual maturity a few days after eclosion) were assigned randomly to adult housing and food treatments immediately after emergence. All flies ($n = 720$) were held in groups of 10 in 1-L cages. Flies from each larval diet (rich and poor) were assigned to either a Fully-Fed (FF) adult diet of sugar and soy protein, or a Dietary-Restricted (DR) adult diet of just sugar. Flies from each larval × adult diet combination were assigned to either same-sex housing (10 males or 10 females) or mixed-sex housing (5 males and 5 females). Each adult treatment was replicated 6 times, for a total of 36 cages within each of the 2 larval diet treatments ($n = 72$ cages in total).

Experimental cages were well-ventilated and provided with sources of water. In fully-fed cages, flies were provided with a 12-mL petri dish spread with a paste of brown sugar and soy protein (resulting in an approximately even, 1:1, protein:carbohydrate ratio), dissolved in hot water in order to ensure thorough mixing and thus

ensure that all flies ingested the same ratio of sugars and protein. In addition, a 70-mL container of moistened cocopeat thoroughly mixed with dissolved brown sugar and soy protein was provided as an additional source of food and oviposition medium. This mixture was then placed into the cages and changed every 10 days. DR cages received a petri dish spread with a brown sugar paste and a 70-mL container of moistened cocopeat mixed with brown sugar. Brown sugar contains 5% protein (a 1:20 protein:carbohyrate ratio). Flies were kept on a 12 h–12 h light–dark cycle using a combination of broad-spectrum fluorescent and incandescent lighting, at an approximately constant temperature of 25 °C at 50% humidity. Cages were checked for dead flies every 3 days.

2.2.1. Male reproductive performance assay

In order to measure the effect of the different diet and sex ratio treatments on male fecundity, one male was removed from each cage at 23 days of age and placed into a 250-mL cage with a 21-day-old virgin female reared on rich larval medium and provided with sugar, yeast and rich oviposition medium ad libitum until the time of the assay. All cages contained dishes of cocopeat mixed with sugar. Males were kept with females for 24 h, and then returned to the same cage from which they were removed. The oviposition medium was checked for eggs at 0, 48 and 72 h after male removal. If eggs were found, up to 20 eggs were transferred to a 250-mL container of poor larval medium. Containers of eggs were placed into an environment chamber set at an alternating 12 h–12 h cycle of 25 °C and 23 °C at 70% humidity, and eclosing adults were counted. Upon eclosion in the environment chamber, flies were frozen and later sexed, photographed under a microscope with their wings removed to provide a clear image of the thorax. Image J software (National Institutes of Health) was used to measure thorax length for all flies. All photographs and measurements were performed by MIA to ensure consistency.

The male reproductive performance assay captured variation in a male's ability to achieve mating with a female in a no-choice setting, fertilize her eggs, and induce rapid oviposition. In *T. angusticollis*, mating is brief and only tiny ejaculates are transferred (Bonduriansky and Head, 2007), but males will often attempt to mate repeatedly with the same female (Bath et al., 2012). Following mating, a female may lay one or more eggs immediately, delay oviposition by up to several days, or never oviposit at all (M.I. Adler, E.J. Cassidy, C. Fricke and R. Bonduriansky, unpublished data). The number of eggs laid by females after a 24-hour exposure to a male is therefore likely to depend on the number of matings that the male achieved, and the quality and quantity of the accessory-gland proteins and sperm transferred by the male at each mating. Thus, although this assay does not capture variation in male ability to defeat other males in combat, it is a snapshot likely to capture much of the variation in male performance in pre- and post-copulatory interaction with females.

2.2.2. Effects on female oviposition rate

To determine whether females on all diet combinations could produce eggs, we provided DR oviposition medium to all mixed-sex cages for 24 h, when the flies were 21 days old. This medium was then removed and checked for the presence or absence of eggs, and the normal medium was replaced.

2.3. Effects of larval diet on female egg development

In a separate experiment, eggs were transferred to rich and poor larval medium, as described above (see Section 2.2). Once the flies emerged, females were collected and placed into 1-L cages, each containing 10 flies ($N = 6$ cages of rich larval-diet flies and 6 cages of poor larval-diet flies, for a total of 12 cages and 120 flies). In a pilot study, approximately 15 females from each larval diet, held under DR as adults, were dissected at 21 days of age, and all had completely undeveloped ovaries (see Fig. 3). Since it was thus determined that females without protein in the adult diet were incapable of producing eggs (also see Results), all flies in the Female Fecundity experiment were fully fed. At least 10 flies from each treatment were frozen at days 0 (eclosion), 4, 8, 12, 16, and 20. Later, the flies were thawed, photographed and measured for thorax length as described above, and then dissected. If eggs were present, all mature eggs from each ovary were counted. As the maximum number of mature eggs that can be produced at any one time is limited by the number of ovarioles, ovariole number tends to be highly correlated with total lifetime fecundity in flies (Ellers and Jervis, 2003; Wayne and Mackay, 1998). All photographs, measurements, dissections and egg counts were performed by MIA.

2.4. Starvation resistance experiments

In two separate experiments, we measured the ability of flies reared on Rich and Poor larval diets to withstand adult starvation in early- and late-life. For both experiments, we transferred eggs to rich and poor larval medium, as described above, and each adult was moved into an individual 250-mL cage. In the early-life starvation experiment, 142 flies from the rich larval diet (72 males and 70 females) and 83 flies from the poor larval diet (41 males and 42 females) were housed in individual cages from eclosion. Each cage was provided with water but no food. In the late-life starvation resistance experiment, 29 flies from the rich larval diet (15 males and 14 females) and 30 flies from the poor larval diet (15 males and 15 females) were housed individually and provided with the same food and oviposition medium as DR flies in the main experiment. Then, at 28 days of age, all food and oviposition medium was removed from the cages, leaving only a source of water. In both experiments, cages were checked for dead flies daily (with the exception of the first day of the early-life starvation resistance experiment). Flies from the late-life starvation experiment were frozen at death, and later photographed and measured for thorax length.

2.5. Statistical analysis

Analyses of variation in lifespan were based on cage means as observational units, and on individual flies for the starvation resistance experiments (in which all flies were housed individually). In the main experiment, the effect of sex was tested in two different ways: First, we restricted the analysis to same-sex groups and included sex as a between-subjects factor. Second, we restricted the analysis to mixed-sex groups, and included sex as a within-subjects factor. The effect of social environment (sex ratio) was tested in males and females separately, with sex ratio (same-sex vs. mixed-sex groups) included as a between subject factor. When necessary, lifespan data were log-transformed to make the data fit the assumptions of ANOVA. For both mixed-sex and same-sex groups, we found no significant three-way interaction (larval diet × adult diet × sex), so it was removed from the models.

In testing for an effect of body size on late-life starvation resistance, we standardized body size (i.e. transformed to Z-scores) within sex and diet, as these factors influence body size. In testing for effects of male larval diet, adult diet, and social environment on the egg-to-adult viability (as the proportion of eggs that survived to adulthood), we arcsine-transformed the data.

Data on female egg number and oviposition were non-normally distributed (there were many zeros), so we used non-parametric tests to compare groups. In order to examine the effects of larval diet, adult diet and social environment on oviposition (number of eggs laid within 72 h of mating) in the male reproductive performance assay, we fit a generalized linear model with a negative binomial distribution using the MASS package (Venables and Ripley, 2002) in R version 2.14 (R Development Core Team). For all other analyses, we used SPSS Statistics version 20.0.

3. Results

3.1. Effects on lifespan

Lifespan means for each treatment group are reported in Table 1, and lifespan analysis results are summarized in Table 2. Adult DR resulted in an increase of approximately 65% in mean lifespan in both same-sex and mixed-sex groups (Fig. 2). There was no overall effect of larval diet on mean lifespan for same-sex or mixed-sex groups (Fig. 2). However, we observed a significant larval diet × sex interaction for longevity of flies housed in same-sex groups, whereby males lived longer than females on a rich larval diet but not on a poor larval diet. The larval diet × sex interaction was non-significant for mixed-sex groups. There was no significant larval × adult diet interaction effect on lifespan for either same-sex or mixed-sex groups. Results remain qualitatively identical if non-significant interactions are removed from the models.

In a separate analysis (not represented in Table 2), social/mating environment (sex ratio) had no overall effect on lifespan for males (ANOVA: $F_{1,\ 40} = 0.002$, $P = 0.961$) or females (ANOVA: $F_{1,\ 40} = 0.388$, $P = 0.537$), and there were no significant interactions of social environment with adult or larval diet on lifespan for either sex (all $F_{1,\ 40} < 1.59$; all $P > 0.21$).

3.2. Effects on female fecundity

Females deprived of protein as adults (DR) were unable to produce any eggs. A sub-group of females from each of the larval × adult diet treatments dissected at 21 days of age revealed entirely undeveloped ovaries in all of the females deprived of adult protein (Fig. 3). Moreover, while females in all fully-fed cages had laid eggs, none of the females in DR groups laid any eggs (Mann–Whitney *U*-Test: $N = 12_{\text{Fully-Fed}}$ and 12_{DR}; $Z = -4.80$, $P < 0.0001$). The absence of eggs in the DR cages must be a female, not a male, effect, since DR males can produce viable sperm and offspring (see below).

We were unable to examine interactions of larval and adult diets on female fecundity, given that adult DR rendered females infertile. Among females fully-fed as adults, we detected effects of larval diet on fecundity. Females on a rich larval diet had more eggs in their ovaries than those on a poor larval diet, pooling across all age groups examined (Mann–Whitney *U*-test: $N = 43_{\text{Poor}}$ and 31_{Rich}; $Z = -4.83$, $P < 0.0001$; Figs. 3 and 4). The same holds true when considering only mature females, with developed ovaries (Mann–Whitney *U*-test: $N = 15_{\text{Poor}}$ and 24_{Rich}; $Z = -4.014$, $P < 0.0001$). Rich larval diet females also developed eggs (i.e. reached reproductive maturity) at younger ages, starting at Day 8, compared with those on a poor larval diet that first had eggs at Day 16 (Fig. 4). The total number of eggs within the ovaries of a female increased with body size within each larval diet, and overall (linear regression: $F_{1,\ 71} = 48.872$, $P < 0.0001$).

Table 1
Lifespan means (days), with standard error (SE), for each larval diet × adult diet × social environment treatment combination, reported separately for each sex.

Larval diet	Adult diet	Social environment	Sex	Mean lifespan	SE
Poor	DR	Mixed-sex	F	58.13	2.48
Poor	DR	Mixed-sex	M	55.76	4.15
Poor	DR	All-female	F	58.75	2.27
Poor	DR	All-male	M	55.68	2.38
Poor	Fully-Fed	Mixed-sex	F	33.63	4.08
Poor	Fully-Fed	Mixed-sex	M	37.00	2.67
Poor	Fully-Fed	All-female	F	34.88	1.65
Poor	Fully-Fed	All-male	M	35.68	2.71
Rich	DR	Mixed-sex	F	62.67	6.04
Rich	DR	Mixed-sex	M	63.10	6.58
Rich	DR	All-female	F	52.58	2.67
Rich	DR	All-male	M	60.77	3.81
Rich	Fully-Fed	Mixed-sex	F	31.83	4.74
Rich	Fully-Fed	Mixed-sex	M	32.19	2.24
Rich	Fully-Fed	All-female	F	33.36	1.17
Rich	Fully-Fed	All-male	M	37.49	1.95

Table 2
ANOVA for effects of larval diet, adult diet and sex, on lifespan (cage means), for same-sex and mixed-sex group cages.

Predictor	Same-sex groups[a]		Mixed-sex groups[b]	
	F	P	F	P
Larval diet	0.005	0.946	0.109	0.745
Adult diet	155.834	**<0.0001**	43.336	**<0.0001**
Sex	1.691	0.201	0.059	0.811
Larval diet × adult diet	0.020	0.890	1.344	0.260
Adult diet × sex	0.022	0.884	0.590	0.452
Larval diet × sex	4.194	**0.047**	<0.001	0.978

Values in bold are statistically significant.
[a] Error DF = 41.
[b] Error DF = 20.

3.3. Effects on male fecundity

Male adult DR had no overall effect on female oviposition 24, 48 or 72 h after mating (Mann–Whitney *U*-tests: $N = 24_{\text{DR}}$ and $24_{\text{Fully-Fed}}$; $|Z| < 1.25$, $P > 0.20$). Adult DR had no overall effect on male fecundity. There was no overall effect of male adult diet on the egg-to-adult viability of eggs laid by females mated to experimental males in the male reproductive performance assay (ANOVA: $F_{1,\ 35} = 0.331$, $P = 0.569$) or on the mean adult body size of resultant offspring (ANOVA: $F_{1,\ 32} = 0.061$, $P = 0.81$).

Larval diet also had no overall effect on male fecundity in terms of offspring body size (ANOVA: $F_{1,\ 32} = 0.930$, $P = 0.342$) or egg-to-adult viability (ANOVA: $F_{1,\ 35} = 0.891$, $P = 0.352$), nor an interaction of larval and adult diet on these factors (both $F < 0.41$, both $P > 0.5$). There was also no overall effect of male social/mating environment on either of these factors (both $F < 0.6$, both $P > 0.45$).

Despite finding no overall male fecundity costs of any diet or social/mating environment treatments, we found that the total number of eggs laid in the first 72 h after mating was affected by a three-way adult diet × larval diet × social environment interaction ($\chi^2_{1,\ 40} = 4.50$, $P = 0.0255$). This result is reflected in the fact that at least half of the females that were mated to males from every treatment combination produced eggs within 72 h of mating, except for

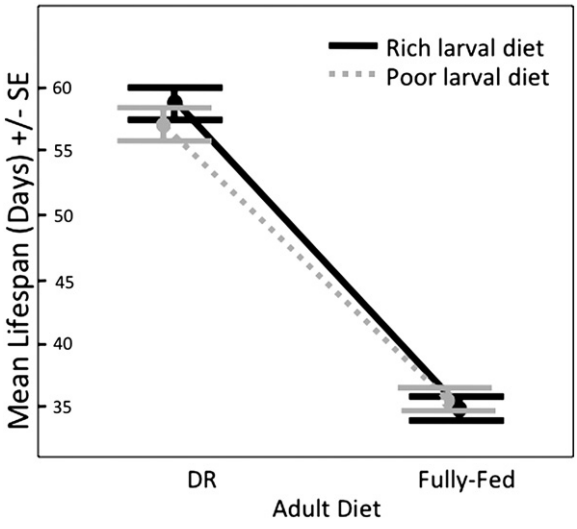

Fig. 2. DR increases lifespan, regardless of larval diet. Mean lifespan of flies maintained on adult DR and Full-Feeding regimes, when reared on both rich and poor larval diets.

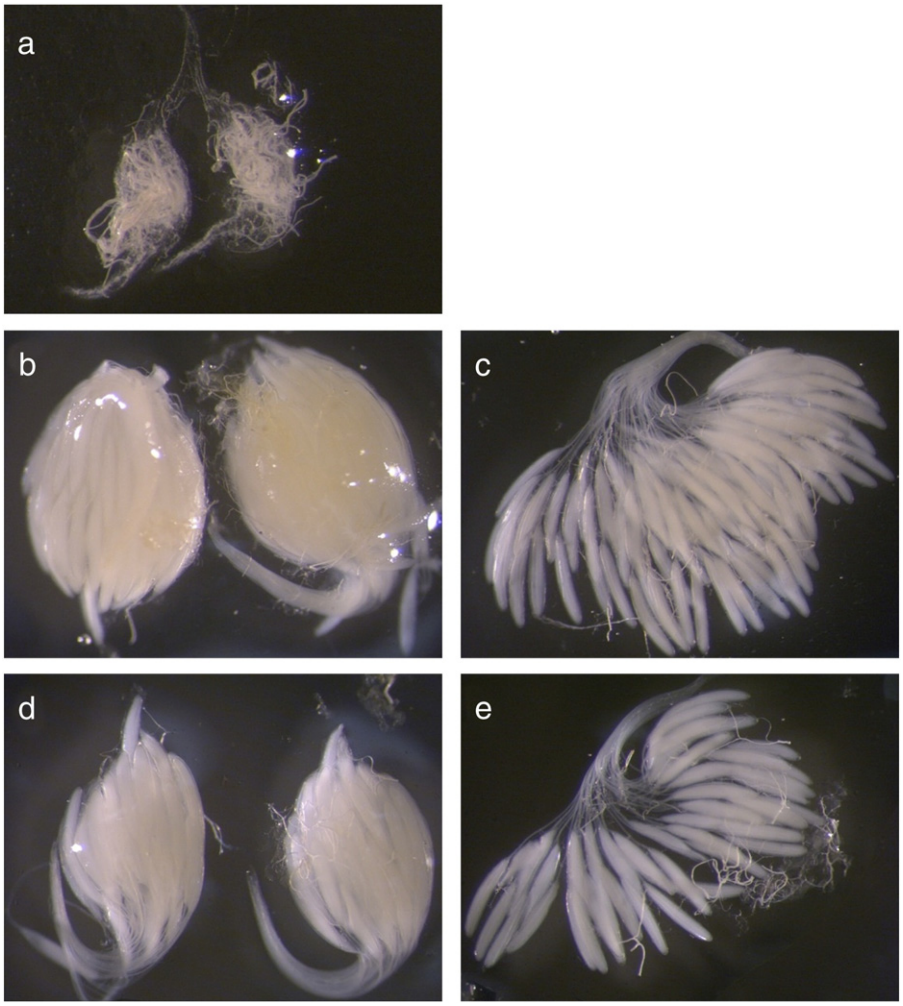

Fig. 3. Adult DR renders females infertile, while larval nutrients constrain egg number. Ovaries dissected out of 3-week old females reared on (a) adult DR — ovaries are undeveloped, vs. (b)–(e) full feeding in adulthood. Photos (b) and (c) are from a rich-larval-diet female, while photos (d) and (e) are from a poor-larval-diet female. Photos (c) and (e) show one ovary pulled apart to reveal the individual eggs.

the rich larval diet × adult DR × mixed sex ratio treatment males, in which none of their mates produced eggs within 72 h of mating (Fig. 5).

3.4. Effect of larval diet on adult starvation resistance

Flies reared on a rich larval diet were more resilient to starvation, both immediately upon eclosion and in late-life. When deprived of food immediately after eclosion, flies reared on a rich larval diet lived longer than flies reared on a poor larval diet (ANOVA: $F_{1,\ 221} = 126.59$, $P < 0.0001$), females lived longer than males (ANOVA: $F_{1,\ 221} = 5.31$, $P = 0.022$), and there was a marginally significant interaction of sex and larval diet, whereby the sex difference in lifespan was greater on a rich larval diet (ANOVA: $F_{1,\ 221} = 3.92$, $P = 0.049$; Fig. 6a).

Likewise, when starved late in life, flies reared on a rich larval diet survived longer than those on a poor larval diet (ANOVA: $F_{1,\ 55} = 64.68$, $P < 0.0001$). However, there was an interaction between sex and larval diet, whereby females lived longer than males on a poor larval diet and males lived longer than females on a rich larval diet (ANOVA: $F_{1,\ 55} = 14.51$, $P < 0.0001$; Fig. 6b). These results persisted when body size was included as a covariate in the model. Body size did not have a significant effect on late-life starvation resistance (ANCOVA: $F_{1,\ 44} = 8.24$, $P = 0.17$). However, we found an interaction of larval diet and body size on late-life starvation resistance, whereby lifespan of poor larval-diet flies under late-life starvation increased with body size, while lifespan of rich larval-diet flies was not related to body size (ANCOVA: $F_{1,\ 41} = 4.96$, $P = 0.031$). Although slopes relating lifespan to body size are not homogeneous within the two larval diet treatments, the interpretation of the effects of categorical predictors is straightforward because these slopes do not intersect within the range of the data.

4. Discussion

4.1. Main findings

We asked whether the lifespan extension response that generally results from dietary restriction (DR) is necessarily accompanied by a reduction in reproductive capacity, by examining the effects of DR in a range of environmental combinations, and in both sexes. Our results indicate that reproductive costs of DR are highly context- and sex-dependent, suggesting that a re-allocation of resources may not underlie the lifespan extension response to DR. This study is the first of which we are aware to incorporate developmental (larval) diet, adult diet, and adult social/mating environment in a full-factorial design, and to measure effects on a range of fitness-related traits in both sexes. Our results suggest a need to re-evaluate the nature and evolutionary implications of responses to DR.

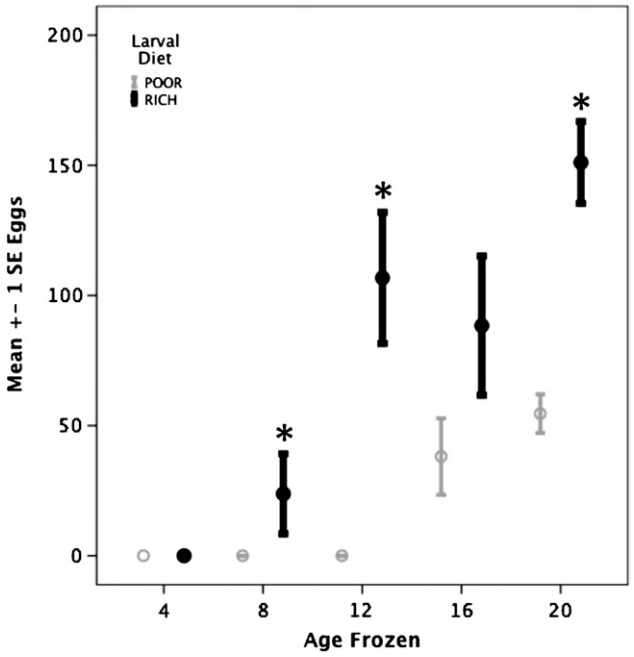

Fig. 4. Rich larval diet females mature faster and have more eggs in their ovaries. Mean number of eggs from females on rich and poor larval diets, dissected at a range of ages (days). * $|Z| > 3.55$, $P < 0.001$ (Mann–Whitney *U*-tests comparing mean number of eggs between similarly-aged females reared on rich and poor larval diets).

4.2. Effects on lifespan

In line with previous work, we found a large lifespan extension effect of adult DR. However, we found neither an overall effect of larval diet on lifespan nor a significant larval × adult diet interaction effect.

We detected an interaction effect of larval diet and sex on lifespan, for flies housed in same-sex groups: males lived longer than females, but only when reared on a rich larval diet. Otherwise, social environment did not appear to affect lifespan.

Although we did not detect an interaction of larval and adult diet on lifespan, we did find that nutrient deprivation in development rendered flies less resilient to adult starvation, applied both at eclosion and late in life. This suggests that reserves built up during development can affect longevity at the adult stage under extreme conditions. This result is in line with the prediction represented by Fig. 1c, whereby flies on a poor larval diet are unable to make up for their poor start, in relation to those reared on a rich larval diet. The suggestion here is that even if a small body is less costly to maintain, stored larval reserves from plentiful resources in development will more than compensate for a potentially more costly phenotype. This idea is also supported by the finding that there is an effect of body size on starvation resistance for flies reared on a poor larval diet, whereby larger flies survive longer.

The sexes tended to respond differently to starvation, but this was dependent on both the larval diet and whether starvation was imposed early or late in life. We found no evidence that body size variation was driving the effects of larval diet, sex, or their interaction on survival. The effects may therefore reflect differences in availability of nutrients stored in development and throughout adulthood. Interestingly, the direction of the sex difference in late-life starvation resistance is reversed between the two larval diets. Specifically, for flies reared on poor larval diet and starved late in life, females lived longer than males, while males lived longer than females under late-life starvation when reared on a rich larval diet. This could perhaps reflect an increased relative investment in egg production, and thus increased costs, for females when reared on a rich larval diet. The fact that all flies in the starvation experiments were maintained as virgins would have been unlikely to have a large effect on relative differences in egg production between females from each larval diet, as *T. angusticollis* females will lay unfertilized eggs throughout adulthood, regardless of mating history, although they lay fewer on any given day than mated females (E. Bath, A. Sentinella, M.I. Adler, R. Bonduriansky, in preparation).

4.3. Effects on male and female reproduction

The effect of adult DR on reproductive capacity was also dependent on environment and sex. While adult DR rendered females completely infertile (their ovaries failed to develop), effects of adult DR on male fecundity were subtle, and dependent on both larval diet and adult social environment. Males subject to adult DR were

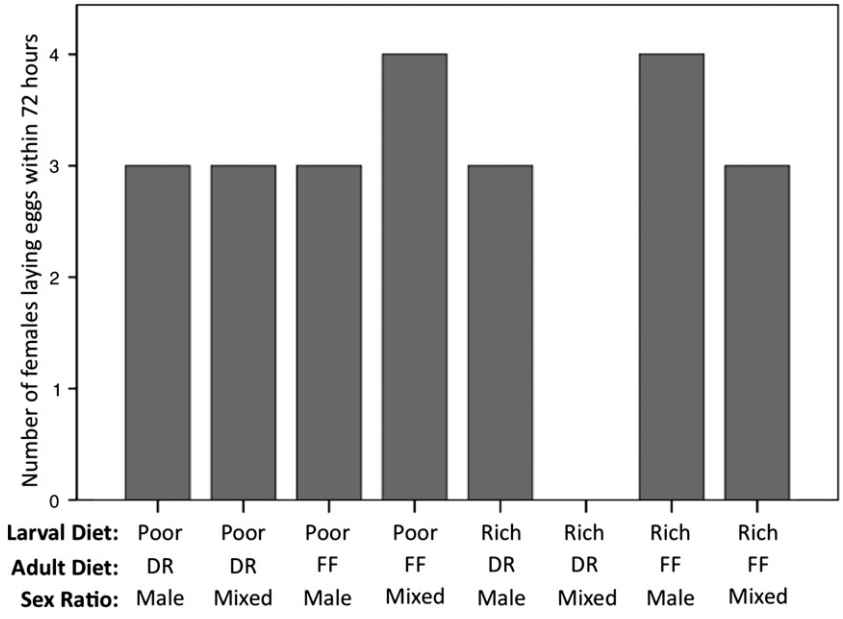

Fig. 5. Male ability to induce oviposition reflects an interaction of larval diet, adult diet and adult social environment. Number of females (out of 6) that laid eggs within 72 h of being mated to males from each combination of larval diet, adult diet and adult social environment treatments.

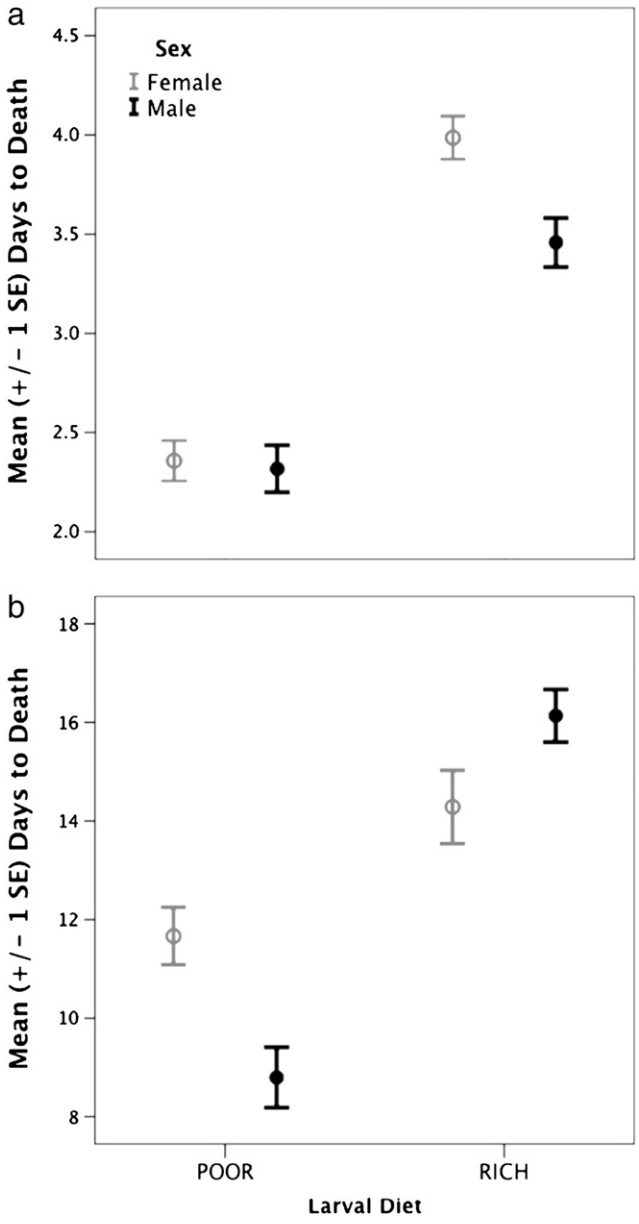

Fig. 6. Rich larval-diet flies survive longer under starvation. Lifespan of males and females on rich and poor larval diets, when deprived of food (a) in early-life (upon eclosion) and (b) in late-life (28 days post-eclosion).

capable of mating and siring viable offspring. Moreover, males on adult DR were not less likely overall to induce oviposition in their mates within 72 h after mating, and their offspring did not differ from those of fully-fed males in egg-to-adult viability or adult body size. However, males reared on a rich larval diet but subject to DR in adulthood and housed with females throughout life were unable to induce any oviposition within 72 h after mating in the male reproduction assay. This environment-dependent male cost of DR is intriguing because it suggests that multiple factors may be at play in both a male's facultative use of resources and the constraints that various environments impose on male fecundity. Males with plentiful larval resources may invest more into large body size and secondary sexual traits, important in competitive interactions in this species (Bonduriansky and Head, 2007), and this may come at the cost of investment in testes and ejaculate quality. However, this combination entailed a measurable cost only when the males had access to females throughout life, which may suggest that costly ejaculate products, such as accessory gland proteins (ACPs) that induce female oviposition, can be depleted, and cannot be replenished when dietary protein is scarce. This type of effect of mating history on male fecundity has been demonstrated in various studies that have found that male ejaculate quality and fecundity decline with each subsequent mating (reviewed in Torres-Vila and Jennions, 2005). Given the importance of body size in male–male competition for access to mates in this species (Bonduriansky and Head, 2007), it would be very informative to measure in the future how DR may affect performance in male–male combat.

We detected no overall effects of larval diet or its interaction with adult diet on any of our measures of male fecundity (i.e., number of eggs laid by females or fitness traits of offspring). In addition, we found no overall effect of social/mating environment on any of these measures. It is noteworthy that environmental effects can be subtle and revealed only in particular interactions. In this study, we found that adult DR revealed fecundity costs only in a particular combination of larval and social environments. The potential for subtle interactions of this kind is also highlighted by other results on male fecundity in this species. In a separate paper (Adler and Bonduriansky, 2012), we report that males with plentiful larval nutrients may have an advantage over males nutrient-deprived as larvae, in terms of offspring size and viability, but this advantage is dependent on the social environment/mating history of the males.

Resource restriction in development reduced female fecundity, both by reducing the number of eggs in the ovaries at all ages examined, and by delaying the age of reproductive maturity. Data from a separate study on this species (C. Fricke, M.I. Adler, R. Brooks & R. Bonduriansky, in prep.) revealed that females fully-fed in development also lay more eggs within 48 h of mating. These findings suggest that, as for males, female fitness is strongly affected by availability of nutrients in development. It is therefore likely that some adult female responses to DR may also interact with the larval diet. Unfortunately, we were not able to examine the interaction of larval and adult diets on other female reproductive parameters, as adult DR rendered females infertile.

On the other hand, it is clear that nutrient restriction in development constrains fitness for both sexes, in a manner that cannot be compensated by access to plentiful adult nutrients. As adult body size and shape are fixed upon eclosion for this and all holometabolous species, the effects of larval nutrient deprivation (smaller body size and lack of secondary sexual characters for males, and smaller ovaries and fewer ovarioles for females in *T. angusticollis*) are likely to carry fitness costs regardless of the adult environment. As with the effects we found of larval diet on starvation resistance, the effects of larval diet on male and female reproductive fitness are also likely to conform to the prediction represented by Fig. 1c. That is, both males and females will be unable to overcome the deficits entailed by a poor quality larval environment, regardless of the adult environment.

4.4. Reconciling DR with life history theory

Our finding that most aspects of male fecundity were unaffected by adult DR – and that indeed the only detectable cost of adult DR for males was dependent on both the larval diet and social environment – suggests strongly that the lifespan–reproduction trade-off expected to be revealed under DR is not straightforward, for males in particular. This finding is supported by other recent work in males of various insect species that has not conformed to classic trade-off predictions (see Introduction). Some of the most convincing evidence to call into question the resource re-allocation effect of DR in males comes from a study by Maklakov et al. (2008) in black field crickets. The authors found that the dietary protein:carbohydrate ratio that maximized female lifespan was very different from that which maximized female reproduction. However, the diet on which male reproductive investment was highest (assayed as nightly and lifetime calling rate) included, in

stark contrast to trade-off predictions, a very similar nutrient ratio to the diet that resulted in the longest lifespans. While Maklakov et al.'s study measured a crucial feature of male reproductive investment in this species, i.e. calling rate, it did not measure the ability to copulate or produce offspring, and it is possible that these functions may be optimized on other diets. Taken together, our findings and those of Maklakov et al. (2008), suggest that adult DR's effects on male reproductive performance are subtle, although such effects can be detected in certain ecologically-relevant environments.

On the other hand, females under adult DR experienced greatly extended longevity accompanied by complete infertility. The contrasting responses of males and females to DR strongly suggest a sex-difference in optimal diet, as has also been concluded by other authors (Maklakov et al., 2008). However, even though our results for females are in line with classic trade-off predictions, they are not sufficient to conclude that a re-allocation of resources from reproduction to somatic functions underlies the extended lifespan observed under DR. Our results, and those of many other studies with similar findings, are also consistent with alternative mechanisms by which lifespan could be extended, notably that DR may simply be associated with reduced costs in comparison with full-feeding regimes. This may be due to a toxic effect of an imbalanced or overly nutrient-rich standard laboratory diet (e.g. Raubenheimer et al., 2005, as discussed in Introduction). While females in this study clearly benefited reproductively from extra protein, it might be the case that a more balanced diet would have restored reproduction without a concurrent reduction in lifespan. In addition to the possibility of toxicity, full-feeding appears to increase costs by activating a number of key nutrient-sensing pathways that down-regulate functions associated with recycling and repair, notably autophagy (Longo and Fontana, 2010). These pathways allow the organism to take advantage of plentiful resources by increasing reproductive potential, but at the cost of long-term survival (reviewed in M.I. Adler and R. Bonduriansky, in review). This scenario does not necessitate a direct re-allocation of resources but sets the stage for trade-offs between reproduction and longevity that are likely to be sensitive to multiple environmental variables.

The results of the present study suggest that reproductive capacity need not trade off with lifespan, as revealed by examining DR's effects across a range of relevant environments, in both sexes, and for a range of performance contexts. This suggests that the lifespan extension effect of DR does not result from a simple resource-allocation trade-off; rather, the optimization of reproduction vs. longevity may be environment- and sex-dependent.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

We are grateful to Mark Brown, Angela Crean, Eddie Aloise King, Michael Kasumovic and Rob Brooks for helpful discussion and comments, Eve Slavich, Bart Adriaenssens, Michael Garratt and David Wharton for statistical guidance and advice, and Bernice & Morton Hunt for copy editing the manuscript. The research was funded through a PhD scholarship to MIA from the University of New South Wales and the Evolution & Ecology Research Centre, and an Australian Research Council Discovery grant to RB.

References

Adler, M.I., Bonduriansky, R., 2011. The dissimilar costs of love and war: age-specific mortality as a function of the operational sex ratio. J. Evol. Biol. 24, 1169–1177.
Adler, M.I., Bonduriansky, R., 2012. Paternal effects on offspring fitness reflect father's social environment. Evol. Biol. http://dx.doi.org/10.1007/s11692-012-9211-6.
Arnqvist, G., Nilsson, T., 2000. The evolution of polyandry: multiple mating and female fitness in insects. Anim. Behav. 60, 145–164.
Baker, R.H., Denniff, M., Futerman, P., Fowler, K., Pomiankowski, A., Chapman, T., 2003. Accessory gland size influences time to sexual maturity and mating frequency in the stalk-eyed fly, *Cyrtodiopsis dalmanni*. Behav. Ecol. 14, 607–611.
Bateman, A., 1948. Intra-sexual selection in *Drosophila*. Heredity 2, 349–368.
Bath, E., Tatarnic, N., Bonduriansky, R., 2012. Asymmetric reproductive isolation and interference in neriid flies: the roles of genital morphology and behaviour. Anim. Behav. 84, 1331–1339.
Bauerfeind, S.S., Fischer, K., 2005. Effects of food stress and density in different life stages on reproduction in a butterfly. Oikos 111, 514–524.
Blay, S., Yuval, B., 1997. Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Anim. Behav. 54, 59–66.
Boggs, C.L., 2009. Understanding insect life histories and senescence through a resource allocation lens. Funct. Ecol. 23, 27–37.
Boggs, C.L., Freeman, K.D., 2005. Larval food limitation in butterflies: effects on adult resource allocation and fitness. Oecologia 144, 353–361.
Boggs, C.L., Ross, C.L., 1993. The effect of food limitation on life history traits in *Speyeria mormonia* (Lepidoptera: Nymphalidae). Ecology 74, 433–441.
Bonduriansky, R., 2007. The evolution of condition-dependent sexual dimorphism. Am. Nat. 169, 9–19.
Bonduriansky, R., Head, M., 2007. Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). Evol. Biol. 20, 2379–2388.
Carey, J.R., Harshman, L.G., Liedo, P., Müller, H.-G., Wang, J.-L., Zhang, Z., 2008. Longevity-fertility trade-offs in the tephritid fruit fly, *Anastrepha ludens*, across dietary-restriction gradients. Aging Cell 7, 470–477.
Chapman, T., 2001. Seminal fluid-mediated fitness traits in *Drosophila*. Heredity 87, 511–521.
Dewsbury, D.A., 1982. Ejaculate cost and male choice. Am. Nat. 119, 601–610.
Dmitriew, C., Rowe, L., 2011. The effects of larval nutrition on reproductive performance in a food-limited adult environment. PLoS One 6, e17399.
Ellers, J., Jervis, M., 2003. Body size and timing of egg production in parasitoid wasps. Oikos 102, 164–172.
Engels, S., Sauer, K.P., 2007. Energy beyond the pupal stage: larval nutrition and its long-time consequences for male mating performance in a scorpionfly. J. Insect Physiol. 53, 633–638.
Fanson, B.G., Fanson, K.V., Taylor, P.W., 2012. Cost of reproduction in the Queensland fruit fly: Y-model versus lethal protein hypothesis. Proc. R. Soc. B Biol. Sci. 279, 4893–4900.
Fricke, C., Bretman, A., Chapman, T., 2008. Adult male nutrition and reproductive success in *Drosophila melanogaster*. Evolution 62, 3170–3177.
Gage, M.J.G., Cook, P.A., 1994. Sperm size or numbers? Effects of nutritional stress upon eupyrene and apyrene sperm production strategies in the moth *Plodia interpunctella* (Lepidoptera: Pyralidae). Funct. Ecol. 8, 594–599.
Gosden, T.P., Chenoweth, S.F., 2011. On the evolution of heightened condition dependence of male sexual displays. J. Evol. Biol. 24, 685–692.
Grandison, R.C., Piper, M.D.W., Partridge, L., 2009. Amino-acid imbalance explains extension of lifespan by dietary restriction in *Drosophila*. Nature 462, 1061–1064.
Gwynne, D.T., Simmons, L.W., 1990. Experimental reversal of courtship roles in an insect. Nature 346, 172–174.
Hunt, J., Brooks, R., Jennions, M.D., Smith, M.J., Bentsen, C.L., Bussiere, L.F., 2004. High-quality male field crickets invest heavily in sexual display but die young. Nature 432, 1024–1027.
Kasumovic, M.M., Brooks, R.C., 2011. It's all who you know: the evolution of socially cued anticipatory plasticity as a mating strategy. Q. Rev. Biol. 86, 181–197.
Kvarnemo, C., Simmons, L.W., 1999. Variance in female quality, operational sex ratio and male mate choice in a bushcricket. Behav. Ecol. Sociobiol. 45, 245–252.
Lee, K.P., Simpson, S.J., Clissold, F.J., Brooks, R., Ballard, W.O., Taylor, P.W., Soran, N., Raubenheimer, D., 2008. Lifespan and reproduction in *Drosophila*: new insights from nutritional geometry. Proc. Natl. Acad. Sci. 105, 2498–2503.
Longo, V.D., Fontana, L., 2010. Calorie restriction and cancer prevention: metabolic and molecular mechanisms. Trends Pharmacol. Sci. 31, 89–98.
Mair, W., Sgrò, C.M., Johnson, A.P., Chapman, T., Partridge, L., 2004. Lifespan extension by dietary restriction in female *Drosophila melanogaster* is not caused by a reduction in vitellogenesis or ovarian activity. Exp. Gerontol. 39, 1011–1019.
Mair, W., Piper, M.D.W., Partridge, L., 2005. Calories do not explain extension of life span by dietary restriction in *Drosophila*. PLoS Biol. 3, 1305–1311.
Maklakov, A.A., Simpson, S.J., Zajitschek, F., Hall, M.D., Dessmann, J., Clissold, F., Raubenheimer, D., Bonduriansky, R., Brooks, R.C., 2008. Sex-specific fitness effects of nutrient intake on reproduction and lifespan. Curr. Biol. 18, 1062–1066.
Masoro, E.J., 2005. Overview of caloric restriction and ageing. Mech. Ageing Dev. 126, 913–922.
Merry, B.J., 1995. Effect of dietary restriction on aging — an update. Rev. Clin. Gerontol. 5, 247–258.
Metcalfe, N., Monaghan, P., 2001. Compensation for a bad start: grow now, pay later? Trends Ecol. Evol. 16, 254–260.
Monaghan, P., Charmantier, A., Nussey, D.H., Ricklefs, R.E., 2008. The evolutionary ecology of senescence. Funct. Ecol. 22, 371–378.
Nakagawa, S., Lagisz, M., Hector, K.L., Spencer, H.G., 2012. Comparative and meta-analytic insights into life extension via dietary restriction. Aging Cell 11, 401–409.
Nylin, S., 1988. Host plant specialization and seasonality in a polyphagous butterfly, *Polygonia c-album* (Nymphalidae). Oikos 53, 381–386.
Partridge, L., Gems, D., 2007. Benchmarks for ageing studies. Nature 450, 165–167.
Piper, M.D.W., Partridge, L., 2007. Dietary restriction in *Drosophila*: delayed aging or experimental artefact? PLoS Genet. 3, e57.
Raubenheimer, D., Lee, K.P., Simpson, S.J., 2005. Does Bertrand's Rule apply to macronutrients? Proc. R. Soc. B 272, 2429–2434.

Shanley, D.P., Kirkwood, T.B.L., 2000. Calorie restriction and aging: a life-history analysis. Evolution 54, 740–750.
Simpson, S.J., Raubenheimer, D., 2007. Caloric restriction and aging revisited: the need for a geometric analysis of the nutritional bases of aging. J. Gerontol. 62A, 701–713.
Torres-Vila, L.M., Jennions, M.D., 2005. Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? Behav. Ecol. Sociobiol. 57, 318–326.
Tu, M.P., Tatar, M., 2003. Juvenile diet restriction and the aging and reproduction of adult *Drosophila melanogaster*. Aging Cell 2, 327–333.
Venables, W.N., Ripley, B.D., 2002. Modern Applied Statistics with S, Fourth ed. Springer, New York.
Wayne, M.L., Mackay, T.F.C., 1998. Quantitative genetics of ovariole number in *Drosophila melanogaster*. II. Mutation variation and genotype-environment interaction. Genetics 148, 201–210.
Wedell, N., Gage, M.J.G., Parker, G.A., 2002. Sperm competition, male prudence and sperm-limited females. Trends Ecol. Evol. 17, 313–320.
Wolfner, M.F., 2002. The gifts that keep on giving: physiological functions and evolutionary dynamics of male seminal proteins in *Drosophila*. Heredity 88, 85–93.
Zajitschek, F., Hunt, J., Jennions, M.D., Hall, M.D., Brooks, R.C., 2009. Effects of juvenile and adult diet on ageing and reproductive effort of male and female black field crickets, *Teleogryllus comodus*. Funct. Ecol. 23, 602–611.
Zajitschek, F., Zajitschek, S.R.K., Friberg, U., Maklakov, A.A., 2012. Interactive effects of sex, social environment, dietary restriction, and methionine on survival and reproduction in fruit flies. Age. http://dx.doi.org/10.1007/s11357-012-9445-3.
Zimmerman, J., Malloy, V., Krajcik, R., Orentreich, N., 2003. Nutritional control of aging. Exp. Gerontol. 38, 47–52.
Zwaan, B., Bijlsma, R., Hoekstra, R., 1991. On the developmental theory of ageing. I. Starvation resistance and longevity in *Drosophila melanogaster* in relation to preadult breeding conditions. Heredity 66, 29–39.