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James R. Carey

University of California, Davis, jrcarey@ucdavis.edu

Lawrence G. Harshman

University of Nebraska - Lincoln, lharshman1@unl.edu

Pablo Liedo

El Colegio de la Frontera Sur, Tapachula, Chiapas, pliedo@ecosur.mx

Hans-Georg Müller

University of California, Davis, hgmuller@ucdavis.edu

Jane-Ling Wang

University of California, Davis, janelwang@ucdavis.edu

See next page for additional authors

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Authors

James R. Carey, Lawrence G. Harshman, Pablo Liedo, Hans-Georg Müller, Jane-Ling Wang, and Zhen Zhang



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Longevity-Fertility Trade-offs in the Tephritid Fruit Fly, *Anastrepha ludens*, across Dietary-restriction Gradients

James R. Carey^{1,2,*}, Lawrence Harshman³, Pablo Liedo⁴, Hans-Georg Müller⁵, Jane-Ling Wang⁵, and Zhen Zhang⁵

¹Department of Entomology, University of California, Davis, CA 95616

²Center for the Economics and Demography of Aging, University of California, Berkeley 94720 USA

³Department of Biological Sciences, University of Nebraska, Lincoln, NE

⁴El Colegio de la Frontera Sur (ECOSUR), Tapachula, Chiapas, Mexico

⁵Department of Statistics, University of California, Davis, CA 95616 USA; current address, Abbott Vascular, Inc., 3200 Lakeside Dr., Santa Clara, CA 95054-2807

Summary

Although it is widely known that dietary restriction (DR) not only extends the longevity of a wide range of species but also reduces their reproductive output, the interrelationship of DR, longevity-extension and reproduction is not well understood in any organism. Here we address the question: “*Under what nutritional conditions do the longevity-enhancing effects resulting from food restriction either counteract, complement or reinforce the mortality costs of reproduction?*” To answer this question we designed a fine-grained DR study involving 4,800 individuals of the tephritid fruit fly *Anastrepha ludens* in which we measured sex-specific survival and daily reproduction in females in each of 20 different treatments (sugar:yeast ratios) plus 4 starvation controls. The database generated from this 3-year study consisted of approximately 100,000 life-days for each sex and 750,000 eggs distributed over the reproductive lives of 2,400 females. The fertility and longevity-extending responses were used to create contour maps (X-Y grid) that show the demographic responses (Z-axis) across dietary gradients that range from complete starvation to both *ad libitum* sugar-only and *ad libitum* standard diet (3:1 sugar-to-yeast). The topographic perspectives reveal demographic equivalencies along nutritional gradients, differences in the graded responses of males and females, egg production costs that are sensitive to the interaction of food amounts and constituents, and orthogonal contours (equivalencies in longevity or reproduction) representing demographic thresholds related to both caloric content and sugar-yeast ratios. In general, the finding that lifespan and reproductive maxima occur at much different nutritional coordinates poses a major challenge for the use of food restriction (or a mimetic) in humans to improve health and extend longevity in humans.

Keywords

cost of reproduction; longevity extension; food restriction; *Anastrepha ludens*; life tables; contour graphs

*Correspondence to: James R. Carey, Department of Entomology, One Shields Ave., University of California, Davis, CA 95616 USA, 530-752-6217, jrcarey@ucdavis.edu.

Introduction

The longevity-extending effects of dietary restriction (DR), known since the seminal work on rats by McCay (McCay *et al.* 1935), are widely documented in research on this laboratory rodent (Davis *et al.* 1984; Masoro 1988) as well as on a wide variety of other species. Excepting diet-restricted house flies (*Musca domestica*) which do not experience a longevity increase (Cooper *et al.* 2004), these species include conventional model organisms such the yeast *Saccharomyces cerevisiae* (Lin *et al.* 2002), the nematode *Caenorhabditis elegans* (Lakowski & Hekimi 1998; Houthoofd *et al.* 2005; Lee & al 2006), the fruit fly *Drosophila melanogaster* (Chippindale *et al.* 1997; Good & Tatar 2001; Clancy *et al.* 2002; Partridge *et al.* 2005b; Piper *et al.* 2005; Pletcher *et al.* 2005; Min & Tatar 2006b), the zebrafish *Danio rerio* (Keller *et al.* 2006), the mouse *Mus musculus* (Weindruch & Walford 1982; Weindruch & Walford 1988; Weindruch 1996; Austad & Kristan 2003; Harper *et al.* 2006), and the rhesus monkey *Macaca mulatta* (DeRousseau 1994; Bodkin *et al.* 2003; Mattison *et al.* 2003; Lane & al 2004), and several non-traditional species including the spider *Frontinella pyramitela* (Austad 1989) and the medfly *Ceratitis capitata* (Carey *et al.* 1998a; Carey *et al.* 2002; Carey *et al.* 2005b). The underlying assumption regarding why this longevity-extension response has been conserved across such a wide variety of species is that it serves as a mechanism for bridging dearth periods by (among other changes) reducing the metabolic demand of reproduction and thereby postponing reproductive senescence (Harrison & Archer 1989; Holliday 1989; Shanley & Kirkwood 2000).

Despite a near-universal agreement among biologists that the longevity-extending effects of DR are the evolutionary outcome of selection for individuals capable of surviving through and reproducing after unfavorable periods, until recently remarkably little research was conducted on the relationship between reproduction, longevity-extension, and DR (Partridge *et al.* 2005a; Lee & al 2006). One reason for the paucity of early work was that much of the DR research involving reproduction was on rodents (e.g. Visscher *et al.* 1952; Merry & Holehan 1979; Bronson & Marsteller 1985; Hamilton & Bronson 1985; Holehan & Merry 1985; Nelson *et al.* 1985; Krackow 1989) and therefore, both cost and time constraints precluded large-scale studies designed to assess the effect of multiple DR treatments on reproduction. With rare exceptions (e.g. Chippindale *et al.* 1997) the majority of DR research involving the joint effects on longevity extension and reproduction using *D. melanogaster* (Good & Tatar 2001; Mair *et al.* 2003; Tu & Tatar 2003; Mair *et al.* 2004; Mair *et al.* 2005; Min & Tatar 2006b; Min & Tatar 2006a; Bass *et al.* 2007; Burger *et al.* 2007) as well as using both the nematode *C. elegans* (Lee & al 2006), and the Mediterranean fruit fly *C. capitata* (Carey *et al.* 1998a; Carey *et al.* 2002; Carey *et al.* 2005b) has been conducted relatively recently.

The results of these and related (Bross *et al.* 2005; Bhandari *et al.* 2007; Burger *et al.* 2007; Ja & al 2007; Libert *et al.* 2007) DR studies in *Drosophila* and other invertebrates underscore the complexity of the relationship. On the one hand, the results of experiments on *D. melanogaster* reveal that lifespan extension via DR is not exclusively due to the reduction in reproductive costs (Mair *et al.* 2004). On the other hand, mortality costs are still inextricably linked to reproduction in that, regardless of how the level of reproduction is manipulated whether through sterilizing radiation (Carey *et al.* 2001; Mair *et al.* 2004) or through access to mates (Partridge *et al.* 1987; Rogina *et al.* 2007), oviposition hosts (Carey *et al.* 1986), or protein-rich diets (Good & Tatar 2001; Piper *et al.* 2005), cohorts that are capable of laying their full complements of eggs nearly always experience higher mortality rates than do cohorts in which egg laying is arrested.

Although these findings shed important light on the complexity of the relationship of DR, longevity extension, and the cost of reproduction, important questions still remain including

the one we use to frame our study: “Under what nutritional conditions do the longevity-altering effects resulting from food restriction either counteract, complement or reinforce the mortality costs of reproduction?” Although the collective DR studies have examined the food restriction response of organisms across a large array of treatments, including measurement of survival in both sexes as well as female reproduction (Graves 1993; Chapman & Partridge 1996; Chapman *et al.* 1998; Good & Tatar 2001; Piper *et al.* 2005), heretofore there have been no previous investigations that have included all of these aspects in a single study. i.e. monitoring daily survival of large numbers of both sexes subjected to multiple DR treatments across both caloric and sugar-yeast gradients, with age-specific and lifetime reproduction measured in females.

In light of this shortcoming in the literature, we initiated fine-grained DR studies on both sexes of the tephritid fruit fly, *Anastrepha ludens*, commonly known as the Mexican fruit fly (Carey *et al.* 2005a), and measured daily survival and female reproduction. The *A. ludens* system was chosen because large numbers of newly-eclosed flies of both sexes were continually available for use in conducting large-scale experiments, the magnitude of both lifetime reproduction and life expectancy varied substantially across restriction treatments, and the range of sugar-yeast combinations elicited changes in longevity and reproduction that were both positively- and negatively-correlated. An additional advantage of this system was that food could be dispensed in micro-quantities using a methodology that, on the one hand, used rodent restriction-like methods (Masoro 1988; Masoro 1995; Shanley & Kirkwood 2000) to limit the quantity of food and, on the other hand, used *Drosophila* restriction-like (dilution) methods to alter the quality of food (Carvalho *et al.* 2005; Partridge *et al.* 2005b). This allowed us to create a 2-dimensional, ‘fine-grained’ treatment grid consisting of yeast restriction on one axis and of sugar restriction on the other. We gathered data on a total of 4,800 flies (see Methods) including daily survival and female reproduction for approximately 100 individuals of each sex fed daily according to a 24-treatment design using 4 compositional levels (sugar-only and 24:1, 9:1 and 3:1 sugar:yeast (SY) ratios) and 6 concentrations relative to the base stock (100%, 75, 50, 25, 10 and 0). Contour maps based on the response surface data (i.e. response plotted on Z axis with respect to variables plotted on the X and Y axes) were constructed for male and female life expectancies and lifetime reproduction in females using local linear fitting techniques (Facer & Müller 2003). We use SY to denote sugar:yeast ratio, CR to denote calorie restriction, and DR to denote the general concept of dietary restriction (i.e. a reduction in food availability).

Results

Longevity extension due to food restriction was evident for both sexes with the highest life expectancy occurring at intermediate levels of both calorie and yeast restriction (Table 1). For example, the highest average life expectancy along the calorie restriction gradient occurred in the 9-to-1 SY treatment cohort where males and females lived 53.1 and 59.5 days, respectively, and along the yeast restriction gradient at the 25% dilution treatment where males and females lived 49.3 and 53.0 days, respectively. The range in female life expectancies varied by nearly 40 days (28.2 vs 67.2 days) and male life expectancy varied by over 32 days (26.4 to 58.8). With the exception of the female cohort subject to the 3:1 SY/10% CR treatment, life expectancy was significantly less in the sugar-only treatments than in the full diet (SY=3:1) treatments at all levels of CR. Indeed, life expectancy for females and males subject to all of the sugar-only (SY=1:0) treatments averaged 17 and 19 days respectively less than for cohorts subject to all of the full-diet treatments.

Lifetime fecundity in females was dependent primarily on yeast composition and secondarily on caloric content (Table 2). For example, per capita egg laying in treatments involving different sugar-yeast levels ranged from 4 to 8 eggs in the sugar-only treatments,

25 to 50 eggs in 24:1 SY treatments, 180 to 280 eggs in the 9:1 treatments, and excepting the 10% CR level, over 850 eggs in the 3:1 SY treatments. In contrast, large differences in lifetime fecundity occurred between treatments in which caloric restriction levels were similar with cohorts maintained on sugar-only diets (across all caloric restriction levels) averaging 6 eggs/female and cohorts maintained on 3:1 SY diets averaging nearly 780 eggs/female. These differences reveal the profound importance of yeast content in the diet inasmuch as lifetime fecundity differed by a maximum of 2-fold across CR treatments but a remarkable 100-fold across the SY gradient.

The event-history graphs (Carey *et al.* 1998b) contained in Fig. 1 arranged by columns (SY) and rows (CR) show the large increase in levels of age-specific reproduction from left-to-right (increasing yeast concentration) but little change from bottom-to-top (increasing caloric levels). Differences in survival across the CR-SY treatments were less pronounced than were differences in reproduction with high survival rates in sugar-only cohorts (left-most column of graphs) at young ages but near-zero survival by 60 days. Broad patterns of survival in the 24:1 yeast restriction treatments were similar except that the near-linear decline was less steep than in the sugar-only (1:0) cohorts. For the 9:1 and 3:1 treatments the survival curves were noticeably more concave due to low mortality at young ages followed by increasing mortality at middle and older ages (i.e. >30 days). The trajectories of the survival curves at the 10% caloric restriction treatments declined rapidly for the first 2 weeks and leveled off to a much slower rate of decrease thereafter. Cohorts that exhibited the highest life expectancies were similar in two respects: i) all were maintained on an intermediate (9:1) SY regime; and ii) for females, all exhibited moderate level lifetime fecundity (i.e. between 210 to 280 eggs/female).

The contour graphs (Fig. 2) that were constructed from the response surface data (supporting online material) reveal the within- and between-sex equivalencies in both levels and rates of change for longevity of both sexes and for female reproduction across the entire nutritional domain. Two nutritional thresholds are also evident: i) a CR threshold (≈ 0.10) below which both female reproduction and survival of both sexes decline precipitously, and ii) a SY threshold ($\approx 5\%$) below which reproduction declines precipitously but where survival decreases gradually. The square contours in the reproductive surface (Fig. 2) show that the yeast level sets a threshold in terms of the range of increasing reproduction due to increasing calories, and calories set a threshold in terms of the corresponding domain where yeast increase leads to reproduction increase.

Reproduction steadily increases in each of the graphs in Fig. 2 from left-to-right (increasing yeast composition) but not from top-to-bottom (or vice versa). More calories at a fixed yeast fraction do not increase reproduction even though absolute yeast intake increases with the increase in calories under these conditions. In contrast to the monotonic changes in reproduction with changes in dietary restriction levels, survival increases and then decreases (is bimodal) as DR levels change.

Contour graphs constructed from the female longevity and reproduction data yielded additional insights into the demographics of DR that were not apparent from the data contained in the tables or visualized in other figures. The intensity of egg laying (Fig. 2; right panel) is mainly regulated via yeast and not calories. Flies need to have access to high quality foods once they have the minimum calories needed to sustain minimum longevity without which reproduction would be low.

Age-specific reproductive schedules were estimated using local linear fitting techniques (Facer & Müller 2003) for points along the transect shown in the inset in Figure 3. These connect the nutritional coordinates corresponding to maximum longevity (blue circle) to the

coordinates corresponding to maximum reproduction (red circle). The color-coded schedules corresponding to the points along the transect show that the changes in age-specific reproduction along this transect (max longevity-to-max reproduction) include an increase in both peak and total reproduction, the emergence of a distinct reproductive peak, a reduction in the mean age of reproduction, and a convergence to a rate of one or fewer eggs per day for females beyond around 70 days for all cohorts. The longevity decrease and reproductive increase along the transect (Figure 3 inset) reveals a mortality cost of reproduction. Conversely, the reproductive decrease with age reveals a reproductive cost of aging—total egg production by day 40 differs by 5-fold across cohorts yet all reproductive schedules decrease rapidly after this age independent of the level of egg production at younger ages.

Discussion

The current study involving fine-grained treatments and measurements of mortality in both sexes and of individual-level female reproduction produced results that shed new light on several aspects of the effects of food restriction on reproductive costs and longevity. One revelation was the difficulty of identifying the right DR combination that maximizes longevity when individuals are allowed to reproduce freely. The greatest longevity (especially for females) occurred in a relatively small nutritional parameter space where reproductive costs for the flies were minimized relative to the maintenance benefits. The longevity isoclines that emerged along the treatment gradients revealed that small changes in longevity may be observed even with large changes in restriction levels. This observation may partly explain why longevity increases are observed in many restriction studies (Harshman & Zera 2006) but not in all (LeBourg & Minois 1996; Carey *et al.* 2002). That similar patterns (isoclines) of longevity change over the same nutritional gradients were observed in both sexes despite the lack of an obvious counterpart in males to the cost of egg laying in females suggests that males also experience an energetically-derived cost of reproduction (Yuval *et al.* 1998). This general finding is similar to the results of a recent study on the effects of DR on sex differences in lifespan and mortality differences in *D. melanogaster* in which (Magwere *et al.* 2004): i) lifespan peaked at an intermediate food concentration; and ii) the relative magnitude of the longevity-extension response due to DR was greater in females. However, unlike their findings that the food concentrations that minimized adult mortality in *D. melanogaster* were different between males and female, they were approximately the same in the current study with *A. ludens*. This may be due either to species-specific differences in the behavior and ecology (Bateman 1972) that impacts sex-specific differences between the species or to differences in the experimental designs. Whereas the *D. melanogaster* study (Magwere *et al.* 2004) involved 8 types of food media (treatments) containing identical sugar-yeast ratios but different agar dilutions that altered the food concentration (i.e. DR dilution method), the protocol in the current study involved 20 treatments based on fixed amounts of food available daily to flies that differed in both sugar-yeast concentrations and ratios.

Another important insight from the current study was that the location of maximum reproduction and maximum female longevity at different nutritional coordinates (i.e. diet amount and composition) suggests that maximum lifespan is not necessary for maximum reproduction, and that the nutritional conditions for maximum reproduction are not conducive to maximum lifespan. It is thus doubtful, even in principle, that a single diet exists in which both reproduction and longevity are maximized—the greatest longevity is achieved at intermediate nutritional levels whereas the highest reproduction is achieved at maximal nutritional levels.

A third important result was the discovery that the effect on longevity extension in *A. ludens* due to CR for the calories that are derived from yeast depended on the caloric content of the

food available. This was evident from the contour graphs (Fig. 2) showing that the effect of increased yeast on longevity was greater when overall restriction levels were high. This result complements recent findings on *D. melanogaster* in which Mair and co-workers (Mair *et al.* 2005) noted that yeast had a much greater effect on longevity extension per calorie than did sugar. Whereas these researchers concluded that calorie intake is not the key factor in determining mortality rate in *D. melanogaster*, our result suggests that the exact impact is conditional on caloric content of the diet and not just calories independent of dietary composition.

A general perspective that was evident from our results is the notion that food restriction regimes that maximize longevity and those that maximize reproduction are fundamentally different. Although negative effects of food restriction on reproductive capacity or performance are well documented including menstrual irregularities in women (Dirks & Leeuwenburgh 2006) and loss of libido in men (Frisch 2002), the importance of the longevity-fertility nutritional conundrum is seldom addressed. Thus one major challenge for research on longevity extension using either food restriction or a mimetic (Ingram *et al.* 2006) is finding a diet (if indeed one single diet exists) and identifying mechanisms that extend longevity but have minimal negative effects on reproduction, health, and quality-of-life components (Heilbronn *et al.* 2006).

Methods

The design for this study was created using the 24 two-way combinations involving 6 caloric restriction (CR) levels on one axis (relative to content of *ad libitum* mother stock) by 4 ratios of sugar-yeast (SY) on the other. The 4 SY ratios were 3:1, 9:1, 24:1, and 1:0 (i.e. sugar-only; no yeast) and the 6 CR levels were 100, 75, 50, 25, 10 and 0% [even though the water-only (0% CR) treatments were redundant, all four were retained for operational simplicity]. The percent yeast contents for the 4 SY ratios were 25, 10, 4, and 0%, respectively. The caloric and yeast content of each of 24 food-restriction treatments used in the study are given in Table S-1.

A 1.5 μ l droplet of the appropriate food treatment and a 6 μ l droplet of water were supplied to individual flies each day on glass slides using separate Eppendorf® needles. Newly-emerged (virgin) individual flies were housed in 4 × 4 × 10 cm plexiglass cages, each of which was part of a 24-unit cage unit. Treatment distribution was made in a randomized block design, using different color codes to identify both the treatment and the food slides. Females and males were placed in alternate cages to eliminate the possibility of eggs from two females overlapping on the egg collection surface. Females laid eggs through organdy mesh fastened to the front of the cage and were counted daily using an electronic image analysis system that automatically records the number of eggs that fall in a 4.5 × 4.5 cm² field. A total of 100 individuals of each sex were used per treatment. Daily mortality and female reproduction were monitored daily throughout the life of each fly. Environmental conditions were 12:12 LD cycle, 24.0° C (\pm 2°) and 65% RH (\pm 9%).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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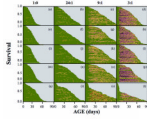


Figure 1.

Event history charts (Carey *et al.* 1998b) for *A. ludens* females showing relationship of cohort survival and individual-level reproduction across treatments. The ratios across the top denote the sugar:yeast ratios and the numbers aligned vertically at the right denote the percent concentration of the original stock solution (see Methods). Each horizontal line within a graph denotes a life line for an individual female, the length of which is proportional to her lifespan and with color-coded segments corresponding to age classes. The age-specific egg laying intensity for each individual female corresponds to the shading: green=zero eggs; yellow=1–20 eggs; red=>20 eggs.

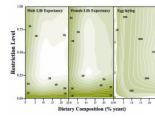


Figure 2.

Contour graphs showing the response surfaces for restriction level (concentration relative to *ad libitum* mother stock) and percent yeast in *A. ludens*— left, middle and right panels shows male life expectancy (days), female life expectancy, and female lifetime egg reproduction, respectively. Local linear fitting techniques (Facer & Müller 2003) were used to produce the numerical values of the response surfaces from the data and DeltaGraph® graphics program was used to generate the contour maps.

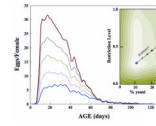


Figure 3.

Age-specific (net) reproduction functions for the surface transect that connects the coordinates corresponding to the longevity (blue dot) and reproduction (red dot) maxima with three equidistant highlighted locations on the transect line in the female longevity contour graph shown in the inset (from Figure 2b). Colors for each transect location correspond to those for the reproductive schedules. Lifetime egg production for the respective schedules are 295, 463, 663, 872, and 1,086 eggs/female, respectively, and the mean age of reproduction are 37.4, 34.0, 32.4, 31.6 and 29.9 days, respectively.

Table 1

Expectation of life (days) for *A. ludens* females and males subject to various combinations of yeast and caloric restriction regimes. The column labels refer to percentage of *ad libitum* food flies were given access to daily and the row labels refer to the dietary composition of sugar-to-yeast. Both linear and quadratic effects of yeast fraction and percentage of *ad libitum* food are statistically significant ($p < 0.001$, see supporting online material for details).^a.

	100%	75%	50%	25%	10%	Means
FEMALES						
Sugar (1:0)	28.2	30.1	31.1	33.4	36.9	31.9
24-to-1	45.5	49.9	51.8	57.4	48.0	50.5
9-to-1	55.6	64.5	63.4	67.2	46.8	59.5
3-to-1	51.3	52.4	55.2	53.9	30.0	48.6
Means	45.2	49.2	50.4	53.0	40.4	47.6
MALES						
Sugar	26.4	27.4	30.2	34.1	33.0	30.2
24-to-1	42.1	39.2	43.3	48.6	46.0	43.8
9-to-1	43.7	52.3	58.6	58.8	52.1	53.1
3-to-1	50.1	56.0	55.1	55.7	39.5	51.3
Means	40.6	43.7	46.8	49.3	42.6	44.6

^alife expectancies for female cohorts maintained on water-only were 4.0, 4.0, 4.1, and 3.9 days for sugar, 24-to-1, 9-to-1, and 3-to-1 diets, respectively and for male cohorts were 4.2, 4.3, 4.4, and 4.3, respectively.

Table 2

Lifetime egg production for *A. ludens* females subject to different combinations of dietary dilution levels and dietary ratios. i.e. 1-to-0 refers to sugar-only, 24-to-1, 9-to-1, and 3-to-1 refers to 24, 9, and 3 parts sugar to one part yeast, respectively. For log (lifetime egg production), both linear and quadratic effects of yeast fraction and percentage of *ad libitum* food are statistically significant (p-values <0.03, see supporting online material for details).

Dietary Ratios	Percent Dietary Content ^a					Means
	100%	75%	50%	25%	10%	
Sugar (1-to-0)	3.8	6.7	7.2	4.7	7.6	6.0
24-to-1	24.6	16.6	18.2	25.0	48.3	26.5
9-to-1	179.3	209.9	241.7	281.8	201.8	222.9
3-to-1	875.6	891.2	1071.4	861.8	191.4	778.3
MEANS	270.8	281.1	334.6	293.3	112.3	258.4

^a no eggs were produced in 0% (water-only) dietary content treatments