2002

Life History Response of Mediterranean Fruit Flies to Dietary Restriction

James R. Carey
University of California, Davis, jrcarey@ucdavis.edu

Pablo Liedo
El Colegio de la Frontera Sur, pliedo@ecosur.mx

Lawrence G. Harshman
University of Nebraska–Lincoln, lharshman1@unl.edu

Ying Zhang
University of California, Davis

Hans-Georg Müller
University of California, Davis, hgmueller@ucdavis.edu

See next page for additional authors

Published in *Aging Cell* 1 (2002), pp. 140–148.

Accepted for publication September 10, 2002.

Life History Response of Mediterranean Fruit Flies to Dietary Restriction

James R. Carey[1], Pablo Liedo[2], Lawrence Harshman[3], Ying Zhang[4], Hans-Georg Müller[4], Linda Partridge[5], and Jane-Ling Wang[4]

1. Department of Entomology University of California, Davis, CA 95616, USA
2. El Colegio de la Frontera Sur, Carreterra Antiguo Aeropuerto Km 2.5, 30700, Tapachula, Chiapas, Mexico
3. School of Biological Sciences, University of Nebraska–Lincoln, Lincoln, NE 68588, USA
4. Department of Statistics, University of California, Davis, CA 95616, USA
5. Department of Biology, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK

Corresponding author – James R. Carey, Department of Entomology, One Shields Avenue, University of California, Davis, CA 95616 USA, email jrcarey@ucdavis.edu

Abstract

The purpose of this study was to investigate medfly longevity and reproduction across a broad spectrum of diet restriction using a protocol similar to those applied in most rodent studies. Age-specific reproduction and age of death were monitored for 1,200 adult males and 1,200 females, each individually maintained on one of 12 diets from *ad libitum* to 30% of *ad libitum*. Diet was provided in a fixed volume of solution that was fully consumed each day, ensuring control of total nutrient consumption for every fly. Contrary to expectation and precedence, increased longevity was not observed at any level of diet restriction. Among females, reproduction continued across all diet levels despite the cost in terms of increased mortality. Among males, life expectancy exceeded that of females at most diet levels. However, in both sexes, mortality increased more sharply and the pattern of survival changed abruptly once the diet level fell to 50% of *ad libitum* or below, even though the energetic demands of egg production has no obvious counterpart in males. We believe that a more

complete picture of the life table response to dietary restriction will emerge when studies are conducted on a wider range of species and include both sexes, more levels of diet, and the opportunity for mating and reproduction.

Keywords: cost of reproduction, life span, life table, male-female mortality, longevity extension, sex-mortality differentials

Introduction

The extension of longevity in organisms maintained on a restricted diet is one of the most intensely studied processes in gerontology. Dietary restriction (DR) is the sole environmental manipulation known to decrease the biological rate of aging (Masoro, 1988; Sohal & Weindruch, 1996; Bertarand et al., 1999), and the response is considered evolutionarily adaptive—natural selection will favor organisms capable of extending their longevity when subjected to dietary stress (Holliday, 1989). DR is currently viewed in gerontology as the "gold standard" against which all other anti-aging strategies are gauged (Kristal & Yu, 1994; Bertarand et al., 1999; Masoro, 2001).

Despite the long history of DR research and the similarity of DR responses across different species (McCay et al., 1935; Weindruch & Walford, 1988; Shanley & Kirkwood, 2000), important demographic questions remain unanswered:

1. What is the relationship of reproductive output and longevity in diet-restricted females? This question is important because experimental animals (particularly rodents) are prevented from mating and reproducing in most DR investigations. With a few exceptions concerning insects (Chapman & Partridge, 1996; Chippindale et al., 1993; Tatar & Carey, 1995), little is known about how reproduction will affect life span in animals subjected to food restriction. Surprisingly, none of the recommendations for future research on caloric restriction (Masoro, 2001) concerned the role of reproduction.
2. How do the specific methods of food restriction affect the longevity response? Most DR studies on rodents and primates involve either proportional reduction of all nutrients relative to *ad libitum* (Bertarand et al., 1999; Bartke et al., 2001; Roth et al., 2002) or reduction in total caloric content (Kristal & Yu, 1994; Sohal & Weindruch, 1996). In contrast, the DR protocols applied to invertebrates include change in the feeding schedule of spiders (Austad, 1989), dilution of nutrients provided in diet supplied in surplus to adult *Drosophila* (David et al., 1971; Chippindale et al., 1993; Bourg & Minois, 1996; Clancy et al., 2002), and dilution of bacteria available to *C. elegans* (Johnson et al., 1990) or of phytoplankton available to rotifers (Kirk, 1997; Kirk, 2001).
3. Do males and females respond similarly to DR even though females require more nutrient to support reproduction? Indeed, most DR investigations focus on only one sex, typically males in the case of rodents, because reproduction is recognized to affect the experimental outcome (Bertarand et al., 1999).

Here we address these issues with the Mediterranean fruit fly (*Ceratitis capitata*) and build upon our previous studies where the timing and composition of diet have been studied with respect to survival and reproduction (Müller et al., 1997; Carey et al., 1998a, 2001).

We apply methods of food restriction similar to those used in the study of rodents, we use treatments across an extremely wide range of diet levels, and we gather survival data on both sexes and on female age-specific reproduction. We ask whether longevity is increased in food-restricted medflies; how sex-specific life expectancy changes with dietary level; and how other components of life history change with diet, including age of first reproduction, lifetime reproduction, frequency of egg laying, and duration of the post-reproductive period?

Results

Pilot studies to define ad libitum

We conducted two pilot trials to determine the diet level equivalent to *ad libitum*. The longevity of 50 females maintained in individual cages were assessed at each of 12 dietary levels ranging from zero (water only) to 80 mg diet day^{-1} (trial 1) and from 12 to 40 mg diet day^{-1} (trial 2). In trial 1, life expectancies (fig. 1) were virtually identical across cohorts maintained on 40–80 mg diet day^{-1}. Since no increase in life expectancy at intermediate diet levels was observed, in trial 2 we narrowed the nutrient range to increase the resolution of the assay. Trial 2 produced the same outcome as trial 1 (fig. 1, inset): life expectancy was not at a local maximum for any intermediary level of diet. Thus, 40 mg diet day^{-1} is taken as *ad libitum* nutrition for an individual medfly since life expectancy is unaffected by any diet of richer concentration.

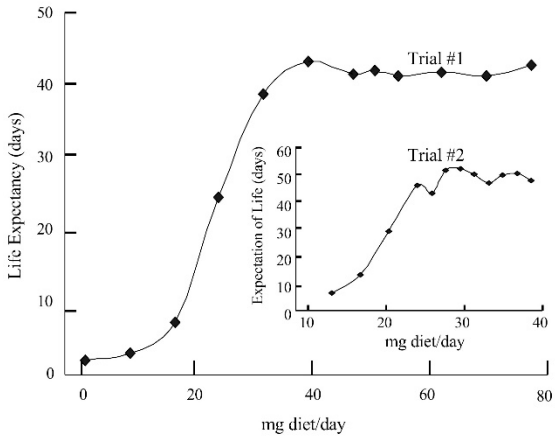

Figure 1. Life expectancy of 50 female medflies in each of 12 diet concentrations assessed during pilot studies. In trial 1 (main graph), dietary levels ranged from 0 mg day^{-1} (distilled water) to 80 mg day^{-1} of diet in 3 mL. In trial 2 (inset), the range of diet levels excluded concentrations of less than 12 mg diet day^{-1} and the redundant (*ad libitum*) concentrations greater than 40 mg diet day^{-1}.

Primary trial: male and female life expectancy

In our primary study, the life spans of 2400 flies were measured across 12 diet levels ranging from 12 to 40 mg day^{-1} (40 mg day^{-1} defines the 100% base diet level). Life expectancy monotonically increased across these treatments but reached a plateau across the higher food levels (fig. 2). The quartile bounds of survival followed this trend. The most striking feature of these data is that the classic "dietary restriction effect" of longevity extension was not observed. For diet levels at or above 65% of base diet, life expectancy varied among cohorts by nearly 10 days in females and by 16 days in males, but without apparent trend. For diet levels below 60% of base, life expectancy decreased markedly with reduced food. The data reveal large differences in the sex-specific longevity—at most diet levels males outlived females across all treatments by 40–90%. The variance in survival rate at the extreme ages also differed markedly among the sexes, as reflected by the broader spread across survival quartiles in males (fig. 2).

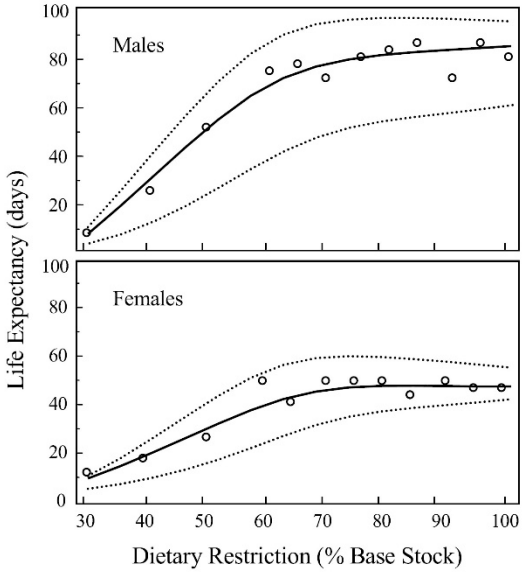

Figure 2. Life expectancy of male and female medflies maintained at one of 12 different levels of diet restriction (30–100%) relative to the base diet stock. Lower and upper dashed lines delineate the life spans for the 25th and 75th percentiles within each cohort.

Table 1. Logrank test of medfly mortality as a function of either sex or diet level

Variable	Statistic	Deviation	χ^2	Probability
Sex	–199.3	13.54	216.5	<0.0001
Dose	15,087.2	511.8	869.1	<0.0001

We applied survival analysis to evaluate the relationship of sex and food level upon mortality (3.2% of events were right censored). Considered independently, both sex and diet level significantly affected mortality (Logrank test, table 1). Considered jointly, the variables were assessed by proportional hazard regression where

$$\lambda(t) = \lambda_0(t) \exp(\beta_1 sex + \beta_2 dose),$$

and $\lambda(t)$ is the observed hazard rate, $\lambda_0(t)$ is the underlying hazard, and β_1 and β_2 are coefficients for sex and diet level, respectively. Accounting for diet, females have significantly higher mortality risk than males (table 2; $\beta_1 = 0.95 \pm 0.05$, where sex = 0 for males, sex = 1 for females, leading to hazard ratio = $\exp(\beta_1) = 2.59$). Accounting for sex, relative mortality decreased by a small but significant amount as a function of diet (table 2; $\beta_2 = -0.031 \pm 0.0013$, hazard ratio = $\exp(\beta_2) = 0.969$).

Table 2. Cox proportional hazards regression for medfly mortality data based on linear coefficients for sex and diet

Variable	d.f.	β	SE	χ^2	Probability	Hazard ratio
Sex	1	0.952	0.04723	406.655	<0.0001	2.592
Diet	1	–0.0312	0.00133	551.095	<0.0001	0.969

Patterns of survival

Among experimental cohorts, three features in pattern of survival are notable. First, at high diet levels, survival was homogeneous across cohorts (fig. 3). Second, at around the 50% diet level the survival curves abruptly shifted from a concave to a convex pattern, and they did so in both sexes. Thus, despite the male advantage in survival in every treatment, their dietary threshold for this change in survival was similar to that of females. This observation is intriguing because the energetic demand of egg production in females has no obvious counterpart in males in the absence of courtship. Third, the survival curves reveal that many males (20–40%) survived to 100 days but few females (1–5%) lived this long. The oldest age attained by a female at any diet level was 126 days whereas that of the oldest male was 174 days. Interestingly, maximal age was less sensitive than life expectancy to the most limited levels of diet. For example, at 40% and 50% diet the maximal age for males was 100 and 147 days, respectively, and for females it was 81 and 94 days, respectively.

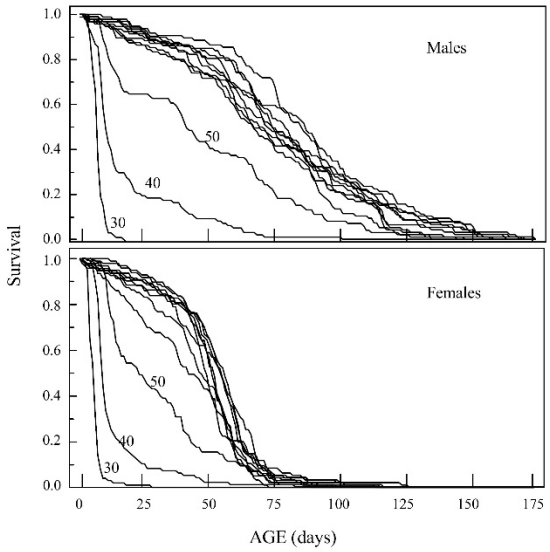

Figure 3. Survival (l_x) curves for male and female medflies maintained at each diet level ($N_0 = 100$, each sex). Survival curves maintained on the lowest three diet levels are labeled 30, 40 and 50, respectively.

Age of first reproduction

The age at which animals first reproduce has long been recognized as an important fitness trait because of its impact on population growth rate. We tallied the age at which female medflies produced their first egg and found that, with the exception of the 30% diet level, there was no relationship of nutrition to first reproduction (fig. 4). Rather, the age of first laid egg was uniform across all cohorts, ranging from 6.2 to 7.1 days.

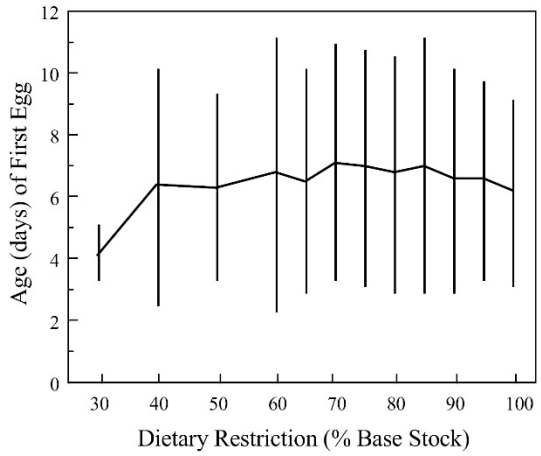

Figure 4. Average age of first egg among females within each diet level (vertical lines: standard deviation).

Lifetime reproduction

Average lifetime reproduction appeared to be graded across diet levels (fig. 5). Lifetime egg production dropped by only 160 eggs/female between the highest value of 1,150 eggs/female (100% diet) to 990 eggs/female at 70% diet. However, females at 60% diet laid approximately 750 eggs, and at lower diet levels fecundity declined sharply. These patterns and the smoothed trend suggest that egg production continuously varies with diet. To assess this observation, we fitted a two-phase regression model with adjoining regression lines assuming a change-point at 75% diet (Draper & Smith, 1998). Slope left of the change-point was 24.9 ($P < 0.0001$), slope right of the change-point was 4.04 ($P < 0.02$), and the change in slope was significant ($P < 0.0001$).

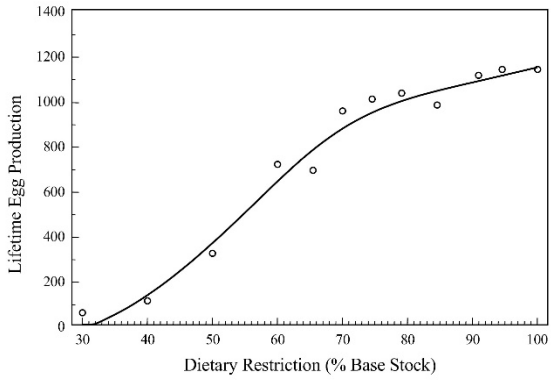

Figure 5. Average lifetime egg production of females within each diet level and the function estimated by smooth curve fit (Müller, 1987).

Analysis of mean fecundity does not capture the inter-female variance for reproduction, which can be substantial. Box plots (fig. 6) reveal that most cohorts contained at least some flies that laid no eggs and some that laid more than 1,800 eggs. A quarter of all females in seven treatments laid at least 1,400 eggs and a quarter of all females in nine treatments laid at least 1,000 eggs. The abrupt shift in mean fecundity across a small change in diet level was also reflected in the contraction of inter-female variance. The overall uniformity in fecundity across this wide range of diet suggests that female medflies, like starved or fasting mammals (Bines, 1999), may be capable of increasing their metabolic and reproductive efficiency when nutrients become limiting.

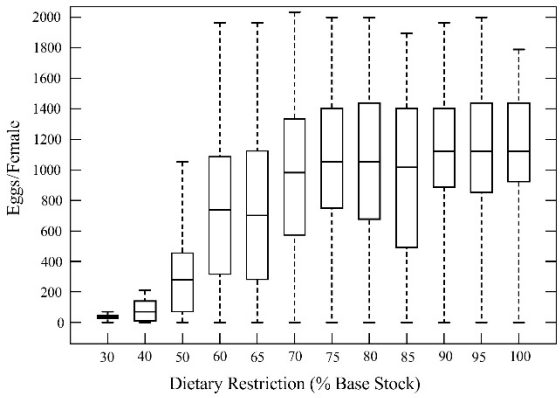

Figure 6. Box plots for lifetime reproduction within each diet level. The high-low extremes of the rectangles and the dashed lines indicate values for the mid-50% mass of the distribution and the minimum and maximum, respectively. The horizontal line indicates the mean.

Event history plots

Event history plots (Carey et al., 1998b) provide useful demographic insight. They visualize multiple dimensions of sampling (i.e., inter- and intra-cohort; inter- and intra-individual) as well as multiple variables and their interactions (e.g., the relationship of longevity to reproductive timing, intensity, and variability). Several observations emerge from the plots applied to each experimental cohort (fig. 7). As noted above, the age of first reproduction was independent of diet level. Furthermore, there were no systematic differences in age of first reproduction between the shortest- and longest-lived individuals within cohorts. Also as noted, the survival patterns in high-level diet cohorts were sigmoidal. By contrast, the survival patterns at intermediate diet levels (60%, 65%, and 75%) tended toward linearity while survival at the lowest diet levels (30%, 40%, and 50%) was distinctly convex. The individual reproductive behavior of each female is embedded within these survival curves and shows that females on low levels of diet reproduce without systematic gaps, in spite of the presumed physiological option of repressing reproduction to increase survival. As well, whereas the majority of females in cohorts maintained at high levels of diet survived for 3–7 days in a post-reproductive state, most females in the food-limited cohorts laid eggs up to the day of death. Females that were maintained at higher food levels appeared to have laid their full complement of eggs. In contrast, in females of the diet-limited cohorts, a fraction of the eggs that would have otherwise been produced at young ages (in *ad libitum* cohorts) was laid at ages which would otherwise have been post-reproductive. This apparent system of food—rather than time—limitation in egg production was also observed in a recent study of medflies subjected to temporal variation in dietary protein (Carey et al., 2002).

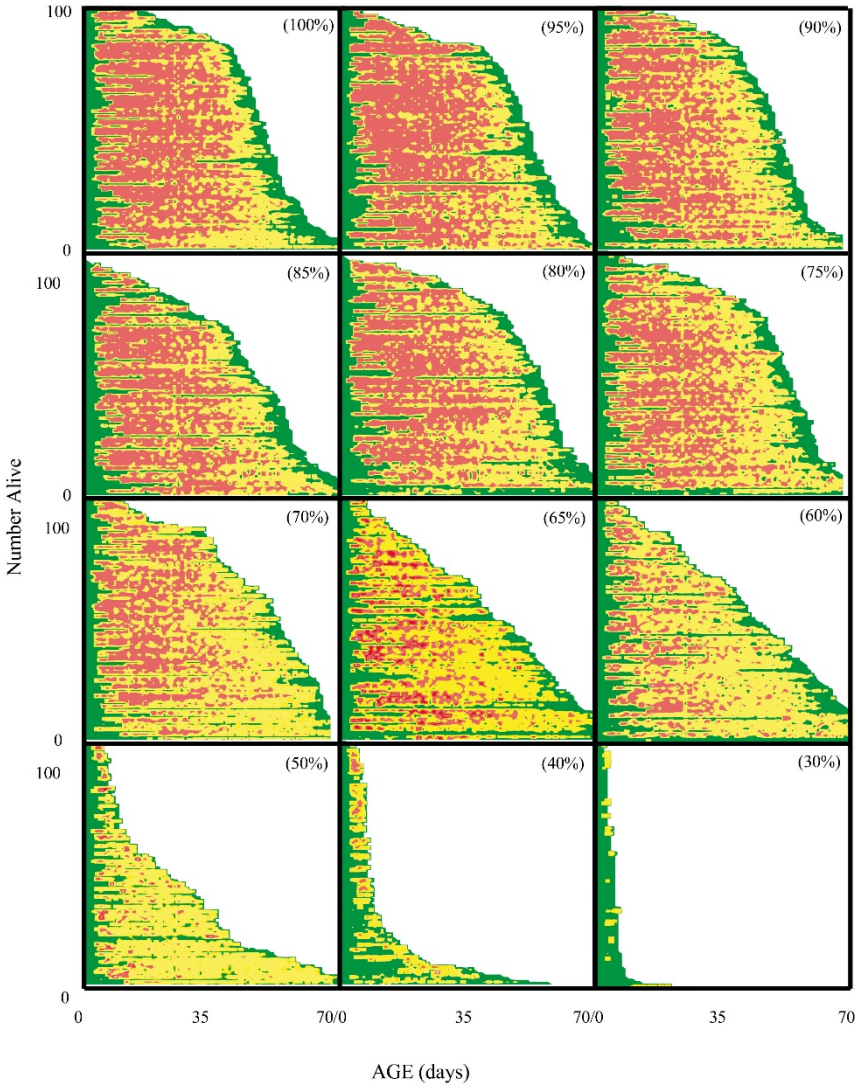

Figure 7. Event-history charts for cohorts of medflies maintained on each diet level. Within a plot, each horizontal line records a female "life-line," where the length is proportional to her life span, and the color categorizes age-specific fecundity (eggs day^{-1}: green = 0, yellow = 1–30, red = >30).

Discussion

Our current results build on previous studies of the demographic response in medflies to diet. A summary of this work will be useful. First, medfly longevity increased when adults were initially maintained on sugar-only diet and then switched to full diet (sugar and yeast) at older ages (Carey et al., 1998a) or when they were subjected to alternating pulses of sugar and full diet (Carey et al., 2002). These cases of increased longevity upon diet

restriction contrast with our current findings where adults were chronically exposed to proportional reduction in complete diet. Second, previous studies revealed that we could not predict the magnitude or the direction of change in life expectancy upon change in diet. For example, knowledge that medfly longevity increased when individuals were maintained on sugar-only diet was, by itself, insufficient to predict the observation that when switched to full diet, mortality rate of females initially decreased but then accelerated (Carey et al., 1998a). Unpredictability is evident in the current study where we anticipated longevity to increase as nutrients were restricted. Third, it is likely that a significant component of this unpredictability involves the interaction of diet, reproduction and longevity. For example, Carey et al. (1998a) observed an apparent survival benefit from reproduction at young ages and when flies reproduced under conditions of full diet. However, reproduction bore a mortality cost as flies continued to lay eggs. Fourth, the sex-specific mortality responses to diet may differ dramatically. This trend, evident in our current data, was especially striking in our earlier study of diet and sterilizing radiation (Carey et al., 2001) where the mortality trajectory of males was nearly identical across treatments but female mortality responded to diet composition, to sterility, and to the interaction of these factors.

Our observation that reduced reproduction is associated with reduced survival differs from the outcomes in other studies of invertebrates. Partridge and co-workers (Partridge & Andrews, 1985; Partridge, 1986) observed decreased mortality in male *Drosophila* denied access to mates. Austad (1989) reported that diet restriction in the spider *Frontinella pyramitela* delayed egg laying, reduced total fecundity and increased survival. Tatar & Carey (1995) showed that the mortality costs of egg production in the beetle *Callosobruchus maculates* were not simply caused by resource allocation between current reproduction and somatic maintenance. Rather the mortality rates during aging depended on the interaction of nutrition and reproduction experienced at early adulthood. Several studies on DR in rodents (Merry & Holehan, 1979; Holehan & Merry, 1985) have also reported that females maintained on a restricted diet at young ages were capable of producing offspring at advanced ages once they were given *ad libitum* diet.

Good & Tatar (2001) suggested that medfly may differ from *Drosophila* in terms of their physiological response to nutritional ecology. Although both species slow reproduction in the absence of yeast, mortality decreases in medflies under these dietary conditions (Carey et al., 1998a) but increases in *Drosophila* (Good & Tatar, 2001). When cohorts of each species were given access to dietary yeast after having been maintained on yeast-free diets, the mortality decreased initially for both species. However, the mortality in *Drosophila* declined to the level of same aged females that had continuous full diet and the change was permanent, as if the cohort had no history of diet restriction. Good & Tatar (2001) suggested that *Drosophila* adults have a more obligatory need for dietary yeast than do medfly, and this shapes different responses to experimental diet restriction. It is likely that interspecific differences in nutritional ecology confounds the effects of different methods used to limit nutrition in these species; *Drosophila* are typically limited by diluting nutrients in a resource supplied in surplus while in the present study medflies were restricted by fixing the total amount of available food.

In the current study, females never completely shut down reproduction when total diet was restricted. Females appeared to balance current reproduction against short- and medium-term survival, as suggested by Carey et al. (2002). Under this model reproductive strategy, a fraction of available amino acids is always held in reserve rather than used for current production of yolk. However, when protein is restricted some fraction of the amino acid reserve is still allocated to current vitellogenesis. Protein acquisition stimulates flies to allocate a greater fraction of the amino acid reserve to egg production. Thus, medfly females adapt to changing dietary conditions by immediately increasing egg production if new sources of protein become available or by immediately reducing egg production if protein becomes scarce. In both situations, females optimize survival relative to reproduction but they never completely shut down reproduction in order to maximize survival.

Carey et al. (2002) also noted that a fraction of a female's reproductive potential appears to be completely lost due to aging alone. Thus, in the presence of at least some dietary yeast, females may produce a few eggs each day across a relatively short life rather than risk dying without any reproduction or facing the prospect of diminished reproductive potential due to reproductive senescence if survival is achieved.

Although our current effort was large relative to most investigations of dietary restriction (Weindruch & Walford, 1988), few studies are definitive and none is all-encompassing. This one is no exception. In future DR studies with medfly several assumptions must be revisited. First, we applied the *ad libitum* conditions assessed in females to males. Although this practice was not calibrated for males, it is of interest that the diet level where the male survival curve changed shape was nearly identical to the level where this occurred for females. Second, individuals of both sexes were maintained as virgins (females lay unfertilized eggs). However, it is virtually certain that male and female survival and female fecundity pattern would change in adults maintained as pairs, because mating alters sex-specific physiology and crowding within cages alters longevity (Carey et al., 1995).

Conclusion

It is nearly 70 years since McCay et al. (1935) first demonstrated that reduced dietary intake increased the life span of rodents, and it is nearly 15 years since Weindruch & Walford (1988) noted that DR lengthens the life in animals as diverse as worms, flies, rodents, and primates. However, our combined studies on the demographic response of medfly to DR suggest that longevity extension is not universal to all types of restriction, that the sexes respond differently to restriction, that reproduction alters the impact of diet restriction upon longevity and that the details of DR methodology affect the actuarial (longevity) outcome. A better understanding of the longevity response of diet-restricted animals requires studies on both sexes and across a number of different species that include a greater number and range of restriction levels, provide the opportunity to mate, conceive, gestate, give birth, lactate and rear offspring, and apply more uniform DR methods across species. Studies on food restriction would also benefit from field studies on rodents (Linduska, 1942; Sage, 1981; Grodzinski, 1985), fruit flies (Bouletreau, 1978; Hendrichs et al., 1993), and other species (White, 1978) to determine natural nutritional conditions for wild populations and help answer the question (Fries, 1980; Masoro, 2001) "Are the life expectancy differentials

the result of improved health due to a protective effect of restriction or to worsened health due to overfeeding?" We believe that a more nuanced and complex but also more complete picture of the life table response to dietary restriction will then begin to emerge, and will provide new insight in to the underlying physiological, cellular, genetic, and molecular mechanisms (Weindruch, 1996; Weindruch & Sohal, 1997; Bertarand et al., 1999; Bartke et al., 2001; Roth et al., 2002).

Experimental procedures

Background

The Mediterranean fruit fly (*Ceratitis capitata*), commonly known as the medfly, belongs to the dipteran family Tephritidae referred to as "true" fruit flies—a group of about 4,000 species distributed throughout most of the world (Christenson & Foote, 1960). Members of this group lay eggs in intact fruit using their sharp ovipositor rather than on decaying fruit as do their distant relatives in the family Drosophilidae. As with all previous medfly research published by Carey and co-workers (Carey, 2003), the current study was conducted at the Moscamed medfly mass rearing facility near Tapachula, Mexico—a facility constructed in 1979 as a joint enterprise funded by the US Department of Agriculture and the Mexico Ministry of Agriculture to rear large numbers (i.e., 500 million to 1 billion per week) of medfly adults for sterilization and subsequent release as a tactical component of a program to prevent the spread of the pest further into Mexico.

The rearing technology involves collecting several liters (i.e., tens of millions) of eggs from reproducing adults each day that are used to "seed" diet-filled trays held in cafeteria-style racks. The mature larvae are separated from the rearing medium after one week and distributed in special holding trays for pupation. A predetermined number of these pupae are removed from the production facility a few days before eclosion (around 2 weeks) for use in the medfly aging studies. General details concerning all aspects of medfly mass rearing at this facility are described in Vargas (1989) and specific details on the use of the medfly model system for aging research are described in Carey & Liedo (1999) and Carey (2003).

Dietary restriction protocol

The experimental protocols used in DR research fall into three general categories (Bertarand et al., 1999), including (1) dietary reduction—scaling total amount of a fixed diet, (2) dietary recomposition—scaling the total amount of calories but ensuring that the diet still contains essential nutrients, and (3) dietary dilution—reducing the amount of calories and key nutrients in the diet but otherwise allowing *ad libitum* feeding. Because studies on rodents have framed the DR paradigm, one of our main objectives was to develop a protocol to study the effects of DR on medfly life histories using a method similar to the dietary reduction protocols commonly applied to rodents.

We provided each individual fly a fixed volume (3 mL) of a liquid diet each day and we varied the total nutritional content of the droplet. Adults fully consume this volume and thus receive a known and controlled quantity of food. A diet stock solution was prepared by mixing "cake" adult diet (3 parts yeast hydrolysate and 1 part sucrose; Vargas, 1989) in

distilled water. Pilot studies, described in the main text, were used to determine what concentration of diet stock represented *ad libitum* nutrition. Twenty-four concentrations were tested with cohorts of 50 flies; 40 mg diet per fly per day delivered in 3 mL was found to be *ad libitum* and was selected as the base diet level (100%) for our primary trial. Diet reduction was implemented by feeding adults one of 12 diets (in 3 mL) with proportionally less total nutrient content relative to the base diet level: 100, 95, 90, 85, 80, 75, 70, 65, 60, 50, 40, and 30%.

Experimental design

The diet stock solution and all dilutions were prepared every other day. Each day, a 3-mL droplet of food and a 6-mL droplet of water were supplied to the flies upon a clean glass slide fixed to each individual cage. Flies were individually housed in 4 × 4 × 10-cm Plexiglas cages, arranged in 24-cage units. A total of 100 units were used to study 1,200 females and 1,200 males. Diet level was assigned in a randomized block design, and females and males were placed in alternate cages within each unit. Females laid eggs through organdy mesh fastened to the front of the cage. Eggs were counted daily using an electronic image analysis system. Life table methods applied to the survival and fecundity data are outlined in Carey (1993, 2001).

Acknowledgments – We thank A. Oropeza, R. Bustamante, E. de Leon, S. Rodriguez, R. Rincón, A. Villela, and G. Rodas for technical assistance, the Moscamed program in Mexico for allowing us the use of their facilities in Metapa, and Roger McCarter for discussion of dietary restriction concepts. Research supported by PO1-08761 from the National Institute on Aging and grant DMS-94–04906 from the National Science Foundation.

References

Austad SN (1989) Life extension by diet restriction in the bowl and doily spider, *Frontinella pyramitela*. *Exp. Gerontol.* 24, 83–92.

Bartke A, Wright JC, Mattison JA, Ingram DK, Miller RA, Roth GS (2001) Extending the lifespan of long-lived mice. *Nature* 414, 412.

Bertrand HA, Herlihy JT, Ikeno Y, Yu BP (1999) Dietary restriction. In *Methods in Aging Research* (Yu BP, ed.). Boca Raton: CRC Press, pp. 271–300.

Bines J (1999) In *Encyclopedia of Human Nutrition* (Sadler MJ, Strain JJ, Caballero B, eds). San Diego: Academic Press, pp. 1779–1786.

Bouletreau J (1978) Ovarian activity and reproductive potential in a natural population of *Drosophila melanogaster*. *Oecologia* 35, 319–342.

Bourg EL, Minois N (1996) Failure to confirm increased longevity in Drosophila melanogaster submitted to a food restriction procedure. *J. Gerontol.* 51A, B280–B283.

Carey JR (1993) *Applied Demography for Biologists*. New York: Oxford University Press.

Carey JR (2001) Insect biodemography. *Annu. Rev. Entomol.* 46, 79–110.

Carey JR (2003) *Longevity: The Biology and Demography of Life Span*. Princeton: Princeton University Press.

Carey JR, Liedo P (1999) Measuring mortality and reproduction in large cohorts of the Mediterranean fruit fly. In *Studies of Aging* (Sternberg H, Timiras PS, eds). Berlin: Springer-Verlag, pp. 111–124.

Carey JR, Liedo P, Harshman L, Liu X, Muller H-G, Partridge L, Wang J-L (2002) Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. *Func. Ecol.* 16, 313–325.

Carey JR, Liedo P, Müller H-G, Wang J-L, Love B, Harshman L, Partridge L (2001) Female sensitivity to diet and irradiation treatments underlies sex-mortality differentials in the Mediterranean fruit fly. *J. Gerontol.: Biol. Sci.* 56A, B1–B5.

Carey JR, Liedo P, Müller H-G, Wang J-L, Vaupel JW (1998a) Dual modes of aging in Mediterranean fruit fly females. *Science* 281, 996–998.

Carey JR, Liedo P, Müller H-G, Wang J-L, Vaupel JW (1998b) A simple graphical technique for displaying individual fertility data and cohort survival: case study of 1000 Mediterranean fruit fly females. *Func. Ecol.* 12, 359–363.

Carey JR, Liedo P, Vaupel JW (1995) Mortality dynamics of density in the Mediterranean fruit fly. *Exp. Gerontol.* 30, 605–629.

Chapman T, Partridge L (1996) Female fitness in *Drosophila melanogaster*: an interaction between the effect of nutrition and of encounter rate of males. *Proc. Royal Soc. London; Series B* 263, 755–759.

Chippindale AK, Leroi AM, Kim SB, Rose MR (1993) Phenotypic plasticity and selection in *Drosophila* life-history evolution. I. Nutrition and the cost of reproduction. *J. Evol. Biol.* 6, 171–193.

Christenson LD, Foote RH (1960) Biology of fruit flies. *Annu. Rev. Entomol.* 5, 171–192.

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

David J, Van Herrewege J, Jouillet P (1971) Quantitative under-feeding of *Drosophila*: effects on adult longevity and fecundity. *Exp. Gerontol.* 6, 249–257.

Draper NR, Smith H (1998) *Applied Regression Analysis*. New York: Wiley.

Fries JF (1980) Aging, natural death, and the compression of morbidity. *N. Engl. J. Med.* 303, 130–135.

Good TP, Tatar M (2001) Age-specific mortality and reproduction respond to adult dietary restriction in *Drosophila melanogaster*. *J. Insect Physiol.* 47, 1467–1473.

Grodzinski W (1985) In *The Ecology of Woodland Rodents: Bank Voles and Wood Mice* (Flowerdew FR, Gurnell, J, Gipps JHW, eds). Oxford: Clarendon Press, pp. 169–192.

Hendrichs J, Lauzon CR, Cooley SS, Prokopy RJ (1993) Contribution of natural food sources to adult longevity and fecundity of *Rhagoletis pomonella* (Diptera: Tephritidae). *Ann. Entomol. Soc. Am.* 86, 250–264.

Holehan AM, Merry BJ (1985) Lifetime breeding studies in fully fed and dietary restricted female CFY Sprague-Dawley rates. 1. Effect of age, housing conditions and diet on fecundity. *Mechanisms Ageing Dev.* 33, 19–28.

Holliday R (1989) Food, reproduction, and longevity: is the extended lifespan of calorie-restricted animals an evolutionary adaptation? *Bioassays* 10, 125–127.

Johnson TE, Friedman DB, Foltz N, Fitzpatrick PA, Shoemaker JE (1990) In *Genetic Effects on Aging* (Harrison DE, ed.), Telford, NJ: Caldwell, pp. 101–126.

Kirk KL (1997) Life-history responses to variable environments: Starvation and reproduction in planctonic rotifers. *Ecology* 78, 434–441.

Kirk KL (2001) Dietary restriction and aging: comparative tests of evolutionary hypotheses. *J. Gerontol.: Biol. Sci.* 56A, B123–B129.

Kristal BS, Yu BP (1994) In *Modulation of Aging Processes by Dietary Restriction* (Yu BP, ed.) Boca Raton: CRC Press, pp. 1–35.

Linduska JP (1942) Winter rodent populations in field-shocked corn. *J. Wildlife Manage.* 6, 353–363.

Masoro EJ (1988) Mini-review: food restriction in rodents: an evaluation of its role in the study of aging. *J. Gerontol.* 43, B59–B64.

Masoro EJ (2001) Special Issue I: Caloric restriction's effects on aging: opportunities for research on human implications. *J. Gerontol.: Biol. Med. Sci.* 56A, 5–75.

McCay CM, Crowell MF, Maynard LA (1935) The effect of retarded growth upon the length of life span and upon the ultimate body size. *J. Nutrition* 10, 63–79.

Merry BJ, Holehan AM (1979) Onset of puberty and duration of fertility in rats fed a restricted diet. *J. Reprod. Fertil.* 57, 253–259.

Müller HG (1987) Weighted local regression and kernel methods for nonparametric curve fitting. *J. Am. Statist. Assoc.* 82, 231– 238.

Müller H-G, Wang J-L, Capra WB, Liedo P, Carey JR (1997) Early mortality surge in protein-deprived females causes reversal of sex differential of life expectancy in Mediterranean fruit flies. *Proc. Natl Acad. Sci.* 94, 2762–2765.

Partridge L (1986) In *Insect Aging* (Collatz K-G, Sohal RS, eds). Berlin: Springer-Verlag, pp. 45–54.

Partridge L, Andrews R (1985) The effect of reproductive activity on the longevity of male *Drosophila melanogaster* is not caused by an acceleration of ageing. *J. Insect Physiol.* 31, 393–395.

Roth GS, Lane MA, Ingram DK, Mattison JA, Elahi D, Tobin JD, Muller D, Metter EJ (2002) Biomarkers of caloric restriction may predict longevity in humans. *Science* 297, 811.

Sage RD (1981) In *The Mouse in Biomedical Research* (Foster HL, Small JD, Fox JG, eds). New York: Academic Press, pp. 40–90.

Shanley DP, Kirkwood TBL (2000) Calorie restriction and aging: a life-history analysis. *Evolution* 54, 740–750.

Sohal RS, Weindruch R (1996) Oxidative stress, caloric restriction, and aging. *Science* 273, 59–63.

Tatar M, Carey JR (1995) Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle, *Calosobruchus maculatus*. *Ecology* 76, 2066–2073.

Vargas R (1989) In *World Crop Pests. Fruit Flies: Their Biology, Natural Enemies and Control* (Robinson AS, Hooper G, eds). Amsterdam: Elsevier, pp. 141–152.

Weindruch R (1996) Caloric restriction and aging. *Scientific Am.* 274, 46–52.

Weindruch R, Sohal RS (1997) Caloric intake and aging. *N. Engl. J. Med.* 337, 986–994.

Weindruch R, Walford RL (1988) *The Retardation of Aging and Disease by Dietary Restriction*. Springfield: Charles C. Thomas.

White TCR (1978) The importance of a relative shortage of food in animal ecology. *Oecologia* 33, 71–86.