

The effect of dietary restriction on the lifespan of males in a web-building spider

Michal Segoli,^{1*} Yael Lubin² and Ally R. Harari^{1,3}

¹Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva,

²Mitrani Department of Desert Ecology, Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus and ³Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel

ABSTRACT

Question: Do web-building spider males respond to dietary restriction by increased lifespan, as is observed in many other organisms?

Hypothesis: Adult males normally do not feed, have extremely high extrinsic mortality rates during mate search, and mate only once, hence they are less likely to benefit from increased lifespan in terms of future reproductive success. Accordingly, they are not expected to respond to dietary restriction by increased lifespan.

Organism: Adult white widow spider (*Latrodectus pallidus*) males.

Methods: Virgin adult males were kept on a low diet or fed *ad libitum*. Longevity of males was recorded.

Results: As opposed to our predictions we found that food-restricted male spiders survived longer than males that fed *ad libitum*.

Keywords: dietary restriction, lifespan, web-building spider.

INTRODUCTION

Dietary restriction – the reduction of nutrient intake without malnutrition – has been shown repeatedly to increase individual lifespan in diverse animal taxa, including nematodes (Houthoofd *et al.*, 2003), insects (Carey *et al.*, 1998), spiders (Austad, 1989), and mammals (Weindruch and Sohal, 1997). The response to dietary restriction is often considered to be adaptive, as it may allow food-restricted animals to postpone reproduction until food is available (Harrison and Archer, 1989; Holliday, 1989; Guarente and Kenyon, 2000), or reduce vulnerability of populations to extinction (Mitteldorf, 2004, 2006). Additionally, life extension may be accompanied by increased stress resistance (Masoro, 2005). Thus, the extension of the organism's lifespan is predicted to be actively mediated by gene regulation of physiological processes involved in ageing (Kirkwood and Shanley, 2005). However, the response to dietary restriction might not be adaptive *per se*, but rather the outcome of reduced reproductive activity, or an indirect

* Author to whom all correspondence should be addressed. e-mail: msegoli@bgu.ac.il

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outcome of reduced damage to soma as a result of decreased food consumption (for discussion on the topic, see Merry, 2004; Guarente and Picard, 2005; Kirkwood and Shanley, 2005; Masoro, 2005; Sinclair, 2005).

We studied the effect of food availability on the lifespan of males of web-building spiders (*Latrodectus pallidus*, Theridiidae). Males of most web-building spiders desert their natal webs soon after maturation and start searching for females (Vollrath and Parker, 1992; Foelix, 1996). Adult males neither build capture webs nor feed during mate search (Vollrath, 1987), but they are able to feed when provided with prey, and may occasionally obtain food from the webs of females by kleptoparasitism (Schneider and Elgar, 1998; Maklakov *et al.*, 2004; Erez *et al.*, 2005). This provided us with an opportunity to test the effect of food consumption on lifespan in typically non-feeding animals. While dietary restriction in feeding animals has been well studied, the effect of different feeding regimes on naturally non-feeding organisms has never been investigated. In particular, we wished to test the general prediction of increased lifespan as a result of decreased food consumption in adult males of a web-building spider.

Web-building spider males generally suffer high extrinsic mortality during mate search (Vollrath and Parker, 1992), such that they are not likely to benefit from a potential increase in lifespan *per se* in terms of future reproductive success. In particular, in the spider *Latrodectus pallidus*, males experience extremely high mortality rates during mate search, and commonly mate with only one female in their lifetime (Segoli *et al.*, 2006). In light of the above, we predicted that the lifespan of *L. pallidus* males would not increase as a result of dietary restriction.

MATERIALS AND METHODS

Our study organism was the white widow spider (*Latrodectus pallidus*). This species, along with other members of the genus, is characterized by extreme female-biased sexual size dimorphism (Levy, 1998). Males court for several hours on females' webs, and mating takes place inside the nest (Segoli, 2004). In this species, cannibalism of males by females occurs in about 20% of courtship and mating events (Segoli *et al.*, in press).

Males were reared from egg sacs laid by field-collected females, under laboratory conditions (natural light period, 24–27°C). Each individual was kept in a plastic cup and fed *ad libitum* with *Drosophila melanogaster* until maturation. At maturation, 44 males were weighed and measured for leg length (tibia + patella of the first leg) using a digital caliper (precision 0.01 mm). Leg length was used as a proxy for body size following Lubin *et al.* (1991). Body condition indices were calculated as the residuals of a least squares regression of body mass on leg length (each individual contributed one data point to this regression) (Schulte-Hostedde *et al.*, 2005). Adult males were then randomly allocated to two treatment groups ($n = 28$ for the abundant-diet treatment; $n = 29$ for the low-diet treatment). Twenty-two males from each treatment were measured and weighed as indicated above. We failed to obtain measurements for 13 males. In the abundant-diet treatment, males were provided with *D. melanogaster ad libitum*, while in the low-diet treatment each male was provided with only three flies per week. Males were kept individually in aerated plastic containers with no access to females throughout the entire experiment, and their mortality was scored daily. Spider feeding was not observed directly. However, some flies were found caught in capture webs and covered with silk inside the containers, indicating that the spiders were feeding. We recorded the date of measurement at maturation and death of each male. Longevity of adult males was determined as the time between these dates.

To test for differences in mortality rate and longevity between the treatment groups, we used survival analysis (Mantel log rank test). This test is appropriate to identify differences in mortality rate between populations. Additionally, we used analysis of covariance (ANCOVA) with leg length and body condition as covariates, since these variables are likely to affect longevity independently of the treatment. Finally, we used linear regression to examine the influence of leg length, body mass, and body condition on longevity in each group separately.

RESULTS

Males on the low diet survived longer than males on the abundant diet (mean $\pm$ standard deviation: 27.8 ± 9.9 vs. 23.6 ± 8.4 days; Mantel log rank test, $\chi^2_1 = 4.19$, $P = 0.041$) (Fig. 1). The difference in longevity was also significant when using leg length and body condition as covariates (ANCOVA, Table 1). Longevity of adult males in the abundant-diet treatment was positively influenced by mass and leg length but not by body condition. Longevity of adult males in the low-diet treatment was positively influenced by mass and body condition but not by leg length (linear regression, Table 2).

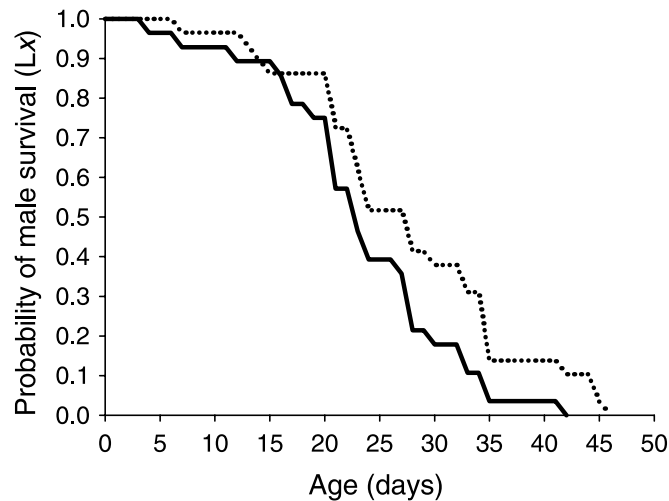


Fig. 1. The survival curves of dietary-restricted ($n = 29$, dotted line) and *ad libitum*-fed ($n = 28$, solid line) adult males. The plot shows the proportion of each cohort alive at age x (Lx), with median ($Lx = 0.5$) and maximum ($Lx = 0$) life expectancies.

Table 1. Results of ANCOVA comparing longevity of adult males in the abundant-diet and low-diet treatments, with body size and body condition as covariates

Source	Sum-of-squares	d.f.	Mean-square	F-ratio	P
Treatment	356.797	1	356.797	7.322	0.010
Body size	464.005	1	464.005	9.522	0.004
Body condition	476.133	1	476.133	9.771	0.003
Error	1949.194	40	48.730		

Table 2. Results of linear regression of adult longevity versus body mass, body size, and body condition of males at time of maturation

Independent variable	Low diet ($n = 22$)	Abundant diet ($n = 22$)
Body mass	Slope = 2405 $R^2 = 0.44$ $P < 0.01$	Slope = 1228 $R^2 = 0.22$ $P = 0.03$
Body size	Slope = 4.8 $R^2 = 0.06$ $P = 0.28$	Slope = 6.9 $R^2 = 0.28$ $P = 0.01$
Body condition	Slope = 1839 $R^2 = 0.26$ $P = 0.02$	Slope = 935 $R^2 = 0.1$ $P = 0.18$

DISCUSSION

Dietary restriction increases the lifespan of adult males, but is this increase adaptive?

Males of widow spiders are not likely to benefit from increased lifespan following low food intake. However, food-restricted spiders in our experiment had a 15% longer lifespan than males that fed *ad libitum*. The difference was significant but smaller than life extension reported in other studies [e.g. 40% in rodents (Weindruch and Sohal, 1997); 30% in *Drosophila* (Carey *et al.*, 1998; Magwere *et al.*, 2004)]. These results suggest that in this species, life extension in response to dietary restriction is due to indirect effects. Alternatively, the response may be adaptive in some species, but not in others, if the general mechanism of this response is evolutionarily conserved (Partridge and Brand, 2005).

There are several reasons to assume that life extension as a result of dietary restriction is not adaptive for males of widow spiders. The small difference in longevity between well-fed and restricted males (~4 days) is unlikely to allow males to delay reproduction until times of plenty (as suggested by Holiday, 1989; Guarente and Kenyon, 2000). Similarly, it is unlikely to allow enough time for population regulation, as suggested by Mitteldorf (2004, 2006).

In a field study of the white widow, it was found that extrinsic mortality rate is high in nature. Most males probably survive no more than several days after they leave their nests to search for females (Segoli *et al.*, 2006). Similar high mortality occurs in natural populations of the congeners *L. revivensis* (Segev *et al.*, 2003) and *L. hasselti* (Andrade, 2003). Low life expectancies were recorded for males of other web-building spiders (Vollrath, 1980, 1985; Singer and Riechert, 1995). Consequently, adult males are not likely to benefit from postponed reproduction (Kirkwood and Shanley, 2005).

Additionally, in nature, adult males do not capture prey on their own and thus are unlikely to benefit from postponing their reproduction to obtain more food. Instead, adult males probably rely on resources accumulated as juveniles, and thus should benefit from reproducing soon after maturation. Most male white widows desert their natal webs and start searching for females within a few days after maturation (Segoli *et al.*, 2006).

In widow spiders, males often lose the end of their embolus (copulatory organ) inside the female spermatheca (sperm storage organ) (Berendonck and Greven, 2000). The tip of the embolus

was shown to function as a copulatory plug resulting in a first sperm priority pattern (Snow and Andrade, 2004). Thus, males of widow spiders that mate early in the season will benefit from greater reproductive success, and this may override any advantages of delayed reproduction even if food is restricted.

One important factor that may determine whether postponed reproduction as a result of dietary restriction is adaptive is the effect of famine on the survival of offspring (Shanley and Kirkwood, 2000). If the effect is strong, it is beneficial to postpone reproduction until conditions improve and the probability of offspring survival is increased. In widow spiders, however, spiderlings emerge long after mating and may hibernate or reduce activity [juvenile diapause (Schaefer, 1987)] for several months until the beginning of next spring. Thus, food availability during mate search is not likely to be a good predictor of offspring survival, and should not affect the timing of reproduction.

Life extension as a result of dietary restriction may be due to several effects

The observed effect of dietary restriction on longevity in widow males may be the result of reduced calories. Caloric restriction is thought to extend life through several possible physiological processes [e.g. reduction in metabolic rate, reduction of body fat, reduction in reactive oxygen species, and attenuation of cell loss (reviewed in Sinclair, 2005)]. Feeding behaviour in spiders includes several energetic costs: the energy required to capture prey, the cost of silk production (silk is used in prey capture), and the cost of producing digestive enzymes for external digestion (Foelix, 1996). In the present study, we did not quantify the cost of feeding, the amount of food consumed or its caloric content, and thus we could not distinguish between these effects.

A study of male field crickets (*Teleogryllus commodus*) found that males reared on a high-calorie diet lived for a shorter period than diet-restricted males, due to earlier and larger investment in reproduction (Hunt *et al.*, 2004). Increased investment in early reproduction may reduce lifespan for two reasons: (1) it may reduce resource allocation to somatic maintenance; and (2) it may cause somatic damage directly (Partridge *et al.*, 2005). Although courtship and mating in widow spiders is very costly and reduces male survival (Segoli, 2004), in the current study males did not have access to females and thus did not pay these costs. It is possible, however, that males fed *ad libitum* invested more in mate-search behaviour, such as increased locomotory activity, or in the production of hormones or pheromones, and thus paid some of the costs of reproduction even though they did not encounter a female (Rausser *et al.*, 2004).

Adaptive life extension as a result of dietary restriction

Although the effect of dietary restriction is not likely to be adaptive for adult males of widow spiders, it may be adaptive in other species. Indeed, there is increasing evidence that in some species the response to dietary restriction is the consequence of an active regulatory program that recognizes food scarcity and programs the resulting effects (Carey *et al.*, 1998; Lin *et al.*, 2000; Guarante and Picard, 2005; Sinclair, 2005). In lower organisms, this strategy extends to cases in which the organism actually builds specialized body forms for survival, for example spores in microbes and dauer larvae in the nematode *Caenorhabditis elegans* (Guarante and Picard, 2005).

Life extension as a result of dietary restriction may also be adaptive to adult females of widow spiders. Females survive for several months in nature and feed throughout their adult

lives (M. Segoli, personal observation). Females of widow spiders may store sperm for long periods after mating (Kaston, 1970). Thus, a female in a poorer body condition may benefit from increased lifespan, allowing her to postpone egg production until prey capture rate increases, although this must be balanced against the probability of mortality through predation or other causes. Additionally, life extension as a result of dietary restriction may be adaptive for widow spiders during the juvenile stages. For example, if a juvenile spider experiences a period of low prey capture, it may delay maturation until its body condition improves. The influence of food availability on the developmental time and on the timing of maturation is known in insects (Hunt *et al.*, 2004) and spiders (Vollrath, 1987), including widow spiders (Kaston, 1970). In general, well-fed spiders undergo fewer moults and mature earlier than poorly fed spiders. Thus, another possible explanation of our results is that the response to dietary restriction is conserved within the species, such that life extension observed in adult males is a by-product of selection acting on females and on both sexes during the juvenile stage, although it does not benefit adult males directly.

So why do adult males feed at all?

In the current study, we found a positive relationship between body mass at maturation and longevity, suggesting that feeding during juvenile stages increases the longevity of adult males. Body size was important to adult survival in the abundant-diet group, while body condition was important in the low-diet group. The difference between the treatment groups is probably due to the ability of abundant-diet males to renew their energy storage.

The effect of body size and condition on longevity, however, is probably less important than their influence on the male's reproductive success. Body size and body condition have been shown to have a positive influence on male reproductive success in many animal taxa (Andersson, 1994), including spiders (Vollrath, 1980; Singer and Riechert, 1995; Ahtiainen *et al.*, 2000; Elgar *et al.*, 2003). In widow spiders, courtship and mating are very costly for males, which lose up to 30% of their body mass in a single mating (Segoli, 2004). There is evidence that females accept larger and heavier males more often (Segoli, 2004) and prefer males that court longer (M.C.B. Andrade, personal communication). Additionally, in *L. pallidus*, females cannibalize smaller and weaker males more often than other males (Segoli, 2004). We suggest that adult males feed when possible by kleptoparasitism, in spite of the fact that feeding will decrease their lifespan, because feeding improves their reproductive success.

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