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SHORT COMMUNICATION

Resource allocation in food-restricted male *Physocyclus mexicanus* Banks, 1898 spiders does not favor proportionally larger testes (Araneae: Pholcidae)

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Abstract. The physiological effects of resource allocation due to dietary restriction in spiders are poorly understood; in fact, the system-wide effects of any environmental stresses on spider physiology remain relatively unstudied. The aim of this study was to show the consequences of dietary restriction in the pholcid spider *Physocyclus mexicanus* Banks, 1898. Male spiders were fed either a high (*ad libitum*) diet ($n = 43$) or low (5–8 *Drosophila melanogaster*/week) diet ($n = 32$) through their penultimate instar. We found significant differences in testis volume, body mass, and tibia-patella length [TPL] between the two groups. Linear regression analysis reveals that the differences in testis volume between the two groups are not solely due to differences in body mass; for any given body mass, the low diet group has a smaller mean testis size than the high diet group. Our results suggest that *P. mexicanus* males allocate resources away from testis volume in times of scarcity.

Keywords: Dietary restriction, testes size, body mass

Organisms maximize fitness for their expected reproductive lifespan by dynamically allocating available energy to growth, maintenance, and reproduction depending on environmental and physical conditions (Sibly & Calow 1986; Stearns 1992). Increased allocation toward one of these three key components necessarily means deprivation to the other two (Stearns 1992; Reznick et al. 2000; Roff & Fairbairn 2001). Resource (energy) allocation is the study of the relative importance of these three at different times in the life history of a species and how changes in allocation depend on the amount of resources available (Jokela & Mutikainen 1995). As has been observed in the male redback spider *Latrodectus hasselti* Thorell, 1870, allocation patterns can shift depending on the environmental context (Kasumovic & Andrade 2006). Resource allocation has been studied in many taxa. For example, wolf spiders that were fed only a limited diet of crickets took longer to reach sexual maturity, were smaller in size, and had higher mortality rates than those fed a well-balanced diet of insects (Uetz et al. 1992). This not only suggests that life cycle readjustments occur in times of famine, but also that life extension as a result of a restricted diet could be an adaptation to dealing with the famine itself (Uetz et al. 1992; Kirkwood & Shanley 2005).

We studied testis size and body size to determine whether metabolic energy was being preferentially used to favor reproduction. Sperm production in spiders, like most investments in reproduction, is a considerable energy cost in males of reproductive age (Greenstone & Bennett 1980; Anderson 1996; Olsson et al. 1997; Hayward & Gilloly 2011). While testis size does not completely correlate with sperm production, larger testes typically correspond to higher levels of sperm production (Schärer et al. 2004; Schärer & Vizoso 2007) or the production of longer sperm (Pitnick 1996). Males with larger testis size can experience greater reproductive success as a result (Schulte-Hostedde & Millar 2004). If an organism were to favor reproduction over survival during times of food restriction, the animal would likely be smaller in size, but its testes would be proportionally larger.

Testis size is correlated to sperm competition between males in species where females mate with more than one male (Gage 1994; Gay et al. 2009; Vrech et al. 2014). Female polygamy acts as the driving force for sperm competition in many spider species, with males partaking in longer copulations, guarding of females, and the production of copulatory plugs in order to prevent the female from mating with other males (Huber 2005). In many cases, these adaptations arose as a result of the behaviors of female spiders and the morphology of their reproductive tracts (Elgar 1998; Eberhard 2005). In more monogamous species, such as the Australian golden orb-web spider *Nephila plumipes* (Latreille, 1804), the males do not adjust their sperm investment based upon sperm competition, and they instead protect their paternity through mate guarding (Schneider et al. 2008). Furthermore, in *Tidarren argo* Knoflach & van Harten, 2001, male spiders abandon spermiogenesis and undergo testes atrophy after their one mating event (Michalik et al. 2010). In more polyandrous species, such as the pholcid spider *Pholcus phalangioides* (Fuesslin, 1775), males spend more time mating with virgin females than already-mated females in order to try and overcome last-male sperm priority and guarantee their parentage (Yoward 1998). Different species of fruit flies (genus *Drosophila*) have varied testis size and sperm lengths, which are due to selective pressures they experience through their environment, life cycle, and mating habits (Pitnick 1996). Scorpion species that experience higher levels of sperm competition have a higher testes mass than their monogamous relatives (Vrech et al. 2014). In yellow dung flies, testis size is influenced not only by sperm competition but also by the number of expected mates, as predicted by the size of the male (Blanckenhorn et al. 2004).

In general, genital size is positively correlated with body size in spiders, though there is little information specifically on testis size in spiders. However, genital size and body size have negative allometry in spiders; i.e., genitals are disproportionally larger in smaller spiders. (Eberhard 2009 and the references therein). Testis size has also been correlated with body size in other arthropods. Butterflies, yellow dung flies, and fruit flies all exhibit positive allometry of testes mass and body size.

Limited resources may lead to increased allocation of resources to survival, reproduction, or growth (Sibly & Calow 1986; Stearns 1992). This can be seen in many different spider species. For example, food-restricted *Latrodectus pallidus* O. P-Cambridge, 1872 males live longer than males fed *ad libitum*, which may be an evolutionary mechanism to ensure survival in order to maximize chances of reproductive success before death (Segoli et al. 2007). This could also result from

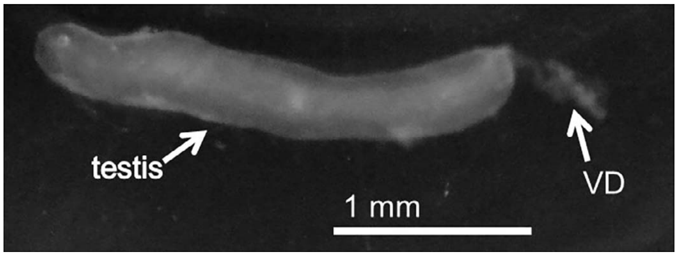

Figure 1.—Testis morphology in *P. mexicanus*, showing both testis and vas deferens (VD). Bar = 1 mm.

the possibility that lab feeding *ad libitum* may be overfeeding; individuals with restricted diets may actually be on a feeding regimen that more closely imitates their foods levels in the wild (Kirkwood & Shanley 2005). Extension of life span has been observed in many other species of spider (Austad 1989; Kleinteich et al. 2015); however, it is not true for all, as redback spiders do not show increased longevity under a restricted diet (Kasumovic et al. 2009). On the other hand, a restricted diet caused female *Tigrosa helluo* (Walckenaer, 1837) to seek mates less and to move to actively seek food more, a shift away from reproduction to survival (Walker et al. 1999). The shift away from reproduction has also been observed in water mites, minnows, and other species (Proctor 1992; Pyron 2000). In the conflict between investment in reproduction and survival, many diverse evolutionary strategies have evolved.

This study focuses on *Physocyclus mexicanus* Banks, 1898, a pholcid spider that can live more than one year (C. Kristensen, pers. comm.). We have observed that *P. mexicanus* continue to produce sperm through adulthood (data not shown), similar to previous observations of the pholcid *Pholcus phalangioides* (Michalik & Uhl 2005). *P. mexicanus* males are iteroparous and typically encounter many reproductive opportunities once they reach sexual maturity (C. Kristensen, pers. comm.). Thus, we hypothesized that they would not divert extra resources to reproduction during times of limited resources due to the likelihood that they would encounter a reproductive opportunity in the future when more resources become available again. As a result, we propose that *P. mexicanus*, like other iteroparous species, is likely to shift resource allocation in a restricted diet away from reproduction in order to prolong survival (Nakatsuru & Kramer 1982). We used testes size to estimate the allocation of resources to reproduction in male *P. mexicanus* spiders under dietary restriction.

Juvenile pre-penultimate-molt *Physocyclus mexicanus* males were collected and identified by C. Kristensen (Spider Pharm) in Yarnell, AZ. One male and one female specimen are deposited in the National Museum of American History, Smithsonian Institution. Spiders were kept individually in 1-pint (470-ml) deli containers with cheesecloth lids. Containers were stacked in aquarium tanks with standard aquarium heaters at approximately 25°C and exposed to natural light and dark cycles. Spiders were randomly assigned to high and low diet groups. A spider in the low diet group was fed 5–8 *Drosophila melanogaster* once per week through its penultimate instar. A spider in the high diet group was fed *ad libitum*, and was provided more than 100 *Drosophila melanogaster* per week through its penultimate instar. We do not have sufficient observations of feeding and life history to determine whether the low diet group represents a near-starvation diet, but food quantity for the low diet spiders was considerably less than individuals in the high diet group received, and led to differences in body mass, tibia-patella length, and testis volume relative to individuals receiving an *ad libitum* diet (see below).

Both treatment groups remained unfed for 5 days after their final molt before dissection to allow the high diet spiders to digest the contents of the gut (Nentwig 2013). Five days after the final molt, spiders were cold-anesthetized for 30 minutes, weighed, and dissected. The front leg was reserved for tibia-patella length measurements. Testes were removed in phosphate buffered saline and imaged using a Nikon (model C-DS) dissecting microscope with a Nikon Coolpix P6000 camera and an eyepiece adapter. Curve analyzing software, Image J 1.48 (Glyn II, 2011) was used to measure testis length and width compared to a ruler. Because *P. mexicanus* testes are nearly cylindrical (Fig. 1), testis volume was estimated using the formula for the volume of a cylinder ($V = \pi r^2 h$).

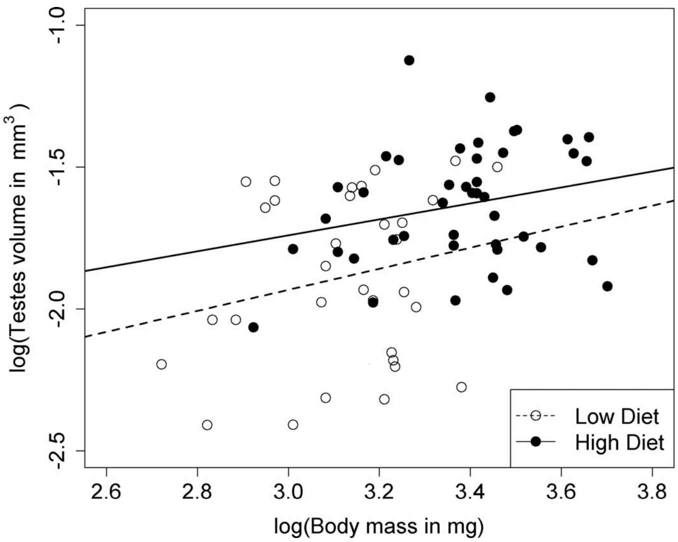

Figure 2.—Allometric relation between log testis volume (in mm^3) and log body mass (in mg) in low and high diet groups. Predicted log(testes volume) at body mass 27 mg = –1.82, slope of low diet group = 0.37 (95% CI = –0.14 to 0.88, P = 0.15), difference in predicted testes volume at body mass 27 mg = 0.166 (95% CI = 0.017 to 0.31, P = 0.029), slope of high diet group = 0.459. Difference in slopes = –0.089 (95% CI = –0.75 to 0.57, P = 0.79). $R^2 = 0.21$.

We compiled the results and used two tailed t-tests to determine whether there was a difference in resource allocation between the low diet (n = 32) and high diet (n = 43) groups. T-tests that yielded results with $P < 0.05$ were considered to show significant differences. The high and low diet groups had significant differences in mean tibia-patella length (TPL—high diet mean = 1.04 cm, low diet mean = 0.85 cm, t = 2.76, $P = 0.0066$), body mass (high diet mean = 29.75 mg, low diet mean = 23.13 mg, t = 5.78, $P = 7.7 \times 10^{-8}$), and testis volume (high diet mean = 0.199 mm^3, low diet mean = 0.158 mm^3, t = 4.04, P = 0.00013).

We fit a log-log regression model to the data, using both body mass and diet group as predictors of testis volume (Fig. 2). Our data show that both the low and high diet groups have low allometric slopes and display negative allometry (both slopes <1), as has been demonstrated for male genitalia in other spiders and insects (Eberhard 2009). While our data show that mean testis volume and mean body mass are both higher in the high diet group than in the low diet group, our analysis shows that the differences in testis volume between the two groups are not due solely to differences in body mass; i.e., for any given body mass, individuals in the low diet group will have a lower testis volume on average than individuals in the high diet group (Fig. 2). These data suggest that *P. mexicanus* invests more heavily in testis size when consuming an *ad libitum* diet than when consuming a restricted diet.

Because the male reproductive physiology of spiders is complex, diet could impact the size of more than one reproductive structure. Sperm are transferred from testes and vasa deferentia to pedipalps prior to mating (Huber & Eberhard 1997; Eberhard 2005). Because the pedipalps play such a critical role in storage and transfer of sperm, it is likely that diet could impact pedipalp volume as well. While we did not measure the impact of diet on pedipalp size in *P. mexicanus*, this could be an interesting future study.

In conclusion, the results above suggest that in times of scarcity, *P. mexicanus* males will allocate resources away from reproduction. This makes sense, because they are iteroparous and can have a long lifespan (more than one year) (C. Kristensen, pers. comm.), allowing for opportunities to reallocate resources to reproduction when food supply improves.

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