

Original Article

The effects of prey lipid on female mating and reproduction of a wolf spider

Qingguo FENG^{a,**}, Lelei WEN^{a,b,**}, Jiayi MA^a, Long YU^a, Changchun LI^{b,*}, and Xiaoguo JIAO^{a,*}

^aState Key Laboratory of Biocatalysis and Enzyme Engineering, Center for Behavioral Ecology and Evolution, School of Life Sciences, Hubei University, Wuhan 430062, China and ^bHubei Key Laboratory of Quality Control of Characteristic Fruits and Vegetables, College of Life Science and Technology, Hubei Engineering University, Xiaogan 432000, China

*Address correspondence to Changchun Li. E-mail: lichangchun@hbeu.edu.cn and Xiaoguo Jiao.

**These authors contributed equally to this work.

E-mail: jiaoxg@hubu.edu.cn.

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Abstract

As predators, the macronutrients spiders extract from their prey play important roles in their mating and reproduction. Previous studies of macronutrients on spider mating and reproduction focus on protein, the potential impact of prey lipid content on spider mating and reproduction remains largely unexplored. Here, we tested the influence of prey varying in lipid content on female mating, sexual cannibalism, reproduction, and offspring fitness in the wolf spider *Pardosa pseudoannulata*. We acquired 2 groups of fruit fly *Drosophila melanogaster* that differed significantly in lipid but not protein content by supplementing cultural media with a high or low dose of sucrose on which the fruit flies were reared (HL: high lipid and LL: low lipid). Subadult (i.e., 1 molt before adult) female spiders that fed HL flies matured with significantly higher lipid content than those fed LL flies. We found that the mated females fed with HL flies significantly shortened pre-oviposition time and resulted in a significantly higher fecundity. However, there was no significant difference in female spiders varying in lipid content on other behaviors and traits, including the latency to courtship, courtship duration, mating, copulation duration, sexual cannibalism, offspring body size, and survival. Hence, our results suggest that the lipid content of prey may be a limiting factor for female reproduction, but not for other behavioral traits in the wolf spiders *P. pseudoannulata*.

Key words: lipid, mating, macronutrients, *Pardosa pseudoannulata*, reproduction, survival.

As one of the most abundant groups of obligate predators, spiders feed upon phytophagous and omnivorous invertebrates, primarily insects. The nutritional quality of an insect prey varies dramatically and significantly affects the survival, growth, and reproduction of spiders (Li and Jackson 1996, 1997; Bilde and Toft 2000; Sigsgaard et al. 2001; Wilder 2011; Toft 2013; Wen et al. 2020). For example, Sigsgaard et al. (2001) found that linyphiid spiders *Atypena formosana* provided with a Collembola diet survived better and developed faster than spiders provided with other types of insect prey

(*Nilaparvata lugens*; *Nephotettix virescens*). Pekar and Toft (2009) reported that the ant-eating *Zodarion* spiders (*Z. atlanticum* and *Z. germanicum*) provided with a diet solely comprising of ants grew larger and had a longer life expectancy than spiders provided only with fruit flies. Mixed prey (i.e., a combination of various prey species) usually promotes spider growth and reproduction (Uetz 1992; Toft 1995; Toft and Wise 1999; Bilde and Toft 2000; Sigsgaard et al. 2001). However, the special prey containing toxic compounds inhibits spider growth and survival (Marcussen et al. 1999; Toft and

Wise 1999; Oelbermann and Scheu 2002; Fisker and Toft 2004; Rickers et al. 2006).

Studies concerning the effects of different or mixed prey species on spider fitness provide a foundation from which to investigate the effects of prey macronutrients on spider fitness (Wilder 2011; Toft 2013). To explore the effects of specific macronutrient contents, such as lipids and protein, on spider fitness, experiments should be well designed to control the influences of prey species and quantity (Mayntz et al. 2003; Philip and Shillington 2010; Kleinteich et al. 2015; Wiggins and Wilder 2018; Wen et al. 2020). In general, there are 2 approaches used to manipulate the macronutrient contents of prey without altering the prey species (Wilder 2011). The first approach simply involves the supplementary feeding of spiders with non-prey food (Vogelei and Greissl 1989; Pollard et al. 1995; Jackson et al. 2001; Taylor and Bradley 2009; Lietzenmayer and Wagner 2017). Therefore, there are some limitations on this approach, including the limited availability of natural non-prey foods for spiders (e.g., pollen and nectar) (Vogelei and Greissl 1989; Taylor and Bradley 2009; Nyffeler et al. 2016). In addition, there are a few spiders species known to consume these non-prey foods, which contain a limited macronutrients, especially carbohydrates (Taylor and Pfannenstiel 2008; Wilder 2011; Nahas et al. 2017). The second approach requires the manipulation of the quantity or ratio of macronutrients present in a single species of prey (Mayntz and Toft 2001; Hawley et al. 2014; Wen et al. 2020). This method was first used by Mayntz and Toft (2001), who cultured fruit flies on a normal medium supplemented with various nutrients (e.g., amino acids and fatty acids). This and subsequent studies demonstrated that altering the macronutrient content of the medium on which larval fruit flies cultured results in the changes in the macronutrient contents of the adult fruit flies. The changes of macronutrient contents of the adult fruit flies influence the survival, growth, and reproduction of spiders (Mayntz and Toft 2001; Mayntz et al. 2003, 2005). However, there are still some limitations on this method. For instance, juvenile spiders, especially the wandering hunters with a long lifespan, always encounter molting problems and subsequently die before reaching adulthood when they are restricted on a monotypic diet of fruit flies (Mayntz and Toft 2001; Wen et al. 2020). Considering the fact that most spider species feed on a variety of prey species to attain nutrition balance for survival and reproduction (Greenstone 1979; Uetz 1992), nutrition enrichment of fruit flies with special macronutrient may of course not succeed in making them perfectly optimal prey for spiders. Therefore, this method was almost only applied to short-term studies on how prey protein and lipid affect spider performance (Toft et al. 2010; Jensen et al. 2011a, 2011b; Hawley et al. 2014; Wiggins and Wilder 2018; Koemel et al. 2019).

Due to the limitations of both approaches above mentioned, only a few studies have determined how macronutrient compositions of a single prey species affect spider fitness depending directly on a performance test (Jensen et al. 2011a, 2011b; Wiggins and Wilder 2018; Wen et al. 2020). These studies have consistently shown that high protein (high ratio of Protein:Lipid) prey has positive impacts on spider growth and survivorship (Blamires et al. 2009; Jensen et al. 2011a, 2011b; Salomon et al. 2011). However, the impact of high lipid (low ratio of Protein:Lipid) prey on spider growth, development, and survival remains controversial. It is evidenced that the effect of prey lipid content (low ratio of Protein:Lipid) on spider growth, reproduction, and survival depends on spider species and life-history strategies (Jensen et al. 2010, 2011a, 2011b; Wen et al. 2020). Wiggins and Wilder (2018) found

that larger body size and heavier juveniles in the jumping spider *Phidippus audax* was tightly associated with high-lipid prey (fruit fly). On the contrary, Mayntz and Toft (2001) demonstrated that prey (fruit fly) with high lipid reduced the survival of juvenile wolf spiders *Pardosa amentata*. Similarly, the negative effects of high lipid on the spider survival were found in the sheet-web spiders *Hylyphantes graminicola* (Wen et al. 2020). In addition, prey (fruit fly) with high lipid reduced the growth of the juvenile wolf spiders *Pardosa prativaga* (Jensen et al. 2011a, 2011b). In terms of reproduction, Wen et al. (2020) demonstrated that female *H. graminicola* fed fruit flies with high lipid throughout their life cycle substantially delayed their egg-laying but not fecundity. However, a number of biochemical analyses on several spider species during the reproductive stage indicated that lipids play an important role in reproduction (Salomon et al. 2008; Blamires 2011; Romero et al. 2018; Laino 2020).

Although there is still no coherent picture of how a spider's lipid requirement changes with life stages, Wilder and Rypstra (2010) did find that female wolf spiders *Hogna helluo* were able to extract nearly all lipid presented in male spiders and crickets. In addition, their data also suggest that lipid, but not protein, may limit egg production of female *H. helluo* (Wilder and Rypstra 2010). In another study, female wolf spiders from 3 species also extracted over 90% of the available lipid in a range of prey items (Wilder et al. 2010). The fact that adult female spiders extract nearly all available lipids in prey suggests that lipid may be a limiting macronutrient for adult females, especially during the shortage of prey (e.g., hibernation), maternal care, and reproduction (Jensen et al. 2010; Salomon et al. 2011; Ruhland et al. 2016; Potts et al. 2020). Yet there is relatively limited direct evidence of the potential effect of lipids on spider mating and reproduction (Wen et al. 2020).

The wolf spider *Pardosa pseudoannulata* (Araneae: Lycosidae) is one of the most important predators against diverse pests found in rice paddies in China (Lou et al. 2014; Xiao et al. 2016; Guo et al. 2018; Hou et al. 2021), and there are 2–3 generations a year (Zhao 1993). In the present study, we aimed to investigate the effects of the lipid content extracted from fruit flies *Drosophila melanogaster* on female mating behavior, sexual cannibalism, and reproductive success in the wolf spider, *P. pseudoannulata* in the laboratory. We first acquired 2 groups of fruit flies *D. melanogaster* varying in lipid but not protein content by manipulating the sucrose content of their culture media. Then the field-collected subadult female wolf spiders were fed with HL or LL fruit flies until they reached maturation, the lipid content of adult female spiders were determined. If the 2 groups of adult female spiders differed in the lipid content, we then examined the effects of the lipid contents of female spiders on their mating behavior (the occurrence of mating, courtship, and copulation), the occurrence of sexual cannibalism, reproductive success (pre-oviposition time and fecundity), and offspring quality (offspring size and survival under food and water stress). We expected to determine not only the effects of lipid content on short-term behaviors but also on long-term reproduction of the wolf spiders *P. pseudoannulata*. Our present study would provide some implications on nutritional requirements for the reproduction of the predatory spiders.

Material and Methods

Acquisition of fruit flies varying in lipid contents

To generate fruit flies *D. melanogaster* varying in lipid contents, we prepared 2 types of culture media with different sucrose contents (32 and 8 g, respectively). We kept other components of the culture media the same and equal quantity (240 mL H₂O, 22 g corn powder,

4 g yeast extract powder, 1.6 g Agar, 0.1 g benzoic acid [dissolved in 2 mL ethyl alcohol], and 1 mL propanoic acid). We termed these 2 groups of fruit flies as Group high lipid (HL) and Group low lipid (LL), respectively. We divided each type of culture media equally into 10 culture tubes (diameter \times length: 50 mm \times 12 mm). After cooling and solidification, we inoculated each culture media tube with approximately 5 pairs of adult fruit flies that had been sub-cultured in the laboratory. After 1 week, we removed the sub-cultured fruit flies from each tube after a large number of fruit flies emerged. When the newly emerged fruit flies in each tube reached the peak, we randomly collected more than 100 flies (a mix of males and females) from each tube as representatives and then measured the percentage (%) of lipid and protein to their dry body mass (HL: $N=9$ tubes; LL: $N=10$ tubes). We measured the lipid content using a gravimetric assay in which chloroform was used to dissolve lipids as described by Wilder et al. (2013). Briefly, the dried samples were soaked in 5 mL chloroform for 24 h after which the chloroform was removed. Each sample experienced 3 soaking periods and then was re-dried and re-weighed. The lipid content was calculated as the difference in dry mass before and after the lipids were extracted by the chloroform. After lipid extraction, the lean samples of fruit flies were immediately immersed in 5 mL of sodium hydroxide solution (NaOH, 0.35 M) and the protein of fruit flies was digested at 37°C (Rho and Lee 2014). The samples also went through 3 successive, sodium hydroxide solution 24-h washes, after which they were re-dried and re-weighed. The protein content of fruit flies was estimated as the difference in dry mass before and after the 3 washes of sodium hydroxide.

Acquisition of female spiders varying in lipid content

We collected the sub-adult female wolf spiders in the rice field in Huazhong Agricultural University, Wuhan, Hubei Province, China, between September and October 2020. They were kept individually in glass tubes (diameter \times height: 3 \times 12 cm) and provided water via a piece of water-dampened sponge at the bottom of glass tubes. We kept them in an incubator with controlled environmental conditions (25 \pm 0.5°C, 60 \pm 10% RH, and 14:10 light:dark regime). Then, we randomly divided the sub-adult females into 2 groups (HL or LL) of at least 100 spiders. We defined the female spiders fed with HL and LL fruit flies as HL and LL groups, respectively. According to its group (HL or LL), each subadult female spider was fed with 20 HL or LL fruit flies each time and twice a week. We monitored the sub-adult female spiders by checking for molting every 12 h until they reached adulthood. We obtained a total of 75 HL and 70 LL adult female spiders. We randomly chose 32 HL and 28 LL adult females from either group of spiders 3 days after their last molt (i.e., 3 days post-maturation) to determine their lipid content. The measurement of the lipid contents of HL and LL adult female spiders was the same as above mentioned (Wilder et al. 2013). The remaining adult female wolf spiders were used for mating and reproduction experiments.

Mating experiments

To determine the effects of lipid on mating of *P. pseudoannulata*, we carried out mating trials for 2 groups of spiders separately. We used a petri dish (12.0 cm in diameter) at the bottom lined with a piece of clean filter paper and covered with an inverted cylindrical glass jar (diameter \times height: 10.5 \times 12.0 cm) as a mating arena. We paired a randomly selected adult female spider aged 3 days post-maturation either from Group HL or Group LL with a randomly selected field-

collected adult male. We used the field-collected males because they are polygynous (Zhao 1993; Gong et al. 2019) and we could ensure all males were of relatively high quality compared with the laboratory-reared males (Zhao 1993; Jiang et al. 2018). We also controlled the possible interaction between female and male diets. Prior to the trial, we first introduced the female into the arena and allowed the spider to acclimate for 30 min. After that, we removed the cover and introduced the male to the mating arena to begin the mating trial. We placed the cover back to avoid the spiders escaping. We recorded courtship latency (the time elapsed from the start of the mating trial to the start of the courtship), courtship duration (time interval in min between the start of the courtship and the onset of copulation), the occurrence of mating, mating duration, and the occurrence of sexual cannibalism for each mating trial. Given that the latency to mate (time interval in min between the start of the mating trial and the onset of copulation) was relatively short, each successful trial lasted for a maximum of 30 min. The mating was deemed successful when the male spider climbed on the female back. However, if the male spider failed to climb on the female's back or was cannibalized by the female within 30 min, the mating was deemed unsuccessful. After mating, the paired male and female were maintained for another 10 min to determine the occurrence of postcopulatory sexual cannibalism. If the female caught the male, we immediately separated them with a soft brush.

Female reproduction and offspring survival

To test the effect of prey lipid content on female reproduction, we put the female into its original glass tube and monitored it every 12 h to determine whether the female laid the egg sac or not. After mating, the feeding regime of these successfully mated females was the same as above mentioned (i.e., fed with HL or LL flies accordingly). We recorded the pre-oviposition period (time interval in days between the end of mating and the first egg sac laying), egg development time (time interval in days between the production of first egg sac and hatching of eggs), and fecundity (i.e., total number of eggs) of all female spiders that had successfully produced the first egg sac. There was no significant difference in carapace width between female spiders from Group HL and Group LL (independent *t*-test: $t_{54} = 1.858$, $P = 0.069$).

We measured the offspring body size (proxy: carapace width) and their survival without food and water. Two days after spiderlings emerged from the egg sacs, 5 spiderlings were randomly selected from each egg sac and their carapace width was measured individually. In addition, another 5 spiderlings were randomly selected from each egg sac to determine their viability in the absence of food and water. They were kept individually in clean and dry glass tubes under 15°C, and their survival was monitored every 12 h.

Data analysis

We performed all statistical analyses using R 4.0.3 (R Core Team 2021). We checked for normality of all the data using the Shapiro-Wilk test. The differences in the lipid contents, protein contents, ratio of lipid to protein of fruit flies, and pre-oviposition duration between Groups HL and LL were tested using independent *t*-tests. The differences in the lipid contents of female wolf spiders, courtship latency, courtship duration, copulation duration, and egg development time between HL and LL female spiders were compared using unpaired Wilcoxon rank sum test. We performed Chi-square tests to compare the differences in the frequency of mating success

and sexual cannibalization of female spiders between HL and LL groups. To examine the effects of lipid content on offspring carapace width and survival under the stress conditions without food and water, we ran general linear models with lipid treatment as a fixed factor and female identity as a random factor.

Results

Lipid contents of fruit flies and adult female spiders

The lipid content of the fruit flies fed on the culture media with high sucrose was significantly higher than that of the fruit flies fed on culture media with low sucrose (independent t -test: $t_{17} = 5.082$, $P < 0.0001$; Figure 1A). However, the protein content was the same between the 2 groups (independent t -test: $t_{17} = 0.499$, $P = 0.6243$; Figure 1B). Therefore, the ratio of lipid to protein of fruit flies was still higher for HL than LL flies (Independent t -test: $t_{17} = 4.281$, $P = 0.0005$; Figure 1C). Similarly, the lipid content of females fed on fruit flies with high lipid was significantly higher than that of females fed on fruit flies with low lipid (Wilcoxon rank sum test: $W = 887$, $N_1 = 32$, $N_2 = 28$, $P < 0.0001$; Figure 1D).

Courtship and mating success

There were no significant effects of female diet on male courtship latency ($W = 361$, $N_1 = 31$, $N_2 = 25$, $P = 0.611$; Figure 2A), courtship duration ($W = 402$, $N_1 = 31$, $N_2 = 25$, $P = 0.813$; Figure 2B), and copulation duration ($W = 367$, $N_1 = 31$, $N_2 = 25$, $P = 0.741$; Figure 2C). Both females with high lipid and those with low lipid had a high rate of mating success (HL: 89% [31/35]; LL: 83% [25/30], respectively), with no significant difference in the mating success between females with different lipid contents ($\chi^2_1 = 0.062$, $P = 0.803$). No female spiders engaged in precopulatory sexual cannibalism. The postcopulatory sexual cannibalization rates of females with high lipid and females with low lipid were 29% (9/31) and 24% (6/25), respectively, and there was no significant difference ($\chi^2_1 = 0.014$, $P = 0.905$).

Reproduction and offspring fitness

Compared with females with low lipid, females with high lipid laid their first egg sac significantly earlier after their mating ($t_{54} = -2.526$, $P = 0.015$; Figure 3A). In addition, females with high lipid produced significantly more eggs than those with low lipid

($W = 507.5$, $N_1 = 31$, $N_2 = 25$, $P = 0.049$; Figure 3B). Eggsacs from all the mated females (31 for females with high lipid and 25 for females with low lipid, respectively) hatched successfully. However, no significant effect of lipid content in female was found on egg development rate ($W = 322.5$, $N_1 = 31$, $N_2 = 25$, $P = 0.269$; Figure 3C).

We found no effect of lipid content from females on their offspring body size (i.e., carapace width) (GLMM: $\beta = -0.004$, $z = 1.13$, $P = 0.257$; Figure 4A). In addition, the spiderlings from females with low lipid had a similar survival time to those with high lipid under the stress conditions without food and water at 15°C (GLMM: $\beta = -0.42$, $z = 0.78$, $P = 0.434$; Figure 4B).

Discussion

Food quality, the components and relative concentrations of macronutrients in food, have important implications for the fitness of animals. Food quality could not be determined just from biochemical analysis because even the same proportion of nutrients in the food may have different quality among different species, if the predator has different nutrition demands. Although the consequences of the macronutritional content of food has been well studied for herbivores (Roeder and Behmer 2014; Dreassi et al. 2017; Wang et al. 2018), relatively few studies have been conducted for predators including spiders (Raubenheimer et al. 2007; Barry and Wilder 2013; Wen et al. 2020). Among the 3 types of macronutrients, previous studies dealing with lipids usually inferred the effects of lipids on spider reproduction (Xu et al. 1994; Salomon et al. 2008; Hyne et al. 2009; Bressendorff and Toft 2011). For example, a few studies showed that adult female spiders could efficiently extract lipids from prey (Wilder et al. 2010; Wilder and Rypstra 2010), while other studies found that lipid content varied significantly for female spiders during reproductive stages through biochemical analyses (Salomon et al. 2008, 2011; Romero et al. 2018). Our first attempt is to determine whether lipid might affect mating and reproduction of spiders directly through a performance test. By acquiring HL and LL fruit flies varying in lipid but not protein contents (Figure 1A–C) and feeding sub-adult *P. pseudoannulata* female spiders with HL and LL fruit flies, respectively, we acquired 2 groups of female wolf spiders varied in their lipid contents when they grow adults (i.e., HL and LL female spiders, Figure 1D). Then, we conducted

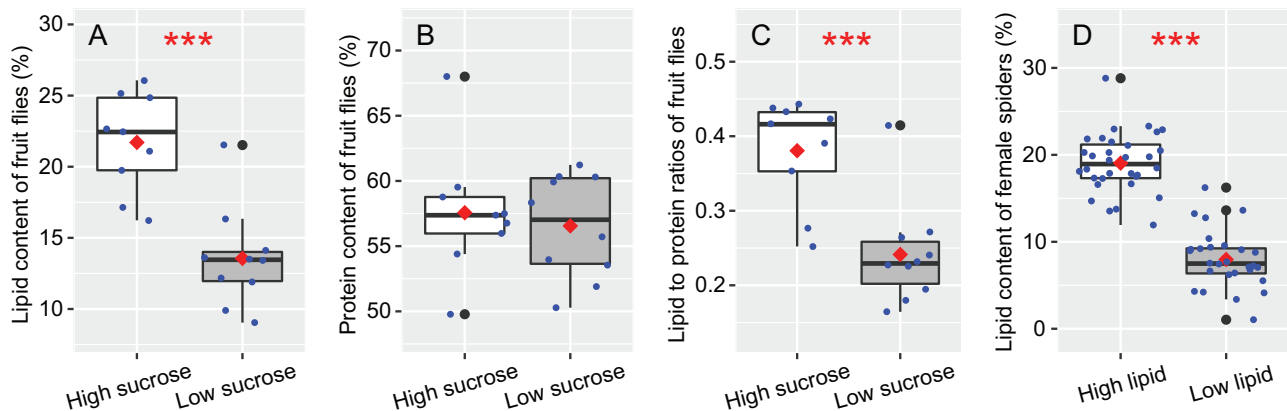


Figure 1. Boxplots of the lipid content (%) (A), protein content (%) (B), ratio of lipid to protein (C) of the fruit flies *D. melanogaster* between the high and low sucrose groups; and (D) female spiders *P. pseudoannulata* that fed on fruit flies *D. melanogaster* varying in the lipid content (HL; LL). Boxplots show the median (central line), first and third quartiles (box), and the minimum and maximum values (whiskers), and the jitter points show the value and corresponding N of each treatment. The red rhombi show the mean values. *** $P < 0.001$.

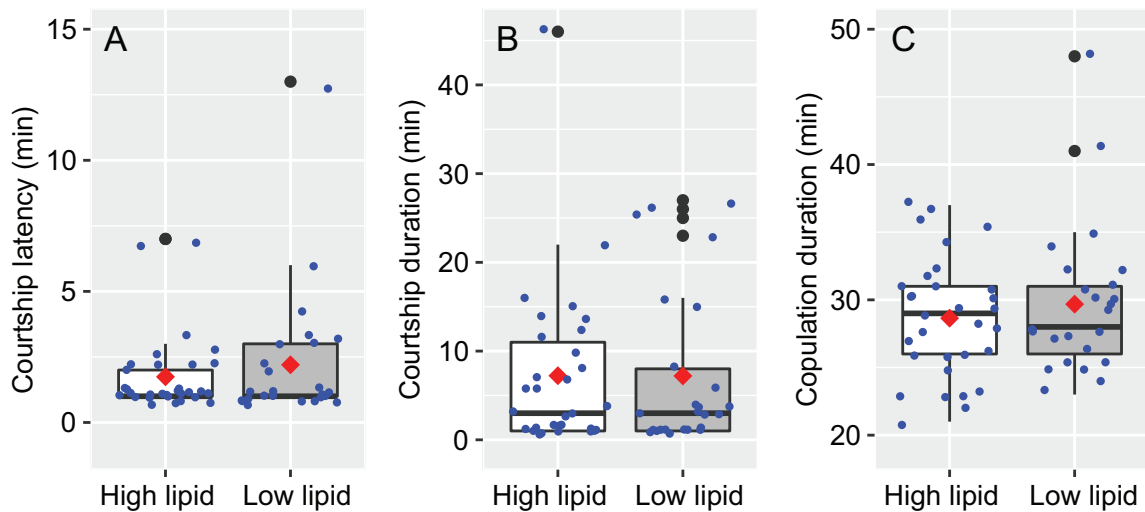


Figure 2. Boxplots of results from mating behavior of *P. pseudoannulata* females from the high-lipid and low-lipid groups. (A) Courtship latency (min); (B) courtship duration (min); and (C) copulation duration (min). Boxplots show the median (central line), first and third quartiles (box), and the minimum and maximum values (whiskers), and the jitter points show the value and corresponding *N* of each treatment. The red rhombi show the mean values.

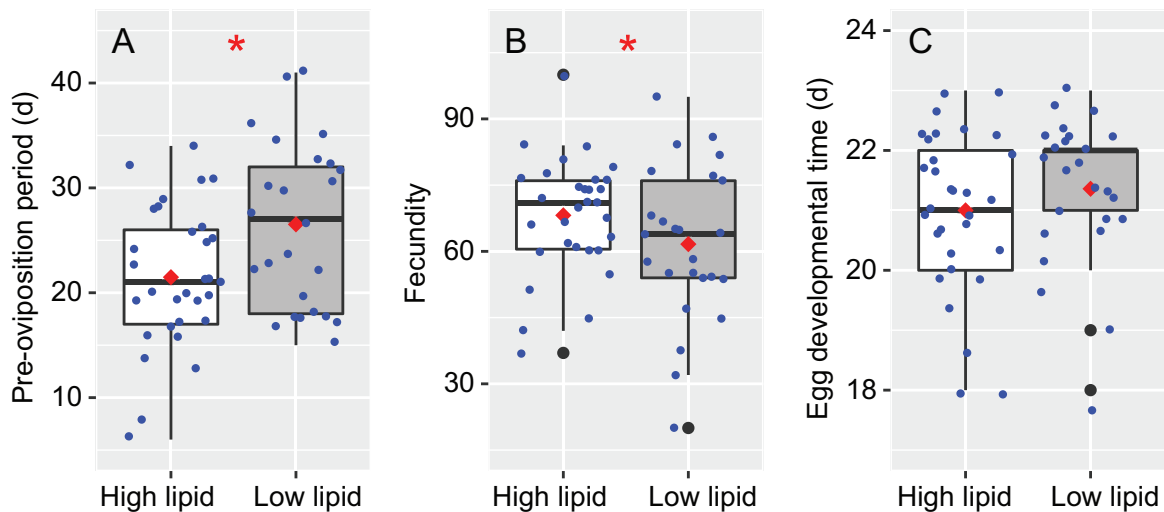


Figure 3. Boxplots of the differences in the reproductive success between high- and low-lipid *P. pseudoannulata* female spiders. (A) Pre-oviposition period (d); (B) fecundity (i.e., number of eggs of the first egg sac); and (C) egg development time (d). Boxplots show the median (central line), first and third quartiles (box), and the minimum and maximum values (whiskers), and the jitter points show the value and corresponding *N* of each treatment. The red rhombi show the mean values. * $P < 0.05$.

performance tests to determine whether the lipid contents in female spiders exerted significant effects on female mating success, sexual cannibalization rate, egg production, and offspring quality.

Our results from the mating experiments show that the lipid content of females has no effect on their courtship latency, courtship duration, mating success, and mating duration (Figure 2). These results are expected since the measured mating behaviors are prominent to be affected by male quality. Male quality is not a confounding factor since all the males used were field-collected and they were randomly paired with either HL or LL females. Wilder and Rypstra (2008) also found there were no significant effect of diet quality on the latency to male courtship, courtship duration, and occurrence of mating in the wolf spider *P. milvina*.

In addition to mating behavior, our results also indicate that there is no significant difference in sexual cannibalistic rate between HL and LL females. Thus, the results rule out the possibility that

female spiders cannibalize males for replenishing the limited lipids in *P. pseudoannulata*. Alternatively, it is also possible that sexual size dimorphism in the wolf spider *P. pseudoannulata* is not large enough for a relatively well-fed female spider to capture a male (Gavín-Centol et al. 2017). Further investigation, using the starved female spiders, is needed to test the hypothesis.

Although no difference was found in mating behavior and sexual cannibalism, our reproduction experiments showed that HL females laid eggsacs significantly earlier and with more eggs than LL females (Figure 3A,B). Field-caught female wolf spiders *P. milvina* maintained on a high-quality diet (fruit flies reared on media supplemented with dog food) also produced eggsacs more quickly (Wilder and Rypstra 2008). Spiders are shown to mainly feed upon protein-rich prey in the field (Wilder 2011; Wilder et al. 2013; Wiggins and Wilder 2018). It seldom happens that the field-collected female spiders were deficient in protein (Wilder 2011; Wilder et al. 2013). In

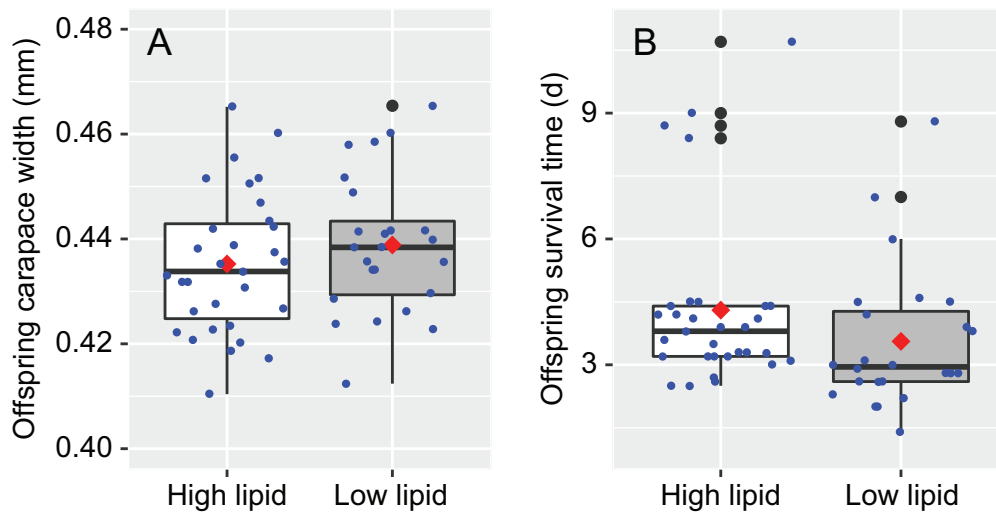


Figure 4. Boxplots of the differences in the offspring performance between high- and low-lipid *P. pseudoannulata* female spiders. (A) Offspring carapace width (mm) and (B) offspring survival time. Boxplots show the median (central line), first and third quartiles (box), and the minimum and maximum values (whiskers), and the jitter points show the value and corresponding *N* of each treatment. The red rhombi show the mean values.

addition, proteins play an important role in promoting the growth of juvenile spiders (Jensen et al. 2011b; Wilder 2011; Laino et al. 2020), but adult spiders might not grow much in size and what an adult spider has to reserve may be lipids to meet the needs of metabolism and reproduction (Jensen et al. 2010; Wilder and Rypstra 2010; Romero et al. 2018; Trabalon et al. 2018), especially for a female spider that has a far higher input in reproduction than a male (Lease and Wolf 2011; Wilder 2011). Thus, it can be inferred that *P. milvina* also benefits from the lipids in the high-quality diet to accelerate egg production. In contrast, our previous study demonstrates that females fed with high lipid prey greatly delayed egg-laying in a sheet-web spider *H. graminicola* (Wen et al. 2020). This discrepancy may be due to several differences between the 2 studies. First, *H. graminicola* was a much smaller spider (Female: about 3–4% wet mass of *P. pseudoannulata*) with an extremely short development duration, thus having a high protein demand for development. In the present study, however, *P. pseudoannulata* females were reared with fruit flies from subadult to adult, whereas *H. graminicola* spiders were constantly fed on high-lipid diet from hatching to maturation. Consequently, *H. graminicola* (Group HL mean: 26% dry mass) matured with much higher lipid than *P. pseudoannulata* (Group HL mean: 19% dry mass). Second, as protein are also important for reproduction (Barry and Wilder 2013; Romero et al. 2019; Montoro et al. 2021), a much higher protein demand for growth and too high lipid reserve at maturation may limit the amount of protein that *H. graminicola* could consume and further invest in egg production. Meanwhile, protein is not a big problem for egg production of female *P. pseudoannulata* with a much larger body to store more protein. Furthermore, in both studies, we are unable to completely rule out the possibility that the inadequacy of other micronutrients, such as phosphorus, vitamins, and minerals, may influence the reproduction of female spiders (Wilder and Rypstra 2008; Wilder 2011; Wilder and Schneider 2017).

During egg-laying, female spider exhibits plasticity in allocating the amount of lipid to their eggs (Anderson 1978; Blamires 2011; Wilder 2013), which may have a potential influence not only on fecundity, but also on egg quality (i.e., egg size and egg energy density) (Trabalon et al. 2018). Moreover, high-quality eggs are often

associated with greater nutritional provisioning (Fox and Czesak 2000; Ameri et al. 2019) and offsprings may hatch earlier, hatching with a larger size, and experience higher survival than offsprings developing from low-quality eggs (Geister et al. 2008; Ameri et al. 2019). However, offsprings from HL females did not show any advantage in quality indexes such as egg developmental rate, body size, and survival (Figures 3C and 4). This is still no wonder as both groups of female spiders fed on fruit flies with different lipid but equal protein contents, the latter was more important for offspring quality. For example, Wilder and Schneider (2017) found that juvenile offspring of female spiders *Argiope bruennichi* supplemented with essential amino acids survived longer than those with fatty acids. Meanwhile, protein contributed principally to the energy expenditure of juvenile wolf spiders *Pardosa saltans* during the period between dismounting off their mother's back until their first exogenous feed (Laino et al. 2020). On the contrary, Anderson (1978) examined the energy content (positive correlation with lipid content) of eggs of 12 spider species from 6 families and found no correlation between energy content per unit egg mass and size of the female parent, egg size, or clutch size. He also found that the variation in mass-specific energy content was less than the variation in clutch size (Anderson 1978). Thus, the high lipid contents of the fruit flies contribute to promoting egg production, but not offspring size and survival of female *P. pseudoannulata*.

In summary, our study demonstrated that female lipid content has no effect on mating behavior, but positively facilitates their reproduction in the wolf spider, *P. pseudoannulata*. The present study sheds some lights on nutritional requirements for the reproduction of the predatory spiders.

Author Contributions

Q.F., L.W., and X.J. participated in the study design. Q.F., L.W., and J.M. collected the data. Q.F., C.L., and L.Y. analyzed the data and wrote the manuscript. X.J. and L.W. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Ethical Statement

Animal husbandry and experimental procedures adhered to the Guidelines for the Use of Animals in Research (ASAB/ABS, 2021). We treated all spiders gently during the experiments to minimize any adverse impacts on their welfare (Kralj-Fišer and Gregorič 2019). After the experiments, the remaining adult spiders and offsprings were released to their original habitats. Animal care in all experiments complied with the current laws and standards of China (Bayne et al. 2018).

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Conflict of interest

The authors declare that they have no conflict of interest.

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