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RESEARCH ARTICLE

High-lipid prey reduce juvenile survivorship and delay egg laying in a small linyphiid spider *Hylyphantes graminicola*

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ABSTRACT

Prey proteins and lipids greatly impact predator life-history traits. However, life-history plasticity offers predators the opportunity to tune the life-history traits in response to the limited macronutrients to allocate among traits. A fast-growing predator species with a strict maturation time may be more likely to consume nutritionally imbalanced prey. Here, we tested this hypothesis by examining the effect of the protein-to-lipid ratio in prey on a small sheet web-building spider, *Hylyphantes graminicola*, with a short life span, using adult *Drosophila melanogaster* as the prey. By manipulating the macronutrient content of the prey to generate three prey types with different protein-to-lipid ratios (i.e. high, intermediate and low), we demonstrated that the majority of the spiders that consumed only these flies could reach full maturity. However, juvenile spiders that consumed high-lipid (low protein-to-lipid ratio) flies had a higher rate of mortality than those consuming medium-protein and high-protein flies. The prey protein-to-lipid ratio had no significant effects on the developmental duration and size at maturity. Although the prey protein-to-lipid ratio had no significant influence on mating behaviour and female fecundity, females reared on high-lipid flies exhibited a significant delay in oviposition compared with those reared on high-protein flies. We conclude that high-lipid prey has negative effects on the survival and reproductive function of *H. graminicola*. Our study thus provides clear evidence that low plasticity with fast development to a certain size means a high nutritional requirement for protein at a cost of lower survival and prolonged time to egg laying when prey have low protein-to-lipid content in *H. graminicola*.

KEY WORDS: Life-history plasticity, Macronutrient content, Protein, Spider, Fitness, Growth, Reproduction

INTRODUCTION

As for all organisms, predators experience trade-offs between life-history traits or fitness components, including growth, reproduction and survival, due to limited resources within a set time frame (Roff, 2002; Stearns, 1992). Selection will shape life-history strategies to maximize fitness in a particular environment and when the environment changes, predators may be selected to alter their life-history strategies to maintain fitness (Nylin and Gotthard, 1998; West-Eberhard, 2003). Predators are both protein and lipid limited in nature (Barry and Wilder, 2013; Fagan et al., 2002; Reifer et al., 2018; Salomon et al., 2011; Toft et al., 2019; Wiggins and Wilder, 2018). However, predators, like other organisms, exhibit life-history plasticity, the capacity to facultatively alter life-history traits in response to a limited pool of macronutrients to allocate among traits (Simpson and Raubenheimer, 2012). This nutrient-mediated life-history trade-off assumes that the different life-history traits cannot be maximized at the same macronutrient intake as each trait needs a specific balance of macronutrients for its maximal performance (Morimoto and Lihoreau, 2019; Rapkin et al., 2018).

Spiders are among the most diverse and abundant carnivorous predators. However, our knowledge of prey nutrient-mediated life-history plasticity of spiders remains poor. Many studies of nutritional ecology in spiders have primarily focused on how macronutrients, such as proteins and lipids, affect spider life-history traits (Toft, 2013; Wilder, 2011). Spiders have traditionally been thought to be lipid limited in nature (Wiggins and Wilder, 2018), but recent studies have shown that spiders also rely on prey protein (Salomon et al., 2011; Wilder and Schneider, 2017). By supplementing basic fruit fly culture medium with a variety of amino acids, fatty acids or dog food, a number of studies have demonstrated the effects of such additives on spider life-history traits and behaviours. For example, *Pardosa amentata* wolf spiders that fed on fruit flies grown on medium supplemented with dog food exhibited more rapid growth and better survival (Mayntz and Toft, 2001). Male *Pardosa prativaga* spiders that fed on fruit flies with a high protein content exhibited a stronger desire for courtship and better mating success (Lomborg and Toft, 2009), while *Pardosa milvina* females that consumed flies with a higher protein content were more aggressive and had a greater egg-laying rate than those that fed on flies with a lower protein content (Wilder and Rypstra, 2008). In contrast, a study of the social spider, *Stegodyphus dumicola*, revealed that a colony that fed on high-lipid prey produced more and larger breeding females than a colony that fed on high-protein prey (Salomon et al., 2008). However, these studies have rarely discussed spider nutritional ecology under a life-history plasticity framework.

Hence, the lipid and protein content of prey can affect a wide range of life-history traits in spiders. To date, much research related to this topic has involved members of a single spider family, Lycosidae, and even a single genus within this family, *Pardosa* (Wilder, 2011), which largely comprises active hunters. In contrast, very little is known about the nutritional ecology of other spider taxa (Hawley et al., 2014; Toft et al., 2010; Wiggins and Wilder, 2018; Wilder, 2011), especially small web-building spiders, which have a short life span and show a 'sit-and-wait' hunting behaviour.

In this study, we investigated the influence of the ratio of protein to lipid content of prey on multiple fitness-related traits, including survival, growth and reproduction, of the money spider, *Hylyphantes graminicola* (Sundevall 1830) (Araneae: Linyphiidae). We chose this species as our study system because it is a small-sized (2–3 mm) species with a short life span (approximately 1 month) and a sit-and-

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wait hunting strategy, and has been widely studied with respect to its life-history traits (Zhao, 1993). We expected that in such a fast-growing, small spider species with a strict maturation time, certain life-history traits would be more affected than others by nutritionally imbalanced prey. This investigation was facilitated by manipulating the macronutrient content of *Drosophila melanogaster* fruit flies, which were grown on culture media containing various concentrations of sucrose or yeast powder. This spider has a palaearctic distribution and is among the most important natural enemies of pests in agricultural ecosystems and forests throughout Asia (Zhao, 1993). We assumed that the extraction and utilization of prey nutrients by *H. graminicola* for survival, growth and reproduction would depend on the variation in the macronutrient composition of the prey. In this experiment, we fed spiders with flies reared on diets containing different ratios of protein and lipid. We hypothesized that within a set time frame, *H. graminicola* could not maximize the different life-history traits at the same protein and lipid intake because they might require a different balance of proteins and lipids for their maximal performance. We predicted that *H. graminicola* might be more plastic in some fitness-related traits than others in response to a diet of certain protein-to-lipid ratio of prey.

MATERIALS AND METHODS

Collection and maintenance

Hylyphantes graminicola juveniles used in laboratory experiments were the first-generation offspring of female spiders caught in a cornfield in Longmen Town (34°34′N, 112°29′E), Luoyang City, Henan Province, China, in June 2018. All adult females had mated in the field before capture and were brought to the laboratory at Hubei University, Wuhan, China. The female spiders were housed individually in glass tubes (diameter×length: 20×60 mm) that were plugged with absorbent cotton. A piece of water-dampened sponge was placed in the bottom of each glass tube to provide water *ad libitum*. All the spiders were housed in an incubator at 25±0.5°C and under illumination for 14 h per day. The spiders were fed every 3 days with 10–15 fruit flies (*D. melanogaster*) reared on Group M cultural medium with a medium protein-to-lipid ratio (see below). Most spiders laid their first egg sac within 1 week of arrival at the laboratory, and the eggs hatched within the subsequent week (mean±s.e.m. hatching time: 6.8±0.2 days; range: 6–8 days; n=19). The hatchlings underwent their first moult within the egg sacs. Only juveniles from the first clutch were assigned to the prey nutrient treatment groups (see below).

Generation of fruit flies with different protein-to-lipid ratios

To generate fruit flies with different protein-to-lipid ratios as prey for *H. graminicola* juveniles, we prepared three types of culture medium. Group M culture medium (intermediate protein-to-lipid ratio), the standard fruit fly culture medium, comprised 240 ml H_2O, 22 g corn powder, 16 g sucrose, 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. Group HL (high-lipid/low protein-to-lipid ratio) culture medium contained 32 g sucrose. Group HP (high-protein/high protein-to-lipid ratio) culture medium contained 10 g yeast extract powder. All other components of Groups HL and HP were identical to those of Group M culture medium.

Each type of culture medium was divided equally into 10 culture tubes (diameter×height: 5×12 cm). After cooling and solidification, each culture media tube was inoculated with approximately five pairs of adult fruit flies that had been sub-cultured in the laboratory. After 1 week, a large number of fruit fly larvae emerged, and the sub-cultured fruit flies were removed. When fruit flies in each tube reached the peak, approximately 100 flies (a mix of males and females) were collected randomly from each tube as representatives, and the percentage of protein and lipid to their dry body mass was measured, to generate a protein-to-lipid ratio per tube per diet (HL: high lipid, N=9 tubes, 100 flies/tube; M: medium, N=10 tubes; HP: high protein, N=10 tubes). The lipid content was measured as described by Wilder et al. (2013), and the protein content was measured as described by Rho and Lee (2014). Both were quantified using a gravimetric assay in which chloroform and sodium hydroxide were used to dissolve lipids and protein, respectively.

Juvenile survival and growth

We first aimed to determine the effects of prey macronutrients on the survival, development duration and mature size of *H. graminicola* spiders. We randomly assigned juveniles (~20–30 individuals) hatched from the first egg sac produced by each female into group HL (N=103), group M (N=199) or group HP (N=108). About twice the number of group M males was used because more M males were needed to conduct mating and reproduction experiments (see below). Each juvenile spider was housed in an individual glass tube (diameter×length: 20×60 mm) as described above. Juveniles were separated on the day of hatching, or the next day, and were fed their first meal on this day. All juveniles were fed four live flies from the corresponding group (e.g. HL juveniles were fed with HL flies) every 4 days and were provided with water *ad libitum*. Newly hatched juveniles that experienced difficulty in consuming live prey were provided with fruit flies that had been frozen to death at −20°C.

During each feeding, we first cleaned the tube to remove exuviae and residual food from the previous feeding, and placed flies at random (i.e. not sex biased) into the tubes. We monitored the juvenile spiders until they reached maturity and recorded the mortality cases every 12 h. We estimated the duration of juvenile development (elapsed time in days from hatching to maturation) and measured the body size (proxy: carapace width) and determined the sex upon maturation. We used a microscope (M205 C, Leica Microsystems GmbH, Wetzlar, Germany) to measure the carapace width to the nearest 0.01 mm. Spiders from the three groups that survived to maturity were used in subsequent mating and reproduction experiments.

Mating and reproduction

To examine whether and how the prey macronutrient contents influenced the mating behaviour and reproduction of *H. graminicola*, we conducted relevant experiments involving mature F1 spiders. For the mating trials, we paired females from group HL, HP or M with males from group M (i.e. HL♀×M♂: n=20; HP♀×M♂: n=20; M♀×M♂: n=42). We recorded the identification code and age of each spider before mating. Mating experiments were conducted in the female's rearing tube. A non-sibling male was selected randomly and introduced gently into the female's rearing tube. If the pair did not mate within 15 min, we defined the pairing as a mating failure and gently removed the male to its original rearing tube.

If mating occurred within 15 min, we recorded the parameters, including the mating latency (elapsed time between the start of the mating trial and copulation), mating duration (i.e. copulation time) and number of mating bouts. During the mating trials, several pairs exhibited a repeating pattern of short separation and re-engagement, indicating multiple mating bouts in a single trial. However, if the duration of separation before re-engagement was ≤20 s, it was not recorded as a new mating bout. A separation of >5 min defined the end of the mating trial. In some cases, the males were cannibalized by the females, the mating duration was <5 min or the male did not

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appear to insert his palps into the female epigynum; these events were also considered mating failures. Although *H. graminicola* is a polygamous species, we did not reuse spiders that had mated successfully in any of the mating trials.

Successfully mated females were transferred into new glass tubes and fed with the same fly types as they had been fed under development as described above. Mature *H. graminicola* females can produce egg sacs, regardless of the mating status (Zhao, 1993). However, viable eggs (i.e. fertilized eggs) would be produced only after mating. Our pre-trials in 2016 demonstrated that the first egg sac produced by a mated female *H. graminicola* spider was fertilized. Therefore, we used the first egg sacs produced by females in this study for reproductive measurements. We recorded the pre-oviposition duration (time interval in days between the end of mating and the first egg sac laying) and fecundity (i.e. number of eggs) of all female spiders that had successfully produced the first egg sac. We also recorded the number of females that produced egg sacs not properly wrapped in silk.

Lipid content of adult female spiders

Next, we examined differences in the lipid levels of adult female spiders that had been collected in the field, as well as those reared to maturity on a diet of HP and HL fruit flies in the laboratory. We did not include females reared on the M diet because we did not have sufficient females to use for testing the lipid levels after mating trials. The lipid levels were evaluated using chloroform extraction described by Wilder et al. (2013).

Statistical analysis

We performed all statistical analyses using SPSS version 19 (IBM Corporation, Armonk, NY, USA). We evaluated the normality of all data using the Shapiro–Wilk test. When necessary, the data were transformed to meet the assumption of a normal distribution. We performed Kruskal–Wallis tests to identify any differences in the protein-to-lipid ratio between the three groups of fruit flies (groups HP, M and HL). If an overall difference was detected, we then performed paired comparisons. We used Chi-square tests for independence to compare the mating success rates in the three mating groups. We used the log rank test within the Kaplan–Meyer test to determine the effects of prey nutrient levels on the survival rate of juveniles. We used a two-way ANOVA to test the effects of prey nutrient levels and sex of spiders, as well as the interaction between prey nutrient levels and sex of spiders on the growth (i.e. development duration and carapace width) of female and male spiders at maturation. We then tested the effects of prey nutrient levels on the mating latency, bouts of mating, pre-oviposition period and fecundity of spiders using Kruskal–Wallis tests. We used one-way ANOVA to analyse the data of mating duration. Finally, we used a one-way ANOVA to test the difference in the lipid content between adult female spiders collected in the field and those reared in the laboratory from hatchlings fed HP and HL fruit flies. All values are reported as means±s.e.m. unless otherwise stated. All reported *P*-values are two-tailed at an α level of 0.05; *P*-values for pairwise comparisons are Bonferroni adjusted. Data are presented in Table S1.

RESULTS

Nutritional content of prey

The protein-to-lipid ratio differed significantly among the three groups of fruit flies grown on different types of nutrient medium (Kruskal–Wallis test: H=7.374, d.f.=2, P=0.025; Fig. 1). Specifically, *post hoc* paired comparisons revealed a significant difference in the protein-to-lipid ratio between HL and HP fruit flies

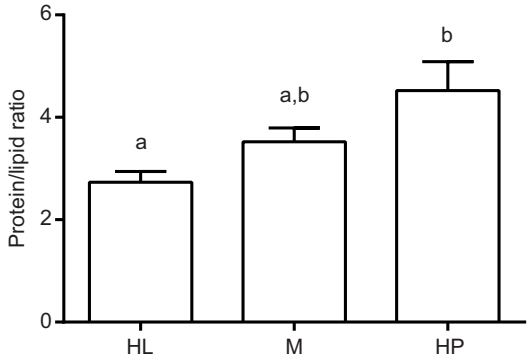

Fig. 1. Mean±s.e.m. protein-to-lipid ratio of the three groups of *Drosophila melanogaster* prey. HL, high lipid (N=9); M, medium (N=10); HP, high protein (N=10). Each sample contained approximately 100 fruit flies. Different letters indicate a significant difference between the two groups from Kruskal–Wallis tests.

(P=0.022), whereas no other comparisons yielded significant results (HL versus M: P=0.233; HP versus M: P=1.000).

Juvenile survival and growth

Prey nutrient content had a significant effect on the survival of juvenile spiders (log rank test: χ^2=8.868, d.f.=2, P=0.012; Fig. 2). Juvenile spiders reared on HL fruit flies had a significantly lower survival rate than those fed on group M fruit flies (log rank test: χ^2=8.675, d.f.=1, P=0.003; Fig. 2). The other comparisons did not yield significant results (HP versus M: χ^2=3.152, d.f.=1, P=0.076; HL versus HP: χ^2=0.920, P=0.337).

Overall, female juvenile spiders had a significantly longer development duration than male juveniles (two-way ANOVA: $F_{1,267}$=9.807, P=0.002; Fig. 3A). However, neither prey nutrient content ($F_{2,267}$=0.193, P=0.824) nor the interaction between prey nutrient content and spider sex ($F_{2,267}$=2.364, P=0.096) had a significant effect on the duration of juvenile development (Fig. 3A). Similarly, neither prey nutrient content ($F_{2,157}$=1.440, P=0.240) nor spider sex ($F_{1,157}$=0.700, P=0.404), nor the interaction between prey nutrient content and spider sex ($F_{2,157}$=1.489, P=0.229) had a significant effect on body size at maturation (Fig. 3B).

Mating and reproduction

Prey nutrient content did not have a significant effect on mating success (χ^2=0.151, d.f.=2, P=0.927), mating latency ($F_{2,37}$=1.971,

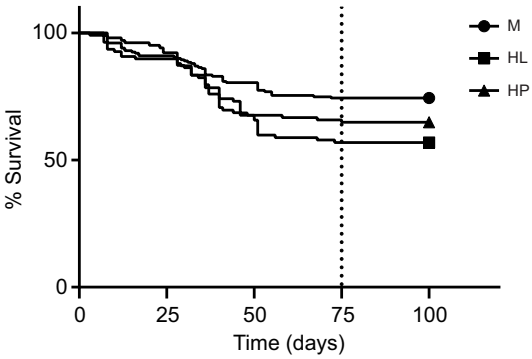

Fig. 2. Effect of diet on survival of *Hylyphantes graminicola* juveniles during development. Kaplan–Meier survival curve of the independent effect of diet on spider mortality (group HL, N=102; group M, N=199; and group HP, N=108). The dotted line indicates when all the juveniles that did not reach maturation died (75 days).

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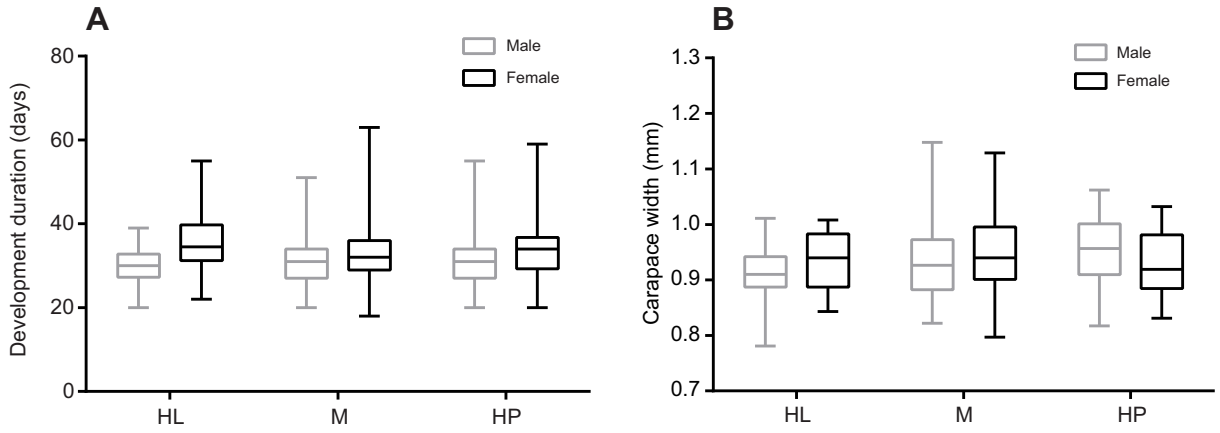

Fig. 3. Effect of diet on developmental duration and carapace width of *H. graminicola* juveniles during development. (A) The developmental duration (HL, *N*=56; M, *N*=148; HP, *N*=69) and (B) carapace width (HL, *N*=40; M, *N*=77; HP, *N*=46) of spiders from the three diet groups. Box plots show median (horizontal line) values, upper and lower quartiles (box) and the minimum and maximum values (whiskers). Female spiders had a significantly longer developmental duration than males ($F_{1,267}$=9.607, *P*=0.002). None of the comparisons revealed significant differences (*P*>0.05).

P=0.154), mating duration ($F_{2,37}$=0.410, *P*=0.667) or number of mating bouts (Kruskal–Wallis test: *H*=0.828, d.f.=2, *P*=0.668; Table 1), and did not lead to significant differences in fecundity ($F_{2,30}$=0.057, *P*=0.944; Fig. 4). However, prey nutrient content did have a significant effect on the pre-oviposition period (Kruskal–Wallis test: *H*=7.373, d.f.=2, *P*=0.025; Fig. 5), such that females in group HL had a significantly longer pre-oviposition period than those in group M (*P*=0.020), whereas no other comparisons yielded significant results (HL versus HP: *P*=0.299; HP versus M: *P*=1.000). Moreover, three of 10 females in group HL produced eggs that were not wrapped in silk and failed to hatch. No such events were observed in groups HP and M.

Lipid levels in adult female spiders

Significant differences in lipid levels were observed when comparing adult *H. graminicola* females that were collected in the field with those reared on HP and HL fruit flies in the laboratory (one-way ANOVA: $F_{2,20}$=58.394, *P*<0.001; Fig. 6). All paired comparisons between groups also yielded significant differences (LSD tests: *P*<0.005). The highest and lowest lipid concentrations were observed in adult females that had fed on HL fruit flies and in field-collected females, respectively.

DISCUSSION

In this study, we used the common strategy of altering the macronutrient content of spider prey by carefully manipulating the quantity of sucrose or yeast extract powder added to *D. melanogaster* media. Consequently, we generated three groups of *D. melanogaster* flies, and observed a significantly higher protein-to-lipid ratio in flies from group HP than in flies from group HL. We then reared juvenile F1 offspring of field-collected *H. graminicola* spiders on fruit flies from the HP, HL and M groups and determined that consuming HL prey was associated with increased spider mortality before maturation and an increased oviposition time after mating. However, we did not detect any significant effects of consuming HP prey on *H. graminicola* survival, growth or reproduction. These results suggest a low plasticity with fast development to a certain size with a high nutrient requirement for protein, and a cost of lower survival and prolonged time to egg laying when prey protein-to-lipid content is low. Therefore, *H. graminicola* appears to be unable to maximize the expression of multiple life-history traits simultaneously, and has to trade off one trait against another when prey proteins are limited.

Although much is known about the beneficial effects of prey protein on spider survival, growth or reproduction, few studies have specifically examined the long-term effects of prey lipids on these processes across an individual's whole life span (Wiggins and Wilder, 2018). Protein is among the most important developmental macronutrients because it forms the building blocks of new tissues (Simpson et al., 2015). Undoubtedly, then, protein would promote spider growth, as demonstrated by previous studies showing better growth in wolf spiders (*P. prativaga* and *P. amentata*) reared on a high-protein diet (Jensen et al., 2010, 2011a,b; Mayntz and Toft, 2001). Wiggins and Wilder (2018) evaluated the effects of the

Table 1. Mean±s.e.m. mating latency, mating duration and number of mating bouts in three mating groups of *Hylyphantes graminicola*

Mating group	Mating latency (s)	Mating duration (s)	Mating bouts
HL♀×M♂ (*N*=10)	6.00±1.32	22.10±3.49	1.3±0.2
HP♀×M♂ (*N*=9)	3.46±0.98	26.33±4.61	1.2±0.2
M♀×M♂ (*N*=21)	3.83±0.77	24.90±1.88	1.2±0.1

Females from group HL (high lipid), HP (high protein) or M (medium) were paired with males from group M (i.e. HL♀×M♂, HP♀×M♂, M♀×M♂; see Materials and Methods). *N* indicates the number of successfully mated pairs. Mating latency was measured as the time required for a mating pair to initiate copulation. There were no significant differences between groups (Kruskal–Wallis tests).

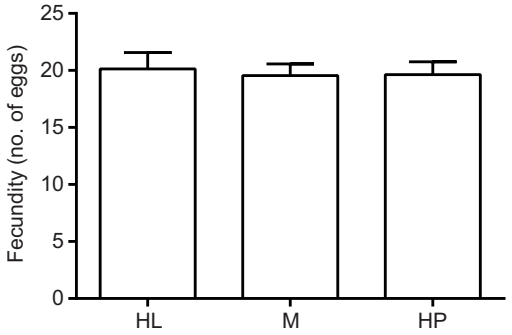

Fig. 4. Effect of diet on mean±s.e.m. fecundity of female *H. graminicola*. Fecundity (number of eggs in the first clutch) was measured in spiders from the three diet groups (HL, *N*=7; M, *N*=18; and HP, *N*=8). None of the comparisons yielded significant results (*P*>0.05).

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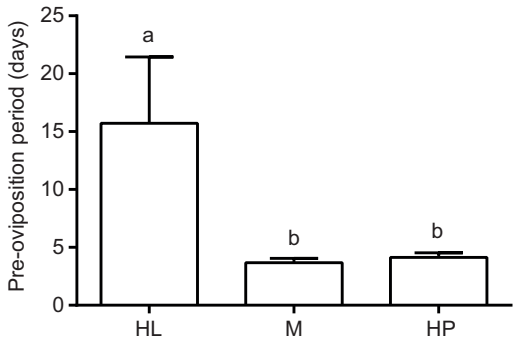

Fig. 5. Effect of diet on mean±s.e.m. pre-oviposition period of female *H. graminicola*. The length of the pre-oviposition period is shown for spiders from the three diet groups (HL, *N*=7; M, *N*=19; and HP, *N*=8). Different letters indicate significant differences between groups from Kruskal–Wallis tests.

quantity and macronutrient content of live prey on the growth of juvenile jumping spiders (*Phidippus audax*) and found that a high-lipid diet was associated with a larger body size (tibia/patella length and posterior lateral eye width) and heavier body mass. Their report may be the first to demonstrate that high-lipid prey can promote spider growth.

In contrast, our results from the present study of *H. graminicola* revealed that a diet of high-lipid prey was associated with increased mortality during development. However, a low prey protein and lipid content had no significant effect on the duration of development or body size at maturity. In other words, our findings suggest that the prey protein-to-lipid ratio negatively affected juvenile survival but not growth in *H. graminicola*. Possibly, all tested groups of fruit flies in our study had sufficient levels of proteins and lipids to ensure the growth of *H. graminicola* juveniles, which have a relatively short developmental duration compared with those of wolf spiders and jumping spiders. These results show that *H. graminicola* is able to prioritize a certain size within a set time frame at the cost of developmental instability and even death if it is not possible to reach this size within the time frame, suggesting no or low plasticity in size and developmental time, but at higher mortality risk, in this species. *Hylyphantes graminicola* spiders probably need to mature quickly at a certain size, for example, in order to reproduce before winter.

In a previous study, it was demonstrated that spiders could extract almost all lipid from prey but had a lower protein absorption rate (Wilder et al., 2010). In other words, it is not difficult for spiders to obtain sufficient dietary lipids for growth (Wilder, 2011), but protein is obtained less efficiently. Recent studies showed that spiders eat only the nutritious body parts of prey but discard the exoskeletons, which contain considerable amounts of nitrogen (Barnes et al., 2019). However, small spiders such as *H. graminicola* may not obtain sufficient amounts of protein within a set time frame when fed a high-lipid diet (e.g. group HL), given the interdependency between lipids and proteins in the body (Foelix, 2011). In our experiments, we could only test the developmental duration and size of individual spiders that survived to full maturity. These spiders might have been able to overcome the negative consequences of a low-protein absorption rate. All the juveniles that died before maturation, especially those in group HL, suffered from a moulting failure (group HL: *N*=44; group HP: *N*=38; group M: *N*=51). It should be noted that the different fly types produced might have differed in micronutrient content such as vitamins, cholesterol and phosphorus, as the yeast used in the fly media in addition to protein also contains a wide range of micronutrients. Although little is known about the effects of these micronutrients on spider fitness (Higgins and Rankin, 1999; Ludwig et al., 2018; Mayntz and Toft, 2001; Wilder, 2011; Wilder and Schneider, 2017), we cannot rule out the possibility of positive effects of higher micronutrient content of yeast in the fly diets on spider fitness-related traits. Furthermore, the different fly types produced may also have had an altered carbohydrate content as glycogen stores of flies change with different sugar/yeast diets. Such a change, although probably small, could have some effect on certain life-history traits.

We did not observe any effect of prey nutrients on fecundity in our study, but *H. graminicola* females reared on high-lipid prey had a significantly longer latency to egg laying than those reared on high-protein prey. These results indicate that there exists a plasticity in egg-laying time but not in reproductive output (i.e. fecundity) in *H. graminicola*. This suggests that when the protein is limited (i.e. when spiders are reared on HL prey), females traded egg-laying time to maintain reproductive output. To some extent, our results contradict the opinion that lipids are beneficial for animal reproduction. Under normal conditions, *H. graminicola* females produce egg sacs wrapped in silk. However, some females that had been reared on high-lipid prey (but not females in the other groups) produced eggs that were not wrapped in silk and thus failed to hatch. As spider silk is almost entirely composed of amino acids, a lipid-rich or protein-limited status may reduce the ability to produce silk (Blamires et al., 2015). A previous isotope tracer experiment revealed the effective accumulation of nitrogen in the egg sacs (Rickers et al., 2006). It is likely that the pre-oviposition period was prolonged because females reared on high-lipid prey are preparing for a more proper egg sac (i.e. one having a higher protein content with more silk wrapping). Web-building spiders with a sit-and-wait hunting strategy may thus experience a higher demand for protein during their short development stage and a relatively lower demand for lipids (which have a higher energy density than protein and carbohydrates) than actively hunting wolf spiders and jumping spiders (Wiggins and Wilder, 2018).

In summary, this long-term study provides evidence that juvenile *H. graminicola* spiders reared on high-lipid prey exhibit reduced survival before maturation and delayed egg-laying among females. These phenomena may be widespread among small, web-building spiders with a sit-and-wait hunting strategy.

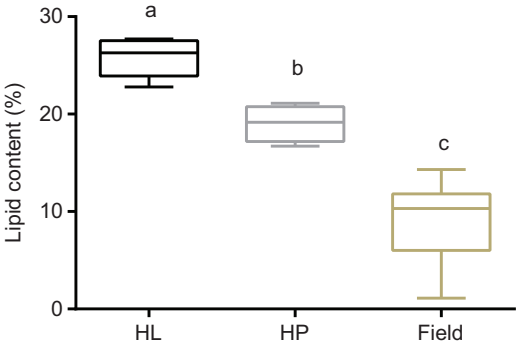

Fig. 6. Effect of diet on lipid content of adult female *H. graminicola*. The percentage of lipid to dry body mass of spiders from the HL (*N*=6) and HP (*N*=4) diet groups versus that of spiders from the field (*N*=13). Different letters indicate significant differences between the groups.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: L.W., X.J., S.Z., D.L.; Methodology: L.W., X.J., F.L., S.Z., D.L.; Software: L.W.; Validation: L.W., D.L.; Formal analysis: L.W., X.J., S.Z., D.L.; Investigation: L.W., F.L., D.L.; Data curation: L.W., D.L.; Writing - original draft: L.W., X.J., S.Z., D.L.; Writing - review & editing: L.W., X.J., F.L., S.Z., D.L.; Visualization: D.L.; Supervision: X.J., S.Z., D.L.; Project administration: D.L.; Funding acquisition: S.Z., D.L.

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Supplementary Table S1

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