SHORT COMMUNICATION

Importance of spider prey for development of a specialized araneophagous predator (Araneae: Palpimanidae)

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Abstract. Specialists possess specialized trophic adaptations. Spiders of the genus *Palpimanus* Dufour, 1820 are araneophagous spiders which also catch alternative prey. They have morphological, behavioral, and venomic adaptations tuned to higher efficacy in the capture and processing of other spiders. Here, we tested the hypothesis whether *Palpimanus* can develop on two prey types. We reared spiderlings of *Palpimanus* spp. on a diet composed of spiders and of insects, and recorded their performance. We found that *Palpimanus* exhibited significantly higher mortality on the insect diet. In fact, only spiderlings on the spider diet were able to develop. The developmental rate was slow, as one instar lasted on average more than 60 days. On the basis of this finding, we estimated that the life cycle is at least biennial. We conclude that *Palpimanus* spiders possess specialized prey-capture adaptations towards spider prey which prevent them to utilize alternative prey.

Araneophagy, i.e., the capture and consumption of spider prey, is a common feeding strategy in many spider species (e.g., Michalko et al. 2022). In several spider genera, such as *Eriauchenius* O. Pickard-Cambridge, 1881 (Archaeaidae), *Ero* C. L. Koch, 1836 and *Mimetus* Hentz, 1832 (both Mimetidae), *Chorizopes* O. Pickard-Cambridge, 1871 (Araneidae), *Cyrba* Simon, 1876 and *Portia* Karsch, 1878 (both Salticidae), and *Lampona* Thorell, 1869 (Lamponidae), araneophagy is the predominant feeding strategy (Pekár & Toft 2015; Pekár et al. 2017). Comparative analysis of spider prey suggested araneophagy to be an ancestral feeding strategy, even in families with representatives that are either generalists or specialised on other prey, such as Gnaphosidae, Salticidae, Thomisidae, and Zodariidae (Pekár et al. 2012; Baydizada et al. 2020).

Species which specialise on the capture of spiders, similarly to other prey-specialists, have evolved several types of trophic adaptations (Pekár & Toft 2015). Specifically, these include morphological adaptations, such as a thick cuticle (e.g., Michálek et al. 2017); behavioral adaptations in the form of specialised prey-capture behavior (Jackson & Wilcox 1990; Michálek et al. 2019a); venomic adaptations such as the possession of more potent venom for preferred prey (Michálek et al. 2019b), and metabolic adaptations in the form of an improved ability to process nutrients from preferred prey (Li & Jackson 1997; Toft et al. 2010).

Spiders of the genus *Palpimanus* Dufour, 1820 (Palpimanidae) are araneophagous specialists. In both field and under laboratory conditions, they have been shown to mainly capture other spiders (Cerveira & Jackson 2005; Pekár et al. 2011). Recent analysis of the natural diets of three different palpimanid genera by means of molecular methods revealed that all three genera are primarily araneophagous (Pekár et al. 2022). Further analysis of morphological trophic traits suggested an araeophagous habit for the whole family (Pekár et al. 2022).

Palpimanus possess several trophic adaptations for araneophagy – specifically, morphological adaptations, such as thick scopulae on forelegs to increase prey capture, and an armored cuticle to prevent counter-attack (Pekár et al. 2011); behavioral adaptations, including a stealthy and very fast attack on prey (Cerveira & Jackson 2005); and venomic adaptations involving faster prey paralysis (Pekár et al. 2018).

Besides capturing other spider species, *Palpimanus* is prone to cannibalism – large individuals can catch small individuals if the body size difference is marked (Liznarová et al. 2018). Observations in the field show there is a large overlap of generations (Pekár, unpublished), presumably because ontogenetic development takes more than one year. *Palpimanus* use stridulation to signal to, and defend themselves from conspecific predators. Experimentally, it was found that the inability to stridulate decreases the spider's chance of surviving an encounter with a larger conspecific (Liznarová et al. 2018). Stridulation thus has a defensive, anti-cannibalistic function.

The aim of the study was to investigate the effect of diet type on the performance of spiders of the genus *Palpimanus*. We reared *Palpimanus* spiders on a preferred diet of spiders and on an alternative diet of insects, and studied their survival, growth, and postembryonic development. We expected that the preferred diet would have a positive effect on fitness parameters, while the alternative diet would have a negative one, indicating the specificity and trade-off of their adaptations. In addition, we studied the spider's ontogenetic development in order to determine the duration of its life cycle.

Spiderlings ($n = 45$) that hatched from one egg-sac of a *Palpimanus potteri* Lawrence, 1937 female collected in Ndumo, South Africa were assigned at random to one of two diet treatments three days after hatching, to study the effect of two diet types. The spiderlings were housed singly in tubes (diameter 10 mm, length 60 mm) with a layer of plaster of Paris at the bottom. The tubes were plugged with rubber-foam and kept in a controlled environment chamber (22 ± 1 °C, L:D = 16:8). The plaster of Paris was kept moistened with a few drops of water at 5-day intervals. The spider diet ($n = 23$) included spiders from the following families: Thomisidae (*Xysticus* sp.), Theridiidae (*Neottiura bimaculata* (Linnaeus, 1767), *Enoplognatha* sp., *Phylloneta* sp.), Zodariidae (*Zodarion* sp.), and Gnaphosidae (*Micaria* sp.). Spiders were collected by hand or by sweeping vegetation around the campus. The body sizes of the spider prey used were smaller (between 50–80% of the spider body) than the body size of *Palpimanus* spiderlings. The insect diet ($n = 22$) included the following prey: *Sinella curviseta* Brook, 1882 (Collembola), *Acheta domesticus* (Linnaeus, 1758) (Orthoptera), larvae and imagoes of *Drosophila hydei* Sturtevant, 1921 (Diptera), and *Reticulitermes* sp. (Isoptera). The body sizes of these prey were similar to, or smaller than the body size of *Palpimanus*

spiderlings. All of these prey came from a laboratory culture. Spiderlings were fed in 7-day intervals with a single prey individual which was released to the tube occupied by the spider, as our previous study showed that *Palpimanus* feeds on average every 14 days (Pompozzi et al. 2019). Type of prey was offered to spiderlings in a random order. Prey remnants were removed the following day. Rearing lasted for more than a year, during which time mortality and molting were checked once a week. Spiders were weighed using a Kern 770 balance with a precision of 0.01 mg once every month or after molting.

To study the ontogenetic development, additional individuals of *Palpimanus* spiders were used—specifically, spiderlings that hatched from one eggsac of *Palpimanus potteri* ($n = 9$) and from *Palpimanus* sp., both collected in Ndumo, South Africa, and from one eggsac of *P. gibbulus* Dufour, 1820 ($n = 13$) collected in Portugal. Spiderlings were housed singly in tubes of the same size and kept under the same conditions as above. They were fed every 7 days only with spiders (same species as above). Spiderlings emerged from the eggsac as the first free instar. The number of instars cited further in the text does not include embryonic or larval development because this took place in the pear-shaped eggsac.

The semiparametric Cox proportional hazard model, from the survival package (Thernau & Grambsch 2000), was used to compare the survival of individuals between the two diet treatments. The longitudinal data on body mass were modelled with semiparametric Generalised Linear Mixed Effect Models (GLMM) with Gamma errors, from the mgcv package (Wood 2006), due to the non-linear relationship between mass and time and heteroscedasticity (Pekár & Brabec 2019). A thin-plate spline with an automatically selected base dimension was used. The ID of the individuals was the random component. All analyses were performed within the R environment (R Core Team 2021).

The diet type had a statistically clear effect on the mortality of *Palpimanus* spiders (Cox proportional hazard, $X^2_1 = 45.8$, $P < 0.0001$): spiderlings had higher mortality on the insect than on the spider diet (Fig. 1A). The median survival time for individuals on the spider diet was 180 days, and on the insect diet, 42 days. Considerable mortality (39%, $n = 23$) in the spider diet group resulted from successful defence by the prey, i.e. prey-spiders killed *Palpimanus*.

Palpimanus spiders showed high mortality on the insect diet as all individuals died before molting to the next instar. *Palpimanus* spiders were not able to catch alternative prey, except for crickets, which were used 26 times. Thus, it seems that spider prey is essential for their ontogenesis. Spiders represent a dangerous prey—in our study more than 40% of individuals fed with this prey died due to counterattack by the spider prey, while in the case of insect diet, no spiderlings were killed by the prey. In the absence of counterattacks, survival on the spider diet might have been considerably higher. Even though *Palpimanus* is able to catch and consume insect prey both in the field and in the lab (Cerveira & Jackson 2005; Pekár et al. 2011, 2022), clearly it is specialized on spiders to such an extent that spider prey cannot be replaced entirely by alternative prey. Similarly, spider-eating *Portia* spiders exhibited higher mortality on an insect diet on which they could not complete their development. However, in contrast to *Palpimanus*, *Portia* spiders reared on insects survived for several instars (Li & Jackson 1997). The nutritional trade-off in this species was revealed by reduced performance with respect to survival and growth on a mixed spider-insect diet compared with a pure spider diet (Li & Jackson 1997). Thus, *Palpimanus* seems to possess more specialised prey-capture adaptations than *Portia*, though it is apparently less strictly specialized than *Nops* MacLeay, 1839 (Caponiidae), which did not even catch alternative prey (García et al. 2018). But the fact that there was also a considerable mortality on the spider diet indicates that *Palpimanus* spiderlings could be either limited in some nutrients which can be provided by insect or local spider prey (which we did not use).
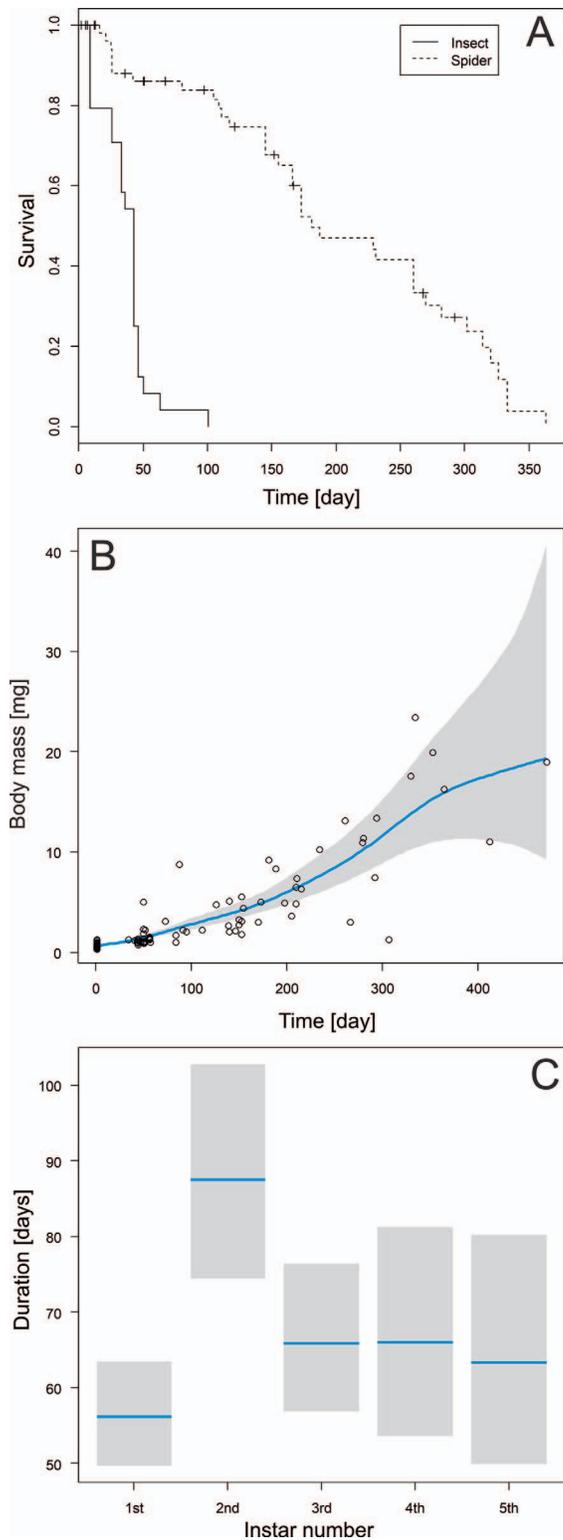

Figure 1.—**A** – Comparison of the survival of *Palpimanus* spiderlings on two diets (insects, spiders) over a period of one year. Plus signs mark censored cases. **B** – Change in the body mass of *Palpimanus* spiderlings reared on the spider-diet for a period of more than one year. Blue line is estimated smoothed model, gray polygons are 95% confidence bands. **C** – Comparison of the durations of five instars of *Palpimanus* spiders on the spider-diet. Blue lines are estimated means, gray boxes are 95% confidence intervals.

Surviving *Palpimanus* spiders used the provided prey and grew in body mass over a period of more than one year. On the insect diet, all individuals died before molting to the next instar; thus, we failed to measure changes in their body mass. The mass of spiders on the spider diet increased significantly (GLMM-g, $F_{2.5} = 283$, $P < 0.0001$, Fig. 1B). The effect of *Palpimanus* species was not statistically significant (GLMM-g, $F_1 = 1.2$, $P = 0.27$).

Spiderlings on the insect diet did not develop to the next instar, while spiderlings on the spider diet went through five instars during the study, yet none have reached adulthood. The durations of inter-molt periods differed significantly among instars (GLMM-g, $F_4 = 5.4$, $P = 0.0007$): the second instar lasted significantly longer (on average, 87 days) than all other instars, which lasted on average 62.8 days (Fig. 1C). The effect of species was not statistically significant (GLMM-g, $F_1 < 0.1$, $P = 0.97$).

The rate of ontogenetic development varies among spider species and is a function of ambient temperature (Li & Jackson 1996). In many spider species, the duration of one instar lasted less than a month, so that the whole life cycle was annual (Schaefer 1987). We found that the duration of a single instar in *Palpimanus* is more than two months on average, when reared under favorable temperature conditions. Similarly long durations have been reported for a few species – for example, *Loxosceles* Heineken & Lowe, 1832 (Sicariidae) (Fischer & Vasconcellos-Neto 2005) and *Arctosa* C. L. Koch, 1847 (Lycosidae) (Dolejš et al. 2014). Such slow development is probably related to a very slow metabolism. While most spiders catch prey every 2–5 days, *Palpimanus* spiders captured prey less frequently (Pompozzi et al. 2019).

Palpimanus potteri spiders achieve a carapace length of 2.5 (males) or 3.5 (females) mm in the adult stage. Similar sized spiders belonging to other families (such as Araneidae, Lycosidae, and Oxyopidae) passed through 7–9 nymphal instars (Benforado & Kistler 1973; Dyke & Lowrie 1975; Dhulia & Yadav 1994). The incubation of eggs together with the larval development of *Palpimanus* lasted 36 days. Thus, at an average temperature of 23 °C, the whole course of ontogenetic development would last approximately 570 days. This shows that the life cycle of *Palpimanus* is at least biennial and explains the considerable overlap of generations in the field.

We show that *Palpimanus* spiders possess specialized prey-capture adaptations for spider prey which constrain use of alternative prey. Their ontogenetic development is slow, resulting in at least biennial life cycle.

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