

MATERNAL CARE

Prolonged milk provisioning in a jumping spider

Zhanqi Chen¹, Richard T. Corlett¹, Xiaoguo Jiao², Sheng-Jie Liu³, Tristan Charles-Dominique¹, Shichang Zhang², Huan Li¹, Ren Lai⁴, Chengbo Long⁴, Rui-Chang Quan^{1*}

Lactation is a mammalian attribute, and the few known nonmammal examples have distinctly different modalities. We document here milk provisioning in a jumping spider, which compares functionally and behaviorally to lactation in mammals. The spiderlings ingest nutritious milk droplets secreted from the mother's epigastric furrow until the subadult stage. Milk is indispensable for offspring survival in the early stages and complements their foraging in later stages. Maternal care, as for some long-lived vertebrates, continues after the offspring reach maturity. Furthermore, a female-biased adult sex ratio is acquired only when the mother is present. These findings demonstrate that mammal-like milk provisioning and parental care for sexually mature offspring have also evolved in invertebrates, encouraging a reevaluation of their occurrence across the animal kingdom, especially in invertebrates.

Parental food provisioning is often indispensable to an offspring's growth, development, and survival (1, 2) and subsequently affects litter size, sex ratio, and offspring body size (3, 4). Foods provided by parents can be classified into three forms: direct food sources, regurgitation feedings, and specialized food sources (4). Specialized foods include mammalian milk, as well as trophic eggs in amphibians and invertebrates (4–6), epidermal mucus in fish (7), and milk in cockroaches (8) and pigeons (9). However, these other foods are very different from mammalian milk in both duration of provisioning and degree of parent-offspring interaction.

Parental food provisioning often stops when offspring acquire foraging ability (4), and provisioning for nutritionally independent offspring has mainly been reported from longer-lived vertebrates (4, 10). Extended food provisioning frequently co-occurs with prolonged parental care, enhancing offspring fitness by providing assistance in foraging (11), teaching skills of foraging and competition (12), or influencing the morphology and behaviors of offspring (13). In extreme cases, parental care is extended to sexually mature offspring (4, 10), but this has only been recorded in long-lived social vertebrates (4). Prolonged care increases offspring fitness by en-

abling them to allocate more time for learning foraging and social skills (14), antipredator behaviors, and defense against nest parasites and for selecting mates (14, 15).

Toxews magnus (Araneae: Salticidae) (16) is a jumping spider that mimics ants. The breeding nest is composed of either several large individuals (fig. S1), with two or more adults, or one adult female and several juveniles. This is a puzzling observation for a species assumed to be noncolonial and suggests that *T. magnus* might provide either prolonged maternal care or delayed dispersal. We thus questioned (i) whether *T. magnus* evolved long-lasting maternal care; (ii) if it has, whether the mother provides food for the offspring; (iii) what benefits could be gained from maternal care; and (iv) how long the mother provides an effective maternal care.

We first assessed how offspring developed and behaved under maternal care in laboratory conditions. No spiderlings were observed leaving the nest for foraging until they were 20.9 ± 1.2 days ($N = 207$, $N_{\text{nest}} = 19$; SEM) old, and the mother was never seen to bring food back to the nest during this period, but offspring body lengths increased continuously (from 0.9 mm at hatching to 3.5 mm at 20 days, $N = 187$). Closer observations revealed that the mother provided a seemingly nutritive fluid, hereafter called milk,

¹Center for Integrative Conservation, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan, 666303, China. ²Center for Behavioral Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan 430062, China. ³Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan, 666303, China. ⁴Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, Yunnan, China.

*Corresponding author. Email: quanrc@xtbg.ac.cn

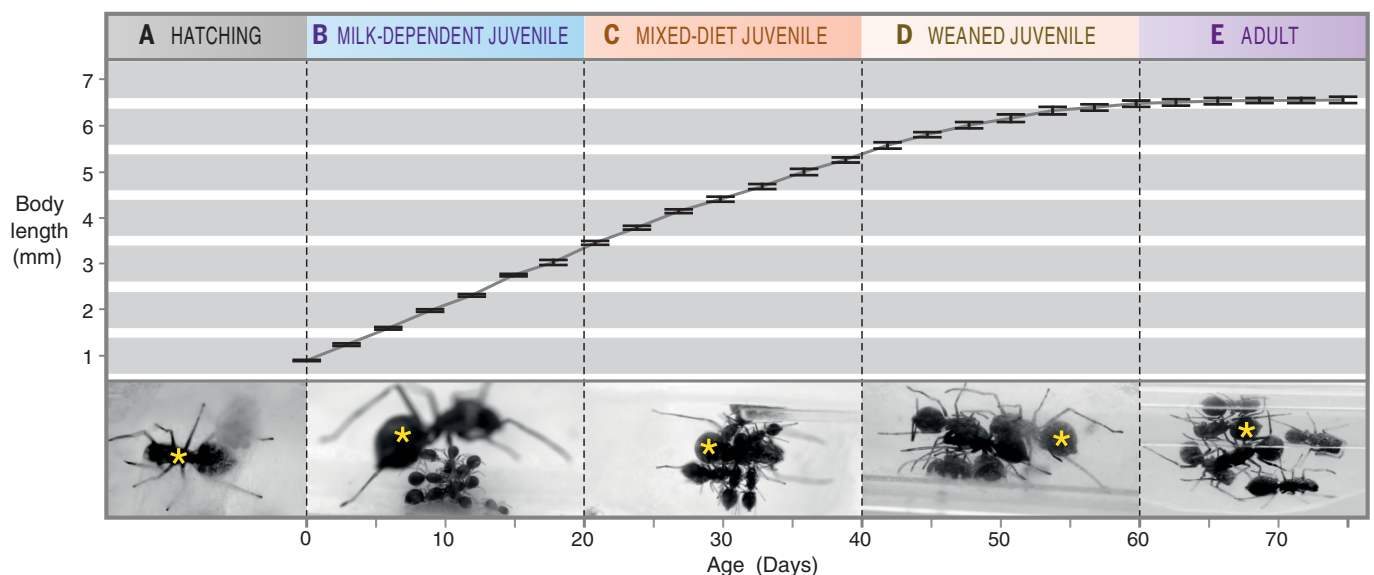


Fig. 1. *T. magnus* offspring body length growth and food resources during development. (A) Egg hatching. **(B)** Absolute milk dependence: Spiderlings do not leave the nest, and the mother releases milk droplets to the nest internal surface. **(C)** Spiderlings forage during

the day and suck milk at night. **(D)** Subadults nutritionally independent but still return to nest. **(E)** Spiderlings reach sexual maturity, but some stay with the mother. *The mother. $N = 207$ offspring, $N_{\text{nest}} = 19$ surveyed nests, error bars (SEM).

to the offspring. During the first week, she deposited milk droplets, excreted from her epigastric furrow, on the nest's internal surface. In response, spiderlings came and sucked the droplets (Fig. 1B). After the first week, milk deposition stopped and the offspring sucked directly from the mother's epigastric furrow (Fig. 1C and movies S1 and S2). Although spiderlings sometimes left the nest to forage from about 20 days, milk sucking continued until they reached the subadult stage at 37.5 ± 1.3 days ($N = 167$) (Figs. 1C), with body length of 5.26 ± 0.06 mm (around 80% of adult body length). From 40 days, milk feeding stopped, but spiderlings still used the nest at night after reaching sexual maturity within the next 20 days. Adulthood was reached at 52.2 ± 0.6 days with a body length of 6.57 ± 0.06 mm ($N = 145$) (Fig. 1D and fig. S2). Milk provisioning in *T. magnus* involves a specialized organ over an extended period, differing markedly from cockroaches, which deposit nourishing substances to the brood sac of developing embryos (8). In these aspects, *T. magnus* milk provisioning is more similar to mammalian lactation.

Observations under the microscope showed droplets leaking from the mother's epigastric furrow where the spiderlings sucked milk (Fig. 2, A and B). Spider milk total sugar content was 2.0 mg/ml, total fat 5.3 mg/ml, and total protein 123.9 mg/ml, with the protein content around four times that of cow's milk (17). We suggest this milk might have evolved from trophic eggs, unviable eggs functioning as a food for newly emerged offspring (18). The epigastric furrow is the egg-laying opening of spiders (6), and trophic eggs have evolved in diverse invertebrate taxa, including spiders (6). Trophic eggs are usually a one-time investment (4, 6), but mothers may also supply progressive trophic eggs, as in the burrower bug *Adomerus triguttatus* (18). However, progressive provisioning usually stops after hatching (19), whereas spider milk is supplied continuously from hatching to subadult.

The mother continued nest maintenance throughout, carrying out spiderlings' exuviae and repairing nest damage. When receiving both maternal care and milk, 76% of the hatched offspring survived to adulthood. We also recorded a highly female-biased adult sex ratio: 84.14% (122/145). Although the mother apparently treated all juveniles the same, only daughters were allowed to return to the breeding nest after sexual maturity. Adult sons were attacked if they tried to return. This may reduce inbreeding depression, which is considered to be a major selective agent for the evolution of mating systems (20–22).

When we blocked the mother's epigastric furrow immediately with correction fluid after hatchling emergence, the hatchlings stopped development and died 10.4 ± 0.5 days later (Fig. 3A and fig. S3), showing their complete dependence on the milk supply. A separate experiment showed that correction fluid did not affect spiderlings' survivorship (fig. S6). We then tested why parental care and milk provisioning were continued after 20 days when the spiderlings were able to

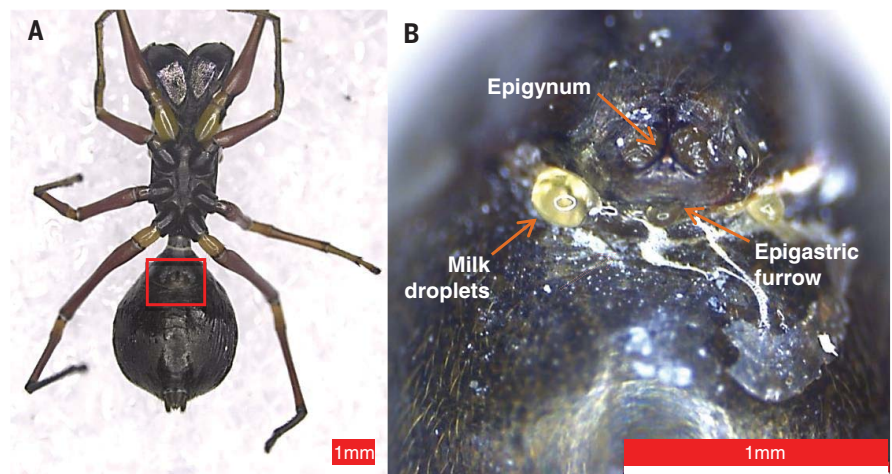


Fig. 2. Spider milk and its secretion site in *T. magnus*. (A) Ventral view of mother. (B) Milk droplets secreted after slight finger pressure on abdomen.

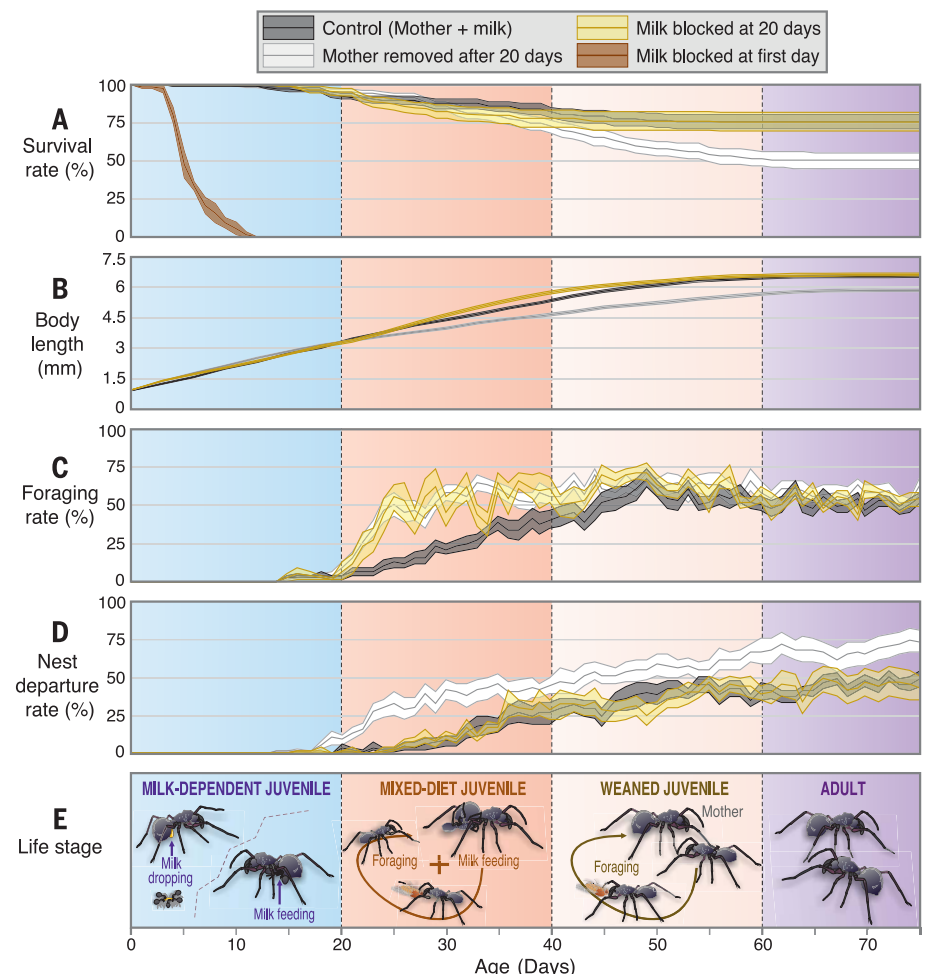


Fig. 3. Offspring development from hatching to independence under different treatments. MC ($N = 187$ offspring, $N_{\text{nest}} = 19$ surveyed nests), MR20 ($N = 255$ offspring, $N_{\text{nest}} = 18$ surveyed nests), MB20 ($N = 101$ offspring, $N_{\text{nest}} = 8$ surveyed nests), and milk blocked on the first day (MB1) ($N = 62$ offspring, $N_{\text{nest}} = 5$ surveyed nests). (A) Survival rate (surviving/hatched spiderlings). (B) Body length growth. (C) Foraging rate (foraged/surviving spiderlings). (D) Breeding nest departure rate (spiderlings that did not return to the breeding nest at night/surviving spiderlings). Error bars (SEM). (E) Life stages and food resource of spiderlings.

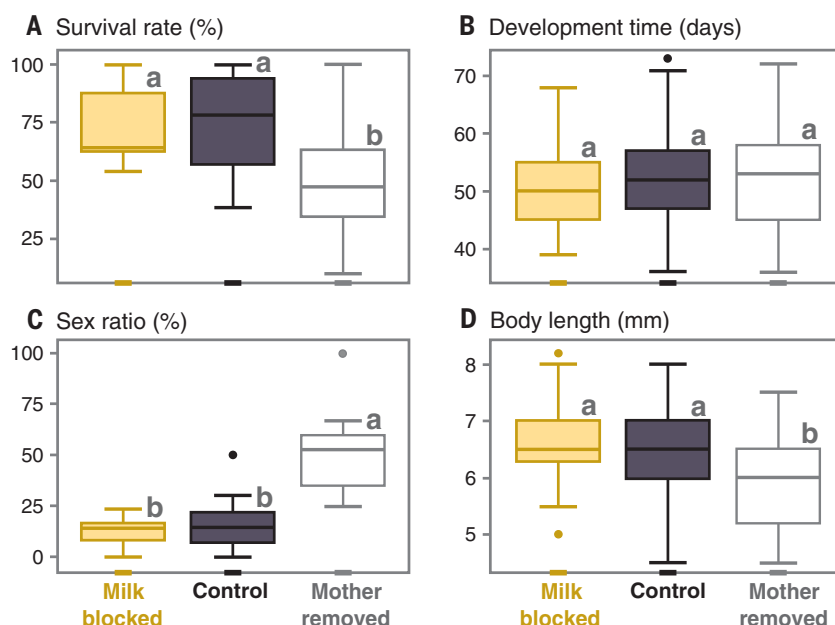


Fig. 4. Adult condition after three different treatments (MC, MB20, and MR20) of juveniles. (A) Survival of offspring to adult stage. (B) Development duration (days from hatching to maturity). (C) Adult sex ratio (mature males/total number of matured juveniles). (D) Adult body length. N_{MC} = 145 offspring, N_{MR20} = 121 offspring, N_{MB20} = 71 offspring, error bars (SEM). The letters “a” and “b” summarize the statistical grouping.

forage for themselves. We assessed the effects on survival, growth, foraging, and nest departure of (i) blocking the milk at this stage but leaving the mother in the nest to assess the effect of maternal care without milk provisioning and (ii) removing the mother from the nest to assess the combined effects of maternal care and milk provisioning. Removing the mother after 20 days significantly reduced survivorship (Fig. 3A) and body size (Fig. 3B) and increased foraging (Fig. 3C) and activities outside the nest (Fig. 3D). Blocking milk significantly reduced survivorship (Fig. 3A), but did not affect body size (Fig. 3B), and increased foraging (Fig. 3C) and activities outside the nest (Fig. 3D) compared with those receiving full maternal care and milk. Among spiderlings not receiving milk, those receiving maternal care survived significantly better (Fig. 3A), grew faster (Fig. 3B), and left the nest more rarely (Fig. 3D) than those without the mother, but both showed similar foraging frequency (Fig. 3C) (table S1 to S4). The milk-blocked spiderlings still attempted to suck milk from the mother (movie S3). These results show that from days 20 to 40, milk provisioning is not essential for spiderlings' survival, as they can compensate by foraging more, but has a positive effect on survivorship and development in lab conditions. In the field, milk provisioning, by reducing time spent outside the nest, might also reduce predation risk, as smaller offspring are more prone to predation (23). When spiderlings did not have milk, the higher survivorship with their mother present might be mediated by parasites. Although not quantified, parasites were observed on juveniles commonly without maternal care but rarely when the mother

was present. Nest cleaning and repairing by the mother may partly explain this reduction in parasite loads. Nest parasites are common in invertebrates (24, 25), and parental care usually plays a crucial role in reducing juvenile parasite infections (4, 10, 25).

Removing the mother at day 20 reduced the percentage of spiderlings reaching maturity [normal maternal care (MC) = $75.7 \pm 4.6\%$; mother removed at 20 days (MR20) = $50.4 \pm 5.3\%$] and reduced their adult body size (by 12%), whereas blocking milk did not change their adult survivorship [milk blocked at 20 days (MB20) = $73.8 \pm 6.4\%$] or body size (MB20: 6.64 ± 0.07 mm) compared with the control (Fig. 4, A and D). Most notably, removing the mother greatly affected the adult sex ratio (female/total: MC = 0.84, MR20 = 0.50), whereas blocking milk did not (MB20 = 0.87) (Fig. 4C). Further analysis showed that the reduction of offspring body size after mother removal is not caused by a sex ratio change coupled with intersexual differences (table S6). No treatment impacted the timing of developmental stages (MR20: 52.5 ± 0.78 days, MB20: 50.5 ± 0.82 days, MC: 52.3 ± 0.60 days) (Fig. 4B and table S5). Thus, milk provisioning after 20 days does not affect adult survivorship, body size, sex ratio, and development time, but the mother's presence plays a key role in assuring high adult survival and normal body size. This may reflect the parasite defense discussed above. The mother's presence is also required for achieving the female-biased sex ratio of adult offspring. Sex role divergence theory suggests that the reproductive value of a population is heavily determined by the sex that invests more in pa-

rental care (26–28). In *T. magnus*, the mother invests much more than the male invests, predicting a female-biased sex ratio to be optimal for reproductive success with a polygamous mating system. Unequal adult sex ratios could be passively induced, as with the selective mortality with respect to sex in the bark beetle *Dendroctonus ponderosae* (29), or maternally induced, as in the spider *Oedothorax gibbosus* (30), but the mechanisms remain largely unclear.

In mammals, lactation is likely an adaptation mitigating an uncertain access to food resource as a juvenile and compensating for low probabilities for future reproduction (31). More generally, food provisioning helps promote offspring fitness by reducing risks linked to self-feeding (32). In nonmammals, we suggest that the most important ecological conditions favoring the evolution of lactation might be predation risk and uncertain food access. As a response, the mother's physiology, behavior, and cognition might have changed to adapt to providing milk and prolonged maternal care as in mammals (33). Extended parental care could have evolved in invertebrates as a response to complex and harsh living environments that require offspring skills (e.g., hunting, predator defense) to be fully developed before complete independence. Another aspect to investigate is whether lactation and extended parental care are accompanied by a reduction in offspring number because of milk production and parental care costs. Lastly, we anticipate that the discoveries presented in this study will encourage a reevaluation of the evolution of lactation and its occurrence across the animal kingdom.

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availability: All data are available in the main text or the supplementary materials.

SUPPLEMENTARY MATERIALS

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Supplementary Text
Figs. S1 to S6
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Spider nursery

Mammals produce milk to feed their offspring, and maternal care often continues well after the young can forage for themselves. Though other cases of milk-like secretions have been found, this combination of ongoing maternal care has largely been considered a uniquely mammalian trait. Chen *et al.* describe an ant-mimicking jumping spider that secretes a nutritious milk-like substance on which its young offspring are entirely dependent. The spider also continues to care for the spiderlings as they mature and become independent. Thus, this type of maternal care may be more widespread than has been assumed.

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