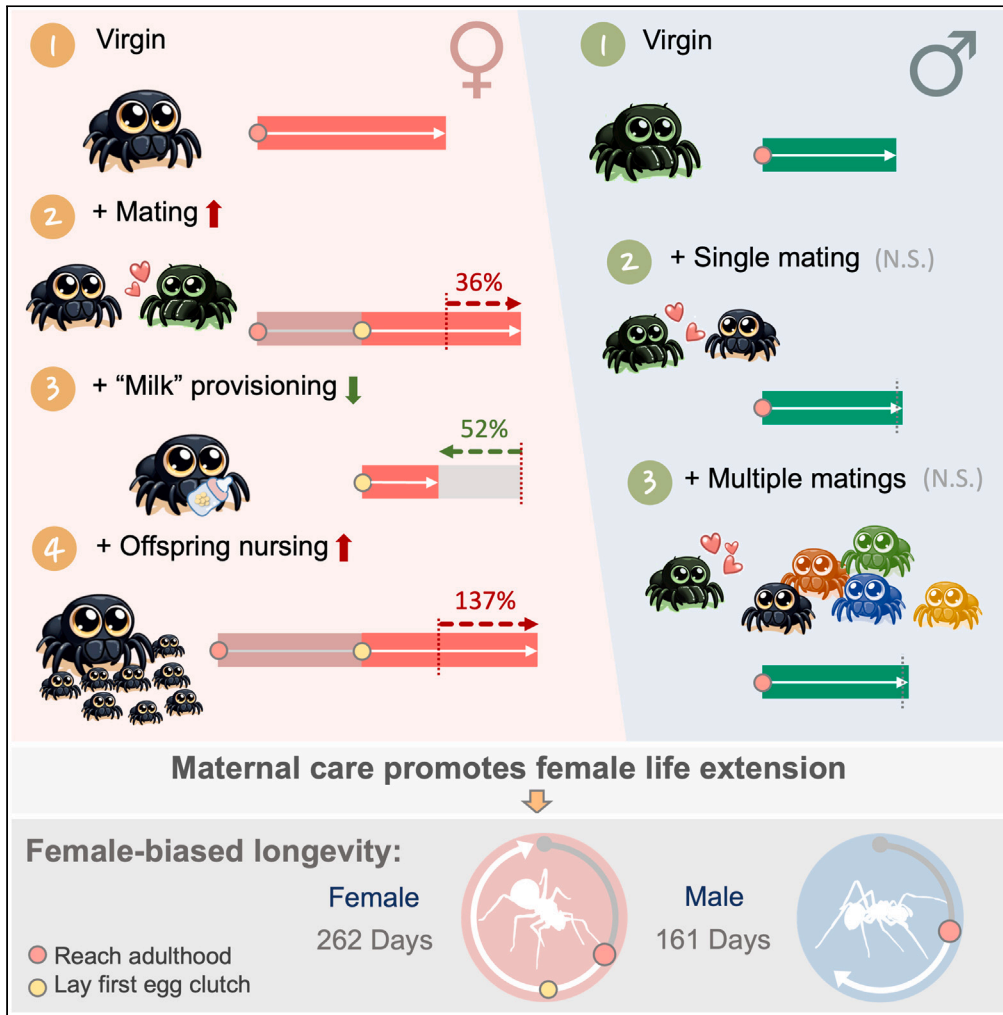


Article

Offspring nursing extends mother's longevity in a long-term maternal cared spider



Chu Jiang, Yi-Rong Wang, Xiaoguo Jiao, Jing-Xin Liu, Zhanqi Chen

chenzhanqi@xtbg.ac.cn (Z.C.)
liujx@xtbg.org.cn (J.-X.L.)

Highlights

Offspring-cared females live 25.5% longer than non-offspring-cared females

"Milk" provisioning decreases 51.8% of the female's reproductive longevity

Copulation extends female's longevity, but does not affect male's longevity

The two sexes' adult longevity difference determines female-biased longevity

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Article

Offspring nursing extends mother's longevity in a long-term maternal cared spider

Chu Jiang,^{1,2} Yi-Rong Wang,^{1,3} Xiaoguo Jiao,³ Jing-Xin Liu,^{1,*} and Zhanqi Chen^{1,4,*}

SUMMARY

Females typically outlive males in animals, especially in species that provide long-term maternal care. However, life history theory predicts that investments in reproduction, such as lactation and offspring nursing, often shorten caretakers' longevity. Aiming to interpret this paradox, we selected the lactating jumping spider *Toxews magnus* to investigate the effects of reproductive activities on longevity for two sexes. We found that: (1) although "milk" provisioning reduces female's longevity, mothers who cared for offspring (provisioned "milk" and nursing) lived the longest compared to virgins and those did not provide care; (2) copulation increased female's longevity but had no effects on males; and (3) the two sexes have comparable developmental duration, but the female adult's longevity was 2.1 times that of male's. This study suggests that the time requirement for offspring dispersal might act as a key selective force favoring females' adulthood extension, which ultimately generates the longer-lived females in maternal cared species.

INTRODUCTION

Reproductive activities often lead to longevity disadvantage of parents, especially to females^{1–4} as they often suffer costs from sexual, gestational, egg/offspring productive, nutritional provisioning, and non-nutritional nursing processes for reproductive success.⁵ However, it is ambivalent that females tend to live longer than their males although they often undertake more reproductive activities,^{6,7} especially for those species that provide maternal care for the young. For example, in primates, if only the females provide the offspring care (including both nutritional provisioning and non-nutritional nursing), they often live longer than their males, while the sex difference in longevity would be reversed if the males provide major offspring care.^{8,9} This suggests that offspring caring process positively contributes to parents' or a parent's longevity, and this may interpret the paradox of why females are often the longer-lived sex although they undertake more reproductive roles than males.¹⁰

As the pattern and magnitude of sex difference in longevity reflect the evolutionary histories of sex-specific longevity requirements,^{9,11} time demand in offspring caring might be the driving force for longer lived caretakers, especially for those species whose offspring rely on long-term maternal care, such as social mammals.^{8,12} However, rigorous experimental studies about the effect of reproductive activities on parental longevity have been conducted mainly either in short-lived arthropods that do not supply post-oviposition caring, such as butterfly *Colias eurytheme*,¹³ fruit fly *Ceratitis capitata*,¹⁴ and spider mite *Tetranychus urticae*,¹⁵ or in biparental care species, such as birds¹⁶ and burying beetle *Nicrophorus vespilloides*.¹⁷ Consequently, inconsistent results have been reported frequently even within the same species.^{9,18} Therefore, to interpret why females often live longer than their males while under costly sufferings of reproductive activities, studies should meet four requirements. First, studied species should exhibit maternal care and the two sexes should be similar in body size (presumably the two sexes exhibit similar developmental duration). Second, effects of reproductive activities such as sexual, egg production, and offspring caring on longevity should be analyzed separately. Third, experiments should be conducted under rigorously controlled conditions to minimize the effects of other ecological factors, such as food resource and predation. Fourth, reproductive costs from the sex role specialization in reproduction have to be involved respectively in the two sexes.¹⁹ To our knowledge, there is no empirical study that satisfies these four requirements simultaneously, probably because of the relative long lifespan and logistical difficulty in conducting studies in those species, such as primates⁶ and elephants.²⁰

Therefore, we conducted experiments using the jumping spider *Toxews magnus* (Araneae: Salticidae).²¹ Similar with most social mammals, male *T. magnus* provides nothing more than gametes for reproduction, while its females have to be involved in all the reproductive phases from sexual, egg production, and "milk" provisioning to post nutritional independence nursing, which enables analyses of differentiated effects on longevity of different reproductive activities.²² In addition, like most arthropods, the developmental time and adult longevity could be accurately recorded, which enables between-sex comparisons in the two periods. These comparisons would reflect intersexual difference in time demands

¹Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Yunnan 666303, China

²University of Chinese Academy of Sciences, Beijing 100049, China

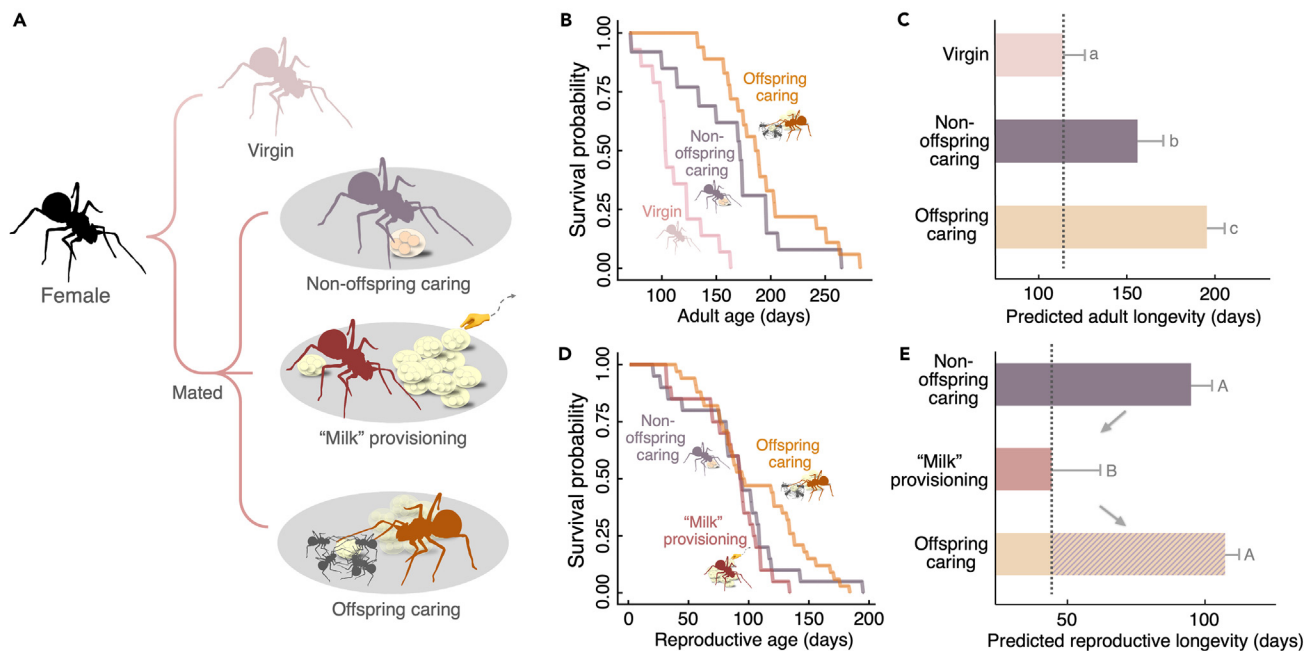
³Center for Behavioral Ecology and Evolution, College of Life Sciences, Hubei University, Wuhan 430062, China

⁴Lead contact

*Correspondence: chenzhanqi@xtbg.ac.cn (Z.C.), liujx@xtbg.org.cn (J.-X.L.)

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for reproductive preparation and accomplishment respectively.⁹ Specifically, we tested: (1) whether egg production, “milk” provisioning, offspring nursing, and offspring number have differentiated effects on female’s adult longevity, (2) whether and how sexual activities affect adult longevity of the two sexes differently, and (3) whether *T. magnus* exhibits female-biased longevity only or mainly in adulthood?

RESULTS AND DISCUSSION

Offspring nursing extends mother’s longevity

To test the overall effects of maternal care activities on the mother’s longevity, we first compared the adult longevity (days from the last molt to death) between offspring-cared (mated females “milk”-fed and nursed offspring, $n = 18$) and non-offspring-cared (mated females never cared for the offspring, $n = 12$) females (Figure 1A, STAR Methods). Results showed that the adult longevity of offspring-cared females (194.8 ± 10.2 days; mean \pm SE) was 25.5% longer than that of non-offspring-cared females (155.2 ± 13.0 days; $t = 2.68$, $p = 0.011$, Figure 1C, and Table S1), and non-offspring-cared females had lower survival probability than offspring-cared females ($z = 2.23$, $p = 0.026$, Figure 1B). Notably, the major difference between the two groups of females was the offspring-cared females hatched eggs and subsequently provided post-hatching care (including “milk” provisioning and non-nutritional offspring nursing); thus, the result indicates that the combined effect of reproductive activities after mating may extend mothers’ longevity in *T. magnus*.

Then, we further investigated the effects of nutritional “milk” provisioning and non-nutritional offspring nursing on the mother’s adult longevity via comparing the reproductive longevities (days from first egg clutch production to death) among only “milk” provided (mated females only provided “milk”, $n = 20$), non-offspring-cared ($n = 20$), and offspring-cared ($n = 55$) females (Figure 1A, STAR Methods). Results

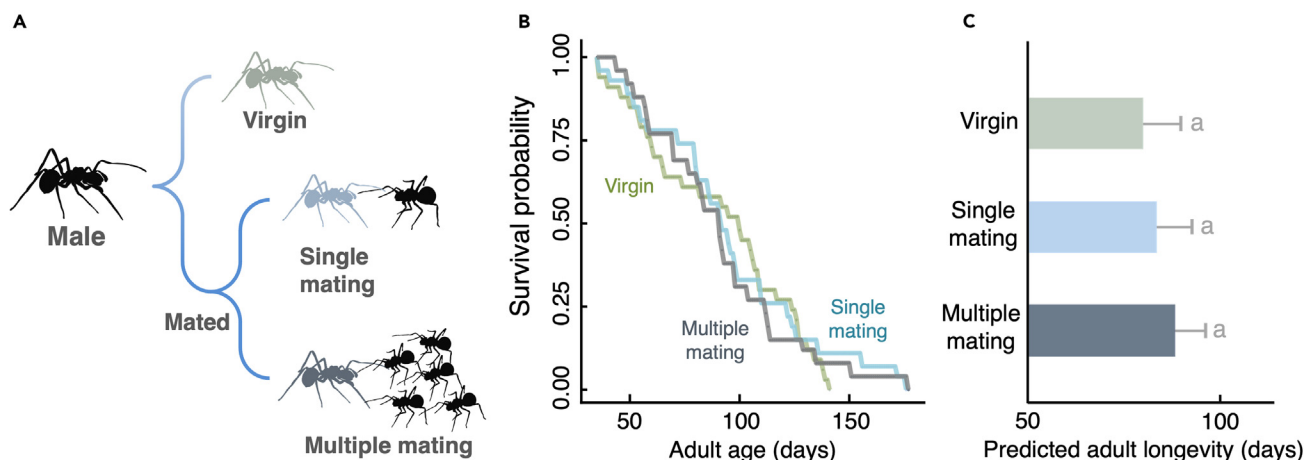


Figure 2. Males of three mating status and their respective adult longevities

(A) Process of group division: 1) virgin: males that never mate until death; 2) single mating: males that only mated once during their whole life; 3) multiple mating: one male mated once respectively with five unrelated females; (B) No significant difference in survival profiles among the three treatments (virgin: green line; singly mated: blue line; multiply mated: gray line; Log rank test: $\chi^2 = 0.140$, $df = 2$, $p = 0.933$). (C) Mating status did not influence their adult longevity (model predicted: mean \pm SE, virgin: 81.2 ± 9.4 days, $n = 33$; single mating: 85.5 ± 8.2 days, $n = 27$; multiple mating: 89.3 ± 8.0 days, $n = 26$; Anova: $F_{2,75.46} = 0.289$, $p = 0.74$).

showed that the three groups of females displayed distinct survival profiles ($\chi^2 = 6.59$, $df = 2$, $p = 0.037$, Figure 1D). Furthermore, (1) the only “milk” provided females’ reproductive longevity (45.4 ± 17.8 days) was only 48.2% of that of non-offspring-cared females’ (94.1 ± 9.5 days; $t = 2.29$, $p = 0.026$), indicating the potential reduction in female reproductive longevity attributable to “milk” provisioning process. (2) The offspring-cared females’ reproductive longevity (107.4 ± 6.8 days) was 2.4 times of only “milk” provided females’ ($t = 3.18$, $p = 0.002$), suggesting a potential extension in reproductive longevity of females resulting from non-nutritional offspring nursing activities. (3) Non-offspring-cared females exhibited a comparable reproductive longevity with offspring-cared females ($t = 1.21$, $p = 0.232$, Figure 1E, and Table S2), indicating that non-nutritional offspring nursing process could completely compensate the negative effect of “milk” provisioning on females’ longevity. Nonetheless, it is imperative to note that in addition to “milk” provisioning, mothers engaged in offspring care also performed various energy-intensive activities, such as egg and nymph attendance, breeding nest cleaning, repairing, and expanding. Consequently, offspring-cared females were anticipated to incur greater reproductive longevity costs compared to those females only providing “milk” for their offspring. Nevertheless, females that cared for their offspring lived remarkably longer than those who were solely responsible for providing “milk”, which suggests that certain non-nutritional nursing behaviors, such as interacting with nymphs, may confer longevity benefits upon mothers.

Offspring number does not affect mother’s longevity

To explore whether and how the number of offspring influences offspring-cared females’ longevity, we correlated the reproductive longevity of *T. magnus* mothers with the number of produced egg clutches, offspring that reached nutritional independence and adult stages. Results showed that there were no correlations between mothers’ reproductive longevity and the number of offspring (egg clutches: $F_{1,42.46} = 0.229$, $p = 0.63$; nutritional independent sub-adult offspring: $F_{1,29.34} = 0.015$, $p = 0.905$; adult offspring: $F_{1,36.04} = 0.198$, $p = 0.659$) (STAR Methods, Figure S1). This suggests that mothers’ reproductive longevity is relatively stable in *T. magnus* regardless of how many offspring they produce and care. Given that a larger brood size often means a slower development of the offspring (Figure S2), it is plausible that an increase in offspring number would not impact mothers’ longevity, otherwise, the mother would not survive until offspring dispersal (from the natal nest).

Copulation extends female’s but not male’s longevity

Aiming to examine the effect of copulation, which tends to affect longevity,³ on each sex’s longevity in *T. magnus*, we first compared the adult longevities between virgin (never met a male until death, $n = 14$) and non-offspring-cared (mated, $n = 12$) females (STAR Methods, Figure 1A). We found that mated females lived 36.4% longer than virgins in adult longevity (virgin: 113.8 ± 12.2 days; mated: 155.2 ± 13.0 days; $t = 2.401$, $p = 0.021$, Figure 1A, and Table S1) and the survival profile of mated females was significantly postponed compared to virgins ($z = 2.71$, $p = 0.007$, Figure 1B). These results suggest mating activity may extend females’ longevity. Then, we compared the adult longevities among virgin ($n = 33$), singly mated ($n = 27$), and multiply mated ($n = 26$) males (Figures 1B and 2A and STAR Methods). Results showed that the three groups of males had similar adult longevities (virgin: 81.2 ± 9.4 days; singly mated: 85.5 ± 8.2 days; multiply mated: 89.3 ± 8.0 days; $F_{2,75.38} = 0.305$, $p = 0.74$, Figure 2C, and Table S3) and survival profiles ($\chi^2 = 0.140$, $df = 2$, $p = 0.933$, Figure 2B). These results suggest that mating activities do not affect males’ longevity.

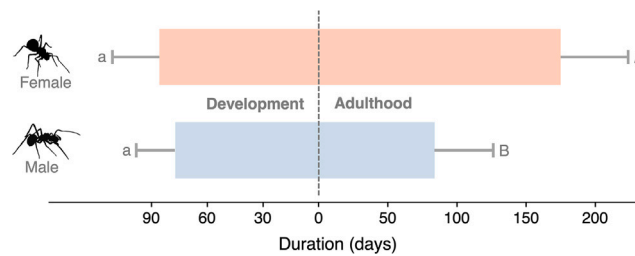


Figure 3. Pattern of sex difference in longevity in *T. magnus*

Females and males live comparable developmental time (female: 84.4 ± 24.9 days, $n = 24$; male: 77.3 ± 20.9 days, $n = 34$; $t = 0.32$, $p = 0.754$), while females live twice longer than males in adult longevity (female: 178.8 ± 46.0 days, $n = 24$; male: 83.9 ± 42.4 days, $n = 34$; $t = 4.48$, $p < 0.001$). Error bars represent standard deviation.

Female-biased longevity only in adulthood but not in development period

The above results illustrate that non-nutritional offspring nursing and copulation might tremendously increase the female's adult longevity, but the male's adult longevity is not affected by copulation (the only reproductive activity of males). These findings suggest that the sex role difference in reproduction might drive the evolution of longer-lived adult females in *T. magnus*. In addition, this spider does not exhibit intersexual body size difference, which suggests the two sexes might need similar development time to reach adulthood.²³ If these assumptions are true, *T. magnus* would exhibit female-biased longevity mainly or only in adulthood rather than in the developmental period. To examine this assumption, we compared the entire longevities (from hatching to death), juvenile developmental durations (from hatching to the last molt), and adult longevities between the two sexes, separately. Results confirmed our prediction, as there was no significant difference in juvenile developmental time between two sexes (females: 84.4 ± 24.9 days, mean \pm SD, $n = 24$; males: 77.3 ± 20.9 days, $n = 34$; $t = 0.32$, $p = 0.754$), while the adult and the entire longevities of females were 2.1 and 1.6 times of males respectively (adult longevity: 178.8 ± 46.0 days, $n = 24$; 83.9 ± 42.4 days, $n = 34$; $t = 4.48$, $p < 0.001$; entire longevity: 261.9 ± 45.7 days, $n = 65$; 161.2 ± 44.5 days, $n = 34$; $t = 7.78$, $p < 0.001$, Figure 3, and Tables S4–S6).

Reproductive activities and longevity in *T. magnus*

For female *T. magnus*, we showed that “milk” provisioning significantly reduced her reproductive longevity, and this reduced longevity should be compensated during non-nutritional offspring nursing process; consequently, the entire reproductive longevity would not be shortened no matter how many offspring they produced and cared for. We also showed that copulation could extend her longevity. The fact that “milk” provisioning significantly shortens mother spiders' longevity could be a result of massive energy investments in “milk” production, which is consistent with those reported in real milk provisioning mammals.²⁴ In addition, as the spider “milk” is derived from a steady provision of eggs (unpublished data), our findings are also consistent with the results in oviparous animals.^{4,25} Long-term non-nutritional nursing activities, although inevitably energy consuming, extended mothers' longevity of *T. magnus*, which might be because the mothers have strong resilience to aging when caring for nymphs and/or mother-offspring social bonds to compensate for the costs.^{10,26} The possible longevity extension mechanism behind mother-offspring social bonds could be early-instar offspring's hormones²⁷ and/or direct social interaction,^{10,26,28,29} which possibly maintain mothers' homeostatic systems and contribute to delay aging.²⁹

To our knowledge, this is the first study to test the effects of nutritional provisioning and non-nutritional nursing (two major components in offspring caring) on females' longevity separately, and showing that the overall effect of offspring caring, i.e., the combined effect of the two components, extends mothers' longevity. Offspring caring, in general, has been shown to reduce caretakers' longevity in various short-term parental cared species (caring for the juvenile offspring only to nutritional independence).^{30,31} However, we showed that for *T. magnus*, a long-term maternal cared species (caring for the offspring at least until adulthood, an extremely rare life history strategy only found in certain longevous mammals with higher sociality^{5,32,33} and few non-eusocial invertebrates^{22,34,35}), mothers' longevities were not reduced by offspring caring. Anyway, for maternal cared species, especially those long-term maternal cared animals, natural selection should favor females that could live long enough to fulfill the time requirement of offspring dispersal to ensure the offspring fitness.^{8,10}

In addition to *T. magnus*, it has been reported that copulated females live significantly longer than their virgin counterparts in various invertebrates,^{36–44} such as fruit flies, stick insects, crickets and spiders. This phenomenon might be a result of beneficial mating^{43,45,46} and/or costly virginity.^{41,44} The possible mechanisms of beneficial mating could be: (1) the nutrients in seminal fluids can be utilized directly by females for somatic maintenance;⁴³ or/and (2) specific proteins within seminal fluids could regulate females' longevity indirectly via immune system and/or endocrine system.^{45,46} Alternatively, virgin females might need to maintain high energetic rates for the egg maintenance and mate attractiveness which could be longevity costly, i.e., “cost of virginity” hypothesis.^{41,44} No matter how costly virginity is maintained, we believe that mating could extend the adult longevity of female *T. magnus* because, from the evolutionary point of view, mated females need to live long enough to accomplish offspring production, which could be one of the vital driving force to promote the females' longevity extension.

For male *T. magnus*, we found that their longevities were not affected by copulation (the only reproductive activity they engage in). This suggests that reproductive activity may not be a selective pressure for longevity in male *T. magnus*. Although copulation has been frequently reported to reduce males' longevity in various species^{47–49} due to the energetic consumption from seminal fluid formation, courtship behaviors and the copulatory process,³⁸ the inconsistent results might be due to male *T. magnus* not incurring significant costs in reproduction. This

could be attributed to their relatively simple courtship display and short copulatory duration (17.2 ± 1.2 min, $n = 47$). Alternatively, the inconsistency may arise because virgin males suffer comparable costs with mated males, as virgins need to transfer many resources to reproductive tissues and prepare to mate.⁵⁰ Regardless of the mechanism, there seems no driving force to extend males' longevity after mating if that is the only reproductive activity for males.

Offspring production and offspring dispersal are the two driving forces for female-biased longevity

T. magnus exhibits no longevity difference in the juvenile period but significant female-biased longevity in adult period. Furthermore, the detailed analysis of major reproductive activities' effects on each sexes' adult longevity (see above analysis) confirmed that the sex role difference in reproduction (i.e., males only engage in mating, while females also produce and attend eggs, and provide long-term offspring caring) could be the driving force of the adult longevity difference.

The non-significant sex difference in developmental time suggests a comparable time demand for reproductive preparation for both males and females in *T. magnus*, which correlates with their comparable adult body size and likely reflects similar prey acquisition strategies during juvenile stage. While the female-biased adulthood longevity pattern reflects females' need to live longer in *T. magnus*. This pattern is unlikely to be caused by a shortened male adulthood longevity, as mating does not affect male's longevity. This is because sexual selection intensity for male *T. magnus* is low and they have a higher possibility of multiple mating. In contrast, mated females lived longer than virgins, and offspring-cared mothers lived significantly longer than non-offspring-cared females. Thus, the female-biased adult longevity pattern reflects that the females suffered from higher pressure on time investment in reproduction than males in *T. magnus*. In other words, although males could die after mating, mated females need additional time for gestation, egg production, egg attendance and post-egg hatching maternal care.

We are surprised to find that, on average, adult female spiders lived 95 days longer than adult males, which is almost the same duration as the period from egg-laying to offspring dispersal (which lasts 91 days,^{22,51} including 10 days for gestation, 14 days from egg-laying to hatching, and 67 days for the post-hatching maternal care until offspring dispersal). The "perfect" match of the data confirmed that the sex role difference in reproduction itself could shape the adult longevity difference between sexes. Furthermore, we propose that the time requirement of offspring dispersal should be a key selective force promoting extension of female adult longevity, which ultimately reflects in female-biased longevity in maternal cared species.

Although maternal care⁸ has been proposed as one of the key factors shaping female-biased longevity,⁹ to our knowledge, this study provides the first experimental evidence showing that maternal care, in general, extends females' longevity and thus shapes the female-biased longevity pattern. Therefore, our findings contribute to explain why long-term maternal cared species often exhibit greater female-biased adult longevity than those species that do not provide extensive offspring care.⁸ It also helps to understand why the paternal cared species often exhibit male longevity advantage, such as in some primates⁸ and birds.⁵²

Lastly, successful reproduction is the ultimate goal of an individual; its whole longevity could be divided into meaningful periods, each having different longevity needs, and different mechanisms may exist to balance the energy expenditures to fulfill the longevity need in each period, especially the adult period. Our study is a case study to illustrate the power of the period-specific analysis to identify the exact period(s) that shape the pattern of longevity difference between sexes and inspire deeper thinking about the evolutionary force and future mechanistic studies. Additional experiments and comparative analyses of the cause-and-effect relationships between parental longevity and parental care will be conducted in future work.

Limitations of the study

In this paper, we illustrated how the sex-specific reproductive role of offspring caring explains the lifespan pattern of female-biased longevity in a maternal cared spider. However, a few limitations should be noticed for this study. First, the non-offspring-cared females were actually those that mated and consumed the only few clutches of eggs they laid but naturally failed to complete the entire reproductive process. Thus, the longevity difference between these females and the offspring-cared females could be a result of either they did not do offspring care or their physiological defects. Offspring adoptive experiment should be conducted for these females to examine whether their longevity will be extended by offspring adoption. Second, we managed to interpret the importance of maternal care in the evolution of female's longevity, yet this study itself is not adequate to confirm this prediction. We suggest a study performs relationship analyses between sexual difference in longevity and forms of parental care, such as maternal care only, paternal care only, biparental care and non-parental care, with as many species as possible regardless their taxonomical relationship. This study will reveal the correlation between longevity and parental care in a more general scale. In addition, phylogenetic analyses are required to examine whether parental care is a key evolutionary force of sexual difference in longevity in animals.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2024.110098>.

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AUTHOR CONTRIBUTIONS

Conceptualization, Z.C., C.J., and J.-X.L.; methodology, Z.C., J.-X.L., and C.J.; investigation, C.J., J.-X.L., and Y.-R.W.; visualization, C.J., J.-X.L., Z.C., and Y.-R.W.; funding acquisition, Z.C.; project administration, Z.C.; supervision, Z.C. and J.-X.L.; writing – original draft, C.J., Z.C., J.-X.L., and X.J.; writing – review and editing, all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Harshman, L.G., and Zera, A.J. (2007). The cost of reproduction: the devil in the details. *Trends Ecol. Evol.* 22, 80–86. <https://doi.org/10.1016/j.tree.2006.10.008>.
2. De Loof, A. (2011). Longevity and aging in insects: Is reproduction costly; cheap; beneficial or irrelevant? A critical evaluation of the "trade-off" concept. *J. Insect Physiol.* 57, 1–11. <https://doi.org/10.1016/j.jinsphys.2010.08.018>.
3. Shi, C., and Murphy, C.T. (2021). Sex and death. *Curr. Top. Dev. Biol.* 144, 353–375. <https://doi.org/10.1016/bs.ctdb.2020.08.004>.
4. Kern, C.C., Townsend, S., Salzmänn, A., Rendell, N.B., Taylor, G.W., Comisel, R.M., Foukas, L.C., Bähler, J., and Gems, D. (2021). *C. elegans* feed yolk to their young in a form of primitive lactation. *Nat. Commun.* 12, 5801. <https://doi.org/10.1038/s41467-021-25821-y>.
5. Royle, N.J., Smiseth, P.T., and Kölliker, M. (2012). *The Evolution of Parental Care* (Oxford University Press).
6. Bronikowski, A.M., Altmann, J., Brockman, D.K., Cords, M., Fedigan, L.M., Pusey, A., Stoinski, T., Morris, W.F., Strier, K.B., and Alberts, S.C. (2011). Aging in the Natural World: Comparative Data Reveal Similar Mortality Patterns Across Primates. *Science* 331, 1325–1328. <https://doi.org/10.1126/science.1201571>.
7. Promislow, D. (2003). Mate choice, sexual conflict, and evolution of senescence. *Behav. Genet.* 33, 191–201. <https://doi.org/10.1023/A:1022562103669>.
8. Allman, J., Rosin, A., Kumar, R., and Hasenstaub, A. (1998). Parenting and survival in anthropoid primates: Caretakers live longer. *Proc. Natl. Acad. Sci. USA* 95, 6866–6869. <https://doi.org/10.1073/pnas.95.12.6866>.
9. Austad, S.N., and Fischer, K.E. (2016). Sex differences in lifespan. *Cell Metab.* 23, 1022–1033. <https://doi.org/10.1016/j.cmet.2016.05.019>.
10. Horn, A.J., and Carter, C.S. (2021). Love and longevity: A Social Dependency Hypothesis. *Compr. Psychoneuroendocrinol.* 8, 100088. <https://doi.org/10.1016/j.cpnec.2021.100088>.
11. Lemaître, J.-F., Ronget, V., Tidière, M., Allainé, D., Berger, V., Cohas, A., Colchero, F., Conde, D.A., Garratt, M., Liker, A., et al. (2020). Sex differences in adult lifespan and aging rates of mortality across wild mammals. *Proc. Natl. Acad. Sci. USA* 117, 8546–8553. <https://doi.org/10.1073/pnas.191199911>.
12. Clutton-Brock, T.H., and Isvaran, K. (2007). Sex differences in ageing in natural populations of vertebrates. *Proc. Biol. Sci.* 274, 3097–3104. <https://doi.org/10.1098/rspb.2007.1138>.
13. Kemp, D.J., and Rutowski, R.L. (2004). A survival cost to mating in a polyandrous butterfly, *Colias eurytheme*. *Oikos* 105, 65–70. <https://doi.org/10.1111/j.0030-1299.2004.12874.x>.
14. Chapman, T., Miyatake, T., Smith, H.K., and Partridge, L. (1998). Interactions of mating, egg production and death rates in females of the Mediterranean fruit fly, *Ceratitis capitata*. *Proc. Biol. Sci.* 265, 1879–1894. <https://doi.org/10.1098/rspb.1998.0516>.
15. Li, G.Y., and Zhang, Z.Q. (2021). Sex-specific response to delayed and repeated mating in spider mite *Tetranychus urticae*. *Bull. Entomol. Res.* 111, 49–56. <https://doi.org/10.1017/S0007485320000292>.
16. Zhao, J.M., Fang, Y., Lou, Y.Q., Swenson, J.E., and Sun, Y.H. (2018). Brood rearing has an immediate survival cost for female Chinese Grouse *Tetrastes sewerzowi*. *J. Ornithol.* 159, 1019–1029. <https://doi.org/10.1007/s10336-018-1578-4>.
17. Stephen, T., and Claudia, M.; Rauter (2014). Juvenile hormone, metabolic rate, body mass and longevity costs in parenting burying beetles. *Anim. Behav.* 92, 203–211. <https://doi.org/10.1016/j.anbehav.2014.04.004>.

18. Aguilani, H. (2015). The mysterious relationship between reproduction and longevity. *Worm* 4, e1020276. <https://doi.org/10.1080/21624054.2015.1020276>.
19. King, A.M., Kirkwood, T.B.L., and Shanley, D.P. (2017). Explaining sex differences in lifespan in terms of optimal energy allocation in the baboon. *Evolution* 71, 2280–2297. <https://doi.org/10.1111/evo.13316>.
20. Robinson, M.R., Mar, K.U., and Lummaa, V. (2012). Senescence and age-specific trade-offs between reproduction and survival in female Asian elephants. *Ecol. Lett.* 15, 260–266. <https://doi.org/10.1111/j.1461-0248.2011.01735.x>.
21. Saito, S. (1933). Notes on the spiders from Formosa. *Trans. Sapporo Nat. Hist. Soc.* 13, 32–60.
22. Chen, Z., Corlett, R.T., Jiao, X., Liu, S.-J., Charles-Dominique, T., Zhang, S., Li, H., Lai, R., Long, C., and Quan, R.-C. (2018). Prolonged milk provisioning in a jumping spider. *Science* 362, 1052–1055. <https://doi.org/10.1126/science.aat3692>.
23. Teder, T. (2014). Sexual size dimorphism requires a corresponding sex difference in development time: a meta-analysis in insects. *Funct. Ecol.* 28, 479–486. <https://doi.org/10.1111/1365-2435.12172>.
24. Gittleman, J.L., and Thompson, S.D. (1988). Energy Allocation in Mammalian Reproduction. *Am. Zool.* 28, 863–875. <https://doi.org/10.1093/icb/28.3.863>.
25. Williams, T.D. (2005). Mechanisms underlying the costs of egg production. *Bioscience* 55, 39–48. [https://doi.org/10.1641/0006-3568\(2005\)055\[0039:MUTCOE\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[0039:MUTCOE]2.0.CO;2).
26. Zhu, P., Liu, W., Zhang, X., Li, M., Liu, G., Yu, Y., Li, Z., Li, X., Du, J., Wang, X., et al. (2023). Correlated evolution of social organization and lifespan in mammals. *Nat. Commun.* 14, 372. <https://doi.org/10.1038/s41467-023-35869-7>.
27. Cho, L.C., Yu, C.C., and Kao, C.F. (2021). Social perception of young adults prolongs the lifespan of aged *Drosophila*. *Npj Aging Mech. Dis.* 7, 21. <https://doi.org/10.1038/s41514-021-00073-8>.
28. Brenman-Suttner, D.B., Yost, R.T., Frame, A.K., Robinson, J.W., Moehring, A.J., and Simon, A.F. (2020). Social behavior and aging: A fly model. *Genes Brain Behav.* 19, e12598. <https://doi.org/10.1111/gbb.12598>.
29. Díaz-del Cerro, E., Ceprián, N., Félix, J., and De la Fuente, M. (2022). A short social interaction between adult and old mice improves the homeostatic systems and increases healthy longevity. *Exp. Gerontol.* 158, 111653. <https://doi.org/10.1016/j.exger.2021.111653>.
30. Dugas, M.B., Wamelink, C.N., and Richards-Zawacki, C.L. (2015). Both sexes pay a cost of reproduction in a frog with biparental care. *Biol. J. Linn. Soc.* 115, 211–218. <https://doi.org/10.1111/bij.12461>.
31. Downing, P.A., Griffin, A.S., and Cornwallis, C.K. (2021). Hard-working helpers contribute to long breeder lifespans in cooperative birds. *Philos. Trans. Roy. Soc. B.* 376, 20190742. <https://doi.org/10.1098/rstb.2019.0742>.
32. Surbeck, M., Mundry, R., and Hohmann, G. (2011). Mothers matter! Maternal support, dominance status and mating success in male bonobos (*Pan paniscus*). *Proc. Biol. Sci.* 278, 590–598. <https://doi.org/10.1098/rspb.2010.1572>.
33. Foster, E.A., Franks, D.W., Mazzi, S., Darden, S.K., Balcomb, K.C., Ford, J.K.B., and Croft, D.P. (2012). Adaptive Prolonged Postreproductive Life Span in Killer Whales. *Science* 337, 1313. <https://doi.org/10.1126/science.1224198>.
34. Dillard, J.R., and Maigret, T.A. (2017). Delayed dispersal and prolonged brood care in a family-living beetle. *J. Evol. Biol.* 30, 2230–2243. <https://doi.org/10.1111/jeb.13190>.
35. Mikat, M., Waldhauserova, J., Frankova, T., Cermakova, K., Broz, V., Zeman, S., Dokulilova, M., and Straka, J. (2021). Only mothers feed mature offspring in European *Ceratina* bees. *Insect Sci.* 28, 1468–1481. <https://doi.org/10.1111/1744-7917.12859>.
36. South, A., and Lewis, S.M. (2012). Effects of male ejaculate on female reproductive output and longevity in *Photinus* fireflies. *Can. J. Zool.* 90, 677–681. <https://doi.org/10.1139/z2012-031>.
37. Boisseau, R. (2022). *The Evolution of Morphological Diversity and Sexual Dimorphism in Stick and Leaf Insects* (University of Montana).
38. Harjoko, D.N., Hua, Q.Q.H., Toh, E.M.C., Goh, C.Y.J., and Puniemoorthy, N. (2023). A window into fly sex: mating increases female but reduces male longevity in black soldier flies. *Anim. Behav.* 200, 25–36. <https://doi.org/10.1016/j.anbehav.2023.03.007>.
39. Wagner, W.E., Kelley, R.J., Tucker, K.R., and Harper, C.J. (2007). Females receive a life-span benefit from male ejaculates in a field cricket. *Evolution* 55, 994–1001. <https://doi.org/10.1111/j.0014-3820.2001.tb00616.x>.
40. Worthington, A.M., and Kelly, C.D. (2016). Females gain survival benefits from immune-boosting ejaculates. *Evolution* 70, 928–933. <https://doi.org/10.1111/evo.12890>.
41. Markow, T.A. (2011). "Cost" of virginity in wild *Drosophila melanogaster* females. *Ecol. Evol.* 1, 596–600. <https://doi.org/10.1002/ece3.54>.
42. Heinze, J., and Schrepf, A. (2008). Aging and reproduction in social insects—a mini-review. *Gerontology* 54, 160–167. <https://doi.org/10.1159/000122472>.
43. Kotiaho, J.S., and Simmons, L.W. (2003). Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*. *J. Insect Physiol.* 49, 817–822. [https://doi.org/10.1016/S0022-1910\(03\)00117-3](https://doi.org/10.1016/S0022-1910(03)00117-3).
44. Stoltz, J.A., Hanna, R., and Andrade, M.C. (2010). Longevity cost of remaining unmated under dietary restriction. *Funct. Ecol.* 24, 1270–1280. <https://doi.org/10.1111/j.1365-2435.2010.01729.x>.
45. Poiani, A. (2006). Complexity of seminal fluid: a review. *Behav. Ecol. Sociobiol.* 60, 289–310. <https://doi.org/10.1007/s00265-006-0178-0>.
46. Avila, F.W., Siro, L.K., LaFlamme, B.A., Rubinstein, C.D., and Wolfner, M.F. (2011). Insect Seminal Fluid Proteins: Identification and Function. *Annu. Rev. Entomol.* 56, 21–40. <https://doi.org/10.1146/annurev-ento-120709-144823>.
47. Partridge, L., and Farquhar, M. (1981). Sexual activity reduces lifespan of male fruitflies. *Nature* 294, 580–582. <https://doi.org/10.1038/294580a0>.
48. South, S.H., Steiner, D., and Arnqvist, G. (2009). Male mating costs in a polygynous mosquito with ornaments expressed in both sexes. *Proc. Biol. Sci.* 276, 3671–3678. <https://doi.org/10.1098/rspb.2009.0991>.
49. Burton-Chellew, M.N., Sykes, E.M., Patterson, S., Shuker, D.M., and West, S.A. (2007). The cost of mating and the relationship between body size and fitness in males of the parasitoid wasp *Nasonia vitripennis*. *Evol. Ecol. Res.* 9, 921–934.
50. Camacho-García, J.C., Pineda, S., and Cordero, C. (2018). Mating has no effect on male longevity in the moth *Amorbia cuneana* (Tortricidae). *J. Lepidopterists' Soc.* 72, 155–158. <https://doi.org/10.18473/lepi.v72i2.a8>.
51. Dong, B., Liu, J.X., Quan, R.C., and Chen, Z. (2022). Mirror image stimulation could reverse social-isolation-induced aggressiveness in the high-level subsocial lactating spider. *Anim. Cogn.* 25, 1345–1355. <https://doi.org/10.1007/s10071-022-01618-4>.
52. Leitner, S., Brighton, R., and Voigt, C. (2021). Barred buttonquail males outlive females. *J. Avian Biol.* 52, e02722. <https://doi.org/10.1111/jav.02722>.
53. RCoreTeam (2022). R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing). <https://www.R-project.org/>.
54. RStudioTeam (2019). RStudio (Integrated Development for R). <http://www.rstudio.com/>.
55. Gómez-Rubio, V. (2017). ggplot2: Elegant Graphics for Data Analysis. *J. Stat. Softw.* 77, 1–3. <http://www.springer.com/gp/book/9783319242750>.
56. Guo, K. (2022). eoffice: Export or Graph and Tables to 'Microsoft' Office and Import Figures and Tables. R package version 0.2.2. <https://CRAN.R-project.org/package=efoffice>.
57. Kuznetsova, A., Brockhoff, P.B., and Christensen, R.H.B. (2017). ImerTest Package: Tests in Linear Mixed Effects Models. *J. Stat. Softw.* 82, 1–26. <https://doi.org/10.18637/jss.v082.i13>.
58. Lüdtke, D., Ben-Shachar, M., Patil, I., Waggoner, P., and Makowski, D. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *J. Open Source Softw.* 6, 3139. <https://doi.org/10.21105/joss.03139>.
59. Fox, J., and Weisberg, S. (2019). An R Companion to Applied Regression, Third Edition (Sage). <https://socialsciences.mcmaster.ca/jfox/Books/Companion/>.
60. Lüdtke, D. (2023). sjPlot: Data Visualization for Statistics in Social Science. R package version 2.8.15. <https://CRAN.R-project.org/package=sjPlot>.
61. Therneau, T.M. (2020). Coxme: Mixed Effects Cox Models. R package version 2.2-16. <https://CRAN.R-project.org/package=coxme>.
62. Therneau, T. (2023). A Package for Survival Analysis in R. R package version 3.5. <https://CRAN.R-project.org/package=survival>.
63. Kassambara, A., Kosinski, M., and Biecek, P. (2021). survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.9. <https://CRAN.R-project.org/package=survminer>.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Experimental data	This paper	Mendeley Data: https://doi.org/10.17632/f876hz684n.1
Experimental models: Organisms/strains		
<i>Toxews magnus</i>	Indoor reared in Xishuangbanna Tropical Botanical Garden (XTBG), Yunnan, China (approx. 21° 55' N, 101° 16' E)	Indoor type
Software and algorithms		
R 4.3.2 in Rstudio	R Core Team	https://www.r-project.org/

RESOURCE AVAILABILITY

Lead contact

Further information and requests should be directed to and will be fulfilled by the lead contact, Zhanqi Chen (chenzhanqi@xtbg.ac.cn).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All the original data are available through the Mendeley data repository, and are publicly available as of the date of publication. DOIs are listed in the [key resources table](#).
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The lactating jumping spider *Toxews magnus* (Araneae: Salticidae)²¹ exhibits an evident sex role difference in reproduction with males only providing gametes, while females providing a long-term maternal care including all the reproductive phases from copulation, egg production, “milk” provisioning to post-nutritional-independence nursing (around three months).²² During the long-term maternal caring period, spiderlings were exclusively dependent on mothers’ “milk” to survive within the first 20 days after egg hatching. Since then, juvenile spiders begin to forage outside the nest and continue “milk” sucking until they are weaning at around 40 days.²² Female offspring would stay in the natal nests with their mother even after sexual maturity and then disperse for breeding (around 20 days after sexual maturity), while male offspring often be evicted from the nest by female companions once they are matured.⁵¹ Female spiders “milk”-feed their early-instar spiderlings by continuously producing eggs (unpublished data), a process that lasts around 40 days.²²

For this study, we collected 47 gravid *T. magnus* females to generate an experimental population from two sites in Xishuangbanna, Yunnan China (42 females in Xishuangbanna Tropical Botanical Garden, 101.272°E, 21.920°N, 584 a.s.l.; five females in Jinuo Village, 101.033°E, 22.043°N, 834 a.s.l.) between August 2020 and July 2021. We brought the field collected females (F0) to the laboratory and housed them individually in transparent plastic boxes (Type1: 15.4 × 11 × 5.8 cm). F0's offspring were F1, and F1's offspring were F2 and so on. Each F0 and all of her progenies were defined as a family. We set up five different social conditions for juvenile offspring to grow from nutritional independence (at 30 days after hatching) to reach their sexual maturity. We randomly assigned nutritional independent spiderlings from each family to rearing groups: 1) mother cared (MC), five nestmates continued living with their mother in Type1 rearing box; 2) mother removed (MR), five nestmates lived together without mother in a new Type1 box; 3) paired living (PR), two nestmates reared together in a smaller transparent plastic box (Type2: 5.7 × 5.7 × 4.2 cm); 4) single reared (SR), one spiderling individually reared in Type2 box; 5) control (CK): all offspring lived with their mother (no manipulations had been performed). Adult females and males from different families were paired to produce offspring. In total, we collected data from four generations (F1-F4) based on the 47 families until the end of our experiments. During the study period, we controlled the temperature (25 ± 2°C), relative humidity (65 - 70%), light: dark regime (light: 8:00 - 20:00) and feeding (every two days we fed fruit flies *Drosophila melanogaster* and houseflies *Musca domestica*). We checked our spiders every two days and cleaned the rearing boxes once a week. During checking, we tried to record the exact date of the egg clutches’ production and hatching, the last molt (reaching adulthood) and the death of each offspring.

METHOD DETAILS

Manipulations of female reproductive status

To ensure completely reaching sexual maturation, about two months after the first male sibling matured in each brood, females were separated from natal nest and individually reared in new Type2 rearing boxes. Then we assigned females with similar developmental time (Figure S3A) to three groups to manipulate their reproductive activities: 1) virgin ($n = 30$): never met males until death; 2) "milk" provisioning ($n = 34$): each of the newly produced egg clutches were immediately removed to induce "milk" provisioning (continuous egg laying) as the "milk" is the continuously produced eggs during maternal care (unpublished data); 3) offspring caring (completed the whole reproductive process): for this group we paired females ($n = 120$) and those successfully mated females were allowed to continue the following reproductive activities naturally (65 of 120). However, another group emerged naturally from the third group, and we named it 4) non-offspring caring group (20 of 120), in which mated females laid only few egg clutches (one to six) after mating and consumed all soon and no longer lay further eggs even after experiencing one or two additional mating, thus no egg clutches hatched and no offspring cared. Detailed descriptions of reproductive activities that four groups of females involved are shown in Table S1. Additionally, twelve out of the 120 individuals did not lay eggs after mating, five individuals did not engage in mating and 18 individuals either died before mating manipulation, escaped during rearing, or experienced unnatural death. All these individuals have been excluded from subsequent data analysis.

To avoid sexual cannibalism during mating, all females were well-fed one day before mating. Mating experiments were carried out between 10:00 h and 17:00 h. We introduced a virgin male into the female's rearing box (Type2) according to inbreeding avoidance. To ensure successful mating and that the mated females received enough sperm, we kept each mated couple overnight, then transferred the female to a new Type1 rearing box in the next day to continue the experiment. If the female did not produce any egg clutches in two weeks after mating, we paired them again with a new virgin male.

Mating regimes for males

Males were separated from natal nest once reached adulthood (at the last molt) and individually reared in new Type2 boxes. One week after the last molt, individually reared adult males with similar developmental time (Figure S3B) were assigned to three mating regimes: 1) virgin ($n = 40$): never met females until death; 2) single mating ($n = 40$): only paired and mated once during the entire life; and 3) multiple mating ($n = 40$): paired with five different females with 7-10 days intervals. To avoid sexual cannibalism, all males were well-fed one day before mating. Mating experiments were carried out between 10:00 h and 17:00 h. We introduced the male into the virgin female's rearing box to ensure a high successful mating probability. Normally a male starts to exhibit courtship display within one minute after being introduced into the female's rearing container, and the receptive female will soon accept, then copulation starts and lasts about 20 minutes. Once copulation concluded, the male was returned to his original rearing container to continue the experiment until his death. We excluded the males who did not exhibit courtship display or were rejected by the female within 30 minutes of this experiment. Finally, we collected reliable data from 33 virgins, 27 singly mated and 26 multiple mated males after excluding the individuals that failed to copulate, escaped during rearing, or experienced unnatural death, defined as those occurring within one week of the last molt or as a result of accidental injury.

QUANTIFICATION AND STATISTICAL ANALYSIS

Before data analysis, the entire longevity (days from hatching to death), developmental time (days from hatching to the last molt), adult longevity (days from the last molt to death) of all individuals (females and males), and the reproductive longevity of females (days from laying the first egg clutch to death) were calculated. We also summarized the number of egg clutches produced, nutritional independent spiderlings, and offspring that reached adulthood per brood.

Effect of maternal caring and mating activities on female's longevity

To examine the effect of maternal caring activities and mating activities on female's longevity, LMM and mixed effects Cox regression (coxme) models were implemented to compare the adult longevities and the adult survival profiles among three groups of females with different reproductive experiences: 1) virgin females ($n = 14$), 2) non-offspring caring females ($n = 12$, these females mated and laid one to six egg clutches that were eaten soon); 3) offspring caring females ($n = 18$). Post-hoc multiply comparisons were further implemented to show the pairwise differences of adult longevities and adult survival profiles among the three groups of females. In the LMM, "reproductive experience" (the major factor we were interested in) and "developmental time" were the fixed factors and "family ID" was the random factor (Table S2). In the coxme model, "reproductive experience" and "developmental time" were included as the predictive factor and "family ID" was the random factor. The model predicted adult longevities and survival profiles of the three groups of female spiders were visualized by bar plots and survival curves respectively (Figures 1B and 1C).

Effects of "milk" provisioning on female's longevity

To examine the effect of "milk" provisioning on females reproductive longevity, LMM and Cox proportional hazards model were implemented to compare the reproductive longevities (to exclude the influence of gestation duration and/or preparation time for egg-laying between different groups) and the reproductive survival profiles among three groups of females with different reproductive experiences: 1) non-offspring caring females ($n = 20$); 2) "milk" provisioning females ($n = 20$, these females mated and laid seven to 41 egg clutches but all of their egg clutches were removed to induce the "milk" provisioning); 3) offspring caring females ($n = 51$). Post-hoc multiply comparisons were further

implemented to show the pairwise differences of reproductive longevity and reproductive survival profiles among the three groups of females. Post-hoc multiply comparisons were further implemented to show the pairwise differences of adult longevity and adult survival profiles among the three groups of females. In the LMM, “reproductive experience” (the major factor we were interested), “number of egg clutches” were the two fixed factors and “family ID” was the random factor (Table S3). In the Cox proportional hazards model, only “reproductive experience” was included as a predictive factor. Model predicted reproductive longevity and survival profiles of the three groups of female spiders were visualized by bar plots and survival curves respectively (Figures 1D and 1E).

Effect of offspring number on mother’s longevity

We further tested how offspring number affects mothers’ longevity by correlating the reproductive longevity of offspring cared females with their number of produced egg clutches ($n = 26$), nutritional independent sub-adults ($n = 26$) and offspring that reached adult stage ($n = 26$) respectively. The relationships between mother’s reproductive longevity and the numbers of their offspring at three stages were visualized by scatter plots (Figure S2). Then three LMMs were implemented to test whether the number of offspring at three stages influences their mother’s reproductive longevity or not. In each LMM, the “family ID” was the random factor, and “generation” was also included as a fixed factor to control the effect of generation difference on the results.

Effect of mating experience on male’s longevity

To examine the effect of mating experience on male’s longevity, LMM and Cox proportional hazards model were implemented to compare the adult longevity and the adult survival profiles among three groups of males with different mating experiences: 1) virgin males ($n = 33$), 2) singly mated males ($n = 27$), and 3) multiply mated males ($n = 26$, twelve mated twice, five mated three times, one mated four times and eight mated five times). In the LMM, “mating groups” (the major factor we were interested in), “juvenile experience” and “developmental time” were the three fixed factors and “family ID” was the random factor (Table S4). In the Cox proportional hazards model, only “mating groups” was included in the predictive factor. The model predicted adult longevity and survival profiles of the three groups of male spiders were visualized by bar plots and survival curves respectively (Figures 3B and 3C).

Sex difference in longevity

To investigate the sex difference in longevity in *T. magnus*, the individuals that completed the whole reproductive process and with exact longevity records were selected, and their entire longevity (male, $n = 34$; female, $n = 65$), developmental time (male, $n = 34$; female, $n = 24$) and adult longevity (male, $n = 34$; female, $n = 24$) were visualized by bar plots (Figure 3) and compared with linear mixed models (LMMs). In the models, “sex” (the major factor we were interested in), “juvenile experience” and “generation” were the three fixed factors and “family ID” was the random factor (Tables S5–S7).

All data visualizations and analysis were implemented in R 4.3.2 through RStudio 2023.9.1.494.^{53,54} The package ‘ggplot2’⁵⁵ was used to produce each plot, and then the package ‘eoffice’⁵⁶ was used to export the plots, which were enhanced in PowerPoint (Microsoft Office 2023). Each LMM was implemented with the function lmer from package ‘lmerTest’⁵⁷ and diagnosed by the function check_model in the package ‘performance’,⁵⁸ then used the Anova function in package ‘car’⁵⁹ to test each fixed factor’s effects on the dependent variable. In the end, the summarized LMM regression table was created by the function tab_model from package ‘sjPlot’.⁶⁰ Mixed effects Cox regression models were implemented with the function coxme in the package ‘coxme’.⁶¹ Cox proportional hazards models were implemented and diagnosed by the function coxph and the function cox.zph in the package ‘survival’,⁶² then we used the function pairwise_survdiff from package ‘survminer’⁶³ to do the multiple comparisons for the survival profiles.