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Females become adults about ten days earlier than males in a phalangiid harvestman *Odiellus aspersus* (Opiliones: Eupnoi: Phalangiidae)

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Abstract. The reproductive phenology of a species of phalangiid harvestmen, *Odiellus aspersus*, was studied at Maruyama Park, Sapporo, Hokkaido, for the difference in adult emergence between males and females. At the timepoint in which 50% of the population had matured to adulthood, more females were represented than males, and adult females developed about 10 days earlier on average than males. There was no difference in adult body size between males that had matured earlier and males that were collected later in the season. This fact would conflict with a plausible hypothesis that males should mature slowly to attain larger body size in this species. Elongation of spermatheca in females of the species may explain the marked difference in the adult emergence in the species, by enhancing sperm priority of the males that copulated with females last.

Keywords: Protogyny, sperm competition, spermathecae
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The dominant species of Japanese harvestmen in Hokkaido, which include 9 bisexual and 2 thelytokous species, were surveyed in 1979 and their phenologies have been reported by Tsurusaki (1986, 2003). The survey showed that all the species studied were univoltine, and eight of the nine Eupnoi species were ones that overwinter as eggs. One phenomenon found in the survey was the tendency towards earlier appearance of females compared to males, or "protogyny" (Tsurusaki 2003) in those univoltine species which overwinter as eggs. This is remarkable because protogyny is very rare in other arthropods, including insects, crustaceans, and spiders (Ridley 1983; Thornhill & Alcock 1983; Austad 1984). In the 1979 survey, protogyny was found in four out of five of the bisexual Eupnoi species that overwinter as eggs (Tsurusaki 2003). Of the four species with female precedence, *Odiellus aspersus* (Karsch, 1881) (Fig. 1) was the species in which the trend was most pronounced (Fig. 2, Tsurusaki 2003). My collection of the species, consisting of more than 5,000 specimens from various sites of Japan, also attests that this is a univoltine species showing protogyny, because juveniles were found only from April to August and adults only from August to October, and the specimens of the first adults that appear in early August are female biased. To estimate how many days earlier females mature compared to males, I made an additional survey of phenology of the species at Maruyama Park in the summer of 1984. Furthermore, to test a plausible hypothesis that males should mature slowly to attain larger body size, I measured and compared adult size for males captured in earlier and later seasons. I will present results of the survey and the measurements and discuss the reason why this species shows the most notable protogyny in harvestmen.

METHODS

The survey was conducted along a path in the Maruyama Park (43.051079 N,141.308865 E), Sapporo City, Hokkaido, in the summer of 1984. The path stretches over the forests along the western foot of Mt. Maruyama, Japan. The path penetrates the border between planted *Cryptomeria japonica* forest and natural broad-leaved deciduous forests of *Cercidiphyllum japonicum*, *Acer mono, Kalopanax pictus*, and other tree species.

The survey was initially planned to start in late July because the first adults were found in early August during the 1979 surveys at Maruyama and Nopporo forests (Fig. 2, and Tsurusaki 2003). The survey was started on July 27, 1984 and harvestmen were collected principally in the morning every alternate day until the end of August. The number of juveniles gradually decreased, as they matured during the survey period. The target number of collecting was set at about 50 for each collection to get reliable composition percentages for juveniles, adult males and adult females in the population. Each collection took about 1 to 2 hours. I used the same path about 1 km long every time for the collecting. Fortunately, no depletion of the individuals occurred even in the later samplings in the survey period, although more than 50 specimens were removed from the path every time. The specimens collected were immediately fixed and preserved in 80% ethanol. Juveniles were dissected to determine sex by checking morphology of gonads (ovaries or testis) to know the difference in the ratio of individuals that attained adulthood between males and females. Adults were sexed by checking presence (males) or absence (females) of ventromesal rows of denticles on the palpal tarsus (cf. fig. 8A in Suzuki & Tsurusaki 1983). Body length and length of femora of the first legs (FIL) of adult males were measured under a stereo-microscope equipped with an ocular micrometer.

For statistical analysis, I used JMP, version 12.0.1 (SAS Institute 2015).

RESULTS

Results of the survey are shown in Figs. 3–5. Probably due to high temperatures in the summer of 1984 in Sapporo (mean highest and mean lowest temperatures in July 1984 at Sapporo were 26.7° and 18.9° compared to the 1974–1983 mean July high of 24.8° and mean July low of 16.8°), three female adults were found on the first day (July 27) of collections despite expectations of only juveniles (Fig. 3). The first adult male appeared on August 1. The number of juveniles progressively decreased after August 5 but the last sample (72 specimens) collected on August 30 still contained a male juvenile.

Fig. 4 shows transitions of adult ratios for males and females. Most of the juveniles were able to be sexed by the morphology of gonads in their abdomens. The difference in the cumulative

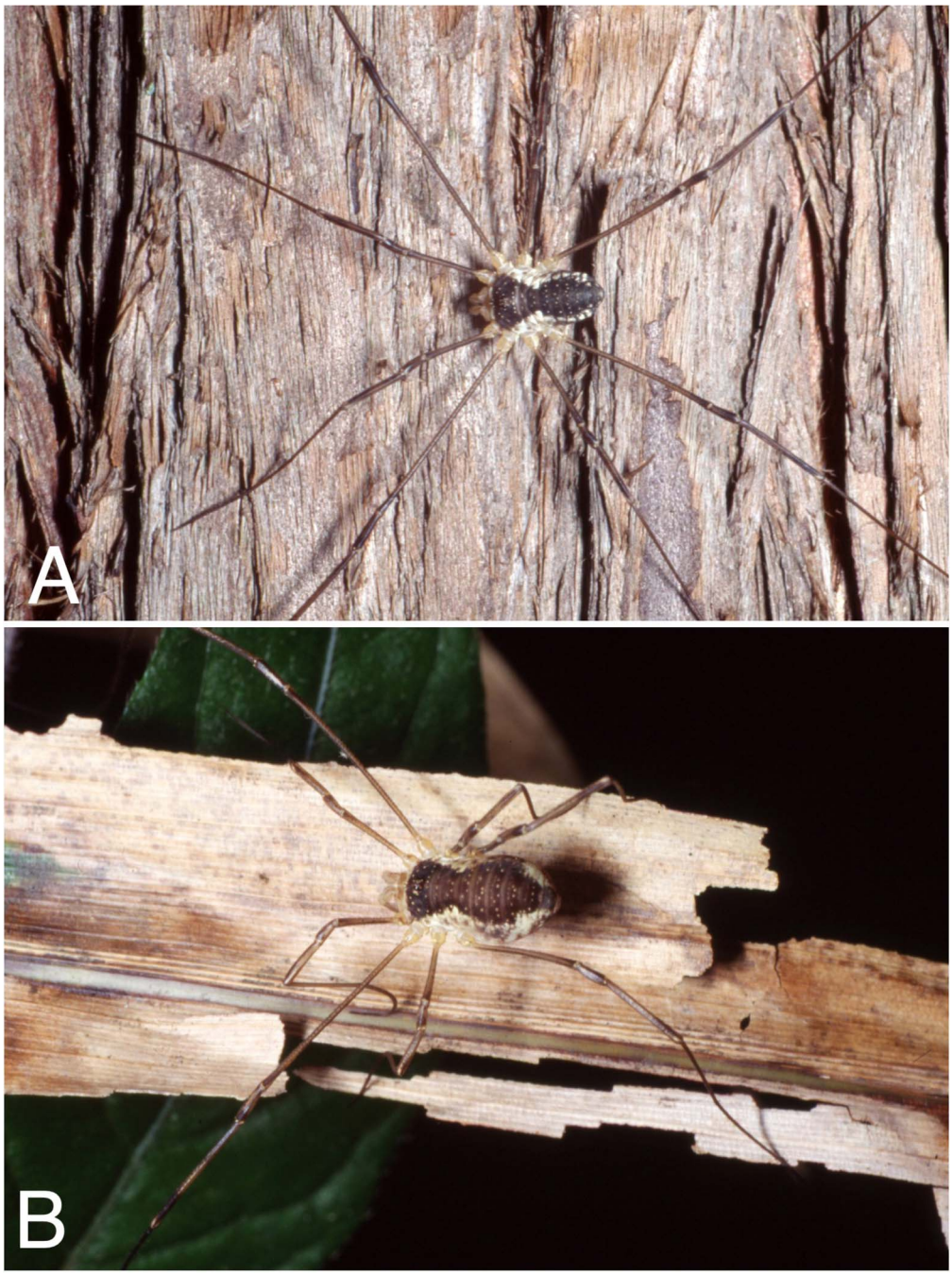

Figure 1.—Adult *Odiellus aspersus*, Maruyama Park, Sapporo (September 1992, Photo by N. Yoshida). (A) male. (B) female.

number between males and females was significant (Kormogolov-Smirnov 2 sample test, <0.001). From the distance between the two curves at the point where 50% individuals attained adulthood, it was found that adult females emerge about 10 days earlier than adult males.

There was no difference between males collected earlier and later in both body length and length of the first femur (FIL) (>0.05 Kruskal-Wallis test). Fig. 5 shows body length in adult males. It is expected that if slower growth in juveniles contributes to larger adult size, this length would be larger in the adult males collected later in the season (those males would consist of both males that matured earlier and later) than males in the earlier season (only males matured earlier are included). However, no such trend in male size was detected (Kruskal-Wallis test, $P > 0.05$).

DISCUSSION

As reported in Tsurusaki (2003), protogyny seems to be prevalent in harvestmen with a univoltine, egg-hibernating life history.

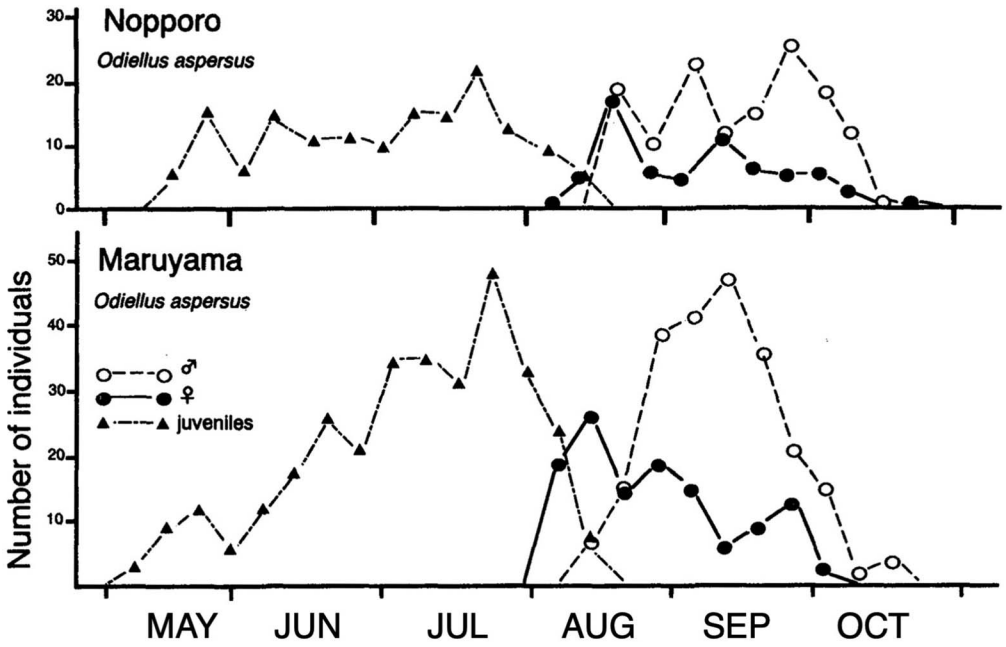

Figure 2.—Phenology of *Odiellus aspersus* surveyed in 1979 at two sites (Nopporo and Maruyama) in and near Sapporo City, Hokkaido (adapted from Fig. 5 in Tsurusaki 2003).

Eupnoi harvestmen mate multiply, and both males and females repeatedly copulate with multiple mates (Machado & Macías-Ordóñez 2007; Fowler-Finn et al. 2018; Brown et al. 2020). *Odiellus aspersus* also easily copulate with adults of the opposite sex when they are placed in the same plastic bag just after collection in the field. It is expected that the last males to copulate would have adaptive advantage if the last male's sperm were preferentially used in the fertilization of eggs. Unfortunately, which male's sperm are preferentially used in the fertilization of eggs when a female mates with more than one male is not confirmed experimentally in harvestmen. However, spermathecae of harvestmen are typical "cul-de-sac"-type, the term coined by Austad (1984), which is essentially a diverticulum of the vagina with a single aperture (Karachiwalla et al. 2020). Sperm must pass through this aperture in its way into the storage organ and then return using the same aperture when eggs are fertilized. Walker (1980) found that there is a trend toward monogamy or low percent sperm displacement in species with spheroid or ovoid spermathecae contrasting with a high percent sperm displacement in species with elongate or tubular sperm storage organs in insects.

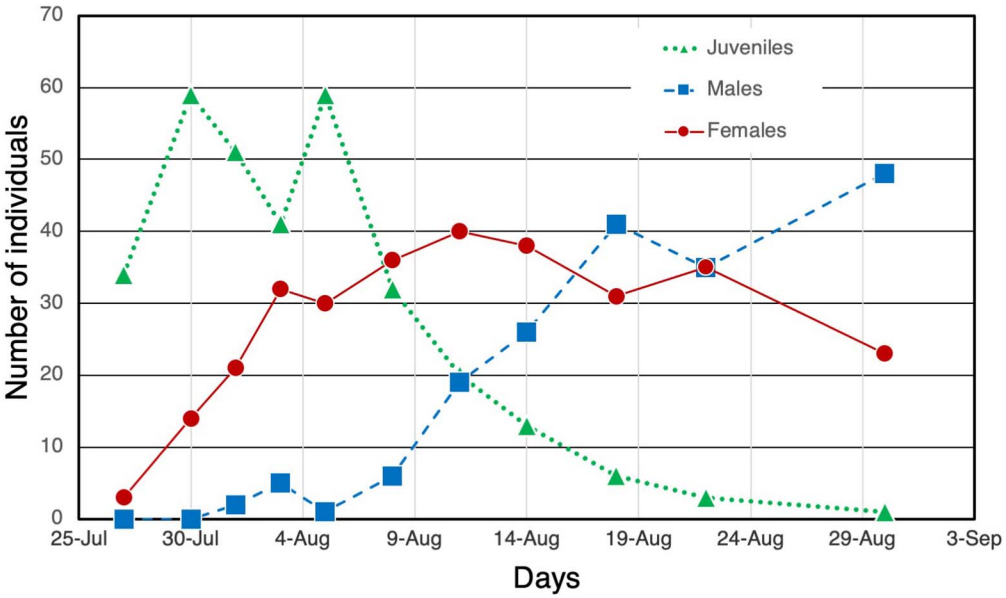

Figure 3.—Phenology of *Odiellus aspersus* at Maruyama in the summer 1984.

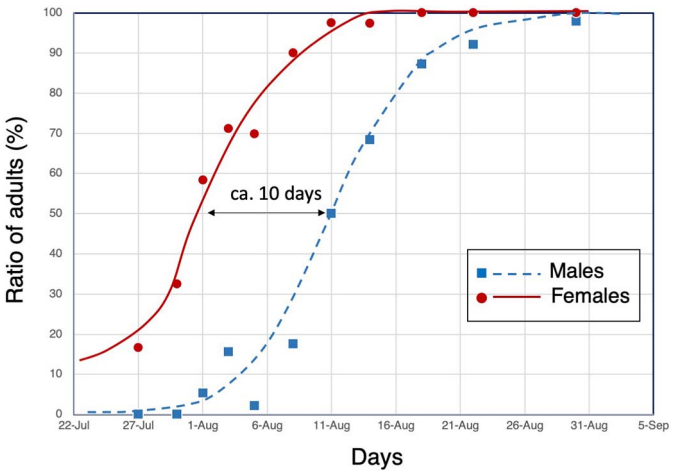

Figure 4.—Transition of adult ratios in males and females of *Odiellus aspersus* at Maruyama in 1984. Adult ratio (%) = number of adult females (or males)/(numbers of juvenile females (or males) + number of adult females (or males)) × 100. Difference in the cumulative numbers between adult males and females (or between immature males and females) is significant (Kolmogorov-Smirnov 2 sample test, <0.001).

Austad (1984) also suggested importance of spermatheca morphology to the sperm priority pattern, precopulatory mate guarding, and evolution of mating plugs in spiders (The mating plugs become adaptive in spiders with conduit-type spermathecae because plugging the orifice of spermatheca does not hamper oviposition of eggs that come out through the different duct in those spiders).

If this reasoning can be applied to other arthropod groups, in harvestmen that have a typical cul-de-sac-type spermathecae, the last male would have an advantage in sperm priority over males that copulate with the mate earlier. Females of *O. aspersus* have elongated sac-like spermathecae in their ovipositors (Fig. 6). This fact may explain why female precedence in maturation is exaggerated in this species, because elongated spermathecae with a narrow opening would prevent a mixture of sperm stored earlier and later. Interestingly, spermathecae are not elongated and seem to have wide openings (Fig. 6) in *Psathyropus tenuipes* L. Koch, 1878 (Opiliones: Sclerosomatidae) that did not show any sign of protogyny in the previous survey in 1979 (Tsurusaki 2003). Further accumulation of the knowledge on the relationship between female spermatheca morphology and tendency towards protogyny would be desirable.

If males that spend longer durations in juvenile stages have other advantages, for example, such as acquiring larger body size, this might also contribute to protogynous maturation. However, body size (both body length and length of the first leg femur) did not differ between males matured earlier and males collected in later season (Fig. 5). Thus, the influence of male body size on the protogynous trend would be excluded from possible explanations for evolution of protogyny in this species.

Protogyny has evolved also in the bug moths (Psychidae) having conduit type of spermathecae (this type of female genitalia is termed "ditrysian" in Lepidopera: Drummond III 1984) which promotes precedence of the first male sperm in fertilization. Degen et al. (2015) showed that protogyny will be the optimal emergence strategy for both males and females when (1) female longevity is considerably greater than that of males, (2) encounter rates between males and females are low, and (3) females mate only once. Life history of Psychidae moths implements these conditions, but that of *Odiellus aspersus* fulfills none of these conditions.

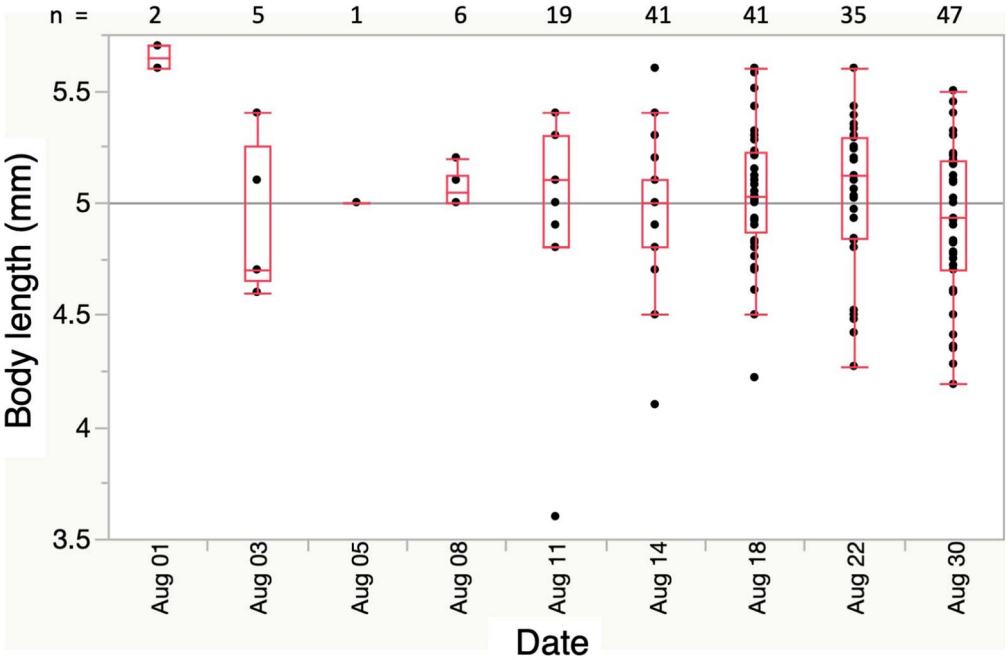

Figure 5.—Body length in adult males collected at Maruyama from August 1 to 30, 1984. Dots are values measured and each box plot consists of a box that represents interquartile range with a median bar and whiskers that represent the range (maximum and minimum values, dots outside the range are outliers). Numerals at the top represent sample size and a horizontal line starting at 5 mm of the Y axis denotes overall mean. No difference was detected among males collected on different dates (Kruskal-Wallis test, $P > 0.05$).

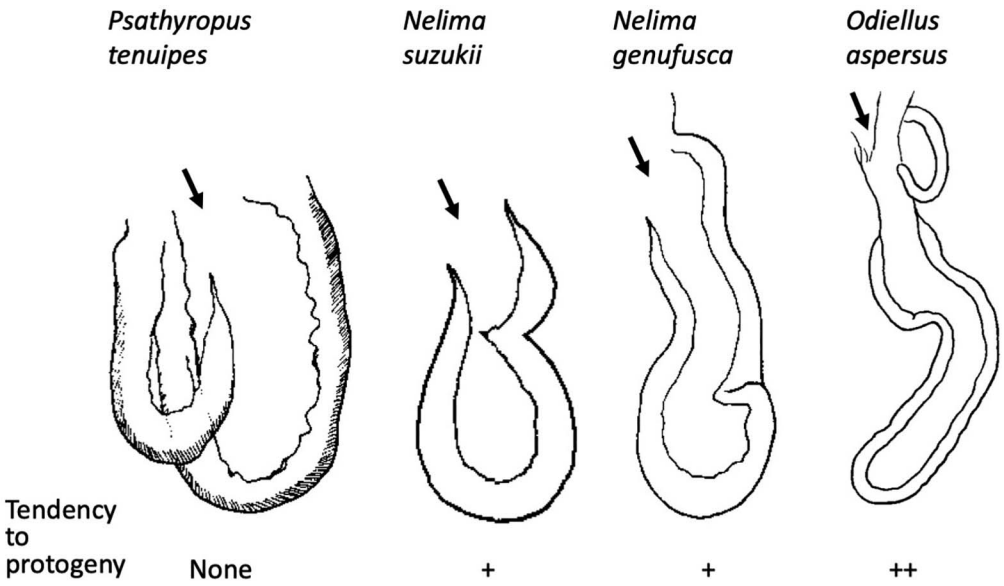

Figure 6.—Spermatheca in female ovipositor of some Eupnoi species found in Hokkaido (compiled from Suzuki & Tsurusaki 1983) and tendency to protogyny. Arrows indicate entrance (and exit) of sperm.

In conclusion, having an elongated spermathecae whose opening is narrow and hence may promote last male's sperm precedence in fertilization is the sole working hypothesis explaining protogyny in *Odiellus aspersus* at present.

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LITERATURE CITED

Austad SN. 1984. Evolution of sperm priority patterns in spiders. Pp. 223–249. *In* Sperm Competition and the Evolution of Animal Mating Systems (Smith RL ed.). Academic Press, Orlando.

Brown T, Tsurusaki N, Burns M. 2020. Genomic determination of reproductive mode in facultatively parthenogenetic harvestmen. *Journal of Heredity* 2021:34–44.

Degen T, Hoverstadt T, Mitesser O, Hölker F. 2015. High female survival promotes evolution of protogyny and sexual conflict. *PLoS ONE* 10(3):e0118354. https://doi.org/10.1371/journal.pone.0118354

Drummond B III. 1984. Multiple mating and sperm competition in the Lepidoptera. Pp. 291–370. *In* Sperm Competition and the Evolution of Animal Mating Systems. (Smith, RL (ed.) Academic Press, Orlando.

Fowler-Finn K, Boyer SL, Ikagawa R, Jeffries T, Kahn PC, Larsen EM et al. 2018. Variation in mating dynamics across five species of leiobunine harvestmen (Arachnida: Opliones). *Biology: Special Issue on the Evolution of Mating Choice* 7:1–15. https://doi.org/10.3390/biology7020036

Karachiwalla Z, deCarvalho T, Burns M. 2020. Spermathecal variation in temperate Opiliones. *Integrative and Comparative Biology*: icaa120. https://doi.org/10.1093/icb/icaa120

Machado G, Macías-Ordóñez R. 2007. Reproduction. Pp. 414–454. *In* The Harvestmen: The Biology of Opiliones (Pinto da Rocha R, Machado G, Giribet G eds.). Harvard University Press, Cambridge, Massachusetts.

Ridley M. 1983. The Explanation of Organic Diversity. The Comparative Method and Adaptations for Mating. Clarendon Press, Oxford.

SAS Institute 2015. JMP. Ver 12.0.1. SAS Institute, USA.

Suzuki S, Tsurusaki N. 1983. Opilionid fauna of Hokkaido and its adjacent areas. *Journal of Faculty of Science, Hokkaido University (VI, Zoology)* 23:195–243.

Thornhill R, Alcock J. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, Massachusetts.

Tsurusaki N. 1986. Parthenogenesis and geographic variation of sex ratio in two species of *Leiobunum* (Arachnida, Opiliones). *Zoological Science* 3:517–532.

Tsurusaki N. 2003. Phenology and biology of harvestmen in and near Sapporo, Hokkaido, Japan, with some taxonomical notes on *Nelima suzukii* n. sp. and allies (Arachnida: Opiliones). *Acta Arachnologica* 52:5–24.

Walker WF. 1980. Sperm utilization strategies in nonsocial insects. *American Naturalist* 115: 780–799.

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