THE FUNCTION OF LONG COPULATION
IN THE WOLF SPIDER *PARDOSA AGRESTIS*
(ARANEAE, LYCOSIDAE) INVESTIGATED
IN A CONTROLLED COPULATION DURATION EXPERIMENT

András Szirányi, Balázs Kiss, Ferenc Samu: Plant Protection Institute of the Hungarian Academy of Sciences, P.O. Box 102. Budapest, H-1525 Hungary. E-mail: samu@julia-nki.hu

Wolfgang Harand: Bundesamt und Forschungszentrum für Landwirtschaft, Vienna, Austria.

ABSTRACT.   Copulation duration varies greatly in wolf spider species, ranging from a few seconds to several hours. In *Pardosa agrestis* (Araneae, Lycosidae), the most common ground dwelling spider in Central European fields, copulation typically takes more than two hours. Since long copulation is likely to entail certain costs, we address the question, ''what is the function of long copulations?'' We investigated the consequences of lengthy copulation in an experimental situation, where copulations either ended spontaneously, or were interrupted after 10 min, 40 min or 90 min. There was no difference in the number of offspring per female when treatments were compared and we conclude that ten minutes of copulation was sufficient to fertilize all the eggs of a female. Long copulations should therefore have other functions than securing the necessary amount of sperm for fertilization. We also found that neither the time until egg production after copulation, nor offspring size was affected by copulation duration. This suggests the lack of transfer of ejaculatory substances that would either stimulate the egg sac formation or increase the size of the spiderlings. We propose that prolonged copulations gain meaning in multiple mating situations and should play a role in sperm competition or other forms of sexual selection. The extra time may be used for copulatory courtship, or for the transfer of surplus sperm or other substances to manipulate the female's willingness to copulate with other males, or to use sperm from them. These hypotheses remain to be tested in multiple mating experiments.

Keywords: Sperm transfer, copulation duration, copulation pattern, sexual selection, wolf spider

Copulation time varies largely in spiders even between closely related species. In the family of wolf spiders many species copulate for just a few minutes, while others copulate for hours. In a survey 30 species of wolf spiders, Stratton et al. (1996) noted that *Arctosa littoralis* (Hentz 1844) copulated for the shortest time, 18 seconds, while *Schizocosa saltatrix* (Hentz 1844) represented the opposite extreme with more than 8 hours. Copulation duration may vary widely even among species within the same genus, e.g. a 20 fold difference was found between the shortest and longest copulating *Hogna* spp. (Stratton et al. 1996). Our own observations on Old World lycosid species showed similar variable patterns. In a pilot study, *Pardosa hortensis* (Thorell 1872) copulated for 20–30 minutes, while *Pardosa agrestis* (Westring 1861) took

on average six times longer to copulate (A. Szirányi unpubl. data).

Given the examples for both short and long copulations, the question arises: what is the function of long copulation duration in wolf spiders, and in particular in the wolf spider, *Pardosa agrestis*? We chose to study *P. agrestis*, because it typically exhibits long copulation, and as the most abundant agrobiont spider in Central European arable fields (Kiss & Samu 2000; Samu & Szinetár 2002), it is the primary model species of our research group. The species builds no retreat and it hunts actively on the ground during the day (Samu et al. 2003). *Pardosa agrestis* has two reproductive peaks, with mating periods mostly in May and in July-early August (Samu et al. 1998). So far, we could not establish whether females remate in nature, but they do so in the labo-

ratory (Kiss 2003). Egg sacs are produced 2–3 weeks after copulation, and they are carried by the female for an average of 3 weeks until hatching.

The main function of copulation is sperm transfer, which may take a long or a short time. Long copulation duration might be necessary for complete fertilization, if sperm transfer is slow. Slow sperm transfer could be a common phylogenetic constraint, however in a taxonomic group in which copulation duration varies widely, this can be ruled out. On the other hand, long copulation has to be evolutionarily maintained, because it is likely to be costly. The time spent copulating is energetically demanding (Watson & Lighton 1994), and it entails a loss of opportunity to copulate with other partners or to forage. In species in which copulation takes place without hiding in a refuge, like in *P. agrestis*, an elevated predation risk can also be expected (Krupa & Sih 1998). Long copulation might also expose the spiders to parasite infection (Scheffer 1992). Thus, lacking a phylogenetic explanation, and considering possible costs, we should look for the adaptive value of prolonged mating.

To find the possible adaptive value of prolonged copulation in *P. agrestis* we created a hypothesis framework, and tested it in single mating experiments where the copulations were interrupted to ensure predefined duration. Three different scenarios of time-use were constructed (Fig. 1). From each scenario, specific predictions can be formulated and tested.

We consider hypothesis A to be the null-scenario, in which sperm is transferred throughout the entire copulation time and it is all used for fertilization. In this scenario sperm transfer is insufficient if copulation time is limited which prevents the fertilization of all ova. Indeed, copulation duration is positively associated with sperm transfer in a number of arthropods (Dickinson 1986; Arnqvist & Danielsson 1999; Stålhandske 2001), but in other cases, like in the spider *Micrathena gracilis* (Walckenaer 1805), this relationship does not exist (Bukowski & Christenson 1997). A prediction from Hypothesis A is that shorter than natural copulation time would result in a reduced number of fertilized eggs and offspring. In the following hypotheses (B and C) the sperm transfer rate

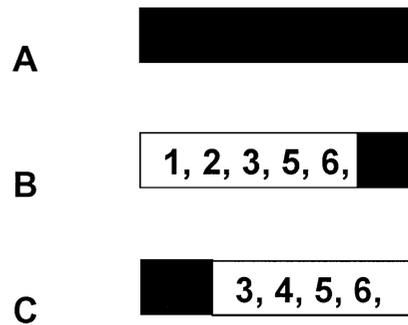## Hypothesis: Copulation pattern:

Figure 1.—Hypotheses concerning copulation pattern. There are two considered components to the pattern: 1. (shaded areas) periods when sperm used for fertilization is transferred; 2. (empty areas) periods when either no sperm is transferred or no such sperm that would be used for fertilization (= non-fertilizing period). Numbers indicate some possible functions of the no-transfer/non-fertilizing period: 1. removing earlier male sperm and/or plug; 2. assessing female virginity; 3. in-copula courtship; 4. transferring extra sperm; 5. transferring materials to accelerate oogenesis; 6. transferring nutritive materials (nuptial gift). See details in text; for further possible functions see Eberhard (1996).

does not limit fertilization, but rather the prolonged copulation duration is maintained by other factors.

With hypothesis B we propose the scenario that the sperm transfer is timed for the end of the copulation. In that case, the first part of the copulation then serves other purposes, such as copulatory courtship. Just as courtship influences female choice, copulatory courtship can influence female choice regarding postcopulation events. In spiders, sperm transfer and fertilization are well separated in time, and during the period between transfer and fertilization females may manipulate which male's sperm is used for fertilization via "cryptic female choice" (Watson & Lighton 1994; Eberhard 1997; Schäfer & Uhl 2002). In Linyphiidae the first part of copulation, the phase without sperm transfer, is often referred to as pseudo-copulation (Helsdingen 1965). Males are able to distinguish virgin from non-virgin females during courtship and pseudo-copulation (Robinson 1982; Suter 1990). This phase of mating can also be used to remove the sperm (Schäfer & Uhl 2002) placed by a pre-
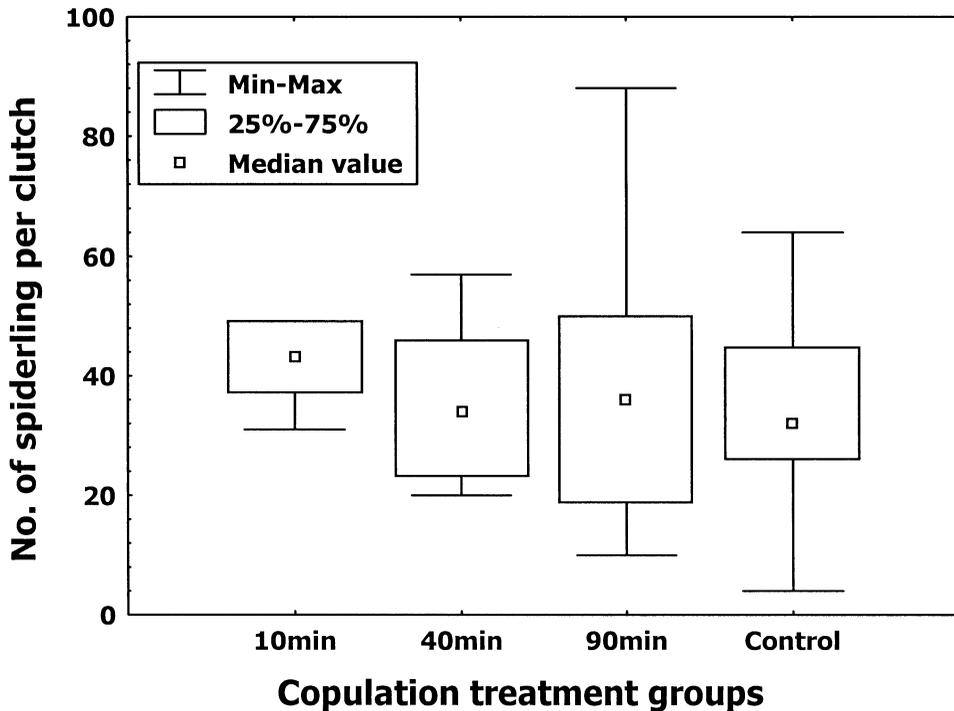
Figure 2.—The number of spiderlings in a clutch as a function of copulation length.

vious mate in the female's genital tract. From Hypothesis B we can predict that copulation interrupted early will result in no sperm transfer, and consequently in no hatched offspring from the egg sacs later.

In Hypothesis C the volume of sperm necessary to fertilize the eggs is transferred at the beginning of copulation and the rest of the time is used for other activities. The remaining copulatory time can, similar to Hypothesis B, serve the purpose of copulatory courtship, or it may simply engage the female long enough, so that competing mates will have reduced chances of copulation (mate guarding). Another possibility is that these activities decrease female receptiveness to the sexual advances of other males (Eberhard 1996). Having transferred the necessary amount of sperm to fertilize all eggs, sperm transfer might continue. Surplus sperm might be advantageous if females mate multiple times, because then a greater volume of sperm can be used in numerical sperm competition (Elgar 1998). In some species after sperm transmission, males create mating plugs in the genital opening of the female to prevent further copulations (Masumoto 1993; Knoflach 1998). Males may

transfer substances to the female that facilitate rapid oviposition, thus leaving less time for the female to meet and mate with competing males prior to egg deposition (Yamaoka & Hirao 1977). Nutritive substances might also be transferred to the female genital tracts (Suter & Parkhill 1990), which increase offspring size, thus increasing parental fitness. Hypothesis C predicts that in a single copulation, the female's reproductive output should remain unchanged after the first part of copulation. On the other hand, the various possible functions of the remaining copulation time generate additional predictions for offspring size and period until oviposition.

We interrupted copulations after three different time intervals to distinguish between the above hypotheses. Here we report the relationship between the artificially set copulation duration and reproductive output. These experiments show which of the originally proposed hypotheses are rejected or supported and cast some light on the function of various phases of copulation.

METHODS

The experiment was conducted from April to September, 1998 at the Plant Protection In-

stitute of the Hungarian Academy of Sciences (near Budapest). *Pardosa* individuals were hand-collected in juvenile or subadult stages in April to ensure virgin adults for the experiment. Animals were kept separately in the laboratory, where they were reared to adulthood under standard conditions (25 °C, long daylight (L:D = 16:8), and *Drosophila melanogaster* ad libitum was provided as food). Basic rearing conditions are explained in Kiss & Samu (2002).

Interrupted copulation experiment.— Following maturation, adult males and females were divided randomly into four groups. Pairs from each group were put together in 17 cm diameter Petri dishes, and the occurrence of copulation was monitored. In the first three treatment groups copulation was interrupted after 10 min ($n = 16$), 40 min ($n = 22$), and 90 min ($n = 16$) respectively. The pairs in the fourth group (Control) were left undisturbed until they finished copulation ($n = 20$). To establish the distribution of uninterrupted copulation duration, additional observations on copulation length were performed ($n = 42$), in which the reproductive consequences were not observed.

After copulation, females were kept in the laboratory under the conditions presented above. We recorded the time between copulation and oviposition (in some cases, exact time of oviposition could not be recorded, which resulted in smaller sample sizes for that variable: $n_{10min} = 9$, $n_{40min} = 7$, $n_{90min} = 7$, $n_{control} = 8$); whether females abandoned or consumed their egg sac, and whether the hatching was successful. We calculated the ratio of abandoned egg sacs as the number of egg sacs abandoned in an interruption treatment / all egg sacs produced in the given treatment. We monitored mothers with egg sacs for hatching daily. If hatching was successful we counted the number of offspring (thus offspring number counts are based only on successful hatches), and placed a sample of 10 spiderlings into 70% ethanol. Later we estimated the prosomal area (length$_{max}$ × width$_{max}$) on this random sample of ten spiderlings of each brood using scaled digital pictures. For the size measurement, we chose to measure the prosoma because it is less prone to the current feeding status (e.g. cannibalizing a littermate) of the spiderling. Voucher specimens were deposited

in the public collection of the Plant Protection Institute, Hungarian Academy of Sciences.

RESULTS

The duration of spontaneously ended (uninterrupted) copulation events was over 2.5 hours (mean = 165.7 min; S.D. = 53.6; range = 90–319 min). Interruption of the copulation did not affect the number of offspring from hatched egg sacs (Fig. 2; ANOVA: $F_{3, 40} = 0.46$, $P = 0.71$; homogeneity of variances assumption tested by Levene's Test: $F_{3, 40} = 2.13$, $P = 0.11$). The prosomal area of the offspring did not differ significantly between copulation duration treatments with the effect of mothers nested within treatment (ANOVA, main treatment effect: $F_{3, 478} = 0.35$, $P = 0.79$). However, the effect of mothers on offspring size was highly significant (ANOVA, effect of mothers nested within treatment: $F_{38, 478} = 6.11$, $P < 0.0001$). The time between copulation and egg sac production was not significantly different among the treatments (overall mean = 20.6 days, S.D. = 3.88, ANOVA: $F_{3, 27} = 0.10$, $P = 0.96$). Egg sac abandonment, on the other hand, occurred unevenly among the treatments (test of homogeneity: $\chi^2 = 8.62$; d.f. = 73, $P = 0.035$). As Fig. 3 illustrates, abandonment ratio was particularly high (0.56) in the 10 min interruption group, significantly higher than in the other treatments (10 minutes vs. other treatments lumped, Fisher's Exact Test: $n = 74$, $P = 0.01$). Among the longer than 10 minutes copulation treatments and the control group, the abandonment ratio was equally low (on average 0.2, test of homogeneity: $\chi^2 = 1.01$; d.f. = 57, $P = 0.6$).

DISCUSSION

In the present study, we wanted to establish the pattern of sperm transfer during the long copulation of *P. agrestis*. Our first hypothesis, Hypothesis A (Fig. 1), was that copulation takes longer because sperm transfer rate is slow. This hypothesis can be rejected, because the interruption experiment demonstrated that sperm transferred even in the first 10 minutes of copulation can be sufficient to fertilize all eggs of a female; offspring numbers were independent of copulation treatments. These results also cause Hypothesis B to be rejected, because this hypothesis predicted zero reproductive output for short copulation treatments.
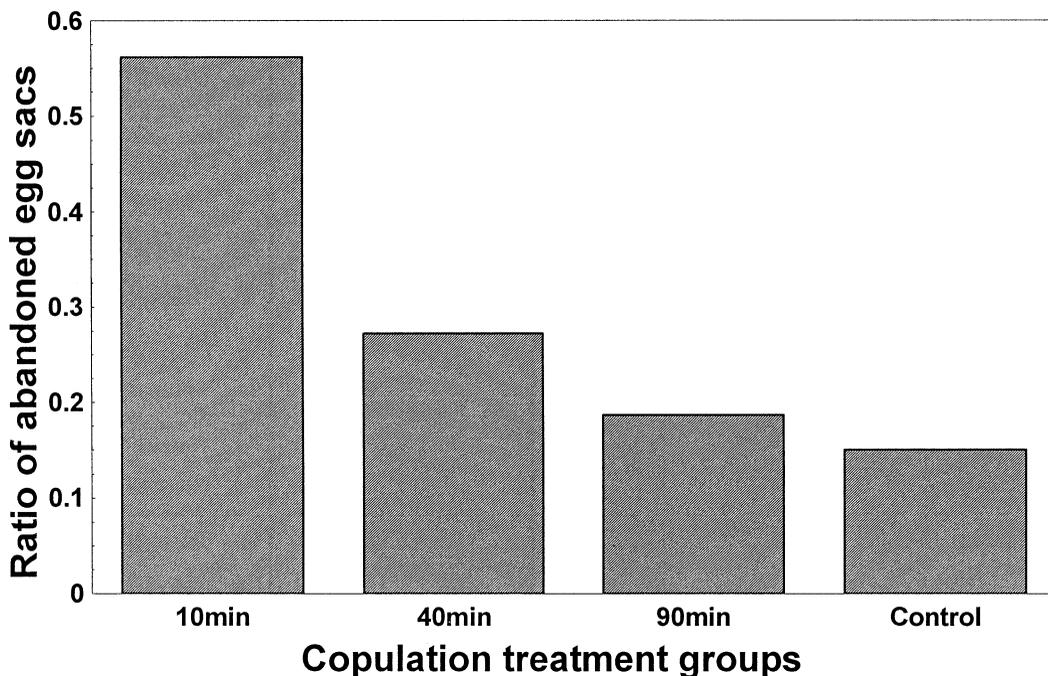
Figure 3.—The ratio of abandoned egg sacs in the copulation duration treatments.

The interruption results are consistent only with Hypothesis C, which proposes a copulation pattern in which the transfer of sperm needed to fertilize the eggs takes place within a short period at the beginning of the copulation.

However, egg sac abandonment was significantly more frequent in the 10 min copulation treatment than in any of the other treatments. Since egg sacs are abandoned when they are sterile (Kiss 2003), this suggests that there is variability in the first 10 minutes of mating. That is, in some cases, during the first 10 minutes enough sperm was transferred to fertilize all eggs of the future egg sac, while in other cases, no sperm was transferred and the egg sacs were sterile. We can speculate that this can happen if the event of sperm transfer is fast, even compared to the 10 min time scale. Sperm transfer seemed to occur with c. 50% probability during the first 10 min, and with near certainty during the first 40 min of the copulation. Since we had no direct observation on the ratio of sterile and fertilized eggs in the abandoned egg sacs, we can only infer from previous observations that they were likely to be sterile. We note a 20% baseline abandonment which occurred in all longer in-terruption treatments, including the control, and which could be either a natural phenomenon, or an artifact caused by the experimental situation. A similar phenomenon has been described in the salticid *Phidippus johnsoni* (Peckham & Peckham 1883), in which copulation duration also had an all-or-none effect on fertility, with short copulations more frequently resulting in the 'none' outcome, and long copulations more frequently resulting in a fully fertilized clutch (an 'all' outcome), whereas no half-sized clutches occurring (Jackson 1980). Jackson (1980) did not provide an alternative explanation to the quick release of a large amount of sperm, with an increasing probability over time.

Thus, the basic copulation pattern of *P. agrestis* corresponds to Hypothesis C: rapid transfer of an amount of sperm that is enough to fertilize all eggs takes place at some point during the first part of the copulation, while the final phase, which is the longer portion of time spent in copula, does not serve the direct purpose of transferring sperm to fertilize the eggs. Given this pattern, the question remains concerning the function of the final phase of copulation. In Fig.1 we list a number of such possible functions. Of these, some can be ex-

cluded based on the present experiment. Since spiderlings were of equal size irrespective of copulation duration, there is no evidence that the male transferred any nutrients during the final phase of the copulation in order to increase parental fitness through offspring size (Suter & Parkhill 1990; Walker et al. 2003). The transfer of materials that could accelerate oogenesis can also be ruled out on the basis of similar egg sac formation times across the copulation duration treatments. We did not find any evidence for the presence of a mating plug. Although, we could rule out a number of proposed functions for the final phase of the copulation, several other functions still remain possible. If surplus sperm are transferred, the sperm could be used to out-compete the sperm of other possible mates. The final phase of copulation may also serve as copulatory courtship or as mate guarding. These functions are not mutually exclusive, and any combination of them is possible.

To summarize, the copulation pattern established for *P. agrestis* seems to be paradoxical in a single mating situation, because much shorter copulations are sufficient to result in full fertilization. Any possible function of a long copulation that was not experimentally excluded here seems to be related to male-male competition and/or female choice, and gains meaning only in a multiple mating situation. Therefore, we tentatively conclude that prolonged copulation is a sexually selected trait in *P. agrestis*. To establish the functional details and exact adaptive advantages, multiple mating studies are needed.

ACKNOWLEDGMENTS

LITERATURE CITED

Arnqvist, G. & I. Danielsson. 1999. Postmating sexual selection: the effects of male body size and recovery period on paternity and egg production rate in a water strider. Behavioral Ecology 10:358–365.

Bukowski, T.C. & T.E. Christenson. 1997. Determinants of sperm release and storage in a spiny orbweaving spider. Animal Behaviour 53:381–395.

Dickinson, J.L. 1986. Prolonged mating in the milkweed leaf beetle *Labidomera clivicollis* (Coleoptera: Chrysomelidae): a test of ''sperm-loading'' hypothesis. Behavioral Ecology & Sociobiology 18:331–338.

Eberhard, W.G. 1996. Female Control: Sexual Selection by Cryptic Female Choice. Princeton University Press, Princeton.

Eberhard, W.G. 1997. Sexual selection by cryptic female choice in insects and arachnids. Pp. 32–57. *In* The Evolution of Mating Systems in Insects and Arachnids. (J. C. Choe & B. J. Crespi, eds.). Cambridge University Press, Cambridge.

Elgar, M.A. 1998. Sperm competition and sexual selection in spiders and other arachnids. Pp. 307–339. *In* Sperm Competition and Sexual Selection. (T. R. Birkhead & A. P. Moller, eds.). Academic Press, New York.

Jackson, R.R. 1980. The mating strategy of *Phidippus johnsoni* (Araneae, Salticidae): II. Sperm competition and the function of copulation. Journal of Arachnology 8:217–240.

Kiss, B. & F. Samu. 2000. Evaluation of population densities of the common wolf spider *Pardosa agrestis* (Araneae: Lycosidae) in Hungarian alfalfa fields using mark-recapture. European Journal of Entomology 97:191–195.

Kiss, B. & F. Samu. 2002. Comparison of autumn and winter development of two wolf spider species (Pardosa, Lycosidae, Araneae) having different life history patterns. The Journal of Arachnology 30:409–415.

Kiss, B. 2003. The autecology of *Pardosa agrestis* (Westring, 1864). PhD dissertation, Veszprém University (Hungary) Pp. 1–115. (In Hungarian)

Knoflach, B. 1998. Mating in *Theridion varians* Hahn and related species (Araneae: Theridiidae). Journal of Natural History 32:545–604.

Krupa, J.J. & A. Sih. 1998. Fishing spiders, green sunfish, and a stream-dwelling water strider: male-female conflict and prey responses to single versus multiple predator environments. Oecologia 117:258–265.

Masumoto, T. 1993. The effect of the copulatory plug in the funnel-web spider, *Agelena limbata* (Araneae, Agelenidae). The Journal of Arachnology 21:55–59.

Robinson, M.H. 1982. Courtship and mating behavior in spiders. Annual Review of Entomology 27:1–20.

Samu, F., J. Németh, F. Tóth, É. Szita, B. Kiss & Cs. Szinetár. 1998. Are two cohorts responsible for bimodal life history pattern in the wolf spider

Pardosa agrestis in Hungary? Proceedings 17th European Colloquium of Arachnology, Edinburgh. Pp. 215–221.

Samu, F. & Cs. Szinetár. 2002. On the nature of agrobiont spiders. Journal of Arachnology 30: 389–402.

Samu, F., A. Szirányi & B. Kiss. 2003. Foraging in agricultural fields: local 'sit-and-move' strategy scales up to risk-averse habitat use in a wolf spider. Animal Behaviour 66:939–947.

Schäfer, M.A. & G. Uhl. 2002. Determinants of paternity success in the spider *Pholcus phalangioides* (Pholcidae: Araneae): the role of male and female mating behaviour. Behavioral Ecology and Sociobiology 51:368–377.

Scheffer, S.J. 1992. Transfer of a larval mantispid during copulation of its spider host. Journal of Insect Behavior 5:797–800.

Stålhandske, P. 2001. Nuptial gift in the spider *Pisaura mirabilis* maintained by sexual selection. Behavioral Ecology 12:691–697.

Stratton, G.E., E.A. Hebets, P.R. Miller & G.L. Miller. 1996. Pattern and duration of copulation in wolf spiders (Araneae, Lycosidae). Journal of Arachnology 24:186–200.

Suter, R.B. 1990. Courtship and the assessment of virginity by male bowl and doily spiders. Animal Behaviour 39:307–313.

Suter, R.B. & V.S. Parkhill. 1990. Fitness consequences of prolonged copulation in the bowl and doily spider. Behavioral Ecolology & Sociobiology 26:369–373.

Walker, S.E., A.L. Rypstra & S.D. Marshall. 2003. The relationship between offspring size and performance in the wolf spider *Hogna helluo* (Araneae: Lycosidae). Evolutionary Ecology Research 5:19–28.

Watson, P.J. & J.R.B. Lighton. 1994. Sexual selection and the energetics of copulatory courtship in the sierra dome spider, *Linyphia litigiosa*. Animal Behaviour 48:615–626.

Yamaoka, K. & T. Hirao. 1977. Stimulation of virginal oviposition by male factor and its effect on spontaneous nervous activity in *Bombyx mori*. Journal of Insect Physiology 23:57–63.