

Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence

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Summary

One male strategy to prevent female re-mating is post-copulatory mate guarding. In the pholcid spider *Holocnemus pluchei* last male's fertilization success is around 74% and females remain receptive after copulation. It is, thus, reasonable to suppose that males should engage in post-copulatory mate guarding. Chronologically, the present study focused on the following aspects: (1) to determine if male permanence near females corresponds to mate guarding. For this, a second male (intruder) was introduced. Time of permanence, distance and behaviour of residents did not change whether or not an intruder was present; (2) to investigate the duration of mate guarding and male distance to the female in a time series intervals after copulation. Males remained close to females during 14 h keeping a distance of less than 15 cm; (3) to evaluate whether guarding duration is influenced by female sexual receptivity. We found that 24 h after the first copulation, when the resident was placed again next to the female, he tried to re-mate; and (4) to examine differences in paternity in relation to whether or not the resident exerted guarding. P2 was higher when second males copulated again within the first 6 h compared to 24 h after the first copulation.

Keywords: mate guarding, sperm competition, sexual behaviour, decision making, Pholcidae.

Introduction

In many species, sperm competition between different males for fertilizing a set of ova has promoted the evolution of different mating strategies in both

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sexes (Parker, 1970). Like many other animals, spiders show great variety of mechanisms that evolved as an answer to polyandry (Arnqvist & Nilsson, 2000). For example, it is assumed that the shape of female sperm storing organs influences sperm priority (Austad, 1984; Eberhard et al., 1993; Elgar, 1998). In this respect, spiders are divided in two groups according to female sperm storage organs (Coddington & Levi, 1991) and male fertilization success will depend on male mating order (Kaster & Jakob, 1997). In haplogyne species there is one single duct for insemination and fertilization and, thus, sperm must enter and leave through the same duct (Foelix, 1982). This results in last male's sperm use during fertilization (Austad, 1984). On the contrary, in entelegyne species, insemination and fertilization ducts are separated so that sperm enters through one duct and leaves through another (Foelix, 1982). This results in first male's fertilization advantage (Austad, 1984).

As indicated by Alcock (1994), males can reduce the probability of female re-mating, and hence, reduce the risk of direct sperm competition, by using certain behaviours: (i) Performing longer matings and maintaining genital contact when sperm transfer has finished. In species performing this strategy, the amount of sperm transfer varies within and between species (Elgar, 1995). (ii) Placing a genital plug after insemination in the female genital operculum to make the access to the female genital tract more difficult to other males. (iii) Maintaining of physical contact in which males hold the female even when the genitalia have already disengaged (contact guarding). (iii) Performing non-contact mate guarding by monitoring males that stay close to females with no physical contact. According to the sperm use during fertilization and male behaviour described above, it is expected that males in entelegyne species guard females before copulation (pre-copulatory mate guarding) (Christenson & Goist, 1979; Austad, 1982) while in haplogyne species, males would perform such guarding after copulation (post-copulatory mate guarding) (Elgar, 1998; Uhl, 1998).

A marked reduction in female sexual receptivity after mating is common in some families of spiders like Pholcidae (Uhl et al., 1995), Anyphaenidae (Huber, 1995), Agelenidae (Singer & Riechert, 1995), Araneidae (Prenter et al., 1994; Elgar & Bathgate, 1996), Linyphiidae (Stumpf, 1990) and Salticidae (Jackson, 1980). Nevertheless such reduction could not be immediate which explains why males guard females for some time after copulation (Elgar, 1998). Then, mechanisms used by males to ensure fertilization success in

sperm competition depend, at least in part, on sperm priority patterns (Elgar, 1998). For example, in *Holocnemus pluchei* (Pholcidae) the last male's fertilization success (P2) is 74% (Kaster & Jakob, 1997) and the female remains receptive after copulation. It is, thus, reasonable to expect in this species that males should engage in post-copulatory mate guarding.

In the present work we tested whether *H. pluchei* males remain close to the females after mating, (1) we first set to investigate whether the male remains close to the female as a way of post-copulatory mate guarding, thus preventing other copulations and increasing male fertilization success. Since our findings indicated that this was the case, we then evaluated functional aspects of guarding: (2) the duration of guarding and male–female distance (i.e., distance between both sexes), and if both depend on female mating status. Due to the existence of last male sperm precedence, we expected no differences in the duration of guarding by males and distance to females; (3) we examined if mate guarding duration is influenced by changes in female sexual receptivity (i.e., re-mating pattern). In addition, we also use the reproductive status of two females to see if this is related to the time that females remain receptive. We expected that males would guard females they had mated with as long as females remain receptive. Finally, (4) we evaluated if males showed differences in paternity according to presence or absence of post-copulatory mate guarding. Our prediction is that males that guarded females they mated with would have a higher fertilization success than those who did not guard.

Our work is novel for two reasons: (i) it confirms for the first time the existence of post-copulatory mate guarding in a haplogyne spider, which is linked to changes in female receptivity and differential rates of paternity for mating males; and (ii) it provides behavioural elements to understand the mating strategies males may practice.

Materials and methods

H. pluchei (Pholcidae) is a spider native of central Europe (Porter & Jakob, 1990; Jakob, 1991). It was introduced in South America (Huber, 2000) and now is very common in central Argentina (Laborda & Simó, 2008). This species inhabits both urban and natural areas. In homes and buildings tends to prefer humid environments such as bathrooms, storehouse or warehouses.

This facultatively group-living spider builds irregular webs that contain from one to 15 spiders of different ages, sizes, and sex (Jakob, 1991). However, males move between the webs, so the chances of multiple matings and sperm competition are abundant (Jakob, 1991). It is common to observe males near females. To copulate, the male introduces both palp copulatory organs in the female's genital opening. In this species, these copulatory organs are loaded with sperm before males seek females (A.V.P., pers. obs.). Like all species of the family, *H. pluchei* is a haplogyne spider, and females have no special sperm storage organs, but the sperm is deposited in a uterus externus (Huber, 1995). Females are polyandric, copulating with several males before oviposition, last male sperm precedence and no apparent post-copulatory male mate guarding (Kaster & Jakob, 1997). In laboratory conditions, adults live up to one year (Peretti & Dutto, unpubl. data).

Collecting and rearing

Penultimate females (55) and 118 adult males were collected in the 'Ciudad Universitaria', Córdoba, Argentina, between September 2007 and April 2009. Each individual was placed in a plastic container (8 × 15 cm height), covered inside with paper (to provide surface for web building) and water, in a photoperiod of 12/12 h. Penultimate juvenile females were maintained to obtain virgin females for experimentation. Males were used 15 days after their capture to reduce effects of potential differences in mating histories. Juveniles and adults were fed on a weekly basis with *Drosophila melanogaster* adults and *Tenebrio monitor* larvae.

Observation of behavioural sequences

All observations were performed in boxes (100 × 60 cm). The edges of the observation boxes were plastic while the inner divisions had woven wire. The copulation took place in a closed compartment. After copulation ended this compartment was opened allowing the male to move freely, in the case he chose not to guard the female he had already copulated with. On the base of the box, a grid with 60 areas (10 × 6 cm each area) was put to estimate the distance between individuals after copulation. Each male was used only once. Each female was used in two reproductive conditions, as virgin and copulated, to determine if this characteristic influenced male guarding. Females were always placed in the box 24 h before males so that females were

able to build the web on which they would copulate. Male–female distance after copulation was estimated. Measurements were taken the day of copulation and the one following day in order to estimate the maximum time and mean distance of the male when he stayed close to the female.

Experimental design

1. Does post-copulatory guarding occur in this species?

To investigate whether males remain close to females as a way of post-copulatory mate guarding, the following experiments were carried out. Fifteen virgin females copulated once, and 10 min after copulation a second male was added to the couple (hereafter referred to as the intruder) to determine ($N = 15$): (i) if time and guarding distance of a recently-mated male (hereafter referred to as the resident) varied, (ii) if there were differences in re-mating frequency or in copulation attempts by resident males in the presence/absence of intruder males, (iii) the frequency of copulation of resident and intruder males, (iv) if the duration of intruder's copulations was different to that of resident's copulations, (v) the number of intruder's copulation attempts avoided by residents and (vi) if there were interactions among males. As differences in male size could affect mating success (Schäfer & Uhl, 2003), in this study males from similar size were used. Size similarity was judged from tibia–patella length which is good indicator of body size in pholcid spiders (Jakob, 1994; Huber, 1996) (mean \pm SD of residents: 12.265 ± 1.420 mm; of intruders: 13.806 ± 1.480 mm; t -test = -2.439 , $p = 0.988$). Males also showed no significant differences in body weight (mean \pm SD of residents: 0.018 ± 0.004 g; of intruders: 0.018 ± 0.003 g; t -test = -0.086 , $p = 0.533$). The criteria considered to define mate guarding were: (i) a close proximity of the male to the female after copulation; and (ii) presence of the resident male directly preventing or making copulation more difficult to the intruder male. To confirm the latter, we carried out experiments in which the resident male was removed from the experimental box after copulation ($N = 18$). From this manipulation we compared copulation duration of intruder (second mating) in presence vs. absence of the resident to determine if the duration of copulation of the intruder was influenced by the resident. These two sets of information (removal of resident males and copulation duration) would allow us concluding that guarding males prevent or male chances to mate by other males more difficult.

2. Duration of mate guarding and male–female distance

To determine behaviour and duration of mate guarding, the distance between male and female was registered during a series of time intervals after copulation both on the same day that copulation took place (10 min, 30 min, 1, 2, 3, 6, 8, 10, 12, 14, 18, 20, 24 h) and in successive day (26, 30, 32, 36, 40 and 48 h). With these data we estimated the time and mean distance between male and female.

Females of different reproductive status were used to test if this influenced male decision to perform mate guarding. For this a group of virgin females that mated once ($N = 22$) and then the same females were re-mate ($N = 18$) at 3 days by other males.

3. Relationship between male guarding and female sexual receptivity

To determine if mate guarding duration is influenced by changes in female sexual receptivity, a male was placed back close to the same female he had already mated with. Then we recorded willingness to copulate in females and males.

Females of different reproductive status were used to determine if female reproductive status influenced the male decision to re-mate. For this we used a group of virgin females that mated once ($N = 15$) and which were consequently re-mated ($N = 15$) 3 days after the first mating and with different males from that first mating.

For females that rejected a new mating, a different male was offered to see if they were still willing to re-mate. In those cases where males did not court females, a virgin female was offered to assess if males were nevertheless willing to re-mate.

4. Relationship between post-copulatory guarding and paternity chances

To evaluate if males showed differences in paternity according to presence or absence of post-copulatory mate guarding, two groups of virgin females were used. In the first group ($N = 18$) after the copulation was finished, the male was removed. Then after a 2–6 h period (Schäfer & Uhl, 2002) a second male was placed. In the second group ($N = 16$), the male was removed after copulation was finished and the second male was put after 24 h. In both groups the second mating male was also removed after the mating ended. These different times of re-mating were related to when mate guarding is still

present (2–6 h) or not anymore (24 h). The second male paternity chances were determined. For this the number of fertilized eggs was calculated for the first and second male (P_1 and P_2 , respectively), using the sterile male technique. We used relative values for paternity success. For example, if 20 out of 40 eggs were fertilized by the last male, the success of this last male was 50%. Usually, 2% of eggs in this species are non-viable in non-experimental conditions (Kaster & Jakob, 1997). The sterile male technique consists of sterilizing one of the two males. Males were exposed to X-rays using a 2000 rad dose (Kaster & Jakob, 1997). This dose does not affect sperm competitive ability as it does not destroy sperm but produces mutations that result in the embryos' death at an early developmental stage. Moreover, a lower dose (i.e., 1000 rad) still gives viable eggs (Kaster & Jakob, 1997). In both treatments, half of the females mated with fertile males in a random fashion: first and second with irradiated males and vice versa. According to the last male sperm precedence documented for *H. pluchei* (74%) (Kaster & Jakob, 1997), it is expected that the number of eggs fertilized by second males in both treatments will be greater than those fertilized by the first males. Our prediction was that P_2 should be greater when second males copulated within the next 6 h following the first copulation. In this period females would have to be guarded by the first males with whom females have already copulated.

Statistics

The statistical packages SPSS 14.0 and NCSS 2007 were used for analyzing results. Continuous variables with normal distribution were analyzed with *t*-tests for independent or paired samples.

Variables that did not adjust to normality were analyzed using non parametric tests (Mann–Whitney *U*-test to independent samples and Wilcoxon signed-rank test for paired samples). Nominal values were analyzed with Binomial and Chi-square tests. Duration of events were recorded in minutes while distance was documented in cm. Results are indicated as mean \pm SD unless stated otherwise.

Results

1. Does post-copulatory mate guarding occur in this species?

In experiments where an intruder male was put near the couple after copulation, time of permanence of resident males close to females did not change

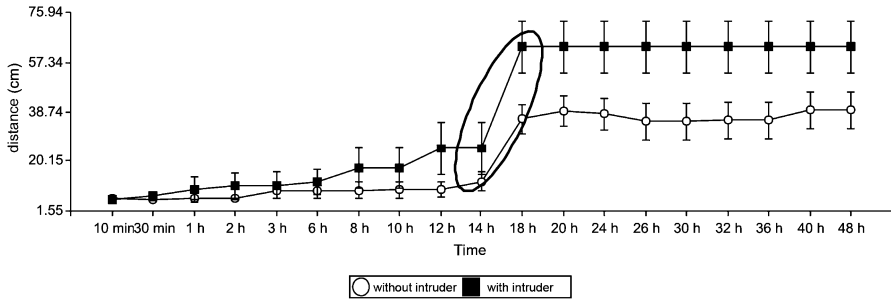


Figure 1. Distance of resident males from females after copulation on the same day and the 1 following days with presence and absence of intruder males. (○) Without intruder, (■) with intruder ($N = 15$). Mean guarding distance of resident males to females at 14 h (guarding period: Mann–Whitney U -test: $z = -0.434$, $p = 0.332$). Mean distance of resident males when guarding was finished at 18 h (Student’s t -test; $t = -2.618$; $p = 0.007$). The time and distance of guarding are emphasized.

compared to trials in absence of an intruder male, and resident males stayed close to females for 14 h. There was no difference in the distance kept by the resident male from the female during the guarding period in relation to whether the intruder was present (25.025 ± 33.981 cm) or absent (12.572 ± 16.928 cm; Mann–Whitney U -test: $z = -0.434$, $p = 0.332$). However, there were differences in distance between resident males and females when the guarding period ended: at 18 h, the distance between residents and females was larger when intruders were present (63.033 ± 33.010 cm) than when intruders were absent (36.173 ± 25.966 cm; t -test for independent samples = -2.618 , $p = 0.007$) (Figure 1). Residents interrupted guarding only in those cases where intruders mated with females (3 out of 15 cases; Binomial test: $p = 0.019$). Those males who remained close to females were the last males to copulate. There was no overt female behaviour during the period that a mating male remained close to her.

There was no difference in re-mating attempts by the resident male in the presence or absence of intruder (intruder present: 0.583 ± 0.790 ; absent: 0.583 ± 0.670 ; Mann–Whitney U -test: $z = -0.161$, $p = 0.436$). Nevertheless only 20% (3 out of 15 cases, Binomial test: $p = 0.019$) of the intruders achieved copulation in the presence of resident males, as residents did not allow intruders to copulate. Thus, 26 out of 35 copulation attempts (2.169 ± 1.947 per experiment) were prevented by residents, by placing themselves between the intruders and the females. While in this position, the resident male touched the intruder constantly with its first pair of legs

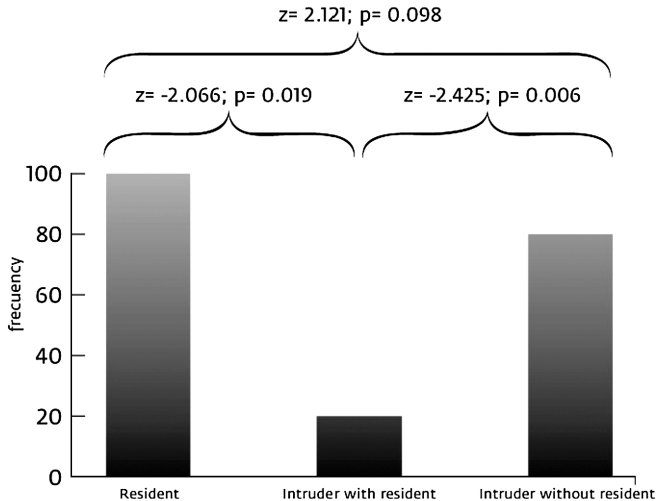


Figure 2. Copulation frequency of resident males with virgin females and recopulations of intruder males with those females, following the first 6 h of the first copula, in the presence of resident male and in its absence. Statistical results on the bars correspond to binomial tests.

and moved towards the intruder so that the latter retreated and moved away from the female. However, when residents were removed, intruders achieved copulation in 80% of the cases (14 out of 18 cases, Binomial test: $p = 0.098$; Figure 2).

We found significant differences in the duration of residents' copulation (first copulation) and that of intruders' copulation (second and last copulation). Copulations lasted longer with the resident (22.707 ± 9.434 min) than with the intruder (3.083 ± 8.649 min; Wilcoxon signed-rank test: $z = 3.059$, $p = 0.001$). One potential cause of such difference is that residents were constantly trying to interrupt intruder's copulations. Indeed, when residents were removed, intruder's copulations took longer (41.670 ± 29.298 min) than those of the resident's (22.713 ± 9.434 min; Wilcoxon signed-rank test: $z = -1.647$, $p = 0.043$). There were also differences in the duration of intruder's copulations in the presence-absence of resident males: when residents were present, copulations were shorter (3.083 ± 8.649 min) than when they were absent (41.673 ± 29.292 min; Mann-Whitney U -test: $z = -4.033$, $p < 0.001$; Figure 3). Regarding interactions between males, residents were more likely to start attack while intruders were more likely to withdraw (Fisher exact test: $p = 0.012$).

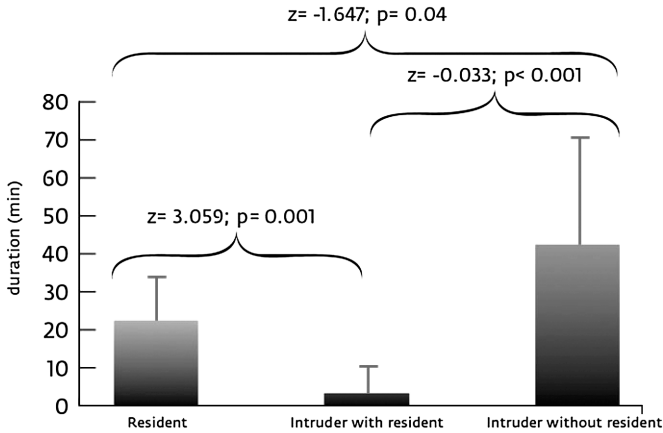


Figure 3. (A) Copulation duration of resident males with virgin females (first copulations) and recopulation of intruder males with those females (second and last copulations) in the presence of resident males (mean resident vs. mean intruder; Wilcoxon signed-rank test) and in the absence of resident males (mean resident vs. mean intruder; statistical results appearing above bars are from Wilcoxon signed-rank test). (B) Difference between intruder males' second copulations in the presence–absence of resident males (mean intruder with resident vs. mean intruder without resident; statistical results above bars correspond to Mann–Whitney *U*-test).

2. Duration of mate guarding and male–female distance

In copulations with virgin females and at 14 h, 65% of males stayed with their mates with a distance of 12.5 ± 16.932 cm. At 18 h, the male moved away 36.17 ± 25.964 cm from females (Wilcoxon signed-rank test: $z = -2.975$, $p = 0.001$). The remaining 35% stayed for a whole day or longer close to females. After inseminating copulated females, 90% of the males remained with females at a distance of 8 ± 2.712 cm. At 18 h, males moved away to 39.125 ± 20.323 cm from females (Wilcoxon signed-rank test: $z = -4.233$, $p < 0.0001$), while the remaining 10% stayed for one day or longer. In any case males moved away from females immediately after copulation, independently of the female reproductive status (Figure 4).

The decision of males to remain close to females was not affected by the mating status of the latter, nor the time they invested in staying close. In both groups males remain close to females for at least half a day.

3. Relationship between male guarding and female sexual receptivity

When males were placed back 24 h after the first copulation both, once copulated females (12 out of 15, Binomial test: $p = 0.999$) and twice

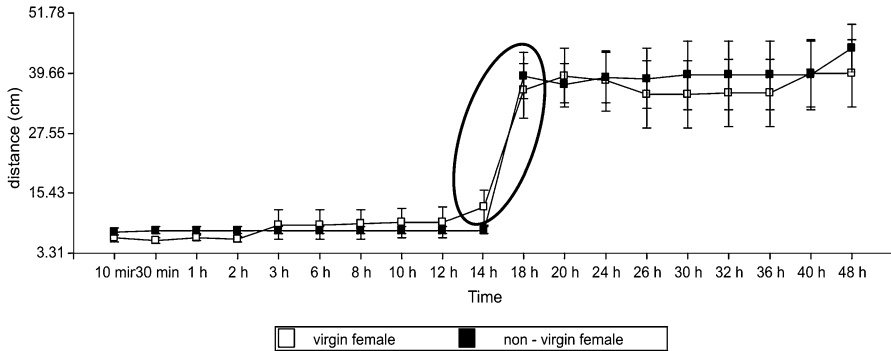


Figure 4. Male–female interdistance after copulation in different time intervals. (A) With once copulated females (mean 14 h vs. mean 18 h; Wilcoxon signed-rank test: $z = -2.975$, $p = 0.001$) ($N = 22$) and (B) with twice copulated females (mean 14 h vs. mean 18 h; Wilcoxon signed-rank test: $z = -4.233$, $p < 0.0001$). (○) Virgin female, (■) no-virgin female ($N = 18$). Significant differences in distances are emphasized.

copulated females (6 out of 15, Binomial test: $p = 0.303$) did not re-mate with these males. Females of both reproductive status that did not re-mate still did not do so when they were placed with different males. One new male was exposed per female, although the new males began to court females, these inseminated females always rejected them.

Eighty percent of males that were removed for 24 h after copulating with virgin females and were placed back, tried to re-mate (12 out of 15, Binomial test: $p = 0.098$). Similarly, 93% of males that copulated with non-virgin females also tried to re-mate (14 out of 15, Binomial test: $p = 0.997$). Female mating status was not related to the decision of males to re-mate when the latter were placed back with females they have mated with 24 h before (χ^2 test = 1.154, $p = 0.283$). Males, who did not try to court, did mate when another virgin female was placed. In these four cases copulation occurred.

4. Relationship between post-copulatory guarding and paternity chances

There was a significant difference in fertilization success between first and second males when the mating time interval was 2–6 h: the percentage of the eggs in the total egg sac fertilized by the first male (0.199 ± 0.112) was lower than that of the last male (0.81 ± 0.11 ; t -test for paired samples: $t = -5.583$, $p = 0.006$) ($N = 18$). Nevertheless when the interval between copulations was of 24 h no difference was found (first male: 0.533 ± 0.110 ; second male:

0.468 ± 0.110 ; *t*-test for paired samples: $t = 1.203$, $p = 0.122$) ($N = 16$). Thus, fertilization success of second males is higher if they copulated again within the first 6 h rather than 24 h after the first copulation.

Discussion

This study clearly demonstrates that males from *H. pluchei* perform post-copulatory mate guarding, staying close to females for approx. 14 h and at a distance of less than 15 cm. This male guarding strategy seems effective to impede competitors' access to recently-mated females for four reasons. First, when an intruder male was added to a couple that had just mated, intruder males were unable to copulate not because females were not receptive (as females accepted a new mating when resident males were removed), but because resident males prevented new matings. Second, copulations with intruder males were less frequent and shorter when resident males were present than when they were absent. Besides, the presence of an intruder close to the female does not modify guarding duration of the male that had first copulated. Also, it does not modify the latter male's behaviour, as re-mating frequency or re-mating attempts by resident males do not change whether or not the intruder is close. Third, resident males actively defended their mates by placing themselves between the intruder and the female. However, such defence can become physical if the intruder gains mating access. Finally, although duration of second copulations was longer than first copulations, if intruder males are attacked (residents dissuade intruders while copulating by touching with legs and vibrating the body, which causes movements of the web) then the duration of second copulations is just of a few minutes. In this case, the first male finally leaves while the last male takes place near the female.

Even though the presence of intruder males does not modify behaviour or distance of resident males from females during mate guarding, once post-copulatory mate guarding ends there is a tendency of resident males to stay away from the female when there are intruders, compared to when no intruders are present. One explanation is that resident males avoid confrontations with other males. Alternatively, males may move away from the female to avoid confrontation with her, although we never observed any aggression of females towards males. Intruder males that were able to copulate in the presence of resident males could do so because they were fast, as they approached

the female immediately and started copulating, giving the resident male no time to prevent copulation. Therefore, as expected for last male sperm precedence in haplogyne spiders (Austad, 1984; Elgar, 1998) and particularly in *H. pluchei* (Kaster & Jakob, 1997), males reduce female re-mating by using post-copulatory mate guarding, but if re-mating takes place then the duration of following copulations is much shorter than the first copulation and second copulation when the resident is absent. We, therefore, conclude that guarding indeed prevents other males from mating.

The male decision to guard does not depend on female mating status as females that mated once or twice were guarded for the same period of time. Such time coincides with the time females continue to be sexually receptive after copulation (e.g., 6 h becoming unreceptive after 24 h). Interestingly, we never recorded any overt female behaviour during this guarding period that may serve as a cue for mating males as for how long they should guard. However, cryptic clues such as chemical signals may also be involved which, nevertheless, have not been studied in this or other pholcids. The idea that males move away when females lower their receptivity is supported because as males were placed again with females after guarding time was over, most females refused to re-mate. Therefore, the duration of post-copulatory mate guarding seems adaptive, as it is adjusted to that time (i.e., up to 14 h) when females would be more likely to re-mate and the paternity benefits would be higher. It is important to remark that in our study the time intervals between the first and second mating were defined considering the time of post-copulatory mate guarding. In one group second copulation occurred six h after first copulation (period of mate guarding, $P_2 > P_1$), whereas in the other group second copulation occurred after 24 h (lack of mate guarding, $P_2 = P_1$). Related to this, Kaster & Jakob (1997) observed a last male sperm precedence of 74% in this species by using an interval of time between first and second copulations less than six h. One possible explanation for why the paternity of the second male is larger when females mate with an interval of 6 h (which is when the female is still guarded by the first male), may be related to male removal ability. Preliminary observations in this species indicate that the second male always removes previous male's sperm from the female genital opening and such removal occurs at the copulation onset (L.C. and A.V.P., unpubl. data).

The timeline at which males continue or leave their mate may represent a balance whose final outcome is maximization of reproductive success.

For example, males may decide what to do in terms of a trade-off between present and future mating opportunities (Alcock, 1994; Elgar, 1998). Other potential sources affecting the decision to guard are the energetic costs incurred by males while repelling other males (e.g., Bel-Venner & Venner, 2006). One future aspect to be uncovered is how *H. pluchei* males optimize such guarding according to mating opportunities and male-male competition.

Post-copulatory mate guarding in *H. pluchei* males agrees with the type of mate monitoring suggested by Alcock (1994). According to this author, males reduce the probability that females re-mate with other males, by preventing any physical contact between mates. Thus, although sperm precedence favours the last male, males that copulate first and perform guarding can fertilize a higher number of eggs than those who do not perform such behaviour (Alcock, 1994). For example in *Gasteracantha minax* (Araneidae) males defend females from other males after copulation, as females remain receptive. However, in this case, guarding continues until the next day when females no longer re-mate. This tactic is carried out to avoid insemination by other males (Elgar & Bathgate, 1996).

Kaster & Jakob (1997) reported that *H. pluchei* males do not perform post-copulatory mate guarding claiming that in natural conditions, population density is so high that this strategy is energetically too costly. Nevertheless, in the field males do perform post-copulatory mate guarding (A.V.P. & L.C.-R., unpubl. data) even in the absence of rival males. Of course, there may be geographical differences in the presence or absence in, or in intensity of mate guarding as documented for other taxa (e.g., Carroll & Corneli, 1995; Shine, 2003).

Mate guarding has been studied in a variety of spiders (Jackson, 1986; Elgar, 1998), especially in males of entelegyne species, in which mate guarding is pre-copulatory (Jackson, 1980; Austad, 1982, 1984; Brown et al., 1985; Miller & Miller, 1986; Toft, 1989; Stumpf, 1990; Watson, 1990, 1991; Eberhard et al., 1993; Prenter et al., 1994; Fahey & Elgar, 1997; Elgar et al., 2002; Venner & Venner, 2006). However, in haplogyne species mate guarding has been less studied. Merrigan (1995) found that *Crossopriza lyon* (Pholcidae) males carried out post-copulatory mate guarding to females they copulated with, in order to prevent re insemination from other males. Nevertheless, Eberhard et al. (1993) showed that *P. globosus* has no post-copulatory mate guarding. He explained that there is no last male sperm precedence, as P2 (percentage of last male success) is of 55%, meaning that sperm mixture in

female tract existed in this case. Uhl (1998) observed in *P. phalangioides* that after copulation 69% of males stayed a maximum of half a day, but considered that males did not profit from guarding because she assumed there was complete sperm mixture in the female tract. However, last male sperm priority (P2: 88%) was established later (Schäfer & Uhl, 2002).

In conclusion, our study confirms that post-copulatory mate guarding occurs only in those cases in which females remain receptive after being inseminated. This is why the prediction of post insemination receptivity is essential for the evolution of male post-copulatory mate guarding behaviour (Alcock, 1994). Nevertheless, behavioural studies are still needed in a variety of groups of arthropods and other organisms to determine if this prediction can be generalized for all animals. Relevant for pholcids, *H. pluchei* is the only species in this family in which post-copulatory mate guarding has been confirmed. However, more detailed studies are needed to test whether this male behaviour occurs in other pholcids.

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