

A Parental Care-Mating Dilemma? Potential Risks for Offspring in the Pholcid Spider When Egg-Carrying Females Accept Mating

Lucia Calbacho-Rosa¹ · Alex Córdoba-Aguilar² · Roberto Munguía-Steier³ · Alfredo V. Peretti¹

Revised: 20 January 2017 / Accepted: 1 February 2017 /
Published online: 16 February 2017
© Springer Science+Business Media New York 2017

Abstract Many species face a trade-off between additional mating opportunities and the offspring benefits (viability, quality) provided by parental care. Female *Holocnemus pluchei* spiders must abandon their egg-sac, which they otherwise carry with their chelicerae, to copulate. This may involve risks for the offspring, such as predation and fungal infection. We assessed whether (1) males discriminate between egg-carrying females according to the egg development stage, (2) females are influenced by the egg development stage in regard to their proneness to mate, and (3) offspring are less likely to survive, due to high humidity and/or predation, in the absence of egg-carrying females. Apparently, males did not distinguish between females according to the developmental stage of the eggs they carried. However, females were more likely to mate when carrying relatively mature versus immature eggs (14–18 days and 3–6 days post-oviposition, respectively). All egg-sacs hatched successfully when guarded by egg-carrying females because this avoided both fungal infections (at high humidity) and cannibalism by conspecific spiders. Thus, *H. pluchei* females may face a trade-off between mating and parental care. Further research should clarify why egg-carrying females mate and how females prevent their egg-sacs from being infected by fungi.

Electronic supplementary material The online version of this article (doi:10.1007/s10905-017-9604-6) contains supplementary material, which is available to authorized users.

✉ Lucia Calbacho-Rosa
luciacalbacho@gmail.com

- ¹ Instituto de Diversidad y Ecología Animal (IDEA), CONICET – UNC and Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299 (5000), Córdoba, Argentina
- ² Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apdo. Postal 70-275, Ciudad Universitaria, 04510 México, D.F., Mexico
- ³ Unidad de Morfología y Función, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Av. De los Barrios 1, Los Reyes de Iztacala, 54090 Tlalnepantla, Estado de México, Mexico

Keywords Maternal care · parental care benefits · sexual selection · egg-carrying · egg survival · *Holocnemus plucheii*

Introduction

Parental care behavior that increases offspring performance (e.g., viability and quality) frequently represents a cost to parents. Such costs include higher predation risk, the loss of mating opportunities, and deterioration in physiological condition (Clutton-Brock 1991; Smiseth et al. 2012; Requena et al. 2013; Klug 2014). On the other hand, certain types of parental behavior, such as abortion and infanticide, may evolve if they ultimately benefit the parents' lifetime reproductive success (Kim et al. 2000; Klug 2014).

Theory indicates that animals can face a conflict between parental care and mating (e.g. Requena et al. 2013). Stiver and Alonzo (2009) proposed three different mechanisms that underlie such conflict: (1) resource expenditure allocated to offspring care versus mate acquisition (for example, mate attraction or intrasexual competition); (2) time dedicated to parental care versus mating when these cannot be performed simultaneously; and (3) phenotypic traits that are favored by sexual selection and used during mating but are detrimental to parental care.

Female decisions on whether to mate while performing parental care can also be affected by male sexual behavior. For example, male harassment may induce females to mate rather than to continue caring for their offspring (Arnqvist and Rowe 2005). This is in part a consequence of the differences in reproductive interests between the sexes: whereas males can increase their reproductive success with additional well-selected matings, polyandric females maximize their fitness with few matings (Arnqvist and Nilsson 2000). This scenario has produced a coevolutionary race between two factors that are at times in contradiction — male persistence, harassment and coercion versus female preference (Arnqvist and Rowe 2005).

Within this context, the evolution of parental care is influenced by the variation between individuals as well as by factors affecting fitness and costs of care, such as the social environment, the life history of individuals, superior quality males and ecological conditions (Alonzo and Klug 2012). For example, females that are caring for their young may opt to mate if avoiding courting males implies a great cost (Arnqvist and Rowe 2005).

Spiders are highly suitable for studying the sexual conflict that arises between mating and parental care (reviewed by Elgar 1998). The degree of parental care in this group varies from investing energy in wrapping a sparse cover of silk threads around the eggs (e.g. Foelix 2011) to actively guarding eggs against predators and parasitoids (e.g. Gonzaga and Leiner 2012). For example, Whitehouse and Jackson (1998) observed that females of the theridiid *Argyrodes flavipes* protect their eggs and spiderlings against conspecific threats, both by moving the egg-sac to a safe place and behaving aggressively towards approaching individuals. Gillespie (1990) showed that brood-guarding behavior in *Theridion grallator* is essential for preventing egg predation. One well-known case of conflict occurs in the eresid spider, *Stegodyphus lineatus*. Schneider and Lubin (1996, 1997) observed that, when late maturing males search

for sexual partners, they often encounter females that are already guarding an egg-sac. In this situation, *S. lineatus* males try to remove the egg-sac and remate with carrying females.

Pholcid spiders are also good model organisms for testing the possible trade-off between mating and parental care of offspring. Copulation in pholcids (i.e., cellar spiders) takes place only when the female adopts a horizontal position. Prior to intromission, the male rotates his pedipalps by approximately 180°. A proper positioning of the male relative to the female is achieved with the help of his frontal cheliceral apophyses (Huber 1995). After pedipalp rotation and proper positioning, male copulatory organs are inserted simultaneously and coupled with the female (Huber 1995). Copulation in these animals consists of a single long insertion of both male pedipalps at the same time. After mating, pholcid females use their chelicerae to externally carry an egg-sac that is not protected by a silk cover and therefore they cannot remate while carrying. It is possible that this lack of protection by a silk cover may have evolved because the female carries the egg-sac (Huber and Wunderlich 2006). Despite this female vigilance, egg-sacs are known to be parasitized by wasps in the pholcids *Micropholcus baoruco* and *M. hispaniola* (Huber and Wunderlich 2006; Huber et al. 2014).

The aim of the present study was to investigate the trade-off between parental care and mating, using the pholcid *Holocnemus pluchei*. In this species, late maturing males persistently try to mate with mated females even when the latter are carrying an egg-sac (unpublished observations of the authors). An egg-carrying female who decides to mate must leave the egg-sac unattended, possibly incurring costs as carrying an egg-sac may serve to regulate the humidity and aeration of eggs, thereby avoiding fungal infection. They also defend their eggs against homospecific and heterospecific predators (e.g. Requena et al. 2013). For females, parental pay-offs may change according to the time period spent since the female mated, if there is a positive correlation with egg-sac maturation (females would be more likely to mate if the time since the last copulation is longer). In this context, reproductive differences between the sexes may be more intense when egg-carrying females carry sacs with immature than with mature eggs. Reproductive pay-offs for egg-carrying females may thus change over time.

To explore these questions, we first sought to establish whether males discriminate between egg-carrying females based on the maturation stage of the egg-sacs that they carry. Here we used the distance of males from females as a proxy for mating. We predicted that males would be more likely to stay close to females with a mature egg-sac. Second, we determined whether the degree of development of the egg-sac influenced the decision of egg-carrying females to mate with males. Our prediction was that females would be more prone to mate once the egg-sac had developed sufficiently. Finally, since mating implies that an egg-carrying female abandons her egg-sac at least momentarily, we tested whether female guarding behavior enhances egg survivorship. In this respect, we investigated the role of two potentially influential environmental conditions that would affect egg survival — humidity and predation. Our prediction was that, in the absence of female guarding, both conditions would pose a risk for offspring survival.

Materials and Methods

Biology of the Species

H. pluchei (Pholcidae) is a spider native to central Europe (Porter and Jakob 1990; Jakob 1991). It was introduced in South America (Huber 2000) and is now very common in central Argentina (Laborda and Simó 2008). This species inhabits both urban and natural areas. In homes and buildings, it tends to prefer humid environments such as bathrooms, storehouse or warehouses. It is a small-sized spider (ca. 13 mm tibia-patella length), which builds irregular webs that may contain from one to 15 spiders of different ages, sizes and gender (Jakob 1991). Females are polyandrous, and they copulate with several males before oviposition. Females can lay as many as 4 egg-sacs in a single breeding season. However, since fertilizing all eggs in an egg-sac requires the use of most of the sperm stored in the external uterus (Calbacho-Rosa et al. 2013), females must copulate again to lay another egg-sac. Males perform post-copulatory mate-guarding to prevent females from remating with other males for approx. 14 h and at a distance of less than 12 cm. (Calbacho-Rosa et al. 2010). However, males may also perform pre-copulatory guarding with a potential future mate; in natural conditions, it is actually highly common for males to stay close to females when the latter are carrying an egg-sac fertilized by other males (pers. obs. of the authors). *H. pluchei* was found to have one generation per year. Females were observed to take around 20 days from fertilization to egg laying, and then another 20 days passed before the eggs hatched (Calbacho-Rosa 2011).

In *H. pluchei*, female reproductive status (virgin or mated) influences male mating behavior. Copulation duration is highly variable but, broadly, it takes less time with virgin females (22.771 ± 9.031) than with mated females (51.711 ± 22.295). Two pedipalp movement patterns can be observed during mating. In the first, males may move a single pedipalp at a time in a non-rhythmic and alternating pattern (alternating pedipalp movements). This movement was consistently carried out during the first minute when the male is mating with an already mated female, but is less consistent in copulations with females in their first mating. In the second type of movement, males move both pedipalps in a rhythmical and simultaneous manner (simultaneous pedipalp movements) (Calbacho-Rosa et al. 2013). During this movement, both pedipalps contract and relax in a regular fashion. This movement was commonly observed during copulations with already mated females and with females during their first mating. The different types of pedipalp movements are associated with different copulatory functions. While the function of the alternating movement is to remove rival sperm, one function for the simultaneous pedipalp movements is sperm transfer sensu stricto (Calbacho-Rosa et al. 2013).

Collection and Rearing

Male and female adult spiders were collected in the Ciudad Universitaria, in Córdoba, Argentina (-31.40° latitude, -64.18° longitude, 384 m above sea level), between September 2008 and April 2011. We placed each individual in a plastic container (8×12 cm) lined with paper for web building, and with a wet cotton ball as a water source. The containers were maintained in standard conditions (a photoperiod of

approximately 12/12 h, 25 °C, 57% relative humidity). We selected 50 females, which laid their eggs after being collected, and placed them individually in containers with paper for web building and a wet cotton ball as a water source (10 × 15 cm). Males were used 15 days after being captured so as to enable them perform sperm induction, thus reducing the effects of potential inter-individual differences in mating histories (L. Calbacho-Rosa & A. V. Peretti, unpub. Data). Individuals were fed with *Drosophila melanogaster* adults and *Tenebrio monitor* larvae on a weekly basis.

Observation of Behavioral Sequences

All the observations occurred in experimental plastic boxes (60 cm long, 30 cm wide and 10 cm high). A grid (with 1 cm between consecutive lines) was placed at the bottom of the box in order to estimate the distance between individuals. Each female was assigned to an experimental group based on the maturation state of her egg-sac. One group of females had an immature egg-sac, in which the eggs are green (Fig. 1a, $n = 15$) and the other a mature egg-sac, in which the eggs are green but the legs can also be differentiated as white (Fig. 1b, $n = 15$). The respective ages of the egg-sacs were approximately 3–6 days and 14–18 days post-oviposition. Females were placed in the observation box 24 h before males so that they could build their web. Males were

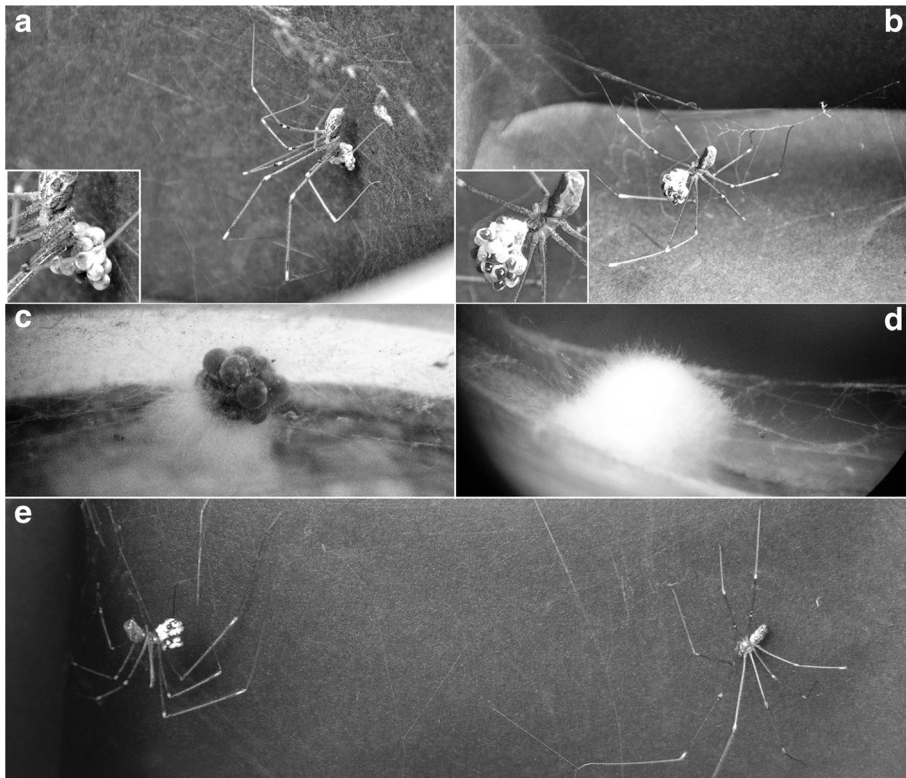


Fig. 1 a Female with an immature egg-sac; b Female with a mature egg-sac; c–d, Egg-sac showing fungal infection; e Male near a female carrying an egg-sac (female is on the left and male is on the right)

placed in the experimental plastic boxes on the opposite side to where the female was located, so males were able to stay away from the females with egg-sacs. Each male was used only once.

Experimental Design

Position of Males near Females Carrying an Egg-Sac, According to the Developmental Stage of the Eggs in the Egg-Sac

A close distance of males to females is a post-copulatory mate-guarding feature in this species. Males stay at a distance of less than 12 cm for approximately 14 h after copulation (Calbacho-Rosa et al. 2010). Preliminary observations showed that the distance between males and juveniles ($n = 20$) was greater than that between males and females. The distance data between males and juveniles were taken under the same conditions as the distance data between males and females with egg-sac. The mean distance between males and juveniles was approximately 20 cm during 48 h of experimentation, which was greater than that observed between males and females with egg-sac (see below). This enabled the distance between males and females carrying an egg-sac to be used as an indicator of the intention of the former to copulate.

Males were placed with females carrying either a mature or an immature egg-sac. Focal observations for each pair were made during two days, at 10 min, 30 min, 1 h, 2 h, 3 h, 6 h, 8 h, 10 h, 12 h, 14 h, 18 h, 20 h, 24 h, 26 h, 30 h, 32 h, 36 h, 40 h and 48 h after the male was placed with the female, in order to measure the distance between the two spiders in centimeters. Data were taken every hour for 48 h, because this is a kind of spider that is not moving all the time, but remains still for periods of time before taking a new position.

For data analysis, we fitted linear mixed models, because the repeated observations of the distance between males and females for each couple over time are not independent. We considered the distance between a male and female as the response variable, and egg development (immature and mature) and time as fixed predictor variables. Time was transformed to natural logarithms ($\log + 1$). Couple identity was considered as a random variable and time as random slope. To assess the significance of the predictor variables, we performed parametric bootstraps and fitted the models using maximum likelihood methods. Parametric bootstraps were carried out with a thousand simulations to assess the significance of each of the predictor variables.

Acceptance of Copulation by Females According to the Maturity of their Egg-Sac

We determined female acceptance to mate in the previously established trial, after 30 min from the start of the experiment, by recording male courtship via movements of pedipalps and legs (see supplementary material, Online resource 1). The behavior males performed was interpreted as pre-copulatory courtship, since it was the same as that which they perform when faced with virgin or mated females (without an egg-sac) to mate. Acceptance was considered as when the female left the egg-sac to mate with the male, and rejection when the female did not leave the egg-sac and thus did not mate. After the experiments, each rejected male was mated to a non-focal virgin female to make sure the males were capable of copulation.

In an additional test, the sexual receptivity of egg-carrying females was tested by removing the immature egg-sac from a group ($n = 15$, individually housed) and adding a male for 30 min, and then recording if each female accepted copulation.

We fitted a generalized linear model with binomial distribution, having the number of females that agreed to mate as the response variable, and the maturation status of the egg-sac as the predictor variable. When all outcomes of a binomial model fall in just one category, a phenomenon occurs known as complete separation. Complete separation causes problems in the estimation of parameters, large confidence intervals and large p values using the standard maximum likelihood methods (Bolker 2015). Since our data is a complete separation case, we employed Bayesian inference using Markov Chain Monte Carlo (MCMC) methods, since these are better suited to analyze this kind of data (Gelman et al. 2014, Bolker 2015). After the model converged, we estimated the probability of mating for each group of females (those with an immature or mature egg-sac), and then the 95% Credible Intervals (CI). Additionally, we made a contrast to assess whether there was a systematic difference in mating acceptance between groups. If the contrast that represents the difference between the expected values for the groups does not have a 95% posterior CI overlapping with zero, we can conclude that there is a difference between the groups. We used prior normal distributions for estimating parameters of the model, with a mean of 0 and a variance of 100.

Are Humidity and Predation Driving Sources of Egg Mortality?

Most females carrying an egg-sac did not mate (see Results). We evaluated two key factors for egg survival: humidity and predation. We chose humidity because it is variable in the natural environment of this species, ranging from 57% to 90% (L. Calbacho-Rosa & A. V. Peretti, unpub. Data). For the evaluation of humidity, guarding females were immobilized by placing them 30 s in the cold, and then their egg-sacs were removed (using dissecting forceps) and placed in the mother's web. Four experimental groups were established according to the development of the eggs in the egg-sac and humidity conditions: (1) immature egg-sacs ($n = 20$) individually exposed to 57% humidity (the environmental humidity at the time), (2) immature egg-sacs ($n = 10$) individually exposed to 90% humidity, (3) mature egg-sacs ($n = 20$) individually exposed to 57% humidity, and (4) mature egg-sacs ($n = 10$) individually exposed to 90% humidity. Humidity levels were controlled by applying water to a cotton base laid on the experimental box floor for 24 h, and were recorded using a digital hygro-thermometer (Schwyz, SETH).

Four control groups consisted of egg-sacs (either mature or immature) carried by females and exposed to the same levels of humidity (57 and 90%; $n = 10$ for each group). We then recorded development and hatching success for these four groups. Development of a sac was considered successful if it was not covered by fungus after 72 h (for an indication of fungal presence, see Fig. 1c–d).

To determine the predation risk for an unguarded egg-sac, immature and mature sacs were removed (as indicated above) and then each sac was individually suspended on its mother's web (the web was already present) in a container (10×15 cm) with: (1) males of *Holocnemus pluchei*, (2) females of *Holocnemus pluchei* (other than the mother), (3) juveniles of *Holocnemus pluchei*, (4) males of *Pholcus phalangioides*, and (5) females of *Pholcus phalangioides*. The latter is a coexisting species (Dutto 2006). There were 15

trials (with each experiment lasting one hour) for each of these 5 groups, resulting in one of three conditions: the sacs remained suspended on the web, had been consumed, or had been thrown to the bottom of the container. Finally, we used a control group consisting of 10 females carrying their egg-sacs that were exposed to the same risks of predation.

We fitted a generalized linear model with binomial distribution employing Bayesian Inference, because Markov Chain Monte Carlo (MCMC) methods are better suited for analyzing data than maximum likelihood (ML) methods (Gelman et al. 2013) when all individuals in one treatment group have a positive or negative result. The response variable was the number of egg-sacs that were preyed upon, while the predictor was a categorical variable of potential predators (males, homospecific females and juveniles). We estimated the probability of predation for the egg-sacs from juveniles, homospecific females and males, and then the 95% CI.

Additionally, we made a contrast to assess whether there was a systematic difference in predation of egg-sacs between groups. Mixed model analyses and graphs of the contrasts were fitted in R (R Core Team 2015; version 3.1.0). We used the lme4 package for fitting the mixed models (Bates et al. 2014) and the pbkrtest package to run the parametric bootstrap tests (Halekoh and Højsgaard 2013). With JAGS software via the r2JAGS interface, we performed Bayesian inference and estimated posterior probabilities of parameters in regard to mating acceptance and predation models. The models ran 3 chains with 120,000 iterations and a 20,000 burn-in period. All the parameters reached convergence using the Gelman-Rubin convergence statistic, \hat{r} -hat, when this was less than 1.01. Prior probability distributions of the parameters came from a normal distribution with a mean of 0 and a variance of 100.

Results

Position of Males near Females with an Egg-Sac

During the 48 h of observation, males stayed near females carrying either immature ($n = 15$) or mature ($n = 15$) egg-sacs (Fig. 1e). Males and females did not move. Generally, the female remained in the same body position before and after males being present. When comparing the two groups for each of the time intervals recorded, the distance of males to females did not differ significantly (Table 1: males near females carrying immature eggs, 10.354 ± 1.321 cm; males near females carrying mature eggs, 12.887 ± 1.838 cm; Fig. 2). Moreover, no tendency was found in either of the two groups for males to increase or decrease their proximity to females over time.

Table 1 Sequential likelihood ratio tests (LRTs) to assess the significance of fixed predictor variables from the null to the global mixed model. We performed parametric bootstraps to estimate p -values since LRTs asymptotically follow a chi square distribution when the number or level of the random variable becomes larger

Predictor variable	Deviance	Df	Bootstrapped p -value
Maturity	0.045	1	0.82
Time (log +1)	2.165	1	0.15

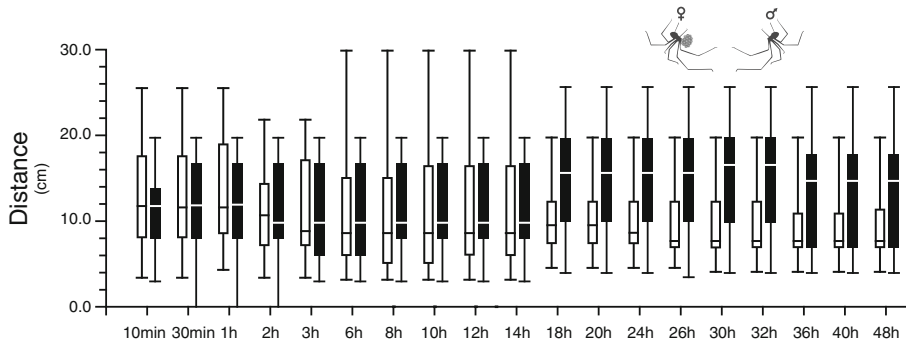


Fig. 2 Inter-distance between male and female, the latter with an immature (in a white boxplot) or mature (in a black boxplot) egg-sac. Distances were measured during two days (at 10 min, 30 min, 1 h, 2 h, 3 h, 6 h, 8 h, 10 h, 12 h, 14 h, 18 h, 20 h, 24 h, 26 h, 30 h, 32 h, 36 h, 40 h, and 48 h) after the male was placed with the female

Acceptance of Copulation by Females According to the Maturity of their Egg-Sacs

None of the 15 females with an immature egg-sac agreed to copulate with the nearby male during the entire experiment. Of the 15 females with a mature egg-sac, 4 accepted copulation with the nearby male. Statistical analyses indicated that females with an immature egg-sac differed in their probability of mating acceptance ($p = 0.010$, CI 95% = 0.000–0.078) compared to females with mature egg-sacs ($p = 0.261$, CI 95% = 0.082–0.503). Thus, females with mature eggs were more prone to mate than females with immature eggs (Contrast: $p = 0.252$, CI 95% = 0.066–0.496).

Females that agreed to copulate left their egg-sac momentarily unattended during copulation (10.25 ± 2.87 min). When copula ended, they returned to recover their egg-sac (see supplementary material, Online resource 1). All males that did not copulate during the mating trials were able to mate successfully with virgin females. Of the females ($n = 15$) that underwent removal of their immature egg-sac before being exposed to a male, none agreed to copulate.

Are Humidity and Predation Driving Sources of Egg Mortality?

All eggs individually placed on the mother's web under 57% humidity successfully developed and hatched, whether from immature or mature egg-sacs ($n = 20$ each). However, none of the eggs hatched when the egg-sac, whether immature or mature ($n = 10$ each), was individually placed on the mother's web under 90% humidity. The eggs under extreme humidity showed signs of fungal infection (Fig. 1 c–d). In contrast, all eggs, whether from mature or immature sacs ($n = 10$ for each), successfully hatched when exposed to extreme humidity but guarded by females.

All eggs in a sac that was unguarded were eaten when exposed to conspecific females other than their mothers ($n = 15$) or to conspecific juveniles ($n = 15$). Unguarded sacs exposed to conspecific males were either eaten by them (5 out of 15) or thrown to the bottom of the box (10 out of 15), where they did not hatch. The probability of predation by juveniles ($p = 0.99$, CI 95% = 0.940–1.000) and females ($p = 0.99$, CI 95% = 0.932–1.000) was higher than that by males (contrast: 0.644, CI

95% = 0.395–0.85). On the other hand, eggs in sacs ($n = 30$) exposed to females or males of *P. phalangioides* all developed and hatched normally, as did eggs in sacs being carried by females (control, $n = 10$) exposed to risks of predation.

Discussion

Holocnemus pluchei males perform post-copulatory mate-guarding of females by staying nearby, at a distance of approximately 12 cm (Calbacho-Rosa et al. 2010). This distance is in fact similar to what we observed in our study for males that stayed close to females carrying an egg-sac. When males are placed near females without an egg-sac (virgin or mated females), the males do not perform pre-copulatory guarding, as they usually mate with these females within a few minutes given the high receptivity and polyandric nature of females (Calbacho-Rosa et al. 2013). However, when males are placed near females bearing an egg-sac (and fertilized by other males), they stay close to them for a longer period of time (48 h approximately). This suggests a form of pre-copulatory guarding even when the chances of mating are reduced. Reviewing whether similar male behavior occurs in other species, we found that, before copulation, males remain near females with an egg-sac, for example in *Physocyclus globosus* (Eberhard et al. 1993) and *Physocyclus dugesi* (Calbacho-Rosa pers. obs.). This characteristic implies a possible pattern of male persistence in mating even when females are not ready.

Interestingly, in *H. pluchei*, the males did not seem to assess copulation chances on the basis of egg-sac development. That is, even though females carrying immature eggs did not accept copulation, males still remained close to them. These females were not sexually receptive, as evidenced by the fact that they did not mate when their egg-sac was removed. The inability of males to discriminate between females on the basis of egg-sac maturity and female mating status is paradoxical, as this ability has been found in other species (e.g., Gaskett 2007; Perampaladas et al. 2008). However, male competition may be so high that it pays to stay close to a female independently of the maturity of her egg-sac.

The fact that few females carrying a sac of mature eggs agreed to copulate suggests that those females are sexually receptive but try to avoid mating. It may be worth exploring whether females that opt to mate when carrying an egg-sac are actually very selective, given that they will incur the risk of losing their offspring. The trade-off for females is between remaining guarding the egg-sac to ensure their survival (see below) vs. copulating again to have enough sperm to fertilize the eggs of the next sac. On the other hand, the reason that males remain near females with an egg-sac is clearly to copulate, since (1) males courted females by movements of pedipalps and legs, and (2) when placed with virgin females (after being exposed to females with an egg-sac), copulation occurred. Nevertheless, males were not very successful at inducing females with an egg-sac to mate, as shown by the fact that mating took place in only 4 out of 30 assays. Although mating frequency in males of this species is high, when males found only females carrying an egg-sac and not with other females without an egg-sac near, as we observe in nature, males stayed near females with an egg-sac, expecting to be able copulate, although the probability that these females leave their egg-sac to copulate was low. So, if this is the only option for copulating, males remain near females with an

egg-sac even when the probabilities of copulation are low. The percentage of sperm present in the external uterus of females with an egg-sac (6 ± 2161) is lower than that present in the external uterus of females that had mated but did not lay an egg-sac ($47, 83 \pm 23,544$) (Calbacho-Rosa et al. 2013). Thus, it is expected that males who mate with females with an egg-sac benefit because their sperm will fertilize a percentage of eggs in the next sac that the female produces. There is no evidence that males of the pholcid species have a limited amount of sperm.

As predicted, females with mature egg-sacs were more likely to mate than those with immature egg-sacs. One hypothesis is that greater egg-sac development is an indicator of a longer time elapsed since the last mating, thus increasing female sexual receptivity. Males can prevent rivals from gaining access to the female by physically repelling, producing a mating plug, or transferring sperm-associated substances that induce a refractory period so that the female is no longer sexually receptive (Thornhill and Alcock 1983; Alcock 1994; Elgar and Bathgate 1996). Therefore, females that have mated earlier (females with mature egg-sacs) may be more receptive than females that have mated more recently (females with immature egg-sacs). Stimulation during copulation could be another factor diminishing female sexual receptivity following mating (Calbacho-Rosa et al. 2013). Furthermore, reducing parental care when the eggs are mature may be less risky for offspring given the advanced developmental stage of embryos. In support of this is the fact that a fungal infection may take days to develop in arthropods (Samson et al. 2013).

Our results indicate that parental care of an egg-sac is key to offspring survival, as they avoid fungal infections when humidity is high and avoid conspecific predation or detachment from the web and disposal by conspecific males. It is well known that high humidity plays a negative role in egg survival (e.g. Boudreaux 1958; Jones 1941; Hieber 1992) because it usually leads to fungal infection in other arthropods (e.g. Mora 1990; Smith 1997; Machado and Oliveira 1998; Kudo et al. 2011). Our results here show that fungal infection is a major factor limiting egg development in *H. pluchei*.

Furthermore, *H. pluchei* live in relatively large groups including up to 15 individuals (Jakob 1991). Thus, abandoning an egg-sac leaves it vulnerable to nearby conspecifics. This same predation pressure has been reported in relation to other species whose females leave their egg-sac in order to mate (Huber and Eberhard 1997; Rodríguez-Márquez and Peretti 2010; Uhl et al. 1995; Calbacho-Rosa & Peretti, pers. obs). Fungal infection and conspecific predation thus act as natural selection forces.

Our results also indicate that conspecifics are a major threat for *H. pluchei* egg-sacs. Egg-sacs were eaten by *H. pluchei* individuals but they were not eaten by *P. phalangioides* individuals. The eggs are more easily preyed upon in our study species because pholcid females do not cover their egg-sac with protective layers of silk, but rather hold them together with a few silk threads (Huber and Wunderlich 2006). It is likely that the lack of a protective covering is an evolutionary result of the protection offered by females because they carry their egg-sac in their chelicerae (Huber and Wunderlich 2006). Nevertheless, this protection disappears and eggs become vulnerable when females abandon them in order to mate.

Spider males may experience significant costs, in terms of energy expenditure and predation risks, in the process of searching for receptive females. This may explain the evolution of male strategies aimed at inducing non-receptive females to accept copulation such as infanticide, where a male kills the offspring sired by another male in

order to increase his own fitness (Schneider and Lubin 1997). The first evidence of infanticide by males in a spider species was that of *Stegodyphus lineatus* (Eresidae) by Schneider and Lubin (1996, 1997). These authors observed that when males search for sexual partners, they often encounter females that are already guarding egg-sacs. This is a case of extreme sexual conflict where the female does not take the decision to leave her egg-sac, but it is the male who tries to remove the egg-sac and induce the female to remate. This does not apply to our study species, as *H. pluchei* females may indeed leave their egg sacs unattended. In *Helvibis longicauda* (Theridiidae), males destroyed half of the unprotected egg-sacs and attacked and consumed unguarded spiderlings (Gonzaga and Leiner 2012). Consequently, maternal protection seems key to ensuring offspring survival in this species, as both eggs and spiderlings are extremely susceptible to predation when the mother is absent (Gonzaga and Leiner 2012). Interestingly, there is another potential risk for a female when leaving her egg-sac to copulate which is that she may not be able to find the sac again. The evidence for this is scarce but comes from a single observation (not from our experimental settings) in which a female, rather than taking an egg-sac back after copulation, took a *Tenebrio* larva that was originally given as food, and provided care to it for several days. The inability of parental females to recognize their own egg-sac seems paradoxical.

We offer three possible non-exclusive explanations for why egg-sac carrying females mate despite the risks documented here involved in not attending egg-sacs. First, females need more sperm because the sperm left in the uterus is not enough to fertilize the eggs of a new egg-sac (Calbacho-Rosa et al. 2013). Second, male persistence is costly, leading females to mate in order to reduce such costs. In many animal species, females suffer a number of costs by being guarded during offspring care (e.g., Bonness et al. 1995; Silk 1999; Weir et al. 2008). Third, females may use male persistence to choose mates. It is possible that females choose males on the basis of their pre-copulatory guarding behavior, if this provides them with cues in the form of direct or indirect benefits related to male fitness (Eberhard 1996; Cordero and Eberhard 2003), such as the ability to defend the female from harassing males. This is precisely the case with *H. pluchei*, as during post-copulatory guarding, males protect females from harassment (Calbacho-Rosa et al. 2010). Finally, a balance between costs and benefits of carrying egg-sacs could occur in females. It is possible that male persistence to mate produces differential effects (from detrimental to beneficial) for females with immature or mature egg-sacs (Wedell et al. 2006; Stiver and Alonzo 2009). Hence, females may benefit from mating if enough time has elapsed since the last copulation. These three explanations await further experimental testing.

In summary, male *H. pluchei* did not show a preference for females according to the developmental stage of the eggs carried by the latter. Females, on the other hand, were more prone to mate when carrying relatively mature versus immature eggs (14–18 days and 3–6 days post-oviposition, respectively). When guarded by parental females, all egg-sacs hatched successfully because both fungal infections (at high humidity) and cannibalism by conspecific spiders were avoided. Thus, *H. pluchei* females may show a trade-off between mating and parental care.

Future research should test whether females with developed egg-sacs would mate after experimental removal of the sac (as was done for females with immature eggs), since this could shed light on female receptivity. However, in this work the level of natural receptivity of these females was demonstrated. It is also important to clarify the reasons why egg-carrying females mate (even though this impairs egg survival) and the way in which they prevent their egg-sacs from being infected by fungi.

Acknowledgements We thank Margarita Chiaraviglio, Cristina Sciocia and Gustavo Requena for suggestions about the manuscript. The authors also acknowledge help from D. Vrech and P. Olivero during collection and rearing. We are grateful for financial support provided by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Fondo para la Investigación Científica y Tecnológica (FONCYT), and Secretaría de Ciencia y Tecnología (SECYT-UNC) of Argentina, and the Consejo Nacional de Ciencia y Tecnología (Conacyt) of Mexico. Additionally, AC-A was supported by a sabbatical grant from PASPA-UNAM. We are also grateful for the most helpful comments made by two reviewers.

Compliance with Ethical Standards This study was conducted in compliance with the “Guidelines for the use of animals in research” as published in *Animal Behaviour* (1991, 41, 183–186) and the laws of the country where the research was conducted”.

Conflict of Interests The authors declare that they have no conflict of interest.

References

- Alcock J (1994) Post-insemination associations between males and females in insects: the mate guarding hypothesis. *Annu Rev Entomol* 39:1–21
- Alonzo SH, Klug H (2012) Paternity, maternity, and parental care. In: Royle NJ, Smiseth PT, Kölliker M (eds) *The evolution of parental care*. Oxford University Press, Oxford, U.K., pp 189–205
- Arnqvist G, Nilsson T (2000) The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav* 60:145–164
- Arnqvist G, Rowe L (2005) *Sexual Conflict*. Press, Princeton University
- Bates D, Maechler M, Bolker B, Walker S (2014) lme4: linear mixed-effects models using Eigen and S4. version 1.16, <http://CRAN.R-project.org/package=lme4>
- Bolker B (2015) Linear and generalized linear mixed models. In: Fox GA, Negrete-Yankelevich S, Sosa VJ (eds) *Ecological statistics: contemporary theory and application*. Oxford University Press, USA
- Bonness DJ, Bowen WD, Iverson SJ (1995) Does male harassment of females contribute to reproductive synchrony in the grey seal by affecting maternal performance. *Behav Ecol Sociobiol* 36:1–10
- Boudreaux HB (1958) The effect of relative humidity on egg-laying, hatching, and survival in various spider mites. *J Insect Physiol* 2:65–72
- Calbacho-Rosa L (2011) *Patrones de selección Sexual en arañas Pholcidae: Comportamiento sexual y Procesos asociados*. PhD thesis. Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Argentina
- Calbacho-Rosa L, Córdoba-Aguilar A, Peretti AV (2010) Occurrence and duration of post-copulatory mate guarding in a spider with last sperm precedence. *Behavior* 147:1267–1283
- Calbacho-Rosa L, Galicia-Mendoza I, Dutto MS, Córdoba-Aguilar A, Peretti AV (2013) Copulatory behavior in a pholcid spider: males use specialized genitalic movements for sperm removal and copulatory courtship. *Naturwissenschaften* 100:407–416
- Clutton-Brock TH (1991) *The evolution of parental care*. Press, Princeton University
- Cordero C, Eberhard WG (2003) Female choice of antagonistic male adaptations: a critical review of some current research. *J Evol Biol* 16:1–6
- Dutto MS (2006) *Análisis funcional del comportamiento de estridulación en *Holocnemus plucheii* (Scopoldi) (Araneae: Pholcidae) femenino*. Graduation thesis, Facultad de Ciencias Exactas. Universidad Nacional de Córdoba, Argentina, Físicas y Naturales, p 56
- Eberhard WG (1996) *Female control: sexual selection by cryptic female choice*. Press, Princeton University

- Eberhard WG, Guzman-Gomez S, Catley KM (1993) Correlation between spermathecal morphology and mating systems in spiders. *Biol J Linn Soc* 50:197–209
- Elgar MA (1998) Sperm competition and sexual selection in spiders and other arachnids. In: Birkhead TR, Møller AP (eds) *Sperm competition and sexual selection*. Academic Press, London, pp 307–309
- Elgar M, Bathgate R (1996) Female receptivity and male mate-guarding in the Jewel spider *Gasteracantha minax* Thorell (Araneidae). *J Insect Behav* 9:729–738
- Foelix RF (2011) *Biology of spiders*. Oxford Univ. Press, New York
- Gaskett AC (2007) Spider sex pheromones: emission, reception, structures, and function. *Biol Rev* 82:26–48
- Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB (2013) *Bayesian data analysis*. CRC press
- Gelman A, Carlin JB, Stern HS, Dunson DB, Vehtari A, Rubin DB (2014) *Bayesian data analysis*. Chapman & Hall/CRC Press, Boca Raton
- Gillespie RG (1990) Costs and benefits of brood care in the Hawaiian happy face spider *Theridion grallator* (Araneae, Theridiidae). *Am Midl Nat* 123:236–243
- Gonzaga O, Leiner N (2012) Maternal care and infanticide by males in *Helvibis longicauda* (Araneae: Theridiidae). *Ethology* 119:20–28
- Halekoh U, Hojsgaard S (2013) pbrtest: Parametric Bootstrap and Kenward Roger Based Methods for Mixed Model Comparison. R package version 0.3–8, URL <http://CRAN.R-project.org/package=pbrtest>
- Hieber CS (1992) The role of spider cocoons in controlling desiccation. *Oecologia* 89:442–448
- Huber BA (1995) Copulatory mechanism in *Holocnemus pluchei* and *Pholcus opilionoides*, with notes on male cheliceral apophyses and stridulatory organs in Pholcidae (Araneae). *Acta Zool (Stockh)* 76:291–300
- Huber BA (2000) New world pholcid spiders (Araneae: Pholcidae): a revision at generic level. *Bull Am Mus Nat Hist* 254:1–348
- Huber BA, Eberhard WG (1997) Courtship, copulation, and genital mechanics in *Physocyclus globosus* (Araneae, Pholcidae). *Can J Zool* 74:905–918
- Huber BA, Wunderlich J (2006) Fossil and extant species of the genus *Leptopholcus* in the Dominican Republic, with the first case of egg-parasitism in pholcid spiders (Araneae: Pholcidae). *J Nat Hist* 40: 2341–2360
- Huber BA, Carvalho LS, Benjamin SP (2014) On the new world spiders previously misplaced in *Leptopholcus*: molecular and morphological analyses and descriptions of four new species (Araneae, Pholcidae). *Invertebr Syst* 28:432–450
- Jakob EM (1991) Costs and benefits of group living for pholcid spiderlings: losing food, saving silk. *Anim Behav* 41:711–722
- Jones SE (1941) Influence of temperature and humidity on the life history of the spider *Agelena naevia* Walckenaer. *Ann Entomol Soc Am* 34:557–571
- Kim KW, Roland C, Horel A (2000) Functional value of matiphagy in the spider *Amaurobius ferox*. *Ethology* 106(8):729–742
- Klug H (2014) What are the benefits of parental care? The importance of parental effects on developmental rate. *Ecol Evol* 4(12):2330–2351
- Kudo SI, Akagi Y, Hiraoka S, Tanabe T, Morimoto G (2011) Exclusive male egg care and determinants of brooding success in a millipede. *Ethology* 117:19–27
- Laborda A, Simó M (2008) First south American records of *Holocnemus pluchei* (Scopoli, 1763) and *Spermophora senoculata* (Duges, 1836) (Araneae: Pholcidae). *Ganaya* 72:261–265
- Machado G, Oliveira PS (1998) Reproductive biology of the neotropical harvestman (*Goniosoma longipes*) (Arachnida, Opiliones: Gonyleptidae): mating and oviposition, brood mortality, and parental care. *J Zool* 246:359–367
- Mora G (1990) Paternal care in a neotropical harvestman, *Zygopachylus albomarginis* (Arachnida, Opiliones: Gonyleptidae). *Anim Behav* 39:582–593
- Perampaladas K, Stoltz JA, Andrade MCB (2008) Mated redback spider females re-advertise receptivity months after mating. *Ethology* 114:589–598
- Porter AH, Jakob EM (1990) Allozyme variation in the introduced spider *Holocnemus pluchei* (Araneae: Pholcidae) in California. *J Arachnol* 18:313–319
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Requena G S, Munguía-Steyer R, Machado G (2013) Paternal care and sexual selection in arthropods. In: *Sexual selection: perspectives and models from the Neotropics* (Macedo RH, Machado G, eds). Elsevier, pp.201–233
- Rodríguez-Márquez I, Peretti A (2010) Intersexual cooperation during male clasping of external female genitalia in the spider *Physocyclus dugesi* (Araneae, Pholcidae). *J Ethol* 28:153–163

- Samson RA, Evans HC, Latgé J P (2013). Atlas of entomopathogenic fungi. Springer Science & Business Media.
- Schneider JM, Lubin Y (1996) Infanticidal male eresid spiders. *Nature* 381:655–656
- Schneider JM, Lubin Y (1997) Male infanticide in a spider with suicidal brood care *Stegodyphus lineatus* (Eresidae). *Anim Behav* 54:305–312
- Silk B (1999) Why are infants so attractive to others? The form and function of infant handling in bonnet macaques. *Anim Behav* 57:1021–1032
- Smiseth P, Kölliker M, Royle N J (2012) What is parental care? Royle NJ, Smiseth PT, Kölliker M (Eds.). pp. 1–18. Oxford University Press
- Smith RL (1997) Evolution of paternal care in the giant water bugs (Heteroptera: Belostomatidae). In: Crespi BJ, Choe JC (eds) The evolution of social behavior in insects and arachnids. Cambridge University Press, Cambridge, pp 116–149
- Stiver KA, Alonzo SH (2009) Parental and mating effort: is there necessarily a trade-off? *Ethology* 115: 1101–1126
- Thornhill R, Alcock J (1983) The evolution of insect mating systems. Harvard University Press, Cambridge
- Uhl G, Huber BA, Rose W (1995) Male pedipalp morphology and copulatory mechanism in *Pholcus phalangioides* (Fuesslin, 1775) (Araneae, Pholcidae) *mn* 10: 1–9
- Wedell N, Kvamemo C, Lessells CKM, Tregenza T (2006) Sexual conflict and life histories. *Anim Behav* 71(5):999–1011
- Weir JS, Duprey NM, Wuersig B (2008) Dusky dolphin (*Lagenorhynchus obscurus*) subgroup distribution: are shallow waters a refuge for nursery groups? *Can J Zool* 86:1225–1234
- Whitehouse MEA, Jackson RR (1998) Predatory behaviour and parental care in *Argyrodes flavipes*, a social spider from Queensland. *J Zool* 244:95–105