Articles

Mating system and exclusive postzygotic paternal care in a Neotropical harvestman (Arachnida: Opiliones)

Tais M. Nazareth [a,1], Glauco Machado [b,*]

[a] Programa de Pós-graduação em Ecologia e Conservação de Recursos Naturais
[b] Departamento de Ecologia, Universidade de São Paulo

This study tests predictions of the hypothesis of evolution of paternal care via sexual selection by using the Neotropical harvestman *Pseudopucrolia* sp. as the model organism. Females use natural cavities in roadside banks as nesting sites, which are defended by males against other males. Females leave the nests after oviposition, and all postzygotic parental care is accomplished by males, which protect the eggs and nymphs from predators. We provided artificial mud nests to individuals in the laboratory and conducted observations on the reproduction of the species. Male reproductive success was directly related to nest ownership time: the longer a male held a nest, the higher his chances of obtaining copulations. All males that succeeded in mating and obtaining one clutch eventually mated with additional females that added eggs to the clutch. Thus, desirable males were not limited to monogamy by paternal care. Experimental manipulations demonstrated that guarding males were more attractive to females than were nonguarding males and also that males guarded unrelated eggs. Finally, we found that females and nonguarding males spent more time foraging than guarding males. We use our data to contrast hypotheses on the origin and maintenance of paternal care and to provide a critical assessment of the hypothesis of the evolution of paternal care via sexual selection.

© 2009 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Numerous factors have been proposed to explain which sex is more likely to provide parental care (Clutton-Brock 1991; Queller 1997). Direct male–male competition for access to females coupled with female mate choice results in low confidence of paternity, especially among species with internal fertilization (Trivers 1972; Williams 1975). Moreover, internal fertilization creates a physical and temporal isolation between males and the eggs they have fertilized (Gross & Shine 1981). Low confidence of paternity and marked variation in mating success may act against the evolution of paternal care because they reduce the benefits and increase the costs that males pay for providing care (Kokko & Jennions 2003). However, it is important to stress that the certainty of paternity per se cannot directly affect the evolution of paternal care because a male cannot increase his paternal confidence by adopting a caring role (Gross 2005).

One of the most robust hypotheses to explain the evolution of paternal care has been proposed by Williams (1975). According to

his hypothesis, females are attracted to suitable oviposition sites that males defend against other males in an attempt to acquire mates. Moreover, males that defend a territory further increase their fitness because they also indirectly defend eggs against conspecific predators. In this case, paternal care does not necessarily decrease the probability of a caring male acquiring additional mating pairs because several females may visit his territory. The 'territoriality hypothesis' presupposes that paternal care has evolved under the pressure of natural selection and it predicts that (1) eggs do not increase male attractiveness and (2) males should not guard unrelated eggs. Even though this hypothesis does not account for the evolution of paternal care in all animal groups, it has nevertheless been proposed as the primary explanation for two groups in which this behaviour is widely distributed, namely fish and anurans (Ridley 1978; Gross & Shine 1981; Lehtinen & Nussbaum 2003; see also Ah-King et al. 2005).

More recently, Tallamy (2000, 2001) analysed all described cases of exclusive paternal care in arthropods, and he proposed that male care has evolved under the pressure of sexual selection. According to his hypothesis, paternal care in arthropods minimizes the physiological costs related to egg guarding by females, allowing them to forage immediately after oviposition. Moreover, paternal care can also provide an honest signal of the male's ability to defend offspring. Under such assumptions, males that provide paternal

care are chosen by females and should obtain a greater number of copulations than males that are unable or unwilling to provide care. The 'sexual selection hypothesis' generates at least two different predictions when compared to the territoriality hypothesis: (1) eggs increase male attractiveness to females and (2) males should guard unrelated eggs. Other important predictions of the sexual selection hypothesis are: (3) paternal care creates more opportunities for females to forage, allowing them to feed more and produce more eggs; (4) females should be iteroparous; and (5) caring males have many mating opportunities during the caring period and copulate more than noncaring males (Tallamy 2000, 2001).

Exclusive postovipositional paternal care is the rarest form of parental investment in arthropods, and has evolved in no more than 17 unrelated taxa (Tallamy 2000, 2001; Nazareth & Machado 2009). All cases of exclusive paternal care in the class Arachnida described so far are restricted to the order Opiliones, in which there are also many cases of nonbrooding species as well as cases of maternal care among Neotropical species (reviewed in: Machado & Raimundo 2001; Machado & Macías-Ordóñez 2007). Observational data obtained for many harvestman species with paternal care support some predictions of the sexual selection hypothesis (reviewed in: Machado et al. 2004; Machado & Macías-Ordóñez 2007). However, experimental studies are necessary for a formal test of this hypothesis, and to better understand the evolution of paternal care in harvestman. Therefore, the objective of this paper was to test all predictions of the sexual selection hypothesis (Tallamy 2000, 2001) using the Neotropical harvestman *Pseudopucrolia* sp. (Gonyleptidae) as the model organism.

METHODS

Study Animal

The *Pseudopucrolia* sp. harvestman is dark brown, has a body length of 6–7 mm (Fig. 1) and belongs to the subfamily Heteropachylinae, which is an early lineage of the family Gonyleptidae (Kury 1994). The systematics of the Heteropachylinae is unresolved and generic boundaries are confusing. The species studied herein is a new taxon that was tentatively placed in the genus *Pseudopucrolia*. A systematic review of the subfamily is currently in progress, and the study species is being formally described (A. C. Mendes,

personal communication). Voucher specimens of males and females are deposited in the arachnological collections of the Museu de Zoologia da Universidade de São Paulo (MZSP, São Paulo state) and Museu Nacional do Rio de Janeiro (MNRJ, Rio de Janeiro state) in Brazil.

Preliminary field data showed that individuals of *Pseudopucrolia* are active only at night, when they were observed walking in the field (mainly females) or standing still at the entrance of natural holes in roadside banks (mainly males). These natural holes had an entrance of 8–41 mm in diameter and 33–92 mm in depth, and were always occupied by adult males. In some of these holes, there were also clutches with eggs covered by a thick layer of sand or mud. The clutches were composed of eggs in different stages of embryonic development, suggesting that several females in succession had contributed eggs to the clutch. Sex ratio in the field was nearly 1:1 and population density was very high, with more than 200 individuals being found on a roadside bank measuring 30 m long and 1.5 m high.

Maintenance in Captivity

The individuals used in this study were collected in an Atlantic Forest fragment, near the municipality of Sooretama (19°06′ S, 39°45′ W), located in the Espírito Santo state, southeastern Brazil. They were found at night on a 30 m long roadside bank located at the edge of the forest fragment. Two field trips were conducted, in June 2003 and June 2005, and the total number of individuals collected was 113 (49 males and 64 females). All harvestmen were brought to our laboratory in the Museu de História Natural of the Universidade Estadual de Campinas, Brazil. To test the prediction that females are iteroparous, 15 gravid individuals were preserved in 70% ethanol and dissected under stereomicroscope. Eggs in the female reproductive tract were counted and classified as mature if they were in the proximal one-third of the ovary, were bright yellow, and had a diameter of at least 1 mm (following Machado et al. 2004). The remaining 98 individuals (49 males and 49 females) were kept alive in three communal terraria (40 × 90 cm base, 20 cm height) with a similar number of individuals and a 1:1 sex ratio. One week before the beginning of behavioural observations, the width of the dorsal scute of all individuals were measured with callipers (to the nearest 0.01 mm) and each one was individually marked on the dorsal scute with colour dots of enamel paint.
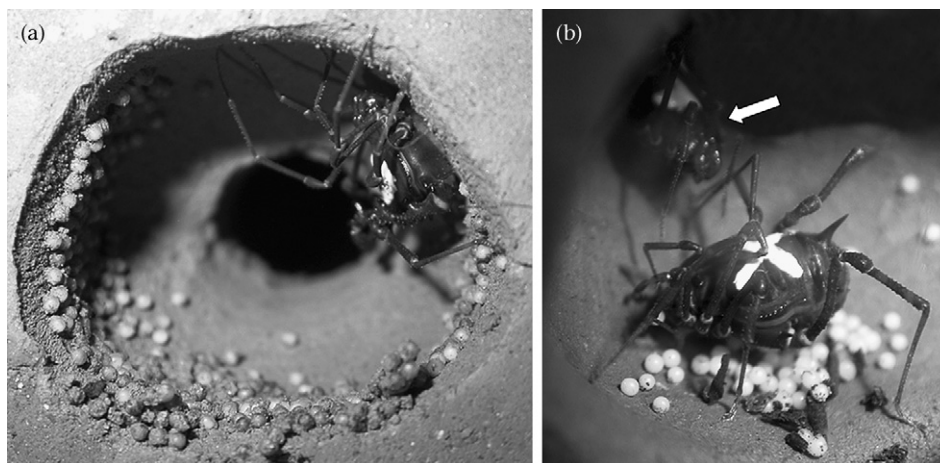

Figure 1. (a) Marked male *Pseudopucrolia* harvestman taking care of a large clutch of eggs laid on an artificial mud nest in the laboratory. Note that the eggs are covered by a coat of debris. (b) Marked *Pseudopucrolia* female everting her ovipositor and manipulating the egg with her chelicerae while scrapping the substrate of the nest with her first pair of legs. Behind her, the guarding male (white arrow) is blocking the nest entrance with his body while she is ovipositing. Both photos were taken throughout the glass wall of the terrarium (photos by B. A. Buzatto).

Three times a week, they were fed pieces of dead cockroaches and an artificial diet for ants (Bhatkar & Whitcomb 1970).

Each terrarium contained soil, small pieces of tree fern trunks and 12 artificial nests built in clay blocks (with 6×2 cm base, 3 cm height). Each artificial nest had a central hole (1 cm in diameter and 2 cm depth) crossing the clay block from side to side. These blocks were placed against the glass wall of the terrarium so that it was possible to observe harvestman behaviour inside the nests (Fig. 1). These artificial nests were immediately used by captive individuals. Moreover, all observed copulations ($N = 36$) and oviposition events ($N = 90$) occurred inside the artificial nests. Thus we concluded that the artificial arena had successfully simulated natural nests. During the study period, mean $\pm$ SD laboratory conditions were $25.5 \pm 1.2\,^\circ$C and $82.0 \pm 5.4\%$ RH, with a 11:13 h light:dark cycle.

Behavioural Observations

The nests of terrarium 1, which contained 17 males and 17 females, were inspected five times a day, at 0830, 1130, 1430, 1730 and 2030 hours. Each time, the identity of the individuals inside each nest was recorded. Continuous recording (sensu Martin & Bateson 1994) was made of all relevant behavioural events, such as fights between males, copulations and ovipositions. Once a day, the eggs in each nest were counted and classified in stages of embryonic development according to their size and coloration (following Hara et al. 2003; Machado et al. 2004). As previously recorded for other harvestmen, recently laid *Pseudopucrolia* eggs are white and small, becoming larger and darker during embryonic development. Daily inspections lasted from May to July 2004, comprising 60 days of sampling. These data were used to provide descriptive information on the reproductive behaviour of the species and to characterize the mating system and the parental activities of the males.

The number of visited nests, nest changes, and records inside the nests were compared between males and females after 60 days of observations using Mann–Whitney tests. The size of guarding and nonguarding males was compared using a t test. Pearson correlations were used to test for relationships between the size of the guarding males, the total number of eggs obtained in their clutches, and the number of females they copulated with. Logistic regressions were used to investigate factors that influenced the probability of males acquiring their first clutch. The independent variables were the number of nests visited by each male until acquiring a first clutch, the number of nest changes made by each male until acquiring a first clutch, and the total number of times that each male was recorded inside the same nest (in this case, the nest where a male acquired his first clutch was used). The dependent variable was the presence (1) or absence (0) of a clutch in the nests of the 17 males.

To test the prediction that paternal care creates more opportunities for females to forage, foraging activities of individuals in terrarium 1 were observed at night, when they were actively exploring and feeding. These samples were taken on 13 nights from 25 June to 17 July 2004. Each sample consisted of 1 h of observation randomly selected from 1800 to 0700 hours, and each time slot was sampled only once. During each sample, the behaviour of all individuals was recorded at 3 min intervals ('fixed-interval time point sampling' sensu Martin & Bateson 1994). Individuals were scored as foraging if they were: (1) walking with the second pair of legs extended sideways or frontward, either touching or not touching the substrate ('exploration' sensu Elpino-Campos et al. 2001); (2) feeding; or (3) walking while carrying food with the chelicerae and/ or pedipalps. Any other behaviour was scored as 'not foraging'. When an individual was not visible in the terrarium (most likely hiding under pieces of tree fern trunks), it was also scored as not foraging. A flashlight with red filter was used for the nocturnal

observations (following Elpino-Campos et al. 2001; Pereira et al. 2004). Food (pieces of dead cockroaches) was always placed in the terrarium 1 h before the beginning of the sampling. The proportion of observations in which females ($N = 17$), and guarding ($N = 6$) and nonguarding males ($N = 11$) were observed foraging during the 13 h of samples was compared using one-way ANOVA. The proportion of time that each individual (P_i) spent foraging was calculated as $P_i = (n_i/T)$, where n_i is the total number of times that an individual i was recorded foraging and T is total number of scans, which was 273 for all individuals (21 scans per one-hour sample $\times$ 13 one-hour samples).

Experimental Manipulation 1: Female Preference

To test the prediction that eggs increase male attractiveness to females, a choice experiment was conducted between July and September 2005. Two males from terraria 2 or 3 were placed in a smaller terrarium (20×10 cm base, 15 cm height) with their respective nests. One of these males had eggs previously deposited in his nest, and the other male was an individual of similar size (paired t test: $t_{13} = 0.430$, $P = 0.674$) that had been a holder of a nest for more than 20 consecutive days but had not obtained any eggs. In the sequence, a gravid female, which had not copulated or laid eggs with any of the experimental males, was placed inside the small terrarium. Females were continuously observed during the first 2 h after their introduction into the experimental terrarium. If copulation and oviposition did not occur during this period, observations were ended and the nests were inspected the following morning to verify whether the female had oviposited in any of the nests. If no oviposition occurred within 1 week, the experiment was staged again with a different gravid female.

After this first phase, males without eggs were returned to their original communal terraria. Males with eggs were kept isolated until all eggs hatched and the nymphs had dispersed. Within 2 months, all first-phase eggs had hatched and all males without clutches in the first phase of the experiment had acquired eggs. Thus, in the second phase of the experiment, the same males were once again paired, but the males without a clutch during the first phase now had eggs and the males that had a clutch during the first phase were now without eggs. Once more, a gravid female was placed in each experimental terrarium and the same procedure as described above was followed. This experiment was replicated 14 times, and the number of males with and without clutches that copulated and acquired eggs in each phase was compared using a Fisher's exact test.

Experimental Manipulation 2: Egg Adoption

To test the prediction that males are willing to guard unrelated eggs, an experiment was conducted in terraria 2 and 3 (containing 16 females and 16 males each) in June 2005. These individuals were left to copulate and oviposit freely during 30 days, after which time 12 males had eggs in their nests. Then, one guarding male was removed from his nest and maintained in a small vial for 4 days. During this period, the experimental nest was monitored ad libitum during daylight, and at 30 min intervals for 4 h from 1800 to 2200 hours. Then new guarding males were removed from the same terrarium, one at a time, but before removing a new guarding male in each case, the guarding male that had been previously removed was returned to the terrarium and placed at the entrance of his nest. The presence, behaviour and residence time of males and females inside the empty nests were recorded at each census. Residence time was calculated as the number of consecutive records of an individual inside the same nest, and if an individual was recorded inside the same nest in two consecutive censuses, it

was assumed that this individual did not leave the nest. The same procedure was adopted if one individual was recorded on the following day inside the same nest where it was recorded in the last census of the previous night.

The frequency of visits in which males and females ate eggs from unattended clutches was compared using a chi-square test. A Fisher's exact test was used to compare the frequency of visiting males and females that defended unattended clutches. Clutch defence was regarded as any aggressive behaviour, such as attacks with the pedipalps or intense leg tapping, which was directed towards another individual. The residence time and the number of eggs consumed by visiting males and females were compared using Mann–Whitney tests. According to the sexual selection hypothesis, the behaviour of males and females in relation to unprotected clutches should differ. Males should adopt unattended eggs, and thus, should remain inside nests longer (more hours or days) than females. Males should also be less eager than females to cannibalize eggs, and only males should show defensive behaviours against potential egg predators, even when the eggs are not genetically related to them.

RESULTS

Mating System

All nests in the terrarium 1 were visited at least once during the study period. Females visited more nests than did males (Table 1), but males were recorded inside the nests more frequently than were females, and males moved among different nests less frequently than did females (Table 1). The same nest could be occupied by more than one individual, but males were less tolerant of other males than they were of females. Whereas females were never observed fighting other individuals (whether male or female) in the same nest ($N = 16$), fights were observed in all cases in which two males were recorded inside the same nest ($N = 12$). As soon as an intruder male entered a mud nest occupied by another male, a brief period of intense mutual tapping with the second pair of legs occurred. After that, the individuals turned their backs to each other and intertwined the fourth pair of legs, which bears many spines and tubercles (Fig. 1a). While in this intertwined position, the males attempted to overturn each other by using a behaviour known as 'nipping 2', which is a sudden upward movement in which one or both males bring their fourth femur close to the body, pinching their opponent's fourth pair of legs (sensu Willemart et al. 2009). The nipping 2 phase generally lasted 30 s, and, in most cases, resident males managed to pull the intruders out of the mud nests ($N = 11$ of 12 fights).

Copulations only occurred inside the nests ($N = 36$). Courtship before, during and after copulations follows a pattern very similar to that described for the related *Chavesincola inexpectabilis* (Nazareth & Machado 2009). There was no size difference between guarding (mean $\pm$ SE $= 7.17 \pm 0.06$ mm; $N = 6$) and nonguarding males (7.02 ± 0.15 mm; $N = 11$) (t test: $t_{15} = 0.910$, $P = 0.382$). A similar result was found when all males from the other two terraria

were included in the analysis (t test: $t_{46} = 1.280$, $P = 0.207$). All males that obtained clutches ($N = 22$) copulated with more than one female, which contributed eggs for multiples clutches. There was no correlation between male size and the total number of eggs that males obtained in their clutches (Pearson correlation: $r_4 = 0.060$, $P = 0.900$) or between male size and the number of females they copulated with ($r_4 = -0.062$, $P = 0.910$). Again, the same pattern was found when all males from the other two terraria were included in the analyses ($P > 0.5$ for both correlations). However, the longer a male's residence time, the greater his chances of acquiring a first clutch (Fig. 2).

Paternal Care

The only form of paternal care shown by the guarding males was egg protection against predation (Fig. 1a). In most attempts of cannibalism observed in the laboratory, guarding males were able to repel intruders (primarily females) before any eggs were eaten
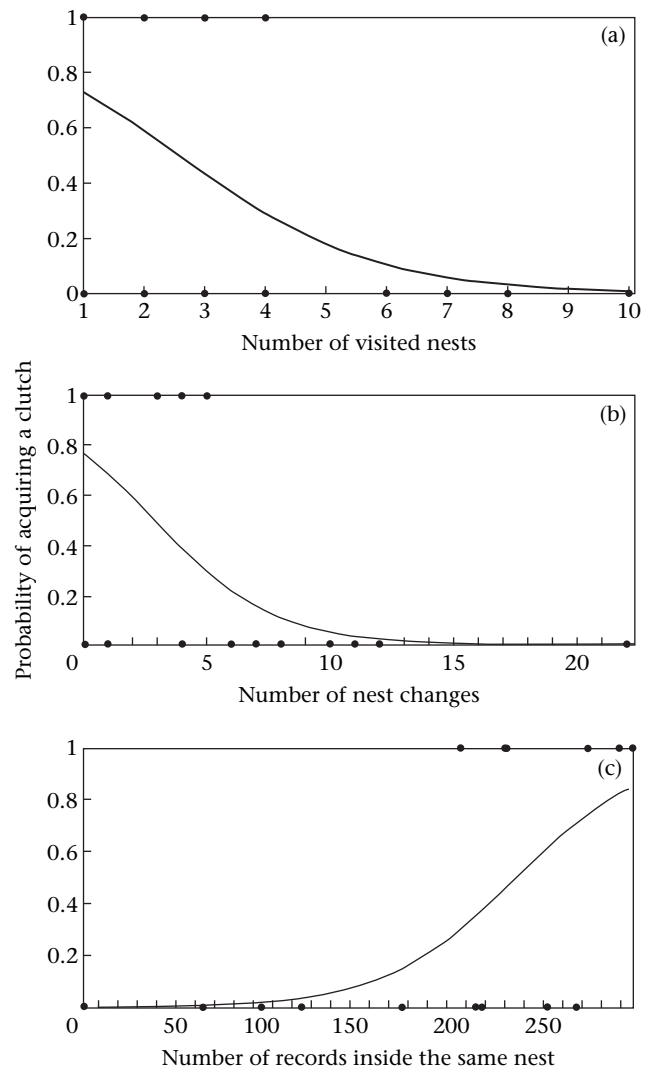
Table 1
Comparison of three parameters of nest use between male ($N = 17$) and female ($N = 16$) *Pseudopucrolia* sp. harvestman reared in captivity

	Males	Females	Statistics
Number of visited nests	4 (1–10)	7.8 (4–10)	$U=40.5$, $P>0.001$
Number of nest changes	5 (0–22)	13 (5–27)	$U=38.3$, $P>0.001$
Number of records inside nests	215 (12–296)	63 (31–162)	$U=56.0$, $P>0.004$

Data are based on 298 point samples taken during 60 days, and are presented as medians (with ranges in parentheses).

Figure 2. Logistic regressions used to investigate factors influencing the probability of *Pseudopucrolia* males acquiring a first clutch. The independent variables were: (a) the number of nests visited by each male until acquiring a first clutch ($\chi^2_1 = 4.903$, odds ratio $= 0.534$, $P = 0.027$); (b) the number of nest changes made by each male until acquiring a first clutch ($\chi^2_1 = 6.517$, odds ratio $= 0.661$, $P = 0.011$); and (c) the total number of times each male was recorded inside the same nest ($\chi^2_1 = 8.690$, odds ratio $= 1.030$, $P = 0.003$).

($N = 8$). In one case, however, a female was observed entering a nest, touching the eggs, and removing some of them with her pedipalps. This female left the nest carrying six eggs, but she was chased by the guarding male. Nearly 20 cm away from the nest entrance, the guarding male grabbed the hind legs of the cannibalistic female, tapped her dorsum with his first legs, and bit her with his chelicerae. The female released five eggs, which were caught by the male and taken back to the nest. After that, the guarding male blocked the nest entrance with his fourth pair of legs for nearly 1 h. In another case, a male managed to grab the hind legs of a cannibalistic female before she left the nest carrying four eggs and, after biting her legs, the guarding male recovered all four eggs. The two females described above entered other nests and laid eggs with different males less than 24 h after the attempted cannibalism, which indicates that they were bearing eggs.

Prediction 1: Females Are Iteroparous

The number of mature eggs in the reproductive tract of the dissected females ranged from 20 to 124 (mean $\pm$ SE $= 67.1 \pm 8.6$ eggs; $N = 15$ females). The number of immature eggs was generally much higher, reaching up 150 eggs (mean $\pm$ SE $= 80.0 \pm 12.0$ eggs). In terrarium 1, seven females copulated with one male, five with two males, and four with three different males. A similar pattern was observed in the other two terraria (data not shown). The mean number of eggs laid in each oviposition event was 39.0 (SE $= 2.1$ eggs; $N = 90$ events in the three terraria), and the mean interval between consecutive ovipositions was 2.1 days (SE $= 0.1$ days; $N = 70$ events).

Prediction 2: Males Have Many Mating Opportunities

Only six of the 17 males in terrarium 1 achieved copulations: two males copulated seven times, three males copulated four times, and one male copulated three times. Thus, clutches of all these males were composed of eggs laid by more than one female. The total number of eggs in the nests was 100, 164, 195, 249, 265 and 291. On two occasions, a guarding male was observed with two females inside his nest. In these cases, while one female was ovipositing, the male courted the other female. When both females were ovipositing, the male alternately touched the dorsum of each female ($N = 1$ observation) or touched both females simultaneously using both legs from the second pair ($N = 1$ observation). A similar observation was made once in the field when individuals were being collected for laboratory work. Males were never seen repelling noncannibalistic females from their nests. Conversely, they generally blocked the entrance of the nest with their body, thus preventing females from leaving after oviposition ($N = 5$ observations; Fig. 1b).

Prediction 3: Paternal Care Creates More Opportunities for Females to Forage

The proportion of time that females spent foraging and feeding was higher than that of guarding males, but similar to that of nonguarding males ($F_{2,31} = 10.846$, $P < 0.001$; Fig. 3). In the great majority of the records (94.2%), females consumed the food items where they had found them. In 58.3% of the records, nonguarding males showed a similar pattern, but in the remaining cases (41.7%), they took the food items back to their nests. Guarding males were recorded feeding only four times, and in all cases the food item was consumed at the nest entrance, but not inside (as nonguarding males did). Guarding males kept the posterior part of the body (including the third and fourth pairs of legs) inside the nest and left the food item outside, probably avoiding contact between eggs and food. In all records of guarding males in exploration activities inside
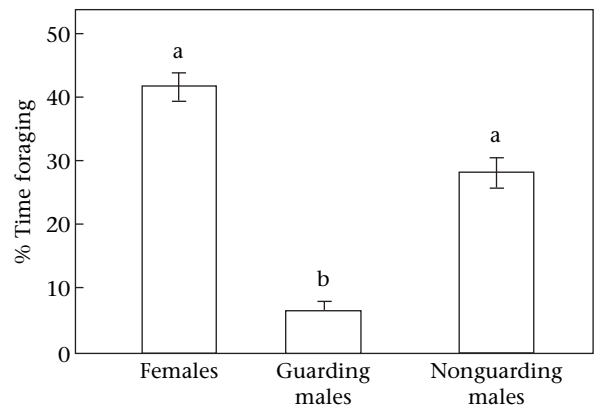

Figure 3. Mean $\pm$ SE percentage of records in which female ($N = 17$) and guarding ($N = 6$) and nonguarding male ($N = 11$) *Pseudopucrolia* harvestman were observed foraging during 13 one-hour samples (total number of records per individual $= 273$). Different letters above the bars indicate a significant difference (Tukey: $P < 0.05$).

the terrarium ($N = 84$), they never walked more than 10 cm away from their nests. Females and most nonguarding males, on the other hand, generally left their shelters (nests or pieces of trunks) and walked through the entire terrarium seeking food.

Prediction 4: Females Prefer to Copulate with Guarding Males

In the first phase of the experiment, 12 males (86%) with clutches were selected by the gravid females and received additional eggs in their clutches. Only two males (14%) without eggs copulated and acquired eggs. In the second phase of the experiment, males with clutches were again preferred by the gravid females: 13 (93%) of them received new eggs in their clutches whereas only one male (7%) without eggs copulated and acquired eggs. Thus, females based their mate selection decisions primarily on the presence of eggs in a male's nest (Fisher's exact test: $P < 0.001$).

Prediction 5: Males Guard Unrelated Eggs

Males and females differed markedly in their responses to unattended, unrelated clutches. Males remained inside unattended nests longer and they cannibalized eggs less often than females did (Table 2). Only 20.4% of the visiting males cannibalized eggs from the nests, whereas 71.1% of the females did so (Table 2). The number of consumed eggs per visit also differed between visiting males and females: males ate up to eight eggs and females ate up to 42 eggs, greatly reducing clutch sizes (Table 2). Finally, only visiting males showed egg-guarding activities, such as repelling intruders (both males and cannibal females) from the nests (Table 2). Females did not show such behaviour and were commonly seen cannibalizing eggs in the company of other individuals, especially females ($N = 40$ observations). However, females never consumed eggs in nests where they had previously laid eggs. Females were seen re-entering nests in which they had laid eggs, inspecting the clutch with the second pair of legs, and leaving the nest without consuming any egg ($N = 5$ observations).

DISCUSSION

Mating System and Paternal Care

Resource defence polygyny occurs when males control females indirectly by defending territories or resources against conspecific

Table 2
Behaviour of male and female *Pseudopucrolia* sp. harvestman that visited 12 experimentally unattended clutches in the laboratory

Behaviour	Males (N=50 visits)	Females (N=83 visits)	Statistics
% Individuals that cannibalized eggs	20.4	71.1	X_1^2=547.59, P<0.001
Median number of cannibalized eggs (min.−max.)	2.5 (1–8)	7 (1–42)	U=141.0, P<0.009
Median residence time (h) (min.−max.)	11.8 (0.02–72)	1.4 (0.02–34.7)	U=1496.0, P<0.009
% Individuals that repelled intruders while inside the nest	34	0	Fisher's exact test: P<0.001

males (Emlen & Oring 1977). Even though arachnids present a high diversity of reproductive strategies (Thomas & Zeh 1984), there have only been three reported cases of resource defence polygyny, all for the order Opiliones (Mora 1990; Macías-Ordóñez 1997, 2000; Buzatto & Machado 2008). In the present study, we briefly describe the polygynous mating system of the paternal harvestman *Pseudopucrolia* sp., whose females use natural cavities in roadside banks as oviposition sites. Males defend these cavities against other males and care for eggs laid by several females. In the laboratory, we were able to simulate the oviposition site of the species using artificial nests built in clay blocks, which allowed us to investigate details of the reproductive biology of this species. Our results indicate that male size does not influence a male's chance of achieving copulations. However, we found that male reproductive success was positively correlated with nest ownership time: the longer a male held a nest, the higher was his chance of acquiring a first clutch. Similar data were obtained for the harvestman *Zygopachylus albomarginis*, in which males build and defend mud nests sought by egg-laying females (Mora 1990). In *Z. albomarginis*, morphological traits of the males or their nests did not account for male reproductive success, but ownership of a mud nest was also crucial for males to achieve copulations (Mora 1990).

Our laboratory observations also suggest that exclusive paternal care in *Pseudopucrolia* has an important role in egg defence. Guarding males always succeeded in repelling aggressive conspecific individuals, and were capable of retrieving eggs that were stolen from their nests by cannibalistic females. Male defence of eggs against female ovicide and egg cannibalism has already been observed in giant water bugs of the subfamily Lethocerinae, whose males care for eggs laid on emergent vegetation (Ichikawa 1990, 1991). In these insects, females destroy the clutches, take over the mate, and lay their own eggs on the oviposition site guarded by the male. *Pseudopucrolia* females, on the other hand, first copulated with the guarding males and then attempted to cannibalize some of the eggs guarded by the males. These females were never observed laying eggs in the nest immediately after cannibalism attempts, suggesting that this is not a case of infanticide to facilitate the production of replacement young, as described for giant water bugs (Ichikawa 1990, 1991). Moreover, the fact that females do not return to mate and oviposit with the resident males that have ejected them from their nests suggests that cannibalism attempts are not used as a strategy for evaluating male commitment to egg defence. Because gravid females are eager to feed on unrelated eggs, one might wonder about the low frequency of attempts of cannibalism in protected nests. It seems that most females do not attempt to eat unrelated eggs because they will be driven off by the resident male and deprived of his care.

Although our experiment on whether males would guard unrelated eggs (prediction 5) was not designed to test the role of males in preventing egg predation, we found that unprotected clutches were severely reduced by conspecifics, primarily by females. In at least two other harvestman species with exclusive paternal care, *Z. albomarginis* and *Iporangaia pustulosa*, male assistance also reduces egg predation (Mora 1990; Requena et al. 2009). In both species, conspecifics are the principal cause of egg mortality in unattended clutches. Contrary to *Zygopachylus* (Mora

1990), whose clutches are frequently attacked by fungi, *Pseudopucrolia* clutches were never attacked by fungi in the field or in the laboratory. Fungal attack on eggs may explain why *Zygopachylus* males manipulate and clean eggs as a part of their brooding behaviour (Mora 1990), whereas *Pseudopucrolia* males do not.

Sexual Selection Predictions

In the present study, we tested predictions from the hypothesis of evolution of paternal care via sexual selection, using *Pseudopucrolia* sp. as the model organism. The first prediction was that the species should be iteroparous because females cannot benefit from males that care for offspring unless they have the physiological capability and ecological opportunity to lay more than one clutch during a breeding season (Tallamy 2000, 2001). The freedom to continue foraging and producing eggs after laying the first clutch provides no advantages for females constrained to semelparity (Tallamy & Brown 1999). In fact, we found that *Pseudopucrolia* females are iteroparous because they produce eggs continuously. The same pattern has been found in other harvestman species with paternal care, such as *Z. albomarginis* (Mora 1990), *Ampheres leucopheus* (Hara et al. 2003), *Gonyleptes saprophilus*, *Iporangaia pustulosa*, *Iguapeia melanocephala*, *Neosadocus* sp. and *Progonyleptoidellus striatus* (Machado et al. 2004). Moreover, in at least one of these, *I. pustulosa*, a long-term study in the field also showed that there is no seasonal interruption in the oviposition activity of the population (Requena et al. 2009). This phenological pattern differs remarkably from that of harvestman species showing maternal care, in which females generally produce only one clutch per reproductive season and the oviposition is seasonal (Machado & Macías-Ordóñez 2007; Buzatto et al. 2007). Field studies with *Pseudopucrolia* are needed to determine whether oviposition under natural conditions occurs continuously throughout the year, or whether females are somehow constrained to a clearly defined reproductive season.

Postovipositional maternal care in arthropods is a costly trait because it reduces lifetime fecundity and foraging opportunities for guarding females during long periods of care, especially in predatory species (Tallamy & Denno 1982; Fink 1986; Gundermann et al. 1997; Tallamy & Schaefer 1997; Stegmann & Linsenmair 2002; Zink 2003; Buzatto et al. 2007). However, care-related reductions in feeding activities are predicted to be less costly for male reproductive potential than they are for female reproductive potential because the production of sperm and other seminal products generally requires fewer nutrients than does the production of eggs (Parker et al. 1972). The 'enhanced fecundity hypothesis' (Maynard Smith 1982) proposes that in species in which postzygotic care is crucial for offspring survival, and in which such care may be provided effectively by either one or two parents, paternal care may minimize the physiological costs related to egg guarding by females, allowing them to forage immediately after oviposition without sacrificing offspring survivorship (Tallamy 2000, 2001). Our behavioural observations showed that *Pseudopucrolia* females are free to forage just after oviposition, and quantitative data demonstrated that females' foraging rates were much higher than their egg-guarding rates.

Additionally, the interval between two consecutive ovipositions was only 2 days, which is almost three times shorter than the interval between two ovipositions in the maternal brooding gonyleptid *Discocyrtus oliverioi*, which was also studied in captivity where food was always available (Elpino-Campos et al. 2001). This 2-day interval is also 10–15 times shorter than the median interval between two ovipositions in three other maternal gonyleptids studied in the field (where food is supposed to be a limiting factor for female fecundity): *Bourguyia hamata* (Machado & Oliveira 2002), *Goniosoma albiscriptum* (Willemart & Gnaspini 2004) and *Acutisoma proximum* (Buzatto et al. 2007). Apparently, the reproductive rate of *Pseudopucrolia* females is higher than that of females of species with maternal care, which is most likely a consequence of their increased foraging rate. However, because the mean number of mature eggs in the ovary of the dissected females was almost two times greater than the mean number of eggs laid at each oviposition event, this difference in reproductive rate may be at least partially explained by the fact that gravid females do not lay all of their mature eggs at once, as a bet-hedging strategy. The number of eggs laid in each oviposition may depend on the courtship skills of the males, so that better courting behaviour by a male will increase the number of eggs a female lays with him (Eberhard 1996).

Exclusive postzygotic paternal care may be favoured through sexual selection because it increases male mating opportunities by simultaneously offering females the direct, fitness-enhancing gift of cost-free care of their offspring (Trivers 1972). Paternal care may also provide an honest signal of the male's quality as offspring defender, and thus guarding males should be selected by females (Tallamy 2000, 2001). Here, we showed that *Pseudopucrolia* males have many mating opportunities, and all clutches in the field and in the laboratory were composed of eggs laid by several females. Moreover, we demonstrated that guarding males are preferred by ovipositing females. Mora (1990) has already shown that females of the harvestman *Z. albomarginis* copulate only with males that have a mud nest, where eggs are laid. However, this is the first experimental demonstration for arthropods where the presence of eggs in the males' nests is a sexually selected trait used by females to evaluate potential mates. Similar results have been reported for some fish in which parental males are preferred over the nonparental males (see references in: Forsgren et al. 1996; Lindström et al. 2006).

Given that the possession of a clutch seems to enhance male attractiveness, intense selection should pressure all males to guard because this is the route to increased mating. In this context, males should not only accept unrelated eggs into their brood, but they should also compete with other males for egg ownership (Tallamy 2000, 2001). Our experiment with unattended clutches of *Pseudopucrolia* demonstrated that the behaviour of visiting males and females differs markedly. As should be expected by the sexual selection hypothesis, the great majority of the visiting females cannibalized eggs from unattended clutches, sometimes drastically reducing clutch size. Additionally, no female showed aggressive behaviours towards other nest visitors in an attempt to protect the clutch from predation. Males, on the other hand, rarely cannibalized eggs, and when they did so, they consumed significantly fewer eggs than did females. The most impressive result, however, was that 34% of the visiting males presented aggressive behaviours towards nest visitors, in a clear attempt to protect the clutch from potential predators. By defending unrelated eggs, *Pseudopucrolia* males are providing an honest signal of their paternal intent for females, and may attract mates that will supply several clutches.

Concluding Remarks

Harvestmen are considered one of the first arachnids to live on land, and fossils from the Devonian indicate that unique morphological features of the group were already present as early as 400 million years ago (Dunlop 2007). It is possible, therefore, that harvestmen were the first land animals to invest in parental care, as well as the first to show exclusive paternal care. Independently of the selective pressures that have favoured the origin of exclusive paternal care in harvestmen, we suggest that this behaviour is currently maintained in *Pseudopucrolia* by two selective pressures: (1) natural selection, favouring males that are able to defend a nest and repel egg predators efficiently, and (2) sexual selection, favouring (a) female preference for guarding males, and (b) adoption of unrelated eggs by nonguarding males (see also Requena et al. 2009). Sexual selection may have also favoured territoriality in *Pseudopucrolia* because males may guard nests to attract mates. Nevertheless, egg-guarding behaviour almost certainly arose secondarily within the territories (see discussion in Zeh & Smith 1985). In fact, another field experiment demonstrated that territorial males of the harvestman *Acutisoma proximum* (Gonyleptidae) temporarily care for clutches that are left unattended by guarding females from their harems (Buzatto & Machado 2009). Facultative male care in harvestmen provides support for the hypothesis that paternal care, even for short periods, is likely to occur when males defend a territory that is also an oviposition site (Williams 1975; Ridley 1978). In the future, comparative studies should test whether exclusive paternal care in harvestmen is more likely to evolve in lineages with a territory-based polygynous mating system, as previously described for fish and anurans (Gross & Shine 1981; Lehtinen & Nussbaum 2003; Ah-King et al. 2005; but see Beck 1998). This information may clarify the selective pressures responsible for the evolutionary origin of exclusive paternal care and the route that has led to the appearance of this rare behavioural trait in harvestmen.

References

Ah-King, M., Kvarnemo, C. & Tullberg, B. S. 2005. The influence of territoriality and mating system on the evolution of male care: a phylogenetic study on fish. *Journal of Evolutionary Biology*, **18**, 371–382.

Beck, C. W. 1998. Mode of fertilization and parental care in anurans. *Animal Behaviour*, **55**, 439–449.

Bhatkar, A. & Whitcomb, W. H. 1970. Artificial diet for rearing various species of ants. *Florida Entomologist*, **53**, 229–232.

Buzatto, B. A. & Machado, G. 2008. Resource defense polygyny shifts to female defense polygyny over the course of the reproductive season of a Neotropical harvestman. *Behavioral Ecology and Sociobiology*, **63**, 85–94.

Buzatto, B. A. & Machado, G. 2009. Amphisexual care in the Neotropical harvestman *Acutisoma proximum* (Arachnida: Opiliones). *Insectes Sociaux*, **56**, 106–108.

Buzatto, B. A., Requena, G. S., Martins, E. G. & Machado, G. 2007. Effects of maternal care on the lifetime reproductive success of females in a Neotropical harvestman. *Journal of Animal Ecology*, **76**, 937–945.

Clutton-Brock, T. H. 1991. *The Evolution of Parental Care*. Princeton, New Jersey: Princeton University Press.

Dunlop, J. A. 2007. Paleontology. In: *Harvestmen: The Biology of Opiliones* (Ed. by R. Pinto-da-Rocha, G. Machado & G. Giribet), pp. 247–265. Cambridge, Massachusetts: Harvard University Press.

Eberhard, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice. Monographs in Behavior and Ecology*. Princeton, New Jersey: Princeton University Press.

Elpino-Campos, A., Pereira, W., Del-Claro, K. & Machado, G. 2001. Behavioral repertory and notes on natural history of the Neotropical harvestman *Discocyrtus oliverioi* (Opiliones: Gonyleptidae). *Bulletin of the British Arachnological Society*, **12**, 144–150.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual selection, and evolution of mating systems. *Science*, **197**, 215–223.

Fink, L. S. 1986. Costs and benefits of maternal behavior in the green lynx spider (Oxyopidae, *Peucetia viridans*). *Animal Behaviour*, **34**, 1051–1060.

Forsgren, E., Karlsson, A. & Kvarnemo, C. 1996. Female sand gobies gain direct benefits by choosing males with eggs in their nests. *Behavioral Ecology and Sociobiology*, **39**, 91–96.

Gross, M. R. 2005. The evolution of parental care. *Quarterly Review of Biology*, **80**, 37–45.

Gross, M. R. & Shine, R. 1981. Parental care and the mode of fertilization in ectothermic vertebrates. *Evolution*, **35**, 775–793.

Gundermann, J. L., Horel, A. & Roland, C. 1997. Costs and benefits of maternal care in a subsocial spider, *Coelotes terrestris*. *Ethology*, **103**, 915–925.

Hara, M. R., Gnaspini, P. & Machado, G. 2003. Male guarding behavior in the Neotropical harvestman *Ampheres leucopheus* (Mello-Leitão 1922) (Opiliones, Laniatores, Gonyleptidae). *Journal of Arachnology*, **31**, 441–444.

Ichikawa, N. 1990. Egg mass destroying behaviour of the female giant water bug, *Lethocerus deyrollei* Vuillefroy (Heteroptera: Belostomatidae). *Journal of Ethology*, **8**, 5–11.

Ichikawa, N. 1991. Egg mass destroying and guarding behaviour of the giant water bug, *Lethocerus deyrollei* Vuillefroy (Heteroptera: Belostomatidae). *Journal of Ethology*, **9**, 25–29.

Kokko, H. & Jennions, M. 2003. It takes two to tango. *Trends in Ecology & Evolution*, **18**, 103–104.

Kury, A. B. 1994. Early lineages of Gonyleptidae (Arachnida, Opiliones, Laniatores). *Tropical Zoology*, **7**, 343–353.

Lehtinen, R. M. & Nussbaum, R. A. 2003. Parental care: a phylogenetic perspective. In: *Reproductive Biology and Phylogeny of Anura* (Ed. by B. G. M. Jamieson), pp. 343–386. Enfield, New Hampshire: Science.

Lindström, K., St Mary, C. M. & Pampoulie, C. 2006. Sexual selection for male parental care in the sand goby, *Pomatoschistus minutes*. *Behavioral Ecology and Sociobiology*, **60**, 46–51.

Machado, G. & Macías-Ordóñez, R. 2007. Reproduction. In: *Harvestmen: the Biology of Opiliones* (Ed. by R. Pinto-da-Rocha, G. Machado & G. Giribet), pp. 414–454. Cambridge, Massachusetts: Harvard University Press.

Machado, G. & Oliveira, P. S. 2002. Maternal care in the Neotropical harvestman *Bourguyia albiornata* (Arachnida, Opiliones): oviposition site selection and egg protection. *Behaviour*, **139**, 1509–1524.

Machado, G. & Raimundo, R. L. G. 2001. Parental investment and the evolution of subsocial behaviour in harvestmen (Arachnida: Opiliones). *Ethology, Ecology and Evolution*, **13**, 133–150.

Machado, G., Requena, G. S., Buzatto, B. A., Osses, F. & Rossetto, L. M. 2004. Five new cases of paternal care in harvestmen (Arachnida: Opiliones): implications for the evolution of male guarding in the Neotropical family Gonyleptidae. *Sociobiology*, **44**, 577–598.

Macías-Ordóñez, R. 1997. The mating system of *Leiobunum vittatum* Say 1821 (Arachnida: Opiliones: Palpatores): resource defense polygyny in the striped harvestman. Ph.D. thesis, Lehigh University, U.S.A.

Macías-Ordóñez, R. 2000. Touchy harvestmen. *Natural History*, **109**, 58–61.

Martin, P. & Bateson, P. 1994. *Measuring Behaviour: an Introductory Guide*. Cambridge: Cambridge University Press.

Maynard Smith, J. 1982. *Evolution and the Theory of Games*. Cambridge: Cambridge University Press.

Mora, G. 1990. Parental care in a Neotropical harvestman, *Zygopachylus albomarginis* (Arachnida: Gonyleptidae). *Animal Behaviour*, **39**, 582–593.

Nazareth, T. M. & Machado, G. 2009. Reproductive behavior of *Chavesincola inexpectabilis* (Opiliones: Gonyleptidae), with the description of a new and independently evolved case of paternal care in harvestman. *Journal of Arachnology*, **37**, 127–134.

Parker, G. A., Baker, R. R. & Smith, V. G. F. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. *Journal of Theoretical Biology*, **36**, 29–553.

Pereira, W., Elpino-Campos, A., Del-Claro, K. & Machado, G. 2004. Behavioral repertory of the Neotropical harvestman *Ilhaia cuspidata* (Opiliones, Gonyleptidae). *Journal of Arachnology*, **32**, 22–30.

Queller, D. C. 1997. Why do females care more than males? *Proceedings of the Royal Society B*, **264**, 1555–1557.

Requena, G. S., Buzatto, B. A., Munguía-Steyer, R. & Machado, G. 2009. Efficiency of uniparental male and female care against egg predators in two closely related, syntopic harvestmen. *Animal Behaviour*, **78**, 1169–1176.

Ridley, M. W. 1978. Paternal care. *Animal Behaviour*, **26**, 904–932.

Stegmann, U. E. & Linsenmair, K. E. 2002. Assessing the semelparity hypothesis: egg-guarding and fecundity in the Malaysian treehopper *Pyrgauchenia tristaniopsis*. *Ethology*, **108**, 857–869.

Tallamy, D. W. 2000. Sexual selection and evolution of exclusive paternal care in arthropods. *Animal Behaviour*, **60**, 559–567.

Tallamy, D. W. 2001. Evolution of exclusive paternal care in arthropods. *Annual Review of Entomology*, **46**, 139–165.

Tallamy, D. W. & Brown, W. P. 1999. Semelparity and the evolution of maternal care in insects. *Animal Behaviour*, **57**, 727–730.

Tallamy, D. W. & Denno, R. F. 1982. Life-history trade-offs in *Gargaphia solani* (Hemiptera, Tingidae): the cost of reproduction. *Ecology*, **63**, 616–620.

Tallamy, D. W. & Schaefer, C. 1997. Maternal care in the Hemiptera: ancestry, alternatives, and current adaptive value. In: *Social Behavior in Insects and Arachnids* (Ed. by J. C. Choe & B. J. Crespi), pp. 94–115. Cambridge: Cambridge University Press.

Thomas, R. H. & Zeh, D. W. 1984. Sperm transfer and utilization strategies in arachnids: ecological and morphological constraints. In: *Sperm Competition and the Evolution of Animal Mating Systems* (Ed. by R. L. Smith), pp. 179–221. London: Academic Press.

Trivers, R. L. 1972. Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man* (Ed. by B. Campbell), pp. 136–179. Chicago: Aldine.

Willemart, R. H. & Gnaspini, P. 2004. Breeding biology of the cavernicolous harvestman *Goniosoma albiscriptum* (Arachnida, Opiliones, Laniatores): sites of oviposition, egg batches characteristics and subsocial behaviour. *Invertebrate Reproduction & Development*, **45**, 15–28.

Willemart, R. H., Osses, F., Chelini, M. C. & Machado, G. 2009. Sexually dimorphic legs in a Neotropical harvestman (Arachnida, Opiliones): ornament or weapon? *Behavioural Processes*, **80**, 51–59.

Williams, G. C. 1975. *Sex and Evolution*. Princeton, New Jersey: Princeton University Press.

Zeh, D. W. & Smith, R. L. 1985. Paternal investment by terrestrial arthropods. *American Zoologists*, **25**, 785–805.

Zink, A. G. 2003. Quantifying the costs and benefits of parental care in female treehoppers. *Behavioral Ecology*, **14**, 687–693.