

Intersexual cooperation during male clasping of external female genitalia in the spider *Physocyclus dugesi* (Araneae, Pholcidae)

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Abstract Sexual conflict may influence the shape and evolution of body structures that males use to grasp females during mating. Not only sexual coercion but also intersexual cooperation may be involved during clasping behavior. Among pholcid spiders, secondary sexual modifications of the male chelicerae, such as apophyses with spines or tooth-like processes, function to grasp the female by specific parts of her external genitalia such as grooves or apophyses of the epigynum. We analyzed how the female and the male respond when their structures for clasping are experimentally modified in the pholcid *Physocyclus dugesi*. We used three treatment groups for virgin females that differed in the manipulation of the epigynum apophyses (uncovered, partially covered, and fully covered by a plaster) and two groups of males (uncovered and fully covered cheliceral apophyses). We found that females are mainly cooperative to courting males not only when the female genital apophyses were experimentally covered but also when the male cheliceral apophyses were covered. The current data also indicate behavioral flexibility in males during courtship, especially when they had difficulty in genital intromission. Our experimental results, together with previous observational studies, support a modulated-cooperative scenario between the sexes for cheliceral clasping and genital intromission in pholcid spiders.

Keywords Sexual dimorphism · Mating · Clasping structures · Sexual conflict · Sexual cooperation · Arachnids

Introduction

Conflict between the sexes over control of copulation may drive the coevolution of complex genitalia and secondary sexual structures (Arnqvist and Rowe 1995; Alexander et al. 1997; Holland and Rice 1999). This coevolution is predicted to result in traits for resistance by females and for coercion by males (Clutton-Brock and Parker 1995; Arnqvist and Rowe 2002; Chapman et al. 2003; Chapman 2006; Parker 2006). Sexual coercion during mating can select for male structures such as claspers to grasp the partner forcefully. Females can use anticlasper devices or resistance behavior to avoid or minimize the costs imposed by forced copulation. For example, among arthropods this kind of interaction occurs in water striders (Arnqvist 1989; Arnqvist and Rowe 1995) and camel-spiders (Peretti and Willemart 2007). In contrast to the sexual conflict hypothesis, the female choice hypothesis suggests a “selective cooperation” from females toward males before and during mating (Cordero and Eberhard 2003, 2005), which may involve sexually dimorphic structures (Eberhard 1996). Moreover, a prediction is that males will use sexual stimulation instead of physical coercion to persuade females to mate (Eberhard 1996, 2004a). Cases of selective behavioral intersexual cooperation during copulation occur in some beetles, flies, and spiders (Eberhard 1996; Huber 1998a; Eberhard 2004a, b).

In many arthropods, the data utilized to support either of these two hypotheses have principally come from comparative morphological studies focused on the male

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grasping structures and the corresponding anchored body region of the female (e.g., Huber 1998a, 1999; Bergsten et al. 2001; Eberhard 2006). However, it is necessary to consider in detail the associated behavior in both sexes. Otherwise, it is difficult to discriminate accurately whether a male utilizes a particular structure in a coercive or cooperative form during copulation (Peretti and Córdoba-Aguilar 2007). In addition, experimental manipulations of these structures (e.g., by modifying the shape or length) have proved useful in determining the function of particular structures (e.g., Thornhill and Sauer 1991; Arnqvist and Rowe 1995; Eberhard 2001, 2002; Ronkainen et al. 2005).

Pholcid spiders of the genus *Physocyclus* show conspicuous and interesting traits to investigate the sexual coercion and sexual cooperation hypotheses. Like other pholcids (Huber 1999), males use cheliceral apophyses and palps to grasp the epigynum (i.e., the chelicerae form one side of the “clamp”). For example, in *Physocyclus globosus* the male chelicerae engage the female epigynum. The bifurcated apophysis on the anterior end of the epigynum is lodged in the paired cavities on the male chelicerae, while the tooth-like processes on the male chelicerae are pressed against the heavily sclerotized bulges of the female epigynum (Huber and Eberhard 1997). This contact persists throughout copulation. In another interesting but still poorly studied species of this genus, *Physocyclus dugesi*, the epigynal bifurcated apophyses are noticeably larger than those of *P. globosus* and the male lacks a paired cavity. In this species, the tooth-like processes of the male chelicerae are smaller than the apophyses of *P. globosus* but more abundant (Huber 1997; A. Peretti pers. obs.). *Physocyclus dugesi* differs from *P. globosus* and the other studied pholcids in that there appears to be sexual conflict over mating since females are very reluctant to copulate more than once prior to the first oviposition, and precopulatory sexual cannibalism by both males and females often occurs (A. Peretti and I. Rodríguez-Márquez, unpub. data). Given these characteristics, *P. dugesi* is an interesting model in which to examine the use and the associated behavior of these nongenitalic (in the male) and genitalic (in the female) apophyses (Huber and Eberhard 1997; Huber 1998a, b, 1999) and to test the sexual conflict and selective cooperation hypotheses.

We consider two principal predictions from sexual coercion and precopulatory selective cooperation to evaluate the pattern of clasping of the female external genitalia: (1) if the male uses the claspers to force the female to copulate, she should not help him during the courtship if he shows difficulty for clasping (e.g., this is apparently the case in *Gerris odontogaster*; Arnqvist 1989). Additionally, it could also be interpreted as precopulatory female choice as this rejection of help could be a female barrier for males

to overcome (thus, she would choose males to father her offspring that were able to pass this barrier). In contrast, if the use of the claspers occurs in a context of mutual sexual cooperation, the female will assist, at least at the beginning of the interaction, the male to achieve the clasping (e.g., in *Sepsis* flies; Eberhard 2001, 2002). (2) If the female structure functions to thwart male mating attempts, it is predicted that once this structure is covered with a plaster to eliminate its functioning, then the male will perform grasping more easily (e.g., by taking less time to anchor the claspers appropriately as was reported in the water strider *G. incognitus*; Arnqvist and Rowe 1995). In contrast, if the female structure functions to aid the male, covering it should result in more difficulties during the clasping attempts of the male (e.g., taking more time, as was also reported in *Sepsis* flies; Eberhard 2001, 2002), including female cooperation at least during an initial tolerance phase (e.g., by body reorientation toward the male instead of a quick withdrawal).

In this context, the present study has three principal objectives: (1) to describe in *P. dugesi* how the male cheliceral apophyses fit with the female epigynal projections, (2) to examine in both sexes the behavior associated with use of these clasping structures and how they respond after manipulating their respective apophyses, and (3) to identify behaviors and traits that indicate presence of sexual coercion or cooperative behavior during clasping, interpreting the data in the light of current hypotheses of sexual selection.

Materials and methods

Study species: collection and rearing

Physocyclus dugesi is found in the central region of Mexico (D.F and near districts) where it lives in close association with manmade buildings. *Physocyclus dugesi* lives at least 2 years and reproductive activity occurs throughout the year, in particular during May–September. Females produce two to four egg sacs per year (A. Peretti, pers. obs.). Juvenile spiders were collected from summer 2002 until autumn 2003 from buildings within the city of Pachuca (Mexico). Animals were kept individually in PVC pots (10 cm diameter × 13 cm height). *Drosophila* sp. and *Musca domestica* flies were provided as food once a week. Moist cotton balls were utilized to maintain humidity. After maturity, females were transferred into separate plastic cups (20 cm diameter × 20 height), in which mating experiments were conducted after females had built a web. Spiders were kept at room temperature (mean 24.50°C ± SE 0.53°C) and in a light:dark cycle of 15 h:9 h.

Morphology of apophyses and clasping mechanism

We fixed ten copulating spiders with “ethyl clorure” that was poured over them, to observe the anchoring mechanism between the male cheliceral apophyses and the epigynal surface. The frozen spiders were transferred into 80% ethanol. We also utilized scanning electron microscopy to describe the fine structure of the external female genitalia and the male cheliceral apophyses. Specimens utilized for electron microscopy were dehydrated in ethanol, dried in a critical point dryer, coated with gold in a sputtering device, and examined in scanning electron microscope.

Effect of manipulation of clasping mechanism on behavior

We investigated the effect of inoperative male and female apophyses on mating behavior by using three independent experimental groups ($N = 20$ individuals in each group): (1) manipulated females by covering the epigynum apophyses with a plaster (water-soluble gum) that impeded genital fitting but did not prevent it (in this group males were not modified); (2) manipulated females, where the entire epigynum including the apophyses was covered with plaster, which prevented genital fitting with the male chelicerae (in this group males were not modified); and (3) manipulated males, where the cheliceral apophyses were covered with a plaster (in this group females were not modified). We did not analyze sexual interactions between a modified male with a modified female because pilot observations showed that the plaster covering both individuals prevented any possibility of body contact around their respective genital area. All manipulations were carried out precisely (i.e., the plaster never covered the female genital aperture and the male mouth). The control group was composed of 20 males and 20 females without any type of modification on their apophyses. We compared variables of sexual behavioral patterns (see below) of the three experimental groups with those of a control group. Individuals of the control group were handled similarly to those of experimental group, except that no plaster was deposited on their bodies. In all groups, each individual was utilized only once. To minimize possible effects of body size of males and females on analyzed variables (e.g., behavioral patterns, clutch size) we always used individuals of similar body size (using cephalothorax width as index; Huber 1996; Peretti et al. 2006) between the different treatment groups (one-way ANOVA of body size between the four groups: males: $F = 1.24$, $P = 0.25$, $N = 20$ in each group; females: $F = 1.08$, $P = 0.30$, $N = 20$ in each group).

All sequences of courtship and copulation were video-recorded with a digital video camera (SONY-DCR-TRV 351) equipped with +6 close-up lenses. Careful positioning allowed close-up views of movements of males and females, in particular of both of the male chelicerae on the female epigynum. Events were transcribed from videotapes by using the analysis of behavior programs Etholog 2.2 (Ottoni 2000) and JWatcher 0.9 (Blumstein et al. 2000). Following criteria assumed by previous studies on mating in pholcid spiders (Schäfer and Uhl 2002; Peretti et al. 2006), we mainly focused our observations in the following variables related to the courtship and copulation. (1) Precopulatory phase: (a) courtship duration, (b) absolute number and rate (absolute number/courtship duration) of male stimulatory patterns (stridulation and courtship with legs), and (c) absolute number and rate of behavior associated with clasping and beginning of genitalic intromission (palpal rotation, palpal re-accommodation, attempts of insertion); and (2) Copulatory phase: (a) copulation duration, (b) absolute number and rate of palpal genitalic movements of the male, and (c) which sex ended copulation first. The male ended copulation first by removing slowly his palps from the female gonopore after a final squeeze. In contrast, the female ended copulation first by moving her body away quickly while the male was squeezing. In every male-female interaction, we paid special attention to female behavior to detect subtle but critical patterns that could facilitate or impede male behavior (e.g., body postures and movement).

Complementary to general ethological analysis, we examined all the video-taped sequences of mating on computer, frame by frame. This step was important to detect whether modified females showed more cooperation or rejection for courting males when the males had difficulty anchoring their cheliceral apophyses in the covered epigynum. In females, willingness to perform a new courtship with a new male (i.e., different from the previous male) was tested daily until the first oviposition.

Effect of clasping manipulation on female fecundity and fertility of eggs

After this first part of the work, we concentrated our attention on the inseminated females. We examined whether modification of apophyses could have effects on immediate female fecundity, a trait under natural selection and usually correlated with female size (Andersson 1994). Other studies in arthropods have shown that morphological manipulation of males can affect variables of fecundity and fertility (Arnqvist and Rowe 2005; Córdoba-Aguilar 2006). For this purpose, clutch size and percentage of fertilized eggs per sac were recorded. Males were removed after

copulation, and females were allowed to lay one egg sac in their respective mating boxes.

Statistics

Mean values are presented \pm one standard deviation. We utilized Kruskal–Wallis test to compare the four studies groups. Subsequently, Mann–Whitney U test was carried out to compare behavior between two groups. Tests were two-tailed with α set to 0.05.

Results

Morphology of clasping: male and female structures

Male apophyses that act as claspers are formed by the frontal area of the chelicerae, which possesses many small tooth-like processes (mean = 33 ± 4) and a large tooth-like process on the lateral basal part of each chelicera (Fig. 1a). Female chelicerae lack these apophyses and processes. The anchoring area of the female epigynum is constituted by a large flattened and sclerotized apophysis that is bifurcated on its posterior end (Fig. 1c). The surface of the apophysis is smooth and, as the frontal area of male chelicerae, is covered by many thin setae.

After approaching the female, the male twists his palps about 90° at the coxa-trochanter joint (similar to that of *P. globosus*; Huber and Eberhard 1997) and then contacts and presses the clasping area of his chelicerae against the surface of the female's epigynal apophyses (Fig. 1b). Once the claspers are anchored in the epigynal apophyses, the male inserts the palps simultaneously into the female

genital aperture. Cheliceral clasping persists throughout copulation, which presents continuous twisting and squeezing movements of the male palps (squeezing pattern is similar to those described in *P. globosus* by Huber and Eberhard 1997).

Effect of manipulation of clasping mechanism on sexual behavior

Precopulatory behavior

There was a highly significant overall effect of apophyses manipulation on duration of courtship in comparison with the control group (Kruskal–Wallis test, $H = 22.82$, $P = 0.00004$). This was more evident in the groups of fully modified females and modified males, in which courtship was longer (Fig. 2; Table 1). Indeed, in the fully modified female group, courtship duration was almost seven times greater than that of the control group. After careful reanalysis of the videos, we detected that the increment in courtship duration among experimental groups, including that of partially modified females, occurred because males could not insert their palps into the female genital aperture to begin copulation. In this situation, the number of palp rotations performed by males prior to genital intromission differed significantly between some groups (Kruskal–Wallis test, $H = 25.03$, $P = 0.00001$; Table 1). The highest frequency of occurrence of this pattern corresponded to mating sequences performed by unmodified males with fully modified females (Fig. 3). In relation to this difficulty during clasping, males performed more of the stimulatory patterns “stridulation” and “courtship with legs” in the experimental groups in comparison with the

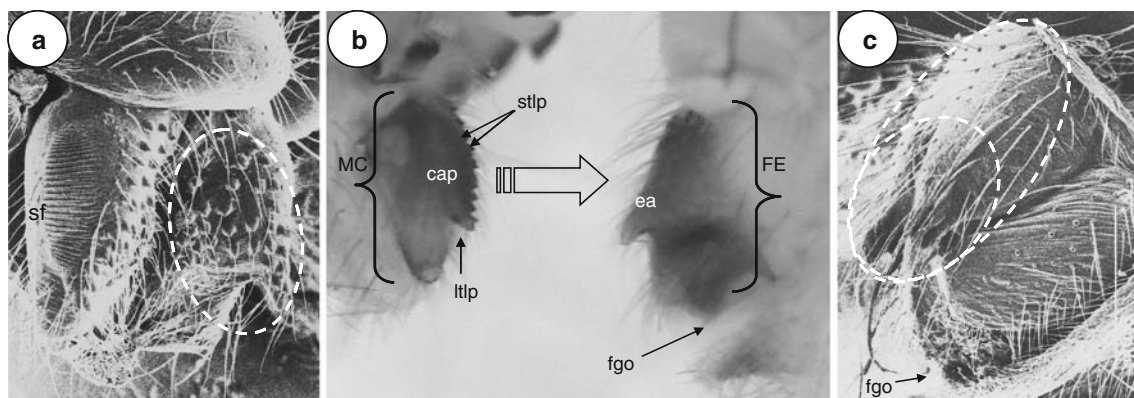


Fig. 1 Male and female structures utilized for clasping in *Physocyclus dugesi*: **a** Scanning electron micrograph of the male chelicerae showing the surface of the spiny apophyses (semilateral view). The white oval indicates the part covered with the plaster (only illustrated for one chelicera). **b** Lateral view of the male chelicera (*left*) and the female epigynum (*right*) immediately before the contact between both regions (position indicated by the *medial arrow*). **c** Scanning electron

micrograph of the female epigynum showing the two elongated apophyses. White ovals indicate the parts covered by the plaster according to the experimental group (small oval: partially modified females; large oval: fully modified females). Abbreviations: *cap* cheliceral apophysis, *ea* epigynal apophyses, *FE* epigynum, *fgo* female genital aperture, *ltlp* large tooth-like process, *MC* male chelicera, *sf* stridulatory file, *stlp* small tooth-like processes

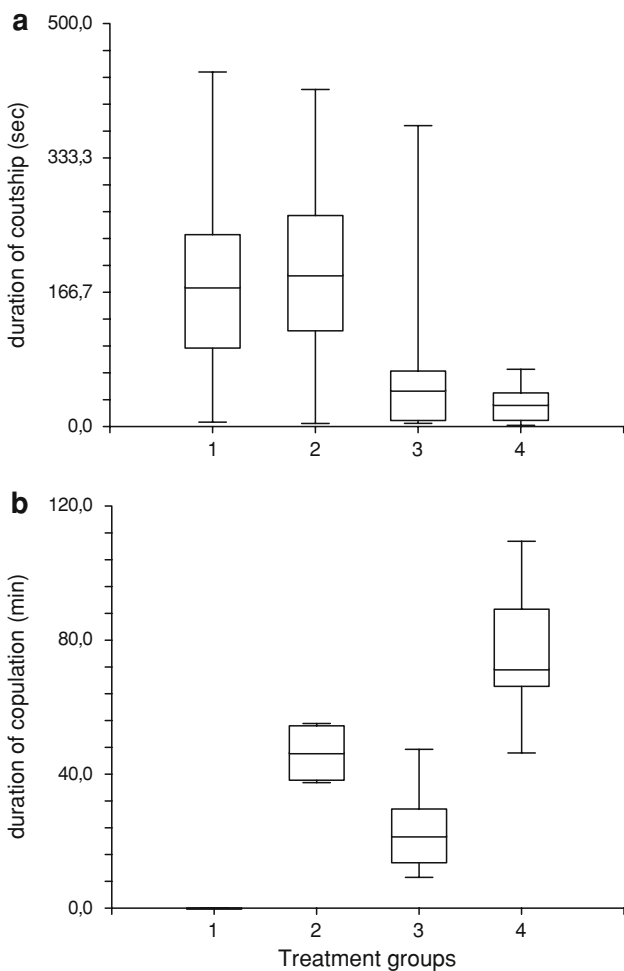


Fig. 2 Effect of manipulation of female and male apophyses on mating duration: courtship (a) and copulation (b). Note that the modification resulted in longer courtship but shorter copulation (or its absence in fully modified females). Group abbreviations: 1 fully modified females, 2 fully modified males, 3 partially modified females, and 4 control group

control group (Kruskal–Wallis test, $H = 19.28$, $P = 0.0002$ and $H = 30.94$, $P = 0.000001$, respectively; Table 1). Unmodified males performed these patterns more times in courtship sequences with fully modified females than with unmodified females. Modified males performed these patterns more times than unmodified males with partially modified females (Table 1). Two other male behavioral patterns, also associated with the intromission of the palps in the female genital aperture, “palpal reaccommodation” and “attempts of insertion,” were also performed significantly more times in experimental groups than in the control group (Kruskal–Wallis test, $H = 21.48$, $P = 0.00008$; $H = 33.98$, $P = 0.0000001$; Fig. 3; Table 1).

A fine-scaled analysis of videos showed that females were nonaggressive to courting males not only when the female genitalic apophyses were experimentally covered

but also when the male cheliceral apophyses were modified. Typically, females showed acceptance by remaining motionless near the male, extending the forelegs laterally so that the opened genital aperture was not covered by any legs, and faced directly to the male during his approach to perform palpal rotation. This positive predisposition was less pronounced in the group of fully modified females, in which 8/15 females closed the genital aperture. However, this closing occurred only after the male had carried out several attempts of palpal insertion.

Copulatory behavior

In 20 out of 20 fully modified females, total covering of epigynal apophyses with plaster resulted in lack of copulation due to males being unable to position their palps properly for intromission. In this experimental group, the end of the mating attempt was initiated by males in 19 of 20 cases. They terminated courtship after performing palpal rotation up to 13 times and many subtle reaccommodations of their chelicerae on the modified females.

Modified males copulated with unmodified females in only 8 out of 20 sequences, whereas all partially modified females copulated (20/20). In these two manipulated groups the duration of copulation was significantly shorter than in the control group (Kruskal–Wallis test, $H = 26.16$, $P = 0.0000002$), with the shortest copulation sequences between unmodified males and partially modified females (Fig. 2; Table 2). Because of this decrease in copulation duration, absolute number of squeezes was higher in control copulations than in this group of manipulated females (Table 2). The squeezing rate (abs. number squeezes/cop. duration) also differed significantly between the three groups: the highest value corresponded to copulations between unmodified males and partially modified females, and the lowest value to the control group (Table 2). In both experimental groups, one palp sometimes disengaged from the female genital aperture during copulation, remaining outside up to 16 min. This occurred indiscriminately for the right palp (5 out of 40 mating sequences) or the left palp (4 out of 40 mating sequences; $Z = 0.353$; $P = 0.36$; Fisher’s exact test: $P = 0.50$). We found no significant effect of modification on which sex terminated copulation since in all groups copulation was ended by the male (they removed their palps slowly from the female genital aperture).

Unusual behaviors during interactions

Although we excluded from the previous analyses those individuals that removed totally or partially the plaster with their chelicerae or legs, it is interesting to mention some particular behaviors because they may represent a certain

Table 1 Total frequency of occurrence of the main behavior patterns in courtship tests between males and females ($n = 20$) of the control and the three experimental groups

Name of stage or behavior pattern	Mean value				Comparison between groups by Mann–Whitney test	Z	P
	Fully modified ♀ (1)	Fully modified ♂ (2)	Partially modified ♀ (3)	Control (4)			
Courtship duration (s)	178.39	194	70.37	28.46	(1)–(2)	–0.601	0.57
					(1)–(3)	2.882	0.003
					(1)–(4)	3.712	0.00007
					(2)–(3)	2.799	0.004
					(2)–(4)	3.629	0.0001
					(3)–(4)	1.326	0.19
Male stridulation							
Abs.	14.07	9.27	7.47	0.47	(1)–(2)	1.816	0.07
					(1)–(3)	2.733	0.006
					(1)–(4)	4.526	0.000006
					(2)–(3)	0.411	0.68
					(2)–(4)	2.304	0.02
					(3)–(4)	2.345	0.02
Rel.	0.0003	0.01	0.004	0.000001	(1)–(2)	0.062	0.94
					(1)–(3)	0.799	0.42
					(1)–(4)	5.005	0.37
					(2)–(3)	0.887	0.000001
					(2)–(4)	3.197	0.001
					(3)–(4)	3.456	0.0005
Palpal rotation (Abs)	6.4	4.47	2.2	1	(1)–(2)	0.501	0.61
					(1)–(3)	2.915	0.003
					(1)–(4)	4.740	0.000002
					(2)–(3)	2.350	0.02
					(2)–(4)	4.222	0.00002
					(3)–(4)	1.724	0.08
Courtship with legs							
Abs.	6.73	7.26	1.6	0.27	(1)–(2)	–0.270	0.78
					(1)–(3)	2.975	0.0029
					(1)–(4)	4.263	0.00002
					(2)–(3)	3.516	0.0004
					(2)–(4)	4.345	0.00001
					(3)–(4)	2.904	0.004
Rel.	0.05	0.0045	0.0014	0.024	(1)–(2)	0.416	0.68
					(1)–(3)	2.817	0.005
					(1)–(4)	2.941	0.003
					(2)–(3)	3.025	0.002
					(2)–(4)	2.983	0.003
					(3)–(4)	1.619	0.10
Palpal re-accommodation (Abs)	4.33	2.13	1.13	0	(1)–(2)	1.345	0.17
					(1)–(3)	2.480	0.01
					(1)–(4)	4.475	0.000008
					(2)–(3)	1.159	0.25
					(2)–(4)	3.711	0.0002
					(3)–(4)	3.465	0.0005
Additional attempts of insertion (Abs)	3.6	3.46	1	0	(1)–(2)	0.419	0.67

Table 1 continued

Name of stage or behavior pattern	Mean value				Comparison between groups by Mann–Whitney test	Z	P
	Fully modified ♀ (1)	Fully modified ♂ (2)	Partially modified ♀ (3)	Control (4)			
					(1)–(3)	3.591	0.0003
					(1)–(4)	4.997	0.000001
					(2)–(3)	2.727	0.006
					(2)–(4)	4.477	0.000008
					(3)–(4)	4.012	0.00006

The frequency of occurrence of each behavior is shown in its absolute value and, when it is useful, the relative rate (absolute value/duration of courtship)

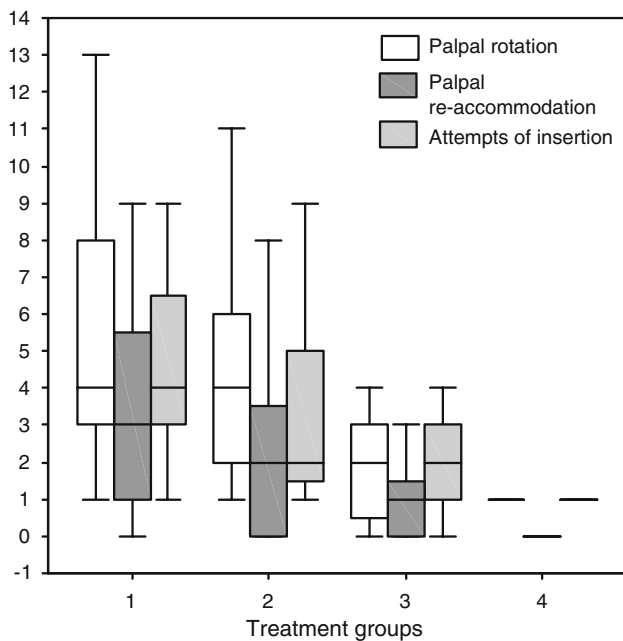


Fig. 3 Effect of manipulation of apophyses in both sexes on the number of occurrences of the main behavior patterns utilized by males to begin copulation. Group abbreviations: 1 fully modified females, 2 fully modified males, 3 partially modified females, and 4 control group

degree of behavioral plasticity during courtship. For example, during our tests to select the final set of individuals, we observed that modified females rarely removed the plaster that covered the epigynum (1 of 26 cases). However, 4 of 26 males were able to do this by using the distal part of the procurus. In 4 of 26 cases they also removed the plaster covering the frontal part of their chelicerae. In four interactions between unmodified males and partially modified females, the males stopped genital intromission and moved the palps in a “pre-rotation position” (as the palps are at the beginning of the courtship) during 13.7 ± 9.6 s. Then the male turned the palps again

in a copulatory position and introduced them into the female genital aperture. During all these cases, females remained motionless and did not close the genital aperture. Finally, 11 males that inseminated partially modified females were tested after 5 days with a new set of partially modified females. Interestingly, five of these males carried out the insertion of the palps into the female genital aperture more easily, needing just one palpal rotation (as was typical in the control group) to start copulation.

Clutch size and fertilization of eggs

Despite the fact that the shortest copulations corresponded to partially modified females, there were no differences between the groups in the mean number of laid eggs [partially modified females group: 38.4 ± 20.54 eggs ($N = 20$), modified males group: 34.25 ± 27.31 eggs ($N = 8$), control group: 33.73 ± 21.86 eggs ($N = 20$); one-way ANOVA: $F = 0.65$, $P = 0.53$] or in the mean rate of fertilized eggs per sac [partially modified females group: 0.95 ± 0.11 ($N = 20$), modified males group: 0.90 ± 0.26 ($N = 8$), control group: 0.89 ± 0.28 ($N = 20$); Kruskal–Wallis test on arcsin-transformed data: $H = 1.78$, $P = 0.41$].

Discussion

Evaluation of the cheliceral clasping mechanism

A first point to remark from our results is that females behaved initially cooperative to courting males not only when their genitalic apophyses were experimentally covered but also when the cheliceral apophyses were modified, indicating that the male cheliceral clasper is not anchored in the female by physical force. This pattern is contrary to that expected by sexual coercion (Clutton-Brock and Parker 1995; Arnqvist and Rowe 2005; Peretti and

Table 2 Effect of manipulation of nongenitalic apophyses on copulatory variables of the control and the three experimental groups

Variables	Mean value				Comparison between groups by Mann–Whitney test	Z	P
	Fully modified ♀ (1) (n = 0)	Fully modified ♂ (2) n = 8	Partially modified ♀ (3) n = 20	Control (4) n = 20			
Copulation duration (min)	–	46.33	22.07	76.47	(2)–(3) (2)–(4) (3)–(4)	2.800 –2.750 –4.624	0.002 0.002 0.000003
Male genital movements (squeezing)							
Abs.	–	214.5	125.33	199.2	(2)–(3) (2)–(4) (3)–(4)	2.600 0.750 –3.630	0.004 0.22 0.001
Rel.	–	4.72	5.92	2.69	(2)–(3) (2)–(4) (3)–(4)	–1.780 3.082 4.742	0.02 0.001 0.000002

Explanation: idem Table 1

Córdoba-Aguilar 2007), which does not predict unforced female cooperation toward males trying to anchor their claspers to start copulation. In fact, in a scenario of forced copulation, females should show indiscriminate resistance toward any mating attempt and males should use physical coercion to overcome forcefully this expected female response. In contrast, our observations indicate that females cooperated with courting males (normal or modified) by keeping the genital aperture opened, moving the genital aperture toward the male palps, and by moving the forelegs laterally in order to facilitate body contact while they increase their stimulatory patterns (e.g., courtship with legs). Therefore, the obtained results fit more closely with predictions of the sexually selective cooperation (Eberhard 2004a, b, 2006) since they involve mutual cooperation to perform grasping prior to copulation. Nevertheless, after remaining cooperative during a part of the courtship, some females rejected males if they continued failing to anchor the cheliceral apophyses against the epigynum. This fact indicates that female cooperation is modulated since it was limited to a brief “tolerance time,” which is in accordance with the implicit selective nature of this cooperation. Hence, this may suggest that females could evaluate males according to their abilities to fit the cheliceral apophyses on the epigynum appropriately (i.e., fast and in the correct position).

Our results did not support the hypothesis that the epigynal apophyses could function to thwart clasping. In contrast, these female genitalic structures acted to facilitate clasping. In fact, partial or total covering of female epigynal region affected the clasping, resulting in longer courtship sequences in which males had to repeat behavior patterns associated with clasping and pre-

intromission many times. Indeed, in *P. dugesi*, intersexual cooperation seems to be reflected in the form of the apophyses. For example, the spiny frontal area of the male cheliceral apophyses facilitate anchoring against the epigynum while, at first glance, the shape of female apophyses seem to be designed to avoid or hinder male clasping. However, detailed analyses of videos and fixed copulating pairs showed that the small tooth-like processes of the cheliceral apophyses anchored against the anterior and medial parts of the epigynal apophyses, which function as wide platforms to ensure the contact without any difficulty. In addition, the two distal large processes of the cheliceral apophyses fit on the two distal bifurcated extensions of the epigynal apophyses. In the other studied *Physocyclus* species, *P. globosus*, the anterior tip of epigynum is small and fits into a groove that the male presents on the upper-medial region of his chelicerae (Huber and Eberhard 1997). Preliminary data on experimental manipulation of these areas in *P. globosus* also showed existence of intersexual cooperation during clasping (A. Peretti, unpub. data). All fully modified females of *P. dugesi* did not copulate since the plaster prevented the males from anchoring their cheliceral apophyses on the epigynum. Indeed, males tried to perform grasping and, even though females were initially cooperative (e.g., remained with the genital aperture opened), they finally rejected males or moved away prior to palpal insertion. Although partial modification of female epigynum did not affect the occurrence of copulation, it resulted in shorter duration of this mating phase, indicating that the epigynal apophyses are crucial cooperative structures to allow male clasping for a long duration.

Alternative explanations and comparison with other pholcid spiders

In *P. dugesi* and *P. globosus* female cooperation for non-genitalic grasping could not be explained only as an “obvious forced act” as clasping always represents an obligatory condition to receive sperm for fertilization of eggs. Indeed, in *P. dugesi*, copulation was also performed in pairs in which apophyses of one sex were modified. Although these copulatory sequences were shorter than those of the control group, females received enough sperm to fertilize all the eggs laid in the first oviposition. By making inoperative the male clasping organ of the scorpion fly *Panorpa vulgaris*, Thornhill and Sauer (1991) observed that copulation duration was drastically reduced, interpreting this result as evidence that the adaptative function of this structure was to prolong mating duration beyond the female optimum. We do not know whether the reduction also observed in copulation duration of manipulated *P. dugesi* males might suggest a similar effect on the female optimum in this spider.

One interesting characteristic of *P. dugesi* is that females never accepted to remate prior to the first oviposition as seems to be the rule in pholcids (Kaster and Jakob 1997; Schäfer and Uhl 2002; Peretti et al. 2006). Indeed, in *P. dugesi*, inseminated females attacked any new courting males also in the sample utilized in this study and in additional females observed by A. V. Peretti (unpublished data from a population in San Jose de Costa Rica: 0/11 inseminated females remated. prior to oviposition). The causes of the lack of female receptivity after a mating in this species are not known. This may occur due to seminal substances transferred by male ejaculate (e.g., as in fruit flies; Chapman and Davies 2004) or because the costs of polyandry to females may be higher than the benefits (Elgar 1998). Interestingly, total lack of sexual receptivity appears not only in females that copulated longer but also in those copulating during less time (A. Peretti, unpub. data). It is interesting to note that observations on typical polyandrous pholcids, such as *P. globosus* and *H. pluchei*, showed that mated females continued cooperating with males to facilitate the cheliceral clasping. (Peretti et al. 2006; S. Dutto and A. Peretti, unpub. data). In *Holocnemus pluchei*, only after the third or fourth mating did females show significantly lower receptivity to remate in comparison with virgin females (S. Dutto and A. Peretti, unpub. data). Therefore, all these data suggest the presence of a cooperative scenario rather than a conflictive “arms race” between the sexes in pholcid spiders, at least in relation to the morphological and behavioral characteristics of the mechanism of the male cheliceral clasping of external female genitalia. In addition, in a recent study, Eberhard (2006) found that in insects, sexually antagonistic

coevolution in clasping structures is associated with only limited morphological diversity. If this is the general rule for other arthropods, then that hypothesis might not be satisfactory applied to pholcid spiders because, in fact, this spider family show a very high morphological diversity in cheliceral claspers and corresponding anchored female (Huber 2000, 2005; Huber et al. 2005).

A basic question arises from these data is: is female cooperation for body clasping the typical pattern in all the spiders? Comparative data from descriptions of the events associated with the beginning of genital intromission suggest this could be a common feature (Foelix 1996; Huber 1993, 1995, 1998a, 2005; Eberhard 2004a, b; V. Méndez and W. Eberhard, unpub. data). However, this does not mean that the female cannot select some particular mates over others (e.g., according to the ability of the male to fit the nongenitalic structures appropriately, etc.). Perhaps an explanation for this possible widespread sexual cooperation for copulation is the particular characteristics of the genital morphology of spiders, in which cooperation in both males and females constitutes an obligatory requisite to transfer and receive sperm, respectively (Schneider and Lubin 1998). However, these explanations do not exclude the possibility that epigynal apophyses facilitate the female’s ability to exercise control of some variables of the mating such as copulation duration, as was indirectly suggested from our results.

In summary, our study supports Huber’s hypothesis that the female epigynal structures appear to be cooperative instead of antagonistic to male claspers (Huber 1998a, 1999; Huber et al. 2005). We think, however, that this does not exclude that a possible key point of precopulatory selective cooperation in pholcid spiders is that females use special traits (such as epigynal apophyses or hoods) in order to choose males that can overcome the obstacles these traits may provide for males. Huber (1999) also suggested that chelicerae may function as “copulatory courtship” devices (Eberhard 1996), whose elaborate morphology is utilized to stimulate or fit the female in a way that increases paternity. We are cautious to affirm that, by elimination, the hypothesis that seems to fit the available descriptive and experimental data best could be the cryptic female choice hypothesis. Further experimental manipulation are needed to test this hypothesis. For example, it is needed to perform a specific study on the effect of variability in morphology and clasping success on the chances of fathering female offspring.

Male behavioral plasticity

During courtship males showed behavioral flexibility, in particular, when they had difficulty with genitalic intromission due to modification of the apophyses. This aspect

is very interesting from a perspective of phenotypic plasticity (West-Eberhard 2003) since in spiders the mechanisms of genital and paragenital contact principally involve accurate and slightly flexible, behavioral patterns (Robinson 1982; Austad 1984; Foelix 1996). Typically, *P. dugesi* males tried to remove the plaster covering the epigynum, indicating that they were able to detect this modification by touching this zone with the chelicerae (directly by sensorial hairs of the chelicerae or indirectly by perceiving the impossibility of starting the clasping). In fact, males improvised rubbing movements with the procurus of each palp and with the frontal part of the chelicerae to clean the female's epigynal surface, a novel behavior that was allowed by females. Detection of the presence of a modification did not imply that males refused to mate. Similarly, modified females did not reduce their sexual receptivity. However, there are no available data from other studies on the capacity of males to improvise behavior when structures utilized as claspers, and their corresponding anchoring surface, are modified.

In our study, the high capacity of males to remove the plaster covering the frontal area of their chelicerae was the principal conditioning factor that prevented us from working with more different degrees of modification of their cheliceral apophyses. In fact, males easily detached small plasters by using the procurus of the palp like a crowbar. Nevertheless, two interesting behavioral aspects arise from this methodological limitation: (1) Evidence of clear behavioral plasticity to resolve, at least in a sexual context as studied, a novel situation that affected the male's ability to achieve a purpose (the initial genital intromission). This plasticity is favored by the presence of a copulatory organ (male palps) which due to its flexibility of movements could be utilized for additional functions such as removal of a strange object from female external genitalia. (2) Given that the procurus was the part utilized to remove the plaster, this observation may reflect the potential role of this genital structure for sperm removal. Indeed, the procurus of each palp is introduced deep in the uterus externus during copulation and it was suggested that, in some Pholcidae, sperm displacement and/or removal might be associated with the continuous and intense movements of these structures inside the female genital tract (e.g., in *Pholcus phalangoides*; Schäfer and Uhl 2002; in *Holocnemus pluchei* L.; Calbacho-Rosa, pers. comm.).

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