



Copulatory dialogue: female spiders sing during copulation to influence male genitalic movements

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Female behaviour during copulation that could function as communication with the male is probably more common than previously appreciated, but its functional significance remains little studied. Stridulation during copulation by the female of the spider *Physocylus globosus* (Taczanowski, 1873), documented here for the first time, is common and noncoercive, thus permitting simple tests regarding its possible function. Males squeezed females rhythmically with their enlarged, powerful genitalia throughout copulation, and more male genitalic squeezes were associated with increased paternity when females mated with two males. Contextual associations suggest that female stridulation represents attempts to induce the male to interrupt genitalic squeezes: female stridulation was more common when the male was squeezing her; females were more likely to stridulate when individual male squeezes were longer, and when the male had not responded to a previous stridulation by loosening a squeeze; females were more likely to refrain from stridulating when the male loosened a squeeze; males were more likely to loosen squeezes when the female stridulated; and female stridulation was associated with rejection of males in other contexts. Males that responded to female stridulation more consistently by loosening their squeezes obtained greater paternity. Possible female communicatory behaviour during copulation is known in other species. Future attention to female as well as to male behaviour, and to possible dialogues during copulation, promises to be valuable in understanding sexual interactions.

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Study of female behaviour before and during copulation has lagged behind that of males, even though appreciation of female roles has resulted in substantially improved understanding (Andersson 1994; Eberhard 1996). Courtship signalling by males, and to a lesser extent by females, has been documented to occur prior to copulation in many species (Andersson 1994). Less explored are the courtship signals (defined as behaviour designed to induce responses in the receiver that are favourable to the signaler: Burghardt 1970; Williams 1992) that are produced during copulation. Both theory and empirical observations indicate that male courtship during copulation

probably serves to bias cryptic female choice in favour of the male (Eberhard 1991, 1994, 1996; Edvardsson & Arnqvist 2000; Schäfer & Uhl 2002; Tallamy et al. 2002a, b; Ortiz 2003; Ramirez 2004). Females also perform possible courtship behaviour during copulation in some species (summary in Rodriguez 1998), but its significance remains little studied. Females probably often have a large degree of control over paternity (Eberhard 1996), so female signals may have crucial effects on the ultimate outcomes of sexual interactions.

Female signalling during copulation is probably more common than has been appreciated. Possible female signaling behaviour occurred during copulation in 10.7% of 131 species of insects and spiders in a survey of copulatory behaviour (Eberhard 1994). This frequency is an underestimate, because subsequent observations of two of these species have revealed possible female signals that were missed; similar female behaviour may well have been missed in other species because of the focus of this

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study (and others) on male behaviour. There are reports of possible female signalling during copulation in other insects and spiders (Cowan 1991; Huber 1994; Kotrba 1996; Crudgington & Siva-Jothy 2000; O'Neill 2001; Arnqvist et al. 2003; Cheng et al. 2004; Eberhard 2005; Sirot et al., in press; D. W. Tallamy, personal communication on the chrysomelid beetle *Diabrotica undecimpunctata howardi*), but little has been established regarding their functions.

One function of possible female signals during copulation is that they serve to induce changes in male behaviour (e.g. Ridsdill Smith 1970; Rodriguez 1998; Knoflach & van Harten 2000; Crudgington & Siva-Jothy 2000; Bloch Qazi 2003; Ortiz 2003), but many female behaviour patterns may instead serve as physical coercion. Possible coercive functions include physically forcing the male to cooperate, such as kicking or otherwise punishing him until it pays him to desist (Crudgington & Siva-Jothy 2000), and testing his ability to tolerate potentially damaging behaviour and or threatening him with such a test (Rodriguez 1998). Evaluation of the signalling and coercion hypotheses is complicated by their possible overlap, and the fact that apparently coercive female movements need not always be coercive in their effects on males (Eberhard 2002; Baena & Eberhard, in press). For instance, abdominal wagging by female *Diaprepes abbreviatus* weevils only sometimes dislodges males, and it is correlated with the number of eggs fertilized by the male (an effect mainly produced by changes in the female's rate of oviposition: Sirot et al., in press). Thus, apparently coercive female behaviour may instead have other functions.

This paper describes a case in which interpretation is simpler. Females of the spider *Physocyclus globosus* (Tac. 1873) stridulate during copulation, a behaviour that is not physically coercive, and that is likely to be an evolved signal rather than an incidental cue (Burghardt 1970; Williams 1992). This behaviour facilitates study of possible messages sent by the female and of possible male responses to them. A second advantage of *P. globosus* is that a large proportion of the male's genitalia remain visible during copulation; the male's stereotyped, rhythmic and sustained squeezing movements (produced by the largest-diameter muscles in his body) can be observed directly (Huber & Eberhard 1997). In addition, the morphological relationships between male and female genitalia during copulation and sperm transfer are known in detail (Huber & Eberhard 1997). This study is the first to demonstrate a dialogue between male and female during copulation, and to determine the reproductive consequences of the female's signals and the male's responses to them.

Mature females of *P. globosus* are visited in nature by multiple males (Eberhard 1992). Preliminary data suggest no effect of mating order (Eberhard et al. 1993). Females lack spermathecae and store sperm in the lining of the oviduct (Huber & Eberhard 1997). Before copulation, the male taps the female with his legs, vibrates his body and stridulates, and a receptive female inflates her membranous genitalia, turns towards the male and assumes a characteristic receptive posture (Huber & Eberhard 1997). Unreceptive females easily avoid copulation by simply turning away or by failing to assume the receptive posture,

and rejected males do not persist in courting. Termination of copulation is usually initiated by the male rather than the female (75.0% of 52 copulations in the present study in which this detail was clear). In captivity, females live for several months and produce several clutches.

METHODS

To evaluate the function of female stridulation during copulation, we recorded male and female behaviour when we mated each of 68 virgin female *P. globosus* to two males and then assessed male paternity. Each female had been maintained on a diet of *Drosophila* sp. flies in captivity and had matured 5–7 days before her first copulation. Copulations were staged on 2 successive days; one of the males was untreated, the other had been sterilized with 50 Gy (5 krad) radiation, a dosage established as appropriate (Eberhard et al. 1993). Control females, which had mated only with a fertile male, laid nearly all fertile eggs (98.2% of 277 eggs in 11 clutches; maximum number of infertile eggs/clutch = 1), but the eggs of females mated only with a sterilized male were all infertile (215 eggs in 9 clutches). Each male was used only once, and the order of mating (sterile–fertile) was alternated with successive females. In all cases, the virgin female had been left overnight to spin a web in a half-gallon (1.89 litre) container in which copulation was staged. Male and female sizes were estimated later, using cephalothorax width of specimens measured in 80% ethanol under a dissecting microscope.

Each copulation was taped in its entirety using a video camera coupled to a dissecting microscope. Careful positioning of the camera allowed close-up views of movements of both of the male's pedipalps in the female's genital opening, and of at least one (usually both) of the female's pedipalps. Events were transcribed from videotapes using Etholog 2.2 (Ottoni 2000) and Jwatcher 0.9 (Blumstein et al. 2000). For analyses of the coordination between squeezing movements of the male's palps and female behaviour, we included only those squeezes in which the angle of viewing as well as the amplitude and duration of the squeeze permitted confident determination of both initiation and termination of the squeeze (most such squeezes were not at the very beginning or very end of copulation). Mean \pm SD values are presented. Nonparametric tests were used when the distribution of the data differed significantly from normality. Some analyses were repeated using individual spiders rather than behaviour patterns as units, to control for possible pseudoreplication when within-individual control values were not available.

Stridulation was recorded in six additional pairs using a Sennheiser MKE2-1053-C microphone positioned 1–2 cm immediately below the female's cephalothorax. The microphone was connected to the audio input of a video camera that simultaneously recorded movements of the female's pedipalps. The fertility of the eggs in the first clutch was checked more than 10 days after oviposition, when fertile eggs had transformed to pre-embryos. Clutches analysed for paternity contained 28.1 ± 10.0 eggs.

Mean paternity of irradiated males did not differ from that of fertile males (47.5 ± 38.4 versus 52.5 ± 38.4 ; Mann–Whitney U test: $Z = -0.823$, $N_1 = N_2 = 52$, $P = 0.21$), as observed previously (Eberhard et al. 1993). Male paternity success was thus quantified directly on the basis of the fractions of fertile and infertile eggs. Possible determinants of paternity success were analysed by comparing the two males that copulated with each female, thus controlling for possible differences between females. Two types of analysis were performed, using arcsine-transformed values for percentages (e.g. paternity) and log-transformed values for other variables. Variables that might affect paternity were compared between the winning male (more offspring) and the losing male (fewer offspring) (the mean paternity value for winners was $90.1 \pm 10.4\%$, range 68.8–100.0%; Table 1). Variables that differed overall between first and second copulations were adjusted before comparisons between winning and losing males. For instance, before comparison, we multiplied the duration of the second male's copulation by the ratio of the mean duration of the first copulation to the mean duration of the second copulation for all females. In all cases in which parametric tests were used, the distributions of the variables did not deviate significantly from normality and homogeneity of variance. As a check, GLM ANOVA analyses were also performed in which combinations of variables (Table 2) were tested for their ability to explain male paternity values.

RESULTS

General Description of Copulation

As in previous observations (Huber & Eberhard 1997), the male's paired secondary genitalia (palps) remained inserted in the female's genital opening throughout copulation ($\bar{X} \pm SD$ duration = 21.20 ± 12.15 min, $N = 68$ pairs). The distal portion of each palp repeatedly twisted medially and flexed towards the male (the 'flexed' position) and then returned to its resting ('relaxed') position. The two

palps flexed and relaxed simultaneously with a regular rhythm. Each flex movement squeezed the anteriorventral surface of the female's abdomen (her epigynal area) between the male's palp and the anterior surface of his chelicerae, which is sexually dimorphic and species specific (Fig. 1c). The rate of squeezing was usually greater early in copulation and later slowed, but patterns varied ($\bar{X} \pm SD = 10.56 \pm 2.92$ squeezes/min and 193.8 ± 71.9 flexed–relaxed cycles/copulation; $N = 136$ copulations). Squeezing movements are produced by the powerful muscles in the greatly expanded basal segments of the male's palps (Huber & Eberhard 1997). Males also performed bursts of dorsoventral abdomen vibration during copulation (2.08 ± 2.05 bursts/min, 34.34 ± 36.26 bursts/copulation), causing both the web and the female to shake perceptibly.

First copulations were longer than second copulations (27.32 ± 12.58 min versus 14.45 ± 7.65 min; two-sample t test: $t_{102} = 6.68$, $P = 0.00001$), and the rate of squeezing was higher in second copulations (12.28 ± 2.48 versus 8.78 ± 2.18 squeezes/min; Mann–Whitney U test: $U = 2398$, $N_1 = N_2 = 52$, $P < 0.00001$). The rate of abdomen vibration (bursts/min) did not differ between first and second copulations.

The female often moved her pedipalps during copulation in short bursts of rapid, alternating dorsoventral movements (80.2% of 136 copulations). The lateral surface of each chelicera of the female has a row of striations (Fig. 1a), and the prolateral surface of the femur of her pedipalp, which is just lateral to her chelicera, has several sharp ridges (Fig. 1b). Sound recordings showed that each burst of movements by the female's pedipalps was associated with a burst of sound, resembling squeaking leather. No sounds occurred when the female's pedipalps did not move this way. Each burst of sound contained several pulses, and each pulse included several peaks (Fig. 2). Similarities between the temporal characteristics of sounds and female palp movements in six video recordings confirmed the connection between sounds and palp movements ($\bar{X} \pm SD$: duration of a burst of sound = 1.7 ± 0.6 s; duration of a burst of palp movements = 1.8 ± 0.8 s;

Table 1. Summary of mean values of winning and losing males

Variable	Winner	Loser	Test of significance	P
Male size*	1.57 ± 0.11	1.55 ± 0.13	$t = 0.364$	0.35
Male size/Female size	1.05 ± 0.11	1.06 ± 0.14	$t = -0.323$	0.37
Order of copulation†	1.48 ± 0.51	1.52 ± 0.51	$Z = -0.208$	0.41
Sterility of male‡	1.43 ± 0.51	1.56 ± 0.51	$Z = -0.625$	0.26
Duration of copulation (min) (adj)	28.63 ± 8.19	24.50 ± 15.62	$t = 1.181$	0.12
Number of squeezes (adj)	246.9 ± 53.7	191.1 ± 93.3	$t = 2.60$	0.006
Rate of squeezing	12.39 ± 2.04	12.74 ± 3.23	$t = -0.435$	0.33
Number of abdomen vibrations (adj)	46.7 ± 37.2	36.1 ± 35.2	$t = 0.914$	0.18
Rate of abdomen vibration	2.12 ± 1.87	2.21 ± 2.16	$t = -0.142$	0.44
Bursts of female stridulation	76.23 ± 75.51	59.40 ± 51.78	$Z = 0.844$	0.20
Rate of female stridulation (adj)	3.35 ± 3.14	3.47 ± 3.07	$t = -0.276$	0.39

Tests of significance: t_1 = paired t test; Z = Wilcoxon matched-pairs signed-ranks test for difference in medians (adj = adjusted for first or second copulation), $N = 51$.

*Cephalothorax width in mm.

†1 = first male, 2 = second male.

‡1 = fertile male, 2 = sterilized male.

Table 2. Summary of results of GLM ANOVA testing the effects of different variables (first male–second male) on relative male fertilization success of eggs in the female's first clutch (first male–second male)

	t_1	P
Morphology		
Male size	-1.23	0.29
Male size/female size	1.02	0.32
Male age	-0.87	0.45
Size of sperm mass expelled	0.56	0.72
Behaviour		
Latency to court	0.43	0.74
Latency to mate	0.89	0.21
Coupling attempts before copulation	0.45	0.68
Duration of copulation	-1.20	0.18
Number of squeezes with palps		
Absolute number	3.21	0.002
Rate (x/min)	0.98	0.34
Bursts of rapid squeezes during last part of copulation		
Absolute number	1.21	0.17
Rate (x/min)	1.45	0.19
Bursts of male abdomen vibration		
Absolute number	0.04	0.92
Rate (x/min)	1.16	0.26
Bursts of female stridulation		
Absolute number	0.42	0.73
Rate (x/min)	0.26	0.81
Male 'responsiveness' to female stridulation	0.92	0.32

number of pulses/burst of sound = 8.2 ± 3.3 ; number of clear palp movements/burst = 6.8 ± 4.2 ; number of peaks/pulse of sound (presumably from a femoral ridge striking a single striation on the chelicera) = 11.2 ± 2.8 , well within the 35–40 ridges on female chelicerae). Female stridulation could presumably be transmitted to the male through the air, through the web lines that both animals gripped or through body contact. Throughout copulation, the male's chelicerae were pressed tightly against the

female's abdomen, his palps were inserted into her reproductive opening, and portions of some of his legs probably touched her legs.

The brisk dorsoventral female palp movements were distinctive and easily recognized, so video recordings of undisturbed pairs observed without a microphone nearby permitted us to determine when female stridulation occurred. The mean \pm SD rate of bursts of stridulation in 109 (of 136) copulations in which stridulation occurred was $3.53 \pm 2.52/\text{min}$, or $68.4 \pm 55.4/\text{copulation}$. Individual females differed consistently in their tendencies to stridulate: the rate of stridulation (bursts/min) in a female's first copulation was positively correlated with the rate in her second copulation (Spearman rank correlation: $r_s = 0.76$, $N = 68$, $P < 0.0001$). There was no relationship between a female's size and her tendency to stridulate ($r_s = 0.184$, $N = 68$, $P = 0.34$).

Triggering Female Stridulation and Male Responses

Female stridulation was more likely to occur while the male was squeezing: the total fraction of the copulation during which the male's palps were in the flexed position was 79.1% in the 52 first copulations in which the female stridulated, and female stridulation during these copulations began while the palps were in the flexed position in 90.9% of 4440 bursts of stridulation (chi-square test: $\chi_1^2 = 8.4$, $P = 0.004$); corresponding values for second copulations were 61.2% and 86.7% of 2796 bursts (chi-square test: $\chi_1^2 = 27.3$, $P < 0.0001$). A second analysis, at the level of individual spiders to avoid possible problems from pseudoreplication, gave a similar result. For each female, the fraction of the copulation during which the male's palps were in the flexed position was compared with the fraction of cases in which female stridulation began while the palps were flexed. In 44 of 52 females, stridulations during the

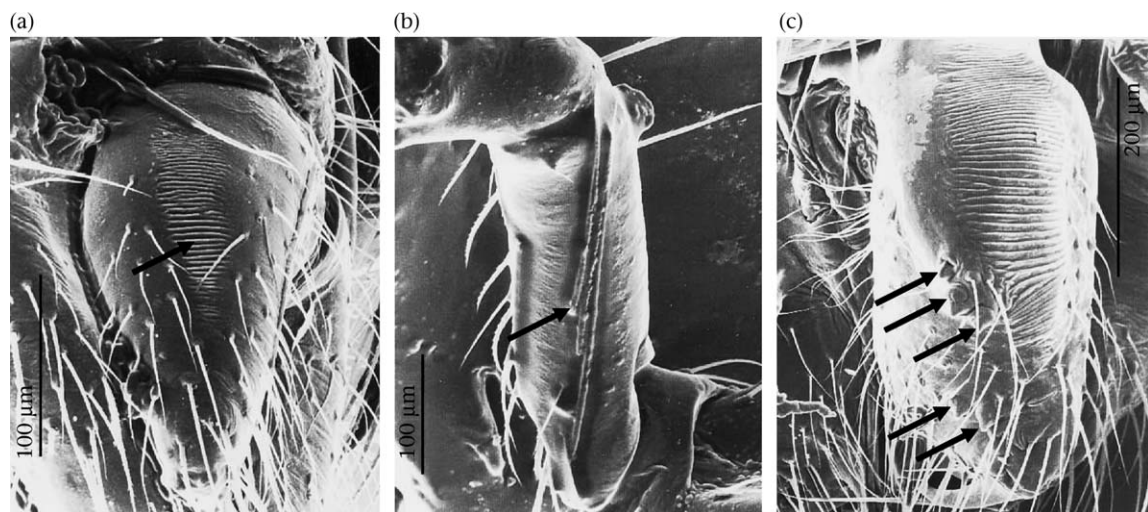


Figure 1. (a) Lateral surface of female chelicera, showing stridulatory striations (arrow). (b) Prolateral surface of femur of female palp showing stridulatory ridge (arrow). (c) Anterolateral surface of male palp, showing striations, and also the knobs and thick setae (arrows) that press against the female during copulation.

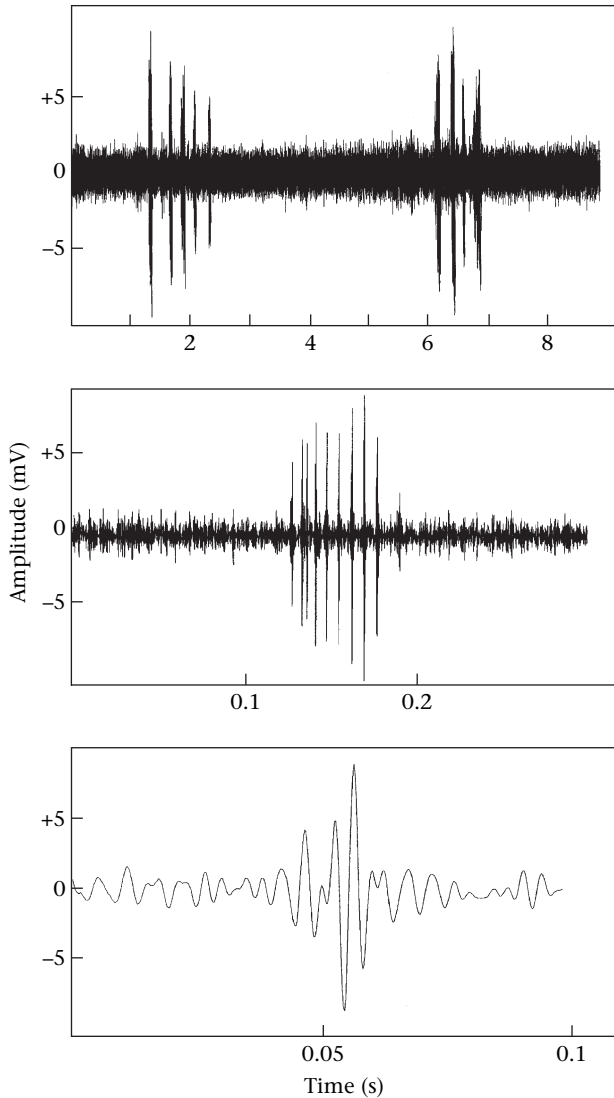


Figure 2. Recordings of female stridulation at different time scales. Each burst of sound contained several pulses, and each pulse included several peaks. The middle record is an amplification of the first burst in the top record.

first copulation began more often than expected during a squeeze (chi-square test: $\chi_1^2 = 24.9$, $P < 0.001$); the corresponding numbers were 47 of 52 in the second copulations (chi-square test: $\chi_1^2 = 33.9$, $P < 0.001$).

We analysed durations during particular lapses of copulations (to correct for gradual changes in durations of squeezes over the length of a copulation) and found that females were more likely to stridulate when the squeeze was longer. In cases in which the female stridulated during one squeeze but did not stridulate during the squeezes either immediately preceding or following it ($N = 784$ in 98 copulations), the squeeze during which stridulation occurred was longer (5.88 ± 3.92 s) than the squeeze immediately before it (5.30 ± 3.7 s; Wilcoxon matched-pairs signed-ranks test: $Z = 4.84$, $P < 0.0001$) or the squeeze immediately after it (5.41 ± 3.75 s; $Z = 3.25$, $P < 0.0001$). The duration of the squeeze prior to the

squeeze with stridulation did not differ from that of the squeeze just after it ($Z = -1.565$, $P = 0.94$). In cases in which one squeeze with stridulation was followed by a second squeeze with stridulation, the durations of the squeezes did not differ (8.59 ± 4.30 s versus 8.60 ± 4.00 s; $Z = 0.710$, $P = 0.761$). Analysis at the level of individual spiders to avoid possible problems with pseudoreplication gave similar results. For each female, we determined the number of times that the squeeze during which she stridulated was longer than the preceding and the following squeezes. In the first copulations of 33 of 52 females, significantly more than 50% of the squeezes during which the female stridulated were longer than both the preceding and the following squeezes ($\chi_1^2 = 3.8$, $P = 0.051$); corresponding numbers for second copulations were 49 of 52 ($\chi_1^2 = 40.7$, $P < 0.001$).

Finally, in cases in which precise determination of the beginning and end of a squeeze was possible, the female was more likely to stridulate again when the male failed to respond quickly (within 2 s) by relaxing his squeeze. In only 0.44% of 675 cases in which the male moved his palps from flexed to relaxed within 2 s after the female began to stridulate was there a second female stridulation within the next 5 s. In contrast, 32.15% of 336 other cases in which the male did not change to the relaxed position within 2 s were followed within 5 s by a second female stridulation (chi-square test: $\chi_1^2 = 230.6$, $P < 0.001$). Analysis at the level of individual spiders, to avoid possible problems of pseudoreplication, gave a similar result. In 29 of 42 females, the frequency with which a second female stridulation followed within 5 s was greater after squeezes in which the male failed to respond to her first stridulation within 2 s than after those in which he did respond (chi-square test: $\chi_1^2 = 6.01$, $P < 0.013$).

The possibility that female stridulation induced the male to shorten his squeezes was tested for 235 squeezes with stridulations by 32 females. In each of these squeezes, we determined the time between the beginning of the squeeze and the beginning of the female's stridulation (denoted as ts_i ; Fig. 3), as well as the stage of copulation (first or second half) during which the squeeze occurred. Then, using data for squeezes that occurred during the same stage of copulation in which no stridulation occurred in this and other pairs, we determined the duration that the squeeze would have been expected to be prolonged beyond ts_i if singing had no effect. This value (' $\text{prolong}_{i(\text{no sing})}$ ') was calculated as the median time elapsed between ts_i and the end of the squeeze in squeezes in which the female did not sing ($t_{i(\text{end of squeeze no sing})} - ts_i$) (Fig. 3). Each such $\text{prolong}_{i(\text{sing})}$ value was then compared with the observed prolongation of the squeeze when the female stridulated ($\text{prolong}_{i(\text{sing})}$) ($=t_{i(\text{end of squeeze sing})} - ts_i$). The mean value of $\text{prolong}_{i(\text{no sing})}$ was significantly greater than $\text{prolong}_{i(\text{sing})}$ (respectively, 0.88 ± 0.61 s, median = 0.73 and 0.67 ± 0.45 s, median = 0.49; $N = 235$ for each) (paired t test: $t_{20} = 2.51$, $P = 0.01$). Alternative analyses also suggested that males responded to female stridulation by relaxing their squeezes. The value of $\text{prolong}_{i(\text{sing})}$ was less than the corresponding expected value of $\text{prolong}_{i(\text{no sing})}$ in 66% of the squeezes with singing ($\chi_1^2 = 26.6$, $P < 0.001$). Analysis

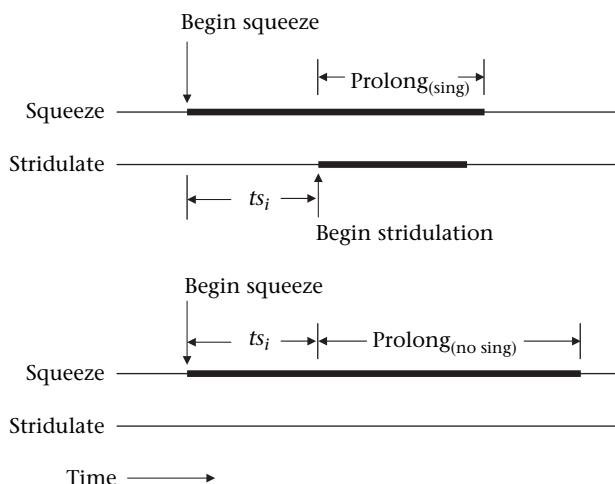


Figure 3. Diagrammatic representation of the time periods that were used to test the effect of female stridulation on the duration of male squeezing behaviour. The data (see text) showed that the amount of time that the squeeze was prolonged after the female began to stridulate ($\text{prolong}_{\text{sing}}$) was shorter than expected, when compared with squeezes in which the female did not stridulate ($\text{prolong}_{\text{no squeeze}}$).

at the level of individual females, to avoid possible problems with pseudoreplication, gave the same result. In 25 of the 32 females, $\text{prolong}_{i(\text{sing})}$ was less than $\text{prolong}_{i(\text{no sing})}$ in more than half of the cases in which the female stridulated ($\chi^2_1 = 10.1$, $P < 0.0015$).

Paternity

Of all the factors analysed separately by comparing the winning and losing males for each female (Table 1), paternity was associated only with more genitalic squeezes (adjusted for copulation number: see Methods; winners: 246.9 ± 53.7 ; losers: 191.1 ± 93.3 ; paired t test: $t_{86} = 2.60$, $P = 0.006$; Fig. 4). The same pattern of differences and lack of differences was found using GLM ANOVA tests of differences (Table 2).

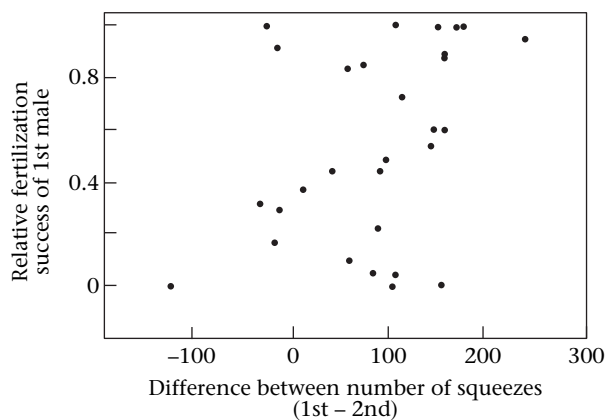


Figure 4. The relative fertilization success of the first male was greater when he made relatively more squeezes with his palps than the second male.

Paternity and Male Responses to Female Stridulation

We calculated an index of 'responsiveness' for each male as the fraction of the times in which he relaxed his squeeze within 2 s after the female began to stridulate. The mean \pm SD responsiveness was 0.66 ± 0.18 , $N = 108$ males. Using only those pairs in which the female stridulated in both copulations (17 pairs discarded), and only the remaining pairs in which the responsiveness of the two males differed strongly (i.e. the difference in indexes was >0.25 ; 31 more pairs discarded), there were 20 cases of the original 68 in which the female's two mates differed sharply in responsiveness. In these pairs, the more responsive male achieved nearly three times more paternity ($\bar{X} \pm \text{SD}$ paternity: more responsive males: $73.2 \pm 22.4\%$; less responsive males: $26.8 \pm 22.4\%$; paired t test: $t_{38} = 3.11$, $P < 0.001$). There was no effect of mating order in this sample of 20 females ($t_{38} = -0.52$, $P = 0.62$). Paternity values did not show a significant difference between more and less responsive males when we included the 31 pairs of males that differed less sharply ($48.3 \pm 36.4\%$ versus $51.7 \pm 21.0\%$; $N = 51$, paired t test: $t_{100} = -0.22$, $P = 0.58$). The GLM overall analysis, which included all pairs, also showed no significant effect of male responsiveness (Table 1).

Female Stridulation in Other Contexts

Females made stridulatory movements with their palps in two other contexts, both associated with rejection of males. The female stridulated in 10 of 17 cases while she chased a male away before courtship. In 50 other cases, the angle of view during precopulatory courtship permitted clear observation of the female's palps after the male initiated courtship: when the female stridulated ($N = 10$), copulation ensued in only 50% of the pairs; in the 40 other pairs, in which the female did not stridulate, copulation ensued in 95% of the pairs (chi-square test: $\chi^2_1 = 9.6$, $P < 0.01$).

DISCUSSION

Female stridulation during copulation fulfilled several criteria for communication: it tended to occur in particular behavioural contexts (during squeezes, and when squeezes were longer); it apparently induced a response by the male that may be beneficial to the female (relaxing the squeeze, which may reduce physical damage, because some setae on the epigyna of nonvirgin females are often broken); and it was more likely to be repeated if the male did not respond. In addition, stridulation contained information of potential reproductive value to the male: males that responded more consistently by relaxing their squeeze had higher paternity rates. The hypothesis that a negative message is conveyed by female stridulation ('cease what you are doing at the moment'; in this case, 'desist from squeezing') is in accord with the association between female stridulation and rejection in other contexts (chasing males away, rejecting courting males).

Female stridulation is also associated with rejection of the male during precopulatory courtship in another pholcid, *Holocnemus pluchei* (A. Peretti, unpublished data).

If females respond negatively to being squeezed by the male's palps by attempting to induce the male to stop, then why do males squeeze them? More squeezing movements were associated with greater paternity, a positive correlation that was also found in another pholcid spider, *Pholcus phalangioides* (Schäfer & Uhl 2002). The male pedipalps of both *P. globosus* and *P. phalangioides* are quite powerful, as is typical of most pholcids (B. A. Huber, personal communication). The diameter of the male's palp femur in *P. globosus* is approximately 4.8 times that of the corresponding female femur, and it is filled with muscle (Huber & Eberhard 1997). The design of the procurus (the portion of the palp that is inserted deepest into the female) also suggests selection to apply increased physical force on the female. The male of *P. globosus* locks his two procuri together during copulation, with the basal projection on one (the 'lateral' procurus) engaging the basal hood of the other (the 'central' procurus); this concentrates the squeezing force of both palps on an area of the female contacted by the 'central' palp (Huber & Eberhard 1997). Male palps are not obviously used in other contexts such as male–male fights (Eberhard 1992). Males stridulate using their palps during precopulatory courtship (Huber & Eberhard 1997), but similar stridulation by the female shows that the male's huge muscles are not needed for this function. Thus, males of this species (and probably many other pholcids) have probably been under selection to squeeze the female's genital area forcefully.

Why does male squeezing increase paternity? One possibility is that it enables the male to remove sperm of other males from the female, and indeed a mass of sperm often emerges from the female in this species (Huber & Eberhard 1997). The details of male and female genitalic morphology and of the process of sperm transfer and storage show, however, that squeezing is not likely to directly remove sperm from the female (Huber & Eberhard 1997). Furthermore, sperm ejection sometimes occurs after copulation is over, and is at least sometimes accompanied by movements of the female genitalia (Huber & Eberhard 1997), suggesting an active female role. Sperm ejection also occurred in some matings with virgin females (29.3% of 58 cases), again arguing against male removal. Another possibility is that powerful squeezing enables the male to push his own sperm to sites within the female where they are more likely to be used to fertilize her eggs. The directions of the principal forces generated by squeezes do not, however, propel the male's sperm transfer structure (the embolus) deeper into the female or past possible internal barriers, but rather squeeze the female's epigynum and the wall of part of her internal reproductive tract (the uterus externus) between the highly modified tip of the male procurus and his chelicerae (Figure 6 in Huber & Eberhard 1997).

Other morphological and behavioural details suggest instead that male squeezing movements with his palps have been under sexual selection to stimulate the female. The male's palps squeeze the female's epigynum against the anterior surface of his chelicerae (Huber & Eberhard

1997), and this surface is elaborately modified in the male (Fig. 1c); its form is species specific in this and many other pholcids (Huber 1997, 2000, 2001). Rhythmic movements of the pedipalps during copulation are also widespread and diverse in pholcids, occurring in all species that have been studied (summary in Huber & Eberhard 1997). An exceptional species with monomorphic chelicerae, *Coryssocnemis viridescens*, is also exceptional in not pressing the male chelicerae against the female during copulation (Huber & Eberhard 1997). Both palp squeezing movements and male cheliceral morphology differ in the closely related *P. dugesi* from those of *P. globosus* (A. Peretti, unpublished data), again suggesting rapid evolutionary divergence. Species specificity is common in traits under sexual selection to stimulate the female (West-Eberhard 1984; Eberhard 1985).

The sharp difference in the paternity of males with strong differences in 'responsiveness' indexes, combined with the lack of difference when all males were included in the GLM analysis, suggests a threshold switching by the female, rather than a continuous linear response to the male's responsiveness.

It is unclear whether female copulatory stridulation is widespread in other pholcids. Females of the congeneric *P. dugesi* lack stridulatory ridges and do not perform stridulatory movements with their palps (A. Peretti, unpublished data). Stridulatory ridges are present, however, on the chelicerae of females in at least four other related pholcid genera (B. A. Huber, personal communication), and female *H. pluchei* stridulate before and during copulation (Huber 1995; A. Peretti, unpublished data). Rhythmic genitalic squeezing by males is widespread in pholcids, and the temporal patterns are diverse (Huber & Eberhard 1997). Thick (presumably muscular) palps are also widespread in this family. In addition, a copulatory position with the epigynal area of the female positioned between the male's chelicerae and his palps, so it can be squeezed as in *P. globosus*, is also typical of pholcids (Huber 1995, 1999; Huber & Eberhard 1997). Apparent female courtship behaviour, a rhythmic lateral swinging, occurs during copulation in the pholcid *C. viridescens* (Huber 1998), and possible female copulatory courtship also occurs in the spider family Theridiidae (Knoflach & van Harten 2000).

The apparently noncoercive nature of female signals suggest that male responses are in the male's own best interests. A signaller will gain from attempting to communicate only if the receiver sometimes changes its behaviour as a result; and the receiver is expected to respond to noncoercive signals only when it is in its own reproductive interest to do so. An alternative hypothesis is that female stridulation represents a sensory trap, producing vibrations that disturb or distract the male, somehow causing him to relax his squeezes. However, males of *P. globosus* gain from responding to female stridulation by siring more offspring, thus arguing against such a trap. Additional morphological and behavioural details of male–female courtship and coupling in this species indicate that female cooperation, rather than male coercion, is crucial for successful copulation (Huber & Eberhard 1997). In summary, conflicts of interests apparently occur in *P. globosus* that may or may not result from male damage to the female; they appear to be

resolved without the physical coercion that is thought to be common in antagonistic coevolution (Alexander et al. 1997; Arnqvist & Rowe 2002).

Do our observations of female communicative responses to male genitalic movements in this spider have general significance for other animals? As noted above, female activities during copulation that could be communicatory are known in a number of other species. The line between energetic female signalling, as opposed to physical coercion of the male by the female (which may incidentally convey information), is not necessarily sharp or easily defined. Apparently, noncoercive female copulatory signals do occur in some other species (beetles: Eberhard 2005; Sirot et al., in press; flies: Oritz 2003; tsetse flies: R. D. Briceño, W. G. Eberhard & A. S. Robinson, unpublished data). Female behaviour may vary along a continuum between signalling and coercion, even during a single copulation. In the lygaeid bug *Ozophora baranowskii*, for example, longer copulations are more likely to result in successful sperm transfer, and gentle female tapping and stroking on the male abdomen early in copulation sometimes grades into forceful kicking and pushing (Rodríguez 1996, 1998). If female copulatory signals are responses to male genitalic movements within the female, they will be difficult to decipher in the many species in which (in contrast to *P. globosus*) the male's genitalia are hidden from view. What is now clear is that attention to female as well as to male behaviour, and to possible dialogues during copulation, can prove valuable in understanding sexual interactions.

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