Investigation of species divergence and reproductive isolation of *Schizocosa stridulans* (Araneae: Lycosidae) from Illinois

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Summary

Schizocosa stridulans Stratton, a North American wolf spider sibling to *S. ocreata* (Hentz) and *S. rovneri* Uetz & Dondale is sympatric with the above species and partially syntopic with *S. ocreata*, but differs from both species in secondary sexual characteristics and courtship behaviour. Courtship behaviour in *S. stridulans* consists of a series of pulses of stridulation of the palps interspersed with tapping of the first pair of legs. Males of *S. stridulans* will sometimes court females of other species, but females of related species are not receptive to them. Females of *S. stridulans* are only receptive to males of their own species. Forced interbreeding of *S. stridulans* with *S. ocreata* produced one viable egg sac from 4 copulations. Seven spiderlings emerged from this one egg sac and lived through 6–7 moults, but none reached adulthood. There were an additional 6 spiderlings that did not emerge and 2 eggs within the egg sac that did not hatch. The chromosomes of *S. stridulans* are very similar in form and number (20 autosomes (2n)$+X_1X_2$ sex chromosomes in the males) to the chromosomes of *S. ocreata* and *S. rovneri*. The above information is used to test models of speciation.

Introduction

Within the wolf spider genus *Schizocosa* Chamberlin, 1904, there are several species pairs that are at least partially sympatric and syntopic. In these species it appears that one of the present functions of species-specific courtship behaviour is species recognition with the secondary effect of reproductive isolation. *Schizocosa rovneri* Uetz & Dondale, 1979 was described as an "ethospecies" (a species behaviourally recognisable but not morphologically distinct), sibling to the widely distributed *S. ocreata* (Hentz, 1844) (Uetz & Dondale, 1979; Uetz & Denterlein, 1979). A third sibling species, *Schizocosa stridulans* Stratton, 1991, has been recognised and described within this species complex. It is slightly smaller than *S. ocreata* and *S. rovneri* but otherwise not morphologically distinct except for secondary sexual characteristics in mature males (Stratton, 1991). The courtship behaviour of *S. stridulans* differs from that of both of the above species, and as reported in this study, the courtship behaviour functions in species recognition and isolation.

Courtship behaviour has been found to be important in distinguishing spider species in a variety of studies. In Philodromidae, Dondale (1964, 1967) recognised two species of *Philodromus* that differed in courtship behaviour. Behaviour was found to be useful for distinguishing genera and even subfamilies of Salticidae (Richman, 1982). Within Lycosidae there are examples in a variety of genera in which courtship behaviour has been shown to be important for both recognising and describing species. For example, *Lycosa carbonelli* Costa &

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Capocasale is a new species sibling to *L. thorelli* (Keyserling) (Costa & Capocasale, 1984); *Pardosa vlijmi* is a sibling species to *P. proxima* (C.L. Koch) (Hollander & Dijkstra, 1974). Suwa (1980) recognised three "forms" of *Pardosa laura* Karsch and showed that these forms are behaviourally isolated and, therefore, worthy of species status. Tanaka (1985) described these forms as species; Tanaka & Suwa (1986) later described three additional species in the *Pardosa laura* complex. Kronestedt (1979) studied both *Pardosa* and *Alopecosa* ethotaxonomically and found species within each of these genera having species-specific patterns of behaviour. In a study involving behaviour and ecology as well as morphology, Kronestedt (1990) recognised two species, *Alopecosa aculeata* (Clerck) and *A. taeniata* (C.L. Koch), both at that time standing as *A. aculeata*. Additional studies (Barthel & von Helversen, 1990; Cordes & von Helversen, 1990; Töpfer-Hofmann & von Helversen, 1990) suggest that further sibling species exist in the wolf spider genera *Pardosa* and *Alopecosa*.

Reproductive isolating mechanisms are frequently divided into pre- and post-mating isolating mechanisms. Interbreeding studies are a means of assessing the relative importance of post-mating isolating mechanisms, as well as a means to test for mechanical compatibility between species. Interbreeding studies have been particularly useful not only in identifying post-mating isolating mechanisms, but also in identifying the genetic basis for the isolating mechanisms (Stratton & Uetz, 1986; Henry, 1985) and as one means of determining the extent of genetic divergence.

Most spiders have two sex chromosomes involved in sex determination. Males have one copy of these while females have two copies. This system is called an X_1X_2O system (White, 1973). Lycosids are no exception to this system, and in lycosids the haploid number of autosomes varies from 10–14 (Hackman, 1948; Gorlov *et al.*, 1995; Tugmon *et al.*, 1990; Gorlova *et al.*, 1997). Chromosomes have been used in studying sex ratios in social spiders (Avilés & Maddison, 1991), but changes in chromosomes have yet to be implicated in speciation events in spiders as has been seen in other taxa (White, 1969; Shaw *et al.*, 1983).

This current study was undertaken to learn more about *S. stridulans* and to evaluate possible modes of divergence of this species. My goals in this study were (1) to investigate pre- and post-mating isolating mechanisms by attempting conspecific and heterospecific crosses (*S. stridulans* × *S. stridulans* and *S. stridulans* × *S. ocreata*), (2) to provide a description of the courtship behaviour and to compare the courtship of those individuals that copulated with those that courted but did not copulate, and (3) to describe the karyotype of *S. stridulans* and its closely related congeners, *S. ocreata* and *S. rovneri*.

Methods

Collecting localities

All *S. stridulans* for this study (for both the behavioural study and the karyotyping) were collected from

an oak hickory forest in Sand Ridge State Forest, Mason Co., Illinois. Spiders were collected in the penultimate or antepenultimate instar and reared to maturity in the laboratory. For the behavioural comparisons of *S. ocreata* and *S. rovneri*, specimens were studied from the same locality as *S. stridulans*. The karyotypes of *S. ocreata* were from specimens collected from Alachua Co., Florida, while those of *S. rovneri* were from Boone Co., Kentucky. Voucher specimens of all species are in the personal collection of the author.

Laboratory conditions

All spiders were housed individually in plastic boxes (7×13 cm) that visually shielded them from one another. Each spider was provided with a paper cage liner (for deposition of silk and pheromone) and a cotton-stoppered glass vial for moisture. Spiders were fed crickets (*Acheta domesticus*) or house flies (*Musca domestica*) twice weekly. Spiders were exposed to ambient light from a bank of windows in the rearing room. Temperature ranged from 22–24°C throughout the study.

Conspecific and heterospecific crosses

As *S. stridulans* is sympatric and syntopic with *S. ocreata* in the northern part of its range (i.e. Illinois and Kentucky (Stratton, 1991)), there is a potential for these two species to interbreed. Crosses were not attempted between *S. rovneri* and *S. stridulans* because in this portion of the range of these two species, they are not syntopic. Two sets of crossbreeding experiments were attempted between *S. ocreata* and *S. stridulans* to test the null hypothesis that there is no difference in response to conspecific versus heterospecific individuals. In the first experiment 10 unmated females of *S. ocreata* and 15 unmated females of *S. stridulans* were presented with courting males of *S. stridulans*. Ten additional females of each species were then presented with courting males of *S. ocreata*. Each individual was used once. If the female mated with the male, she was scored as positive and held for subsequent weeks to see if she would construct an egg sac; if she did not mate with the male, she was scored as negative (thus a negative score for the female could include both aggressive and escape behaviour). The female was given 30 min after courtship began to respond to the male. The 15 pairings involving *S. stridulans* males and females were videotaped (described below) to investigate the details of the courtship behaviour.

In a second experiment, an unmated female was given 30 min to respond to a courting heterospecific male. When there was no response to the male, she was anaesthetised with CO_2 and placed in front of the courting male (method described in Stratton & Uetz, 1981). The male generally continued courtship and attempted to mount the female within a few minutes. When she "awoke", the female would sometimes run and escape from the male; alternatively, she would begin the abdomen rotation typical of the female copulatory

behaviour (a behaviour essential for sperm transfer to occur). If the female ran from the male, she was anaesthetised a second and sometimes a third time. All females (mated or not) were then maintained in the laboratory and observed for egg sac production and spiderling emergence. Heterospecific matings were attempted between six *S. ocreata* males crossed with *S. stridulans* females and five *S. stridulans* males crossed with *S. ocreata* females.

Courtship behaviour of S. stridulans

Courtship and mating behaviours were videotaped using a JVC colour video camera model 6X-N74 with either a 50 mm or 105 mm macrolens connected to a Pentax Video Recorder (Model PV-R1000A) for 15 different pairs of *S. stridulans*. Acoustic recordings were made with a Sony ECM-D15 microphone which was placed on the substrate within the test arena ($18 \times 9 \times 15$ cm plexiglass container). Sounds were analysed with a Kay Digital Sonograph Model 7800 (high dynamic range, hi shape engaged, analysis attenuator at 0 dB; narrow bandwidth); multiple frequency ranges were used (up to 32 kHz). The most informative range was 0–4 kHz. Room temperature varied from 22–24°C, and all videotaping was done between 0900 and 2000h. Females were placed with their cage liners (containing silk and pheromone) on the arena floor and allowed 5 min to settle. Males were then introduced individually and observed for 20 min. Once courtship began, the spiders were recorded on videotape for 30 min. Once copulation began, 30 min of copulatory behaviour was recorded on videotape. If courtship behaviour was not seen within 20 min, the male was scored as a negative and was removed. The following parameters were timed from the videotapes and are summarised for all 15 pairings in Tables 3 and 4: (1) *Total time spent in courtship*: onset of courtship to onset of copulation or to the end of the sampling period. (2) *Latency to chemo-exploratory behaviour*: time from when the male is placed on the female cage card to when he first displays chemoexploratory behaviour. Chemoexploration is the rubbing of the dorsum of the male palp (which is loaded with chemosensory hairs; Tietjen, 1977) on the pheromone-rich substrate. (3) *Latency to courtship*: time from onset of chemoexploration to onset of courtship behaviours. The latter include stridulation of the palp or tapping of the first pair of legs. (4) *Duration of chemo-exploratory behaviour*: average length of a chemoexploratory bout for each individual. (5) *Duration of series of stridulatory pulses:* a single flexion of the tibiocymbial joint of the palp produces a pulse or one discrete sound. A group of these movements or sounds constitutes a series of pulses (average length is reported for each individual). (6) *Rate of stridulation*: reported as number of pulses per second. (7) *Duration of front leg tap*: average length of time required for the quick tap of the front legs. Often the front legs are held in an arched position after the tap. Comparisons were made between courtships that ended in copulation and courtships that did not end in copulation.

Karyotype

Chromosome preparation followed the technique reported by Maddison (1982). A total of 14 males were examined; 7 of *S. ocreata* (plus 3 preparations by W. Maddison), 2 of *S. rovneri* and 5 of *S. stridulans*. Preparations were of penultimate males or males that had recently moulted to maturity. Males were allowed to drink a 0.05% colchicine solution 1–2 days before chromosomes were prepared. Fixation was accomplished by opening the abdomen in 3 parts absolute ethanol:1 part glacial acetic acid. Tests were Feulgen stained (7–10 min, hydrolysis in 1 N HCl at 60°C), then squeezed in 75% acetic acid.

Results

Conspecific and heterospecific crosses

In the non-forced, conspecific crosses, 15 out of 15 males of *S. stridulans* showed courtship behaviour to conspecific females. Six females of *S. stridulans* were receptive to these males (Table 1) and copulated (Table 3). In similar non-forced crosses between *S. ocreata* males and *S. ocreata* females, 42 males out of 53 responded with courtship while 39 females responded with receptive behaviour to those (42) courting males (data from Stratton & Uetz, 1986). Out of 10 *S. stridulans* males, only 2 showed courtship to *S. ocreata* females ($\chi^2 = 18.6$, $p < 0.001$). None of the *S. ocreata* females was receptive to courting *S. stridulans* males. Thus, in 10 heterospecific pairings involving *S. stridulans* males and *S. ocreata* females, none resulted in copulation (Table 1). Eight of 10 *S. ocreata* males responded with courtship to *S. stridulans* females; none of the *S. stridulans* females was receptive to these courting males ($\chi^2 = 26$, $p < 0.001$). Thus, in 10 heterospecific pairings involving *S. ocreata* males and *S. stridulans* females, none resulted in copulation (Table 1).

Six "forced" crosses were attempted between males of *S. ocreata* and females of *S. stridulans*; only 4 resulted in copulation. Of these 4, only 1 female produced an egg sac, and from this egg sac, only 7 spiderlings emerged. The other females all lived many months in the laboratory following the procedure; none of these produced egg sacs (Table 2). Five "forced" crosses were attempted

Pairings	Male response		Female response	
	+	−	+	−
S. stridulans ♂ × *S. stridulans* ♀	15	0	6	9
S. stridulans ♂ × *S. ocreata* ♀	2	8	0	2
S. ocreata ♂ × *S. stridulans* ♀	8	2	0	8
S. ocreata ♂ × *S. ocreata* ♀[1]	42	11	39	3

Table 1: Numbers of conspecific and heterospecific non-forced pairings. Neither males nor females were involved in more than 1 trial. A plus sign for males indicates courtship behaviour was shown. A plus sign for females indicates receptive behaviour was observed; a minus sign indicates there was either no response or there was an aggressive response.

[1]Data from Stratton & Uetz, 1986.

Pairings	*S. ocreata* ♂ × *S. stridulans* ♀	*S. stridulans* ♂ × *S. ocreata* ♀
Matings attempted	6	5
Copulations	4	0
Females producing at least one egg sac	1	0
Egg sacs to hatch	1	0
Offspring produced	7	0

Table 2: Numbers of forced interspecific crosses between male *S. ocreata* and female *S. stridulans* and between male *S. stridulans* and female *S. ocreata*.

between *S. stridulans* males and *S. ocreata* females; none of these resulted in copulation in spite of some of the females being anaesthetised 3 times. In each of these cases, the males were actively courting. These females were also maintained in the laboratory; none of these produced egg sacs. In a similar study (Stratton & Uetz, 1986), a total of 35 out of 38 "forced" crosses between *S. ocreata* and *S. rovneri* resulted in copulation. In the present study, the control of forcing crosses between conspecifics was not attempted as forced conspecific crosses in *S. ocreata* and *S. rovneri* resulted in viable offspring in numbers comparable to non-forced crosses (Stratton & Uetz, 1986).

Courtship behaviour of S. stridulans

Courtship behaviour in *S. stridulans* begins when a mature male encounters silk and pheromone from a mature female. Frequently the male chemoexplores the substrate by rubbing the dorsum of the palp along the silk-laden substrate in the manner described by Tietjen (1977). After chemoexploring, the male assumes a stance with his 8 legs widely spaced and with palps nearly perpendicular to the substrate. In this position he does several pulses of stridulation by rubbing a sclerotised scraper across a file, both of which are located on the male palp (Rovner, 1975). The movement producing the sound is a slight flexing of the distal joint of the male palp. This subtle movement is most easily detected with magnification. In the stridulatory movement, the tip of each palp is held against the substrate by setae located at its tip (note: this species lacks the "macrosetae" found in *S. ocreata*). Stridulation typically occurs as a series of pulses. Each pulse consists of a full flexion of the terminal joint of the palp, requires about 0.2 s to complete, and produces a discrete sound. A series may consist of 5 to 24 pulses with the series lasting from 4 to 15 s (Fig. 1).

The end of one series and the commencement of a new series is frequently punctuated by a quick tap of the front legs followed by the spider holding these legs in an arched position and another series of sounds each of which is shorter than the pulses of stridulation (Fig. 1, second half of sequence shown). The tap also produces audible sounds. Frequently, the male spider alternates between producing a series of pulses of stridulation and tapping the front legs. The male may also alternate between either of these behaviours and chemoexploring. Both the pulses of stridulation and the tapping

1985 date	Spider pair	Total time in courtship		Latency to chemoexplore		Latency to courtship	
		min	s	min	s	min	s
Je 5	1	18	52	1	23	2	28
Je 6	3	43	23	0	11	21	52
Je 10	4	7	34	0	0	0	0
Je 10	5	10	39	0	10	0	27
Je 19	6	15	0	1	9	0	38
Je 19	8	14	21	0	16	0	11
Je 22	11	14	51	12	33	0	0
Je 22	12	15	0	0	1	0	1
Je 23	14	15	0	17	26	0	0
Je 23	15*	14	59	14	59	0	16
Je 5	2*	6	29	2	46	0	7
Je 19	7*	4	38	1	4	0	10
Je 19	9*	6	45	0	2	0	10
Je 20	10*	16	47	0	11	1	49
Je 22	13*	3	43	0	5	0	2
Means		13	52	3	29	1	53
Means for courtships not ending in copulation							
		17	11b	3	41c	2	51
Means for courtships ending in copulation							
		8	54a	3	11c	0	26

Table 3: Summary of courtship parameters for *S. stridulans*. Values followed by different letters are significantly different at $p<0.05$. All pairings were done in June 1985. *indicates courtship ended in copulation.

behaviour normally precede copulation and seem to be necessary components of courtship.

In the minutes immediately preceding mounting and copulation, the relative frequency of the front leg tap increases, and the sound intensity of the pulses of stridulation also increases. The male approaches the female only after the female has done various movements that apparently signal receptivity. These movements include a turning in place ("turn"), a lowering of the prosoma ("settle"), and walking up to 5 cm away from the male and then returning ("pivot") (Table 5). When the male is within a few cm from the female, he slowly approaches holding the first pair of legs in a splayed position, as though to get as close as possible to the female without actually touching her.

The total time spent in courtship varied from 3 min 43 s to longer than 43 min (Table 3). The average time for a courtship that ended in copulation was significantly shorter (8 min 54 s) than the average time for a courtship that did not end in copulation (17 min 11 s) Mann–Whitney U-test, 1-tailed, $p<0.05$). Many of the

latter were not timed for their whole duration due to the a priori decision to sample only the first 30 min of courtship. The few pairs that were watched for longer periods, however, suggested that longer time did not alter the probability of copulation occurring. The parameter, "total courtship time", is as much an indication of the receptivity of the female as it is a measure of the persistence of the male.

Curiously, there were no other significant differences in the measured parameters (including date of trial) when courtships ending in copulation were compared with courtships not ending in copulation (Table 3). The latency to chemoexploratory behaviour varied from 0 (i.e. the male started chemoexploration immediately) to over 17 min. Long latency before the onset of courtship was observed both in courtships ending in copulation and in those not ending in copulation and is, thus, not a good predictor of the eventual occurrence of copulation. There was no difference in mean latency to courtship between both sets of courtship (Mann–Whitney U-test, 1-tailed, $p>0.05$). The latency to courtship from the beginning of chemoexploratory behaviour in most instances was short (0 to 2 min 28 s in 14 pairs), with a mean value for all 15 pairs of 1 min 53 s (S.D.$=5$ min 34 s). In one instance (pair 3) this value was nearly 22 min. If this one value is not included in calculating the mean, the mean drops to 27.0 s (S.D.$=39.0$ s).

The average duration of the episodes of chemoexploring behaviour varied from 12.06 s for courtships that ended in copulation and 8.8 s for courtships not ending in copulation (Table 4, n.s.). There was no correlation between the number of episodes of chemoexploring and their average duration ($r^2=0.0069$, n.s.). Also, there was no correlation between number of episodes and time spent in courtship ($r^2=0.0209$, n.s.). There was no correlation between the average duration of chemoexploratory behaviour and the average duration of stridulatory bouts ($r^2=0.00095$, n.s.).

The average duration of a series of pulses of stridulation for an individual varied from 2.33 to 20.80 s, but there were no differences in this parameter when courtships ending in copulation were compared with those that did not end in copulation (2-tailed t-test, $t=0.1994$, $p>0.05$; Table 4). It was expected that longer stridulatory bouts would be seen in successful courtships, perhaps reflecting a greater intensity of courtship, but this was not the case. There was no correlation between the duration of stridulation and the number of pulses/s ($r^2=0.028$, n.s.).

	Duration chemoexplore	Duration of series of stridulatory pulses	Pulses/s	Duration front legtap
	Mean (s) $\pm$ S.D.	Mean (s) $\pm$ S.D.	Mean $\pm$ S.D.	Mean (s) $\pm$ S.D.
Means for courtships ending in copulation	(n=9) 12.06 $\pm$ 7.61a	8.81 $\pm$ 5.40b	1.66 $\pm$ 0.26d	3.13 $\pm$ 1.16c
Means for courtships not ending in copulation	(n=6) 8.80 $\pm$ 7.72a	9.7 $\pm$ 10.57b	1.44 $\pm$ 0.37d	3.13 $\pm$ 1.05c

Table 4: Averages of courtship parameters for *Schizocosa stridulans*: comparison of courtships ending in copulation and not ending in copulation. Means are indicated $+/-$ S.D. Values followed by the same letters are not significantly different at $p<0.05$.
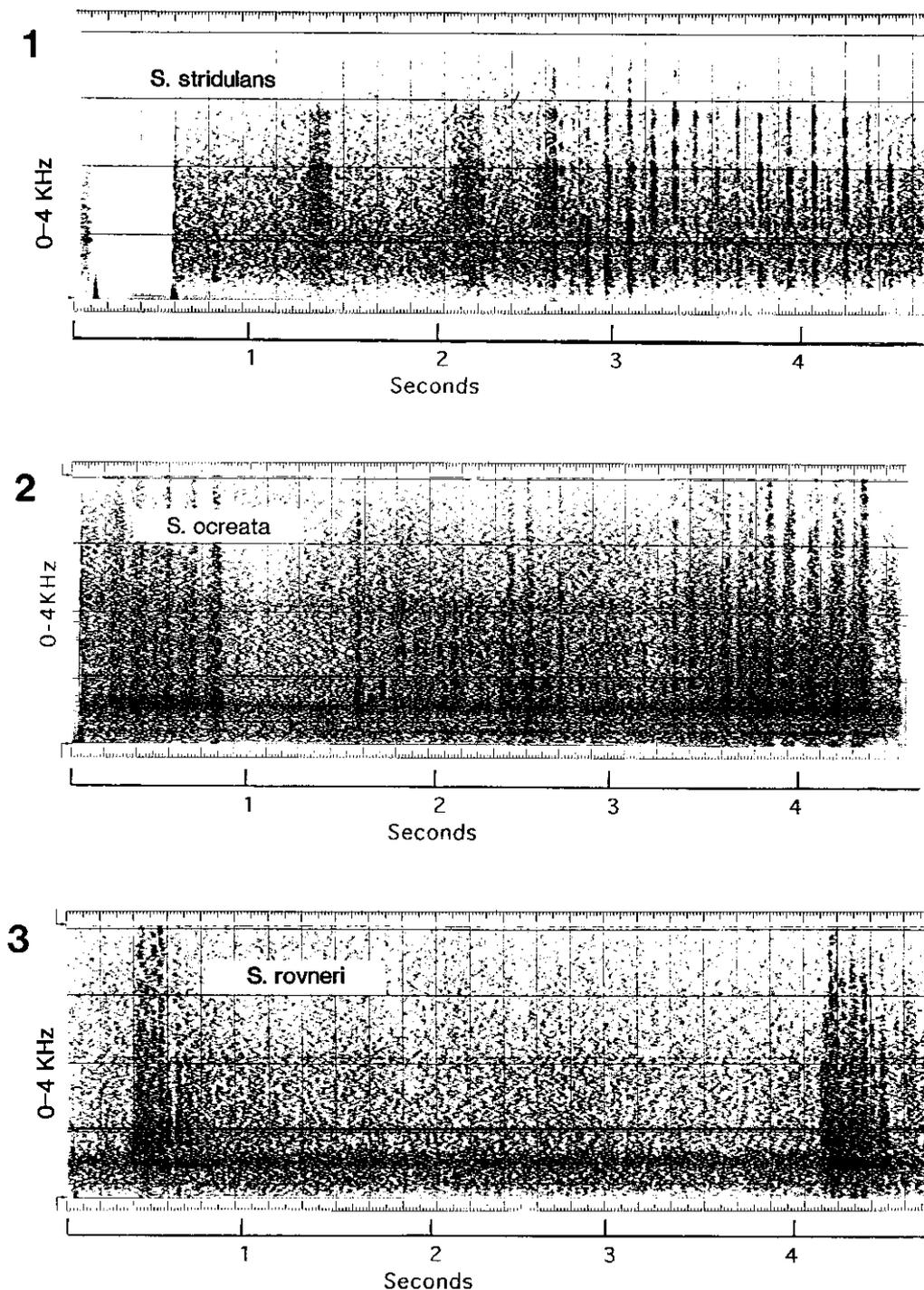
Figs. 1–3: Sonographs of sounds produced by *Schizocosa* males during courtship. Frequency range 0–4 kHz, narrow bandwidth, 4.8 s.
1 *S. stridulans*: the first 2 sounds are pulses of stridulation; at about 2.6 s there is a leg tap followed by a very quick "trill" of pulses.
2 *S. ocreata*: this demonstrates nearly continuous sound production over the time period displayed. **3** *S. rovneri*: the two pulses of sound are produced by abdominal "bounces".

The sounds produced by male *S. stridulans* are distinctive and easily recognisable, although they are barely audible to the human ear without amplification (Fig. 1). The duration of an individual pulse of stridulation varies from 0.14 to 0.24 s (mean=0.189; n=12). The greatest intensity of sound in this pulse is between 300 and 2,000 Hz. Above this point, the intensity of the sound drops drastically but may extend up to 6,000 Hz. The pulses of stridulation occur at fairly equal intervals (mean pulse/s=1.66 for courtships ending in copulation and 1.44 for courtships not ending in copulation; Table

4). Leg tapping produces a much more transient sound, with the sound occurring at some intensity from 300 to 3,000 Hz. Some of the taps, particularly early in a series, extend to 7,000 Hz, although the higher frequencies become progressively fainter. The duration of the tapping was virtually identical when courtships ending in copulation were compared with those not ending in copulation (Table 4; n.s.).

Female receptive behaviour in *S. stridulans* is very similar to that which has been observed and reported for *S. rovneri* and *S. ocreata* (Uetz & Denterlein, 1979;

Stratton & Uetz, 1981, 1983). Typically, if a female is receptive she lowers her prosoma, waves her front pair of legs, and turns or rotates 90 or 180°. She often walks a short distance from the male then returns to her original position. Females indicate receptivity on vertical as well as horizontal surfaces. In one instance, the female was on the underside of a leaf (upside down) when the male mounted and began insertions.

S. stridulans *copulatory behaviour*

Copulatory behaviour is typical of this genus (Rovner, 1973; Stratton *et al.*, 1996), with the male inserting his palp several times on one side before switching to the opposite side for insertions. During each insertion there is a single expansion of the haematodocha. The duration of copulation ranged from 54 to 127 min with an average of 85 min. This is similar to the duration of copulation in many *Schizocosa* species (Stratton *et al.*, 1996).

Comparison of courtship behaviour in S. stridulans, S. ocreata *and* S. rovneri

Although all 3 species show some behaviours in common (i.e. chemoexploratory behaviour, female receptive behaviours and copulatory behaviour), each species is unique with respect to the major elements of male courtship (Table 5) including sounds produced in courtship (Figs. 1–3). The courtship of *S. ocreata* is very active, with the spider walking and stridulating nearly continuously. While walking, the male spider also frequently arches and taps the first pair of legs which have conspicuous tibial bristles (Montgomery, 1903; Kaston, 1936; Uetz & Denterlein, 1979; Stratton & Uetz, 1981). The male of *S. ocreata* also does a "cheliceral bounce" in which the spider slams its body to the substrate, with the fangs making the first contact with the substrate (Miller *et al.*, in press), making a very transient sound. In *S. rovneri*, the male does a series of body slams (or "bounces") in which the whole body contacts the substratum, producing a loud and discrete sound (Fig. 3). The bounces are done very rhythmically at a rate of 2 bounces every 10 s (Uetz & Denterlein, 1979).

The sounds produced by the pulses of stridulation of *S. stridulans* (Fig. 1) are qualitatively different from the nearly continuous stridulation in *S. ocreata* (Fig. 2) and the series of bounces seen in *S. rovneri* (two bounces shown in Fig. 3). The sounds in all three species show a similar frequency range (up to 6 kHz), but the pattern of each species is distinct.

Karyotype

In each of the 3 species examined, *S. stridulans*, *S. ocreata* and *S. rovneri*, males have a haploid number of 10 autosomes plus X_1X_2 sex chromosomes (Figs. 4–12). Although females were not examined in this study, it is expected that the females of these species would have a haploid number of 10 autosomes plus two copies of the X_1X_2 sex chromosomes. This number of chromosomes is consistent with that reported by Hard (1939) for *S. crassipes* (now *S. ocreata*) and is similar to other reports for Lycosidae. Prophase I is shown for each of the species (Figs. 4, 7, 10). The X chromosomes are most clearly visible in Figs. 6, 10, 12. There do not appear to be differences in the size or number of chromosomes when these 3 species are compared.

Discussion

Schizocosa stridulans differs from its closely related congeners *S. ocreata* and *S. rovneri* in its courtship behaviour and in the secondary sexual characteristics, but not in its karyotype. Since this species will not interbreed with the species with which it is sometimes syntopic, *S. ocreata*, its species status is confirmed. Each of these species has unique courtship elements (Table 5).

S. stridulans is apparently not genetically compatible with *S. ocreata*, as only 1 in 4 egg sacs ever hatched and none of the very few hybrids from the "forced" cross between these 2 species survived to maturity. This suggests that there is a greater genetic difference between *S. ocreata* and *S. stridulans* than between *S. ocreata* and *S. rovneri*; the latter pair are nearly fully interfertile using these same techniques (Stratton & Uetz, 1981). However, the possibility cannot be ruled out that

Character	S. stridulans	S. ocreata	S. rovneri
Amount of locomotory movement in courtship	Courtship while male is mostly stationary	Courtship with constant locomotion	Courtship while male is mostly stationary
Major components of male courtship behaviour (including sounds produced)	Pulses of stridulation with palps, leg taps with front legs	"Jerky walk," tapping and arching with front legs	Series of "bounces"
Sounds produced in courtship	Occurs in pulses	Nearly continuous sound	Loud, discrete sounds from bounces
Major components of female behaviour	Pivot, turn, settle	Pivot, turn, settle	Pivot, turn, settle
Copulatory behaviours	Series of insertions of palp on one side, switch sides, series of insertions on other side	Series of insertions of palp on one side, switch sides, series of insertions on other side	Series of insertions of palp on one side, switch sides, series of insertions on other side
References	Present study Stratton, 1991	Montgomery, 1903 Kaston, 1936 Stratton & Uetz, 1981	Uetz & Denterlein, 1979 Stratton & Uetz, 1981 Stratton & Uetz, 1983, 1986

Table 5: Comparison of major elements of courtship in *S. stridulans*, *S. ocreata* and *S. rovneri*.
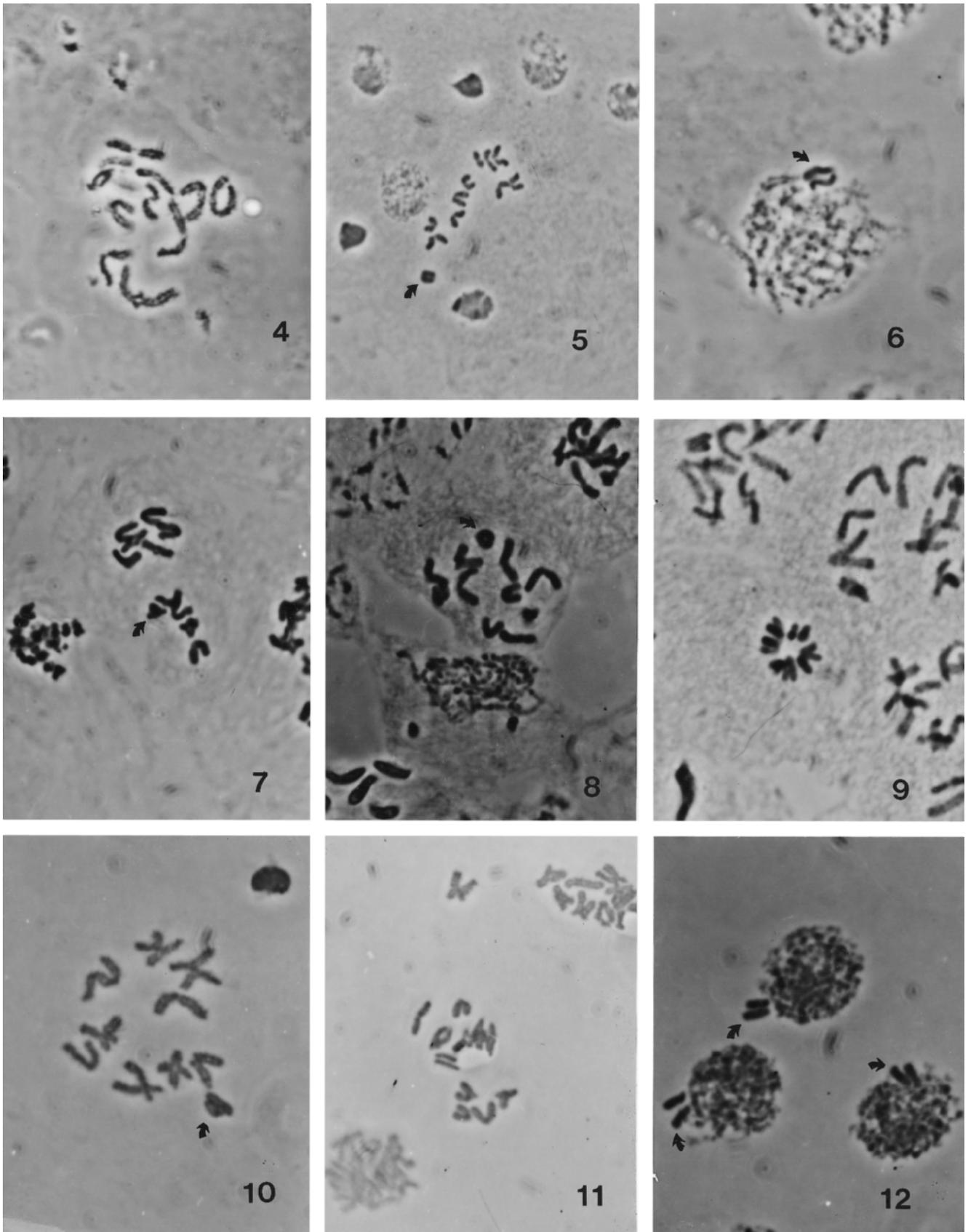
Figs. 4–12: Karyotypes of *Schizocosa* species. **4–6** *S. stridulans*, mature male (Illinois, Mason Co., Sand Ridge State Forest, 6 June 1985). **4** Diplotene stage of prophase I; **5** Metaphase II, arrow points to X chromosomes; **6** Early prophase I, arrow points to X chromosomes. **7–9** *S. ocreata*, mature male (Florida, Alachua Co., chromosome preparation by W. P. Maddison). **7** Diplotene stage of prophase I; X chromosomes (shown by arrow) are slightly more darkly stained than autosomes; **8** Metaphase II, arrow indicates X chromosomes; **9** Prophase I. **10–12** *S. rovneri*, mature male (Kentucky, Boone Co., 22 Oct. 1981; moulted to penultimate instar 30 Nov.; preparation by W. P. Maddison). **10** Diplotene stage of prophase I, X chromosomes shown by arrows; **11** Diakinesis; **12** Early prophase I (pachytene stage); 3 nuclei are visible, each showing condensed X chromosomes (indicated by arrows).

S. stridulans is more sensitive to the manipulations of anaesthetisation than *S. ocreata* and *S. rovneri*.

Although the general pattern of courtship behaviour is easily recognised, there is some variability both within and between individuals in the measured courtship parameters (Table 4). None of the measured parameters is correlated with the occurrence of copulation, thus these parameters provide no evidence of female choice in this laboratory setting. Perhaps a female's ability to detect a potential mate is more important than "choosing" a particular individual. It is not known how frequently a female is courted in the field nor how many times she may copulate. It remains unknown what elements of courtship (if any) or characteristics of a male (if any) a female is choosing. Studies involving manipulation of visual and acoustic signals produced by the male should prove fruitful.

Many models have been presented to explain how species diverge into groups that will not interbreed and to explain how species maintain their reproductive isolation once the differences are established (Dobzhansky, 1940; Mayr, 1963; White, 1969, 1973; West-Eberhard, 1983). The hypotheses to explain speciation can be divided into three categories: (1) divergence of populations in allopatry with subsequent selection against hybrids when the populations rejoin (sometimes called the reinforcement hypothesis; Fisher, 1930; Dobzhansky, 1940; Butlin, 1987, 1995), (2) divergence by sexual or social selection (Darwin, 1871; West-Eberhard, 1983), and (3) divergence by changes in chromosome number or form such as by deletions or fusions (White, 1973, 1978).

The divergence mechanism of this species remains unknown although the present study provides preliminary data for testing some hypotheses. The reinforcement hypothesis suggests that some differences would be the result of selection against hybrids, and some differences would be the result of divergence in allopatry. This model has some support from this study, since there is apparently genetic incompatibility between *S. ocreata* and *S. stridulans* as evidenced by their inability to produce viable offspring and their unwillingness to interbreed. This suggests that there is some genetic divergence. However, a study by Steiner *et al.* (1992) showed that there were *no* differences in the genetic variability of *S. ocreata* and *S. stridulans* as measured by electrophoresis and that there were no diagnostic loci for *S. stridulans*. While *S. stridulans* and *S. ocreata* are sympatric and syntopic over at least part of their range (Stratton, 1991), it is not known if their differences would be less pronounced where they do *not* co-occur. A more thorough test of the reinforcement hypothesis should include such a comparison.

The sexual selection hypothesis predicts that the main differences between species would be epigamic, or those traits associated with courtship and reproduction. This hypothesis does not require other differences to exist. These species do differ most visibly in the epigamic characters (dark pigmentation on front legs and courtship behaviour; Stratton, 1991). Sexual selection generally works in two contexts: intraspecific competition and interspecific choice. This study has only addressed interspecific choice; the epigamic characters could also be more important in the context of male–male competition.

In the current study, it would appear that females of *S. stridulans* are being "choosy" (only 6 out of 15 mated in conspecific pairings), but it is not clear what (if anything) the female is choosing. The sexual selection hypothesis may have some support in that the primary differences are epigamic, but it is not clear from this study if the females are providing selection for the epigamic characters. Other parameters to be investigated could include variability of female receptivity over time (demonstrated in *S. ocreata*; G. W. Uetz, pers. comm.), size and weight of males, effects of substrate on effectiveness of courtship, relative importance of acoustic, chemical and vibratory signals and the relative importance of intra-sexual competition. The present study will allow for future quantitative comparisons.

The chromosomal divergence hypothesis suggests that changes in the chromosomes can "drive" divergence. Since there are no differences in the number of chromosomes of these 3 species, the model of chromosomal speciation at the level of changes in number or form of the chromosomes is not supported.

Future studies will look in more detail at the sexual communication of this species in its natural environment. Studies focusing on the details of female choice and male competition should also be productive.

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