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New findings on the courtship behaviour of *Pardosa wagleri* (Hahn, 1822) and *P. saturatior* Simon, 1937 (Araneae, Lycosidae), a pair of sibling species

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Abstract

Pardosa wagleri and *P. saturatior* are sibling species previously studied by Tongiorgi (1966) and Barthel & von Helversen (1990). Tongiorgi (1966) established the validity of the two species on the basis of differences in ecology, phenology, coloration and size. Barthel & von Helversen (1990) provided preliminary evidence for their separation by reproductive isolation mechanism and morphometric parameters. In this paper the courtship displays of the two species are described for the first time and compared by the examination of the sequence successions and optical flow, both based on video analysis. Intra-individual, intra- and inter-specific differences were tested with classical statistical analysis, including Anova, Nested-Anova and Paired t-test. The two displays are characterized by two units, the first (A) involving several parts of the spider body and the second (B) the male's palps only. Main differences between *P. wagleri* and *P. saturatior* displays are found in unit A, which is characterized by two sub- units (A1 and A2) involving synchronic movements of palps, legs and abdomen. According to Nested Anova, unit B resulted identical for the two species. In conclusion our study deepens the work of Tongiorgi (1966) and Barthel & von Helversen (1990), providing new insight (description and contrast of the two displays) on the biology of the two species.

INTRODUCTION

Species-specific recognition and sexual selection are two issues related to animal communication resulting from the female's ability to respond to a change in male traits (Ryan & Rand 1993).

Secondary sexual characters are particularly developed in those groups showing high specific variation, suggesting that sexual selection could be implicated in the phenomena of speciation.

Communication in sexual selection is often based on courtship display performed by males involving different sensory channels (Johnstone 1995). Lycosids represent an excellent model for studies on sexual communication due to multimodal signaling. This is particularly true for the spiders of the genus *Pardosa* in which visual, vibratory and chemical signals are simultaneously involved in courtship.

Species belonging to this genus are very abundant and common in Europe and North

Name	Species	Date	Locality	UTM	Habitat
Sat1	*P. saturatior*	30/06/07	Conca del Prà, Alta val Pellice. Bobbio Pellice (TO)	034495 4959313	Pebble river bank (1732m s.l.m.)
Sat2	*P. saturatior*	30/06/07	Conca del Prà, Alta val Pellice. Bobbio Pellice (TO)	034495 4959313	Pebble river bank (1732m s.l.m.)
Sat3	*P. saturatior*	30/06/07	Conca del Prà, Alta val Pellice. Bobbio Pellice (TO)	034495 4959313	Pebble river bank (1732m s.l.m.)
Sat4	*P. saturatior*	30/06/07	Conca del Prà, Alta val Pellice. Bobbio Pellice (TO)	034495 4959313	Pebble river bank (1732m s.l.m.)
Sat5	*P. saturatior*	30/06/07	Conca del Prà, Alta val Pellice. Bobbio Pellice (TO)	034495 4959313	Pebble river bank (1732m s.l.m.)
W1	*P. wagleri*	27/06/07	Parco delle Capanne di Marcarolo. Lerma (AL)	047777 4940421	Pebble river bank (293m s.l.m.)
W11	*P. wagleri*	04/03/07	Fondovalle Tanaro. Farigliano (CN)	041348 4928905	Pebble river bank (263m s.l.m.)
W12	*P. wagleri*	04/03/07	Fondovalle Tanaro. Farigliano (CN)	041348 4928905	Pebble river bank (263m s.l.m.)
W14	*P. wagleri*	04/03/07	Fondovalle Tanaro. Farigliano (CN)	041348 4928905	Pebble river bank (263m s.l.m.)
W15	*P. wagleri*	04/03/07	Fondovalle Tanaro. Farigliano (CN)	041348 4928905	Pebble river bank (263m s.l.m.)

Table 1. Sampling data, locality and habitat of the five males of *Pardosa saturatior* and the five males of *Pardosa wagleri*.

America and show a high degree of diversification (Foelix 1996). Moreover, in several cases the systematics is still unclear and characterized by the presence of groups containing species that share some common characteristics such as the external pattern or the shape of the palp (i.e. *P. monticola* and *P. lugubris* group).

Pardosa wagleri and *P. saturatior* are sibling species. They both have a uniform grey body coloration. In some cases yellow or red spots can be visible on their abdomen while their legs are characterized by yellowish annulations. Generally, *P. saturatior* can be distinguished from *P. wagleri* by its larger body size and the darker general coloration. The differences in the shape of the palps are not minor. The study of Barthel & von Helversen (1990) has highlighted the differences in dimensional relations among the single palpal components.

Paradosa wagleri and *P. saturatior* are also separate ecologically, more precisely *P. wagleri* lives in river banks below 1200 m while *P. saturatior* lives mainly in river banks and stony places over 1400 m. Contact areas between the two species are found between 1200 and 1400 m. The presence of an ecological barrier and the results obtained by Barthel & von Helversen (1990) demonstrate that the two species are separate, as previously stated by Tongiorgi (1966).

In this work we describe and compare the courtship behaviours of the two species to find out if they may contribute to the observations made by Barthel & von Helversen (1990). In their work they only mentioned the diversity of two displays and the consequent ethological separation but they never published any description of the behaviours.

	P. saturatior			*P. wagleri*		
	A1	A2	B	A1	A2	B
A1	7.6%	6.7%	7.1%	37.0%	12.7%	8.5%
A2	9.8%	40.6%	5.4%	4.1%	7.0%	5.7%
B	1.3%	7.6%	13.8%	14.8%	0.1%	10.1%

Table 2. Sequence analysis of the events in *Pardosa saturatior* and *Pardosa wagleri*. Values in the table are referred to linkages among different BUs. Columns precede in the time sequence rows (example: subBU A1 precedes subBU A2 in 6.7% of the cases; 9.8% vice-versa).

MATERIAL AND METHODS

Twenty specimens have been field collected. Five males and one female of both species were used for the visual display analysis (see Tab. 1). Voucher specimens are stored in Isaia's collection at Dipartimento di Biologia Animale e dell'Uomo of Turin University.

Obtaining the displays

The reproductive behaviour of the two species was obtained by placing the female and the male into a circular glass arena of 20 cm diameter. An absorbent cloth paper was placed inside the arena to ensure that chemical traces, mainly produced by females, could last long and guarantee an optimal transmission of the vibrations produced on the substrate by both male and female (Rypstra et al. 2003).

Display recording

The recordings of the courtships were made with three cameras (Sony DCR-HC17E, Canon MV700 and Canon MV800i, recording speed 25 frames/s), placed perpendicularly at the same height in order to observe a sufficiently broad area. The movies were acquired with Windows Movie Maker software (resolution: 720 x 576 at 25 frames/s).

Observation of the displays and behavioural units (BUs) identification

To obtain a sufficient number of information, several data sheets were compiled for each individual. One set of data sheets corresponds to each camera.

Data sheets have the purpose to evaluate a number of aspects which characterize the display, but also to define the behavioural units (BUs) that are the succession of the events repeated in the courtship sequence. Following the terminology adopted by Lehner (1998), with the term "event" we mean an instantaneous change of state, not clearly measurable in a time scale. Events are thus defined according to the different movements of the anatomical parts, particularly the abdomen, the palps, the first pair of legs and the general body movement. A small program was developed using Matlab 7 in order to study the links between the different BUs.

Optical flow analysis

We followed the approach of Elias et al. (2005) for the analysis of the optical flow. This kind of analysis evaluates the changes at the pixel intensity that compose the video frame. We developed an application software using Matlab, to study the male courtship behaviour. Unlike Elias et al. (2005), we implemented a number of options that simplify and make the video analysis easier. That is an intuitive graphical interface and the possibility of cropping the area of interest in the movie. A further application software was developed in Matlab to analyse the optical flow in a more detailed way. The program detects the duration of a single BU and measures the interval between a single BU and the following one.

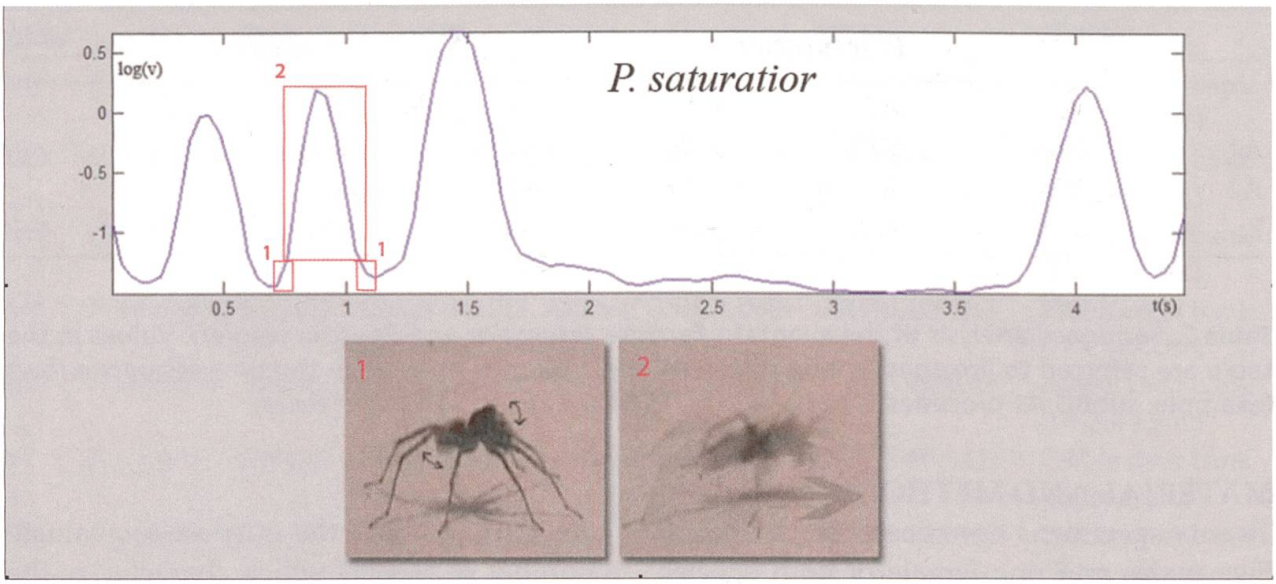

Fig. 1. *Pardosa saturatior:* SubBU A2, optical flow analysis. 1: initial vibration of the palps and abdomen; 2: movement of the male towards the female.

RESULTS

Description of the displays

Both *P. saturatior* and *P. wagleri* show two distinct BUs (A and B) and two sub-behavioural units (subBUs) for BU A (A1 and A2) within a single display:

- In *P. saturatior,* BU A involves the general movement of the body. The male begins to vibrate palps and abdomen with increasing intensity until their movement is turned into a wide fluctuation. The male remains motionless (subBU A1). When reaching the maximum intensity the male moves towards the female very quickly, lowering the body against the substrate, vibrating the abdomen and raising and lowering the first pair of legs at increasing frequency (subBU A2). Afterwards the male stops and eventually repeats subBU A1.
  In BU B the male rapidly raises and drops the palps alternately. This behaviour can be repeated from one (raising and lowering of one single palp) to four times (two palps alternately). In some cases the lowering of the palps may be slow, especially when the female changes position inside the arena. The whole courtship display before copulation may last 10–15 minutes.
- In *P. wagleri,* the first BU (A) is mainly characterized by a rapid and clear movement of palps and body. The display begins with a palpal pulse that gradually increases in intensity to finally evolve into a wider movement. The palps are moved simultaneously upwards and then lowered very quickly and in some cases they can touch the substrate (subBU A1). When the intensity of vibration increases, the male moves forward in the direction of the female, whipping the first pair of legs (subBU A2).
  BU B is equivalent to BU B of *P. saturatior* (see later paragraph on ANOVA results).

Sequence analysis (Tab. 2)

The most distinctive behaviour observed in *P. saturatior* is the repetition of subBU A2 (40.6% of cases) that may be followed by A1 (9.8%) or, rarely, by B (5.4%). In a few cases A2 is preceded by A1 (6.7%) or B (7.6%). The repetition of subBU A1 is not very frequent (7.6%); BU B is repeated more often (13.8%). B does not frequently follow A1 (7.1%) and the rarest behaviour observed is the linkage between B and A1 (1.3%).

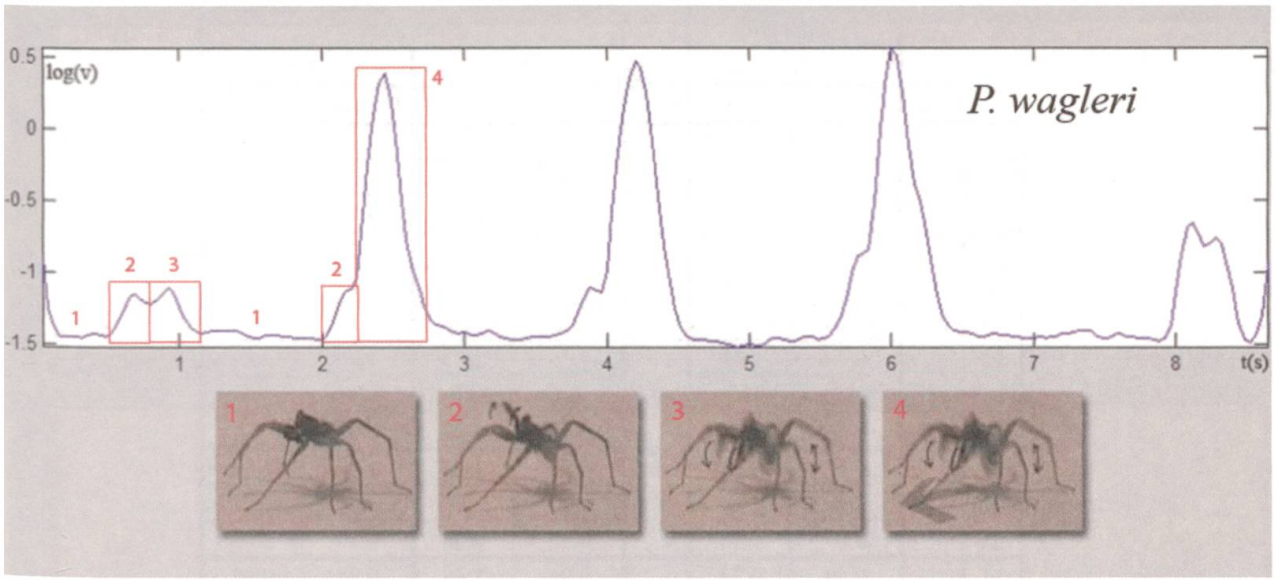

Fig. 2. *Pardosa wagleri:* BU A, optical flow analysis. 1: the spider is motionless; 2: first palpal raising and body inclination of about 45 ° from substrate level; 3: rapid lowering of both palps and the consequent vibration of the body; 4: the previous behaviour plus the movement towards the female, characterized by the whipping of the first pair of legs. SubBU A1 corresponds to 2+3; SubBU A2 corresponds to 2+4.

The display of *P. wagleri* is mostly characterized by the repetition of subBU A1 (37.0%), which may be followed by both A2 (12.7%) or B (8.5%). The repetition of subBU A2 is not frequent (7.0%). SubBU A2 seldom precedes subBU A1 (4.1%) or B (5.7%). The sequence B→A2 has never been observed whereas the repetition of BU B or the linkage B→A1 are quite frequent (10.1 and 14.8% respectively).

Optical Flow

BEHAVIOURAL UNIT A

- *Pardosa saturatior* (Fig. 1): The first upslope (indicated in the figure by "1") can be observed in correspondence to the initial vibration of the palps and abdomen. This movement is graphically represented by the beginning of the curve that will compose the peak. The peak ("2") corresponds to the rapid and wide movement of the male toward the female. Each curve represents subBU A2. SubBU A1 is more rare and it has not been analyzed with the program (the movement resulted too rapid for 25 frame/sec resolution).
- *Pardosa wagleri* (Fig. 2): In this species subBUs A1 and A2 can be distinguished more easily. At the beginning ("1") the spider is motionless. The first peak ("2") corresponds to the first palpal raising and to the body inclination of about 45° from the substrate level. The nearby peak may be related to (a) a rapid lowering of both palps and to the consequent vibration of the body ("3") and (b) the previous behaviour plus the movement towards the female, characterized by the whipping of the first pair of legs ("4").

The analysis considers the duration (in seconds) of subBUs A1 and A2 and the interval between the successive BUs (Tab. 3). *Pardosa saturatior* shows significant differences at intervals between the successive BUs ($f = 3.29$, sig.= 0.02). *Pardosa wagleri* shows differences in the duration of subBUs A1 ($f = 7.10$, sig.= 0.00) and A2 ($f = 4.22$, sig.= 0.01).

BEHAVIOURAL UNIT B (Fig. 3)

This BU is very similar in the two species. Initially the male is motionless ("1"). The first peak ("2") corresponds to the rapid raising of one of the palps while the second peak ("3") corresponds to its rapid lowering. This behaviour is also repeated with the other

		P. saturatior			*P. wagleri*			
		A2			A1		A2	
	Ind.	Duration	Interval	Ind.	Duration	Interval	Duration	Interval
M	tot	0.45	0.66	tot	0.97	2.39	0.97	2.09
N		77	51		54	40	27	17
DV		0.11	0.36		0.27	2.59	0.14	2.09
M	sat1	0.43	0.95	w11	1.02	3.30	0.78	0.93
N		15	10		13	9	2	1
DV		0.11	0.54		0.22	2.36	0.11	.
M	sat2	0.45	0.56	w12	1.18	2.08	1.20	5.18
N		15	10		14	10	2	1
DV		0.11	0.32		0.18	2.29	0.08	.
M	sat3	0.42	0.50	w14	0.88	1.20	1.00	2.34
N		14	9		3	2	10	7
DV		0.09	0.15		0.23	0.08	0.10	2.09
M	sat4	0.48	0.74	w15	0.96	3.43	0.85	0.98
N		19	14		11	7	3	2
DV		0.11	0.30		0.32	4.22	0.07	0.07
M	sta5	0.45	0.50	w1	0.73	1.57	0.97	1.85
N		14	8		13	12	10	6
DV		0.12	0.12		0.14	1.81	0.14	2.43
f.		0.66	3.29		7.10	1.00	4.22	0.76
sig.		0.62	**0.02**		**0.00**	0.42	**0.01**	0.57

Table 3. ANOVA optical flow analysis results for BU A. For *Pardosa saturatior* only subBU A2 is shown (see text). M: mean value, N: number of cases, DV: standard deviation.

palp, raised ("4") and lowered ("5") very quickly. In some cases it is possible that the male raises the palp in two steps. The small initial peak ("I") observed in the diagram can be related to this behaviour. The valley observed between the two main peaks ("II"), indicates the short period during which the palp is held for a short time.

The analysis has been performed in terms of the duration (in seconds) of the movement of the palps (tab.4). In *P. saturatior* the movements of the palps (2–3 and 4–5) show a significant difference in time length (t = -2.601, df = 19, P = 0.018).

In *P. wagleri* the movement of the first palp (2–3) shows intraspecific variability (f=4.80, sig.=0.01). A difference similar to *P. saturatior* in the duration of 2–3 and 4–5 was observed in *P. wagleri* (t = -2.413, df = 15, P = 0.029).

No significant difference has been found by comparing the two species with a nested-Anova: duration (2–3): (F = 2.79, df = 1, 8.89 P = 0.13), intervals: (F = 2.16, df = 1, 17.6, P = 0.16), duration (4–5): (F = 0.502, df = 1, 11.3, P = 0.493).

DISCUSSION

Despite the similarities regarding the anatomical parts involved in the courtship display, the two species behave differently. As demonstrated by the description of the display, the differences between the quantitative analysis of the sequences and the optical flow analysis are clearly evident at BU A. This is probably the main BU of the display which gives the information needed for a correct species-specific recognition.

BU B is identical in the two species. The results of nested-Anova actually confirmed that there are no significant differences between the two behaviours. BU B may be better interpreted as an "alarm posture", prob-

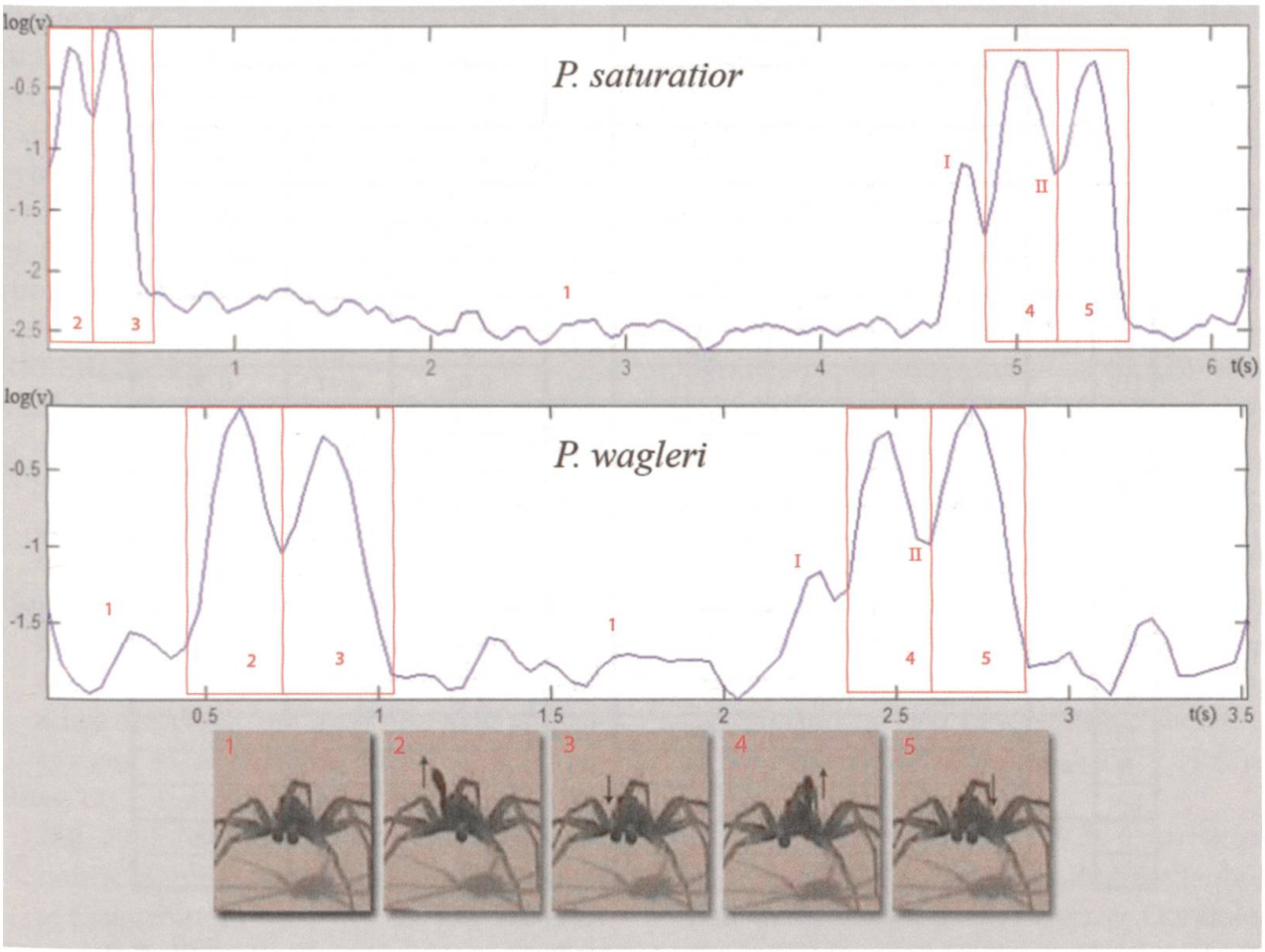

Fig. 3. *Pardosa saturatior* and *Pardosa wagleri:* BU B, optical flow analysis. 1: the spider is motionless; 2: rapid raising of the first palp; 3: rapid lowering of the first palp; 4: rapid raising of the second palp; 5: rapid lowering of the second palp. I: peak related to the raising of the palp in two steps; II: short period during which the palp is held.

ably adopted to capture the attention of the female. The display of this behaviour in both species may indicate the absence of information regarding species-specific recognition in BU B.

Several intraspecific differences have been observed. Individuals do not always display a homogeneous behaviour within the same species, which causes changes in significance. For example, in one individual of *P. saturatior* (SAT1) a double timing in the interval between the different BUs was observed. On the other hand the duration of subBU A2 is extremely homogeneous within individuals (nearly half a second in all cases).

It is interesting to note that one individual of *P. wagleri* (W1) coming from a different population showed different values in most parameters.

The difference between the two displays demonstrates the presence of reproductive isolation mechanisms based on ethological barriers that avoid the gene flow, which further confirm the separation of the two species as stated by Barthel & von Helversen (1990) and Tongiorgi (1966). Probably the ancestral population was separated by a physical barrier and, only afterwards, the two species diverged in different habitat conditions (i.e. shorter season and lower temperature for *P. saturatior*) due to genetic drift and later selection.

The high diversity of courtship behaviour in BU A and, more generally, in spiders, could be a result of selection from potential

		P. saturatior				*P. wagleri*		
		B				B		
	Ind.	Duration (1)	Interval	Duration (2)	Ind.	Duration (1)	Interval	Duration (2)
M	tot	0.52	1.54	1.07	tot	0.62	0.87	0.65
N		25	20	20		20	16	16
DV		0.26	1.50	1.08		0.12	0.35	0.11
M	sat1	0.51	2.25	0.95	w11	0.62	0.86	0.71
N		5	5	5		5	5	5
DV		0.17	1.63	0.57		0.02	0.47	0.09
M	sat2	0.38	1.71	0.84	w12	0.60	0.98	0.65
N		5	5	5		5	5	5
DV		0.10	1.95	0.87		0.13	0.22	0.11
M	sat3	0.72	1.53	2.03	w14	0.70	.	.
N		6	5	5		2	.	.
DV		0.42	1.42	1.66		0.03	.	.
M	sat4	0.46	0.61	0.47	w15	0.78	0.36	0.81
N		5	4	4		3	1	1
DV		0.21	0.84	0.25		0.13	.	.
M	sat5	0.49	0.94	0.49	w1	0.51	0.86	0.57
N		4	1	1		5	5	5
DV		0.15	.	.		0.06	0.32	0.06
f.		1.42	0.67	1.62		4.80	0.86	3.06
sig.		0.26	0.62	0.22		**0.01**	0.49	0.07

Table 4. ANOVA optical flow analysis results for BU B. M: mean value, N: number of cases, DV: standard deviation.

sexual cannibalism, species recognition, female choice, or, more probably, a combination of all these factors (Delaney et al. 2007; Foelix 1996; Persons & Uetz 2005). The male has to communicate to a female that he is a conspecific and that he is a male of good quality, avoiding cannibalism behaviour (Persons & Uetz 2005; Delaney et al. 2007). The consequences of all these forces are sexual dimorphism and complex courtship displays involving more than one sensory channel (Delaney et al. 2007). These kinds of display are very common in the family Lycosidae and they can act as a systematic character. Hollander and Dijkstra (1974) studied reproductive barriers in the genus *Pardosa* and described a new species, *Pardosa vlijmi*, separate from *Pardosa proxima* only on the basis of the courtship behaviour. More recently Töpfer-Hofmann et al. (2000) separated six species of the *P. lugubris* group on the basis of the reproductive behaviour with the description of two new ethospecies (*P. saltans* and *P. pertinax*). Moreover these ethospecies can also be found syntopically.

Schizocosa is one of the most studied genera regarding courtship behaviour and speciation. Uetz and Denterlein (1979) separated *S. ocreata* from *S. rovneri* on the basis of the courtship display. These differences cause strong prezygotic isolation but hybridization can also occur. Stratton and Uetz (1986) produced F_1 hybrids that showed an intermediate behaviour, switching between behaviours typical of each parental species (both visual and vibratory behaviour).

Preliminary experiments performed by Barthel & von Helversen (1990) on heterospecific cross matings showed that females of *P. wagleri* never accepted heterospecific males while *P. saturatior* females accepted males of *P. wagleri* in 20% of cases. Further

studies on *P. wagleri* and *P. saturatior* will focus on reproductive isolation mechanisms between populations of the two species occurring in contact areas, in order to obtain hybrid generations and study their behaviour. Both species will be also investigated on the genetic variability at population and species level.

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