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Differences in sexual behavior of two distant populations of the funnel-web wolf spider *Aglaoctenus lagotis*

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Abstract Generally, sexual repertoire within a species is conserved, but frequencies of occurrence of sexual behavioral acts often vary, and wide geographic distributions may favor these variations. *Aglaoctenus lagotis* is distributed along South America and belongs to Sosippinae, a subfamily of wolf spiders that builds funnel webs. Previous reports, based on different populations, suggested variations in sexual behavior and life cycle. Our objective was to describe and compare the sexual behavior of two populations of *A. lagotis*: 'Southern Uruguay' (SU) and 'Central Argentina' (CA). We carried out intrapopulation trials, in the laboratory, with 15 female–male pair matings. The most representative courtship acts in SU were web-stretching, striding-forward and forelegs-elevating, whereas in CA they were alternate-waving, web-stretching and leg-tapping. Juddering, forelegs-elevating and striding-forward were exclusive for SU, while alternate-waving and simultaneous-waving were exclusive for CA. We also found differences in copulatory characteristics such as frequencies of insertions and ejaculations. We documented body-shaking during copulation for the first time. Some sexual acts were exclusive of each population, while the shared ones differed in pattern and frequencies of occurrence. In addition to variations in sexual periods, these differences may favor divergence processes or an exceptional high level of flexibility in the sexual behavior of *A. lagotis*.

Keywords Courtship · Copulation · Intraspecific variations · Sedentary · Lycosidae

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Introduction

In animals with sexual reproduction, mating is the mechanism that allows the union of gametes and exchange of genes. Classically, this swap is expected between members of the same species, which are reproductively isolated from populations of other species (Mayr 1942, 1998) and show an identifiable genotypic cluster (Mallet 1995). In agreement with this, the behavioral acts involved with mating (defined as 'sexual repertoire', following Lehner 1996) will appear in the same 'pattern' (defined as 'linkage of several acts in a predictable and stereotyped way' by Lehner 1996) in individuals from the same species and, at the same time, will differ from the patterns of other species (Barlow 2002). These sexual behavioral patterns, especially during

courtship, play roles in recognition between mates, as well as in synchronization, orientation, persuasion and assessment of the mate (Tinbergen 1953; Verrell 1999; Verrell and Mabry 2003).

In spite of the specificity in sexual repertoire and behavioral patterns, we can expect certain levels of intraspecific variation in sexual behavior (Herberstein 2011), mostly related to the frequencies in which individuals perform the behavioral acts. Variations may be associated with physiological conditions of individuals (Kotiaho et al. 1996; Mappes et al. 1996), with preferences for mating partners (Parker 1983; Kotiaho et al. 1996; Hoikkala et al. 1998; Singer et al. 2000; Parri et al. 2002; Cratsley and Lewis 2003; Peretti and Eberhard 2010; Dutto et al. 2011), with the complexity of the microhabitat, favoring different communication modalities (Jackson 1997; Wilczynski and Ryan 1999; Clarck and Biesiadecki 2002; Taylor et al. 2005; Su and Li 2006; Gordon and Uetz 2011; Wilgers and Hebets 2011), with the existence of alternative mating strategies (Gross 1996; Brockmann 2001; Rios-Cardenas et al. 2007), or with personality (Trillmich and Hudson 2011). Another source of variation, often closely associated with the above-mentioned, can be the wide geographic distributions that some species show (Macías-Ordóñez et al. 2013).

Widely distributed species are often good candidates to develop intraspecific variation in sexual behaviors. Large areas of distribution favor the existence of gradients in environmental conditions (temperature, vegetation, and substrates, among others), which could facilitate the origin of differences in the frequencies of occurrence of sexual behavioral acts (Claridge et al. 1985; Wilczynski and Ryan 1999; González-Zuarth and Macías-García 2006; Macías-Ordóñez et al. 2013). Less commonly, environmental gradients are related to deeper variations in sexual behavior, such as in the patterns that develop in behavioral acts (Zann 1976; Miller et al. 1998; Wilczynski and Ryan 1999) or in the acts that compose the repertories themselves (Elias et al. 2006; Ishikawa et al. 2006). Sometimes, these differences between populations coexist without affecting the occurrence of matings (Saarikettu et al. 2005; González-Zuarth et al. 2008), but in other cases, these intraspecific variations promote a speciation process and prevent interpopulation matings (Zann 1976; Hoikkala and Welbergen 1995; Miller et al. 1998; Lachlan and Servedio 2004). In spiders, we have found few studies reporting interpopulation variations in sexual behavior. The salticid *Habronattus pugillis* shows geographical differences in courtship behavioral acts related to the seismic channel (Masta and Maddison 2002; Hebets and Maddison 2005; Elias et al. 2006), and Miller et al. (1998) reported variations in behavioral pattern and frequencies of occurrence of behavioral acts during courtship for *Schizocosa crassipes*.

Our objective in the present study was to describe, analyze, and compare the sexual behavior (courtship and copulatory behavior) of two distant populations of *A. lagotis*, one from Southern Uruguay and the other from Central Argentina, thought to occur in a continuous range. The former is located at the southernmost point given for the species distribution, whereas the second is located intermediately between the former population and the Brazilian ones from which Sordi (1996) and Stefani et al (2011) have described ecology and sexual behavior. In southern Uruguay, the temperatures are lower, humidity is higher, and winters are longer than in Central Argentina. Grassland vegetation is dominant in Southern Uruguay while hills with scrubby vegetation predominate in Central Argentina (Cabrera 1971, Grela 2004). Considering these different environmental conditions in chosen localities, we could expect differences in durations and frequencies of occurrence of behavioral acts in courtship and copulation, as has been reported for other widely distributed species. This study provides information about the degree of intraspecific variation in sexual behavior of *A. lagotis* and, additionally, offers useful data about the effects of ecogeographical variations in divergence processes of the species, sexual compatibility, and cohesion of the species.

Materials and methods

The genus *Aglaoctenus* belongs to Sosippinae, a small subfamily long considered as basal for Lycosidae, which includes many sedentary species, contrary to the vagrant habits of the family (Foelix 2011). *Aglaoctenus* is typical of South America (Piacentini 2011) and has a long and confusing taxonomic history, including several changes in the number of species (Santos and Brescovit 2001). *Aglaoctenus lagotis* (Holmberg 1876), a funnel-web builder, is the most studied species from the genus and one of the few that has been studied in this subfamily (Bucher 1974; Capocasale 1982; Sordi 1996; Garabini and Gonçalvez 2001; Santos and Brescovit 2001; Rubio and Meza-Torres 2003; Piacentini 2011; Stefani and Del-Claro 2011; Stefani et al. 2011). The holotype specimen of the species, cited for Northern Buenos Aires (Argentina) by Holmberg (1876), is lost (Capocasale 1982). The geographical distribution of *A. lagotis* is wide and extends from Uruguay to Colombia (Capocasale 1982; Piacentini 2011). This area of the Neotropical region is characterized by its complex and diverse fauna prone to show intraspecific variations associated with the environmental conditions gradient (Macías-Ordóñez et al. 2013). Santos and Brescovit (2001) showed intraspecific variations in female genitalia of *A. lagotis* and Capocasale (1982) reported the presence of the species in different habitats and vegetation strata. Additionally, when

reviewing the scarce information reported for the species' sexual behavior (a summary by Fernando G. Costa is included in Capocasale 1982, and Stefani et al. 2011), we also detected variations in the descriptions reported for the two populations.

Collecting and housing

We collected adult and subadult individuals of *A. lagotis* in two sites: grasslands from Piedras de Afilar, Canelones, Southern Uruguay (SU; 34°36′44.83″S, 54°27′24.22″W), from March to April 2010–2011 and the hillsides of Sierras Chicas, Western Córdoba, Argentina (CA; 30°57′00.10″S, 64°10′00.28″W), from August to October 2010 (Fig. 1). The collection and trial periods were consistent with the sexual period of each population (unpublished data). We performed the trials in laboratories at each locality (Laboratorio de Etología, Ecología y Evolución, IIBCE, Montevideo, Uruguay, and Laboratorio de Biología Reproductiva y Evolución, UNC, Córdoba, Argentina, respectively). We captured the spiders during daylight, by manually blocking their silk tubes. We obtained 43 subadult individuals from Uruguay and 35 subadult individuals and 7 adult males from Argentina. Adult and subadult spiders were individually housed in Petri dishes (diameter 9.5 cm, height 1.5 cm) with a piece of cotton embedded in water. We fed all the individuals twice a week with mealworm larvae, *Tenebrio* sp. (Coleoptera; Tenebrionidae) and small crickets *Acheta domestica* (Orthoptera; Gryllidae). We monitored individuals daily and recorded the occurrence of molting in the subadults, to determine the exact date that they reached adulthood. We deposited voucher specimens (10 females and 10 males of each population) in the scientific arachnological collections of the Facultad de Ciencias, UdelaR, Montevideo, Uruguay, and the Cátedra de Diversidad Animal I, Facultad de Ciencias Exactas, Físicas y Naturales, UNC, Argentina.

Observation and analysis of sexual behavior

For the trials, we used virgin females of at least 10 days of adult age, ensuring the genital sclerotization and the chemical sexual attractiveness (Papke et al. 2001; Baruffaldi and Costa 2010). We used males after 7 days of reaching adulthood or with at least 7 days after capture in the field, to be sure of cuticle hardiness of individuals and acclimatization to captivity conditions. Males from the field were captured in their own webs, before they leave them for searching for females (M. González, personal observation; reported for other funnel-web spiders by Singer and Rietchert 1995). So, they were expected to be virgin males of no more than 10 days, the average time that they stay in their webs after reaching adulthood (M. González, personal observation). We did not reuse individuals. We performed the trials during daylight, according with our observations of matings at the field in the SU population (F.G. Costa and M. González, unpublished data). We carried out the experimental trials in glass cages (length 30 cm, width 16 cm, height 20 cm), with a layer of 2 cm of sand and 2 cm of wood-chips as substrate, simulating leaf litter. We also added Y-shaped small branches for refuge and web support, and a water source in small lids. We placed each virgin female in the arena 5 days before the trial to allow funnel-web construction. We sprinkled water over each web, simulating dew, to ensure a water supply.

We exposed 15 females of each population to males from the same population. We selected the male randomly and placed it on the margin of the female web. The occurrence and duration of the behavioral acts of both individuals were registered. We considered an act as

Fig. 1 Locations of the studied populations of *A. lagotis*: Southern Uruguay population (Piedras de Afilar, Canelones, Uruguay) (*hexagon*), and Central Argentina population (Sierras Chicas, Córdoba, Argentina) (*circle*). Also shown are the Brazilian population described by Stefani et al. (2011) (Araguari, Minas Gerais) (*square*), and two nearby populations reported by Sordi (1996) from São Paulo (Campinas) (*triangle*)
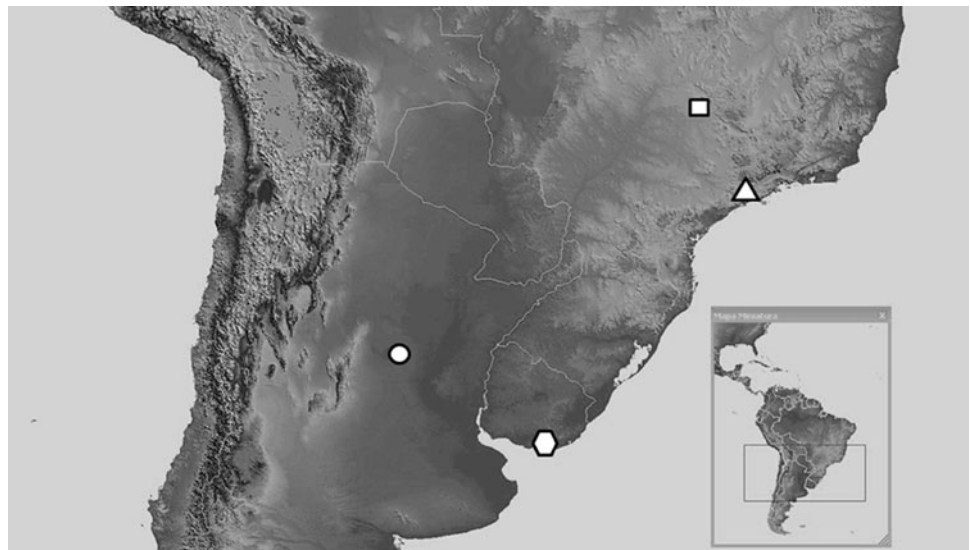
'representative of courtship' if it appeared in all the trials of a population. Each of the sequences of rubbing and leg-tapping (courtship behavioral acts; see Table 1) was considered as one rubbing bout and one leg-tapping bout, respectively. The duration of courtship latency was the period between positioning the male on the web and the first occurrence of a courtship behavioral act. We discarded the trials when male courtship did not occur after 30 min or the copulation did not occur after 60 min since placing the male on the female web. The number of ejaculations was estimated by the hematodochal expansions or by the erection of the spines in male hindlegs. We recorded the trials with a Sony DCR-SR85 digital video camera and analyzed videos with JWATCHER software (Blumstein et al. 2000). After each trial, we measured carapace width, a common measure of body size in spiders (Eberhard et al. 1998; Foelix 2011), and abdominal width of all adult individuals. We considered the ratio between abdominal width and carapace width as representative of body condition (Moya-Laraño et al. 2003). During the trials, room temperature averaged 22.81 °C (±2.07 SD, range 18–27 °C) for the SU population and 23.70 °C (±2.87,

range 19–30 °C) for the CA population. We did not find significant differences in temperature between SU and CA ($U = 329$; $P = 0.31$).

We analyzed the results using the statistical packages PAST v.1.18 (Hammer et al. 2003) and WINPEPI v.1.6 (Abramson 2004). We checked data for normal distribution with the Shapiro–Wilk test and homogeneity of variances with the Levene test. We used the Student's t test (parametric data) and Mann–Whitney U test (nonparametric data).

Results

Sexual behavior

Southern Uruguay

We describe courtship behavioral acts in Table 1 as well as providing durations and frequencies of occurrences of them in Table 2. The most representative acts of males were web-stretching (8.00 ± 3.2 times/individual), striding-

**Table 1** Description of courtship behavioral acts of Southern Uruguay and Central Argentina populations of *Aglaoctenus lagotis*

Behavior	Description of behavior	Performed by
Both populations		
Rubbing (R)	Ipsilateral skim between forelegs (first against second pair of legs) or hindlegs (third against fourth pair)	Female and male
Web-stretching (WS)	Pulls of the silk web by flexing all legs alternately while raising the body. The abdomen also vibrates (up and down movements) at high frequencies	Male
Leg-tapping (LT)	Semi-flexed forelegs (sometimes also palps) touching the web quickly and alternately	Female
Stationary (S)	Absence of noticeable movements	Female and male
Forelegs-interplaying (FI)	The male and the female, facing each other, wave their forelegs contacting repeatedly (immediately before mounting)	Female and male
Approaching (A)	Locomotion towards the other individual through the web sheet	Female and male
Mounting (M)	Climbing of male on the female back, taking the typical copulating posture of wolf spiders	Male
Abdominal-vibrating (AV)	Fast sagittal shakings of the abdomen during mating	Male
Body-shaking (BS)	Short vigorous female hops producing oscillations of the copulating couple	Female
Southern Uruguay population only		
Juddering (J)	Abrupt vibration of the male body over the web, causing oscillations of the web	Male
Forelegs-elevating (FE)	The individual raises and extends his forelegs	Male
Striding-forward (SF)	A large step with raised forelegs (immediately after foreleg elevation)	Male
Central Argentina population only		
Alternate-waving (AW)	Alternate up-and-down movements of extended forelegs, while the leg tips touch the silk	Male
Simultaneous-waving (SW)	Semi-flexed forelegs shake simultaneously, touching the silk layer, immediately before foreleg interplay	Male

Table 2 Comparisons of courtship characteristics between the studied populations of *A. lagotis*: Southern Uruguay (SU) (Canelones, Uruguay) and Central Argentina (CA) (Córdoba, Argentina)

	Southern Uruguay	Central Argentina	Statistics
Courtship latency (min)	12.58 ± 11.17 ($n = 15$)	6.37 ± 5.44 ($n = 15$)	$U = 100, P = 0.06$
Courtship duration (min)	2.91 ± 3.33 ($n = 15$)	9.47 ± 6.10 ($n = 15$)	$U = 31, P < 0.01$
No. of rubbings bouts/min	1.39 ± 1.83 ($n = 7$)	0.53 ± 0.64 ($n = 17$)	$U = 37, P = 0.22$
No. of web-stretching/min	9.80 ± 12.60 ($n = 15$)	1.31 ± 0.64 ($n = 15$)	$U = 28, P < 0.01$
No. of leg-tapping bouts/min	5.62 ± 8.80 ($n = 7$)	3.33 ± 3.78 ($n = 15$)	$U = 57, P = 0.90$

We only considered the individuals that performed the acts (number in parentheses). Data are presented as mean $\pm$ SD
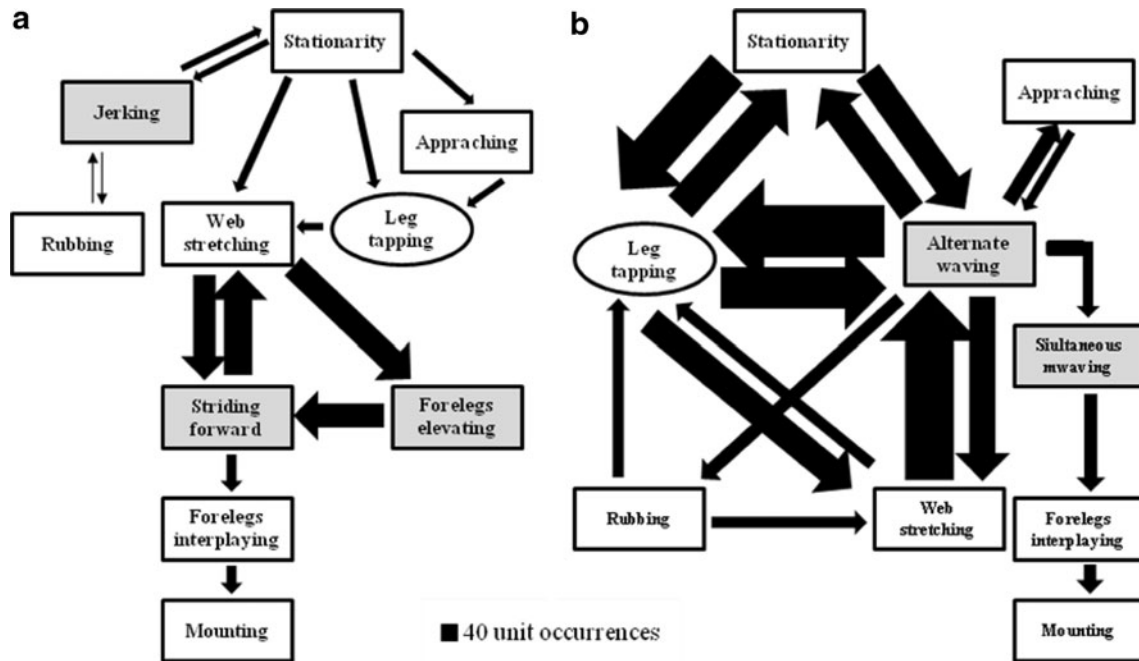
Fig. 2 Pattern of courtship behaviors in *A. lagotis* from Southern Uruguay population (Piedras de Afilar, Canelones, Uruguay) (**a**) and Central Argentina population (Sierras Chicas, Córdoba, Argentina) (**b**). *Arrow width* represents the succession frequency of the behavioral acts in time. Acts exclusive of each population are *shaded*. Those behavioral acts that occurred less than ten times are not included in this diagram

forward (6.57 ± 3.61 times) and forelegs-elevating (3.25 ± 2.0 times); all males performed them at least once during each trial. The typical behavioral pattern was: web-stretching, forelegs-elevating, and striding-forward, in this order (Fig. 2a; Supplementary Material Video 1). In 60 % of the trials, web-stretching was the courtship behavioral act that lasted the longest. Forelegs-elevating, striding-forward, and juddering occurred exclusively in this population. Six males performed juddering (2.00 ± 1.26 times) and seven females performed leg-tapping (2.00 ± 0.89 times). Rubbing was performed by four males (1.50 ± 1.00 times) and three females (1.33 ± 0.57 times) ($U = 136$, $P = 0.75$).

We show the copulation characteristics of SU in Table 3. Copulation occurred at the entrance of the tube of the funnel-web (Fig. 3). The copulatory position was the typical for Lycosidae, with the male mounting on the female dorsum, facing the tube and opposite to the female. Males performed several ejaculations per insertion (Table 3; Supplementary Material Video 1). Males vibrated their abdomen during copulation and, although the vibrations were more notorious between two ejaculations, videos analyzed frame by frame showed that males performed vibrations during the entire copulation. We also observed female body-shakings during copulation (Table 3). The dismounting always consisted of a quick jump backward and a distancing movement of the male from the female. Ten times, females chased males while they were trying to achieve the funnel-web periphery. Two females tapped their palps on the web after dismount and before chasing males.

Table 3 Comparisons of copulation characteristics of the studied populations of *A. lagotis*: Southern Uruguay (SU) (Canelones, Uruguay) and Central Argentina (CA) (Córdoba, Argentina)

	Southern Uruguay ($n = 15$)	Central Argentina ($n = 15$)	Statistics
Copulation duration (min)	60.9 ± 55.5	7.84 ± 3.54	$t = -8.61, P < 0.01$
No. of insertions	29.43 ± 12.34	7.06 ± 3.72	$t = 7.31, P < 0.01$
No. of ejaculations	88.90 ± 27.25	29.10 ± 17.10	$t = -7.02, P < 0.01$
No. insertions/min	0.55 ± 0.27	1.05 ± 0.69	$t = -3.47, P < 0.01$
No. ejaculations/min	1.50 ± 0.61	4.28 ± 2.58	$U = 6.0, P < 0.01$
No. ejaculations/ no. insertions	2.97 ± 1.08	4.53 ± 2.12	$t = -2.40, P = 0.02$
No. of body-shaking/min	0.10 ± 0.01	3.57 ± 2.58	$U = 1, P < 0.01$

Data are given as mean ± SD

Fig. 3 a Copulation of *A. lagotis* in the funnel-web entrance in the field (photo Luciana Baruffaldi). **b** Copulation under laboratory conditions, showing the insertion of male left palp (*black arrow*). Note the spine erection during ejaculation
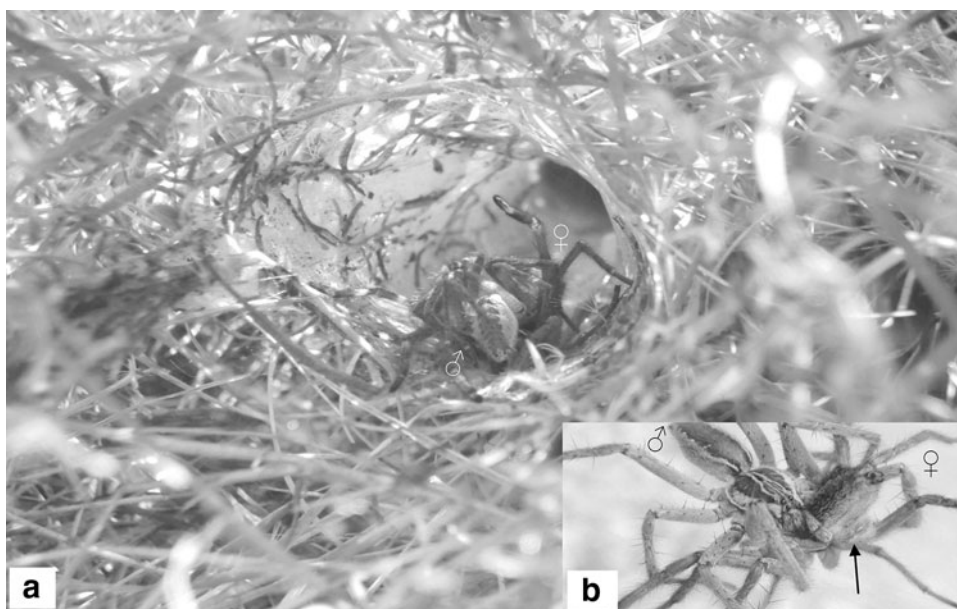
Central Argentina

We describe courtship behavioral acts in Table 1 as well as providing durations and frequencies of occurrences in Table 2. The most representative behavioral acts of this population were alternate-waving (21.12 ± 14.77 times), leg-tapping (20 ± 11.53 times), and web-stretching (10.90 ± 7.10 times), performed by all the individuals of the corresponding sex. The typical behavioral pattern was leg-tapping, web-stretching, and alternate-waving in this order (Fig. 2b; Supplementary Material Video 2). In 75 % of the trials, leg-tapping was the courtship behavioral act that lasted the longest. Males always performed simultaneous-waving preceding mounting attempts (2.53 ± 2.03 times). Alternate-waving and simultaneous-waving occurred exclusively in this population. Fourteen males performed rubbing (3.44 ± 2.37 times), but only three females performed this behavioral act (0.35 ± 0.99 times, $U = 24, P < 0.01$).

We show copulation characteristics of CA in Table 3. Copulation occurred at the entrance of the tube of the funnel-web (Supplementary Material Video 2), showing the same copulatory pattern described for SU. The dismounting also consisted of a quick jump backward and a distancing movement of the male from the female. Ten times, the females chased the males while they were trying to achieve the funnel-web periphery. In two cases, the female caused the male to pull down the web immediately. In a single opportunity, the female cannibalized the male while he was dismounting.

Interpopulation comparisons

There were significant differences in behavior between the two populations.

We compare courtship characteristics of them in Table 2 as well as showing the behavioral patterns in Fig. 2. As we mentioned above, some behavioral acts were exclusive to

each population and there were differences in the sequences in which they were performed (behavioral patterns). The SU population showed shorter courtship duration than CA and higher frequencies of web-stretching compared to CA (Table 2). The frequencies of occurrence of rubbing did not differ between populations when considering males and females together. However, a higher number of males, and at higher frequency, performed this act in CA population compared to SU population ($U = 59$, $P = 0.01$). Courtship latency was at the limit of significance, with longer durations for SU than for CA.

Durations of copulations were longer and highly variable in the SU population, and included more insertions and ejaculations compared with the CA population (Table 3). Frequencies of insertions (number of insertions per minute), frequencies of ejaculations, number of ejaculations per insertion, and frequencies of body-shakings (performed by the females during copulation) were also higher in the CA compared with the SU population. We did not find significant differences in female body size (carapace width: SU = 4.91 ± 0.52 mm, CA = 4.84 ± 0.85 mm; Student's t test: $t = -0.37$, $P = 0.71$) or male body size (SU = 4.89 ± 0.44 mm, CA = 4.09 ± 0.64 mm; Mann–Whitney U test: $U = 1297$, $P = 0.06$) between both populations. Neither female body condition indexes (SU population: 0.91 ± 0.17; CA population: 0.93 ± 0.18; Mann–Whitney U test: $U = 503$, $P = 0.75$) nor male body condition indexes (SU population: 0.74 ± 0.07; CA population: 0.75 ± 0.10; Student's t test: $t = -0.35$, $P = 0.73$) showed significant differences between populations. We observed differences in body pigmentation patterns between populations: SU individuals resembled to the pattern reported by Capocasale (1982) for the species and CA individuals to the description presented by Santos and Brescovit (2001).

Discussion

Unexpectedly, the analyzed populations of *A. lagotis* showed consistent differences between them in qualitative and quantitative aspects of their sexual behaviors, involving the acts that composed the repertoire, the patterns in which they occurred, and the frequencies at which they occurred. Several behavioral acts were specific to one of the given populations, as well as being the most frequently performed by individuals of that population. Additionally, although the pattern of copulation did not differ between the populations, all measured characteristics showed significant differences in frequencies or durations. These interpopulation variations could be related to high levels of intraspecific flexibility in sexual behavior of *A. lagotis*, with a divergence process, or with

the existence of two different species that have appeared as very similar.

The SU population showed similar characteristics in sexual behavior to those described by Capocasale (1982) for Uruguayan specimens from Canelones, Lavalleja and Maldonado, localities situated a few kilometers from the Uruguayan population studied here. However, the sexual behaviors observed seem different from those reported by Stefani et al. (2011) for the population from Minas Gerais (Brazil). Members from this Brazilian population performed particular acts not observed in SU or CA (e.g., palpal vibration) whereas they did not show other acts reported for SU and CA (e.g., web-stretching or striding-forward). Also, courtship durations (16.41 ± 3.14 min) and copulation durations (2.70 ± 2.38 min) reported by Stefani et al. (2011), as well as the number of insertions (generally only one per palp) and ejaculations (36.23 ± 9.22), are quite different to data observed in this study, for both SU and CA populations. Most sexual behavioral variables had shorter durations and higher frequencies in the Brazilian population. We have not found any previous reports for *A. lagotis* about body-shakings during copulation (which produce the abrupt and violent movement of the couple), such as we observed in both studied populations.

Interpopulation differences seem to have also reached to life cycles. Preliminary information about phenology in SU (M. González, A.V. Peretti and F.G. Costa, unpublished data) suggests a sexual period in autumn and a period of oviposition and of offspring not beginning until the spring. At the same time, reports of populations from Sao Paulo (Brazil) (Sordi 1996), Northern Argentina (Bucher 1974) and even our observations in Central Argentina (M. González, A.V. Peretti and F.G. Costa, unpublished data) showed the sexual period and oviposition and offspring period occur together, in spring and summer. Santos and Brescovit (2001) reported semelparity for the *Aglaoctenus* genus, as well as the existence of variations in the reproductive season (starting earlier or later in relation to latitude), as has been mentioned for other spiders (Schaefer 1987). However, previous studies did not mention the occurrence of the sexual period temporarily detached from the oviposition and offspring period.

Geographical variations in sexual behavior have been reported in many taxa, like frogs (Ryan et al 1990; Prohl et al. 2006), fishes (Ishikawa et al. 2006), snails (Kuppernagel and Baur 2011), insects (Claridge et al. 1985; Saarikettu et al. 2005; Briceño et al. 2007), and spiders (Miller et al. 1998; Elias et al. 2006). Generally, such differences refer to courtship and copulation durations and variations in frequencies of occurrence of sexual behavioral acts. Less commonly, variations can also reach the sequences of acts (the behavioral patterns) of courtship and copulation. In the lycosid spider *Schizocosa crassipes*,

Miller et al. (1998) reported variations among populations in the frequencies and pattern of sexual behavioral acts, observing fewer interpopulation copulations when compared to intrapopulation ones. In the salticid spider *Habronattus pugillis,* Masta and Maddison (2002) reported that sexual selection was leading to the divergence of phenotypes potentially crucial to the speciation process. Later, Elias et al. (2006) added the existence of differences in the seismic signals of two populations of that species and the preference of females for foreign males with the most complex signals. These authors strongly suggested an incipient divergence process between populations. While we have not yet tested female preferences in relation to variations in male courtship, the interpopulation variations we report here could be an example of a divergence process associated with geographical conditions. However, we cannot discard the existence of an uncommon high level of intraspecific flexibility in the sexual behavior of this species. We hope that the study of areas where individuals of both populations apparently overlap will help us to clarify this scenario.

Santos and Brescovit (2001) revised the *Aglaoctenus* genus and considered only 2 species of the 12 originals to be valid, based on characteristics of genital morphology. In the same publication, the authors considered all the populations mentioned above as members of *A. lagotis,* although at the same time reporting polymorphism in female genitalia. While intraspecific variations in genitalia are not common, examples in invertebrates (Hausmann 1999; Sakurai et al. 2012), spiders included (Pérez-Miles 1989; Heinemann and Uhl 2000; Crews 2009), have been reported, albeit usually associated with divergence processes (Huber and Pérez-González 2001; Jocqué 2002). In the past, some behaviors were largely assumed to be invariant within species, particularly those behavioral signals involved in mate choice or in species recognition (Barlow 2002). However, nowadays, behavior, like morphology and other traits, are considered a phenotypic characteristic, the expression of the combined effects of genotype and environment (Magurran 1999; Verrell 1999; West-Eberhard 2005), and behavioral variations within species, even in the sexual context, can be expected (e.g., Bansemer et al. 2002; Rissler et al. 2004; Cocroft et al. 2006; Herberstein 2011). However, when differences in sexual behavior of a species accumulate (in frequencies, patterns, or even in the acts composing them), as happens between the populations studied here, they may be taken as cues of more or less advanced divergence processes (Gross 1996; Kotiaho et al. 1996; Hoikkala et al. 1998; Wilczynski and Ryan 1999; Singer et al. 2000; Brockmann 2001; Parri et al. 2002; Cratsley and Lewis 2003; Su and Li 2006; Rios-Cardenas et al. 2007; Peretti and Eberhard 2010; Dutto et al. 2011 Gordon and Uetz 2011).

Finally, when comparing *A. lagotis* with typical wolf spiders, courtship patterns found for this species seem to have visual displays present in the family (e.g., elevation, tapping, and waving forelegs) (Rovner 1968; Costa 1975). However, males also perform web-stretching, behavior typical of web spiders that give priority to seismic channels (Robinson and Robinson 1980). These 'mixed' modalities of communication would answer to historical and environmental forces that could have affected this singular wolf spider. As the position of the subfamily in the phylogeny of Lycosidae is unresolved, we are still not capable of knowing if the web is a secondary acquisition in this family, suggested by Murphy et al. (2006), or a residual component from basal members, as is more commonly considered (Dondale 1986; Jocqué 1995; Foelix 2011).

We still do not know if all differences encountered in relation to mating are arbitrary or adaptive, but sexual selection could be an important force explaining them. To determine whether there are differential sexual preferences between populations, we are developing heterotypic trials, as in recent studies developed in other spider species by Masta and Maddison (2002), De Busschere et al. (2010), and De Busschere et al. (2012). Knowledge of populations history, populations genetic studies, and more data from populations with intermediate geographical location could bring valuable information about the actual situation of *A. lagotis* and the factors that could have caused and maintained this scenario.

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