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Original Article

A sexual network approach to sperm competition in a species with alternative mating tactics

Danilo G. Muniz,^{a,b} Paulo R. Guimarães Jr,^b Bruno A. Buzatto,^c and Glauco Machado^b

^aPrograma de Pós-graduação em Ecologia, Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, no. 321, São Paulo 05508-900, Brazil, ^bLAGE do Departamento de Ecologia, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, Travessa 14, no. 321, São Paulo 05508-900, Brazil, ^cCentre for Evolutionary Biology, School of Animal Biology, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

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Alternative mating tactics are common among species exhibiting resource defense polygyny. While large territorial males aggressively defend harems, small sneaker males generally invade these harems to mate furtively. The result is a sexual network that provides information on the sperm competition intensity (SCI) faced by males of both morphs. Here, we use metrics derived from the network approach to compare SCI between sneakers and territorials of the male-dimorphic harvestman *Serracutisoma proximum*. We also tested hypotheses about the influence of harem size and spatial distribution of harems on the SCI faced by territorial males. Sneakers faced, on average, higher levels of SCI than territorials, while the SCI faced by territorials was more variable than that of sneakers. Owners of large harems faced less intense sperm competition than owners of small harems, suggesting that sperm competition is more diluted among females in large harems. At the population level, sneakers concentrated their invasions on neighboring harems that were spatially aggregated. We argue that the spatial distribution of harems is an important element influencing the topology of the sexual network, and that the spatially explicit approach we used here can bring new insights to the study of sperm competition and mating systems in a wide range of organisms.

Key words: mating system, Opiliones, resource defense polygyny, sexual selection, social network, spatial distribution.

INTRODUCTION

In polygynous mating systems, a few males often monopolize access to most females, while most males have no access to females. In these systems, it is common for some males to adopt alternative mating tactics, such as female mimicry and/or sneaking into territories to copulate (Shuster and Wade 2003). Males adopting alternative mating tactics may be younger or smaller individuals, but several species present alternative male morphs (Oliveira et al. 2008). In these species, there is usually a large and armed morph known as major or territorial that defends territories and/or females, and a smaller and generally unarmed morph known as minor or sneaker that does not defend territories or females (Dominey 1984; Gross 1996; Oliveira et al. 2008). The presence of males adopting alternative mating tactics increases the likelihood that females will mate with multiple males, creating

the opportunity for the sperm of 2 or more males to overlap inside the female's reproductive tract and thus compete for the fertilization of ova. This process, known as sperm competition, is widely recognized as an important process modulating the total strength and direction of sexual selection (Kvarnemo and Simmons 2013). In species with different male morphs, sneakers are frequently subject to sperm competition, since they usually mate with already mated females. Territorials, on the other hand, are subject to sperm competition only when sneakers invade their harems (Parker 1990).

Sperm competition has been quantified in 2 different and complementary ways: sperm competition risk and sperm competition intensity (Simmons 2001). Sperm competition risk is the probability of facing sperm competition resulting from female promiscuity, while sperm competition intensity is determined by the number of different males engaged in competition for a single set of ova (Simmons 2001). Theoretical models predict that within-species relative ejaculate investment should increase with increasing sperm

Address correspondence to G. Machado. E-mail: glaucom@ib.usp.br.

competition risk and decrease with increasing sperm competition intensity (Parker et al. 1996, 2013; review in Kelly and Jennions 2011). In species with different male morphs, the prediction is that there should be an asymmetry in ejaculate investment between morphs. We would expect, therefore, sneakers to have proportionally higher ejaculate investment because they generally face higher sperm competition risk. However, if sneakers are too common or too effective in acquiring copulations, most territorials will also face sperm competition. As a result, the asymmetry in sperm competition disappears, and the prediction is of no difference in ejaculate investment between male morphs (Parker 1990; Simmons 2001).

Two important factors that could influence the sperm competition intensity faced by individual territorial males are the spatial distribution of harems in the population and the number of females in the harems (harem size). Larger harems are more easily invaded because territorials cannot monopolize all females effectively (e.g., Shuster 1987; Byers and Kitchen 1988; Munguía-Steyer et al. 2012). Thus, territorials whose harems have many females should be at higher sperm competition risk and intensity than territorials with fewer females in their harems. Also, when harems are spatially aggregated and sneakers are highly vagile, the same sneaker can invade several harems sequentially, imposing sperm competition on a great number of territorials (e.g., Gross 1991; Buzatto et al. 2011). Harem size and spatial distribution may also interact, so that a territorial defending a small harem close to large and highly attractive harems may be at higher sperm competition than a territorial defending an isolated small harem.

To evaluate the influence of harem size and spatial distribution of harems on the sperm competition intensity faced by territorials, we used a sexual network approach (Fortuna et al. 2008; McDonald et al. 2013), according to which a mating population can be viewed as a network where males and females are connected by copulations (Figure 1). Each individual may be characterized by its number of connections (i.e., sexual partners) and by the number of connections of its mating partners, which brings information about the sperm competition intensity (Figure 1). Additionally, the network

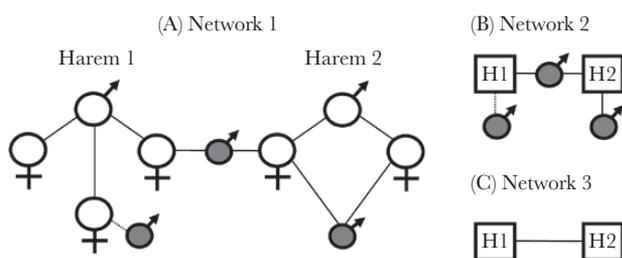


Figure 1 Hypothetical sexual network depicted in the 3 different ways used in our study. (A) Network 1: male–female network in which females and territorials are depicted in white and sneakers in gray. This is the most detailed network, from which we can extract information on sperm competition at the individual level. (B) Network 2: a harem–invader network, in which each square is a harem and sneakers are again depicted in gray. In this network, we lose information of which females are connected to each male, but it becomes easier to evaluate harem invasion. (C) Network 3: harem–harem network in which each harem is depicted as a square. In this network, we retain only the information about males that establish sexual connections between harems. In the networks (A) and (B), solid lines depict cases in which males were actually observed copulating with the females. Dotted lines depict cases in which invaders were seen close to the females, but no copulation was observed. In networks (B) and (C), H1 and H2 represent harem 1 and harem 2, respectively.

structure that emerges from the interactions between all individuals in the population is a quantitative descriptor of the mating system, characterizing the degree of promiscuity of both males and females (Sih et al. 2009; McDonald et al. 2013; Pinter-Wollman et al. 2014). By combining detailed behavioral observations in the field with a multi-scale network analyses, we therefore addressed questions on sperm competition intensity from the perspective of individual males and also at the population level, incorporating the spatial structure of the harems.

Our model organism was the harvestman *Serraculisoma proximum* (Opiliones: Gonyleptidae), in which dimorphic males exhibit different reproductive tactics. Large males (hereafter “territorials”) use their long and sexually dimorphic second pair of legs in fights for the possession of territories on the vegetation along river margins (Buzatto and Machado 2008). Territories are visited by 1 or several females, who copulate with the territorial, lay their eggs on leaves and remain caring for the clutch (Buzatto et al. 2007). Small males (hereafter “sneakers”), on the other hand, invade territories and furtively mate with egg-guarding females. This tactic is only possible because oviposition may last up to 2 weeks and, although 80–90% of the eggs are laid in the first 24 h after copulation with the territorials, sneakers may sire some offspring if they successfully copulate with females that still retain unfertilized eggs in their ovaries (Buzatto et al. 2011). Even though sperm precedence and the forms of sperm use by females in harvestmen are unknown, sneakers usually copulate with already mated females (Buzatto et al. 2011). Moreover, 82% of the females in the population lay only 1 clutch of eggs during their lives (Buzatto et al. 2007), thus most of the sperm competition imposed on territorials by sneakers is restricted to a single set of unfertilized eggs present in the females’ ovaries.

The first hypothesis we tested was that sperm competition intensity should be higher for sneakers than for territorials. We also predict that sperm competition faced by territorials should be more variable (Munguía-Steyer et al. 2012). Considering that sneaker vagility is limited and that harems are widely scattered along river margins (Buzatto et al. 2011), our second hypothesis was that the sexual network should be poorly connected and clearly arranged in many components, that is, groups of nodes that are mutually interconnected (Proulx et al. 2005). Given that large harems are more likely to be invaded by sneakers (Munguía-Steyer et al. 2012) and that the same sneaker can invade several harems (Buzatto et al. 2011), our third hypothesis was that both sperm competition intensity for territorials and intensity of harem invasion should increase with harem size and the proximity to other harems. Finally, our fourth hypothesis refers to the entire population and postulates that females from nearby harems should mate with the same sneakers, thus establishing sexual connections between these harems. In this sense, aggregated harems should be hotspots of sneaker invasions when compared with isolated harems.

MATERIALS AND METHODS

Study population and dataset

The studied population of *S. proximum* occurs in an Atlantic Forest area at Interales State Park (24°14’S, 48°04’W; 800 m a.s.l.), state of São Paulo, southeastern Brazil. Specifically, our data describes the population living on the vegetation flanking the stream that follows the Caçadinha track. In the season when the data were collected, the frequency of sneakers in this population was of 20%

(Buzatto et al. 2011). Males were classified as sneakers or territorials following Knell (2009) and details of the procedure can be found in Buzatto et al. (2011).

The data we used is a subset from the data of Buzatto et al. (2011), comprising the behavioral observations from the period between January and February 2005, which corresponds to the peak of *S. proximum* mating season (Buzatto et al. 2007; Buzatto and Machado 2008). These behavioral observations were conducted in a 200 m transect with marks every 10 m on the vegetation flanking the stream. The exact location of 29 harems in this transect (to the nearest meter) was determined as the distance between each harem and the beginning of the transect. Each of these harems was inspected 6 times a day: twice between 08:30 and 12:00h, twice between 14:00 and 17:30h, and twice between 20:30 and 00:00h. In each inspection, it was recorded female mating activities (with territorials or sneakers), mate guarding (always performed by territorials), distance between males (territorials and sneakers) and all females in the harem, and harem invasions (for more details on the sampling procedure, see Buzatto et al. 2011).

In total, the behavioral observations comprised nearly 260 h, and included information on 47 males (13 of which are sneakers) and 72 females that oviposited in the 29 harems monitored in the transect. For 2 females it was not possible to identify the territorial male with which they copulated, and thus these females were ignored in the harem level analyses, but not in the individual level analyses (see below). One of these females was observed near a territorial male only once, and thus her status as part of his harem could not be confirmed. The other female was never observed near a territorial male, and it is possible that she mated with a territorial male that died prior to the field observations.

Sexual networks

A network is a set of elements, known as nodes, with connections between them, known as links (Newman 2003; Costa et al. 2007). To perform a multi-scale analysis, going from the individual to the population level, we used 3 networks describing different aspects of the mating system of *S. proximum* (Figure 1). Although the definition of nodes and links in these 3 networks differ, we will refer to them collectively as *sexual networks*. In the first network, nodes are males and females, and links represent the probability of copulation (Figure 1A). We used this network to investigate the patterns of sperm competition intensity faced by individual males, including both territorials and sneakers. In the second network, nodes are harems and invader males, and a link represents the invasion of a harem by a male (Figure 1B). In this second network, used to investigate harem invasion, the invader males were usually sneakers, but some territorials may also invade neighboring territories and establish sexual connections between harems. Finally, in the third network, nodes are harems and links represent that 2 harems share at least 1 invader male (Figure 1C). We used this network to investigate the pattern of harem invasion at the population level. In what follows we elaborate on the analyses performed on each of these networks.

Male–female network and sperm competition intensity

We built a weighted network in which nodes depict males and females in the population. In a weighted network, links between individuals assume continuous values representing how strongly connected are 2 elements (Costa et al. 2007). We built a sexual network

in which the link weight represents the probability that 2 individuals mated, which ranges from 0 to 1. All territorials received a link weight of 1 with all females within their respective harems because we know that *S. proximum* females only lay eggs in a territory after copulating with its owner (Buzatto and Machado 2008). If a sneaker was observed copulating with a female, the link between these individuals also received a weight of 1 because we were sure they mated (solid lines in Figure 1A,B). When a sneaker was observed within 20 cm of a given female, we considered him to have a probability c of copulating with that female (dashed lines in Figure 1A,B). Given that harvestmen are unable to perceive long-range chemical, acoustic, and visual stimuli (Willemart et al. 2009), we suppose that the minimum distance for female detection is approximately 2 times the length of the sneaker's second pair of sensory legs (i.e., 20 cm). Moreover, our field observations indicate that: 1) sneakers directly move toward egg-guarding females within this distance, suggesting that they are able to locate potential mating partners using close range cues, 2) all sneakers that approached egg-guarding females attempted to copulate with them, and 3) sneakers were never rejected by the females (see Buzatto et al. 2011).

If the sneaker was observed more than once near the same female, his probability of copulating increased. We derived this probability using the binomial distribution as follow: $w_{ij} = 1 - (1 - c)^{N_{ij}}$, where w_{ij} is the link weight between the sneaker i and female j , and N_{ij} is the number of times the sneaker i was seen near (<20 cm) female j . The value of w_{ij} increases toward 1 with increasing values of N_{ij} . The probability c of a sneaker copulating with a given female once he is observed less than 20 cm from that female was defined as 0.7 because nearly 70% of the sneaker approaches to females observed in the field resulted in successful copulation (Buzatto et al. 2011). On the few occasions in which a territorial was observed invading a territory, we considered this male to have the same chance as a sneaker of copulating with a female and calculated their strength of interaction in the same way. The probability of copulation was set to 0 if the female closest to the invader (either sneaker or territorial) was mate guarded by the resident territorial male. During mate guarding, the territorial male remains less than 20 cm from a female, periodically touching her with his long second pair of legs (see Fig. 3b in Buzatto and Machado 2008), and most of the cases of sneaker detection and repulsion by territorials occurred when females were mate guarded (Buzatto et al. 2011).

Our approach to build the male–female network incorporates the uncertainty surrounding whether some interactions between males and females indeed culminate in copulation, and is solidly based on the reproductive biology of *S. proximum*. Sneakers usually invade a harem, copulate with a female, and then promptly abandon the area (Buzatto et al. 2011). In fact, according to our field observations, from all the times that a sneaker was observed copulating with a female ($n = 32$), in 26 cases he was not recorded inside the harem in the previous inspection. Moreover, from all the times that a sneaker was recorded less than 20 cm from a female ($n = 24$), in 17 cases (70%) he was not recorded close the female in the subsequent inspection, and in 6 cases (25%) he was recorded copulating with the female in the subsequent inspection (in only 1 case, the sneaker remained close to the female during 2 consecutive inspections). These findings support the notion that mating interactions between sneakers and females are fast and have low probability of being detected. Therefore, considering only the copulations actually observed in the field would greatly underestimate the role of sneakers on the network topology. To assess the sensitivity of our results to copulation probability, we performed all the analyses

described below varying the value of c from 0 to 1 (with regular intervals of 0.1). Using the value 0 we are considering only the actually observed copulations in the networks, and all other values of c consider that a sneaker observed close to a female has some chance of copulating ranging from 10% to 100%.

In a weighted network, the *strength* of an individual node is the sum of the weights of all its links (Costa et al. 2007). In a sexual network context, the strength of an individual can be interpreted as a measure of its mating success or promiscuity (McDonald et al. 2013). Given that sperm competition arises when a female copulates with more than 1 male within a limited period of time, allowing the ejaculates to overlap inside the female's reproductive tract, the strength of females in our analysis is an estimator of sperm competition. Hence, the mean strength of the partners of a male is an estimate of the sperm competition intensity faced by that male. This metric is known as *mean neighbor strength* in the network approach (Costa et al. 2007), but as a mnemonic device we will call it SCI (after "sperm competition intensity") from now on.

To test the first hypothesis that sperm competition risk should be higher for sneakers and that its variation should be higher for territorials, we calculated the SCI of all males in the network. We compared the mean SCI of territorials and sneakers using a Welch's *t*-test, and compared the variances in SCI using a variance ratio test (Zar 2010). To test the hypothesis that SCI for territorials should increase with harem size and the distance to other harems, we calculated the mean distance of each harem to the 5 closest neighboring harems. The number 5 is an arbitrary choice, but the results do not change qualitatively with the number of harems varying from 3 to 10 (Supplementary Table S1). We evaluated the effect of harem size and distance to other harems on SCI using a multiple linear regression. We standardized all predictor variables, thus regression coefficients are comparable between each other and can be interpreted as effect sizes (Schielzeth 2010).

To test the second hypothesis that the male–female network should be poorly connected due to spatial constraints and arranged in many components, we conducted a randomization analysis. We compared the number of components of the observed network with the number of components of an ensemble of 5000 simulated networks generated by a null model. To build the null model, we kept the number of connections of each sneaker and assumed that he could be connected with any female in the population. Using this approach, we created a scenario where there is no constraint in the capacity of individual sneakers to impose sperm competition to any territorial male in the population, that is, there was no effect of the spatial distribution of harems on the sneaker copulations. For each simulated network we counted the number of components, generating a null distribution of this metric. We calculated the *P*-value as the proportion of simulated networks in which the number of components was equal or higher than the observed network. We ran this analysis in 2 different ways: firstly considering only the observed copulations as links, and secondly including both the observed and the probabilistic copulations.

Harem–invader network and harem invasion

Based on the male–female network, we built a second network where the nodes were harems and invader males (Figure 1B). In this network, a harem and an invader male were connected if the invader had a link weight higher than 0 with at least 1 female in that harem. We defined invader male as any male that was not the territorial resident of a harem, so that both sneakers and territorials from neighboring territories could be scored as invaders. We

calculated the link weight w_s between a harem and an individual male as the sum of the link weights w_f for all g females with which the invader was connected according to: $w_s = \sum_{f=1}^g w_f$. Therefore, the

strength of each harem is a measure of the intensity of harem invasion and is calculated as the sum of all its link weights.

To test the hypothesis that the intensity of harem invasion should increase with harem size and the proximity to other harems, we used again the mean distance to the 5 closest harems as a measure of distance to other harems. Then we used a multiple linear regression and standardized the predictor variables as described in the previous topic.

Harem–harem network

To investigate the effect of the spatial distribution of harems on the connections between them, we built another network in which the nodes were the harems. In this network, 2 harems were connected when at least 1 male mated with females in the 2 harems (Figure 1C). The link weight (w_{kl}) between harems k and l was calculated with 2 different approaches. In the first approach, we used

the equation $w_{kl} = \sum_{s=1}^m (w_{sk} + w_{sl}) \cdot a_{kl}$, where w_{sk} and w_{sl} are the link

weights between the male s and the harems k and l . The term a_{kl} is equal to 1 if $w_{sk} > 0$ and $w_{sl} > 0$, and 0 otherwise. Thus, the link weight between the harems k and l is the sum of w_{sk} and w_{sl} for all m shared males between the 2 harems. This first link weight indicates how strongly connected are 2 harems, since it increases with the number of shared males and the number of females with which these males are connected to. In the second approach, we built an unweighted network, in which we considered 2 harems to be connected if at least 1 invader male had a $w_s > 0$ in both harems. Thus, in this second approach a link indicates if 2 harems share at least 1 invader male.

To test the hypothesis that spatially aggregated harems should share the same invaders, we built a matrix of distances between all harems in the population. We calculated the distance between each pair of harems as the length (in meters) of the shortest path between the 2 harems through the vegetation at the river margins. When 2 harems were located on different sides of the river we considered the distance between the margins to be 5 m (Buzatto and Machado 2008) and considered that the individuals could only cross to the other side in places where the foliage connects the 2 margins (see scheme in Figure 2). Since a network can be represented as a matrix, we tested our hypothesis using a Mantel test (Manly 2006) correlating the matrix of distances between harems and the matrix describing the harem–harem network. We used the Pearson correlation coefficient as the test statistic and ran 5000 permutations to determine the *P*-value. Since differences in the way we calculated the link weight between 2 harems led to differences in the topology of the harem–harem network, we performed 2 Mantel tests, 1 for each topology of the harem–harem network.

Software used in the analyses

We performed all calculations and analyses described above using the software R 3.0.1 (R Development Core Team 2013).

RESULTS

Male–female network: sperm competition intensity

The male–female network of *S. proximum* was composed of 27 harems (Figure 2). Nearly 91% of the territorials (31 out of 34) and

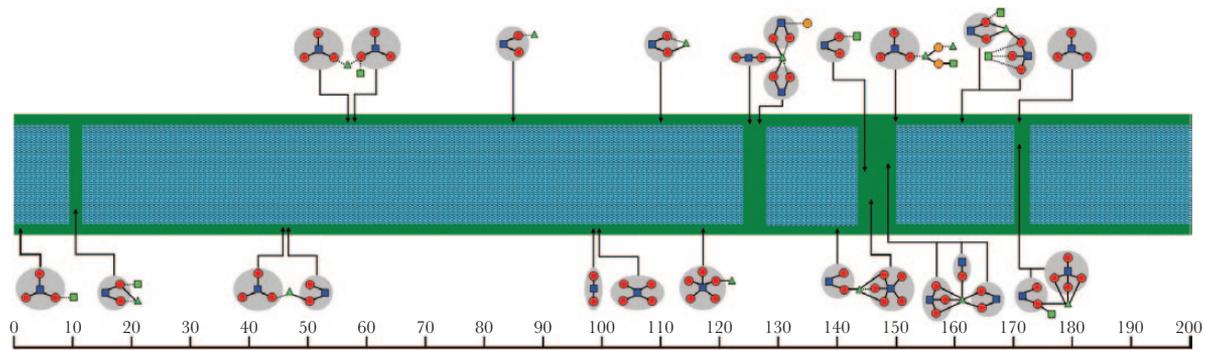


Figure 2

Spatially explicit schematic view, from above, of the male–female network of the harvestman *Serracutisoma proximum* in which each dot represents an individual: circles = females, triangles = sneakers, and squares = territorials. The red circles depict females in harems where a territorial owner was unequivocally identified, and the orange circles depict females without an assigned territorial owner. The blue squares depict territorials that held a harem (delimited by a gray shadow), and the green squares depict territorials that did not hold a harem and acted as invaders. Following the same rationale presented in the Figure 1, solid lines connecting males and females represent cases in which copulations were observed, and dotted lines represent cases in which invaders were seen close (<20 cm) to the females, but no copulation was observed. The specific position of each harem in the transect is indicated by an arrow. The green background color represents the marginal vegetation, whereas the blue background color represents the river. Notice that there are some vegetation bridges that connect the 2 margins of the river. The ruler at the bottom indicates the distance (in meters) from the beginning of the study transect. To calculate proximity between harems, we always considered the shortest walking distance through the vegetation.

all sneaker males faced some level of sperm competition (i.e., $SCI > 1$). Among males holding territories, 89% (23 out of 27) faced some sperm competition. The mean SCI of sneakers was 13% higher than that of territorials when $c = 0.7$ ($t = 2.11$, degrees of freedom [df] = 42.39, $P = 0.02$; Figure 3). The same qualitative result was obtained when $c = 0$ and when $c \geq 0.5$ (Figure 3; Supplementary Table S2). The standard deviation of territorials was 100% higher than that of sneakers when $c = 0.7$ ($F = 0.23$, $df = 12$; 33, $P = 0.005$; Figure 3). Once more, the same qualitative result was obtained when $c = 0$ and when $c \geq 0.4$ (Figure 3; Supplementary Table S2).

The SCI of territory owners was related only to harem size when $c = 0.7$, but the observed effect was not in the direction we predicted (Table 1): territorials with larger harems faced less intense sperm competition (Figure 4). The negative relationship between SCI and harem size was also significant when $c \geq 0.3$, and marginally significant when $c < 0.3$ (Figure 4; Supplementary Table S3).

Male–female network: effect of the spatial structure

Considering only copulations actually observed, the male–female network was arranged in 20 components (Figure 2). Fifteen components were exclusively composed of a territorial male and 1–5 females inside his harem, which may or may not have been invaded by other males (Figure 2). The other 5 components were composed of 2 ($n = 3$) or 3 harems ($n = 2$) interconnected by invader males (Figure 2). When considering the probabilistic copulations ($c > 0$), the harems were arranged in 17 components (Figure 2), being 8 of them composed of 2 ($n = 6$) or 3 harems ($n = 2$) interconnected by invader males (Figure 2). The number of components in the observed male–female network was higher than expected by our null model, both when we considered only the observed copulations (median = 14 components; range = 11–19; $P < 0.001$) and when we included the probabilistic copulations (median = 10 components; range = 4–16; $P < 0.001$).

Harem–invader network

From all harems where we could identify a territorial resident, 24 (88.9%) were invaded by other males, 13 of them belonging

to the sneaker morph and 7 to the territorial morph (Figure 2). Individual sneakers invaded up to 3 harems, but did not necessarily mate with all females within each harem (Figure 2). No harem was invaded by more than 1 sneaker, but the same harem could be invaded by a sneaker and a territorial male ($n = 4$, Figure 2). The only female that mated with 2 sneakers was in 1 of the 2 harems where we could not identify the territorial resident. For $c = 0.7$, harem invasion was not correlated with harem size or with the distance to other harems (Table 1). However, for $c \leq 0.3$, harem invasion was negatively correlated with the distance to other harems (Supplementary Table S4).

Harem–harem network

The mean (\pm SD) distance between harems in the population was 64.9 ± 51.5 m, but the mean distance between harems that shared at least 1 invader male was only 3.8 ± 6.9 m. All 8 males that connected harems were sneakers (Figure 2). For all values of c , the matrix of distances among harems was negatively correlated to the 2 matrices of harem–harem network (Table 2). Thus, nearby harems were more likely to be invaded by the same males and become sexually connected.

DISCUSSION

Here, we used a network-based approach to investigate the influence of harem size and spatial distribution of harems on the sperm competition intensity (SCI). We used a network metric based on the mean neighbor strength of each male as a proxy of SCI, and found that average SCI faced by sneakers of the harvestman *S. proximum* was slightly higher than that faced by territorials. However, the variation in the SCI was much higher for territorials than for sneakers. These results support both predictions of our first hypothesis. We also found that the male–female network is arranged in many isolated components, supporting our second hypothesis that there is a strong effect of the spatial distribution of harems on the sneaker copulations. Owners of large harems faced less SCI than owners of small harems, which contrasts with our third hypothesis that predicted that SCI for territorials should increase with harem

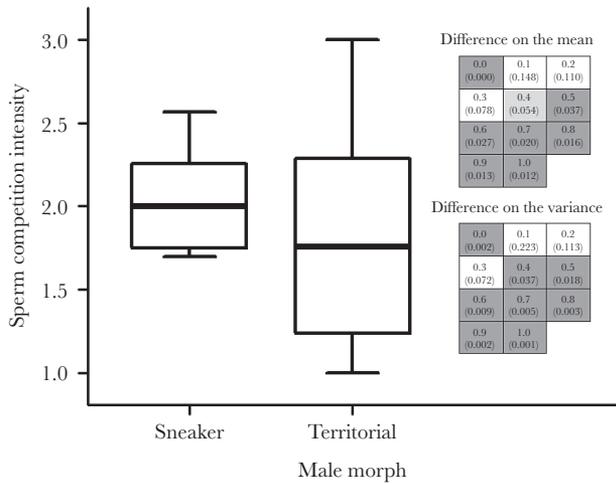


Figure 3

Comparison of sperm competition intensity faced by territorial and sneaker males of the harvestman *Serracutisoma proximum*. The bold line represents the mean, the box represents the standard deviation, and the whiskers indicate the range. The boxes on the right show the results of the sensitivity analysis on the copulation probability when a male was observed close (<20 cm) to a female. Each box shows a copulation probability value and between parentheses the *P*-value. Values are shown for the tests of difference between the means and the variances. Lower significance values are highlighted with darker boxes.

Table 1

Summary of the liner models relating SCI faced by territorial males of the harvestman *Serracutisoma proximum* and harem invasion to the mean distance to the 5 closest harems (D) and to the harem size (H)

| Response variable | Predictor | Coefficient | <i>t</i> -Value | <i>P</i> -value |
|-------------------|-----------|--------------|-----------------|-----------------|
| Sperm competition | Intercept | 1.63 ± 0.07 | 22.52 | <0.01 |
| | H | -0.19 ± 0.09 | -2.21 | 0.04 |
| | D | -0.12 ± 0.07 | -1.6 | 0.12 |
| | H:D | -0.09 ± 0.12 | -0.76 | 0.46 |
| Harem invasion | Intercept | 1.49 ± 0.19 | 7.95 | <0.01 |
| | H | -0.02 ± 0.22 | -0.11 | 0.92 |
| | D | -0.27 ± 0.19 | -1.41 | 0.17 |
| | H:D | -0.46 ± 0.3 | -1.55 | 0.13 |

D × H is the interaction between distance and harem size. SCI was estimated as the mean neighbor strength in a male–female sexual network. Harem invasion was estimated as the strength of each harem in a harem–invader sexual network. Predictor variable coefficients are presented as estimate ± standard error.

size. Additionally, the distance to other harems had no influence on the SCI and on the intensity of harem invasion. However, sneakers concentrated their invasions on neighboring harems, which supports our fourth hypothesis that predicted that aggregated harems should be hotspots of sneaker invasions when compared with isolated harems. These results emerge when we build the male–female network including as links only the copulations actually observed in the field, and also most values of copulating probability. In what follows, we explore the implications of these findings in terms of sperm competition and sexual selection in mating systems with alternative mating tactics.

According to our field observations, 89% of territory owners experienced some level of sperm competition, despite the fact

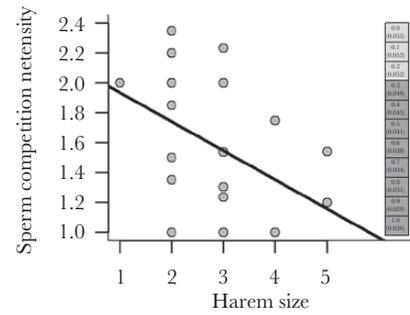


Figure 4

Relationship between sperm competition intensity faced by territorial males of the harvestman *Serracutisoma proximum* and harem size (number of females per harem). The line represents predicted values from the model presented on Table 1. The boxes on the right show the results of the sensitivity analysis on the copulation probability when a male was observed close (<20 cm) to a female. Each box shows a copulation probability value and between parentheses the *P*-value for the relationship between sperm competition intensity and harem size.

Table 2

Results of the Mantel test correlating the matrix of distances among harems and 2 matrices of representing the harem–harem network

| Copulation probability (<i>c</i>) | Pearson coefficient (<i>r</i>) | |
|-------------------------------------|----------------------------------|-----------|
| | Network 1 | Network 2 |
| 0.0 | -0.19 | -0.19 |
| 0.1 | -0.2 | -0.22 |
| 0.2 | -0.2 | -0.22 |
| 0.3 | -0.21 | -0.22 |
| 0.4 | -0.21 | -0.22 |
| 0.5 | -0.21 | -0.22 |
| 0.6 | -0.21 | -0.22 |
| 0.7 | -0.21 | -0.22 |
| 0.8 | -0.21 | -0.22 |
| 0.9 | -0.21 | -0.22 |
| 1.0 | -0.21 | -0.22 |

In the Network 1, the link weight between 2 harems indicates how strongly connected by invader males are 2 harems, and in the Network 2, the link weight indicates if 2 harems share at least 1 invader male. Copulation probability indicates the probability of a sneaker copulating with a female once he invades a territory, but no actual copulation is observed. All correlations are highly significant ($P < 0.001$).

that sneakers composed only 20% of the males in the population (Buzatto et al. 2011). Therefore, even a small percentage of sneakers in the population may increase the SCI faced by territorials. In fact, the average SCI for territorial males was 1.76 (Figure 3), suggesting that most females in the harems mate polyandrously. When the probability of sneak copulations is high, sperm competition is expected to be intense for both territorials and sneakers, and asymmetries in gonadal investment should be low between the 2 morphs (Parker 1990; Simmons 2001). A previous study with several populations of *S. proximum* (including the 1 we studied here) indeed showed that gonadal investment did not differ between morphs (Munguía-Steyer et al. 2012). Moreover, gonadal investment was not influenced by the relative frequency of sneakers, suggesting that population-level measures may not be appropriate proxies of SCI in *S. proximum*, and perhaps other species with similar mating systems (Munguía-Steyer et al. 2012).

Contrary to Munguía-Steyer et al. (2012), our results do not indicate that larger harems are more likely to be invaded. This difference probably occurs because our definition of invasion includes only cases in which an invader male was observed less than 20 cm from a female, whereas Munguía-Steyer et al. (2012) defined invasion as any male found less than 1 m from a female. Although our definition is more rigorous, our results still support the prediction made by Munguía-Steyer et al. (2012) that SCI should be more heterogeneous among territorials (Figure 3). Territorials should, therefore, respond strategically adjusting their ejaculate expenditure in response to perceived SCI (Simmons 2001). Based on the results presented here (Figure 4), and also on the theory of strategic ejaculation (Parker et al. 1996), the increased intensity of sperm competition faced by males holding small harems should result in decreased sperm numbers transferred to newcomer females. However, although the pattern predicted by the theoretical model has already been observed in some species (e.g., Simmons and Kvarnemo 1997), a recent meta-analysis of strategic ejaculation indicates that ejaculate expenditure responds positively to sperm competition risk, but not to SCI (Kelly and Jennions 2011). The contrast between theory and empirical results is probably related to the fact that the underlying assumptions of the models are rarely checked in the studied species (Kelly and Jennions 2011). As we stressed before, sperm precedence and the forms of sperm use by females in harvestmen are unknown, and without this information it is not possible to robustly test hypotheses of strategic ejaculation in the group.

The use of a sexual network approach circumvents the assumption that sperm competition is evenly distributed among all males in the population, allowing us to investigate the factors that influence inter-individual variation in SCI. We showed that territorials owning large harems experienced less SCI (Figure 4). The simpler explanation to this unexpected finding is that not all females of a large harem copulate with invaders (Figure 2). Thus, SCI seems to be diluted in large harems, which leads to the prediction that mean paternity per clutch should increase as harem size increases. This prediction contrasts with the pattern reported for some mammals, in which males holding the largest harems do not have the highest reproductive success (e.g., Kaseda and Khalil 1996; Heckel et al. 1999). Since mammals usually give birth to 1 or few offspring at a time, whereas arthropods lay a clutch containing many eggs, the negative impact of multiple paternity on the reproductive success of harem holding males should be different in these 2 groups. In mammals, each individual offspring sired by an invader promotes great reduction in the reproductive success of the harem holding male. In arthropods, invaders may sire only a small fraction of the eggs laid by each female. Assuming a scenario where 1) invaders are unable to copulate with all females in a harem, 2) an increase in harem size does not lead to an increase in the number of invaders, and 3) successful males do not suffer from sperm depletion, we expect that the mean proportion of eggs sired by territorial males should increase as harem size increases. In a harem containing 1 female, for instance, an invader that sires 15% of the eggs promotes a decrease of 15% in the territorial reproductive success. In a harem containing 3 females, an invader that sires 15% of the eggs of 1 female promotes a decrease of only 5% in the territorial reproductive success (considering that all females lay the same number of eggs).

The presence of alternative mating tactics allegedly decreases the total intensity of sexual selection among males, since the number of males without any copulation decreases (Webster et al. 1995;

Jones et al. 2001; Shuster and Wade 2003). However, the influence of the sneaker tactic on the total intensity of sexual selection can follow different directions depending on which females the sneakers copulate with (McDonald et al. 2013). Based on a previous study of *S. proximum* (Munguía-Steyer et al. 2012), we expected that harem invasion would be positively correlated with harem size, so that the most successful territorials would also be the ones facing the strongest sperm competition, which weakens the total intensity of sexual selection (Sih et al. 2009; McDonald et al. 2013). However, as explained above, territorials with larger harems faced lower SCI (Figure 4). In this sense, if territorials with smaller harems are also the ones losing more fertilizations to sneakers, the presence of the alternative tactic in *S. proximum* may indeed increase the total intensity of sexual selection among territorials. Further quantifications based on the number of offspring sired by each male in the population are necessary to test this hypothesis.

Contrary to our expectations, both the SCI faced by individual territorials and the intensity of harem invasion were not influenced by the distance to other harems. Even the most isolated harems were found and eventually invaded at least once (Figure 2), suggesting that sneakers are highly effective in detecting harems. However, the number of harems invaded by each sneaker was limited by the spatial distribution of harems, so that only neighbouring harems were invaded by the same individual sneakers (Figure 2). The result was a poorly connected sexual network, divided in more components than would be expected if there was no spatial constraint on sneaker movements. Most of the invader males were sneakers, but 7 harems were invaded by males that unequivocally belonged to the territorial morph (Figure 2). These territorials could be defending territories without females close to the invaded harems and, at some point of the breeding season, they adopted the sneaker mating tactic after failing to attract females. This implies behavioral plasticity that allows territorials to switch between territorial defense and invasion. Similarly, in damselflies and dragonflies, the costs of territoriality may only be affordable to attractive males that are large and/or in good condition (e.g., Tsubaki and Ono 1986, 1987; Forsyth and Montgomerie 1987).

At the population level, we found a negative correlation between the distance between any 2 harems and the probability of these harems being sexually connected by sharing at least 1 invader male (Table 2, Network 2). Given that some harems are spatially aggregated along the river margins (Figure 2), once a sneaker finds a harem, he probably invests some time searching for additional mates in the nearby areas. Therefore, the spatial distribution of harems in the population plays an important role influencing the topology of the sexual network, as has been previously demonstrated for an insect-pollinated plant in which close mother trees shared the same pollen donors (Fortuna et al. 2008). In resource defense mating systems, the proximity to other harems may increase the number and strength of sexual connections in the network. In fact, our results supports this prediction and show that spatially aggregated harems are more strongly connected (Table 2, Network 1), which may have important implications for the spread of sexually transmitted diseases (Proulx et al. 2005).

In conclusion, the network approach we employed allowed us to investigate SCI at multiple scales, ranging from individual males to the whole population. To the best of our knowledge, this is the first empirical study to use a spatially structured sexual network to make inferences on sperm competition. By incorporating the spatial structure of the population in studies of sperm competition, we can start thinking about a sperm competition landscape in

which harems are analogous to habitat patches. In landscape ecology, theory predicts that the closer the patches, the more intense the transit of individuals among them if the matrix is permeable (Forman 1995). In the same way, the more aggregated the harems, the more likely invasions by sneakers should be. Permeability in this case could be viewed as the mate-searching costs (especially mortality) paid by sneakers while moving among harems. Moreover, in the same way that large patches attract more immigrants and bear populations with higher genetic variability (Forman 1995), large harems may attract more invaders and increase the opportunity for females to mate polyandrously, which could bring benefits such as increased genetic variability in the offspring (Arnqvist and Nilsson 2000; Kvarnemo and Simmons 2013). In this sense, it would be advantageous for females to join large harems, which could explain some cases of female copying in species with resource defense polygyny (e.g., Shuster and Wade 1991). These are exciting new ideas that could be tested and expanded to other species exhibiting alternative mating tactics.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at <http://www.beheco.oxfordjournals.org/>

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