



Does silk mediate chemical communication between the sexes in a nuptial feeding spider?

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Abstract

Chemical signals play a crucial role in reproduction as a means for locating mates and/or gaining information about their quality, ultimately affecting mating system dynamics and mate choice. In spiders, one of the potential sources of chemical signalling is silk. However, while female silk is known to attract mates and/or elicit courtship, due to sex-specific roles in mate searching, male silk-related signals are often neglected. In the hunting spider *Pisaura mirabilis* (Pisauridae), both sexes leave silk draglines during movements while males additionally use silk to wrap nuptial gifts (food donations to females at mating). We explored the potential for both silk types (draglines and gift silk) to release signals and tested the hypothesis that chemical compounds bound to gifts' silk serve to elicit female attraction. We conducted behavioural choice assays for dragline and gift silk, and their putative transmission mode (airborne or contact) by testing (i) male and female attraction towards draglines of the opposite sex and (ii) female attraction towards gift silk. Whereas males were attracted to female draglines (contact cues), females did not respond to male silk of any type. We suggest that females use draglines for advertisement to secure copulation and foraging of nuptial gifts. If these signals ease male mate searching, attractive male draglines are unnecessary. Overall, males may not invest in chemical stimulation but rather exploit female foraging interests through gift giving. Alternatively, they may release signals that prime other female sexual behaviours or towards which females may have evolved resistance.

Significance statement

Animals commonly use chemical signals to communicate during reproduction, and spiders have the potential to release such signals from their silk. We investigated whether two silk types, draglines released during movements and silk covering male nuptial gifts (prey offered to females at mating) are attractive to the opposite sex in a hunting spider. While males were attracted to female draglines, females did not respond to male silk of any type. Females may be using silk to advertise themselves to secure matings and food through reception of nuptial gifts. If males can successfully locate females, attracting females through draglines may be unnecessary. The finding that males do not release attractant signals in the silk cover of their nuptial gifts further suggests that rather than attempting to increase their attractiveness by using chemical stimulation, males may be uniquely exploiting females' interest in food through gift giving behaviour.

Keywords Silk · Sexual selection · Chemical signals · Nuptial gifts

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Introduction

Chemical signalling is considered to be one of the most ancient and widespread forms of communication (Bradbury and Vehrencamp 2011; Wyatt 2003), and its central role in sexual reproduction has been acknowledged for over a century (Darwin 1871). Studies across taxa show that animals use pheromone signals for mate location, sex recognition and mate choice (Johansson and Jones 2007; Wyatt 2009). Sex pheromones are sex-specific semiochemicals, known to attract mating partners (Eisner and Meinwald 1995) but also

convey information on potential mates, such as body condition (Rantala et al. 2003), age (Nieberding et al. 2012), dominance (Moore et al. 1997) or mating status (Thomas 2011). Since males generally perform mate searching (Kasumovic et al. 2006; Fromhage et al. 2016), selection may favour advertisement in females and fine-scale sensory detection mechanisms in males, leading to sex-specific pheromone emitter and detector roles. Nevertheless, male pheromones are also described, and can be considered elaborate sexual traits shaped by selection through female choice (Rantala et al. 2002; Howard et al. 2003; Thomas and Simmons 2009), or by sexual conflict, where by soliciting an acceptance response in the receiver, they may be used to manipulate the opposite sex into mating beyond its optimum (Arnqvist and Rowe 2013).

Spiders represent a particularly well-suited taxonomical group for studying the evolutionary function of pheromone communication due to the large amount of behavioural studies providing evidence for their role in mating (Huber 2005; Gaskett 2007). Spider pheromones, which can be emitted not only from the animals' body but also from its silk, can enable the exchange of information between emitter and receiver at long distances through airborne chemicals, or through direct interaction with short-range contact chemicals (Schulz 2004; Gaskett 2007). Most of our knowledge of sex pheromone use in spiders comes from studies investigating female chemical signals bound to silk. Stationary females of web-building species primarily rely on airborne emission to be located by males (Cory and Schneider 2016) through which they provide detailed information about their quality, e.g. sexual maturity (van Helsdingen 1965) and mating status (Tuni and Berger-Tal 2012). In webless wandering species, silk draglines may instead have a higher relevance for both mate searching and mate attraction (Baruffaldi et al. 2010), inducing male courtship (Rypstra et al. 2003, 2009; Roberts and Uetz 2005) and providing information about female quality, e.g. mating status (Stoltz et al. 2007) and body condition (Havrilak et al. 2014). Despite their potential role in shaping mating dynamics, fewer studies have instead investigated male pheromones, which have been found to trigger courtship behaviour in females in species with sex role reversal (Aisenberg et al. 2010), or mutual mate assessment (Cross and Jackson 2009). These studies specifically focus on volatile body compounds, leaving male silk pheromones poorly understood.

Nuptial gift-giving spiders provide an interesting system to investigate chemical signalling through silk. The mating of these webless hunting species is characterised by the presence of nuptial gifts consisting of prey wrapped in dense silk layers offered by males and eaten by females during copulation. Therefore, while both sexes leave silk draglines during movements, males additionally use silk for mating, providing two distinct media (draglines and gift silk) through which spiders

can potentially release chemical compounds and use signals in different modalities. Nuptial gifts in spiders are rare, with only two well-studied species, the Palearctic *Pisaura mirabilis* (Pisauridae) and the Neotropical *Paratrechalea ornata* (Trechaleidae) (Albo et al. 2013). Interestingly, chemical cues are found bound to the silk cover of *P. ornata*'s gifts and are responsible for inducing female gift acceptance (Brum et al. 2012), suggesting an attractant role and/or possibly serving as a primer for other female behaviours during mating. Since these animals are nocturnal, chemical sensory channels may play a crucial role in mating if vision is reduced. Although the two species are not closely related and gift giving is suggested to be a convergent trait, chemical signals in nuptial gifts' silk have also been suggested for *P. mirabilis* (Bilde et al. 2007). *P. mirabilis* is diurnal, with some studies implying that the silk cover is noted visually by females (Stålhandske 2002), and others reporting lack of evidence for female's preference for silk-wrapped gifts compared to unwrapped ones (Bilde et al. 2007; Albo et al. 2012). The silk cover prolongs copulation by extending female feeding duration (Lang 1996; Stålhandske 2001), provides males with a better hold on the gift during transportation and mating (Andersen et al. 2008) and allows the contents of the gift to be concealed (Ghislandi et al. 2017). Indirect evidence also suggests that the silk may contain chemical substances that manipulate female reproductive behaviour; gift-offering males rejected by females commonly deposit additional silk layers to gifts, resulting in female acceptance during subsequent courtship attempts (Bilde et al. 2007; Stålhandske 2002). Females are polyandrous and gain additional nutritional benefits from the gift by mating multiply (Tuni et al. 2013), with satiated females accepting fewer matings than food-limited ones (Bilde et al. 2007). By freshly depositing additional silk layers, males may be adding chemical signals to entice females to accept the gift and copulate, either by manipulating reluctant females to mate above their optimal mating rates or by adding information about their underlying quality to facilitate female mate choice.

Draglines may also convey signals. While male draglines remain entirely unexplored, female draglines are suggested to contain chemical information, as several studies on male gift construction report that males exposed to silk threads of adult females become sexually excited and initiate silk wrapping (Bristowe and Locket 1926; Lang 1996; Stålhandske 2001; Albo et al. 2011a; Tuni et al. 2013). However, these studies rely on males being exposed to complex substrate-borne chemical cues, which may consist of a combination of female silk, excreta and traces of body cuticles rather than silk alone. Since males and females wander in the field, it may be beneficial for both sexes to be located via draglines left during movements, thus enhancing the probability of encounter rates and mating. Females would also benefit particularly from mate location due to the nutritious food donation received by males at mating (Tuni et al. 2013; Toft and Albo 2015).

We conducted a series of experiments to test whether the silk of male and female *P. mirabilis* spiders may be used as a means for chemical communication in a sexual context, with the additional aim of contributing to our general understanding of the evolutionary function of male silk wrapping of nuptial gifts. We investigated the presence of chemical signals in different silk types (dragline and gift silk) and their putative mode of transmission (airborne or contact cues) by conducting choice assays testing (i) male and female attraction towards draglines of individuals of the opposite sex and (ii) female attraction towards silk of nuptial gifts. We hypothesised that both males and females would respond to each other's draglines: airborne attractants would ease long-range mate location and sex recognition, whereas contact compounds would provide short-range information on the individual's quality and condition. If males add enticing chemical compounds to the silk of their gifts, we expect females to respond preferentially to silk of gifts compared to dragline silk, most likely through contact pheromones, due to the close-range gift display from males during courtship.

Material and methods

Animal collection and rearing

Juvenile *P. mirabilis* of both sexes (approximately 100) were collected in grass fields near the Ludwig Maximilians University of Munich (Planegg-Martinsried, Germany) in April 2016 and brought to the laboratory, where they were reared at natural photoperiod (16-h light:8-h dark) and room temperature (approx. 25 °C). Spiders were housed individually in plastic vials (5 cm diameter × 10 cm height), covered with a sponge lid and filled with a layer of moss. Every 3 days, we sprayed the moss with water and fed spiders either 10 fruit flies (*Drosophila melanogaster*), one housefly (*Musca domestica*) or one cricket nymph (*Gryllus bimaculatus*). Vials were inspected every second day, moulted exoskeletons were removed and the date of the moulting was noted to control for spider age. All experiments were conducted 1–2 weeks after spiders reached adulthood.

Experimental design and procedures

The study consisted of two distinct experiments. We conducted choice assays (details below) to investigate the presence of airborne or contact chemical cues in male and female dragline silk, by testing the attraction of each sex towards draglines of the opposite sex compared to a control with no silk (experiment 1). We then investigated the presence of airborne or contact chemical cues in male gift silk, by testing female attraction towards silk of gifts compared to either a control with

no silk, or male dragline silk (experiment 2). Spiders were randomly selected for use in the trials.

Choice assays

Airborne cues To test for the presence of airborne cues in silk, we used an open-field olfactometer. The device consisted of two petri dishes (3 cm height × 15 cm in diameter each) affixed on top of each other. In the lower part, we placed standardised silk samples, as described below. The lower chamber was separated in two compartments by a plastic wall (3 cm height × 15 cm length), and each side was covered with absorbent paper. The bottom of the top chamber consisted of a fine metal mesh preventing any appendages from protruding into the lower chamber. The focal individual was placed on this mesh, allowing any volatile chemical cues from the bottom chamber to reach the spider, while preventing direct contact with the silk. A hole (7-cm diameter) in the top of the upper chamber allowed the spider to be inserted into the olfactometer via a vial, and ensured that all spiders began each trial in the centre of the olfactometer (Fig. 1a).

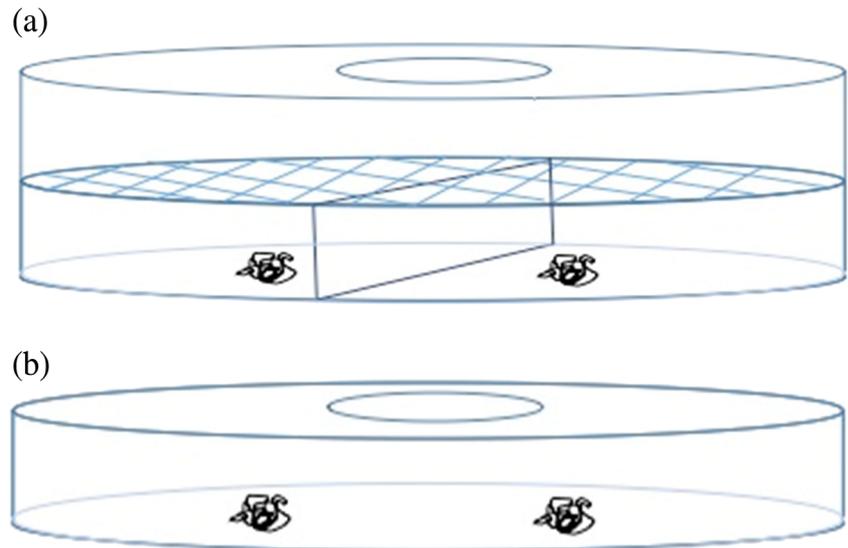
Contact cues To test for the presence of contact cues, we used a petri dish (3 cm height × 15 cm in diameter) without a dividing wall. The bottom was covered with absorbent paper. As above, a hole (7-cm diameter) for inserting the vial was cut through the plastic lid. Silk samples were placed on the absorbent paper, allowing the spider to directly contact the silk (Fig. 1b).

At the start of each assay, once the silk was placed in a standardised position on one or both sides of the lower chamber, a vial containing the spider was positioned top down in the hole and the animal prevented from exiting for 1 min for acclimation. The vial was then gently removed and the petri dish covered with its lid. The spider was allowed to move freely inside the device. Trials were video-recorded for a total of 16 min using web cameras (Logitech HD Pro Webcam C920) fixed at a distance of 40 cm from the test arena, and each connected to a laptop. After each trial, males were returned to their housing vials and olfactometers washed using 70% ethanol and dried completely before re-use. Preference for either stimulus was determined by assessing the relative residency time on each side of the olfactometer (see video scoring, below).

Experiment 1—chemical cues in male and female draglines

Dragline silk was obtained by allowing spiders to freely move inside an arena. Since males released fewer draglines compared to females, we used different procedures to collect silk from each sex. Adult females were placed inside a plastic petri dish (15 cm diameter × 1.5 cm height) with the bottom

Fig. 1 Schematic representation of olfactometer used. **a** Presence of airborne silk cues was tested for in an open-field olfactometer which allows volatile chemical cues from the bottom chamber to reach the spider placed on top of the mesh, while preventing direct contact with the silk. **b** Presence of contact silk cues was tested by placing both spiders and silk samples on the bottom chamber, allowing direct contact with the silk



covered in absorbent paper and left to move freely for 30 min. They were then returned to their housing vials and silk was collected from the papers' surface using forceps and used immediately for behavioural assays. Males were instead placed inside a transparent plastic box (11 cm × 19 cm × 11 cm) with the bottom covered in absorbent paper and left overnight. On the following day, males were returned to their housing vials and silk collected using forceps and used immediately in assays. The amount of silk used for trials was standardised to approximately 2 mm³.

Males were given the choice between (i) female draglines vs. no silk, testing for airborne cues ($n = 22$) and (ii) female draglines vs. no silk, testing for contact cues ($n = 25$). Females were given the choice between (i) male draglines vs. no silk, testing for airborne cues ($n = 30$) and (ii) male draglines vs. no silk, testing for contact cues ($n = 25$).

Experiment 2—chemical cues in silk of nuptial gifts

Silk from nuptial gifts was obtained by allowing males to wrap gifts and then remove the silk. Adult males were placed in a plastic box (20 cm × 20 cm × 20 cm) in which a female was previously kept for 15 min as a sexual stimulus (Lang 1996). The male was then offered a cricket nymph as prey to wrap into a nuptial gift. Males of this species generally capture and kill prey, and deposit silk in a series of wrapping episodes. We allowed males to wrap their gifts for a total of 15 min (sum of all wrapping episodes) in order to standardise the amount of silk deposited on the gift, as silk wrapping duration correlates with silk amounts (Lang 1996; Ghislandi et al. 2017). If males failed to catch a prey item or did not wrap it in silk, they were returned in their housing vial and tested again on the following day. Once silk-wrapping was completed, the gift was taken from the male using forceps and placed under a stereomicroscope to remove silk following established protocols

(Ghislandi et al. 2017). Briefly, we used fine scissors to remove the outer silk layers. If any contamination from prey (e.g. through body fluids) occurred, the silk was excluded from testing. The collected silk (standardised as above) was then immediately used for behavioural tests, with the entire procedure from silk collection to choice assays of silk occurring within 10 min.

Females were given the choice between (i) gift silk vs. no silk, testing for airborne cues ($n = 24$), (ii) gift silk vs. no silk, testing for contact cues ($n = 25$), (iii) gift silk vs. male draglines (collected as described for experiment 1), testing for airborne cues ($n = 18$) and (iv) gift silk vs. male draglines, testing for contact cues ($n = 19$). When comparing two silk types in the same test, silk was taken from the same male.

Video scoring

All videos were scored using the video tracking software EthoVision XT 10 (Noldus). For the scoring, we selected the whole olfactometer as the arena (focus point for the scoring software) with two different zones (silk vs. no silk or silk type1 vs. silk type2). As soon as the spider started moving into the arena, the software measures accumulated residence time of the tested individual in each zone. A total of 15 videos could not be scored using EthoVision due to bad video quality, and these were excluded from further analyses.

Statistical analyses

Data were analysed using generalised linear mixed-effect models in *R* (R Core Team 2014) using the 'lme4' package (Bates et al. 2014). We excluded spiders that did not move throughout the entire data collection period (5 females and 1 male). Proportion data (relative time spent on focal side of test arena) were logit-transformed (Shi et al. 2013). We

constructed simple models using a Gaussian distribution family for each comparison (see Table 1), containing only the silk source as a random effect as spiders were used more than once as focals, and a fixed value (1) as a proxy predictor variable. Thus, a preference for one cue source or another is described by the model intercept. The model formula structure used was thus:

$$\text{Logit.proportion.time.in.field} = 1 \\ +(\text{silk donor ID as a random effect})$$

Data availability All data generated or analysed during this study are included in this published article (in its [supplementary information files](#)).

Results

Experiment 1—chemical cues in male and female draglines

Males spent more time near the female dragline silk when able to make physical contact with it, but not when only airborne cues were available (Fig. 2; Tables 1, 2). By contrast, females did not respond to male dragline silk, neither in airborne or contact cue tests (Tables 1, 2).

Experiment 2—chemical cues in silk of nuptial gifts

Females did not respond to male gift silk when tested against no silk, showing no preference during both contact and airborne cue tests (Tables 1, 2). Similarly, females did not respond to male gift silk when tested against male dragline silk, again showing no preference during both contact and airborne cue tests (Tables 1, 2; Fig. 3).

Table 1 Average proportion of time (%) spent by tested individuals (males and females) on each field of the testing device during tactile and volatile cue tests

	Average time (%)			
	Tactile		Volatile	
Experiment 1				
Males tested	Female dragline	No silk	Female dragline	No silk
	64.2	35.8	51.4	48.6
Females tested	Male dragline	No silk	Male dragline	No silk
	49.3	49.7	47.6	52.4
Experiment 2				
Females tested	Male gift silk	No silk	Male gift silk	No silk
	57.2	42.8	54.4	45.6
	Male gift silk	Male dragline	Male gift silk	Male dragline
	51.2	48.8	40.4	59.6

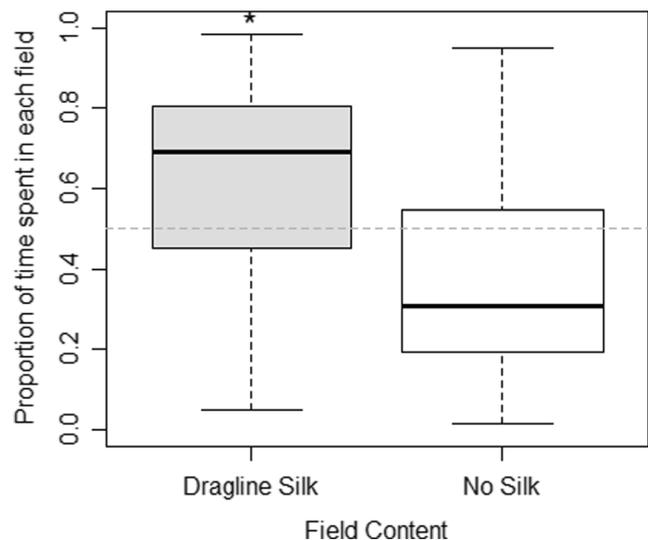


Fig. 2 Proportion of time spent by males on the side of the test arena containing either dragline silk or no silk while testing for contact cues. Males are attracted to female dragline silk

Discussion

Our study revealed sex differences in *P. mirabilis* responses to silk, with males being attracted to female dragline silk but females not responding neither to dragline nor to silk of nuptial gifts. These findings highlight that there is variation in the use of silk as a source of biologically relevant cues between the sexes, and between silk types.

Males responded to the dragline silk of females, suggesting the presence of chemical cues bound to silk that likely function as female advertisement. Females across taxa adopt several strategies to attract mating partners, such as visual signalling through bright coloration in fish (Amundsen and Forsgren 2001), acoustic signalling in bush crickets (McCartney et al. 2012) or chemical signals in spiders' silk (Gaskett 2007). Selection should promote female advertisement when it counterbalances the ecological costs associated with signalling, as

Table 2 Statistical analysis of all experiments. Note that estimates are logit-transformed. Estimates refer to the estimated intercept of the model, representing the modelled proportion of time spent on the focal olfactometer side. For example, in the first row, the estimate represents the logit-transformed proportion of time spent on the female dragline silk side of the olfactometer. Italics denotes significance

		Estimate	Estimate standard error	Z value	P value	
Experiment 1						
Males tested	Female dragline vs. no silk	0.78	0.34	2.31	<i>0.021</i>	
	Tactile					
Females tested	Female dragline vs. no silk	-0.52	0.73	-0.71	0.48	
	Volatile					
	Male dragline vs. no silk	-0.19	0.31	-0.63	0.53	
	Tactile					
Females tested	Male dragline vs. no silk	-0.25	0.31	-0.80	0.42	
	Volatile					
	Experiment 2					
	Females tested	Male gift silk vs. no silk	0.27	0.46	0.59	0.56
Tactile						
Male gift silk vs. no silk		0.21	0.44	0.47	0.64	
Volatile						
Male gift silk vs. male dragline		0.33	0.86	0.39	0.70	
Tactile						
Females tested	Male gift silk vs. male dragline	-0.59	0.54	-1.1	0.27	
	Volatile					

signal emission will inevitably reveal the emitters' presence also to unwanted eavesdroppers such as predators, parasitoids (Jones et al. 2002; Umbers et al. 2015) or harassing males (Arnqvist and Rowe 2013). In *P. mirabilis*, ensuring reproduction and avoiding the risk of mating failure may represent the driver for female chemical advertisement (Rhainds 2010; Umbers et al. 2015). Furthermore, in this species, signalling through draglines may also serve to supplement the female's own foraging by soliciting additional food gifts (Prokop and Maxwell 2009; Tuni et al. 2013) and gaining indirect genetic benefits via matings with multiple partners (Tuni et al. 2013). As a result, if mate location is successfully achieved through male mate search alone, mutual detection via dragline silk

may not be necessary. This may explain our finding on no female response towards male silk.

The lack of female attraction towards male silk may suggest that males do not release chemical signals. Signal production may carry survival and metabolic costs (Jones et al. 2002; Wyatt 2003) which will add to the general costs associated with gift production. These include predation risks while hunting for prey, missed meals, costly silk proteins for wrapping (Nentwig and Kuhn-Nentwig 2013) and mobility-related costs due to carrying of a gift (Prokop and Maxwell 2012). As such, males may preferentially invest in traits related to nuptial gift production, reducing energetic investment in pheromone emission. It may however also imply that despite containing

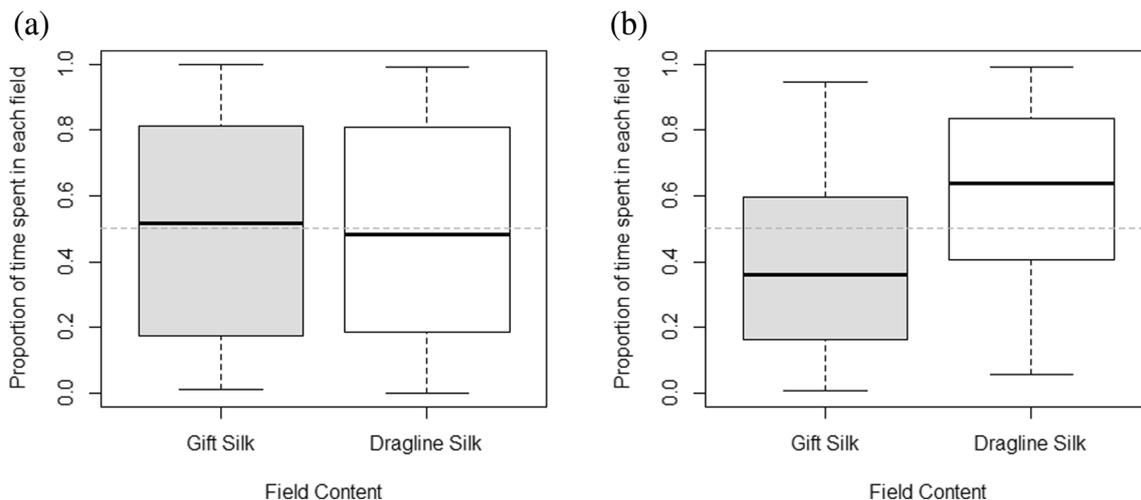


Fig. 3 Proportion of time spent by females on the side of the test arena containing either gift silk or dragline silk, while testing for contact (a) and airborne (b) cues. Females are not attracted to male silk

chemical signals, females do not respond to them by showing attraction (i.e. spending more time in the presence of male silk). Rather than releasing observable female behaviour, male cues could, for example, prime female response to important courtship interactions and acceptance of the nuptial gift (Brum et al. 2012). Alternatively, silk chemicals may exert other functions. For example, during courtship, male *Agelenopsis aperta* induce a quiescent state in females through the use of airborne pheromones, facilitating the mating position (Becker et al. 2005). A similar function could be favoured to reduce *P. mirabilis* females' aggressive and cannibalistic behaviour (Toft and Albo 2016). Lack of silk attractants in the gift however go against our initial expectations, based on evidence for female acceptance of gifts wrapped at close range (Bilde et al. 2007). Such female behaviour may represent a response to the male wrapping performance per se, which may ultimately indicate good body condition in males, with male phenotype rather than silk being the target of female choice (Albo et al. 2012). Indeed, *P. mirabilis* females may have evolved resistance to the information carried by gift silk in response to males evolving efficient methods by which to deceive females into mating (Albo et al. 2011b; Ghislandi et al. 2014). It is well established that males can reduce the nutritional value of the gift by feeding on the prey prior to silk-wrapping (Ghislandi et al. 2017). These males successfully cheat females into mating by donating a gift that in appearance resembles a genuine food gift due to the silk cover (Albo et al. 2011b; Ghislandi et al. 2017). Lowering the content quality of the gifts facilitates males' reproductive interests, but by reducing female fitness benefits derived from the gift nutrients, it should also select for female discrimination abilities to resist deception (Arnqvist and Rowe 2013). Thus, females may no longer respond to silk traits, such as chemical cues, even if present.

Although we can only speculate on the information content of female silk cues, it is worth considering several possible scenarios. Females may be advertising their presence and location by providing a directional signal, as dragline silk is generally considered instrumental for spider mate location (Hegdekar and Dondale 1969; Roland and Rovner 1983; Taylor 1998; Baruffaldi et al. 2010). Since both sexes of our study species are mobile and are generally found wandering in high numbers in relatively small areas (CT personal observation), it may be likely that encounters with female draglines will successfully lead to location of the mating partner in the field. Female draglines are also known to provide males with important information about their quality (e.g. condition, mating status) (Roberts and Uetz 2005; Havrilak et al. 2014). This information is crucial in determining male strategic decisions on courtship and/or sperm allocation to partners (Wedell et al. 2002; Andersson and Simmons 2006; Tuni et al. 2017), especially in males that invest in costly matings, such as *P. mirabilis* with their energetically expensive nuptial gifts (Albo et al. 2011a). Finally, although we did not assess male

courtship behaviour, our findings strongly suggest that the large indirect evidence for female substrate-borne cues eliciting sexual excitement (i.e. jerking of the body, rapid rubbing of the front pair of legs, trembling movements) and gift-wrapping in *P. mirabilis* males (Lang 1996; Tuni and Bilde 2010; Albo et al. 2011a; Tuni et al. 2013) is indeed due to exposure to dragline silk. Female silk also seems to be an important stimulus eliciting silk-wrapping behaviour in the gift-giving spider *P. ornata* (Albo et al. 2009). Hence, males may be chemically enticed to prepare a nuptial gift in order to be ready to court females upon encounter. In the field during the mating season, males are commonly found carrying silk-wrapped gifts in their mouthparts while moving across the vegetation (Prokop and Maxwell 2012; Ghislandi et al. 2017). Moreover, there is evidence that the ratio of adult males carrying a gift increases with female availability, which may be signalled through silk traces (Ghislandi et al. 2017, in revision). The functional roles of female dragline cues suggested above require empirical corroboration and represent interesting avenues for research.

Since males responded exclusively to dragline contact cues, we further improved our understanding of how chemical information is conveyed in spiders. This finding suggests short-range cues that cannot be used to attract males over long distances. Long-range airborne cues bound to silk are commonly reported in most web-building species, where selection may favour this form of transmission due to the stationary lifestyle of females and the large web surface area, which is likely an efficient medium for signal release (Kasumovic and Andrade 2004; Xiao et al. 2009). Since airborne cues in wandering spiders, such as wolf spiders, may also be released from the female's body cuticle (Searcy et al. 1999), a combination of cuticle and contact silk cues is likely sufficient for female advertisement. The lack of response towards airborne cues may however also be due to limitations in the methodology adopted. As already mentioned, silk may trigger responses other than attraction that may be revealed by scoring different behavioural variables from the one chosen in our design (e.g. female grasping behaviour (Brum et al. 2012)), or other types of choice tests may prove more efficient, as for example those using distinct chambers containing samples, and airflow crossing them to reach the test animal. Additionally, volatile chemical compounds are known to fade rapidly (Wyatt 2003), requiring further investigations to understand whether signal persistence was reduced during the timeframe of our experimental tests.

To conclude, we found sex differences in chemical communication. Female silk is attractive to *P. mirabilis* males, but not the reverse. The risk of reproductive failure for females and/or the gains from soliciting more gifts to meet the female's nutritional needs may outweigh the costs of signalling (predation, male harassment) and select for the use of chemical information bound to female silk. The high energetic investment

in costly matings sustained by males may instead reduce allocation to chemical emission, with resources preferentially invested in gift-giving behaviour acting on female foraging motivation, rather than exploiting the females' chemical sensory channels. Male signals may be present but prime female sexual behaviour in ways that are difficult to detect from our study, or alternatively, females may have evolved to ignore the information content of the silk due to an existing arms race between male deceptive strategies and female resistance to deception.

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Author contribution CT conceived the study, MB and TC designed the experiments, MB collected the data, TC analysed the data and CT led the writing of the manuscript with all authors contributing relevantly to the draft and giving final approval for publication.

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