Web reduction by courting male black widows renders pheromone-emitting females' webs less attractive to rival males

Catherine Scott[*], Devin Kirk, Sean McCann, Gerhard Gries

Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada

Male adaptations that limit sperm competition include guarding females, applying mating plugs and chemically reducing the attractiveness or receptivity of females. In many web-building spider species, females attract males with silk-borne volatile pheromones. In widow spiders (Latrodectus, 30 species), the courting male often engages in web reduction behaviour during which he excises and bundles sections of the female's web and wraps them with his own silk. Hypothesized functions of this wide-spread behaviour include sexual communication (e.g. through dissemination of male sex pheromone) and/or decreasing the female's attractiveness to rivals. The latter function was previously demonstrated in a single spider species, Neriene litigiosa, but the extent to which web reduction may decrease male–male competition has never been quantified in the field. In a dense population of western black widows, Latrodectus hesperus, we ran mate attraction experiments to test the hypothesis that web reduction and/or male silk addition decrease web attractiveness to potential rivals. Webs reduced by males attracted three times fewer males than intact webs; webs with a similar proportion of silk experimentally removed attracted as many males as intact webs. However, the experimental addition of male silk did not affect the attractiveness of intact webs. We conclude that web reduction in black widows limits male–male competition by reducing the attraction of rival males to females' webs. This effect is probably mediated through targeted excision of pheromone-laden silk by courting males, possibly in combination with the male's silk forming a physical barrier to pheromone emission.

© 2015 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

In polyandrous animals, males have diverse adaptations that reduce sperm competition, including preventing or impeding female remating by mate guarding, applying a mating plug, or chemically altering female attractiveness or receptivity (Birkhead & Møller, 1998; Parker, 1970). These adaptations may conflict with the female's interests if they have costly side effects, such as restricting the female's mobility or foraging opportunities, or if the female benefits from remating (Arnqvist & Rowe, 2005; Stockley, 1997). Conversely, females sometimes benefit from such adaptations; for example, Pieris (Pieridae) butterfly males limit costly harassment of mated females by transferring an antiaphrodisiac pheromone during copulation (Andersson, Borg-Karlson, & Wiklund, 2000). Therefore, studying adaptations that limit sperm competition can provide insights as to how potentially conflicting male and female sexual strategies shape mating systems.

In many web-building spider species, males are attracted to volatile pheromones released from the bodies or silk of females and use chemical cues to discriminate between virgin and mated females (Gaskett, 2007; Schulz, 2013; Uhl, 2013). Whether post-copulatory changes in female attractiveness are under female or male control is not yet clear. Regardless, males should be able to avoid sperm competition if females become unattractive to rival males immediately after mating. However, male spiders may face fierce competition for access to females and/or lengthy courtship and copulation, during which another male may usurp their position. If so, the female becoming unattractive only after mating will not be sufficient to prevent sperm competition. One solution may be to reduce female attractiveness before mating. For example, when males of the sierra dome spider Neriene litigiosa (Linyphiidae) enter the pheromone-emitting webs of highly receptive virgin females (those that have remained unmated for 6–10 days post-maturity), they immediately excise large areas of those females' webs and pack the silk into dense bundles. Watson (1986) showed in laboratory experiments that such reduced webs are then less attractive to other males than are intact webs. Mated females rebuild their webs without attractive pheromone; consequently, a

* Correspondence: C. Scott, Department of Biological Sciences, Simon Fraser University, 8888 University Drive, Burnaby, BC V5A 1S6, Canada.
E-mail address: ces14@sfu.ca (C. Scott).

male that performs web reduction decreases the risk of competitors arriving at a female's web both during and after copulation (Watson, 1986). Web reduction in *N. litigiosa* may thus be considered a defensive mating tactic against sperm competition. However, the extent to which web reduction decreases competition in the field has not been quantified.

The mechanism underlying decreased attractiveness of reduced *N. litigiosa* webs has never been studied, but the compaction of the web into tight bundles is thought to limit release of the female's pheromone by decreasing the exposed surface area of silk (Watson, 1986). One nonexclusive alternative suggested for another linyphiid spider, *Florinda coccinea*, is that males add antiaphrodisiacs to the female's silk during web reduction, and that these chemical cues repel rivals (Willey Robertson & Adler, 1994). The ability to detect and avoid females' webs that are occupied, or have been previously occupied, by other males should be favoured in species with strong first-male sperm precedence and when costs of mate searching do not preclude choosiness. Studies on spider chemical communication have focused on female pheromones (Gaskett, 2007; Schulz, 2013; Trabalon, 2013; Uhl, 2013) and although male spiders also emit pheromones from their bodies or silk (e.g. Becker, Riechert, & Singer, 2005; Cross & Jackson, 2013), these pheromones have received little attention.

Web reduction behaviour is common in sheet weaver (Linyphiidae) (Rovner, 1968; Stålhandske & Gunnarsson, 1996; Van Helsdingen, 1965) and tangle-web (Theridiidae) spiders (Breene & Sweet, 1985; Locket, 1927; Whitehouse & Jackson, 1994). During web reduction in *Latrodectus revivensis*, the male dismantles sections of the female's web, bundles and wraps the sections with his own silk, and then often leaves the bundles hanging near the female's retreat (Anava & Lubin, 1993). This may be a means of disseminating male sex pheromone during intersexual communication (Anava & Lubin, 1993), or chemicals on the male's silk may render the female's pheromone unattractive (Willey Robertson & Adler, 1994). Males of the orb weaver *Nephila senegalensis* (Nephilidae) avoid webs that have previously been visited by another male based on cues associated with his silk draglines (Schneider, Lucass, Brandler, & Fromhage, 2011). Similarly, the curtailed attractiveness of reduced webs may be due to males avoiding silk deposited by a courting male.

Web reduction is a common element of long, normally nocturnal precopulatory displays in black widows (*Latrodectus* spp., Theridiidae) (Breene & Sweet, 1985; Forster, 1995; Harari, Ziv, & Lubin, 2009; Segoli, Arieli, Sierwald, Harari, & Lubin, 2008). A courting *Latrodectus* male typically removes approximately half of a female's web during web reduction, wrapping the bundled silk extensively with his own (Anava & Lubin, 1993; Scott, Vibert, & Gries, 2012; Fig. 1). Male western black widows, *Latrodectus hesperus*, produce a silk-borne contact pheromone that elicits female courtship behaviour (Ross & Smith, 1979), and immature males of a congener adjust their development based on volatile chemical cues from conspecific males (Kasumovic & Andrade, 2006). Whether web reduction in black widows or other theridiid spiders curtails male−male competition or serves in intersexual communication has never been studied. Here, we investigate the function of web reduction in *L. hesperus*, in which both direct competition for mating opportunities and sperm competition have probably shaped male reproductive strategies. Males prefer virgin to mated females based on long-range pheromone cues (MacLeod & Andrade, 2014), and some males guard subadult females (Ross & Smith, 1979). Males should benefit from adaptations that improve their ability to monopolize females because polyandry occurs in the field and copulatory plugs (the broken-off tips of male intromittent organs) are often ineffective at preventing subsequent mates from siring offspring (MacLeod, 2013).

Figure 1. Photographs of a male *Latrodectus hesperus* during web reduction. During precopulatory courtship on the female's web, the male excises sections of silk, bundles them up and wraps them with his own silk, resulting in a (a) rope or (b) ball of silk. Note that in each image the male is pulling wrapping silk from his spinnerets with the last pair of legs.

In studies of a dense field population of *L. hesperus* (Salomon, Vibert, & Bennett, 2010) with the potential for intense male−male competition, we tested the hypothesis that web reduction decreases the attractiveness of females' webs to rival males. We also tested two hypotheses for the mechanism underlying this effect. First, we asked whether a decreased silk surface area alone is sufficient to reduce web attractiveness, and second, we asked whether males avoid the silk deposited during web reduction.

METHODS

Study Area and Animals

Our study site was a 20×400 m area of coastal sand dunes above the high-tide line at Island View Beach, on the Saanich Peninsula of Vancouver Island, British Columbia, Canada (48°34′N, 123°22′W; elevation 3−5 m). At this site, *L. hesperus* females build their webs under driftwood logs and other woody debris. The population is dense, with two to three subadult or adult females/m² of suitable habitat during each mating season (Salomon et al., 2010).

On 26 May 2013, we collected 50 mated female *L. hesperus*. We reared their offspring to maturity in the laboratory on a diet of house crickets (*Acheta domestica*) and blow flies (*Lucilia sericata* and *Phormia regina*). When not being used for experiments, we kept all spiders individually in petri dishes (150 × 25 mm for females; 100 × 25 mm for males) at 20–25 °C on a reverse 12:12 h light:dark cycle. Once spiders had reached maturity, we fed females at least one large cricket or four blow flies per week. We provided adult males with a few drops of water weekly but did not feed them because they normally do not forage as adults (Foelix, 2011). For web building, we used virgin females that were 2 weeks–6 months postmaturity. Each female built one web for each of our two experiments. For web reduction, we used males 6 days to 3 weeks postmaturity. Each male courted on only one female's web. Males that are 1–3 weeks postmaturity usually engage in courtship behaviour upon contact with silk of virgin females, and the webs of well-fed virgin females that are 1 week–6 months postmaturity usually elicit web reduction by males (Scott & Gries, n.d.).

Mate-attraction Assays in the Field

At our field site, we examined attraction of males to screen cages containing webs from which females had been removed.

General experimental methods

We built cages (26 cm diameter × 20 cm tall; see Fig. 2a) from aluminium window screening (18 × 16 mesh; New York Wire, Hanover, PA, U.S.A.) and plastic plates (26 × 26 cm; Solo Squared ™, Solo Cup Company, Lake Forest, IL, U.S.A.). We surrounded each cage with strips of waxed cardboard (10 cm wide) coated with a thin layer of adhesive insect trap coating (Tangle-trap, Contech Enterprises, Victoria, BC, Canada), so that attracted males would be captured before making contact with the silk inside a cage. In the laboratory, we placed females individually in wire-mesh cages to establish webs. Seven days later, we transported these cages to our field site and established them as traps. We did not feed the spiders until after the experiment so that no prey odour would be present in cages.

At our field site we established a 380 m transect adjacent to a walking path and placed trap replicates approximately every 20 m along the transect (Fig. 2b, c). We selected locations that were ~1 m away from at least one driftwood log, which is suitable black widow habitat (Salomon et al., 2010). We placed traps on the beach at sunset and checked them every 3 h for 24 h. We counted and collected all males that we found on the sticky strips surrounding each cage, and occasionally on the cages themselves, and preserved them in 95% ethanol. The few males not stuck to the adhesive strips probably reached cages via silk lines from nearby vegetation.

Our experiment was designed to ensure that any males we captured responded primarily to volatile chemical cues associated with female or male silk inside cages. Because widow spiders are nocturnal and have poor vision (Foelix, 2011), visual cues are not likely to have affected the males' responses (also see MacLeod & Andrade, 2014).

Experiment 1: effect of natural and experimental web reduction on attractiveness of females' webs

Less than 3 h before starting the field experiment, we removed females from their webs and prepared 20 replicates of each of the following four treatments: (1) cages containing intact webs (no manipulation); (2) cages containing male-reduced webs (on average ~50% of web silk bundled and wrapped with male silk; see below); (3) cages containing mechanically reduced webs (~50% of web silk excised); (4) cages containing no webs (control). We included no-web control cages to confirm that captures of males on
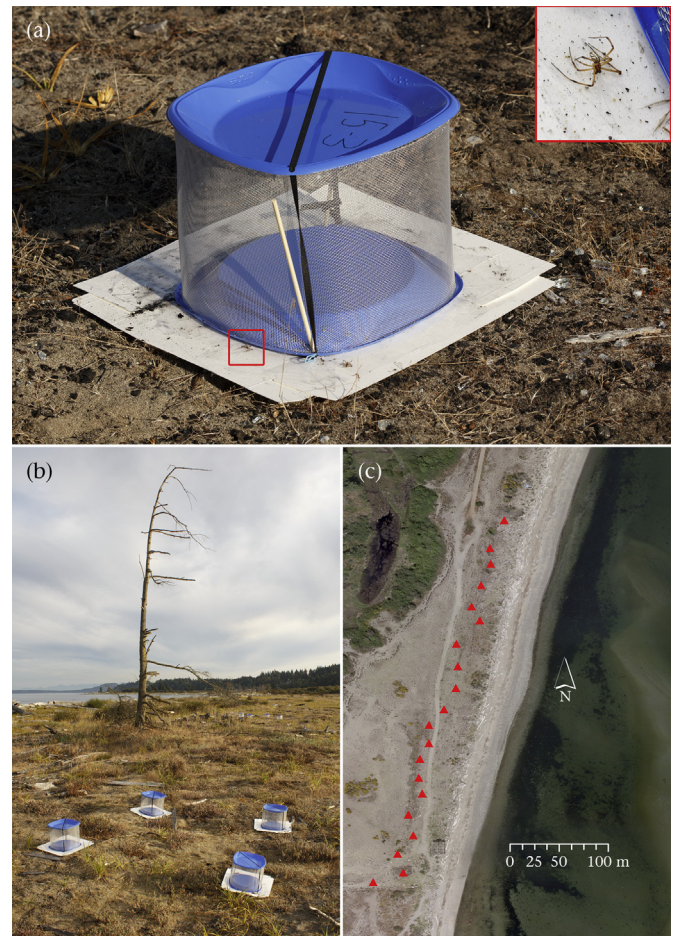

Figure 2. (a) Photograph of a wire-mesh cage containing the web of a *Latrodectus hesperus* female. The cage is surrounded by adhesive strips for capturing males that are attracted to silk-borne pheromone. Inset: a captured *L. hesperus* male. (b) Photograph of one of 20 blocks tested in experiment 1, illustrating four wire-mesh cage traps spaced 1 m apart and each containing one of four treatments (for details of experimental design, see General experimental methods). (c) Aerial view of the Island View Beach field site on the Saanich Peninsula of Vancouver Island, BC, Canada (image courtesy of the Capital Regional District). Red triangles indicate the location of experimental replicates (blocks) placed approximately 20 m apart in experiments 1 and 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

adhesive strips around cages resulted from attraction of males to female silk rather than random mate searching.

We used a randomized complete block experimental design, with each treatment appearing once in each block. We randomly assigned treatments to the four vertices of a randomly oriented 1 × 1 m square. We chose this spacing to match the actual density of females at our field site (see Study Area and Animals). To minimize variation in web attractiveness within each block, we used webs spun by females that matured within 14 days of one another and whose body masses (mean ± SD = 343.6 ± 56.8 mg) differed as little as possible. The mean largest mass difference among the three females within each block was 39.3 ± 32.5 mg.

To obtain male-reduced webs, we introduced a male onto each of 20 empty webs at the farthest possible location from the female's retreat and allowed him to engage in courtship for 1 h. Males readily court on empty webs of virgin females (Ross & Smith, 1979; Vibert, Scott, & Gries, 2014). Males usually begin web reduction within the first 15 min of courtship and continue to engage in this behaviour for up to 1 h (Scott & Gries, n.d.). If after 15 min a male

had not moved at all, or had not begun web reduction behaviour, we replaced him with a new male who was allowed to court for 1 h. The extent to which males reduced webs was variable, ranging from no visible web reduction to most of the web being reduced to a dense rope or ball (Fig. 1). There is no evidence that variation in male age is associated with variation in web reduction when females are well fed (Scott & Gries, n.d.). Based on visual inspection of reduced webs, we estimate that on average 50% of the web area was removed and wrapped with male silk ($\leq$25% reduced: 5 webs; ~50% reduced: 9 webs; $\geq$75% reduced: 6 webs). We removed males from reduced webs immediately before transporting cages to trap locations on the beach.

We prepared mechanically reduced webs by cutting webs in half with scissors along a straight line extending outward from the female's retreat. Although we do not know where the female deposits pheromone on her web, this method ensured that on average, across replicates, we removed 50% of the pheromone-laden silk. The silk threads of the web are usually most concentrated around the female's retreat, and the web becomes less dense with increasing distance from the retreat. We randomly selected the right or left half of the web for silk removal. Because tangle-webs have a three-dimensional structure, the remaining half of the web remained intact, with minimal changes in structure due to reduced tension in some of the threads. We chose to remove approximately 50% of the web because this is the typical extent of web reduction during *Latrodectus* courtship (Anava & Lubin, 1993; Scott et al., 2012).

Experiment 2: effect of male silk on attractiveness of females' webs

To test whether the male's silk on females' webs affects the attraction of males, we conducted a second experiment 3 weeks later, using the same design as above except that our 20 replicates included a paired-treatment design with cages containing either (1) intact webs (no manipulation), or (2) intact webs with male silk added (see below). To minimize within-replicate variation in web attractiveness, we chose two females for each replicate that had matured within 14 days of one another and whose masses (331.5 ± 63.4 mg) differed as little as possible (mean mass difference: 16.6 ± 14.6 mg). We used a third group of 30 females (mean mass: 350.45 ± 54.0 mg) to obtain male silk produced in the process of web reduction. Less than 3 h before the experiment, we removed all females from their webs, introduced a male onto each of the 30 empty webs and allowed each male to court for 1 h. For collection of male silk, we then chose 20 visibly reduced webs with an obvious ball or rope of female web wrapped in male silk (Fig. 1). We used scissors to excise these balls or ropes of silk, and we used forceps to place them in the centre of a web randomly selected from each pair of intact treatment webs. Thus, webs with male silk added comprised an entire female's web plus an unknown quantity of a second female's web wrapped with male silk. In each replicate, we randomly assigned the intact web and the intact web with male silk added to one of two trap locations, with 1 m spacing between paired traps and approximately 20 m spacing between trap pairs (Fig. 2c). We chose 1 m spacing between traps to match the actual density of females at our field site (see Study Area and Animals).

Ethical Note

We conducted this study in accordance with the guidelines of the Simon Fraser University Animal Care Committee and the Canadian Council on Animal Care (no protocol number because approval is not required). We treated experimental spiders as well as possible given the constraints of our study objectives and experimental design. We know of no alternative effective method of live trapping spiders that would have allowed us to determine the first choice of males and prevent them from making contact with silk inside traps other than constant human surveillance of all traps and collection of spiders by hand. Pitfall traps are not likely to be effective at capturing male black widows, because these spiders can scale smooth walls with the aid of their draglines. We therefore used adhesive Tanglefoot to capture spiders. It is not clear whether spiders and other arthropods experience pain (Sneddon, Elwood, Adamo, & Leach, 2014) and thus we cannot assess whether this method caused undue suffering. We limited the time spiders spent trapped in Tanglefoot by checking traps and euthanizing captured spiders in ethanol every 3 h. Checking traps more often would have been logistically difficult and would have resulted in excessive disturbance of trap sites during the experiments. During experiments, we kept spiders used in preparation of silk treatments in 50 ml Falcon centrifuge tubes (females; Thermo Fisher Scientific, Waltham, MA, U.S.A.) or their usual petri dishes (males; see Study Area and Animals), and then returned them to the laboratory colony.

Statistical Analyses

For each experiment we used a generalized linear model with a negative binomial distribution (suitable for overdispersed count data) and log link to assess the effects of treatments and blocks on the number of male spiders captured over 24 h. For experiment 1, we ran post hoc tests to determine whether there were differences between treatments, and we adjusted P values for multiple comparisons using the Tukey method for four means (raw data provided in Supplementary Material file S1). For experiment 2, we excluded one block that did not capture any spiders from the analysis (raw data provided in Supplementary Material file S2). We used R 3.0.2 for all data analyses (R Core Development Team, 2013), the function glm.nb in the MASS package (Venables & Ripley, 2002) to run the models, and the lsmeans package (Lenth, 2014) for calculations of least squared means and 95% confidence intervals, and post hoc comparisons between means (see Supplementary Material file S3, for analyses).

RESULTS

Experiment 1: Effect of Natural and Experimental Web Reduction on Attractiveness of Females' Webs

We captured 230 *L. hesperus* males over 24 h across all traps. Most (155) of these males responded during the first 6 h of the experiment, between 2100 and 0300 hours (Table 1, Fig. 3). The GLM indicated that there was a significant effect of treatment ($F_{3,57} = 10.71$, $P < 0.001$), but not block ($F_{19,57} = 0.72$, $P = 0.79$), on the number of male spiders captured. Male-reduced webs attracted fewer males than did either intact or mechanically reduced webs. Intact webs attracted 3.07 ± 0.84 (least-squared mean ± SE) times as many males as did male-reduced webs ($t_{57} = 4.09$, $P < 0.001$), and mechanically reduced webs attracted 2.31 ± 0.65 times as many males as did male-reduced webs ($t_{57} = 2.99$, $P = 0.02$). In contrast, the attractiveness of intact and mechanically reduced webs did not differ significantly ($t_{57} = 1.173$, $P = 0.65$). All three web-containing treatments captured more males than did the no-web controls ($P < 0.01$ for all three comparisons).

Experiment 2: Effect of Male Silk on Attractiveness of Females' Webs

In experiment 2, we captured 461 *L. hesperus* males over 24 h across all traps. Most (268) of these males responded during the first 6 h of the experiment, between 2030 and 0230 hours (Table 2).

Table 1
Summary statistics describing the number of *Latrodectus hesperus* males captured on adhesive strips around wire-mesh cages during experiment 1

Treatment	N	First 6 h (2100–0300 hours)				First 12 h	24 h
		Mean	Median	Range	Total	Total	Total
Intact web	20	3.80	3.5	0–13	76	103	105
Male-reduced web	20	1.05	0	0–5	21	34	36
Mechanically reduced web	20	2.65	2	0–8	53	82	83
No web	20	0.25	0	0–2	5	5	6
Total	80	1.94	1	0–13	155	224	230

The 24 h field trapping experiment commenced at 2100 hours on 21 August 2013. The four cages within each of the 20 experimental replicates (*N*) were randomly assigned one of the four treatments.
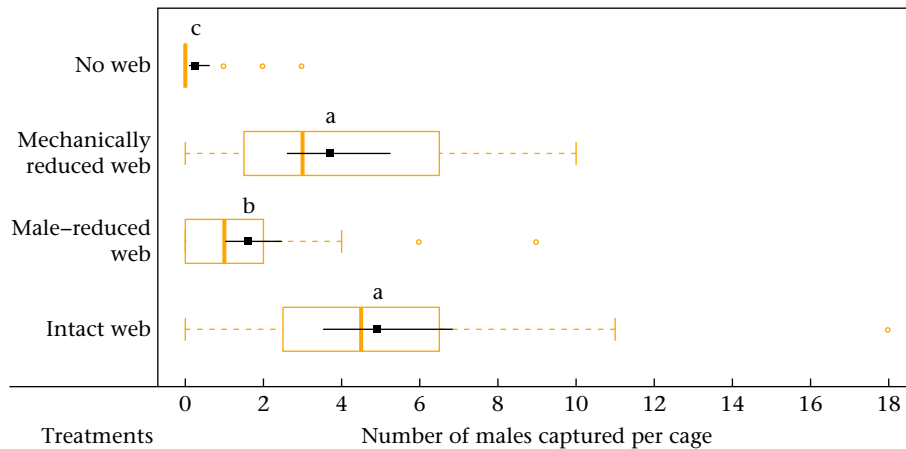

Figure 3. Number of *Latrodectus hesperus* males attracted over 24 h to cages containing (1) intact webs of virgin females, (2) experimentally reduced webs (with 50% of the silk removed by us), (3) male-reduced webs (with, on average, 50% of the silk bundled up and wrapped with silk by a courting male) and (4) no-web controls in a randomized complete block field-trapping experiment with 20 replicate blocks. Box plots display the median (vertical line), interquartile range (box), the most extreme data points within 1.5 times the interquartile range from the box (whiskers) and outliers (open circles). Backtransformed marginal means and 95% confidence intervals from a negative binomial GLM including the effects of treatment and block are also shown (solid squares with solid lines). Different letters indicate means that were significantly different from one another (*P* < 0.05 after adjusting for multiple comparisons using the Tukey method).

Intact webs and webs with male silk added captured 242 and 219 male spiders, respectively (Fig. 4). There was no effect of treatment ($F_{1,18} = 0.11$, $P = 0.74$) or block ($F_{18,18} = 0.49$, $P = 0.93$) on the number of male spiders captured.

DISCUSSION

Our field experiments demonstrate that web reduction by male *L. hesperus* results in a three-fold decrease in web visits by rival males, thereby reducing male–male competition. Below, we discuss the fitness consequences of web reduction for both sexes, arguing that the benefits of web reduction for females may outweigh the costs, even though conflict may arise over web damage. Web reduction with male silk deposition decreased web visits by males whereas experimental removal of half of the web did not. However, the addition of male silk to intact webs had no

effect on their attractiveness. Based on these results, we conclude that neither a simple reduction in silk surface area, nor the addition of male silk alone, is sufficient to decrease web attractiveness. We speculate that targeted excision of pheromone-laden web sections, coupled with male silk wrapped around these excised sections becoming a barrier to pheromone emission, is the most likely mechanism by which web reduction curtails web attractiveness.

Consequences of Web Reduction

Our results provide evidence of strong male–male competition in a field population of western black widow spiders. The median number of males arriving at a single female's intact web within the first 6 h was 3.8 (range 0–13) and 7.5 (range 0–21) in the first and second experiment, respectively. Because courtship and copulation in *L. hesperus* typically last several hours (MacLeod, 2013; Scott

Table 2
Summary statistics describing the number of *Latrodectus hesperus* males captured on adhesive strips around cages during experiment 2

Treatments	N	First 6 h (2030–0230 hours)				First 12 h	24 h
		Mean	Median	Range	Total	Total	Total
Intact web	20	7.05	7.5	0–21	141	192	242
Intact web plus male silk[a]	20	6.35	5.5	0–21	127	189	219
Total	40	6.70	6.5	0–21	268	381	461

The 24 h field trapping experiment commenced at 2030 hours on 9 September 2013. The two cages within each of the 20 experimental replicates (*N*) were randomly assigned one of the two treatments.
[a] Entire female's web plus an unknown quantity of a second female's web bundled and wrapped with male silk.
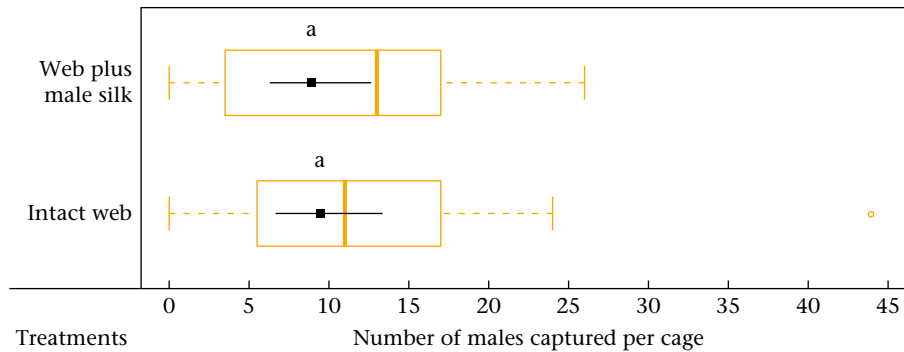
Figure 4. Number of *Latrodectus hesperus* males attracted over 24 h to cages containing (1) intact webs of virgin females and (2) intact webs of virgin females with a male silk-wrapped bundle or ball from a separate reduced web in a randomized complete block field-trapping experiment with 20 replicate blocks. Box plots display the median (vertical line), interquartile range (box), the most extreme data points within 1.5 times the interquartile range from the box (whiskers) and outliers (open circles). Backtransformed marginal means and 95% confidence intervals from a negative binomial GLM including the effects of treatment and block are also shown (solid squares with solid lines). The same letters indicate that the means were not significantly different from one another ($P=0.74$).

et al., 2012), the arrival of multiple males at a female's web within hours of one another poses a substantial risk that the courtship or copulation of an early-arriving male will be interrupted by rivals. In contrast, the median number of males arriving within the first 6 h at male-reduced webs was 0 (range 0−5), showing that males engaging in web reduction immediately upon entering a virgin female's web could limit the number of rival males arriving at the web, and thus greatly restrict direct competition over access to the female.

Latrodectus males can limit sperm competition by depositing mating plugs in the female's reproductive tract (Berendonck & Greven, 2000; MacLeod, 2013; Snow, Abdel-Mesih, & Andrade, 2006). Although this tactic contributes to a general pattern of first-male sperm precedence in *L. hesperus*, it often fails to prevent subsequent males from siring offspring (MacLeod, 2013). Consequently, a male that prevents rivals from arriving on the web of a female both during courtship and after mating will significantly reduce his probability of facing sperm competition. Female *Latrodectus hasselti* cease production of contact sex pheromone immediately after mating (Jerhot, Stoltz, Andrade, & Schulz, 2010; Stoltz, McNeil, & Andrade, 2007), and *L. hesperus* males discriminate against mated females in favour of virgin females based on volatile pheromone cues alone (MacLeod & Andrade, 2014). Thus, the effect of web reduction is likely to be long lasting because mated females will rebuild their webs without attractive pheromones

Web reduction may be a particularly efficient means of guarding against female remating because it does not require the physical presence of a male on a female's web. Mate searching is generally risky for *Latrodectus* males (Andrade, 2003; Segoli, Harari, & Lubin, 2006), severely limiting opportunities for polygyny, and favouring investment in a single mating. However, *L. hesperus* males can mate multiple times, even after genital damage (MacLeod, 2013). Moreover, the dense population of female *L. hesperus* at our field site (Salomon et al., 2010) makes it likely that a mated male can readily locate a second mate with minimal energetic cost and predation risk. In this system, males may have the potential to increase their fitness by both mating with more than one female and remotely guarding those females via web reduction.

Not all *L. hesperus* males engage in web reduction during courtship (Scott et al., 2012), and there is considerable variation in the extent to which webs are reduced (this study). Silk is costly to produce (Craig, 2003) and is likely a limiting resource for *Latrodectus* males who do not forage as adults (Foelix, 2011). Web reduction may have two distinct functions: (1) limiting the arrival of competitors (Watson, 1986) and (2) signalling to the female

(Anava & Lubin, 1993; Ross & Smith, 1979). We speculate that the former may depend on the amount of web reduced, whereas the latter may depend on the amount of male silk added. Males might then adjust their investment into reducing a web and depositing their own silk according to the competitive environment, their own condition and the female's receptivity. If *L. hesperus* males assess their competitive environment using chemical cues as *L. hasselti* males do (Kasumovic & Andrade, 2006), this could explain why males did not invariably avoid reduced webs in the field. A male that locates and enters a web that is still in the process of being reduced may be able to sneak copulations with the female while the other male invests in lengthy courtship behaviour (Stoltz & Andrade, 2010; Stoltz, Elias, & Andrade, 2008).

That *Latrodectus* females tolerate web reduction by males is somewhat puzzling because web damage appears to conflict with female interests through lost opportunities for prey capture, energy and silk required for web repair, and increased predation risk (Schneider & Lubin, 1998). Fewer web visits by male suitors as a result of web reduction may also be costly if females benefit from mating with, or choosing among, multiple males. Conversely, if repeated courtship and remating are costly for females (Arnqvist & Rowe, 2005), mated females should benefit from changes that limit the arrival of additional males. Silk of virgin females remains attractive and continues to elicit courtship behaviour in males for several days, even in the female's absence (Andrade & Kasumovic, 2005; Ross & Smith, 1979). Web reduction may benefit females by allowing them to cease attracting males immediately upon mating, and then to remain unattractive by rebuilding their webs without pheromones. Web reduction may not necessarily limit opportunities for mate choice or polyandry, however, if several males arrive at a web within hours of one another, as found at our study site. Furthermore, the effect of web reduction on a female's attractiveness need not be permanent if females can readvertise their receptivity several months after mating, as do *L. hasselti* females (Perampaladas, Stoltz, & Andrade, 2008).

Proximate Mechanisms: How Does Web Reduction Alter Web Attractiveness?

Our results do not support the hypothesis that males avoid silk deposited by another male during web reduction, but they do not rule out the possibility that male silk plays a role in the decreased attractiveness of reduced webs. We may not have seen an effect in this study because we tested male silk in the presence of female sex pheromone emanating not only from an entire intact web but

possibly also from the added male silk-wrapped bundle of female silk. The dragline silk that males deposit all over the web during courtship may also function in decreasing web attractiveness but this silk was absent in our experiment. Conceivably, males detect and avoid male silk on a female's web only when a portion of her web has been destroyed and wrapped with male silk, perhaps limiting pheromone emission and resulting in a distinct ratio of male to female silk cues. With strong competition for access to females at our field site, and first-male sperm precedence in *L. hesperus* (MacLeod, 2013), the best strategy for males might be to avoid webs that have been largely reduced (high ratio of male to female silk cues) and therefore present a risk that mating is in progress or already complete, but enter webs that are still being reduced by a recently arrived male (low ratio of male to female silk). The possibility that mate-seeking male spiders respond to chemical cues from male silk deposited during web reduction warrants further study.

Our results in experiment 1 suggest that web reduction may not decrease a female's attractiveness simply by decreasing the silk surface area available for pheromone release. Webs with half of the silk experimentally excised were visited by as many males as were intact webs, indicating that lowering pheromone emission by 50% has no effect. This is expected, given that in the spider *Agelenopsis aperta* the amount of female sex pheromone must be lowered more than 100-fold to reduce male responses by half (Papke, Riechert, & Schulz, 2001). In our study, the webs from which we excised 50% of the silk attracted more than twice as many males as did webs from which males had cut, bundled and wrapped 50% of the silk (on average). Even if pheromone is not evenly distributed throughout the web, mechanically reduced webs had (on average) 50% of the female's sex pheromone removed entirely, whereas male-reduced webs retained the full complement of female pheromones. In his study of web reduction by *Neriene litigiosa*, Watson (1986) did not notice male silk addition to the female's web and concluded that compaction of a female's silk into a tight mass limits pheromone release by reducing the exposed silk surface area. The web bundles formed by *L. hesperus* males during web reduction are often rather loose (see Fig. 1), and thus a decrease in silk surface area exposed to air alone does not seem likely to explain the large decrease in attractiveness of male-reduced webs. We speculate that targeted excision of pheromone-rich sections of the web, coupled with a barrier to pheromone release formed by the male's added silk, is the most likely mechanism by which web reduction curtails web attractiveness in widow spiders. It is also plausible that chemicals associated with male silk alter or neutralize pheromone on the female's silk. The likelihood of these potential mechanisms is unclear because we know little about how spider pheromones are incorporated into the silk or how they are sensed (Uhl, 2013).

Conclusions

Through web reduction, black widow males appear to 'mute' the attractive signals produced by females, perhaps also altering them through addition of silk-borne chemicals. Male manipulation of female signals by chemical deposition is common in insects, and may occur by direct contact with the female, or by transfer with the ejaculate or a mating plug (Thomas, 2011). Web reduction is unusual in that it takes place before the male makes contact with the female; male alteration of female attractiveness or receptivity prior to copulation is rarely reported for insects (but see Ablard, Schaefer, & Gries, 2013). Web reduction in black widows appears to be a means by which a male can monopolize a female during a long courtship display and continue to remotely guard her after copulation, complementing other defences against sperm competition including mating plugs. Our results provide the basis for comparative studies of the function of web reduction in other spider species. Among the widow spiders alone, there is extensive variation among mating systems and population densities. Studying this behaviour and the mechanisms by which it functions in mate monopolization and intra- or intersexual communication will provide new insights into how mating tactics, sexual selection and conflict shape mating systems.

Supplementary Material

Supplementary material associated with this article is available, in the online version, at http://dx.doi.org/10.1016/j.anbehav.2015.06.009.

References

Ablard, K. M., Schaefer, P. W., & Gries, G. (2013). An alternative reproductive tactic: a parasitoid wasp gathers and guards a harem by pheromone-tagging virgins. *Behavioural Processes, 94*, 32–40. http://dx.doi.org/10.1016/j.beproc.2012.11.015.

Anava, A., & Lubin, Y. (1993). Presence of gender cues in the web of a widow spider *Latrodectus revivensis*, and a description of courtship behaviour. *Bulletin of the British Arachnological Society, 9*, 119–122.

Andersson, J., Borg-Karlson, A., & Wiklund, C. (2000). Sexual cooperation and conflict in butterflies: a male-transferred anti-aphrodisiac reduces harassment of recently mated females. *Proceedings of the Royal Society B: Biological Sciences, 267*, 1271–1275. http://dx.doi.org/10.1098/rspb.2000.1138.

Andrade, M. C. B. (2003). Risky mate search and male self-sacrifice in redback spiders. *Behavioral Ecology, 14*, 531–538. http://dx.doi.org/10.1093/beheco/arg015.

Andrade, M., & Kasumovic, M. (2005). Terminal investment strategies and male mate choice: extreme tests of Bateman. *Integrative and Comparative Biology, 45*, 838–847. http://dx.doi.org/10.1093/icb/45.5.838.

Arnqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton, NJ: Princeton University Press.

Becker, E., Riechert, S., & Singer, F. (2005). Male induction of female quiescence/catalepsis during courtship in the spider, *Agelenopsis aperta*. *Behaviour, 142*, 57–70. http://dx.doi.org/10.1163/1568539053627767.

Berendonck, B., & Greven, H. (2000). Morphology of female and male genitalia of *Latrodectus revivensis* Shulov, 1948 (Araneae, Theridiidae) with regard to sperm priority patterns. In S. Toft, & N. Scharff (Eds.), *European arachnology 2000* (pp. 157–167). Aarhus, Denmark: Aarhus University Press.

Birkhead, T. R., & Møller, A. P. (1998). *Sperm competition and sexual selection*. London, U.K.: Academic Press.

Breene, R., & Sweet, M. (1985). Evidence of insemination of multiple females by the male black widow spider, *Latrodectus mactans* (Araneae, Theridiidae). *Journal of Arachnology, 13*, 331–335.

Craig, C. L. (2003). *Spiderwebs and silk*. Oxford, U.K.: Oxford University Press.

Cross, F. R., & Jackson, R. R. (2013). The functioning of species-specific olfactory pheromones in the biology of a mosquito-eating jumping spider from East Africa. *Journal of Insect Behavior, 26*, 131–148. http://dx.doi.org/10.1007/s10905-012-9338-4.

Foelix, R. F. (2011). *Biology of spiders* (3rd ed.). Oxford, U.K.: Oxford University Press.

Forster, L. (1995). The behavioral ecology of *Latrodectus hasselti* (Thorell), the Australian redback spider (Araneae: Theridiidae): a review. *Records of the Western Australia Museum, Supplement, 52*, 13–24.

Gaskett, A. C. (2007). Spider sex pheromones: emission, reception, structures, and functions. *Biological Reviews of the Cambridge Philosophical Society, 82*, 27–48. http://dx.doi.org/10.1111/j.1469-185X.2006.00002.x.

Harari, A. R., Ziv, M., & Lubin, Y. (2009). Conflict or cooperation in the courtship display of the white widow spider, *Latrodectus pallidus*. *Journal of Arachnology, 37*, 254–260. http://dx.doi.org/10.1636/St08-97.1.

Jerhot, E., Stoltz, J. A., Andrade, M. C. B., & Schulz, S. (2010). Acylated serine derivatives: a unique class of arthropod pheromones of the Australian redback spider, *Latrodectus hasselti. Angewandte Chemie International Edition, 49,* 2037–2040. http://dx.doi.org/10.1002/anie.200906312.

Kasumovic, M. M., & Andrade, M. C. B. (2006). Male development tracks rapidly shifting sexual versus natural selection pressures. *Current Biology, 16,* R242–R243. http://dx.doi.org/10.1016/j.cub.2006.03.017.

Lenth, R. V. (2014). *lsmeans: Least-squares means (R package version 2.00-5).* http://www.cran.r-project.org/web/packages/lsmeans/index.html.

Locket, G. H. (1927). XII. On the mating habits of some spiders in the family Theridiidae. *Annals and Magazine of Natural History, 20,* 91–99.

MacLeod, E. C. (2013). *New insights in the evolutionary maintenance of male mate choice behaviour using the western black widow* Latrodectus hesperus (Doctoral Dissertation). Toronto, ON: University of Toronto.

MacLeod, E. C., & Andrade, M. C. B. (2014). Strong, convergent male mate choice along two preference axes in field populations of black widow spiders. *Animal Behaviour, 89,* 163–169. http://dx.doi.org/10.1016/j.anbehav.2013.12.023.

Papke, M. D., Riechert, S. E., & Schulz, S. (2001). An airborne female pheromone associated with male attraction and courtship in a desert spider. *Animal Behaviour, 61,* 877–886. http://dx.doi.org/10.1006/anbe.2000.1675.

Parker, G. (1970). Sperm competition and its evolutionary consequences in the insects. *Biological Reviews, 45,* 525–567. http://dx.doi.org/10.1111/j.1469-185X.1970.tb01176.x.

Perampaladas, K., Stoltz, J. A., & Andrade, M. C. B. (2008). Mated redback spider females re-advertise receptivity months after mating. *Ethology, 114,* 589–598. http://dx.doi.org/10.1111/j.1439-0310.2008.01513.x.

R Core Development Team. (2013). *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/.

Ross, K., & Smith, R. L. (1979). Aspects of the courtship behavior of the black widow spider, *Latrodectus hesperus* (Araneae: Theridiidae), with evidence for the existence of a contact sex pheromone. *Journal of Arachnology, 7,* 69–77.

Rovner, J. (1968). Territoriality in the sheet-web spider *Linyphia triangularis* (Clerck) (Araneae, Linyphiidae). *Zeitschrift für Tierpsychologie, 25,* 232–242.

Salomon, M., Vibert, S., & Bennett, R. G. (2010). Habitat use by western black widow spiders (*Latrodectus hesperus*) in coastal British Columbia: evidence of facultative group living. *Canadian Journal of Zoology, 88,* 334–346. http://dx.doi.org/10.1139/Z10-004.

Schneider, J., & Lubin, Y. (1998). Intersexual conflict in spiders. *Oikos, 83,* 496–506.

Schneider, J. M., Lucass, C., Brandler, W., & Fromhage, L. (2011). Spider males adjust mate choice but not sperm allocation to cues of a rival. *Ethology, 117,* 970–978. http://dx.doi.org/10.1111/j.1439-0310.2011.01960.x.

Schulz, S. (2013). Spider pheromones: a structural perspective. *Journal of Chemical Ecology, 39,* 1–14. http://dx.doi.org/10.1007/s10886-012-0231-6.

Scott, C., & Gries G. (n.d.). *Factors affecting web reduction behaviour in the western black widow.* Manuscript in preparation.

Scott, C., Vibert, S., & Gries, G. (2012). Evidence that web reduction by western black widow males functions in sexual communication. *Canadian Entomologist, 144,* 672–678. http://dx.doi.org/10.4039/tce.2012.56.

Segoli, M., Arieli, R., Sierwald, P., Harari, A. R., & Lubin, Y. (2008). Sexual cannibalism in the brown widow spider (*Latrodectus geometricus*). *Ethology, 114,* 279–286. http://dx.doi.org/10.1111/j.1439-0310.2007.01462.x.

Segoli, M., Harari, A. R., & Lubin, Y. (2006). Limited mating opportunities and male monogamy: a field study of white widow spiders, *Latrodectus pallidus* (Theridiidae). *Animal Behaviour, 72,* 635–642. http://dx.doi.org/10.1016/j.anbehav.2005.11.021.

Sneddon, L. U., Elwood, R. W., Adamo, S. A., & Leach, M. C. (2014). Defining and assessing animal pain. *Animal Behaviour, 97,* 201–212. http://dx.doi.org/10.1016/j.anbehav.2014.09.007.

Snow, L. S. E., Abdel-Mesih, A., & Andrade, M. C. B. (2006). Broken copulatory organs are low-cost adaptations to sperm competition in redback spiders. *Ethology, 112,* 379–389. http://dx.doi.org/10.1111/j.1439-0310.2006.01163.x.

Stålhandske, P., & Gunnarsson, B. (1996). Courtship behaviour in the spider *Pityohyphantes phrygianus* (Linyphiidae Araneae): do females discriminate injured males? *Revue Suisse de Zoologie, 2*(hors série), 617–625.

Stockley, P. (1997). Sexual conflict resulting from adaptations to sperm competition. *Trends in Ecology & Evolution, 12,* 154–159. http://dx.doi.org/10.1016/S0169-5347(97)01000-8.

Stoltz, J. A., & Andrade, M. C. B. (2010). Female's courtship threshold allows intruding males to mate with reduced effort. *Proceedings of the Royal Society B: Biological Sciences, 277,* 585–592. http://dx.doi.org/10.1098/rspb.2009.1554.

Stoltz, J. A., Elias, D. O., & Andrade, M. C. B. (2008). Females reward courtship by competing males in a cannibalistic spider. *Behavioral Ecology and Sociobiology, 62,* 689–697. http://dx.doi.org/10.1007/s00265-007-0493-0.

Stoltz, J. A., McNeil, J. N., & Andrade, M. C. B. (2007). Males assess chemical signals to discriminate just-mated females from virgins in redback spiders. *Animal Behaviour, 74,* 1669–1674. http://dx.doi.org/10.1016/j.anbehav.2007.03.011.

Thomas, M. L. (2011). Detection of female mating status using chemical signals and cues. *Biological Reviews of the Cambridge Philosophical Society, 86,* 1–13. http://dx.doi.org/10.1111/j.1469-185X.2010.00130.x.

Trabalon, M. (2013). Chemical communication and contact cuticular compounds in spiders. In W. Nentwig (Ed.), *Spider ecophysiology* (pp. 125–140). Berlin, Germany: Springer-Verlag. http://dx.doi.org/10.1007/978-3-642-33989-9.

Uhl, G. (2013). Spider olfaction: attracting, detecting, luring and avoiding. In W. Nentwig (Ed.), *Spider ecophysiology* (pp. 141–157). Berlin, Germany: Springer-Verlag. http://dx.doi.org/10.1007/978-3-642-33989-9.

Van Helsdingen, P. J. (1965). Sexual behaviour of *Lepthyphantes leprosus* (Ohlert) (Araneida, Linyphiidae) with notes on the function of the genital organs. *Zoologische Mededelingen, 41,* 15–44.

Venables, W. N., & Ripley, B. D. (2002). *Modern applied statistics with S.* New York, NY: Springer.

Vibert, S., Scott, C., & Gries, G. (2014). A meal or a male: the 'whispers' of black widow males do not trigger a predatory response in females. *Frontiers in Zoology, 11*(4). http://dx.doi.org/10.1186/1742-9994-11-4.

Watson, P. (1986). Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa* (Linyphiidae). *Science, 233,* 219–221. http://dx.doi.org/10.1126/science.3726530.

Whitehouse, M., & Jackson, R. R. (1994). Intraspecific interactions of *Argyrodes antipodiana*, a kleptoparasitic spider from New Zealand. *New Zealand Journal of Zoology, 21,* 243–268. http://dx.doi.org/10.1080/03014223.1994.9517993.

Willey Robertson, M., & Adler, P. H. (1994). Mating behavior of *Florinda coccinea* (Hentz) (Araneae: Linyphiidae). *Journal of Insect Behavior, 7,* 313–326. http://dx.doi.org/10.1007/BF01989738.