Presence of gender cues in the web of a widow spider, Latrodectus revivensis, and a description of courtship behaviour

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Summary

We describe the seasonal behaviour of males in webs of females and the courtship behaviour of a Negev desert widow spider, *Latrodectus revivensis* Shulov (Theridiidae). Adult males remained in webs of juvenile and adult females for up to 28 days. Courtship behaviour was similar to that described for other species of *Latrodectus*. Males responded with courtship behaviour to silk from webs of adult females, but not from webs of subadult females. Females responded aggressively to silk from webs of other females, but not to silk produced by males. We postulate that information about gender and male quality may be obtained by females from the courtship behaviour of males, particularly during stages of web-reduction.

Introduction

Silk plays an important chemical role in courtship and mating in many species of spiders (Foelix, 1982). The webs or nests of spiders may contain information about the reproductive status of the occupant in the form of pheromones carried on the silk (Pollard *et al.*, 1987). Silk-borne chemicals that attract males have been demonstrated in females' webs (Linyphiidae: Watson, 1986; Araneidae: Blanke, 1975; Theridiidae: Ross & Smith, 1979; Dictynidae: Jackson, 1978); dragline threads (Lycosidae: Hegdekar & Dondale, 1969); and nests (Salticidae: Jackson, 1986a, 1987). Aggregations of males on webs of recently-matured females (Robinson, 1982) and "guarding" of subadult females by adult males (Austad, 1984; Jackson, 1986b; Toft, 1989) have been observed in different families, suggesting that silk-borne pheromones are widespread in spiders. By attaching a pheromone to web silk, females may attract many males over a long period, possibly enhancing inter-male competition and improving the chances of mating with better males (Watson, 1990).

The female's web or nest alone may be sufficient to elicit courtship, even without the female's presence. Ross & Smith (1979) showed that males of a widow spider, *Latrodectus hesperus* Chamberlin & Ivie, distinguish between silk derived from webs of males and females. Males respond with courtship behaviours to silk of sexually mature females (virgin or mated) and even to silk of juvenile females. Females also responded with courtship behaviour to silk of males, leading the authors to suggest

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the presence of a "complementary male pheromone" in this species.

We observed adult males of a desert widow spider, *Latrodectus revivensis* Shulov, in or near the nests of juvenile and adult females. Males are considerably smaller than females, and the females' webs are widely dispersed in the habitat. Because of this dispersion pattern and the small size of males, we regard it as unlikely that males would find females' webs by random search of the habitat. Instead, pheromones present in web silk may facilitate the location of females' nests. In the present study, we test the hypothesis that the web silk provides cues about gender and reproductive status in this species, and discuss possible functions of these pheromones. We describe the courtship behaviour of *L. revivensis* and examine the responses of adult males and females of *L. revivensis* to web silk of both sexes as well as to silk from sexually-immature females.

Methods

Natural history

Latrodectus revivensis occurs in the central Negev Desert of Israel (Levy & Amitai, 1983), where an average annual rainfall of < 100 mm occurs in the winter months (November–March). The spiders have an annual or subannual life cycle. Adults are present throughout the summer and reproduction occurs from April to September. The young overwinter and may remain active through part of the winter. Larger juveniles reach maturity and reproduce early in the summer, and their offspring may mature and produce a second generation in the same summer. Males are considerably smaller than females and mature at an earlier instar (male body length: 3–6 mm, female body length: 9–17 mm; Lubin *et al.*, 1991).

Unlike many species of *Latrodectus* which construct concealed nests, the conical nests of *L. revivensis* are built near the tops of shrubs (Shulov, 1948; Szlep, 1965). The capture web is at the edge of the shrub and is connected by long bridge threads to the nest on one side and to the ground or to another shrub on the other side. Non-sticky threads surround the nest and are attached to the bridge threads and capture platform. The spiders engage in web-maintenance and prey capture at night; during the day they generally remain hidden in their nests. Juvenile males build their own webs which are similar in structure to those of females. Adult males leave their juvenile webs and either build small retreats attached to the nests of juvenile or adult females, or move into the nests of juvenile or adult females (Lubin *et al.*, 1991).

Field and laboratory observations

Webs of marked and unmarked *L. revivensis* were observed during March–September of 1988–1990 as part of a study of web-site selection (Lubin *et al.*, 1991). The study site was a sparsely-vegetated rocky slope of a small wadi (dry watercourse) near Sede Boqer in the central Negev highlands. Webs occurred mainly in shrubs of *Zygophyllum dumosum, Hammada scoparia, Artemisia herba-alba* and *Noaea mucronata*. Males and females were marked with

dots of enamel paint on the dorsal side of one or two legs. In 1988, males occupying webs of females were marked and the duration of stay in the females' webs was noted.

In April 1991, 19 adult and juvenile females and 11 males were collected from the vicinity of Sede Boqer. Juvenile females were all one moult from adult, and henceforth are referred to as subadult females. Females were kept in screened cages and terraria large enough for them to build a complete web and nest in the *Hammada* branches provided. Males were kept in plastic cups where they produced a tangle of silk threads attached to branches. Partial or complete courtship sequences were observed in the laboratory ($n = 31$). In each case, a male was introduced during the day into the cage containing a mature, unmated female and placed at the edge of her web furthest from the nest. In most cases, we removed the male before copulation occurred. The same female was used in observations with other males.

Web-discrimination experiments

To determine whether males and females distinguished silk derived from different sources, we adopted an experimental technique used by Ross & Smith (1979). We eliminated the possibility of a response to web structure by rolling silk on to clean, thin wooden dowels. The dowels were then presented to males and females, and their responses observed over a period of 10–20 min. The procedure for males and females differed slightly. The male was placed in a plastic cup, on a small branch supporting the experimental dowel, which was fixed into a clay base with the silk side up. Females were tested *in situ* by presenting the silk end of the dowel at the entrance to the female's nest. Dowels with clean cotton-wool were used as controls. In all cases, the order of presentation was randomised, and at least one hour was allowed to elapse between each series.

Results

Field observations

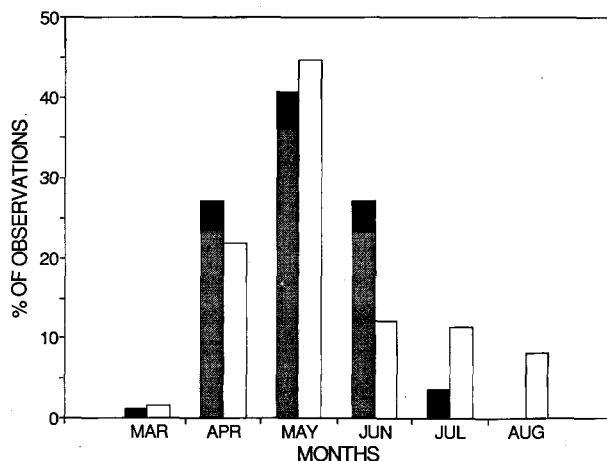Males were observed in webs or nests of females in the Sede Boqer area from March to August (1988: 28 March–

Fig. 1: Percent occurrence per month of males in webs of females. Dark bars: 1988, $n = 81$ observations; clear bars: 1989, $n = 123$ observations.
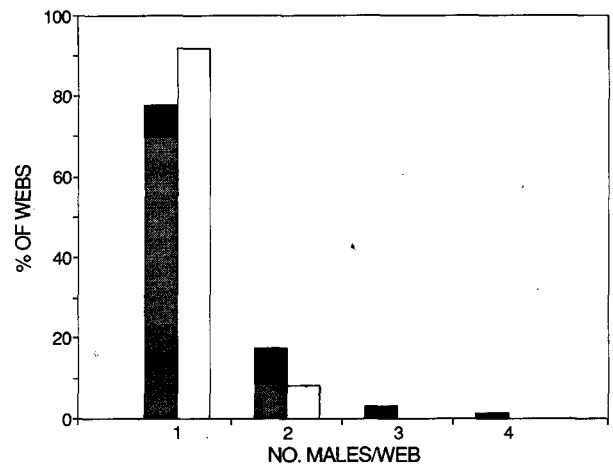
Fig. 2: Percent occurrence of one to four males in webs of females. Dark bars: 1988, $n = 31$ female webs; clear bars: 1989, $n = 36$ females.

4 August; 1989: 21 March–6 July). However, more than 80% of the observations of males were during April–June (Fig. 1). In most instances (87%) there was one male per web, but as many as four males were observed together in a single female's web (Fig. 2). When two or more males were present, only one was found inside the nest with the female while the others sat among the frame threads or at the edge of the nest.

Marked males were difficult to track because of their small size. Three out of 81 marked males moved at least once and one of these was observed in 4 different webs. Males sometimes remained in females' webs for considerable periods of time. Individually-marked males were observed in webs of females for up to 28 days, although 79% remained only 1–5 days.

Courtship and mating

Males that were introduced into cages with webs of female spiders became active within 1–10 s of encountering silk of the female's web. The duration of courtship was highly variable, lasting 2–80 min from first activity to the start of copulation.

The male first approached the female's nest with a jerking and bouncing movement, caused by alternately flexing and relaxing the legs, and with rapid, up-and-down vibrations of the abdomen. Often the male did not reach the edge of the nest, but followed his approach immediately with "web reduction" behaviour. The male walked beneath the platform and among the barrier threads above the platform, cutting threads and wrapping them into compact bundles, and replacing the threads of the female with a matrix of thin threads of his own. The thread bundles formed from the cut lines were left hanging in various locations in the web, but generally near the nest. As much as 50% of the barrier threads were removed by a single male during the web reduction phase. At intervals during this phase, the male approached the nest in the jerky-bouncing walk described above. Often the female responded by coming out of the nest, pulling on barrier-web threads with her first pair of legs, and sometimes chasing the male away from the nest. Chases became less frequent as courtship progressed. The web-reduction phase lasted up to 30 min, during which time the male gradually moved closer to the nest entrance.

Web reduction was performed by most males (69%, $n = 16$ total observations), regardless of whether they were first or second males on the female's web. We found that five of seven males that were presented to females as first males, and six of nine males presented as second males performed web reduction. Second males performed web reduction even when, in some cases, there was very little of the female's web left to remove.

Web-reduction ceased abruptly when the male began short-range courtship. The male entered the nest, usually through the normal entrance (though in 3 instances males cut holes in the nest and entered through these holes), and approached the female while alternately jerking and vibrating his abdomen. The male tapped the female with his first pair of legs, and usually the female responded by trying to push him away with her first two pairs of legs, or even chasing him out of the nest. In about a third of the observations, the male walked around the female and wrapped her with loose threads, applying the silk primarily to the female's hind legs and abdomen. The female did not seem to adopt a particular mating posture; instead a successful male eventually appeared to just climb on to the female's ventral side and insert a palp. The male copulated several times in succession, each palpal insertion lasting usually < 1 min.

Web discrimination experiments

Web discrimination by males: Silk from fresh webs of adult and subadult females, and of adult males, was presented to adult males. Males that jerked the web, with or without abdomen vibrations, or approached the silk were considered to have shown a positive response; the alternative, no reaction, describes no visible response to the stimulus (Table 1).

Males responded positively to web silk of adult females (78%, $n = 18$ trials) but did not respond to silk from subadult females (all nine trials), other males (eight of nine trials) or to the controls (all nine trials). The response of males to silk of adult females was significantly different from the responses to subadult female or male silk or to

cotton wool (pairwise χ^2 tests, $p < 0.001$). There was no significant difference between the responses to silk produced by males or subadult females or to the controls ($p > 0.1$).

Web discrimination by females: Females were presented with silk of other adult females, subadult females and adult males, and with cotton-wool controls.

Females often responded to disturbance with "pull ups" or bouncing in slow motion, in which the body was slowly raised and then lowered by flexing and relaxing all legs simultaneously. This behaviour was observed in nature when a female invaded the web of another, when a large wasp (*Vespa orientalis*) attempted to steal prey from a web, and in response to repeated poking of females by the investigator. Repeated disturbance caused the spider to bounce vigorously, attempt to bite or throw sticky silk on the intruder, or to run away. In these experiments, any of the above behaviours (pull-ups, bouncing, attack or escape) were considered negative responses. The alternative was no visible reaction to the stimulus (Table 1).

Most adult females gave no response to silk from webs of males (89%, $n = 27$) or to the controls (85%, $n = 26$). They responded negatively to silk from other females, both of adults (91%, $n = 32$) and subadults (79%, $n = 14$). There were significant differences between responses to males and adult females and between responses to adult females and controls ($p < 0.005$ in both cases). Pull-ups were observed in 55% of the negative responses, attacks combined with pull-ups in 30% and an escape response in 15% of the cases. When given silk from their own webs, four adult females showed no reaction.

Subadult females were tested with silk from webs of adult females, other subadult females and males. We observed no differences in the responses of subadult females to these three sources ($n = 6$ presentations each; Fisher's exact test, $p > 0.5$). Overall, 72% of the responses were negative, but this was not significantly different from random ($\chi^2 = 3.56$, $0.05 < p < 0.1$).

Discussion

The courtship and mating behaviours of *L. revivensis* are similar in most respects to those of other species of *Latrodectus* described by Kaston (1970) and Ross & Smith (1979). Unlike *L. hesperus* (Ross & Smith, 1979), however, female *L. revivensis* did not exhibit active courtship responses to male displays. Previous descriptions of the web-reduction phase of courtship in *Latrodectus* fail to mention that the male not only cuts out portions of the female's web, but also wraps them with his own silk, forming silk bundles which are usually left in proximity to the female's nest.

Males of *L. revivensis* responded with courtship behaviours to silk of adult females' webs, but in contrast to the results of Ross & Smith (1979), males did not respond positively to silk from juvenile (subadult) females' webs. Nonetheless, in nature, adult males of *L. revivensis* occurred frequently in webs of juvenile females. Are webs of juvenile females found by random search, or is a different web-borne pheromone responsible for long-distance attraction of males? The phenomenon bears further investigation.

	No. of Responses		
Males	+	**N.R.**	**N**
Adult female silk	14	4	18
Subadult female silk	0	9	9
Male silk	1	8	9
Control	0	9	9
Females	**N.R.**	−	**N**
Adults			
Adult female silk	3	29	32
Subadult female silk	3	11	14
Male silk	24	3	27
Control	22	4	26
Subadults			
Adult female silk	2	4	6
Subadult female silk	1	5	6
Male silk	2	4	6

Table 1: Web discrimination by males, adult females and subadult females. Responses are positive (+), negative (−) and no response (NR), as defined in the text. N = total number of trials.

Males may benefit from finding and remaining in webs of subadult females for at least two reasons:

1. Nutrition. — Males were observed feeding on prey caught in the female's web, either together with the female or after the remains were abandoned by her.

2. Mate guarding. — A male may guard a female approaching the moult to maturity, thus increasing his chances of being the first to mate with her (Austad, 1982). In the few cases investigated in spiders, sperm of the first male to mate fertilises most of the eggs (first-male sperm priority; Austad, 1984; Watson, 1991).

Virgin females did not respond with courtship behaviours either to male-produced silk or to males introduced on to their webs. Initial courtship attempts of males often elicited aggressive responses from females, but the presentation of male silk did not. Thus, male silk alone does not induce female aggression. However, females reacted aggressively to silk of other females, whether adult or subadult. Competition among females for web-sites may explain this behaviour. Indeed, juvenile females move to new web-sites after each moult, or at the most two moults (Lubin et al., submitted), and we have observed web usurpation on at least three occasions following such moves.

One of the most puzzling aspects of male courtship is "web reduction" behaviour. Watson (1990) showed that in *Linyphia litigiosa* Keyserling (Linyphiidae), webs that have been reduced in volume by a male are less attractive to successive males. He suggested that compaction of the silk reduces the surface area over which the pheromone is distributed, and therefore lowers its effectiveness as an attractant. Similar reasoning may apply to the behaviour of *L. revivensis*, in which a significant portion of the female's web is removed. However, some aspects remain unexplained:

1. Successive males placed on the web of a virgin female continue to perform web reduction, even when there is little or no web left to remove.

2. The compacted web bundles are left hanging in the vicinity of the female's nest, rather than discarded from the web (which would remove the pheromone source altogether).

Our observations of courtship in *L. revivensis* lead us to suggest an additional function of web reduction that is related to inter-sexual selection. During web-reduction, the male replaces the silk produced by the female with his own threads, and deposits his own silk on the bundles of silk compacted from the female's web. In the contact phase of courtship, the male also covers the female with threads of his own silk. Pheromones associated with the male's silk may act to stimulate the female to mate. In addition, these behaviours may provide the female with cues for assessing male quality. Potential cues are the time and energy invested by the male in web-reduction and in laying down silk, or the nature of silk-borne pheromones produced by the male himself (Ross & Smith's (1979) "complementary male pheromone"). A threshold-criterion decision tactic (choose the first male who meets a minimum criterion; Wittenberger, 1983) may enable the female to ensure fertilisation by an adequate male. Further work is required to test this hypothesis and to understand the role of sexual selection in shaping courtship behaviour in *Latrodectus*.

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