24. HTLV-III/LAV–infected and uninfected macrophages were detached from the surface of plastic flasks as follows. Macrophage layers were washed with PBS, Ca^{2+}-Mg^{2+}–free Hanks balanced salt solution was added, the flasks were incubated on ice for 15 to 30 minutes, and then the cells were gently scraped into the Hanks solution, washed with PBS, and processed for indirect immunofluorescence assay as described (*10*).
25. Virus production was detected in culture fluids by RT assay (*11*). RT activity was measured at 3- to 5-day intervals and expressed in counts per minute per milliliter of harvested culture fluid. Cell cultures were considered to be positive for virus infection only if an increase in RT activity was observed in culture fluids harvested from the same cultures during subsequent RT assays (see Fig. 4). In all cases analyzed, there were unambiguous correlations between positivity for RT activity in culture fluids and the percentage of cells positive for HTLV-III/LAV p17. Similarly, cultures exposed to the virus inoculum were considered negative for infection if no or only low and decreasing RT activity was observed during at least 4 weeks of cultivation in vitro.
26. Samples of high molecular weight DNA were prepared by standard methods (*27*). The samples were subjected to electrophoresis in 0.7% agarose gels, transferred to nitrocellulose (*28*), and hybridized as described (*14*). The probe used was either the Sst 1–Sst 1 region from the BH-10 clone (*29*) or a mixture of the 5′ and 3′ ends of the HXB-2 clone (*14*), each representing an 8.9-kb-long fragment of the HTLV-III_B genome.
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8 April 1986; accepted 6 June 1986

Transmission of a Female Sex Pheromone Thwarted by Males in the Spider *Linyphia litigiosa* (Linyphiidae)

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When a male Sierra dome spider (*Linyphia litigiosa*) encounters a virgin female that has been sexually mature for 7 to 10 days, he rapidly packs the silk of her web into a tight mass. This behavior hinders evaporation of a male-attractant chemical that such highly receptive females apply to their webs. The male thereby reduces the likelihood that his mating partner will attract rival males.

IN MANY ANIMAL SPECIES, MALES COMPETE intensely for sexually receptive females. Since aggressive interactions with rival males are likely to entail energetic expenditures and physical risks, males who are able to minimize such costs while still achieving matings will be at a selective advantage. One tactic males may use to reduce direct confrontations with rivals is to counteract sexually attractive signals being issued by females they locate.

Certain male beetles (*1*), moths (*2*), solitary bees (*3*), and garter snakes (*4*) produce an odor that renders females less attractive to other males. In these cases, male scent contributions probably signal rival males who detect the female that she has already mated or is about to copulate. Males would be deterred by such a signal only in species whose females become nonreceptive after copulation or are invulnerable to takeover attempts (*5*). In any other circumstance, the female's signal must be completely blocked. However, as far as I know, examples of animals truly muting communicative signals of conspecifics as a means of concealing sexual partners from potential usurpers, particularly cases involving clear costs to the blocked individual, are nonexistent. Here I report a novel behavioral mechanism used by male Sierra dome spiders (*Linyphia litigiosa*) to thwart transmission of a male-attractant pheromone facultatively produced by highly receptive virgin females.

The Sierra dome spider occurs throughout mountainous regions of western North America. I studied the reproductive behavior of this spider along Flathead Lake, Montana, from 1979 to 1985. Here the Sierra dome breeds from late June through early September. During this time, females are solitary and sedentary, constructing semipermanent dome-shaped webs used in prey capture and in which all sexual encounters occur.

Mature males are nomadic throughout the breeding season, wandering in search of webs of potential mates where they pause to guard and court the resident female. Intense combat for possession of females is common. In my dense study population (*6*), 96% ($n > 250$) of all females are guarded by a male at the time of their final molt, when they become sexually mature. These females inevitably copulate with the male currently guarding them. Although most females mate more than once, their sperm utilization pattern is one of strong first-male priority (*7*).

Table 1. Sections of the T-rod (Fig. 2A) from which males descended to the table during exposure to the three categories of females. Numbers in parentheses are expected frequencies.

Female in cage	Frequency of male descent: Near cage	Center	Far from cage
Virgin, intact web	22 (17.00)	7 (6.50)	4 (9.50)
Nonvirgin, intact web	2 (5.67)	3 (2.17)	6 (3.17)
Virgin, reduced web	10 (11.33)	3 (4.33)	9 (6.33)

Females were forced to remain unmated in the laboratory for 7 to 10 days after maturation; such an unmated condition might occur regularly in low-density populations. When these females were brought to the field and introduced onto webs from which mated females had been expelled (*8*), they elicited two intriguing male responses: (i) a greatly increased rate of male visitation compared to simultaneously monitored webs inhabited by mated females and (ii) a behavior by early-arriving males, termed web reduction, in which the male rapidly excised and gathered large portions of the dome of the female's web into a dense rope or ball (Fig. 1). Web reduction typically was completed within 15 to 30 minutes of the male's arrival upon the female's web and, without exception, was immediately followed by mating.

Introductions of mature virgin females onto individual webs in the field were performed 26 times. Mean male visitation rates were an order of magnitude greater for webs inhabited by these virgin females than for 184 simultaneously monitored webs occupied by mated females [0.29 ± 0.07 (SEM) and 0.03 ± 0.01 males per hour, respectively ($P < 0.01$)] (*9*). Sixty-nine percent of the 29 males arriving at the webs of mature virgin females performed web reduction, but none of the 63 males entering webs occupied by mated females displayed the behavior. Additionally, I monitored more than 625 natural intersexual pairings of Sierra dome spiders involving more than 200 previously mated females and never saw evidence of web reduction. Introductions of more than 175 mated females onto vacant webs never resulted in either an increased rate of male attraction or web-reduction behavior.

Researchers (*10, 11*) have described web reduction in natural populations of two other species of linyphiid spiders. They suggested that web reduction is triggered by a behavioral or pheromonal cue from highly

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receptive females. They also speculated that the adaptive significance of web reduction for the male might be: (i) to restrict the female's freedom of movement during courtship (*11*), (ii) to reduce the territorial area the resident male needed to defend from rivals (*10*), or (iii) to reduce the likelihood that copulation would be disrupted by prey becoming ensnared in the web (*10*). However, these explanations of web reduction cannot account for the behavior's rigid associations with (i) an increased rate of male visitation and (ii) the presence of a female that had been denied mating opportunities during early adult life.

My observations suggest a different explanation for web reduction. Females in a situation where mating opportunities do not occur for a number of days after sexual maturation begin incorporating a volatile male attractant pheromone onto the silk of their web. Males attracted to the web ball up the silk on which the pheromone is located to hinder its evaporation (*12*). This reduces the probability that rivals will detect the virgin female's location and challenge the earlier arriving male during the long process of copulation (*13*). Several lines of evidence support this hypothesis.

To test whether web reduction is elicited by a web-borne factor rather than by the female herself, I performed experiments with mature virgin females that had been released into a large room and allowed to construct webs. First, a male was released onto one of these webs with the female present; this was done to determine whether the female and her web could elicit web reduction. Twenty-eight percent of the trials (*n* = 50) resulted in web-reduction behavior by this first male within 15 minutes of his introduction. In these cases, as soon as the first male had created a 2-cm hole in the dome of the web, both he and the resident female were removed and a new male was immediately introduced. Web reduction by the second male, in the female's absence, occurred in 11 out of 14 (79%) trials. Web-reduction behavior by males in the absence of females was indistinguishable from that seen with females present.

I also elicited web-reduction behavior after applying an extract from the webs of 84 mature virgin females to the unoccupied web of a mated female (*n* = one male). The extracted webs had been assayed as able to elicit web reduction just before collection. Within a minute of his introduction onto the treated web, the male reduced precisely that portion of the dome that had been sprayed with the extract. Four control webs sprayed with the solvent only (normal hexane) did not elicit web reduction (*n* = eight males), nor did a web treated with an extract from webs of 56 mature virgin females that had failed to elicit web reduction before they were collected (*n* = two males).

The ability of the pheromone to evaporate from the web surface, thereby attracting

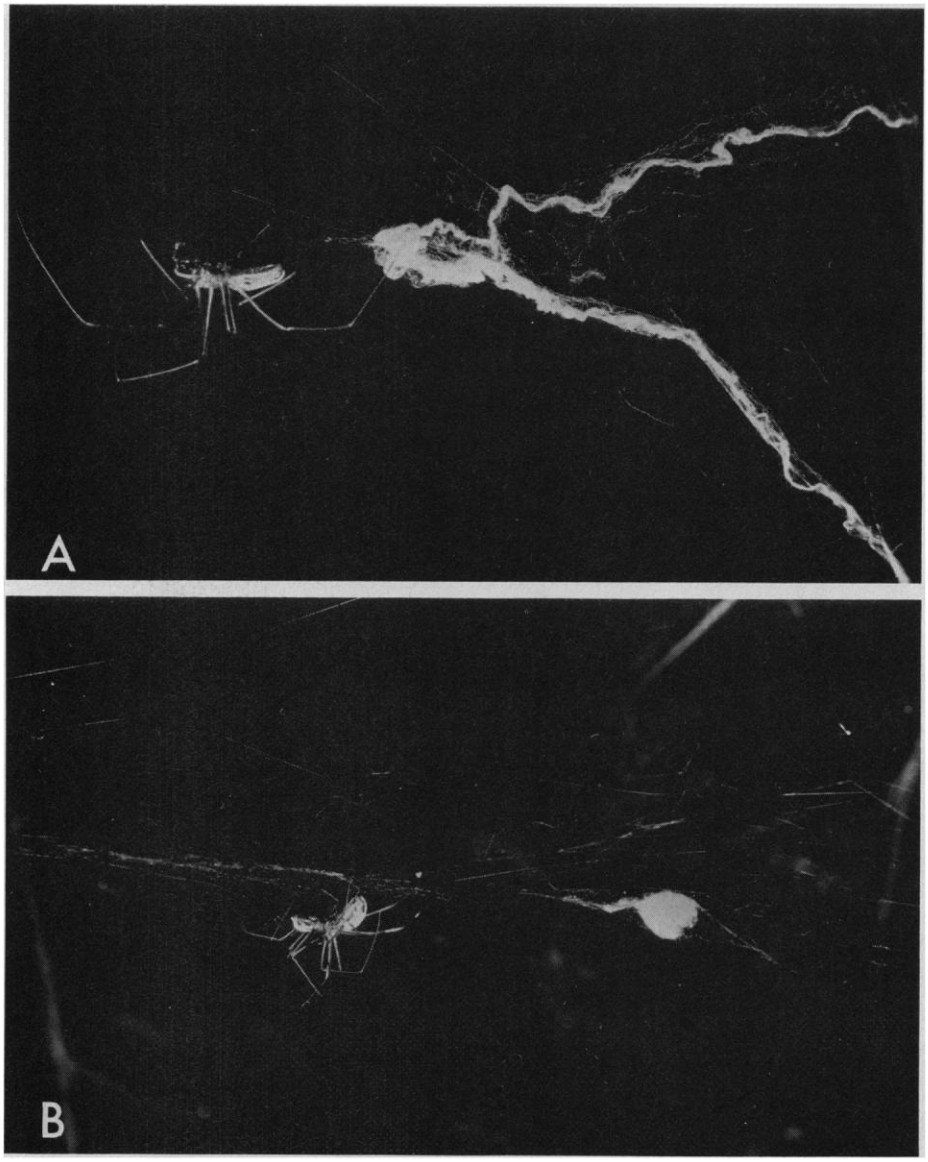

Fig. 1. (A) Male Sierra dome spider packing the silk of a mature virgin female's web. (B) Web of a Sierra dome female after completion of web reduction. The support lines of the web remain intact. Most of the dome has been packed by the male into a dense ball visible at center right. The male and female are in copula at center left.

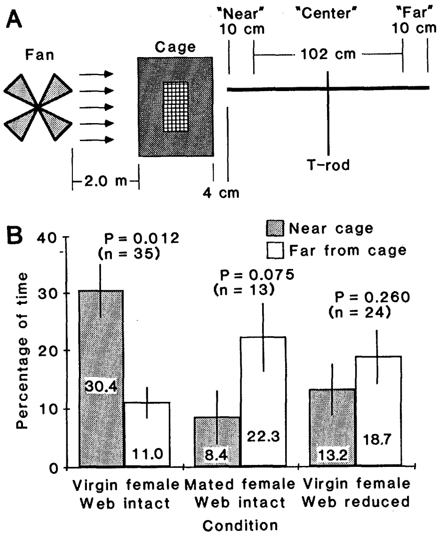

Fig. 2. (A) T-rod apparatus used to test the airborne nature of the male attractant pheromone and the efficacy of web reduction. (B) Percentage of total exposure time males spent within the 10-cm sections at each end of the T-rod during exposure to the three categories of females. Shaded bars represent the sector of the T-rod near the cage and open bars the sector far from the cage. Sample sizes (*n*) are the number of different males exposed to each condition. Error bars represent 1 SEM. Significance levels concern differences in the percentage of time spent at the two ends of the T-rod in each of the three conditions (Wilcoxon tests); see the text for differences between conditions.

males from long distances, and the efficacy of the male's web-reduction behavior in reducing the pheromone's detection by rivals were documented in experiments testing the attraction of males to three types of caged females with webs: (i) virgin females with intact webs, (ii) mated females with intact webs, and (iii) virgin females with reduced webs. The testing apparatus is shown in Fig. 2A.

In each trial, a single male was released atop the central vertical support of the T-rod and exposed to a breeze reaching him through the cage. Two sets of data were recorded: (i) the percentage of the male's total exposure time spent within each of the two 10-cm terminal sections of the T-rod near and far from the cage and (ii) the section of the T-rod from which the male finally descended, by means of a dragline, to the table surface (*14*).

Only when exposed to a virgin female on an intact web did males spend significantly more time at the 10-cm portion of the T-rod near the cage than at the 10-cm portion of the T-rod far from the cage. In addition, under this condition males spent a greater percentage of their total exposure time within the 10-cm section of the T-rod near the cage than males exposed to mated females with intact webs or virgin females with reduced webs (Mann-Whitney test; $P = 0.002$ and $P = 0.008$, respectively). The percentage of exposure time spent by males near the cage did not differ significantly between these latter two conditions (Mann-Whitney test; $P = 0.279$) (Fig. 2B). Males exposed to intact webs of virgin females descended from the 10-cm section of the T-rod near the cage more often than those exposed to nonvirgins with intact webs or virgins with reduced webs (Table 1; $P = 0.046$) (*15*).

Thus, oriented searching behaviors were exhibited by males exposed to virgin females with intact webs, while in the other two conditions males wandered about the T-rod apparatus without obvious orientation. The data show that male Sierra dome spiders are attracted to the webs of mature virgin females by an airborne emission, but web reduction neutralizes the attractiveness of these webs.

These results illustrate the complex blend of mutual and conflicting interests that can exist in intersexual mating strategies. Female Sierra dome spiders probably benefit by producing a male attractant pheromone to ensure mating in circumstances where rates of male visitation are very low. Competition among attracted males could further benefit females by resulting in a higher quality mate. But, the first male to enter the web of a pheromone-producing female avoids combat with other males by quickly canceling the female's signal through web reduction. This cost of web reduction to the female is added to that associated with the destruction of a large portion of her web, which represents a nonrecoverable protein investment (*16*) and the female's sole means of foraging and defense against predators.

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6. The density of adult females on my study site during the middle of the breeding season was about one individual per 6 m^2.
7. This finding is based on a five-locus electrophoretic paternity exclusion analysis using the mates and progeny of 20 Sierra dome females. These females were allowed multiple matings at will in the field under natural circumstances.
8. Females are easily introduced onto webs, where they behave normally. A female promptly adds silk of her own to the web onto which she has been introduced.
9. The average male visitation rate is a minimum estimate based on a total of 57 hourly scans of the webs of each class of female. The significance level is based on a two-tailed Wilcoxon test ($T = 45$; $n = 27$).
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12. Web reduction would have this effect because the male's matting of the silk drastically decreases the concentration gradient of pheromone molecules between the silk and open air.
13. Since the mean male visitation rate at the webs of mature virgin females is approximately one arrival per 3-hour period, and copulations last 3 to 6 hours, many matings would be disrupted by one or more rivals if the early-arriving male failed to perform web reduction.
14. Females constructed normal webs within their cages (cage size: 30 by 30 by 40 cm, all sides screened). Each female was tested for attractiveness with three different males, but males were used in only one trial each. Tests on females with reduced webs were arranged by releasing a male into the cage of a mature virgin female, allowing completion of web reduction, and then removing the male from the cage. Such trials were begun minutes after web reduction was complete and involved only webs that clearly had been attractive to males immediately before web reduction.
15. Separate 2 × 3 analyses show that frequency distributions differ between virgins with intact webs versus mated females with intact webs ($P = 0.020$), and virgins with intact webs versus virgins with reduced webs ($P = 0.048$), but not between nonvirgins with intact webs versus virgins with reduced webs ($P = 0.338$). The average distance from the tip of the T-rod near the cage at which males in the "center" category descended was 60 cm (SEM = 4.0).
16. Linyphiid spiders do not ingest web silk.
17. I thank M. Apple, V. Demas, D. Glaser, B. Jacobs, D. Lee, E. Smith, and D. Watson for assistance with observations; J. Stanford and the staff of the University of Montana Biological Station for hospitality during my time in the field; S. T. Emlen, P. W. Sherman, T. Eisner, R. Charif, and J. Crawford for discussions and comments; and M. Miyakado for performing web extractions. Study supported in part by NSF Dissertation Improvement Grant BSR-8311331 and NIH grant 5T32MH15793.

27 December 1985; accepted 15 April 1986

A Toxic Dipeptide from the Defense Glands of the Colorado Beetle

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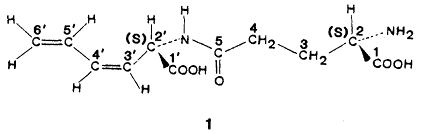
The Colorado beetle is protected against predators by the secretions of defensive glands located on the pronotum and the elytra. The single major compound of the secretion was identified as γ-L-glutamyl-L-2-amino-3*(Z)*,5-hexadienoic acid by spectroscopic and chemical methods. This compound, which contains a nonprotein β,γ-unsaturated amino acid, is toxic to ants *(Myrmica rubra)* at a concentration 10^{-2} molar, which is less than its estimated concentration in the secretion (1.8×10^{-1} molar).

The Colorado beetle (*Leptinotarsa decemlineata*), a notorious pest of potato plants, is chemically well protected against predators (*1, 2*). Like many toxic insects, it has a bright coloration, which provides a visual signal for predators that hunt by sight, such as birds (*3*). The beetle produces a secretion from which we have isolated a toxic dipeptide, γ-L-glutamyl-L-2-amino-3*(Z)*,5-hexadienoic acid (**1**).



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The discovery of such a dipeptide containing a nonprotein amino acid in the defensive secretion of the Colorado beetle was unexpected on the basis of the known defensive chemistry of adult chrysomelids. The compounds so far isolated constitute a biosynthetically diverse group that includes cardenolides, from several *Chrysolina* spp. (*4–6*); polyoxygenated steroidal glucosides, from *Chrysolina hyperici* (*7*); and isoxazolinone glucosides, from several species be-

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