second-order schedule of intravenous injection. Average rates of responding often exceeded one response per second, and monkeys pressed the lever as many as 250 times per single injection of nicotine. The maintenance of responding was unequivocally the result of the consequent injections of nicotine, since responding could be extinguished by either saline substitution or mecamylamine treatment. Although responding ultimately depended on injections of nicotine, the brief visual stimulus associated with injections played an important role in the maintenance of persistent responding, since rates of responding were about twice as high when the brief stimulus was presented as when it was not.

There has been a continuing need for a sensitive laboratory method for evaluating the reinforcing effects of nicotine. In this study, persistent behavior was maintained at high rates under a second-order schedule of intravenous nicotine injection. Furthermore, the behavior was highly sensitive to both environmental and pharmacological intervention. Second-order schedules of nicotine injection may therefore provide a useful experimental technique for examining environmental and pharmacological factors that contribute to the maintenance of tobacco use by humans.

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8. The chair was similar to the one described by D. F. Hake and N. H. Azrin [*J. Exp. Anal. Behav.* **6**, 297 (1963)].
9. The volume of each injection was 0.20 ml, infused over 200 msec. Nicotine hydrogen (+)-tartrate was dissolved in 0.9 percent saline; doses are expressed as the salt.
10. Training procedures and apparatus were similar to those described by S. R. Goldberg [*J. Pharmacol. Exp. Ther.* **186**, 18 (1973)].
11. Training procedures were similar to those described by Goldberg and Spealman (*3*).
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13. Overall response rates were computed by dividing total responses in the presence of the green light by total time the green light was on. (Responses and time during 1-second amber lights were not included in computations.)
14. Local response rates were computed as the mean rate from the first to last response in each FR unit. (Pause time before the first response was not included in computations.)
15. This dose was selected on the basis of a previous study, which showed that 1.0 mg of mecamylamine per kilogram of body weight can block the behavioral effects of nicotine in squirrel monkeys but does not alter schedule-controlled behavior when given alone [R. D. Spealman, S. R. Goldberg, M. L. Gardner, *J. Pharmacol. Exp. Ther.* **216**, 484 (1981)].
16. Supported in part by PHS grants DA02658, DA00499, MH07658, MH02094, and RR00168.

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Acoustic Communication and Reproductive Isolation in Two Species of Wolf Spiders

Abstract. *Sound production by male wolf spiders during courtship is critical for behavioral reproductive isolation of two sibling species. Females only respond to and copulate with conspecific males, and acoustic signals through a substrate are necessary to induce receptivity. No reproductive barriers that could arise during mating (such as genital or mechanical incompatibility) or after mating (infertility) are in effect between the species, since forced interspecific matings produce viable offspring.*

Sound production in spiders is more common than is generally realized (*1*), although in only a few instances have spider sounds been recorded (*2–4*). Recently, Rovner (*3*) found a substrate-coupled sound-producing apparatus in wolf spiders (family Lycosidae) and demonstrated, by playback techniques, the communicatory function of these sounds during courtship and agonistic display (*4*). Our study provides evidence that communication by substrate-coupled stridulation is critical for the reproductive isolation of two sibling species of wolf spiders.

Schizocosa ocreata (Hentz), common in deciduous forest leaf litter throughout the eastern United States (*5*), is identical to *Schizocosa rovneri* Uetz and Dondale with respect to genital characters, body size, general morphology, and color. These species were previously considered to be a single species (*6*). The females are indistinguishable, but male *S. rovneri* lack the prominent tufts of black bristles present on the tibiae of the first pair of legs in mature male *S. ocreata*. These two species exhibit a high degree of overlap in geographic range, microhabitat, and seasonality (*7*).

Table 1. Behavioral responses of male and female wolf spiders in experimental courtship pairings. Symbols: +, courting by male and receptiveness by female; −, no response, avoidance, or agonistic behavior.

Courtship pairing	Males		Females	
	+	−	+	−
Schizocosa rovneri				
Conspecific	52	1	45	8
Heterospecific	38	3	0	53
χ^2	N.S.*		78.2	
			$P < .005$	
Schizocosa ocreata				
Conspecific	21	1	34	9
Heterospecific	29	1	1	40
χ^2	N.S.		50.71	
			$P < .005$	

*N.S., not significant.

The courtship behavior of mature male *S. ocreata* has been described (*8, 9*) as an active tapping of the first pair of legs in unison, accompanied by movements of the pedipalps. Sounds (vibrations) are produced by a stridulatory organ on opposing segments of the palpal tibiotarsal joint and are conducted through the substrate via stout spines at the distal ends of the palps (*3*). In the later phases of courtship, the display consists of raising and extending the first pair of legs in addition to stridulation.

The courtship behavior of *S. rovneri* differs considerably from this. The male executes a "bounce" several times in succession at 3- to 5-second intervals, then moves to another location. The bounce involves movement of the entire body; the cephalothorax and abdomen are raised up and thrust downward between the legs, sometimes hitting the substrate. Rotating movements of the

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palps occur and stridulation is clearly audible at this time.

The behavior of receptive females is the same in both species, and involves lowering the cephalothorax, extending the forelegs on the substrate, and rising and turning (90° to 180°) several times. Although males of both species court conspecific and heterospecific females (*9*), females copulate only with conspecific males (Table 1). Thus the two species appear to be reproductively isolated by their courtship behavior.

Knowing that their genitalia are identical, we tested the two species for mechanical reproductive barriers and interfertility by forced copulations. Females anesthetized with CO_2 (*10*) were placed in front of heterospecific males and moved slowly forward with the front legs extended. The males would court, mount, and begin scraping the side of the female's abdomen in the manner typical for wolf spiders. The females remained anesthetized for about 2 minutes. Once "awake," they responded normally, swiveling the abdomen to facilitate insertion of the male palp.

Offspring were produced in all the interspecific crosses. There was no significant difference in egg production or hatching success between conspecific and heterospecific matings, and the offspring were not sterile. Thus it seems that no mechanical or other reproductive barriers, such as gamete incompatibility or zygote mortality, are in effect.

A series of experiments was conducted to isolate and test the relative importance of the various communication modalities used by *S. rovneri* during courtship. The male's response to female silk (containing pheromone) was tested by putting the liner from a female's cage in a paper arena and placing the male on top of it. His behavior was then observed and recorded. The male was also tested in the presence of the female and her silk. The female was placed under a plastic bubble and her cage liner was placed next to her. In each instance the female's behavior was also noted, particularly any display of receptive behavior. In some cases the male and female were allowed to come into contact.

Table 2. Percentage of male and female *Schizocosa rovneri* responding to various stimuli during courtship.

Stimulus	*N*	Percent responding
Males		
Control	39	38.5
Visual	20	25.0
Chemical*	29	93.1
Visual and chemical	36	91.6
Females		
Control	39	0.0
Visual	16	37.5
Auditory	19	78.9
Visual and auditory	18	88.9

*Silk with pheromone.

The male's response to visual stimuli was tested by placing the female in a plastic bubble (cleaned with alcohol to remove any trace of pheromone) and sealing her in with Vaseline. The arena was also cleaned with alcohol. The female's response to the sight of the male was tested by sealing a female in a plastic bubble with Parafilm and hanging the bubble slightly above the substrate (*11*). Presumably this eliminated substrate-coupled vibratory communication and chemical communication.

The female's response to substrate-coupled sound production was tested by sealing her in a bubble with Parafilm and visually isolating her from the male with opaque paper. The bubble was placed directly on the arena on which the male was courting. This allowed vibrations of the paper substrate to be felt by the female.

The data indicate that for the male, pheromones are the most important stimuli in eliciting courtship behavior (Table 2). The pheromone alone—whether airborne (*12*) or contained in the female's silk (*13*) or both—was sufficient to induce courtship. However, this stimulus appears not to be species-specific: the silk of either species induced courtship. Visual contact with the female triggered courtship only if the female moved but was necessary for the male to orient to the female (*14*). Substrate-borne sounds alone were capable of inducing female receptivity (Table 2) (*4*). Acoustic communication by substrate-coupled stridulation appeared to be a critical factor in the response of the female and thus in determining whether copulation would occur.

It seems that these species differ mainly in the temporal patterning of sound production during stridulation. Dondale (*15*) indicated that both species have 30 ridges on the stridulatory file. However, the number of strums per bout of stridulation is quite different between the two species, with *S. ocreata* producing sounds continuously for a much longer period than *S. rovneri*. This suggests that the two species are using the same device to make different courtship songs.

Vibration recordings of stridulating spiders were made (*16*) and were used to produce oscillograph tracings (*17*). Oscillograms of the sounds produced by courting male *S. ocreata* show complexity, without clear temporal patterning. A single episode of sound production can be as long as 10 seconds, and is followed by a period of silence. Apparently there are many strums (20 or more) in a single episode (Fig. 1, A and B). A single wave train may last 30 msec (Fig. 1C). The example given in Fig. 1C shows 24 waves of variable amplitude (fundamental frequency, about 800 Hz).

During a series of bounces by courting male *S. rovneri*, the palpal joint appears to rotate several times, although definite passes of the scraper across the file are not easily discernible in either high-speed films (54 frames per second) or oscillograms. Since a bounce occurs about every 3.5 seconds (Fig. 1D), the sounds made by *S. rovneri* are much more regular than those made by *S. ocreata*. The sound produced during a single bounce lasts about 0.25 second (Fig. 1E) and probably includes several strums. A single wave train shows high-amplitude waves and lasts about 25 msec (Fig. 1F). As there are 13 high-amplitude waves in this particular sequence, the fundamental frequency is about 520 Hz.

Communication between male and female spiders is important in reducing cannibalism as well as in species recognition. Sound production would seem to be a better way to achieve communication in the environment of leaf litter than a

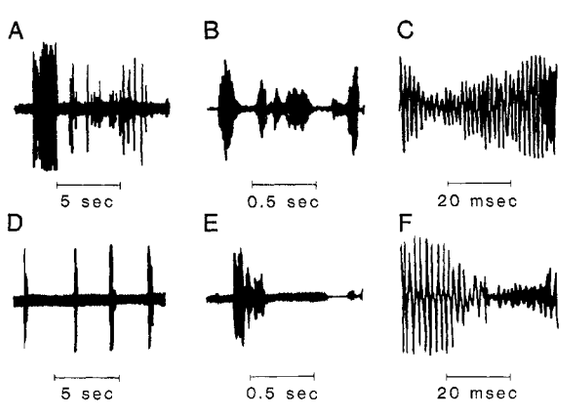

Fig. 1. Oscillograms of sound produced by male *Schizocosa ocreata* (A to C) and male *S. rovneri* (D to F) during courtship.

visual display. Leaf litter could serve to amplify vibrations of courting males (*3*, *18*); thus communication by substrate-coupled stridulation would be particularly effective in this environment.

Differences in courtship behavior are clearly important in maintaining the otherwise incomplete isolation of these two species and hence their genetic integrity. We conclude that *S. rovneri* is an ethospecies, reproductively isolated from its sibling species by its unique courtship behavior.

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7. Specimens confirmed to be *S. rovneri* have been collected in Illinois, Kentucky, and Ohio. Individuals suspected to be *S. rovneri* (that is, specimens lacking the tufts of bristles but otherwise indistinguishable from *S. ocreata*) have been collected in Delaware, Arkansas, North Carolina, and Texas. Both species are found in forest litter near water, mature between May and July, and breed during that time. Eggs are laid in July and August, and the offspring overwinter in the immature state.
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14. Males never oriented to a motionless female; 71 percent of males oriented to females that moved receptively.
15. C. D. Dondale, personal communication.
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17. Recordings were played back into a Tektronix 510 3N recording oscilloscope, kindly loaned by E. S. Kaneshiro. Photographs were taken of the oscilloscope screen.
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Ganglioside Stimulation of Axonal Sprouting in vitro

Abstract. *Bovine brain gangliosides were applied to primary and established neuronal cultures to examine the role of gangliosides in neuronal development. Media containing gangliosides enhanced the degree of axonal elongation exhibited by sensory ganglia neurons and increased the length and number of Neuro-2a neuroblastoma cell processes. Ganglioside-supplemented media caused a twofold increase in ornithine decarboxylase activity in both culture systems. These experiments suggest that gangliosides function as acceptor molecules for growth-promoting substances in embryonic and tumor-derived neurons.*

Gangliosides are cell membrane–associated molecules that play a role in a variety of cellular events, including differentiation (*1–3*), defense (*4*), growth (*3*, *5*, *6*), regeneration (*7*), and transformation (*7*). The striking changes in the distribution and quantity of gangliosides during cephalogenesis (*8*) suggest that they are important in neuronal development. Since gangliosides are mainly associated with cellular membranes, it has been suggested that they act in the transfer of information across these membranes (*9*). Gangliosides function as receptors on the cell surface for glycoprotein hormones (*10*), interferon (*11*), and serotonin (*12*).

To investigate the role of gangliosides in neuronal development, we exposed primary cultures of sensory ganglia and an established neuroblastoma line to media containing mixtures of bovine brain gangliosides. As evaluated morphologically and biochemically, the ganglioside mixtures enhanced neurite development and metabolic activity of both sensory ganglia and Neuro-2a neuroblastoma cells. Thus it appears that gangliosides mediate development in the nervous system.

Dorsal root ganglia from $8\frac{1}{2}$-day-old chick embryos (White Leghorn) were cultured on collagen-coated cover slips, as double cover slip lying-drop preparations, in medium 199 (Gibco) supplemented with 10 percent heat-inactivated fetal calf serum (Irvine Scientific) or in serum-free HI-WO_5/BA_{2000} medium (International Scientific Industries). Neuro-2a murine neuroblastoma cells (CCL-131, American Type Culture Collection) were maintained in plastic and glass petri dishes with Eagle's minimum essential medium containing Hanks balanced salt solution (Gibco) and supplemented with 10 percent fetal calf serum, 10 mg of

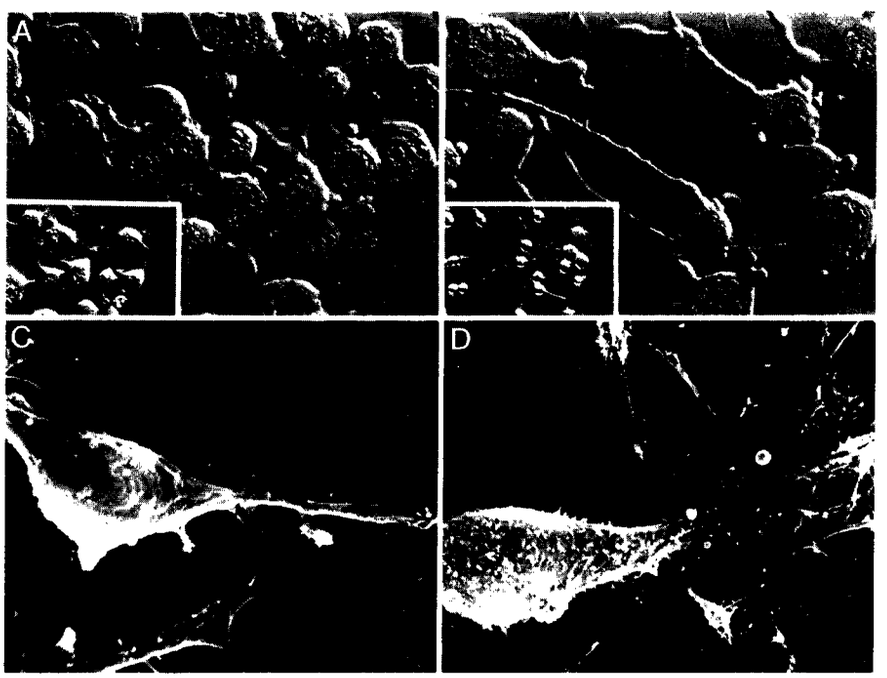

Fig. 1. (A and C) Photomicrographs of Neuro-2a cells grown in control medium for 40 hours. Few processes are seen and the cell surface is relatively smooth, with only occasional microspikes. (A) Nomarski optics (× 300; inset × 130). (C) Scanning electron microscopy (× 1400). (B and D) Photomicrographs of Neuro-2a cells grown for 40 hours in media supplemented with 250 μg of bovine brain gangliosides per milliliter (*13*). Extensive sprouting can be seen on cell surfaces. Numerous microvilli and blebs are also visible on the perikaryon. (B) Nomarski optics (× 300; inset × 85). (D) Scanning electron microscopy (× 1300).

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