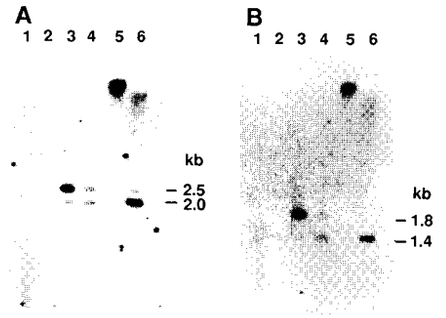

Fig. 3. Southern blot analysis of fractionated nucleohistone DNA and nucleoprotamine DNA from human sperm chromatin. After removal of histones (lanes 1), the chromatin was washed in Bam HI buffer (lanes 2), restricted with Bam HI, and centrifuged. The supernatant (lanes 3) was removed, and the pellet was reextracted with NaCl (lanes 4). DNA in the supernatants (lanes 1 to 4) and pellet (lanes 6) was purified and restricted with Bam HI as described in the text, separated according to size on a 1% agarose gel along with NP DNA that was not restricted after DNA purification (lanes 5), and transferred to nitrocellulose filter membrane. The filter was baked at 80°C for 2 hours and then prehybridized overnight in 5× Denhardt's solution, 3× saline sodium citrate (SSC), and 0.5% SDS with 0.2 mg/ml of salmon sperm DNA at 60°C (*13*). (**A**) A 2.5-kb NH clone and a 2.0-kb NP clone were labeled with ^{32}P by nick translation and simultaneously used as hybridization probes. After hybridization at 60°C for 16 hours, the filters were washed in 0.5× SSC, blotted dry, and autoradiographed. (**B**) The filter was stripped of radioactive DNA by washing in 0.1% SDS at 90°C for 5 minutes. A 1.8-kb NH clone and a 1.4-kb NP clone were used as hybridization probes as described above. In both (A) and (B) the NH clones preferentially hybridized to the NH DNA (lanes 3), and the NP clones preferentially hybridized to the NP DNA (lanes 6).

may also be involved. On occasion, Bam HI failed to cleave the DNA even after removal of the histones, indicating that factors other than histone protection may also contribute to the inhibition of NH DNA restriction.

We conclude that human sperm DNA is packaged into nucleohistone and nucleoprotamine in a sequence-specific manner. The nucleohistone component does not contain a full genomic equivalent, and therefore cannot be exclusively derived from contaminating somatic cells. Our results indicate the nucleohistone component is present in most (not necessarily all) ejaculated sperm. Thus, the bulk of the DNA is condensed and inactivated by protamines, but the remaining DNA is complexed with histones in a fashion that may resemble active rDNA chromatin (*8, 9*). Even though these results do not accurately quantitate the relative distribution of DNA in these two domains, the percentage of NH DNA solubilized by Bam HI digestion (10 to 15%) is consistent with that reported to be complexed with histones by others (*1*).

The biological significance of the nucleohistone component is unknown. The DNA complexed as nucleohistone could be functional either structurally or transcriptionally in the final stages of spermatogenesis or in the early stages of embryonic development. For example, the nucleohistone could designate initiation sites for chromatin decondensation, thus serving a structural role. Furthermore, paternal-specific gene products have been detected in early mammalian development by the two-cell stage (*15*), and this nucleohistone component could contain genes programmed for very early expression.

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Chemical Mimicry: Bolas Spiders Emit Components of Moth Prey Species Sex Pheromones

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Field studies have indicated that bolas spiders attract male moth prey, apparently by mimicking the odor of female moth sex pheromones. Three moth sex pheromone compounds, (*Z*)-9-tetradecenyl acetate, (*Z*)-9-tetradecenal, and (*Z*)-11-hexadecenal, were identified in volatile substances emitted by hunting adult female *Mastophora cornigera* spiders. These compounds are components of pheromone blends that attract some of this spider's moth prey species.

SPIDERS IN THE GENUS *Mastophora* ARE notable because of their minimal "bolas" web (Fig. 1a) and because they capture only one type of prey: male moths. Prey approach spiders from downwind and, in experiments, are attracted into traps that contain hunting spiders. Spiders can be hand-fed a wide variety of moths, but in the field they capture only males of a few species. This evidence suggests that spiders mimic the odor of the sex pheromones emitted by female moths (*1–5*).

We have identified three compounds, identical to sex pheromone components of some moth prey species, in volatile substances collected from hunting *M. cornigera*. To the best of our knowledge, this is the first chemical evidence to support the chemical mimicry hypothesis. Volatiles collected from different spiders varied in blend composition and ratio, which suggests that individuals may alter their blends or that different individuals may produce different blends. The compounds were not found in freshly spun webs, which indicates that they are emitted from an undetermined part of the spider's body. This study furthers our understanding of the chemical ecology of moth-attracting spiders and their possible influence on the evolution of chemical communication in moths.

Volatiles were collected from mated adult female *M. cornigera*. Twenty-four individuals were reared from egg cases laid by female spiders from San Diego, California

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(*6*). One additional individual was collected in South El Monte, California. Collections were made from spiders that maintained a hunting stance while holding a bolas (*7*).

Because these spiders do not initiate hunting inside collection chambers, they were kept under an approximation of natural conditions (*8*). Each spider that started hunting, that is, spun a web on its small "spinning platform" (Fig. 1a), was gently transferred (with its platform) into a collection chamber. Each collection was relatively short—spiders stopped hunting 22 minutes, on average, after they were put in a chamber. Volatiles were collected repeatedly from spiders if they reinitiated hunting after removal from the chamber. Collections were made between 2 and 11 hours after sunset (*6*).

In each "push-pull" volatile collection chamber (Fig. 1b), a pressurized air tank pushed air through purifying charcoal filters into the chamber; at the same time a vacuum pump pulled air from the chamber through a charcoal entrapment filter at a rate of 1.3 liter/min. "System blank" volatiles were collected in an identical, parallel control chamber while volatiles were being collected from spiders. Chambers were cleaned and oven-dried to minimize background contamination. After a platform had been introduced into a chamber, the chamber was purged with three or more volumes of cleaned air before starting volatile collection. Positive pressure of clean air was maintained in each chamber at all times that air was pulled through the entrapment filter. Volatiles were eluted from entrapment filters with methylene chloride and stored in hexane (*6, 9*).

In preliminary work, single night–single spider collections were made from each of two individuals. We pooled all subsequently collected material. Volatiles emitted by spiders were collected from eight individuals during the course of 2 months (September and October), with a total collection period of 18.4 hours. This material was first combined into four "initial pools" by using a protocol described below. One-fourth of each initial pool was set aside for separate analysis. The remaining three-fourths of all initial pools were combined into a "final pool." All system blank material was combined into a single pool.

The volatiles in the final pool and the system blank pool were analyzed by capillary gas chromatography (GC) on OV-101 and CPS-1 columns (*10*). On the OV-101 column there were four peaks present in the spider volatiles that corresponded in retention time to peaks produced by the authentic standards (*Z*)-9-tetradecenal (Z9-14:Al), (*Z*)-9-tetradecenyl acetate (Z9-14:Ac), (*Z*)-11-hexadecenal (Z11-16:Al), and (*Z*)-11-hexadecenyl acetate (Z11-16:Ac). The system blank clearly lacked the latter three compounds, but it did contain impurities with retention times similar to that of Z9-14:Al. Analysis on the CPS-1 column established that peaks corresponding exactly in retention time to all four compounds were present in the spider volatiles and absent in the system blank.

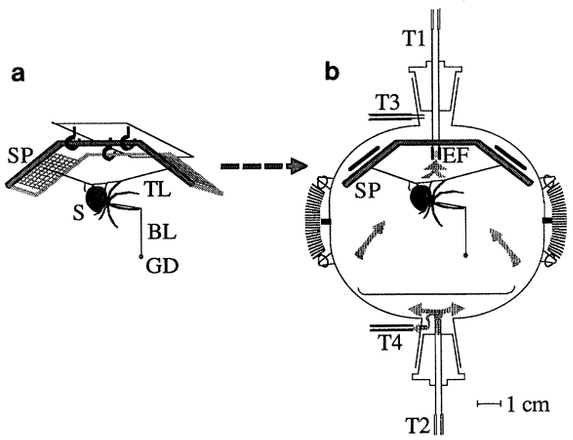

Fig. 1. (**a**) Oblique view from below of a spider (S) hunting in the web it has spun on a spinning platform (SP). (**b**) A push-pull volatile collection chamber with spinning platform and spider transferred inside. Arrows show airflow. Abbreviations: TL, trapeze line; BL, bolas line; GD, glue droplet; EF, entrapment filter; T1, vacuum; T2, pressurized air; T3, purge; and T4, positive pressure bleed.

Portions of the final pool and the system blank pool were also analyzed by chemical (methane) ionization gas chromatography–mass spectrometry (GC-MS) (*10*). In the first GC-MS analyses, selected ion monitoring (SIM) was used. Peaks were present in the final pool of volatiles, which corresponded in retention time and in selected ions to authentic standards of Z9-14:Ac, Z9-14:Al, and Z11-16:Al. A peak corresponding to Z11-16:Ac could not be detected, probably because there was too little material. No peaks or specific ions for the four compounds were present in the system blank. The remainder of the final volatile pool was analyzed in the same GC-MS system under the same conditions except that data were collected over the range of mass-to-charge ratio of 60 to 350. Diagnostic ions observed in these analyses confirmed the presence of the three compounds detected by SIM (*10*).

Since the CPS-1 capillary column separates both positional and geometric isomers of C_{14} and C_{16} acetates and aldehydes (*11*), these data prove that the spiders emitted Z9-14:Ac, Z9-14:Al, and Z11-16:Al. The substantiation of spider emission of Z11-16:Ac awaits further data. The quantity of the compounds in the final pool per hour of volatile collection was about Z9-14:Ac, 1 ng/hour; Z9-14:Al, 1 ng/hour; and Z11-16:Al, 2 ng/hour. This is similar to release rates observed in many moths (*12*).

Preliminary analyses of earlier single night–single individual volatile collections indicated that the composition of the spider volatiles varied. However, most individuals yielded only small amounts of material. Therefore, our subsequent collection and pooling protocol was designed to (i) maximize the amount of material for GC and GC-MS analyses, but (ii) still allow some

Table 1. Relative amounts of three moth sex pheromone compounds in different spider volatile collections. The most abundant compound is normalized to one. Pools combined material from more than one individual (named by letters). Two of the single spider–single night collections are included: those that were analyzed on CPS-1 columns (*10*) and contained at least 5 ng of the three compounds (combined). The asterisk indicates that an inconclusive peak was present at the correct retention time for this compound. The amount of the compound was at most one-fourth the amount of the most abundant compound.

Analyzed material		Ratio of compounds		
Description	Composition: individuals (collection minutes)	Z9-14: Al	Z9-14: Ac	Z11-16: Al
Initial pools				
A	N (120), P (99), W (31)		*	1.0
B	N (101), P (73), W (11)	1.0	*	*
C	Z (174), N (100)			1.0
D	N (135), U (94), V (54), L (46), Z (40), Q (21), W (7)		1.0	*
Final pool	N (456), Z (214), P (172), U (94), V (54), W (49), L (46), Q (21)	0.5	0.5	1.0
Individuals				
EE	EE (150)	1.0	0.1	
EF	EF (90)	1.0		0.4

Table 2. The moth sex pheromone compounds (MSPCs) present in spider volatiles and in attractant blends for all moth prey species for which information is available. The check marks indicate all MSPCs that were found in spider volatiles in this study. The evidence for Z11-16:Ac is incomplete. Plus signs indicate all MSPCs that are necessary for attraction or that constitute more than 10% of known prey attractant blends (*18*). All such compounds belong to the set of eight compounds shown here, a biosynthetic series in moths. Minus signs indicate, for moth blends, all reported attraction inhibitors that belong to this set of compounds (*18*). The first seven species are noctuids, the last a plutellid.

Spider volatiles	Pheromone compounds							
	Z5-10:		Z7-12:		Z9-14:		Z11-16:	
	Al	Ac	Al	Ac	Al	Ac	Al	Ac
					√	√	√	?
Moth prey attractant blends								
*Peridroma saucia**						+	−	+
Pseudaletia unipuncta						−	−	+
*Leucania phragmatidicola**						+		
Scotogramma trifolii								+
*Heliothis phloxiphaga**							+	
*Euxoa messoria**						−		+
*Euxoa olivia**		+						
Plutella xylostella†					−	−	+	+

*Attractant information based solely on field screening of blends. †This moth species is known only from the prey of subadults. Most prey species are caught by both adults and subadults (*4*).

assessment of variability in spider volatile composition. To meet the first goal, it was necessary to combine material from different individuals and different nights, in the initial as well as the final pools (*13*). To meet the second goal, two initial pools (A and B) were created by using material from the same individuals, and two pools (C and D) by using material from different sets of individuals.

The four initial pools were analyzed on CPS-1 columns by GC and by SIM GC-MS under the same conditions used for the final pool. The GC and the SIM analyses were in agreement on the composition of each initial pool (Table 1). Pools A and C contained only or predominantly Z11-16:Al. Pool D contained predominantly Z9-14:Ac, and pool B, Z9-14:Al. Pools A and B were quite different in volatile composition, even though the same individuals contributed material in similar amounts. These results suggest intra- or interindividual variation in volatile composition. Pools differed in a number of ways that might mediate volatile composition changes by individuals: the time of night and time of year that collections were made, and the hunting history of the spiders before collection (*6*).

Exhaustive collections were made from freshly spun webs taken from spiders (6 webs from two individuals in one collection, and 11 webs from three other individuals in another) (*6*). The GC analyses of these collections on CPS-1 columns (*10*) did not reveal any of the compounds obtained from hunting spiders. The only peaks present were also present in the system blank.

Attractant information is available for eight moth species captured by *M. cornigera* (Table 2). Male-attracting blends can be made for each of these prey species by using mixtures of compounds from the set: Z5-10:Ac, Z9-14:Ac, Z9-14:Al, Z11-16:Ac, and Z11-16:Al. These five compounds are part of the same biosynthetic series in moths (*14*) (Table 2). This study demonstrates that spiders produce at least three of these compounds. We hypothesize that spiders attract male moths with blends of these (and other, not yet identified) female moth sex pheromone compounds (*15*).

In the field, the spiders prey on male moths of at least 19 species, with single individuals catching up to 9 species (*4*). It is unlikely that a single blend of sex pheromone compounds could attract all 19 moth species, particularly because the attraction of some prey species is inhibited by compounds that are apparently necessary for the attraction of other species (Table 2). Prey records indicate that individual spiders often catch two or more moth species with apparently incompatible pheromone blends. This study provides preliminary evidence that the composition of spider volatiles varies. We hypothesize that spiders produce different blends of compounds that attract different sets of prey species.

The source of prey attractant compounds is unknown. In the field, glue droplets from bolas webs alone are not attractive to prey, whereas hunting spiders without droplets are attractive (*1*). This finding and the fact that we did not find volatile compounds in collections from fresh *M. cornigera* webs (glue droplets plus all the silk lines) indicates that the attractants are emitted by the body of the spider (*16*).

Aggressive mimicry of moth sex pheromones by spiders is apparently widespread. Spiders in the genus *Mastophora* and at least seven other araneid genera prey on male moths of at least seven families (*1–5*). The spiders are found in temperate and tropical habitats worldwide. The relative densities of hunting spiders and pheromone-emitting female moths are not known. If the densities are comparable in some habitats, then the spiders may have influenced the evolution of sex pheromone communication in several major moth groups (*17*).

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7. It is uncertain whether spiders attract moths while they maintain the hunting stance without the bolas (*1, 4*).
8. Spiders were kept inside a greenhouse where fans provided a constant, gentle flow of outside air. Even so, 11 out of 19 individuals never spun in captivity and were not collected from; most spider individuals do not readily engage in hunting behavior when they are not in long-occupied sites in the field.
9. The collection technique is derived from the one used by J. H. Tumlinson, E. R. Mitchell, P. E. A. Teal, R. R. Heath, and L. J. Mengelkoch [*J. Chem. Ecol.* **12**, 1909 (1986)].
10. Analyses of spider volatiles and corresponding system blanks were performed under identical conditions. The GC analyses were performed on 50-m fused silica columns (inside diameter, 0.25 mm) in a Varian model 3700 GC equipped with a split-splitless capillary injection system and a flame ionization detector. Linear flow velocity of the helium carrier gas was 19 cm/sec. Columns and conditions were: OV-101, 60°C for 1 minute, then temperature programmed (TP) at 10°C per minute to 225°C; CPS-1 (Quadrex), 60°C for 1 minute, then TP at 10°C per minute to 165°C. Injections were made in the splitless mode with a 30-second delay before switching to split mode. The quantity of each compound present was estimated by GC peak area integration and comparison with an internal standard. The GC-MS analyses were performed on the CPS-1 column (under the conditions given above), in a Varian 6000 GC interfaced to a Nermag model R1010 mass spectrometer. The ions monitored during SIM analyses (89, 167, 193, 195, 211, 221, 223, 227, 239, 255, and 283), included the $(M+1)^+$ ions for monounsaturated (C_{12}, C_{14}, and C_{16}) acetates and (C_{14} and C_{16}) aldehydes, the $(M+1-60)^+$ ions for the acetates, and the $(M+1-18)^+$ ions for the aldehydes. The following ions were observed during SIM (only the ions indicated by an asterisk) and total ion GC-MS analyses: for the Z9-14:Ac peak, 255* $(M+1)^+$, 283 $(M+29)^+$, 195* $(M+1-60)^+$, and 61 $(CH_3COOH_2)^+$; for Z9-14:Al, 211* $(M+1)^+$, 239 $(M+29)^+$, and 193* $(M+1-18)^+$; for Z11-16:Al, 239* $(M+1)^+$, 267 $(M+29)^+$, and 221* $(M+1-18)^+$.
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14. L. B. Bjostad and W. L. Roelofs, *Science* **220**, 1387 (1983). Four additional *M. cornigera* moth prey species belong to taxonomic groups (Hadeninae in the Noctuidae, and Crambinae in the Pyralidae) for which, for almost all studied species, members of this biosynthetic series are the principal components of the attractant blend [Y. Tamaki, in (*12*), pp. 145–191]. Some moth prey species are known for two other members of the *M. cornigera* species group: *M. dizzydeani* (*1, 2*) and *M. hutchinsoni* (*5*). Attractant blends for all four prey species for which information is available have as their major components members of the set: Z9-14:Ac, Z11-16:Al, and Z11-16:Ac—a subset of the equivalent set for *M. cornigera* prey. Very different sex pheromone compounds are used by some of the moth species captured by other *Mastophora* species (*2, 3*).
15. This study is the first step in our research program of (i) characterizing spider volatiles, (ii) reproducing spider volatile blends with synthetic compounds, and (iii) measuring the attractivity of these blends for various prey species. This is a more practical approach to understanding how spiders attract moths than bioassaying spider material directly. Spider material is very difficult to obtain; volatile collection is labor-intensive, and extraction of these difficult-to-find, hard-to-raise spiders is not practical. Until the factors controlling the variation in spider blends are understood, it will be necessary to perform assays with a variety of moths. Reproducing blends with synthetic compounds alleviates the difficulty of obtaining sufficient assay material.
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17. See (*3*) for a review of chemical mimicry systems and the possible effects of mimic-model coevolution on the composition of mimic and model signal blends.
18. W. Steck, E. W. Underhill, M. D. Chisholm, *J. Chem. Ecol.* **8**, 731 (1982); M. S. Mayer and J. R. McLaughlin, *Handbook of Insect Sex Pheromones* (CRC Press, Boca Raton, FL, in press). The effect of a component on the attractiveness of a moth sex pheromone blend is not simply determined by its presence or absence; blend attractiveness usually depends on the ratio of two or more components [W. Steck, E. W. Underhill, M. D. Chisholm, *Environ. Entomol.* **9**, 583 (1980); (*3*)].

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Carbon Tetrachloride at Hepatotoxic Levels Blocks Reversibly Gap Junctions Between Rat Hepatocytes

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Electrical coupling and dye coupling between pairs of rat hepatocytes were reversibly reduced by brief exposure to halogenated methanes ($CBrCl_3$, CCl_4, and $CHCl_3$). The potency of different halomethanes in uncoupling hepatocytes was comparable to their hepatotoxicity in vivo, and the rank order was the same as that of their tendency to form free radicals. The effect of carbon tetrachloride (CCl_4) on hepatocytes was substantially reduced by prior treatment with SKF 525A, an inhibitor of cytochrome P-450, and by exposure to the reducing reagent β-mercaptoethanol. Halomethane uncoupling occurred with or without extracellular calcium and did not change intracellular concentrations of calcium and hydrogen ions or the phosphorylation state of the main gap-junctional protein. Thus the uncoupling appears to depend on cytochrome P-450 oxidative metabolism in which free radicals are generated and may result from oxidation of the gap-junctional protein or of a regulatory molecule that leads to closure of gap-junctional channels. Decreases in junctional conductance may be a rapid cellular response to injury that protects healthy cells by uncoupling them from unhealthy ones.

HEPATOCYTES IN SITU OR SOON after dissociation into cell pairs communicate with each other through gap junctions (*1, 2*). One of the functions proposed for gap junctions is mediation of metabolic cooperation among coupled cells under physiological conditions. Liver gap-junctional channels can be closed by various treatments (*2*); uncoupling could serve to disconnect an unhealthy cell from healthy ones (*3*) and thereby protect them from loss of metabolically important substances and from spread of toxic molecules.

Cell death in a variety of pathological conditions is commonly attributed to a cascade of cytochemical changes that lead to the formation of an excess of oxidants (*4*). Halogenated hydrocarbons—a prime example is carbon tetrachloride (CCl_4)—are usually considered to be toxic in the liver by virtue of free-radical generation (*5, 6*), and the toxicity of several hepatotoxins is prevented by prior treatment with α-tocopherol, a free-radical scavenger (*7*). Cytochrome P-450 catalyzes the one-electron reduction of CCl_4 to yield as products chloride anion and the trichloromethyl radical (*8*). We report here that halogenated hydrocarbons decrease junctional conductance between hepatocytes. The effect may be due to an oxidant stress caused by free radicals since it is decreased by a blocker of cytochrome P-450 or a reducing agent, and it is not seen in cells in which the halogenated hydrocarbons presumably do not cause oxidant stress.

Dissociated hepatocytes were obtained with a collagenase perfusion technique (*2, 9*). Carbon tetrachloride and the other halomethanes tested were prepared as 5% (by volume) stock solutions in either pure ethanol or dimethyl sulfoxide (DMSO) and then diluted with Dulbecco's phosphate-buffered saline (PBS, Gibco) to the concentration used to perifuse the hepatocytes. At higher concentrations, solutions had to be used within 20 minutes or they lost potency because of separation of the halomethane from the aqueous phase. Electrical coupling was recorded under current clamp with four intracellular microelectrodes (resistance typically 10 to 20 Mohms when filled with 3*M* potassium chloride or 3*M* potassium citrate). Input and transfer resistances were used to calculate the conductance of junctional (g_j) and nonjunctional (g_{nj}) membranes by means of the pi-T transform (*10*).

When hepatocytes were perifused with CCl_4 (650 μ*M*) electrical coupling was blocked almost completely within 1 minute (Fig. 1). This uncoupling was due entirely to closure of gap-junctional channels; g_j decreased to less than 1% of its initial value while g_{nj} decreased slightly, the latter tending to increase coupling. Coupling returned when CCl_4 was washed out, although it appeared at a slower rate than that of the development of uncoupling. The incidence of dye coupling, evaluated by injecting Lucifer yellow into one cell of each of a large sample of pairs, went from 100% before treatment to 0% when CCl_4 was applied and reversed to 100% when CCl_4 was washed out (Fig. 2A).

Three additional halomethanes were tested, and two were found to block hepatocyte coupling (Fig. 2B). The relative potency of the halomethanes in uncoupling was $CBrCl_3 > CCl_4 > CHCl_3 > CH_2Cl_2$; the last was ineffective at the highest dose treated. The order of potency was the same as their

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