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Evidence for the Sensory Receptor(s) of the Mounting Sex Pheromone in Two Species of Ixodid Ticks, *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles

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by

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ABSTRACT

Evidence for the Sensory Receptor(s) of the Mounting Sex Pheromone in Two Species of Ixodid Ticks, *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles

James Stephen Phillips
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Director: Dr. Daniel E. Sonenshine

The foreleg claw sensilla were determined to be the receptors of the female contact sex pheromone, MSP (mounting sex pheromone), in *D. variabilis*, *D. andersoni* and *A. americanum* male ticks. In all three tick species, the claw sensilla consists of six anteriorly-directed setae arranged in three symmetrical pairs, two each on the opposite sides of the apotele of the claw and one on the ventral side. Behavioral bioassays and morphological study of these setae revealed that only the dorsal and middle (= lateral) pairs of claw sensilla are mechanogustatory, while the ventral pair are strictly mechanoreceptors. The dorsal and middle sensory setae exhibit a single pore-like structure located at their tip, a feature characteristic of gustatory or mechanogustatory sensilla similar to those found on the palps that are believed to function as pheromone receptors. Similarities in structure and function with contact chemosensilla of insects also are discussed.

In all three tick species, male mounting and post-mounting behaviors were suppressed only when the dorsal and middle pairs of claw sensilla were ablated or covered with gelatin; normal behavior was restored when the gelatin was removed. Dose-response bioassays were conducted with *D. variabilis* males to authenticate the results of the gelatin tests. The results of these bioassays demonstrated that the gelatin did not permit molecular penetration of the pheromone. Results of transfer of stimulus bioassays, in which only the MSP, cholesteryl oleate, was applied to inanimate objects (beads), demonstrated that male ticks responded only to the pheromone and mating behavior could be regulated by covering or uncovering the foreleg claw sensilla with gelatin. The significance of these findings for an understanding of mating behavior in ixodid ticks is discussed.

DEDICATION

To my parents, Arlington and Constance Phillips, no one could ask for more understanding parents, lo these many years; and especially to my wife Christine, whose constant love and support not only makes life bearable but makes every day a wonderful experience.

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INTRODUCTION

Semiochemicals (information-bearing compounds) are the basis of chemical communication among animal species. These chemical compounds are divided into three major categories that are classified according to their biological rather than their chemical composition. Allomones are compounds released by an individual of one species that alter the behavior of individuals of another species in a manner that benefits the emitter, such as defensive secretions. Conversely, kairomones are compounds released by an individual of one species that influence the behavior of individuals of another species in a manner that benefits the recipient, such as attractant odors. Pheromones, the most common type of semiochemical, are compounds released by one individual that affect the physiology and behavior of other individuals of the same species (Blomquist and Dillwith, 1983; Sonenshine, 1984a, 1985).

Within the phylum Arthropoda, the Insecta utilize all three types of semiochemicals to form a complex "chemical language". By comparison, the Acari appear to have a rather limited chemical communication system; however, this is probably due to the fact that the study of acarine chemical communication is still in its infancy. The true status of

the chemical language of this subclass is probably somewhat more complex than is presently known.

In insects, pheromones regulate many biological functions; they serve as food-finding and territory markers, intruder defense, aggregation and arrestant chemicals, sex attractants and ovipositional stimulants and deterrents. Acarine pheromones, in contrast with those of insects, appear to regulate fewer biological functions. The acarine pheromones are divided into: (1) alarm pheromones, which when released, usually due to injury, excite dispersal of conspecific individuals, (2) assembly pheromones which induce clustering of free-living individuals in favorable parts of the environment to ensure their survival during the nonparasitic life phase, (3) aggregation-attachment pheromones which regulate attachment of unfed individuals, on a host, by previously attached individuals, and (4) sex pheromones which, when released by individuals of one sex, regulate the mating behavior of the opposite sex. Ixodid and argasid ticks are highly dependent upon these chemical cues for assembly, aggregation-attachment (*Amblyomma* spp. only) and mating behaviors (Sonenshine, 1984b; Sonenshine *et al.*, 1986); thus, perception of these "messages" is crucial to the survival of the species.

Pheromones may be composed of a single chemical compound or mixtures of different compounds and may be volatile or

non-volatile. Sex pheromones foster mating between conspecific individuals although this is not always successful. In ticks, they include (1) volatile attractant sex pheromones which are perceived via olfaction, and (2) contact sex pheromones which elicit a response only when physical contact is made. The same pheromonal compound or compounds may be used by many species or they may be highly species specific (Sonenshine, 1986). For example, the common ixodid attractant sex pheromone (ASP), 2,6-dichlorophenol (2,6 DCP), is known to be present in at least 14 species of ticks in 5 genera (Khalil *et al.*, 1983; Sonenshine *et al.*, 1985) and provides little or no species-specific recognition. In contrast, differences in the concentration of the contact genital sex pheromone (GSP) of *Dermacentor variabilis* and *Dermacentor andersoni* prohibits interspecific mating between these two sympatric species (Allan *et al.*, 1989).

Much has been learned about the biology of pheromone perception, in addition to the pheromones themselves. In arthropods, most pheromone perception research has been conducted on insect olfaction. Olfactory sensilla are used to detect volatile compounds, even in extremely minute concentrations. For the detection of non-volatile pheromonal compounds, insects must make direct, physical contact with the chemical using gustatory sensilla. These sensilla usually require higher thresholds of the stimulant molecules

for detection as compared to olfactory sensilla. Olfactory and gustatory chemosensilla generally appear in the form of setae, but gustatory sensilla also occur as pits. Ticks, like insects, possess both types of chemosensilla for pheromone perception; however, much less is known concerning the function of acarine chemosensilla (Sonenshine, 1991).

The general structure of chemosensory sensilla is similar in most arthropods that have been studied. In insects, the chemosensilla are formed by trichogen and tormagen cells. They generally are composed of a seta, or hollow hair-like structure, which has branched or simple, unbranched pores along the wall or at the tip of the structure. The seta is situated in a socket, which is a pore in the cuticle that ends in an enlarged, rounded cavity at the dorsal surface. Bipolar neurons with dendrites are surrounded by thecogen cells which form a sheath, or scolopale, to insulate the neuron cell bodies. The dendrites are divided into the inner dendritic segment at the base of the sensillum and the outer dendritic segment which, surrounded by sensillum lymph, enters the setal shaft and is situated close to the pores. Axons from the receptor neuron cell bodies, located at the base of the seta, extend through the epidermis to fuse with other axons to form sensory nerves. A similar sensillum organization is believed to occur in ticks; however, specific trichogen, tormagen and thecogen cells have not been described. In contrast to insects, tick

chemosensilla are innervated by many neurons, presumably the result of extensive fusion, while only two or three normally are found innervating insect chemosensilla (Steinbrecht, 1987; Sonenshine, 1991); however, Zacharuk and Shields (1991) state that many insect multiporous sensilla are composite structures containing large numbers of neurons.

Two major types of olfactory sensilla occur in insects. Single-walled (SW) multiporous sensilla (sensilla basiconicum) facilitate entry of stimulant molecules through a pore-tubule system. Numerous pores occur along the wall of the sensillum. Each pore subdivides into several pore tubules that open into the sensillum lymph. The dendrites are branched and in close proximity to, but normally not in contact with the pore tubules. The pores contain plugs of an unknown material that resists the entry of stimulus molecules. Double-walled (DW) multiporous sensilla (sensilla coeloconicum) have spoke-channel systems for stimulus conduction. Very few pores occur in these sensilla and the dendrites are unbranched. Ticks also have the same two major types of olfactory sensilla that occur in insects; however, although the single-walled sensilla contain numerous pores on the sensillar shaft, the pore tubules appear to be absent. Ticks seem to lack branched wall pores. Thus, the function of the pores may be different from that of insects. Single-walled sensilla containing simple pores also may occur. The pores lead directly into

the cavity of the sensillum which contains branched or unbranched dendrites. The double-walled sensilla are similar to those of insects. Gustatory sensilla also are similar in both insects and ticks. These sensilla are terminal pore (TP) contact chemoreceptors that are often multifunctional, combining mechanoreception with their primary function. The sensilla are generally setiform with a single pore at or near the tip of the shaft. The pore leads directly into the cavity of the sensillum in which generally two dendrites are found (Steinbrecht, 1987; Sonenshine, 1991).

In insects, the pore-tubule/sensillum lymph transport model is widely accepted as the mechanism of stimulus molecule transport. The molecules are believed to dissolve in the surface lipids, diffuse along the cuticular surface to a pore, enter the pore-tubule system and bind to a specific protein which functions as a carrier to transport the molecule to the dendritic membrane (Steinbrecht, 1987; Sonenshine, 1991). Vogt (1987) proposes that specialized receptors on the dendritic plasma membrane bind to the stimulant molecules and elicit a receptor potential which initiates the nerve impulse. Enzymes (especially esterases), which are present in the sensillum lymph, degrade the stimulatory molecule enabling the membrane to repolarize and respond to a new stimulus. This system is

probably applicable for a wide range of gustatory and olfactory chemicals. Chemicals that are readily soluble in aqueous media could easily diffuse in the sensillum lymph to the dendritic membrane. In ticks, lipophilic stimulants could be transported across the sensillum lymph to the dendritic membrane by binding proteins and degraded by enzymes; thus, although it has not been investigated in mites or ticks, acarine chemoreception is believed to follow the insect model because of the similarities in their morphology (Sonenshine, 1991). However, during a study of tick olfactory sensilla ultrastructure, Leonovich (1987) noted that the pores of single-walled sensilla lacked pore tubules and contained well-developed central plugs of unknown material. These plugs were surrounded by thin annular membranes. Thin filaments from the membrane contacted the dendrites. This suggests that stimulant molecules could be relayed directly from the pores to the dendrites via the filaments, without the aid of carrier proteins. Such a mechanism might allow for more rapid transport of stimulant molecules but with considerably less specificity. Further study is needed to clarify the differences in chemosensory perception of odorants between ticks and insects.

There are three organs known to be used by ticks to detect pheromones. Haller's organ, on the dorsal surface of the first leg tarsi, is the most thoroughly studied and complex of the receptors. Olfaction is believed to be the

major function of this organ, although several of the anterior pit sensilla exhibit morphology consistent with a gustatory role. In ixodid species, Haller's organ consists of an anterior trough containing six or seven setiform sensilla of at least four distinct types (multiporose, grooved, fine and no-pore sensilla), alphanumerically labelled Ap (anterior pit) 1 through 6 or 7, and a posterior capsule with two types of sensilla. The anterior trough is completely exposed to the atmosphere, while the posterior capsule is exposed only by a narrow slit or small pore. There is also a group of setae on the ridge anterior to the trough which are similar to the Ap_1 sensillum (single-wall, multiporose) of the anterior trough (Foelix and Axtell, 1971, 1972; Chow and Wang, 1975; Homsher and Sonenshine, 1975, 1977, 1979; Leonovich, 1977; Hess and Vlimant, 1982, 1983a; Waladde and Rice, 1982; Sonenshine, 1991). The Haller's organ of argasid tick species is generally similar to that of ixodid ticks in setal morphology and ultrastructure (Roshdy *et al.*, 1972); however, there is considerable variation in the number of Ap sensilla (Roshdy *et al.*, 1984; Hoogstraal *et al.*, 1984).

The function of the Haller's organ sensilla has been tested in a number of studies. Haggart and Davis (1979), using electrophysiological techniques, demonstrated the presence of two types of NH_3-sensitive neurons in the anterior trough of and on the ridge anterior to Haller's organ in

Rhipicephalus sanguineus. In a later study, Haggart and Davis (1981), using the same methods, demonstrated that the Ap_1 sensillum in the anterior trough in *Amblyomma americanum* was highly sensitive to the volatile ASP, 2,6 DCP, and was moderately stimulated by other phenols. Waladde (1982), using advanced tip-recording techniques to obtain electrophysiological responses, showed that both the Ap_1 sensillum and the Md_3 seta on the anterior ridge detected 2,6 DCP in *R. sanguineus* and *Amblyomma variegatum*. Haller's organ also is responsible for detection of the male originated aggregation-attachment pheromone of *Amblyomma* spp.; however, the specific sensilla used to perceive the pheromone are unknown (Sonenshine *et al.*, 1986).

Less is known concerning the detection of pheromones by the other two organs, the palps and cheliceral digits of ticks. The terminal segment of the palps, article IV, of ixodid ticks, contains a cluster of gustatory setiform sensilla surrounded by larger mechanosensory setae located along the lateral edges of the segment. The palpal article IV, of adult ixodid ticks, is situated in a cavity on the ventral surface of article III (Ivanov and Leonovich, 1983), whereas, in argasid ticks and ixodid larvae, the segment forms the terminal end of the palps (Sonenshine, 1991).

Sensilla on the palps have been implicated in pheromone perception. Feldham-Muhsam and Borut (1971) stated that

male ixodid ticks would not copulate if the palps were amputated, but Sonenshine *et al.* (1984) reported that the ixodid tick *D. variabilis* copulated readily when one palp was removed; however, if both palps were ablated, copulations decreased. In argasid ticks, the palps are known to perceive assembly pheromones (Leahy *et al.*, 1975). Graf (1975) also reported palpal perception of assembly pheromones in the ixodid tick, *Ixodes ricinus*.

The cheliceral digits of ixodid ticks function in pheromone perception, but only in the recognition of the genital sex pheromone (GSP). These organs contain chemoreceptive, mechanoreceptive and probably thermoreceptive sensilla (Waladde and Rice, 1977, 1982; Sonenshine *et al.*, 1984). In *Boophilus microplus*, the chemoreceptor sensilla of the cheliceral digits play an important role in the perception of blood meal quality (Waladde and Rice, 1977, 1982). In *D. variabilis* and *D. andersoni*, copulation does not occur until the contact sex pheromone (GSP), in the female's anterior reproductive tract, is perceived by the chemosensory receptors on the male's cheliceral digits (Sonenshine *et al.*, 1984, 1985; Allan *et al.*, 1988, 1989). Recently, Allan *et al.* (1991) demonstrated that the same behavior occurred in *A. americanum* males. However, no such behavior occurred in *Amblyomma maculatum* males, a finding which led Allan *et al.* (1991) to conclude that a GSP was absent in

that species. Taylor *et al.* (1991), using electrophysiological techniques, demonstrated sensitivity of male *D. variabilis* and *D. andersoni* cheliceral digits to high doses of ecdysteroids that partially comprise the contact GSP of these species.

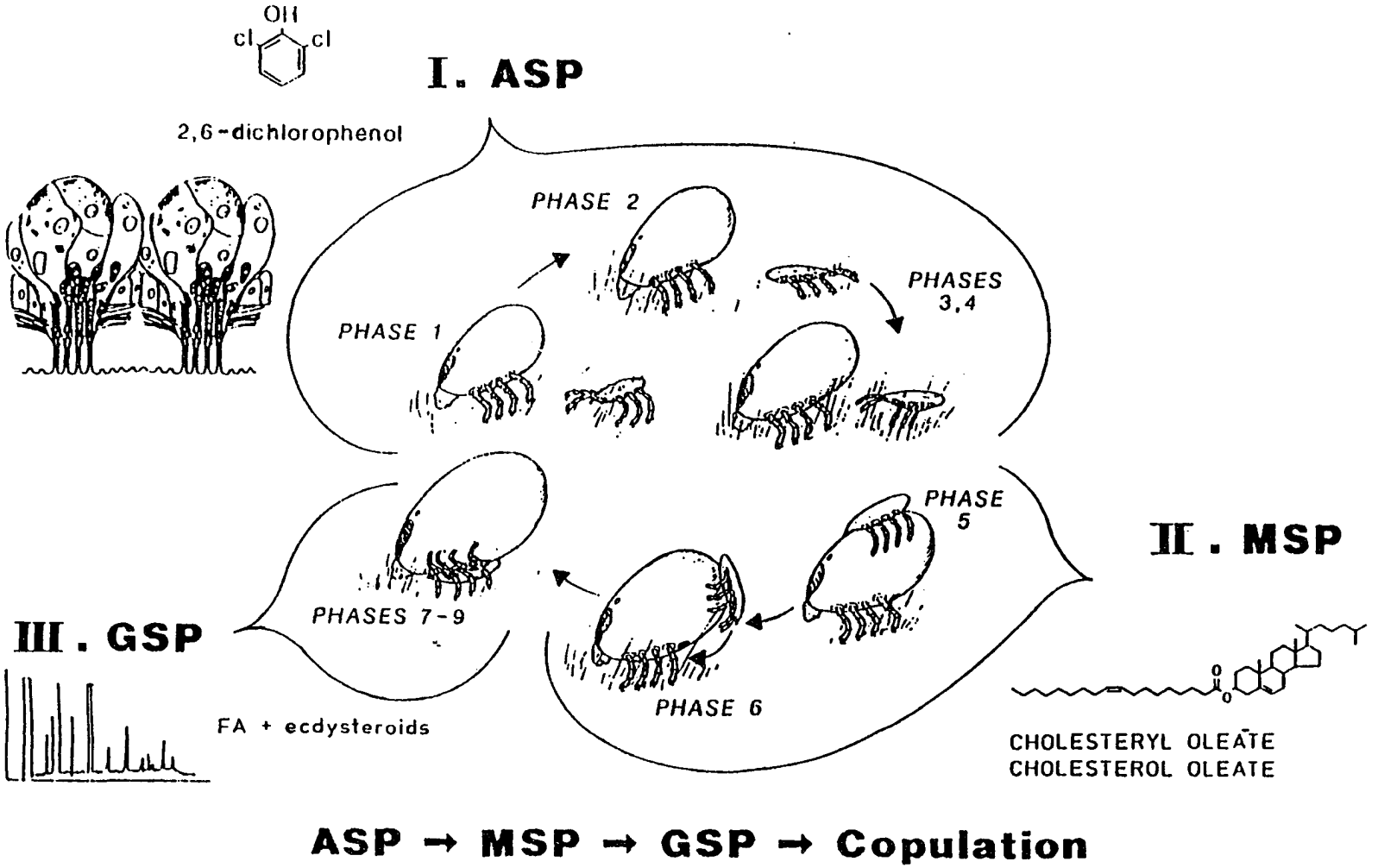
Recently, there have been an increasing number of reports describing new tick pheromones (eg., *R. evertsi* volatile and non-volatile assembly pheromones (Gothe and Neitz, 1985); aggregation-attachment pheromones of *A. variegatum* and *A. hebreum* (Schöni *et al*, 1984; Norval *et al*, 1989, 1991a, 1991b); GSP of *A. americanum* (Allan *et al*, 1991)), which suggests that more chemical cues remain to be discovered. The chemical composition of many of these pheromones has not been identified; however, even less is known about the dynamics of their perception by the recipient ticks. Hess and Vlimant (1986) note that most research on perception in ticks has thus far been limited in scope (except for Haller's organ) and only recently investigated. The authors also cite the near total neglect of work done on the great number of sensory hairs on the legs (other than the tarsi of the first legs) and body of the tick. To date, there has been little functional and behavioral experimentation performed with these sensilla to determine if they are receptors of pheromones; most studies have dealt solely with the sensillum morphology and ultrastructure.

Successful mating in ticks, as in most arthropods, requires a specific behavioral process, involving a complex hierarchy of individual components, regulated by chemical or physical stimuli. The *Dermacentor* spp. model of courtship behavior, which displays the typical pattern of mating behavior in most ixodid ticks, distinguishes nine discrete stages of sexual activity that are regulated by pheromones (Fig. 1). Until recently, it was believed that two different sex pheromones, produced by the female tick, regulated this behavior.

The first six stages, involving excitation of the male, orientation toward and mounting of the female, orientation on the dorsum and movement to the venter of the female, were believed to be controlled by the volatile ASP, 2,6 DCP, which the female secretes during blood-feeding (Sonenshine *et al.*, 1976). This chemical is detected by the sensilla in and, in some species, adjacent to the Haller's organ on the first leg tarsi of the male tick (Waladde, 1982; Sonenshine *et al.*, 1986).

The final three stages, gonopore location, recognition of species identity and mating, are regulated by a contact GSP (Sonenshine *et al.*, 1982) which is composed of fatty acids (Allan *et al.*, 1988, 1989) and ecdysteroids (Taylor *et al.*, 1991). During mating, this pheromone is detected by chemosensory receptors on the cheliceral digits of the

Figure 1. Model illustrating the behavioral stages that occur during courtship in *Dermacentor variabilis* and *Dermacentor andersoni*. Phase 1: Feeding female secretes the volatile attractant sex pheromone (ASP), 2,6-dichlorophenol (2,6 DCP), from the foveae dorsales, exciting the attached feeding male to detach. Phase 2: The detached male begins searching for the emitting female. Phases 3 and 4: The sexually excited male orients to and approaches the pheromone secreting female. Phase 5: The male contacts the dorsum of the female, perceives the contact mounting sex pheromone (MSP), cholesteryl oleate, secreted onto the cuticle as a byproduct of the blood meal, mounts and moves anteriorly to the region of the foveae dorsales. Phase 6: The male turns, moves posteriorly on the dorsum of the female and proceeds to the venter. The female lifts its body to facilitate the male's movements. Phases 7-9: The male encounters the contact genital sex pheromone (GSP), a mixture of fatty acids and ecdysteroids, secreted from the genital tract and positions itself at the genital aperture, places its legs between those of the female and flexes its capitulum to probe the aperture. The male's chelicerae are inserted into the aperture and copulation ensues.



male's mouthparts when they are inserted into the vulva of the anterior reproductive tract of the female, as noted above.

Experimentation by Hamilton and Sonenshine (1988) and Hamilton *et al.* (1989) demonstrated the presence of a third pheromone, the mounting sex pheromone (MSP), which when perceived by the male tick enabled it to recognize a fed female as a prospective mating partner. The MSP, was determined to be a contact sex pheromone which regulates the male tick behaviors of mounting, dorsal orientation and searching for the female gonopore (Fig. 1; Phases 5-6). The regulation of these behaviors previously had been attributed to the ASP.

The MSP has been identified in *D. variabilis* as cholesteryl oleate, a steryl ester which is secreted onto the female cuticle during blood-feeding (Hamilton *et al.*, 1989). Experiments by Sonenshine *et al.* (1991) have indicated that the MSP occurs not only in the genus *Dermacentor* but also in the genera *Amblyomma* and, possibly, *Rhipicephalus*.

Although the receptors that perceive the ASP and GSP have been determined in previous studies, nothing is known regarding the means by which ticks detect the MSP. The fact that the MSP is a contact pheromone suggests that a gustatory sense organ is used by the male to perceive the chemical. The sensilla on the chelicerae and palps are the only known chemoreceptive structures that detect contact pheromones in

ticks that have been studied. However, experiments by Sonenshine *et al.* (1984) demonstrated that excision of the palps and chelicerae of *D. variabilis* would not eliminate the earliest stages of mate recognition, namely, orientation, mounting and the search for the gonopore. Clearly, these structures are not the receptors of the MSP in this species (no experiments were conducted with *D. andersoni*). Thus, until this study's report, the manner in which the MSP of ticks is perceived was unknown.

The purpose of this study was to, (1) identify the sensory site(s) of MSP perception in *D. variabilis* and *D. andersoni* males, (2) describe and characterize the sensillum(a) involved, determining whether the receptor is of a known category or a new, previously undescribed type, (3) determine to what degree the receptor responds to the MSP and its significance in the courtship behavior of the two species and (4) determine if a species from a different genus, namely, *A. americanum*, also known to use MSP in its courtship activity (Sonenshine *et al.*, 1991), perceives the pheromone with the same sensory site(s) as species of the genus *Dermacentor*.

Materials and Methods

Ticks

Three species of ticks were used in this study; all were reared in the laboratory in accordance with previously described techniques (Sonenshine et al., 1976). *Dermacentor variabilis* (Say), the American Dog Tick, and *Amblyomma americanum* (L), the Lone Star Tick, were colonized from wild specimens collected near Suffolk, Virginia. *Dermacentor andersoni* Stiles, the Rocky Mountain Wood Tick, was colonized from specimens obtained from the U.S. Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana. *D. variabilis* larvae and nymphs were fed on albino rats (*Rattus norvegicus*), adults on laboratory rabbits (*Oryctolagus cuniculus*). All life stages of *A. americanum* were fed on laboratory rabbits. Larvae of *D. andersoni* were fed on hamsters (*Mesocricetus auratus*), nymphs and adults on laboratory rabbits. Except when feeding on hosts, all ticks were held in an Aminco-Aire Climate Laboratory Incubator (American Instrument Co., Silver Spring, MD) at 27 $\pm$ 1°C and 92 $\pm$ 2% relative humidity.

Behavioral Study

D. variabilis, *D. andersoni* and *A. americanum* male and female adult ticks were blood-fed on laboratory rabbits for seven days prior to studying their mating behavior. Consequently, at this time, female ticks were producing pheromones and the males were sexually active.

The behavior of 30 male ticks, of each species, to conspecific females was observed using a Bausch and Lomb MonoZoom 7E dissecting microscope (7x magnification) (Bausch and Lomb, Rochester, NY) connected by a Panasonic color camera (Model WV-CD110A) to a Panasonic color video monitor (Model CT-2010Y) (Matsushita Communications Industrial Co., Ltd., Japan) with a screen size of 30.48 cm (height) x 40.64 cm (width). A female tick was taped, by the anterior portion of its body (not covering the 2,6 DCP-secreting foveae dorsales), in the center of a petri dish (9 cm diameter) which was placed on the dissecting microscope stage. A conspecific male tick was placed $\leq$2 cm from the female and the tick behavior was viewed on the monitor. Using this system, the parts of the male tick's body that were in contact with the female during the mounting phase and subsequent steps of the mating process were identified.

After studying the courtship behavior of the ticks, the males were taped, on their dorsal sides, to a microscope slide. A stereoscopic microscope (Wild Heerbrugg, Switzerland) and a Nikon Optiphot differential interference contrast microscope (Nippon Kogaku K. K., Tokyo, Japan) then were used, because of their higher magnification and resolution, to locate, identify and enumerate the structures of the males which were in contact with the females.

Specimen Preparation for Scanning Electron Microscopy (SEM)

Prior to SEM preparation, unfed, adult, male and female ticks, of all three species, were cleaned in the following manner to remove any debris that might adhere to the cuticle or setae of the tick's body. Ticks were immersed in a beaker of acetone and "scrubbed" with a fine, soft-bristled brush for 7 minutes. At this time, the ticks were removed, placed into a separate beaker of acetone for 10 minutes and subsequently sonicated for 5 minutes. The ticks then were rinsed with distilled water (DI) and placed in a separate beaker of DI water for 10 minutes. The ticks were immersed in acetone again and the sonication and DI rinse were repeated. This procedure was performed a total of three times (3x) and ultimately resulted in the death of the tick. When the procedure was concluded, the ticks were blot-dried on filter paper. Unfed nymphs, of all three tick species, were prepared in the same manner; however, the brush "scrubbing" was excluded due to the fragile nature of this immature life stage.

Cleaned ticks were transferred to 2% osmium tetroxide (OSO_4) (Polysciences Inc., Warrington, PA) and osmicated for 2 hours at room temperature (27°C). After osmication, the ticks were rinsed three times (3x), at 10 minutes per rinse, in DI water. The ticks were processed through the following graded dehydration series in ethanol: 30% (10 min), 50% (10 min), 70% (10 min), 95% (10 min) and 100% (2x, 15 min each).

The ticks then were immersed in acetone two times (2x), at 15 minutes per immersion, and subsequently transferred to a Denton critical point dryer (CPD) (Denton Vacuum Inc., Cherry Hill, NJ). CPD was used to alternatingly soak and flush the specimens with liquid carbon dioxide (CO_2) three times (3x), each for 5 minutes. Following CPD, the ticks were mounted, on their dorsal sides, on double-sided carbon tape on top of aluminum stubs. The ticks were viewed with a dissecting microscope, and using forceps, all of their tarsi were positioned so that they were perpendicular to the stub. The ticks then were placed in a Polaron Autocoating Unit (E5200) (Bio-Rad Microscience Division, Cambridge, MA) and sputter-coated with gold/palladium (Au/Pd). The coating thickness on the adult ticks was approximately 150 Å (angstroms), while on nymphs it was approximately 80-100 Å.

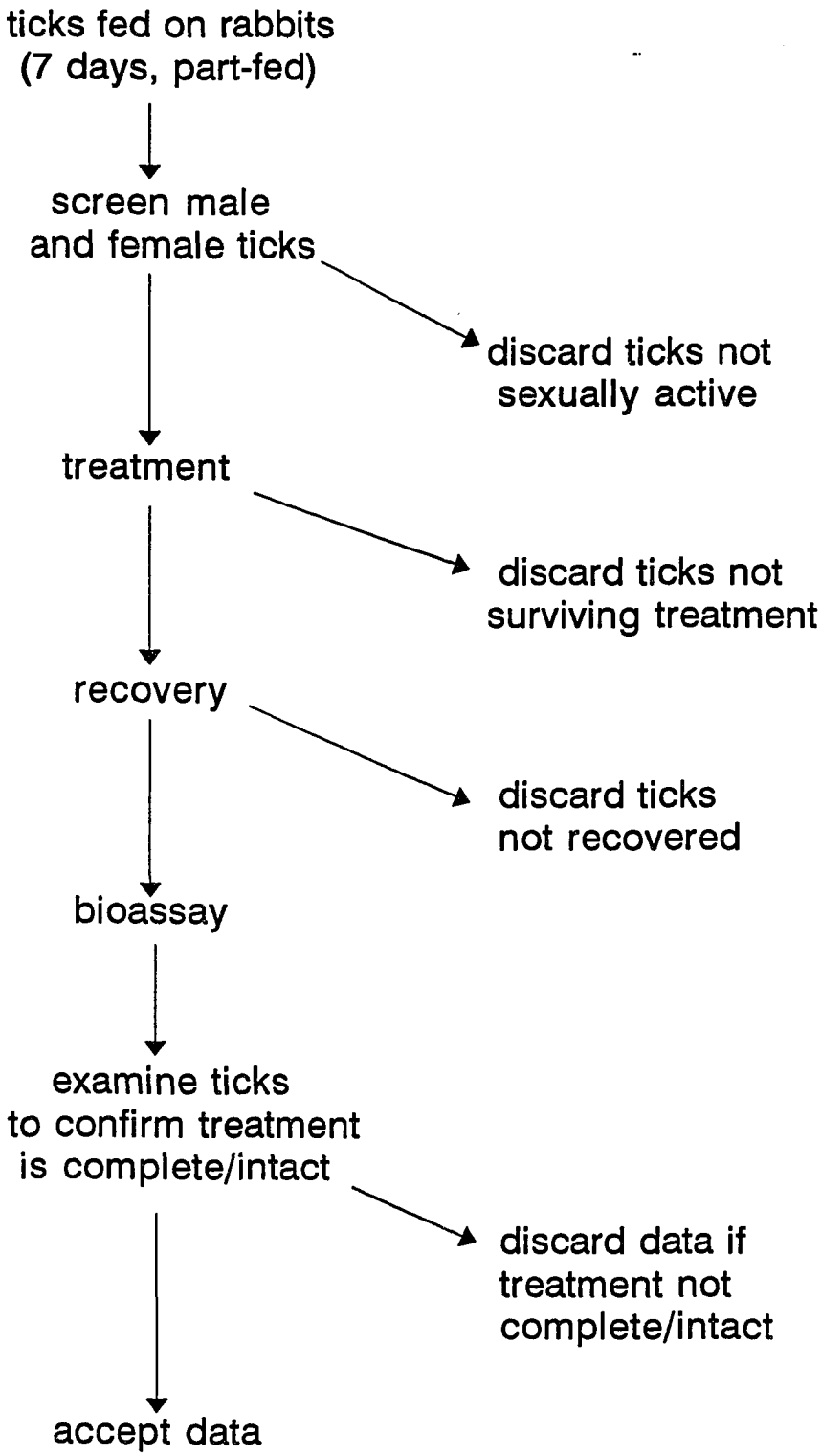
Morphological structures of the tick that were involved with the mounting behavior were viewed and photographed using two scanning electron microscopes. A Cambridge Stereoscan 100 (Leica Instruments Inc., Edison, NJ), set at 5 and 10 kilovolts (KV), was used to take low magnification photomicrographs, while a Philips SEM 515 (Philips Export B.V., Eindhoven, Netherlands) was used to take high magnification photomicrographs. Images were collected on Polaroid type 55 positive/negative (PN) film (Polaroid Corp., Cambridge, MA). When the specimens were not being used, they were held in a desiccating cabinet to prevent contamination.

Bioassays

All species and sexes of adult ticks were blood-fed on laboratory rabbits (*O. cuniculus*) for seven days prior to the bioassay. At this time, female ticks are known to produce pheromones (Sonenshine *et al.*, 1974; Kellum and Berger, 1977) and the males are sexually active. To ensure these conditions before experimentation, both male and female ticks were screened. Screening consisted of a female tick being placed in a 15 cm diameter petri dish in the manner previously described (see Behavioral Study). Males were released near conspecific females and allowed to proceed through the stages of courtship behavior to the gonopore-probing phase, at which point they were removed. If the male's response was positive to the female, then the male was used for testing. In this manner, the male acted as its own control. These male ticks, however, were not used as controls in the analyses; separate males were used as controls. A positive response also indicated that the female was producing pheromones and could be used for experimentation.

The male ticks then were prepared for bioassay as described in the following sections, Categories and Treatments. Generally, the bioassay procedure (Fig. 2) consisted of a conspecific female tick taped, by the anterior portion of its body (not covering the 2,6 DCP-secreting foveal glands), in the center of a petri dish (15 cm diameter). A

Figure 2. Flow diagram of bioassay procedure.



"treated" male tick was released ≤2 cm from the female to test its mating behavior response at one or more of the behavioral steps included in the courtship stages for which the MSP is responsible: initial contact with the female, mounting the posterior dorsum of the female, dorsal anterior orientation to the female's foveae dorsales, dorsal posterior orientation and ventral orientation. Each male received three opportunities to proceed through the courtship behavior step(s). Responses were considered positive if the males completed the behavioral step(s) and negative if, after three attempts, the male failed to complete the behavior(s). The length of time that it took for the male to complete the behavioral step(s) was recorded, to be analyzed in the event that more than one receptor was responsible for perceiving the MSP. This would be indicated by a statistically significant response when more than one receptor was tested. All screening and bioassays were conducted in a controlled environment chamber (Western Environmental, Napa, CA) at 28 ± 2.0°C and 90 ± 2% relative humidity.

Categories

The following bioassays were conducted on *D. variabilis* and *D. andersoni* male ticks to identify the sensory receptor of the MSP:

Category 1: Structures located on the most distal portion of the tarsus on the first pair of legs were bioassayed at

initial contact with the female to determine if the male tick would proceed with the mounting behavior. The structures tested were the claw, claw sensilla and first pair of ventral setae, all of which were determined, through behavioral studies, to initially contact the female. A single lateral seta on each first leg tarsus (la I; Hess and Vlimant, 1986), located posteriorly to the claw sensilla, also were bioassayed, although generally they do not appear to contact the female during this phase of the courtship ritual. Observations of thirty male ticks, of both species (see Behavioral Study), indicated that these lateral setae are directed anteriorly at either a 90° (horizontal) or 45° angle and are normally not long enough to establish initial contact with the dorsum of the female. During mounting and throughout the post-mounting behaviors, due to the angle of the male's tarsus, as well as the angle of the lateral setae and their varying length, the lateral setae may come into contact with the body of the female tick more frequently than they did during initial contact. To ensure their function in regards to MSP perception, however, the lateral setae were tested in all of the bioassay categories. The receptors listed above also were bioassayed using *A. americanum* to determine if this species perceived the MSP with the same receptor(s) as the species in the genus *Dermacentor*.

Category 2: Structures that were not involved with initial contact of the female were bioassayed. Due to the location of these structures, potential contact could be made as the male mounted the female; however, based on the behavioral studies, most of the structures bioassayed rarely contacted the female during this phase of the courtship ritual. The structures bioassayed were all eight claws, the claw sensilla of the second, third and fourth pair of legs (the last three pairs of claws and the claw sensilla of legs 2-4 do not initially contact the female), the lateral seta on the tarsus of each first pair of legs (see Category 1), the mouthparts and the setae of the venter and ventral and lateral portions of all eight legs.

The first pair of dorsal setae on the tarsus of the first pair of legs are located posteriorly to the lateral seta, on the ridge anterior to the Haller's organ. The setae are angled vertically. Due to the distal location of these setae, from the point of initial contact, they are not involved with the initial mounting behavior; however, because of the position of the male's tarsus during mounting and the subsequent courtship behaviors, potential contact might be made with the body of the female tick, although the behavioral study determined this to be extremely rare. To ensure their function in regards to MSP perception, the first pair of dorsal setae were tested. The dorsal region of the tarsus between the claw sensilla and the first pair

of dorsal setae was bioassayed in conjunction with the first pair of dorsal setae in the event that any known or unknown slit sense and stretch receptor-like organs, that may function as contact chemoreceptors, could be eliminated as a receptor of the MSP.

The tarsi of legs 2-4 differ from those of the first pair of legs in that they lack a Haller's organ and have fewer dorsal setae. Due to the position of the male's tarsus during mounting and post-mounting behaviors, the setae of the tarsi of legs 2-4, as well as any known or unknown slit sense organs that might be present, also were bioassayed.

The bioassays were conducted from initial contact through mounting of the posterior dorsum of the female. The tests were conducted to determine if any other receptors assisted the tick in MSP perception while mounting the female, after initial contact had been established.

Category 3: The same structures that are listed in Category 2 were bioassayed; however, testing began with dorsal anterior orientation, after the male had mounted the female, and proceeded through dorsal posterior and ventral orientations. These bioassays were conducted to determine if any other receptors assisted the male in MSP perception, after mounting of the female, that would in turn initiate the subsequent behavioral steps. In Category 3, a

greater number of bioassayed structures were commonly in contact with the body of the female tick than there were with the same structures in Category 2, especially with *D. andersoni*. *A. americanum* bioassays were not conducted in Category 2 or 3.

Treatments

Category 1:

I.) Ablation of the claw, claw sensilla and first pair of ventral setae

Male ticks of each species were taped, on their dorsal sides, to microscope slides and placed in a refrigerator (≈ 4.0°C) for 1 to 2 minutes, to minimize leg movement while the ablations were performed. A thin strip of tape was placed across the first pair of legs, posterior to the Haller's organ, to further immobilize the legs. This treatment avoided loss of the Haller's organ which is essential for perception of 2,6 DCP. The distal portion of the tarsus of the first pair of legs, including the claw, claw sensilla and the first pair of ventral setae, was ablated, using a razor blade and microsurgical scalpel, while viewed with a Wild stereoscopic microscope. If any other portion of the tarsus was damaged during the ablation, the tick was not used in the experiment. The treated ticks were placed in an enclosed petri dish in a controlled environment chamber (see Bioassay section), for two hours, to recuperate and become active (to recover from possible traumatic effects of the

treatment that might affect experimentation) before being bioassayed. At the time of testing, those ticks that did not orient to the female, after three attempts, were discarded. After the bioassays were completed, the ticks were viewed with a stereoscopic microscope to ensure that all of the structures were ablated. If this condition was not met, the tick and the corresponding data were discarded. Controls were ticks in which the distal portion of the tarsus of the first pair of legs was not ablated.

Ia.) To determine if the ablation of the distal portion of the tarsus created a traumatic effect from which the tick could not fully recover, and thus would result in the occurrence of a false negative response to mounting, the same treatment was performed on the same portion of the tarsus of the second pair of legs.

II.) Claw ablation

The same procedure used for the ablation of the distal portion of the tarsus (see I above) was used to ablate the claw from the first pair of legs of male ticks of each species. The claw was ablated at the tarsal juncture while the claw sensilla and first pair of ventral setae remained intact. If the tarsus, claw sensilla or first pair of ventral setae was damaged during the ablation, the tick was not used in the bioassay. The ticks were placed in a controlled environment chamber, for 1 to 1.5 hours, to

recuperate and become active before being tested. Ticks that did not orient to the female during the bioassays, after three attempts, were discarded. Controls were ticks in which the claws were not ablated.

III.) Claw sensilla ablation

The claw sensilla (and the claw) were ablated from the tarsus of the first pair of legs of male ticks of each species. This was accomplished by ablating the tip of the tarsus containing the claw-tarsal juncture and the claw sensilla. The same procedure, as described above, was used to perform the ablations. The ticks were allowed to recover in a controlled environment chamber for 1 to 1.5 hours before being bioassayed. Ticks damaged during the ablation and those that did not orient to females after the ablation were discarded. Controls were ticks in which the claw sensilla were not ablated.

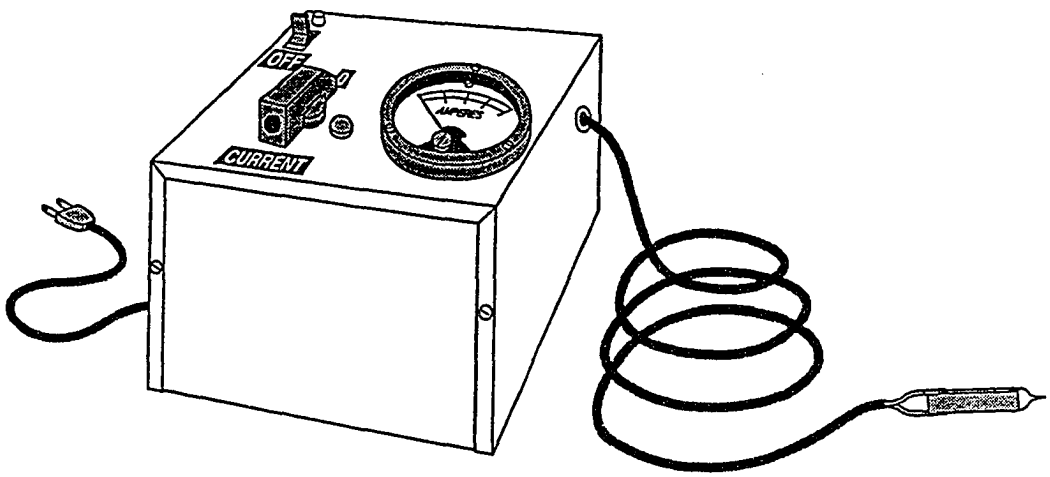
IV.) Ablation of the first pair of ventral setae

The first pair of ventral setae were ablated from the tarsus of the first pair of legs of male ticks of each species. The ablation preparation was identical to those listed above; however, the ablation was performed by using a heated wire attached to a voltage regulator (Fig. 3). The temperature of the wire was controlled by regulating the current it received. When the heated wire contacted a seta, it was depilated ("burned-off") to the point of attachment

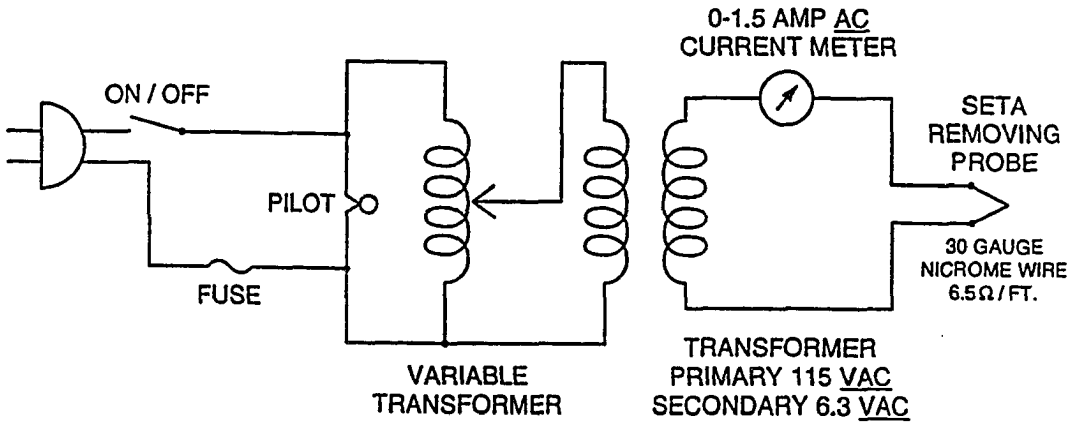
Figure 3-A. Schematic of the voltage regulator used to depilate setae.

Figure 3-B. Schematic of the electrical system of the voltage regulator.

A



B



on the leg, without damaging the leg. If the leg was damaged during the ablation, the tick was not used in the experiment. The ticks were placed in a controlled environment chamber, for 2 hours, to recuperate and become active before being bioassayed. At the time of testing, those ticks that did not orient to the female, after three attempts, were discarded. Controls were ticks in which the first pair of ventral setae on the tarsus of the first pair of legs were not ablated.

V.) Ablation of the lateral setae

The lateral seta was ablated from each tarsus of the first pair of legs of male ticks of each species using the depilation method described previously. The males were taped, on their ventral sides, to microscope slides. The ablation preparation, treatment and recovery were identical to those listed above. At the time of testing, those ticks that did not orient to the female, after three attempts, were discarded. Controls were ticks in which the lateral seta of each tarsus of the first pair of legs was not ablated.

Category 2 & 3:

The same treatments (detailed below) were conducted on male ticks in both Category 2 and 3; however, tick behavior in Category 2 was recorded from initial contact through mounting of the posterior dorsum of the female,

whereas, in Category 3, tick behavior was recorded from dorsal anterior orientation through ventral orientation. In both categories, if the "treated" male tick did not orient or attain the initial behavioral stage from which it was being tested, the male was discarded.

I.) Claw ablation

The ablation preparation, treatment and recovery of male ticks of both species were identical to those of ticks in which the claws of the first pair of legs were ablated in Category 1; however, in Category 2 and 3 all eight claws were ablated. Controls were ticks in which none of the claws were ablated.

II.) Ablation of the claw sensilla of legs 2-4

The ablation preparation, treatment and recovery of male ticks of both species were identical to those of ticks in which the claw sensilla of the tarsus of the first pair of legs were ablated in Category 1; however, in Category 2 and 3 only the claw sensilla of the second, third and fourth pair of legs were ablated. The claw sensilla of the first pair of legs remained intact. Controls were ticks in which none of the claw sensilla of legs 2-4 were ablated.

III.) Ablation of the lateral setae

The lateral seta of each tarsus of the first pair of

legs of both species of male ticks was depilated as described in Category 1 (see Ablation of the first pair of ventral setae). The ablation preparation, treatment, recovery and controls also were the same as those found in the section on the ablation of the lateral setae in Category 1.

IV.) Mouthpart ablation

Male ticks were taped, on their dorsal sides, to microscope slides. The refrigerator preparation (see Treatments, Category 1) was performed to limit movement of the tick during the ablations. Using a Wild stereoscopic microscope, razor blades and microsurgical scissors and scalpels, the following ablations were performed on males of both species:

(1) left palp to the basis capitulum

(2) right palp to the basis capitulum

(3) both palps to the basis capitulum

(4) left chelicera (sheath and digits)

(5) right chelicera (sheath and digits)

(6) both chelicerae (sheaths and digits)

(7) palps (to basis capitulum) and chelicerae (sheaths and digits)

(8) hypostome to basis capitulum

(9) all mouthparts (palps, chelicerae and hypostome to basis capitulum)

The ticks were placed in a controlled environment chamber, for 1 to 1.5 hours, to recuperate and become

active, before being bioassayed. At the time of testing, the ticks that were not active, were discarded. If after testing it was noted (via a microscope) that the ablation was not complete, the tick and corresponding data were discarded. Controls were ticks in which none of the mouthparts were ablated.

V.) Covering of the setae of the venter and legs with gelatin

The gelatin used in these treatments was a warm 10% solution (using distilled water (DI)) of bovine skin type III (≈ 225 bloom: Sigma Chem. Co., St. Louis, Mo.). This gelatin was selected for its hydrophobic properties and virtually non-porous state after application. Due to these properties, the gelatin coating created an impenetrable barrier to contact pheromone perception. The gelatin also was easily applied to the tick and once hardened could be removed (peeled and/or gently scraped) using a forceps without damage to the surface of the tick and the setae. This treatment was divided into two parts and male ticks of each species were treated in each of the following manners; (1) setae and the surface of the venter, including the basis capitulum, coxae, coxal spurs and venter proper, were covered with a thin coating of gelatin, so that neither the setae nor any known or unknown slit sense and stretch receptor-like organs would contact the body of the female tick (although all known slit sense organs are a type of

mechanoreceptor with no chemoreceptive function (Hess and Vlimant, 1984, 1986) the surface of the venter was covered with gelatin to ensure the authenticity of the results); (2) the lateral and ventral setae and these surfaces (including the first pair of ventral setae of the first leg and the claw sensilla of legs 2-4 but not leg 1; the lateral setae of leg 1, located between the claw sensilla and Haller's organ, were tested previously and thus were not tested along with the other lateral setae of leg 1 during this experiment) of all the legs were covered with a thin coating of gelatin so that neither the setae nor any known or unknown slit sense and stretch receptor-like organs would contact the female tick body during the courtship behavior. The joints of all of the legs were covered in such a manner as to allow for limited leg movement. Male ticks of both species were taped to microscope slides on their dorsal sides. Thin strips of tape were placed vertically over all of the tick's legs to immobilize them while gelatin was applied to the venter. Conversely, a thin strip of tape was placed vertically over the length of the tick's body when the tick's legs were covered with gelatin. The gelatin was applied to the tick with a fine straight pin while viewed with a Wild stereoscopic microscope. The ticks were air-dried (gelatin solidified) at room temperature ($\approx$ 24 °C), for 0.5 hours, to recuperate from the procedure and become active; at this time they were bioassayed. The ticks

that were not active, at the time of testing, were discarded. Upon completion of the bioassay, the ticks were viewed under a microscope to ensure that the gelatin coating was intact (i.e., it did not break apart or setae did not protrude through the gelatin) and it was not loosened by the tick's movement during the bioassay. If any of these conditions was noted, the tick and corresponding data were discarded. Controls were ticks in which none of the setae and surface of the venter or legs were covered with gelatin.

VI.) Covering of the first pair of dorsal setae with gelatin

The first pair of dorsal setae, located on the ridge anterior to Haller's organ on the first pair of legs, and the dorsal surface of the leg between the claw sensilla and the first pair of dorsal setae were covered with gelatin. The surface of the leg was covered with gelatin in the event that any known or unknown slit sense and stretch receptor-like organs, that might function as contact chemoreceptors, could be eliminated as receptors of the MSP. Male ticks of both species were taped on their ventral sides to microscope slides and a thin strip of tape was placed across the first pair of legs, posterior to Haller's organ. The previously described gelatin treatment was used in applying the gelatin to the dorsal setae and leg surface of the first pair of legs. The ticks were air-dried (gelatin solidified) at room temperature ($\approx$ 24°C) for 0.5 hours, at which time they

recuperated from the procedure and became active. The ticks that were not active or did not orient to the female, at the time of testing, were discarded. Upon completion of the bioassay, the ticks were viewed with a Wild stereoscopic microscope to ensure that the gelatin coating was intact (i.e., it did not break apart or the setae did not protrude through the gelatin) and it was not loosened by the tick's movement during the bioassay. If any of these conditions were noted, the tick and corresponding data were discarded. Controls were ticks in which the first pair of dorsal setae and dorsal tarsal surface were not covered with gelatin.

VII.) Covering of the dorsal tarsal surface and setae of legs 2-4

The dorsal tarsal surface and setae of legs 2-4 were covered with gelatin using the treatment previously described. The dorsal surface was coated to account for any slit sense and stretch receptor-like organs in that region. The ticks were taped on their ventral sides to microscope slides and a thin strip of tape was placed vertically over the length of the tick's body to further immobilize the tick while the gelatin was applied with a fine straight pin. The gelatin drying, recovery and bioassay procedures were like those previously described. Controls were ticks in which the dorsal tarsal surface and setae of legs 2-4 were not covered with gelatin.

Additional Mounting Behavior Bioassays

I.) Ablation of the claw sensilla from either the right or left first leg

These bioassays were conducted to determine whether the claw sensilla of both first legs were necessary to perceive the MSP. *D. variabilis* and *D. andersoni* male ticks were treated in either of the following manners; (1) the claw sensilla were ablated from the right first leg of the tick while the claw sensilla of the left first leg remained intact; (2) the claw sensilla were ablated from the left first leg of the tick while the claw sensilla of the right first leg remained intact. The ablations were conducted as previously described (see Category 1, claw sensilla ablation). Controls were ticks in which the claw sensilla of neither first leg were ablated.

II.) Ablation of the ventral pair of claw sensilla from the first pair of legs

These bioassays were conducted to determine whether the ventral pair of claw sensilla of the first pair of legs, which have been reported in previous studies to be mechanoreceptive setae (Chu-Wang and Axtell, 1973; Axtell, 1974; Hess and Vlimant, 1986), were necessary to perceive the MSP. The setae were depilated, from *D. variabilis* and *D. andersoni* male ticks, in the manner previously described. The bioassay procedure first consisted of each male tick being tested with a conspecific female with the ventral pair of claw sensilla of the first pair of legs intact. The male

was scored as positive if it mounted the female and negative if, after three attempts, it failed to mount the female. In these bioassays, the male acted as its own control. The ventral claw sensilla on both first legs then were ablated and each male placed in a separate vial so as to identify it. The male was placed in a controlled environment chamber, for 2 hours, to recover from the depilation. Following recovery, the male was tested again for its mounting behavior with a conspecific female; scoring was identical to that described above. In this manner, it was determined if a male tick which mounted a female with the ventral claw sensilla intact, likewise was able to mount the female after these setae had been ablated.

Additional Post-Mounting Behavior Bioassays

I.) Ablation of the claw sensilla of the first pair of legs, with placement of the male on the dorsum of the female

These bioassays were conducted to determine whether the claw sensilla of the first pair of legs were necessary for the male tick to complete the post-mounting behaviors or if some other receptor is utilized. It was noted in previous experiments (Phillips, unpublished data) that mounting behavior could be circumvented artificially if a pre-screened, sexually active male was placed directly onto the posterior dorsum of a partially-fed (7 day) female. Four different behavioral patterns occurred: (1) the male moved

directly to the female's foveae dorsales, (2) the male moved erratically, in a random direction, on the female's dorsum, then proceeded to the foveae dorsales; or in some cases, the male would move from the dorsum to the venter, then return to the dorsum and proceed to the foveae dorsales, (3) the male tick withdrew from the female and then mounted in the normal manner, (4) the male, with little or no dorsal movement, withdrew from the female and did not remount. It was noted that behaviors 1 and 2 occurred more frequently than behaviors 3 and 4. This wide range of behavioral results may indicate the involvement of another receptor, besides the claw sensilla, regulating dorsal and ventral orientation behavior. The ablations were conducted on *D. variabilis* and *D. andersoni* male ticks, as previously described (see Category 1, claw sensilla ablation). The bioassay consisted of a male tick, with the claw sensilla of the first pair of legs ablated, being placed on the posterior dorsum of a conspecific female. The subsequent behavior was scored as positive if the male completed dorsal and ventral orientation and negative if, after three attempts, the male failed to complete the courtship ritual. Controls were ticks in which the claw sensilla of the first pair of legs were not ablated and the tick was placed on the posterior dorsum of the female.

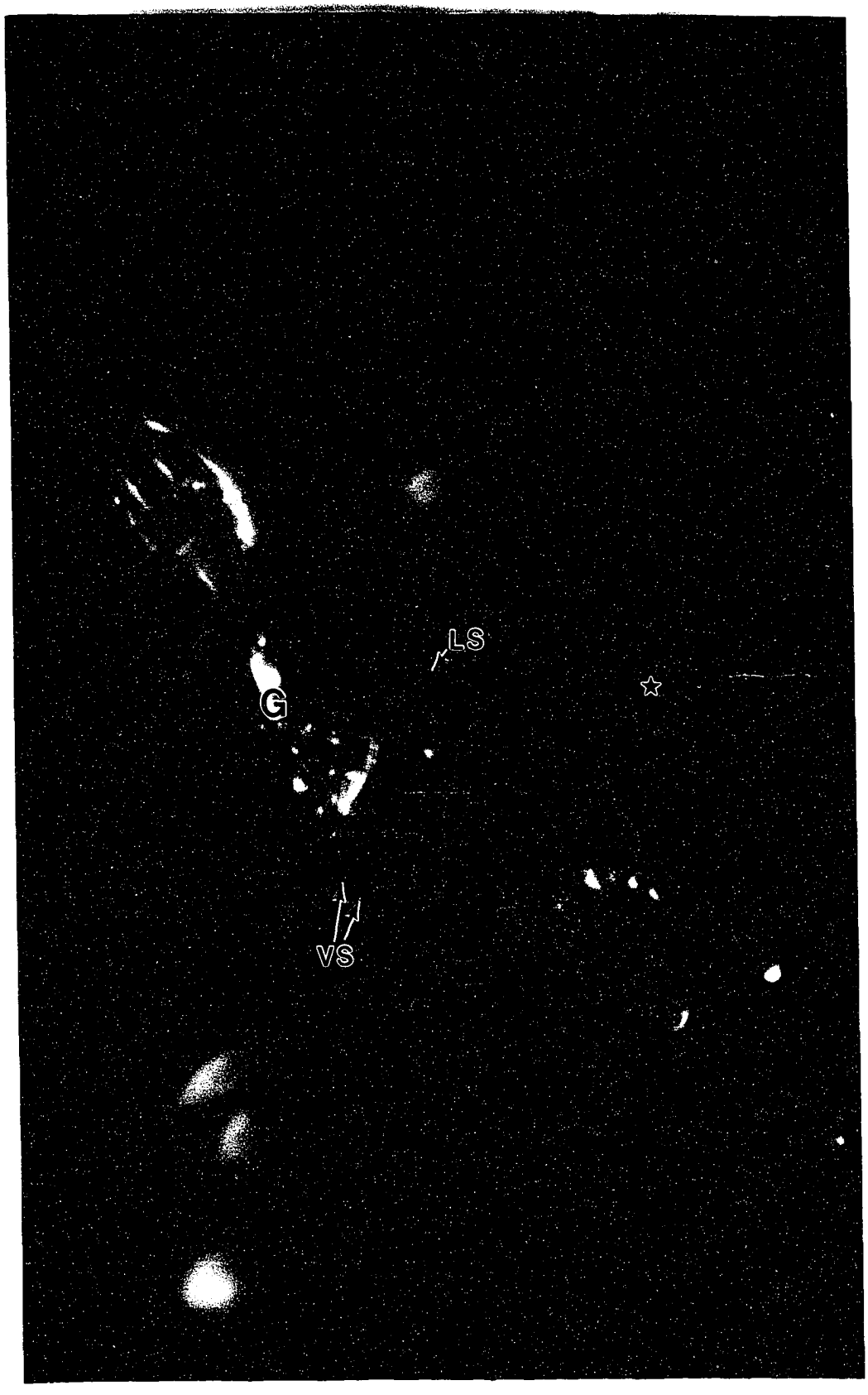
Bioassays Covering the Claw Sensilla with Gelatin

The following bioassays were conducted on *D. variabilis*, *D. andersoni* and *A. americanum* male ticks to identify the receptor of the MSP. These treatments did not involve ablations of the setae (and part of the leg) of the claw sensilla; rather, gelatin was used to cover the claw sensilla (and in most treatments the claw) (Fig. 4). Thus, the claw sensilla were intact and the gelatin could be removed, without damage to the claw sensilla, so that it could be used in further testing.

I.) Covering the claw sensilla of the first pair of legs with gelatin

Male ticks were taped on their dorsal sides to microscope slides and a thin strip of tape was placed across the first pair of legs, posterior to the Haller's organ, to immobilize them. Gelatin was prepared as previously described (see Category 2 & 3, covering of the setae of the venter and legs with gelatin) and applied to the claw sensilla (and claw) with a fine straight pin, while viewed with a stereoscopic microscope. The ticks were placed in a controlled environment chamber for 0.25 hours, at which time the gelatin solidified and the ticks recovered from the procedure. The hardened gelatin formed a cap over the tip of the leg. The ticks that were not active or did not orient to the female, at the time of testing, were discarded. Upon completion of the bioassay, the ticks were viewed with a stereoscopic

Figure 4. Light micrograph of gelatin covering the claw and claw sensilla of the leg I tarsus of a *D. andersoni* male tick. Note that no other setae are covered by the gelatin. G = gelatin coating. VS = first pair of ventral setae. LS = lateral seta. ★ = Haller's organ.



microscope to ensure that the gelatin coating was intact and not loosened by the tick's movement during the bioassay. If either of these conditions was noted, the tick and the corresponding data were discarded. Controls were ticks in which the claw sensilla of the first pair of legs were not covered with gelatin.

Ia.) To determine if the application of gelatin to the distal portion of the tarsus, containing the claw and claw sensilla, created a traumatic effect from which the tick could not fully recover, and thus would result in the occurrence of a false negative response to mounting, the same treatment was performed on the same portion of the tarsus of the second pair of legs.

II.) Bioassays with the claw sensilla of the first pair of legs covered with gelatin and upon removal of the gelatin from the claw sensilla

Untreated male ticks were bioassayed and scored for mounting behavior with conspecific females. Responses were considered positive if the males completed mounting and negative if, after three attempts, the males failed to mount. In this manner, males acted as their own controls. The males then were placed in separate vials so as to identify them. The same ticks were prepared and the claw sensilla of the first pair of legs covered with gelatin, as described above. Following the gelatin coating, the ticks were placed in a controlled environment chamber for 0.25

hours, at which time the gelatin solidified and the ticks recovered from the procedure. The treated males then were bioassayed and scored for mounting behavior with conspecific females. Upon completion of the bioassay, the gelatin was examined to ensure that it was still intact. The male ticks then were separated and taped to microscope slides on their dorsal sides. A thin strip of tape was placed across the first pair of legs, posterior to the Haller's organ, to immobilize them. While viewing the ticks with a stereoscopic microscope, a pair of forceps was used to remove the gelatin. The males were placed in a controlled environment chamber for 0.25 hours to recover from the procedure and subsequently bioassayed and scored for mounting behavior with conspecific females. After each bioassay, in which either gelatin was placed on or removed from the claw sensilla, the ticks were examined to verify that the gelatin was intact or had been completely removed.

Dose Response Bioassays

An important concern addressed in this study was the possibility that the gelatin coating did not completely obstruct passage of chemical compounds to the sensilla dendrites. Dose response bioassays were conducted with *D. variabilis* male ticks to determine whether some molecular penetration of the gelatin occurred. If so, it was expected that this would result in an increasing number of males that

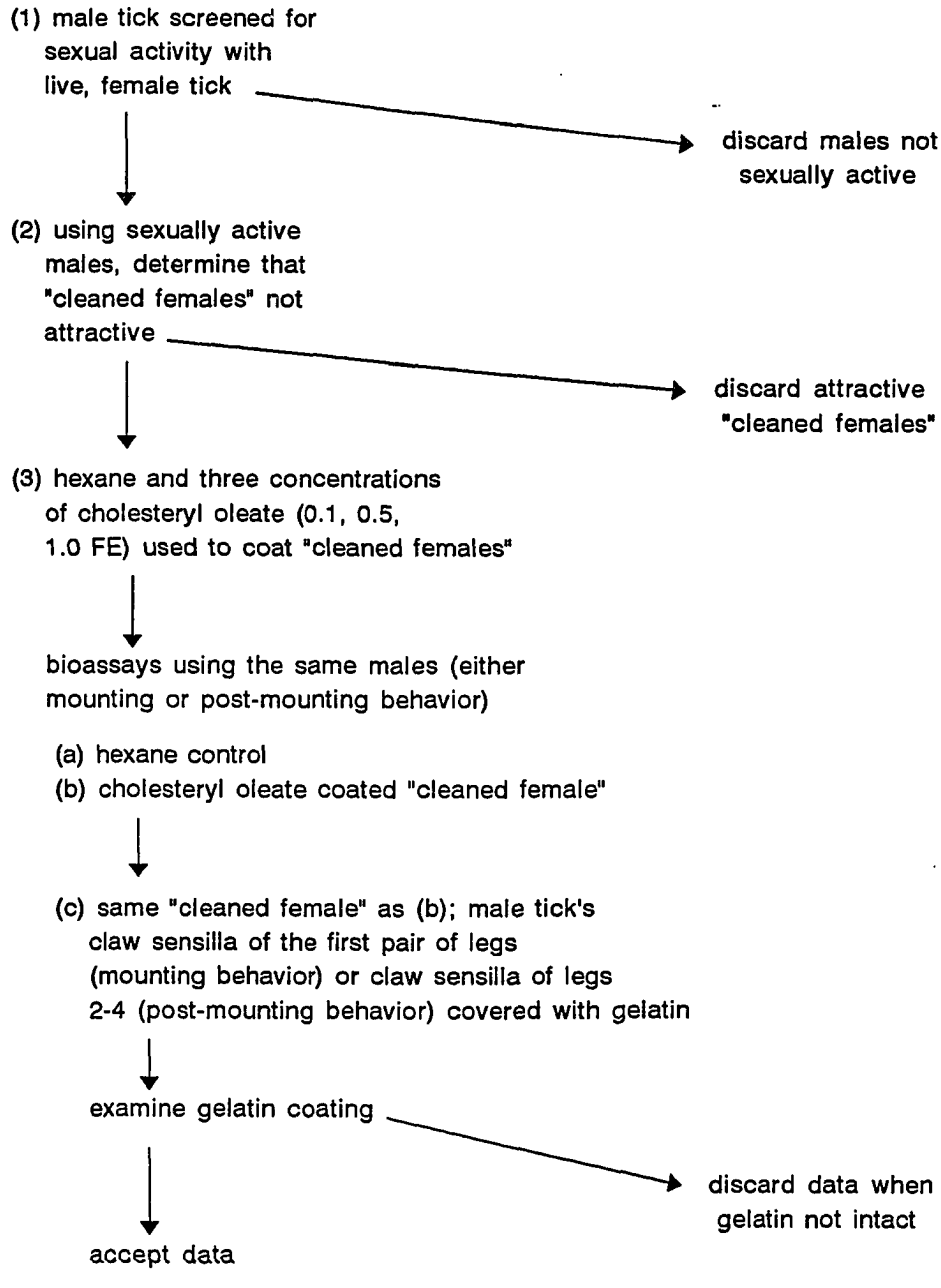
could mount females as the dosage of the MSP was increased. Three experiments were conducted to test the gelatin.

I.) Part-fed (7 day) male ticks were screened with part-fed conspecific females to determine that the males were sexually active. The males then were bioassayed with conspecific females that had fed on a host for 1 day; the claw sensilla of the first pair of legs of the males were not covered with gelatin (the male acted as its own control). The claw sensilla of the first pair of legs of the same males then were covered with gelatin (see Category 2, covering of the setae of the venter and legs with gelatin, for procedure) and the males bioassayed with the same conspecific females. Responses were considered positive if the male mounted the female and negative if, after three attempts, the male failed to mount the female. Likewise, additional male ticks, which had been screened, were bioassayed on conspecific females that had been fed for 1 day; however, for the second set of tests the claw sensilla of legs 2-4 were covered with gelatin while the claw sensilla of leg 1 remained intact. The males were tested for post-mounting behavior from posterior dorsal orientation to ventral orientation. Scoring was similar to that described above. This test was used to determine whether the claw sensilla on legs 2-4 acted as receptors of the MSP, once the male had mounted the female. The same bioassays, using the same number of male ticks, were conducted daily

for 6 subsequent days. The day that the female was used in the bioassay was equal to the number of days it had fed on a host (i.e., the bioassays conducted on day 4 used females that had fed for 4 days). By the seventh day, females are usually part-fed and generally sexually active males mate with them at this time; also, it is believed that as the female's blood meal increases daily, a greater concentration of MSP is secreted onto the female's cuticle.

II.) "Cleaned female" *D. variabilis* ticks were used in bioassays (Fig. 5) in which known concentrations of cholesteryl oleate (MSP), to which males previously were shown to respond, were added to the female cuticle. Cleaned females were part-fed ticks in which the cuticular lipids were removed from the body surface using hexane (Hamilton and Sonenshine, 1988; Hamilton, 1989; Hamilton *et al*, 1989). This was accomplished by immersing the female tick in hexane and "scrubbing" it with a fine, stiff-bristled brush for 10 minutes. The female then was immersed in a beaker of hexane for 1 hour. Following this "soaking", the female was scrubbed again for 10 minutes in the solvent. This scrubbing-soaking procedure was repeated for a total of three times (3x) and invariably led to the demise of the female tick. After the final soaking, the female was placed in a controlled environment chamber and the solvent allowed to air-dry.

Figure 5. Flow diagram of the "cleaned female" dose response bioassay procedure.



Part-fed *D. variabilis* male ticks were screened for sexual activity with live, part-fed, conspecific female ticks. The same males also were screened with the cleaned females to ensure that they were not attractive to the males. This was done by adding 6 ng of 2,6 DCP (Aldrich Chemical Company, Inc., Milwaukee, WI) in 10 μl of hexane, with a microcapillary pipet, to the foveal gland region of the cleaned female. Those females that were not sufficiently cleaned, and thus able to excite a male mounting response, were discarded.

Cholesteryl oleate (99% pure) (Sigma Chemical Co., St. Louis, MO.) was prepared in hexane at concentrations of 0.1, 0.5 and 1.0 female equivalents (FE) (1 FE = 10 μg cholesteryl oleate). Cholesteryl oleate was applied to cleaned female ticks in 10 μl of hexane. Hamilton *et al.* (1989) determined that cholesteryl oleate at concentrations from 0.1 to 1.0 FE stimulated *D. variabilis* male mounting responses that were indistinguishable from natural controls. One FE of cholesteryl oleate yielded the highest results in response to the chemical.

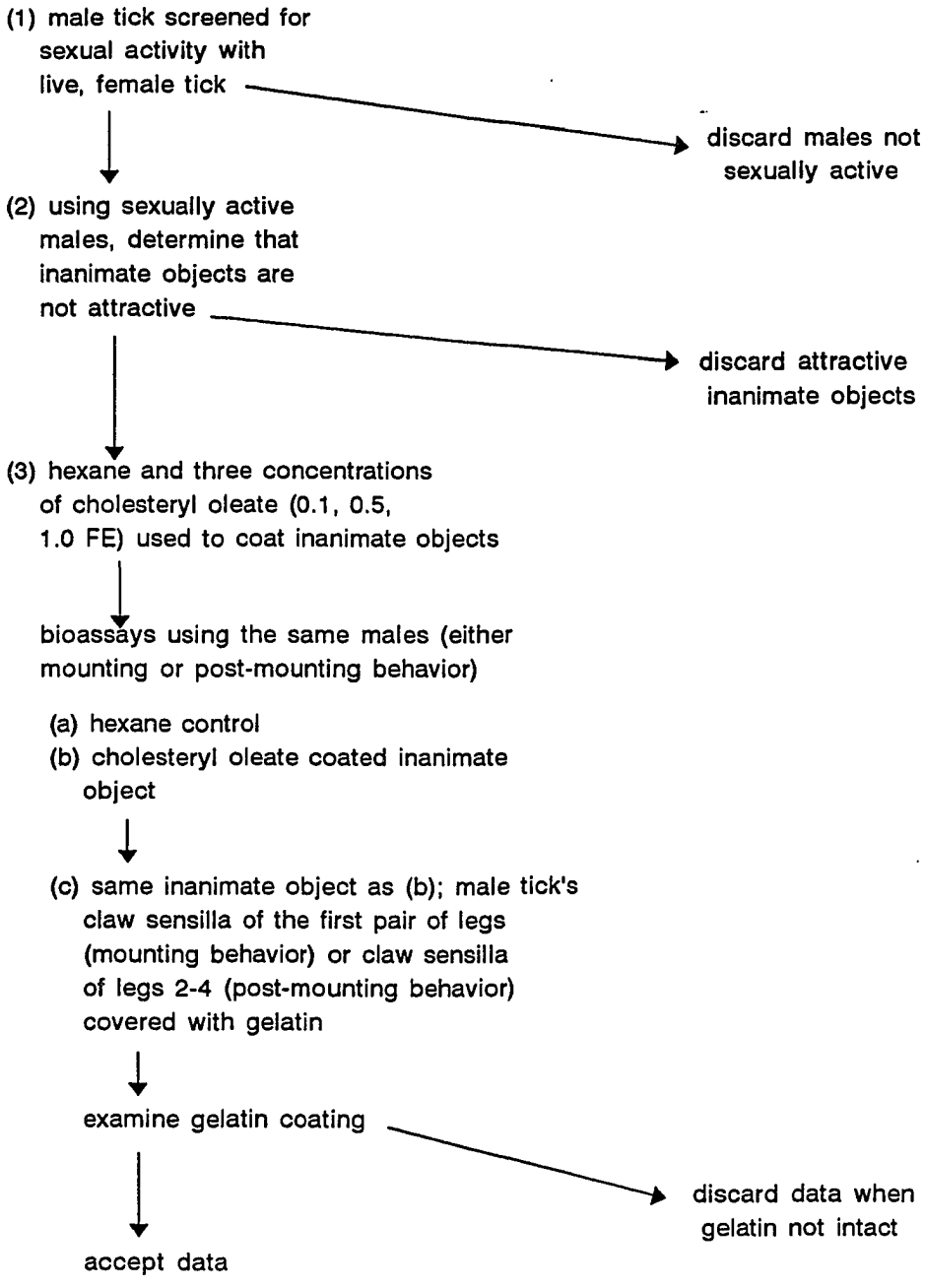
Cleaned female ticks were coated with 0.1, 0.5 and 1.0 FE of cholesteryl oleate using a microcapillary pipet. Following the coating, the ticks were placed in a controlled environment chamber to allow complete solvent evaporation. Cleaned female control ticks were coated with hexane. Male

ticks were bioassayed with the cleaned female control ticks and scored (see experiment I above). These tests were conducted using the same number of male ticks each for mounting and post-mounting behavior. The same males then were bioassayed with the cleaned females coated with 0.1, 0.5 or 1.0 FE of cholesteryl oleate for mounting or post-mounting behavior. Following these bioassays, the claw sensilla of the first pair of legs of the males were covered with gelatin (for preparation of gelatin and recovery time of ticks, see Category 2, covering of the setae of the venter and legs with gelatin) and the males again were tested with cleaned females coated with the same concentration of cholesteryl oleate for mounting behavior. Likewise, the claw sensilla of legs 2-4 of other male ticks were covered with gelatin and the males were tested with cleaned females coated with the three concentrations of cholesteryl oleate for post-mounting behavior. Upon bioassay completion, the ticks were viewed with a stereoscopic microscope to ensure that the gelatin coating was intact and it was not loosened by the tick's movement during the bioassay. If either of these conditions was noted, the tick and the corresponding data were discarded. In all of the bioassays, 6 ng of 2,6 DCP also was added to the cleaned females to stimulate and orient the males to the females. Scoring of the mounting and post-mounting behaviors were like those

described above. In summary, the same male tick was bioassayed with (1) a cleaned female control tick coated with hexane (the male acted as its own control), (2) a cleaned female tick coated with one concentration of cholesteryl oleate and (3) the same cleaned female tick, however, the claw sensilla of the first pair of legs or those of legs 2-4 of the male tick were coated with gelatin, depending upon the behavior that was being tested.

III.) Inanimate objects or "dummy ticks" were used in bioassays (Fig. 6) in which known concentrations of cholesteryl oleate (MSP) were added to the object's surface. The concentrations applied were the same number of FE previously described in the dose response bioassay section. Hamilton (1989) noted that MSP extracts placed on inanimate objects would stimulate sexually active males to mount them. The inanimate objects were inert, spherical, plastic beads. The same study determined that the size and shape of the bead did not affect the behavioral response of the male to MSP with which the beads were coated. Thus, the size of the spherical bead chosen for the present study was one with a radius of 3.85 mm (surface area 186 mm^2); this was similar to the size of a part-fed (7 day) *D. variabilis* female tick. The bead was coated with one of the concentrations of the MSP, the solvent allowed to volatilize and the bead placed in the center of a petri dish (15 cm diameter). A copper wire, inserted through the center of the bead, was hooked

Figure 6. Flow diagram of the inanimate object ("dummy tick") dose response bioassay procedure.



over the rim of the petri dish, thus immobilizing the bead for the bioassay.

D. variabilis male ticks were screened for sexual activity with live, conspecific, female ticks. The same males also were screened with inanimate objects, to which only 6 ng of 2,6 DCP was added, to ensure that the objects were not attractive to them. The same bioassays were conducted using the same number of male ticks and concentrations of cholesteryl oleate as those in the cleaned female dose response bioassays: (1) male ticks were tested with inanimate objects coated with hexane (controls); (2) the same male ticks were tested with inanimate objects coated with one concentration of cholesteryl oleate; (3) the same male ticks were tested with the same inanimate objects, however, the claw sensilla of either the first pair of legs or legs 2-4 were covered with gelatin, depending upon the behaviors that were being tested (i.e., claw sensilla of leg 1 for mounting behavior and the claw sensilla of legs 2-4 for post-mounting behavior). Scoring also was identical to that described in the cleaned female bioassays.

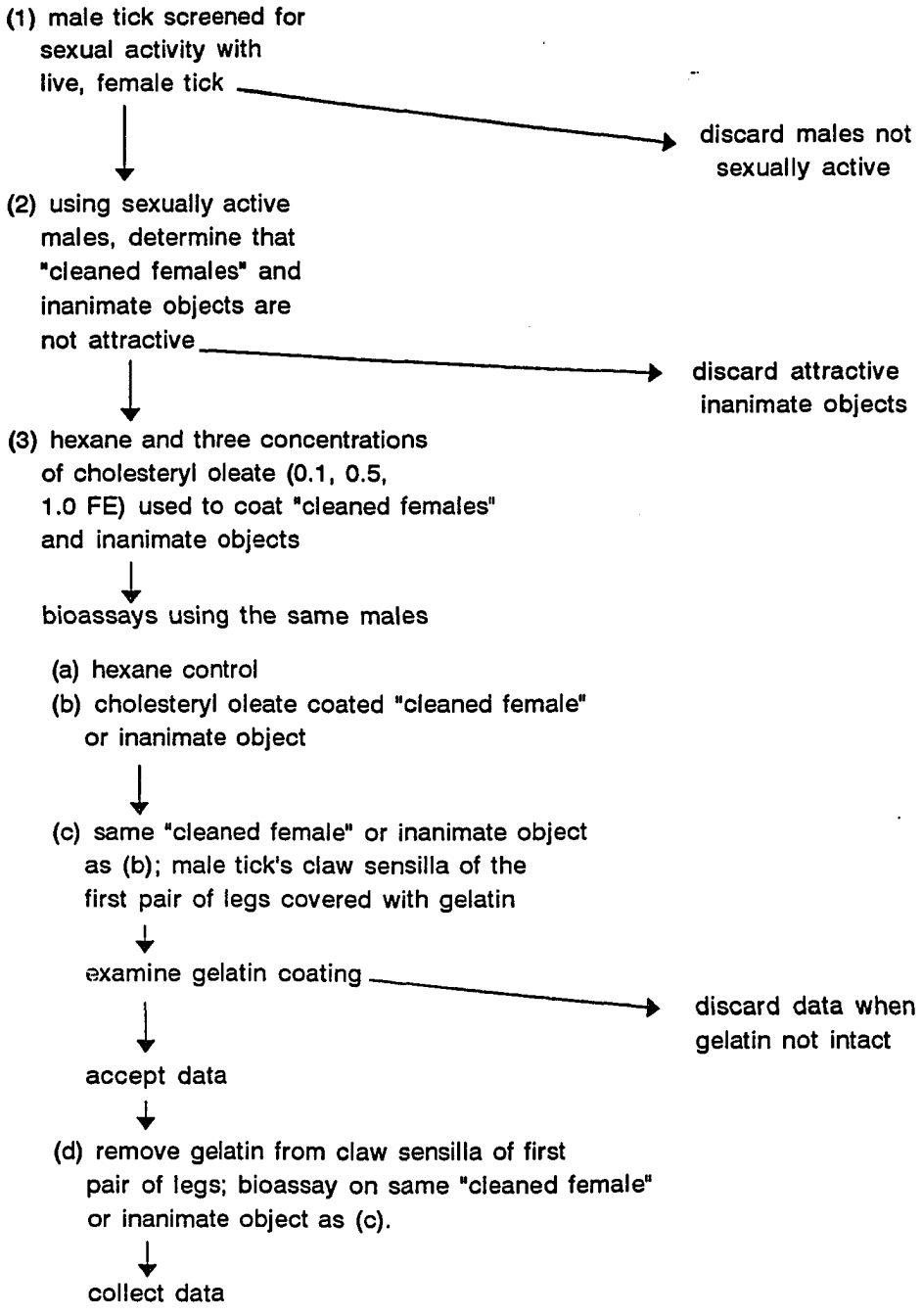
Transfer of Stimulus Bioassays

Two sets of experiments with *D. variabilis* and *D. andersoni* male ticks were conducted using cleaned female ticks and inanimate objects (dummy ticks) to authenticate the positive male mounting response to the MSP of live,

conspecific, female ticks demonstrated in the previous bioassays. Male ticks were bioassayed with cleaned female ticks and inanimate objects that were coated with the MSP, cholesteryl oleate. These bioassays determined whether the male behavior could be transferred to objects other than live, female ticks by moving the MSP stimulus to the objects. With only cholesteryl oleate to which the male ticks could respond, the bioassays demonstrated whether the claw sensilla of the first pair of legs were the receptors of the MSP and moreover, that only the pheromone was responsible for the male's mounting behavior. These bioassays also determined whether the male mounting response could be regulated by covering the claw sensilla of the first pair of legs with gelatin and likewise, by removing the gelatin from the claw sensilla. The bioassays were conducted similarly to those using the same objects in the dose response bioassay section above. Male ticks were screened for sexual activity with live, conspecific females, as well as cleaned females and inanimate objects with 6 ng of 2,6 DCP added, for an attraction stimulus, to ensure that these objects alone were not attractive to the males (Fig. 7).

The concentrations of cholesteryl oleate used to coat the two types of objects were the same as those in the dose response bioassay section above. These tests were conducted using the same number of male ticks in each bioassay of the different concentrations of cholesteryl oleate. The same

Figure 7. Flow diagram of the transfer of stimulus bioassay procedure.



male was bioassayed with (1) a cleaned female tick or inanimate object coated with hexane (control), (2) a cleaned female tick or inanimate object coated with one concentration of cholesteryl oleate, (3) the same cleaned female tick or inanimate object at the same concentration of cholesteryl oleate, however, the claw sensilla of the first pair of legs of the male were covered with gelatin and (4) the same cleaned female or inanimate object at the same concentration of cholesteryl oleate, however, the gelatin was removed from the claw sensilla of the first pair of legs of the male. Scoring was identical to that previously described in the dose response bioassay section above.

Statistical Analyses

A Model II (fixed for one criterion), Test of Independence, G-statistic incorporating a Williams correction factor (Sokal and Rohlf, 1981) was used to determine the significance of the bioassay treatments to controls in Category 1, 2 and 3. This test also was used to determine the significance of the additional mounting and post-mounting behavior bioassays, excluding those in the section, ablation of the ventral pair of claw sensilla from the first pair of legs. In the bioassays that used gelatin to cover the claw sensilla, the G-statistic was used in the section, covering the claw sensilla of the first pair of legs with gelatin, as well as the trauma bioassays performed in conjunction with those tests. Runs tests with unequal sample

sizes (Sokal and Rohlf, 1981) were performed to determine the randomness of data in selected treatments of Category 2 and 3. The same test was conducted on the results of the bioassays in which the claw sensilla of the first pair of legs of male ticks were ablated and the male was placed on the dorsum of the female to test its behavior.

The McNemar Test for Significance of Changes incorporating a Williams correction factor (Conover, 1980; Sokal and Rohlf, 1981) was performed to determine the significance of the results in the dose response and transfer of stimulus bioassays, as well as the sections, bioassays with the claw sensilla of the first pair of legs covered with gelatin and upon removal of the gelatin from the claw sensilla and ablation of the ventral pair of claw sensilla from the first pair of legs.

In the dose response bioassays, significance was determined between the results of male ticks tested with (1) controls and cholesteryl oleate and (2) cholesteryl oleate in which the claw sensilla of the first pair of legs (or legs 2-4 depending upon the behavior which was being bioassayed) were intact and then covered with gelatin. The two groups of tests were conducted using the same male ticks at the three concentrations of cholesteryl oleate. Significance, in the transfer of stimulus bioassays, was determined between the results of males tested with (1) controls and

cholesteryl oleate, (2) cholesteryl oleate in which the claw sensilla of the first pair of legs were intact and then covered with gelatin and (3) cholesteryl oleate in which the claw sensilla of the first pair of legs were covered with gelatin and after the gelatin was removed. There were no post-mounting behavior tests conducted with the male ticks in the transfer of stimulus bioassays.

In the bioassays that ablated the ventral pair of claw sensilla from the first pair of legs of male ticks, significance was determined between the results of males with the ventral pair of claw sensilla intact and the same males after the ventral pair of claw sensilla had been ablated. For the bioassays in which the claw sensilla of the first pair of legs of male ticks were covered with gelatin and tested, then the gelatin was removed and the ticks retested, significance was determined between the results of males tested with (1) controls and with gelatin covering the first pair of claw sensilla and (2) gelatin covering the claw sensilla and after the gelatin was removed. The same males were tested in the two groups.

Results

Behavioral Study

The courtship stages mediated by MSP in *D. variabilis*, *D. andersoni* and *A. americanum* male ticks, can be divided into five steps: (1) initial contact with the female, (2) mounting, (3) dorsal anterior orientation to the female's foveal glands (foveae dorsales), (4) dorsal posterior orientation and (5) ventral orientation. During initial contact in all three species, only the most distal portion of the tarsus of the male's forelegs contacts the posterior dorsum of the female (Figure 8; 5a). This section of the tarsus includes the claw, claw sensilla and the first pair of ventral setae and is the same in all three species. The lateral seta is located posterior to the claw sensilla on each foreleg tarsus of the males in all three species. Depending upon its angle and length, the seta may initially contact the female; however, this does not generally occur.

After the male tick has contacted the female with the distal portion of its first pair of legs, the male proceeds with mounting behavior (Fig. 8; 5b). Using the most distal portion of all eight legs, these males move anteriorly over the dorsum and then proceed to the ventral surface. Like the forelegs, the distal tarsi of legs 2-4 contain claw sensilla and a first pair of ventral setae in all three species; however, the claw sensilla of legs 2-4 differ

Figure 8. A representation of the behavioral stages and structures associated with the perception of the mounting sex pheromone (MSP) in *D. variabilis* and *D. andersoni* male ticks. The following stages are situated between Phases 4 (male orientation to pheromone-secreting (2,6 DCP) female) and 6 (movement of the male to the female's venter) of the *Dermacentor* spp. courtship behavior model. During initial contact (5a), in both tick species, only the most distal portion of the tarsus of the male tick's first pair of legs contacts the posterior dorsum of the female. The structures that comprise the distal portion of the foreleg tarsi are the claw and claw sensilla (I) and the first pair of ventral setae (II). Both tick species exhibit the same number and arrangement of claw sensilla (Ia; clear circles); six anteriorly-directed setae arranged in three symmetrical pairs, two each on the opposite sides of the apotele and one on the ventral side (Ia; stippled circle represents the apotele of the claw). The first pair of ventral setae (IIa) are located posteriorly to the claw and claw sensilla, but protrude anteriorly over a small, ventral spur. During mounting (5b), the claw sensilla and first pair of ventral setae of the tarsi of all eight legs are in contact with the female. The claw sensilla of legs 2-4 contain seven setae (Ib; clear circles); thus, differing in structure from that of the first pair of legs (Ia). The first pair of ventral setae of legs 2-4 (IIb) are identical in location and arrangement to those on the first pair of legs (IIa). Dorsal orientation of the male to the female's foveae dorsales (5c) differs between the two species. Most *D. variabilis* males (D.v.) exhibit "tip-toeing" behavior in which the body is raised off the female dorsum and only the most distal portion of the tarsi of all eight legs contact the female (for information concerning the labelled tarsus and associated structures, see above; the leg tarsus illustrated is leg 1). All *D. andersoni* males (D.a.) exhibit "sliding" behavior in which the tick contacts the female with the entire ventral surface of all of its legs and venter as it proceeds through dorsal orientation. The legs of the males are positioned so that the distal section of all of the tarsi also contact the female (for information concerning the labelled tarsus and associated structures, see above). *D. andersoni* males also may palpate the female's cuticle during dorsal and ventral orientation.

from those of the forelegs in number, arrangement and morphology. The location and arrangement of the first pair of ventral setae on legs 2-4 is identical to that of the forelegs in all three species of male ticks. The first lateral and medial setae, located posterior to the claw sensilla, on legs 2-4 in all three species are anteriorly-directed at a 45° angle away from the leg. Depending upon the angle of the tarsus and the setae, as well as the length of the setae, the first lateral and medial setae may contact the female's dorsum. It was noted that two (6.7%; n=30) *D. variabilis* males mounted females after establishing contact with only one leg of the first pair of legs; however, in the subsequent behavioral steps, both of the first legs contacted the female's dorsum. Furthermore, it was noted that male ticks, of all three species, would not mount a female if any leg(s) other than the first pair, came into initial contact with the female, despite the similarity of the setal arrangement on all of the legs.

After mounting the female, male ticks of each species proceed anteriorly to the region of the female's foveae dorsales (Fig. 8; 5c). The male then moves posteriorly in a searching mode from the dorsal to the ventral surface of the female.

After mounting, *D. variabilis* males exhibited one of three different behavioral postures as the ticks moved from

the dorsal to the ventral surface of the female; namely (1) "tiptoeing" behavior, in which most males (90%; n=30) proceeded through dorsal orientation to the ventral surface of the female with only the most distal portion of the tarsi of all eight legs in contact with the female; no other parts of the males' bodies were in contact with the female dorsum; (2) "sliding" behavior, in which males extend their legs to position themselves flat against the female's body. In this type of behavior the males (6.7%; n=30) contacted the females with the entire ventral surface of all of their legs and venter as they proceeded through dorsal and ventral orientation; the legs of the males were positioned so that the distal section of all of the tarsi also contacted the female; and (3) "waving" behavior, in which males (3.3%; n=30) raised their forelegs off of the female dorsum as they moved anteriorly to the region of the foveae dorsales; once at the foveal gland region, the male lowered its legs to contact the female and resumed "tiptoeing" behavior. In contrast to *D. variabilis*, "sliding" behavior was the only type of posture noted in *D. andersoni* males (100%; n=30), while *A. americanum* males exhibited only "tiptoeing" behavior (100%; n=30).

Fifty percent (n=30) of the *D. andersoni* males exhibited palpating of the female cuticle from dorsal through ventral movement. This behavior was observed rarely in *D. variabilis* males (6.7%; n=30) and only occurred when

the male tick initiated the "sliding" posture.

A. americanum male ticks did not palpate the female cuticle (n=30).

Scanning Electron Microscopy (SEM)

During initial contact in the courtship behavior, only the most distal portion of the tarsus of the first pair of legs of the male tick contacts the posterior dorsum of the female. This section of the tarsus contains the claw, claw sensilla and first pair of ventral setae, as well as the lateral seta which, in general, does not initially contact the female (Fig. 9). These same setae are found on the legs of the female ticks, as well as the males. The location, arrangement and morphology of these setae are identical in *D. variabilis, D. andersoni* and *A. americanum* males and females; however, the setae differ in length among individuals of the same species and there are some generic differences in the length of some setae.

The first pair of ventral setae are mechanoreceptors. These are sharply-pointed setae which lack pores (both on the sensillar shaft and at the tip) and are slightly curved (upward). These setae are located posterior to the claw sensilla but protrude anteriorly over a small ventral spur in all three species and sexes of ticks. The lengths of the individual setae of the pair are identical (or approximately the same). The setae range from 60.0-80.9 μm in

Figure 9. Scanning electron micrograph of the first leg tarsus of a *D. variabilis* male tick. The labeled structures are those that (may) initially contact the posterior dorsum of the female tick during the courtship ritual. CL = claw. PV = pulvillus. → = lateral seta. VS = first pair of ventral setae. CS = claw sensilla (3 pairs). The Haller's organ (★) is labeled for orientation purposes, it does not initially contact the female. 374x. Measurement bar = 100 μm.

D. variabilis males (n=10 pairs), 73.0-95.0 μm in *D. andersoni* males (n=10 pairs) and 84.6-123.2 μm in *A. americanum* males (n=7 pairs); the lengths of the first pair of ventral setae of the females of each species were within the range of those found in the conspecific males. While the first pair of ventral setae are approximately the same length in the two *Dermacentor* species, those of *A. americanum* are generally longer.

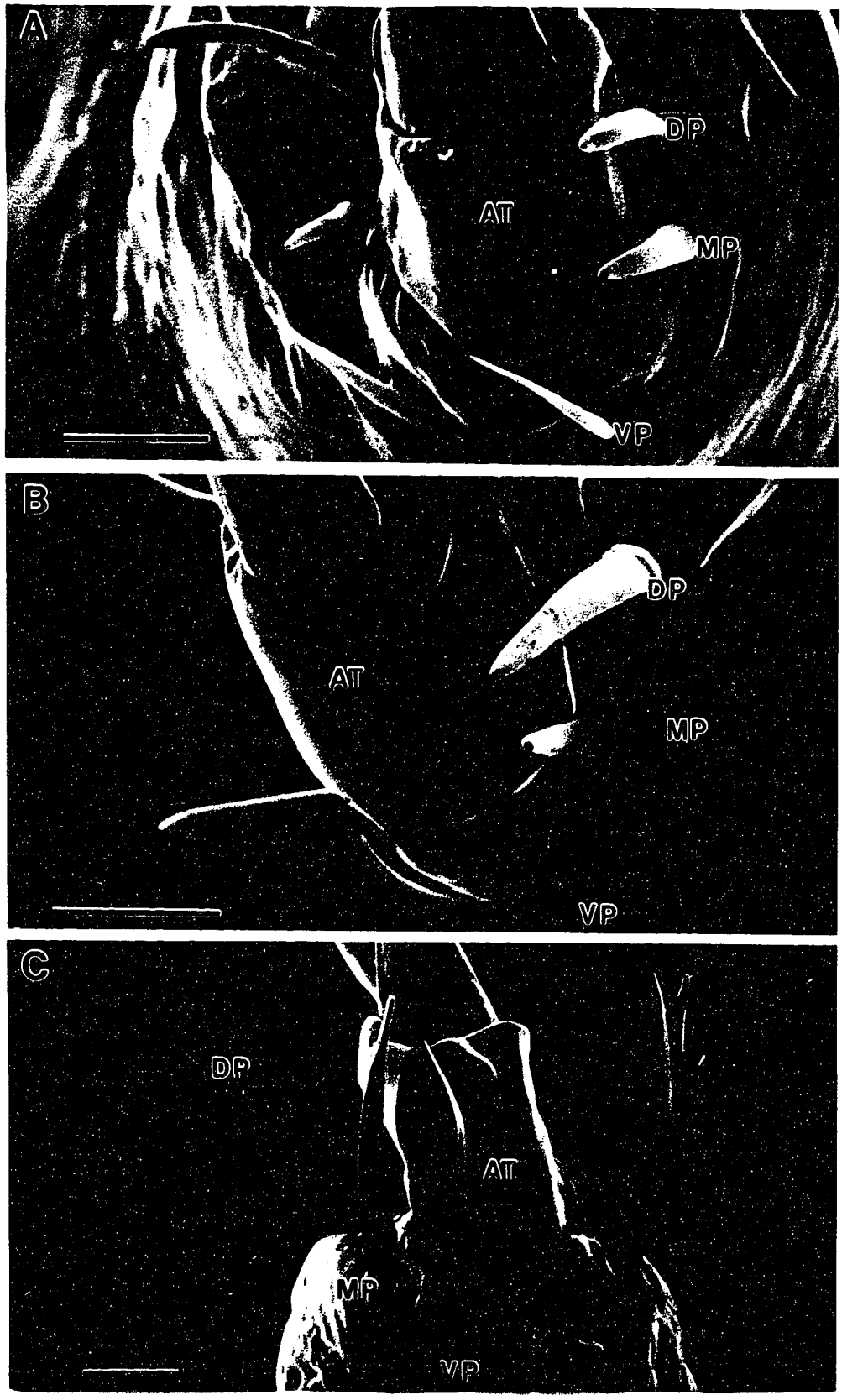
A single lateral seta is located on each foreleg between the claw sensilla and first pair of dorsal setae on the ridge anterior to Haller's organ and is directed anteriorly at a 45° to 90° angle. This seta also is a mechanoreceptor. The length of the seta ranges from 60.0-75.0 μm in the three species (n=5 setae/species of male).

The claw sensilla are located at the distal end of the tarsus (Fig. 10). In all three species and sexes of ticks, the claw sensilla consist of six anteriorly-directed setae arranged in three symmetrical pairs, two each on the opposite sides of the apotele of the claw and one on the ventral side. The individual seta of each pair is identical (or approximately the same) in length to the other seta of that pair. This description is based on observations of a total of 60 adult ticks (of the three species) and takes into account distortions of the position of the claw sensilla due

Figure 10-A. Scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the first leg of a *D. variabilis* male tick. AT = apotele of claw. DP = dorsal pair of claw sensilla. MP = middle pair (lateral) of claw sensilla. VP = ventral pair of claw sensilla. Note the blunt tips of the dorsal and middle pair of claw sensilla (darkened tips indicate the presence of depressions and/or pore-like structures) and the pointed tips of the ventral pair of claw sensilla. 989x. Measurement bar = 25 μm.

Figure 10-B. A scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the first leg of a *D. variabilis* female tick. The location, arrangement and setae are identical to that of the male tick. AT = apotele of claw. DP = dorsal pair of claw sensilla. MP = middle pair (lateral) of claw sensilla. VP = ventral pair of claw sensilla. 1,136x. Measurement bar = 25 μm.

Figure 10-C. Scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the first leg of an *A. americanum* male tick. The claw sensilla differ from those of the *Dermacentor* species only in that the dorsal claw sensilla are generally the longest in the group (in *Dermacentor* species the ventral pair of claw sensilla are the longest). AT = apotele of claw. DP = dorsal pair of claw sensilla. MP = middle pair (lateral) of claw sensilla. VP = ventral pair of claw sensilla. Note the blunt tips of the dorsal and middle pair of claw sensilla and the pointed tips of the ventral pair of claw sensilla. Ventral view of tarsus. 670x. Measurement bar = 25 μm.



to the angle of the specimen when it is viewed, as well as distortion due to SEM preparation.

In all three species and both sexes of ticks, the dorsal claw sensilla are blunt-tipped and lack pores along the sensillar shaft. A subterminal depression is located near the tip of each seta; inside the depression is a pore-like structure (Fig. 11). The seta is a mechanogustatory receptor (contact chemoreceptor). These setae range from 48.0-68.0 μm in *D. variabilis* males (n=10 pairs), 44.5-60.0 μm in *D. andersoni* males (n=10 pairs) and 72.0-101.1 μm in *A. americanum* males (n=7 pairs). The length of the same setae in female ticks is comparable to the conspecific males.

The middle (= lateral) pair of claw sensilla, in all three species and both sexes of ticks, are blunt-tipped and lack pores along the sensillar shaft. A pore-like structure is located at the tip of the seta (Fig. 12). This seta also is a mechanogustatory receptor (contact chemoreceptor) like the dorsal pair of claw sensilla; however, the pore-like structure is terminal, not subterminal, in location and it is not situated in a depression. The middle pair of claw sensilla are shorter than the dorsal pair in all three species and both sexes of ticks. The setae range from 40.0-57.8 μm in *D. variabilis* males (n=10 pairs), 36.7-51.1 μm in *D. andersoni* males (n=10 pairs) and 55.0-70.2 μm in

Figure 11. A scanning electron micrograph of the terminal end of one mechanogustatory seta of the dorsal pair of claw sensilla of a *D. variabilis* male tick. Note the blunt tip of the seta, the subterminal depression and the pore-like structure (→) within the depression. 13,000x. Measurement bar = 1 μm.

Figure 12. A scanning electron micrograph of the terminal end of one mechanogustatory seta of the middle pair (lateral) of claw sensilla of a *D. variabilis* male tick. Note the blunt tip of the seta and the pore-like structure (→) at the tip of the sensillar shaft. 8,060x. Measurement bar = 2.5 μm.

Figure 13. A scanning electron micrograph of the sensillar shaft and terminal end of one mechanoreceptive seta of the ventral pair of claw sensilla of a *D. variabilis* male tick. Note the tapered end (tip turned upward) of the seta and the absence of a pore at the tip. 20,800x. Measurement bar = 1 μm.

A. americanum males (n=7 pairs). The same setae of the females were comparable in length to those of the conspecific males.

The ventral claw sensilla in all three species and both sexes of ticks are mechanoreceptors. The setae are sharply-pointed, lack pores (both on the sensillar shaft and at the tip) and are slightly curved (upward) (Fig. 13). The setal length ranges from 54.8-86.8 μm in *D. variabilis* males (n=10 pairs), 60.0-96.1 μm in *D. andersoni* males (n=10 pairs) and 66.0-97.2 μm in *A. americanum* males (n=7 pairs). The same setae of the females are comparable in length to those of the conspecific males.

Fine grooves (canals) were noted on the sensillar shaft of all six claw sensilla in all three species and both sexes of ticks; however, no pores were found in the grooves. The grooves also did not extend to the terminal end of the shaft on any of the claw sensilla.

A pattern exists concerning the lengths of the pairs of claw sensilla on the forelegs of male and female ticks that is different between the two genera. *Dermacentor* males and females exhibit a pattern in which the ventral claw sensilla (mechanoreceptors) are the longest, the middle pair (mechanogustatory receptors) the shortest and the dorsal pair (mechanogustatory receptors) intermediate to the others in length. In *Amblyomma* males and females, the

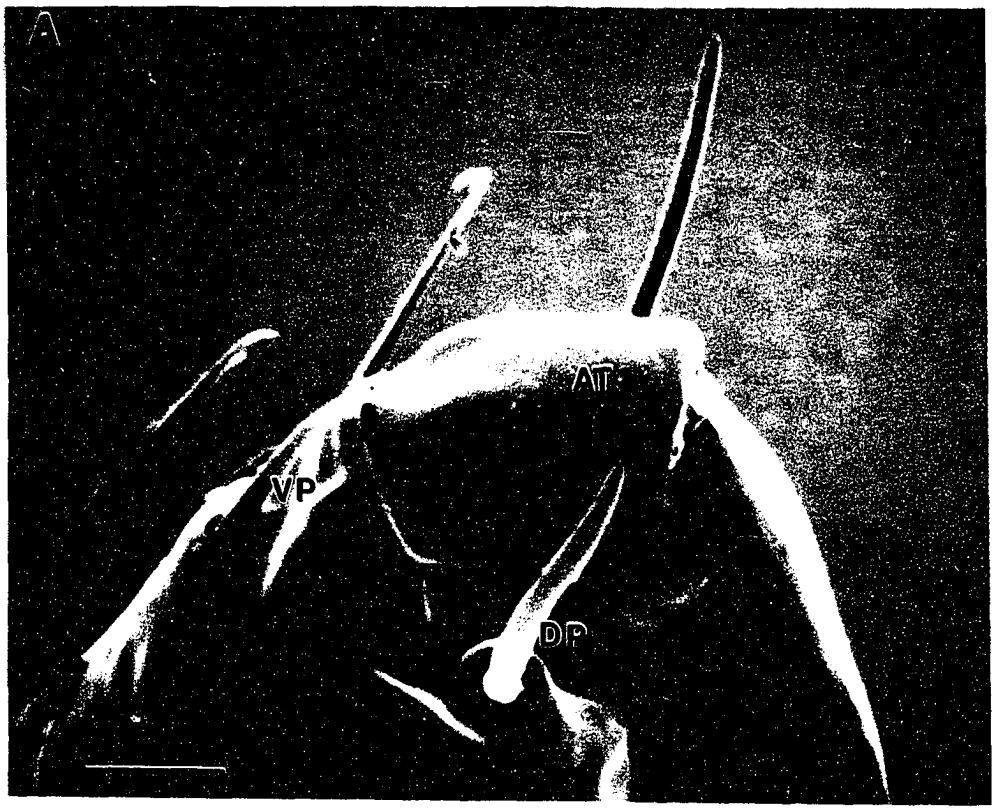
dorsal pair of claw sensilla are approximately the same length as, or longer than, the ventral pair. The middle pair of claw sensilla, like those in the *Dermacentor* species, are the shortest.

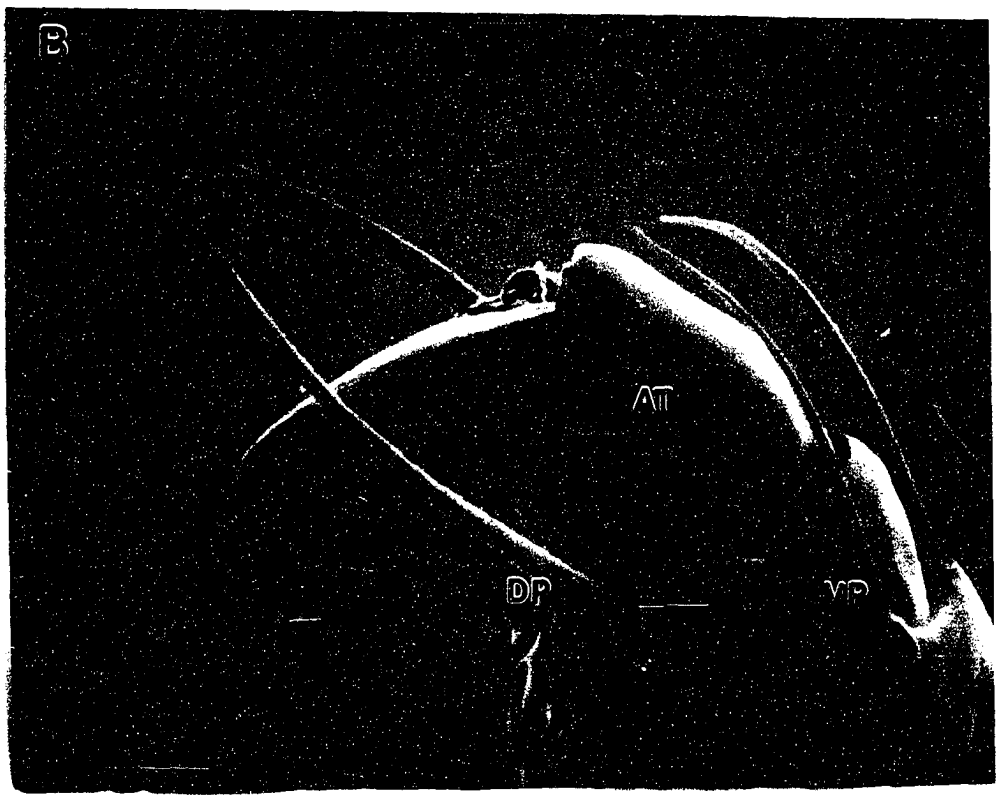
There are only two pairs of claw sensilla in the nymphal stage of all three species of tick (Fig. 14). The dorsal pair of claw sensilla are located slightly above the apotele, are blunt-tipped and lack pores on the sensillar shaft; therefore, they most closely resemble the dorsal pair of claw sensilla of the adults. The other pair of claw sensilla are located below the apotele, are sharply-pointed and lack pores on the sensillar shaft and terminal end. These claw sensilla are identical to the ventral pair of claw sensilla in the adults. The middle pair of claw sensilla found in the adults is absent in the nymphs.

The most distal portion of legs 2-4 of the male ticks of all three species contacts the female's cuticle during mounting and dorsal and ventral orientation. The claw sensilla of the distal tarsi of legs 2-4 are identical in all three species and both sexes of adult ticks. Six anteriorly-directed setae are arranged in three symmetrical pairs around the apotele of the claw in the same manner as the claw sensilla of the forelegs; in addition, an unpaired lateral seta also is located between the dorsal and middle

Figure 14-A. A scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the first leg of a *D. andersoni* nymph. AT = apotele of claw. DP = dorsal pair of claw sensilla. VP = ventral pair of claw sensilla. Note the blunt tips of the dorsal pair of claw sensilla, the pointed tips of the ventral pair of claw sensilla and the absence of the middle pair (lateral) of claw sensilla. 1,932x. Measurement bar = 10 μm.

Figure 14-B. A scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the first leg of an *A. americanum* nymph. Note the similarity of the claw sensilla to those of the *D. andersoni* nymph (Fig. 14-A). AT = apotele of claw. DP = dorsal pair of claw sensilla. VP = ventral pair of claw sensilla. 1,484x. Measurement bar = 10 μm.





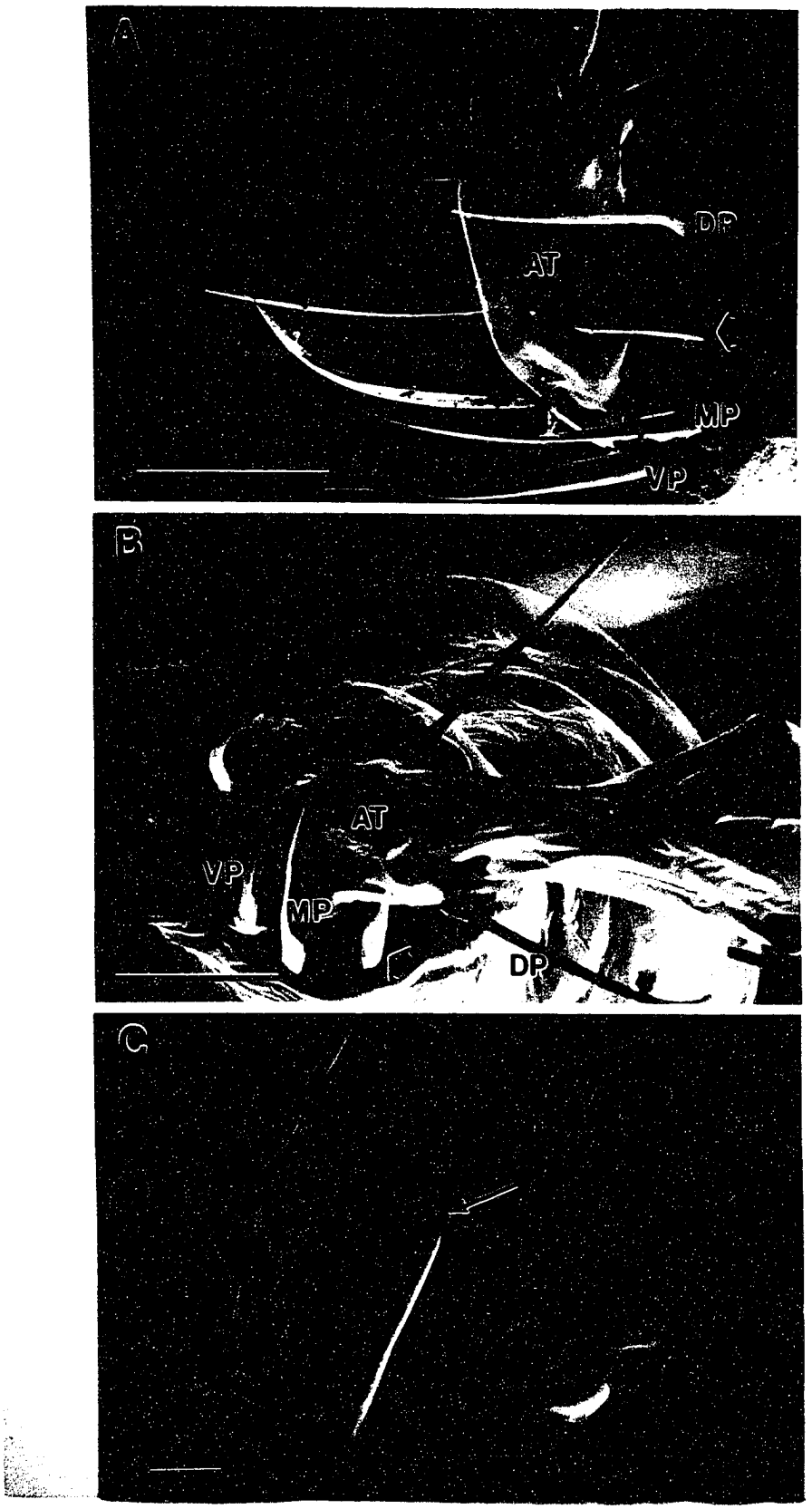
pairs of claw sensilla (Fig. 15). The three pairs of claw sensilla on these posterior legs are mechanoreceptors; they are sharply-pointed setae that lack pores (both on the sensillar shaft and at the terminal end). Fine grooves were noted on the sensillar shaft; however, the grooves did not extend to the terminal end of the shaft. No pores were found in the grooves. In contrast to the claw sensilla of legs 2-4, the claw sensilla of the forelegs of all three species and both sexes of ticks contain only one pair of mechanoreceptors; the dorsal and middle pairs of claw sensilla are mechanogustatory with a pore-like structure at or near the terminal end of the setae.

The individual seta of each pair is identical (or approximately the same) in length to the other seta of that pair. No pattern was found to exist between the lengths of the six setae of legs 2-4 in *Dermacentor* males and females as there was with the claw sensilla of the forelegs in the same species. The six setae on legs 2-4 range in length from 55.5-120.1 μm in *D. variabilis* male ticks (n=30; 10 pairs/leg) and 46.0-110.0 μm in *D. andersoni* males (n=30; 10 pairs/leg). The lengths of the six setae in the female ticks are comparable to those of the conspecific males. In *Amblyomma* male and female ticks, a similar pattern of setal length was noted in the claw sensilla of legs 2-4 like that of the claw sensilla of the forelegs. The dorsal pair of claw sensilla are approximately the same length as, or

Figure 15-A. A scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the fourth leg of a *D. andersoni* male tick. AT = apotele of claw. DP = dorsal pair of claw sensilla. MP = middle pair (lateral) of claw sensilla. VP = ventral pair of claw sensilla. → = unpaired lateral seta. Note the six paired mechanoreceptive setae, tapered to a pointed tip and lacking a terminal pore, and the shorter, unpaired lateral seta located between the dorsal and middle pair of claw sensilla. The "wick-like" tip of the seta has been destroyed in the preparation. 579x. Measurement bar = 50 μm.

Figure 15-B. A scanning electron micrograph of the claw sensilla at the distal end of the tarsus of the third leg of an *A. americanum* male tick. The number, location, arrangement and morphology of the claw sensilla around the apotele of the claw are identical to those described for the *D. andersoni* male (Fig. 15-A); however, note the longer length of the dorsal pair of claw sensilla in the *A. americanum* male as compared to those of the *D. andersoni* male. AT = apotele of claw. DP = dorsal pair of claw sensilla. MP = middle pair (lateral) of claw sensilla. VP = ventral pair of claw sensilla. → = unpaired lateral seta. 504x. Measurement bar = 50 μm.

Figure 15-C. A scanning electron micrograph closeup of the "wick-like" tip of the unpaired lateral seta of the claw sensilla of the second leg of a *D. variabilis* male tick. 1,100x. Measurement bar = 10 μm.



longer than the ventral pair; the middle pair of claw sensilla are shorter. In *Amblyomma* males, the dorsal setae range from 100.7-128.0 μm (n=5 pairs), the middle setae from 69.7-98.4 μm (n=5 pairs) and the ventral setae from 88.4-106.0 μm (n=5 pairs). The same setae of the females are comparable in length to those of the males.

The unpaired lateral seta is similar in morphology and length on legs 2-4 in all of the species and both sexes of ticks. There are no pores on the sensillar shaft and the structure of the terminal end of the seta is "wick-like" (in most SEM preparations, the fragile wick-like tip was destroyed) (Fig. 15). This seta is generally the shortest of the seven claw sensilla on legs 2-4 in each species; it ranges from 36.6-49.1 μm in *D. variabilis* males (n=5 setae), 39.0-50.3 μm in *D. andersoni* males (n=5 setae) and 50.1-76.2 μm in *A. americanum* males (n=5 setae). The seta of the females is comparable in length to those of the conspecific males.

In *D. variabilis* and *D. andersoni* nymphs, the claw sensilla of legs 2-4 are arranged in three symmetrical pairs around the apotele of the claw as they are in the adults of these species; however, they lack the short, unpaired lateral seta of the adults. In contrast to the *Dermacentor* species, *A. americanum* nymphs exhibit the short, unpaired lateral seta on legs 2-4 which is located between the dorsal

and middle setae of the claw sensilla (same as in the adults). The morphology of the three symmetrical pairs of claw sensilla in all three species is similar to the same claw sensilla of the adults. All of the setae are sharply-pointed mechanoreceptors which lack pores on the sensillar shaft and at the terminal end of the seta. The morphology of the short, unpaired lateral seta found in *A. americanum* nymphs is similar to the same seta of the adults; the terminal end is wick-like and no pores are found on the sensillar shaft.

Bioassays

At initial contact, *D. variabilis* and *D. andersoni* male ticks displayed a significant difference ($p=0.001$; $G_{adj}=37.930$ and $G_{adj}=36.720$, respectively) from the controls in the mounting of conspecific females, when the claw, claw sensilla and first pair of ventral setae were ablated, as a group, from the male's first pair of legs (Category 1). Most treated males of both species failed to mount the females. Trauma ablations conducted on the same setae on the male's second pair of legs demonstrated that both species of ticks readily mounted the females. These non-significant results indicated that the ablation treatment of the distal portion of the leg and claw did not cause undue stress to the tick from which it could not recover; thus, the treatment did not alter the mounting behavior of the male tick (Table 1). Individual ablation bioassays of the

Table 1. Responses of *D. variabilis* (DV), *D. andersoni* (DA) and *A. americanum* (AA) male ticks to sexually-active, conspecific females following the ablation of various structures on the male's forelegs. Male tick behavior was tested from initial contact through mounting of the posterior dorsum of the female.

Bioassay	species	n*	χ^2 value
ablation-cl/cs/vs	DV	35	37.930†
	DA	41	36.720†
trauma♦-cl/cs/vs	DV	39	1.075‡
	DA	38	1.141‡
	AA	40	1.540‡
ablation-cl	DV	48	0.190‡
	DA	48	0.887‡
	AA	38	0.440‡
ablation-cs(w/cl)	DV	36	38.410†
	DA	39	35.890†
	AA	42	31.930†
ablation-vs	DV	35	0.730‡
	DA	38	0.000‡
	AA	40	0.640‡
ablation-ls	DV	48	0.190‡
	DA	46	0.000‡
	AA	40	1.540‡

*n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

♦trauma: conducted using the second pair of legs (see text).

†: Significant at p=0.001. G-statistic with Williams correction factor.

‡: Not significant. G-statistic with Williams correction factor.

cl = claws

cs = claw sensilla

vs = first pair of ventral setae

ls = lateral seta

claw, first pair of ventral setae and lateral seta did not alter the mounting behavior of both tick species; however, when the claw sensilla were ablated, both tick species demonstrated a significant difference ($p=0.001$; $G_{adj}=38.410$ in *D. variabilis* males and $G_{adj}=35.890$ in *D. andersoni* males) in the mounting of conspecific females compared to the control males (Table 1). Most males of both species failed to mount the female. The claw also was ablated with the claw sensilla because it was noted that extensive damage was done to the leg if the claw remained intact while the claw sensilla were removed, and the claw previously had been shown not to be a receptor of the MSP. Likewise, most *A. americanum* male ticks failed to mount conspecific females only when the claw sensilla (along with the claw) were ablated from the first pair of legs ($p=0.001$; $G_{adj}=31.930$). The males also were not affected significantly by the trauma ablation treatment of the claw sensilla of the second pair of legs (Table 1).

Ablation of the claws and mouthparts of *D. variabilis* and *D. andersoni* male ticks, conducted from initial contact through mounting of the posterior dorsum of the female (Category 2), had no effect on mounting behavior (Table 2). Likewise, bioassays, conducted through the same behavioral stages (Category 2), using gelatin to cover the male tick's setae and surfaces of the venter and the lateral and ventral portions of all eight legs, first pair of dorsal setae and

Table 2. Mounting responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks to sexually-active, conspecific females following the ablation of the claws (all eight legs) and mouthparts of the males. n*=60.

Bioassay	species	χ^2 value
claws	DV	1.202‡
	DA	1.584‡
left palp	DV	0.000‡
	DA	0.000‡
right palp	DV	0.000‡
	DA	0.000‡
both palps	DV	0.837‡
	DA	1.584‡
left chelicera	DV	1.584‡
	DA	1.584‡
right chelicera	DV	0.342‡
	DA	0.342‡
both chelicerae	DV	0.187‡
	DA	0.342‡
chelicerae & palps	DV	1.584‡
	DA	0.837‡
hypostome	DV	0.837‡
	DA	1.202‡
all mouthparts	DV	0.837‡
	DA	1.584‡

* n = 50 treated ticks and 10 control ticks used in each bioassay.

‡: Not significant. G-statistic with Williams correction factor.

distal tarsal surface of leg 1 and the setae and dorsal tarsal surface of legs 2-4 yielded non-significant results in both species (Table 3). The results of ablation bioassays of the lateral seta and the claw sensilla of legs 2, 3 and 4 (each claw sensilla ablated in separate treatments) of *D. variabilis* and *D. andersoni* male ticks, conducted from initial contact through mounting of the posterior dorsum of the female (Category 2) also were not significantly different from the controls (Table 3).

In the bioassays in which male tick behavior was tested from initial contact through mounting of the posterior dorsum of the female (Category 2), Runs tests were performed on specific bioassays in which the number of male ticks that did not complete the behavior was greater than five (suggesting some other factor affected the behavior) but the alpha value was not significant. These tests were conducted to determine whether the data occurred in a random sequence or if it was a function of some other factor, in which case, since the data is not significant, it would be expected that the Chi-square value would be decreased further.

Runs tests were calculated on the results of the ventral and lateral leg setae and surfaces bioassays of both *D. variabilis* and *D. andersoni* males, as well as the results of the venter setae and surface bioassays of *D. variabilis* males (Table 3). The results of the bioassays of the

Table 3. Responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks to sexually-active, conspecific females following the occlusion (with gelatin) or ablation of setae and portions of the male's body that come into contact with the female during mounting.

Bioassay	species	n*	χ^2 value
venter setae and surface-gelatin	DV	50	1.974‡
	DA	50	1.584‡
ventral and lateral leg setae and surfaces-gelatin	DV	50	2.377‡
	DA	50	3.639‡
first pair of dorsal setae and distal tarsal surface (leg 1)-gelatin	DV	50	0.000‡
	DA	50	0.837‡
setae and dorsal tarsal surface of legs 2-4-gelatin	DV	45	0.000‡
	DA	44	0.000‡
lateral seta (leg 1)-ablation	DV	45	0.000‡
	DA	45	0.000‡
claw sensilla leg 2-ablation	DV	48	0.887‡
	DA	40	1.540‡
claw sensilla leg 3-ablation	DV	45	0.947‡
	DA	39	0.643‡
claw sensilla leg 4-ablation	DV	44	0.000‡
	DA	42	0.203‡

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

‡: Not significant. G-statistic with Williams correction factor.

ventral and lateral leg setae and surfaces of both species of male ticks were found to be random (t_s<1.960; t_s=1.800 in *D. variabilis* males and t_s=1.100 in *D. andersoni* males); however, the *D. variabilis* male venter data was determined to be nonrandom (t_s>1.960; t_s=2.410), thus indicating the influence of some other unknown factor or event.

Following ablation of the claws and mouthparts of *D. variabilis* and *D. andersoni* male ticks, tests of the post-mounting behavior of the treated males (Category 3) from dorsal anterior through ventral orientation, did not show any significant differences from the controls (Table 4). Bioassays conducted through the same behavioral stages (Category 3), of males of both species, using gelatin to cover all setae that contact the female except the leg 1 claw sensilla also did not reveal any significant differences from the controls (Table 5). Likewise, the results of ablation bioassays of the lateral seta and the claw sensilla of legs 2, 3 and 4 (each claw sensilla ablated in separate treatments) of males of both species were not significantly different from the controls (Table 5). Runs tests were calculated on the bioassay results of the tests with the left and right palps of *D. andersoni* males during post-mounting behaviors (Table 4). The results of both sets of data determined that the responses of the ticks were random (t_s<1.960; left palp, t_s=0.024; right palp, t_s=1.610).

Table 4. Post-mounting responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks to sexually-active, conspecific females following the ablation of the claws (all eight legs) and mouthparts of the males.

Bioassay	species	n*	χ^2 value
claws	DV	48	0.000‡
	DA	47	0.000‡
left palp	DV	48	0.612‡
	DA	50	2.377‡
right palp	DV	48	0.887‡
	DA	48	3.340‡
both palps	DV	46	0.669‡
	DA	43	1.429‡
left chelicera	DV	45	0.000‡
	DA	44	0.000‡
right chelicera	DV	44	0.000‡
	DA	45	0.000‡
both chelicerae	DV	47	0.000‡
	DA	47	0.000‡
chelicerae & palps	DV	44	0.000‡
	DA	44	0.000‡
hypostome	DV	48	0.190‡
	DA	43	0.000‡
all mouthparts	DV	45	0.000‡
	DA	45	0.000‡

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

‡: Not significant. G-statistic with Williams correction factor.

Table 5. Responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks to sexually-active, conspecific females following the occlusion (with gelatin) or ablation of setae and portions of the male's body that come into contact with the female during the post-mounting behaviors.

Bioassay	species	n*	χ^2 value
venter setae and surface-gelatin	DV	44	0.000‡
	DA	44	0.000‡
ventral and lateral leg setae and surfaces-gelatin	DV	43	0.607‡
	DA	41	0.607‡
first pair of dorsal setae and distal tarsal surface (leg 1)-gelatin	DV	48	0.190‡
	DA	48	0.190‡
setae and dorsal tarsal surface of legs 2-4-gelatin	DV	45	0.000‡
	DA	45	0.000‡
lateral seta (leg 1)-ablation	DV	45	0.000‡
	DA	45	0.000‡
claw sensilla leg 2-ablation	DV	45	0.000‡
	DA	45	0.200‡
claw sensilla leg 3-ablation	DV	43	0.604‡
	DA	37	0.273‡
claw sensilla leg 4-ablation	DV	43	0.214‡
	DA	37	0.273‡

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

‡: Not significant. G-statistic with Williams correction factor.

All of the G-statistic data for the initial contact, mounting and post-mounting behaviors violated the rule of heterogeneity because at least one cell of the 2x2 contingency table lacked three or more samples. This is due to the fact that almost all screened, sexually-active, untreated (control) and treated male ticks which contact the female's cuticle with the claw sensilla of the forelegs intact mount the female and complete the post-mounting behaviors (within three attempts). Conversely, ticks that have the claw sensilla of the forelegs treated in some manner so that they do not come into contact with the female's cuticle rarely mount the female. It is for these reasons that at least one cell of the table lacks a minimum of three samples. In this study a Williams correction factor was used with the G-statistic. The Williams correction factor is the preferred statistic for data in which cells have less than five samples; the G-statistic approximates the Chi-square distribution more closely with the Williams correction factor than without it, or if a different correction factor is used with the G-statistic (Sokal and Rohlf, 1981). There was no evidence of secondary receptors of the MSP, since no significant differences were noted in the results of the tests with other sensory structures of these ticks as compared to their controls; thus, the recorded time that it took for the treated ticks to complete their behaviors was not used.

D. variabilis and *D. andersoni* male ticks were able to mount conspecific females and complete the post-mounting behaviors with the claw sensilla of one of the first pair of legs ablated and the other leg (with claw sensilla) undamaged. No significant difference was observed between controls and ticks with either the right or left foreleg claw sensilla ablated (Table 6). This G-statistic data also violated the rule of heterogeneity (see above). Male ticks of both species also were able to mount conspecific females and complete the post-mounting behaviors when the ventral pair of claw sensilla were ablated (depilated) from the first pair of legs (G_{adj}=0.000 for both species: n=21 *D. variabilis* males and n=15 *D. andersoni* males). Bioassays in which the claw sensilla of the forelegs of the males were ablated and the male placed on the posterior dorsum of the conspecific female to test male post-mounting behavior revealed a significant difference (p=0.001) between controls and treated *D. variabilis* and *D. andersoni* male ticks. Most males of both species failed to complete the post-mounting behaviors (Table 7). The data for these bioassays also violated the rule of heterogeneity (see above). Runs tests were performed on the results of the bioassays of the *D. variabilis* males. The results determined that the responses of the ticks were random (t_s<1.960: t_s=1.700).

Table 6. Mounting responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks to sexually-active, conspecific females following the ablation of the claw sensilla from one of the male's forelegs.

Bioassay	species	n*	χ^2 value
ablation-right leg claw sensilla (left leg intact)	DV	45	0.200‡
	DA	45	0.200‡
ablation-left leg claw sensilla (right leg intact)	DV	45	0.561‡
	DA	43	0.214‡

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

‡: Not significant. G-statistic with Williams correction factor.

Table 7. Post-mounting responses of *D. variabilis* (DV) and *D. andersoni* (DA) male ticks with the claw sensilla of their forelegs ablated and the male placed on the posterior dorsum of sexually-active, conspecific females.

Bioassay	species	n*	χ^2 value
ablation-claw sensilla: place treated male on posterior dorsum of female	DV	46	17.250†
	DA	33	17.360†

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

†: Significant at p=0.001. G-statistic with Williams correction factor.

When gelatin was used to cover the claw sensilla of the first pair of legs in *D. variabilis*, *D. andersoni* and *A. americanum* male ticks, most males of all three species failed to mount conspecific females. There was a significant difference between the treated males and the controls ($p=0.001$; $G_{adj}=37.926$ in both *D. variabilis* and *D. andersoni* male ticks and $G_{adj}=37.241$ in *A. americanum* males). However, there was no significant difference in mounting behavior in the trauma bioassays between the male ticks which had the claw sensilla of the second pair of legs covered with gelatin and the controls (Table 8). The gelatin treatment did not cause undue stress to the tick from which it could not recover; thus, the mounting behavior of the male ticks was not altered. The data from these bioassays also violated the rule of heterogeneity for the reasons stated previously.

Covering the claw sensilla of the first pair of legs of *D. variabilis*, *D. andersoni* and *A. americanum* male ticks with gelatin suppressed mounting behavior in most cases; when the gelatin was removed from the claw sensilla, mounting behavior in the three species was restored in almost all cases (Table 9). The male tick mounted the conspecific female when the claw sensilla of its forelegs were intact but failed to mount the female when the claw sensilla were covered with gelatin (by leaving the claw sensilla intact and then covering them with gelatin, the male acted as its

Table 8. Mounting responses of *D. variabilis* (DV), *D. andersoni* (DA) and *A. americanum* (AA) male ticks to sexually-active, conspecific females following the occlusion with gelatin of the claw sensilla of the first or second pair of legs of the males.

Bioassay	species	n*	χ^2 value
claw sensilla of	DV	35	37.926†
first pair of	DA	35	37.926†
legs-gelatin	AA	36	37.241†
claw sensilla of	DV	40	0.000‡
second pair of legs	DA	40	0.273‡
(trauma)-gelatin	AA	40	0.273‡

* n = the number of treated ticks used in the bioassay calculated with 10 control ticks per bioassay.

†: Significant at p=0.001. G-statistic with Williams correction factor.

‡: Not significant. G-statistic with Williams correction factor.

Table 9. Mounting responses of *D. variabilis* (DV), *D. andersoni* (DA) and *A. americanum* (AA) male ticks to sexually-active, conspecific females when the claw sensilla of the forelegs of the males were occluded with gelatin and following removal of the gelatin from the claw sensilla.

Bioassay	species	n*	χ^2 value
no gel on cs/gel on cs	DV	45	28.130†
gel on cs/gel off cs	DV	45	28.130†
no gel on cs/gel on cs	DA	43	28.130†
gel on cs/gel off cs	DA	43	28.130†
no gel on cs/gel on cs	AA	43	26.070†
gel on cs/gel off cs	AA	43	26.760†

* n = the number of males used in the bioassay. The same male was tested three times (3x): no gelatin on the claw sensilla, gelatin on the claw sensilla and gelatin removed from the claw sensilla.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

no gel on cs = no gelatin on claw sensilla

gel on cs = gelatin on claw sensilla

gel off cs = gelatin removed from claw sensilla

own control); this resulted in a significant difference (p=0.001) between the two bioassays. Likewise, there was a significant difference (p=0.001) in the results between the gelatin-treated vs gelatin-removed males; the former failed to mount the females while the latter did so readily (Table 9).

All of the dose response bioassays that were conducted with *D. variabilis* males demonstrated that the gelatin used in these (and other) bioassays did not permit molecular penetration of the pheromone. No increases in male mounting activity occurred that would indicate a dose response, regardless of the increase in the concentration of the pheromone. Similarly, when the claw sensilla of legs 2-4 were covered with gelatin and subjected to increasing concentrations of pheromone, there was no increase in the number of males that completed the post-mounting behaviors that would indicate a dose response relationship. These same bioassays also determined that the claw sensilla of legs 2-4 of *D. variabilis* male ticks were not necessary for the male to complete the post-mounting behaviors.

In the dose response bioassays in which *D. variabilis* male ticks were tested for their mounting behavior of females that had fed on a host for one to seven days, there was a significant difference (p=0.001) in mounting between the same male when the claw sensilla of the first pair of

legs were not covered with gelatin and when the same claw sensilla were covered with gelatin (Table 10). On all seven days, male ticks with the claw sensilla intact could mount the female; however, when the claw sensilla were covered with gelatin most of the same male ticks failed to mount the female. Only one male tick, on both days four and six, was able to mount the female when the foreleg claw sensilla were covered with gelatin (Fig. 16). In contrast, using the same type of dose response bioassay, all *D. variabilis* male ticks with gelatin covering the claw sensilla of legs 2-4 were able to complete the post-mounting behaviors.

In the bioassys with the "cleaned", conspecific females, *D. variabilis* males failed to mount "cleaned" control females coated with hexane; however, the same males mounted "cleaned" females coated with 0.1, 0.5, or 1.0 FE of cholesteryl oleate. This resulted in a significant difference ($p=0.001$) between the two bioassays. When the claw sensilla on the forelegs of the same males were covered with gelatin and the test repeated, most males failed to mount the cholesteryl oleate-coated females. At all concentrations, there was a significant difference ($p=0.001$) between the mounting behavior of the male to the control and the cholesteryl oleate-coated female, as well as between the cholesteryl oleate-coated female when the male's foreleg claw sensilla were intact or covered with gelatin (Table 11). In each

Table 10. Mounting results, in dose response bioassays, of *D. variabilis* male ticks to conspecific females after occluding the claw sensilla of the male's forelegs with gelatin. The bioassays were conducted daily with females feeding for 1-7 days. n*=30.

No. of Days Females Fed	χ^2 value
1	20.382†
2	20.382†
3	20.382†
4	19.076†
5	20.382†
6	19.076†
7	20.382†

* n = the number of males used in each bioassay, day 1-7. The same male was tested two times (2x): (1) with conspecific female tick fed for 1-7 days; the claw sensilla of the first pair of legs of the male tick were not covered with gelatin, (2) with the same conspecific female tick fed for 1-7 days; claw sensilla of the first pair of legs of the male tick were covered with gelatin.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

Figure 16. Mean mounting behavior responses (dose response bioassays) of *D. variabilis* male ticks with the claw sensilla of the first pair of legs covered with gelatin to conspecific females, i.e., the number of male ticks that completed mounting. The bioassays were conducted daily with females feeding for one to seven days (eg., the bioassays with the males conducted on day four used females that had fed for four days). Bars represent standard error of the mean. (n=30).

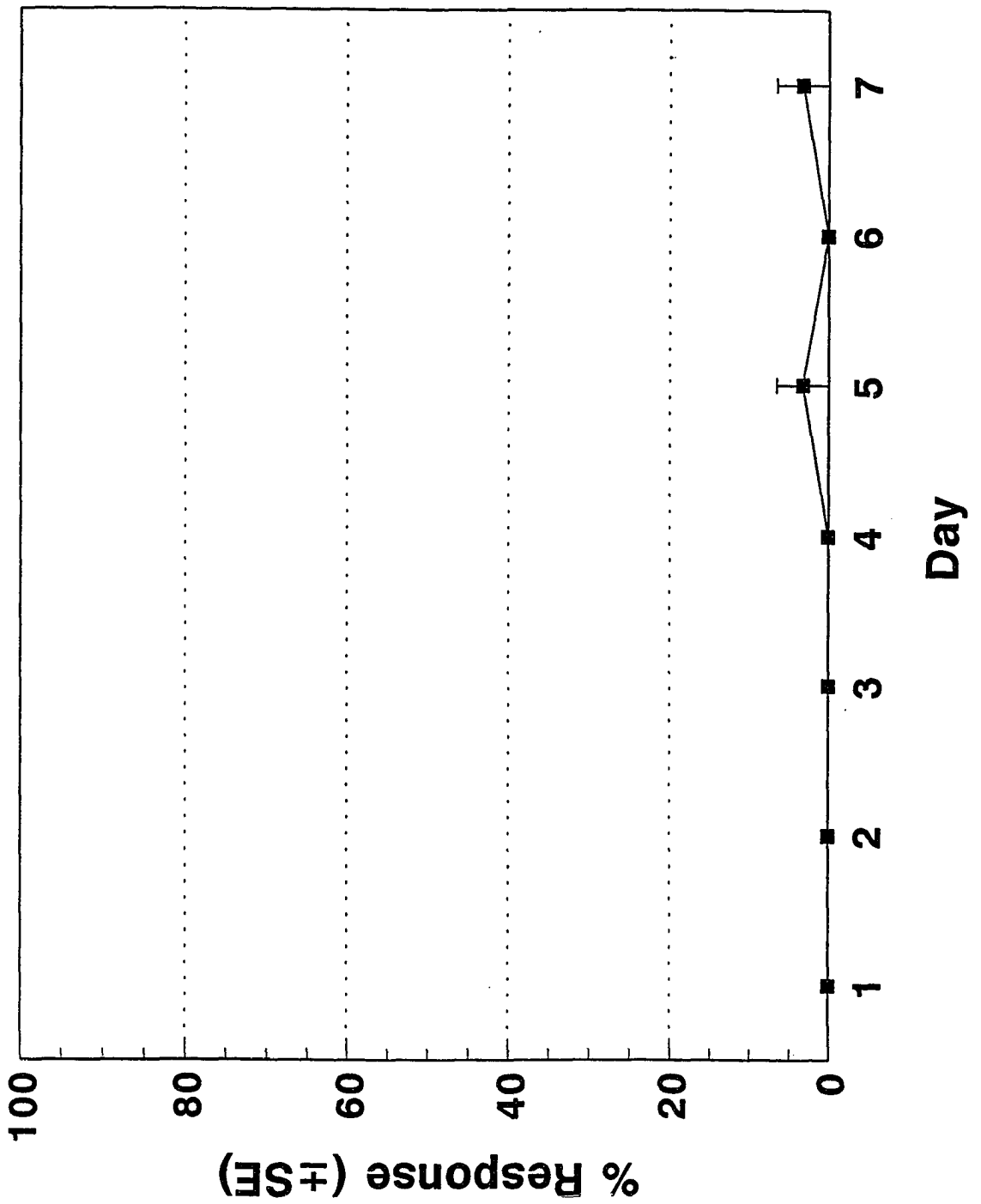


Table 11. Mounting results, in dose response bioassays, of *D. variabilis* male ticks to "cleaned" (dead), conspecific females after occluding the claw sensilla of the male's forelegs with gelatin. The "cleaned" female tick was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Concentration (FE)	Bioassay	χ^2 value
0.1	hexane/co	19.076†
0.1	co/gel	19.076†
0.5	hexane/co	19.076†
0.5	co/gel	19.076†
1.0	hexane/co	19.024†
1.0	co/gel	19.076†

* n = the number of males used in each bioassay. The same male tick was bioassayed three times (3x): (1) with a "cleaned" female control tick coated with hexane, (2) a "cleaned" female tick coated with one concentration of cholesteryl oleate and (3) the same "cleaned" female, with the claw sensilla of the first pair of legs of the male tick covered with gelatin.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = "cleaned" female control

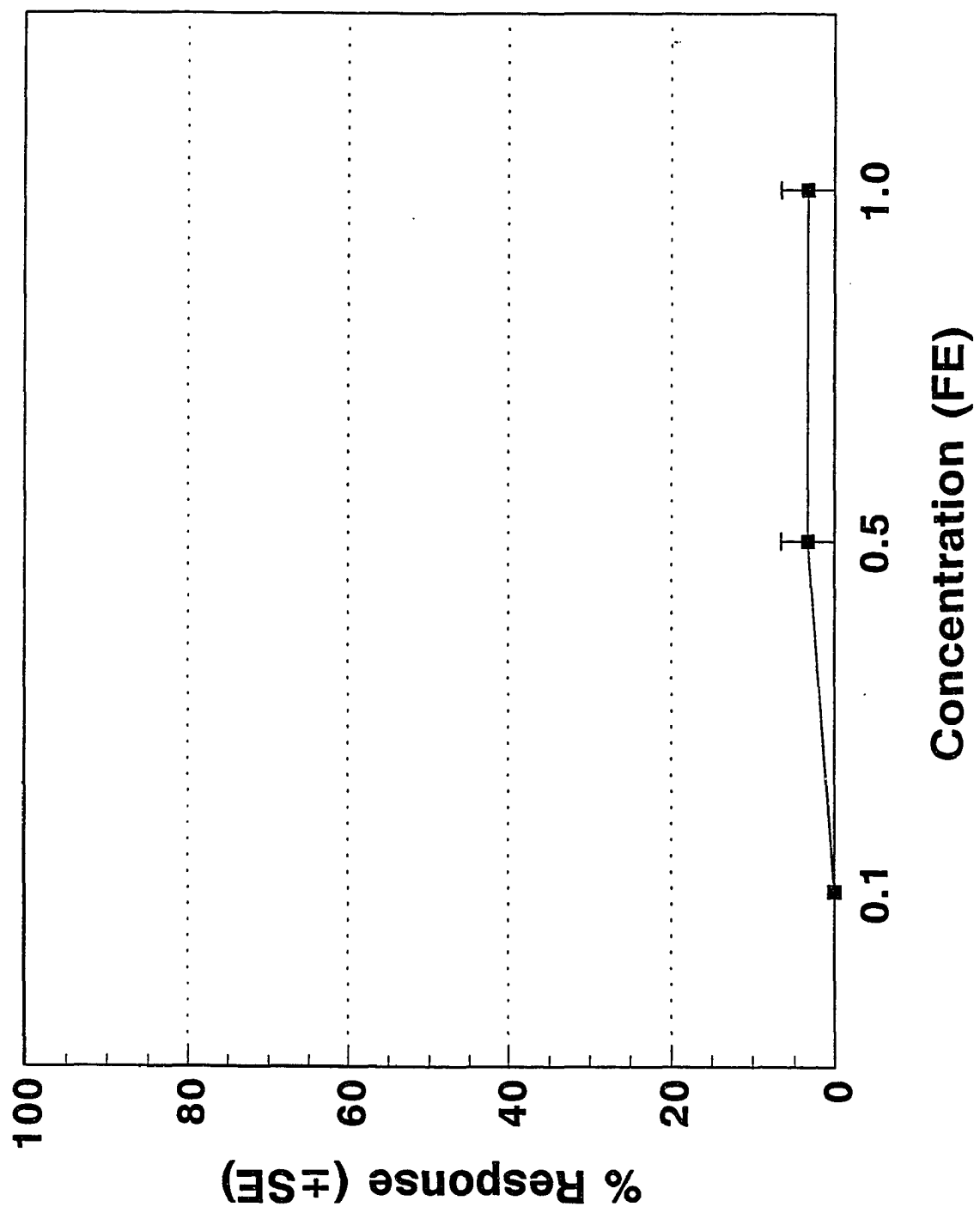
co = "cleaned" female coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of the first pair of legs of the male tick

case, only one male tick with gelatin covering the claw sensilla of the forelegs mounted the females coated with 0.5 and 1.0 FE of cholesteryl oleate (Fig. 17).

When male ticks were tested for post-mounting behavior in the "cleaned" female and inanimate object dose response bioassays, the hexane control data included males that contacted but did not mount the controls, as well as those that mounted the controls. In the dose response bioassays of mounting and post-mounting behaviors, few males (14 of 360) mounted the hexane controls (no male tick proceeded beyond dorsal anterior orientation) as compared to those "cleaned" females or inanimate objects coated with cholesteryl oleate (MSP). In order to determine whether hexane, the solvent for cholesteryl oleate, was attractive to the ticks during the post-mounting behaviors, male ticks were included that were attracted to and contacted but did not mount the controls. This was contrary to the data used in the previous post-mounting behavior bioassays, in which live, conspecific, female ticks were used, because almost every sexually active male that detected the MSP, with the claw sensilla on their forelegs, would mount the female and could be used as controls in tests of the post-mounting behaviors.

Figure 17. Mean mounting behavior responses (dose response bioassays) of *D. variabilis* male ticks with the claw sensilla of the first pair of legs covered with gelatin to "cleaned" (dead), conspecific females, i.e., the number of male ticks that completed mounting. The "cleaned" female tick was coated with either 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. Bars represent standard error of the mean. (n=30).



In the dose response bioassays for the post-mounting behaviors of *D. variabilis* males to "cleaned" conspecific females, a significant difference ($p=0.001$) was exhibited, at all three FE concentrations, between the males' failure to complete the post-mounting behaviors on the controls (for the reasons previously stated) and the completion of the behaviors by the same males on "cleaned" females coated with cholesteryl oleate. There was no significant difference, at all three FE concentrations, in the ability of the same male to complete the post-mounting behaviors on a "cleaned" female coated with cholesteryl oleate, regardless of whether legs 2-4 of the male were gelatin-coated (Table 12). Only five male ticks ($n=90$) with the claw sensilla of legs 2-4 covered with gelatin were unable to complete the post-mounting behaviors on "cleaned" females coated with the three FE concentrations of cholesteryl oleate (Fig. 18).

In the inanimate ("dummy" tick) object dose response bioassays *D. variabilis* males failed to mount hexane-coated controls; however, the same males mounted inanimate objects coated with cholesteryl oleate at all three FE concentrations. This resulted in a significant difference ($p=0.001$) between the two bioassays. When the claw sensilla on the forelegs of the same males were covered with gelatin and the males were tested with the same inanimate object coated with cholesteryl oleate, most males failed to mount the inanimate object (Table 13). Only one male tick in this treatment

Table 12. Post-mounting results, in dose response bioassays, of *D. variabilis* male ticks to "cleaned" (dead), conspecific females after occluding the claw sensilla of the legs 2-4 of the male with gelatin. The "cleaned" female tick was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Concentration (FE)	Bioassay	χ^2 value
0.1	hexane/co	18.334†
0.1	co/gel	0.150‡
0.5	hexane/co	19.024†
0.5	co/gel	0.000‡
1.0	hexane/co	20.382†
1.0	co/gel	0.000‡

* n = the number of males used in each bioassay. The same male tick was bioassayed three times (3x): (1) with a "cleaned" female control tick coated with hexane, (2) a "cleaned" female tick coated with one concentration of cholesteryl oleate and (3) the same "cleaned" female, with the claw sensilla of legs 2-4 of the male tick covered with gelatin.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

‡: Not significant. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = "cleaned" female control

co = "cleaned" female coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of legs 2-4 of the male tick

Figure 18. Mean post-mounting behavior responses (dose response bioassays) of *D. variabilis* male ticks with the claw sensilla of legs 2-4 covered with gelatin to "cleaned" (dead), conspecific females, i.e., the number of male ticks unable to complete post-mounting behaviors. The "cleaned" female tick was coated with either 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. Bars represent standard error of the mean. (n=30).

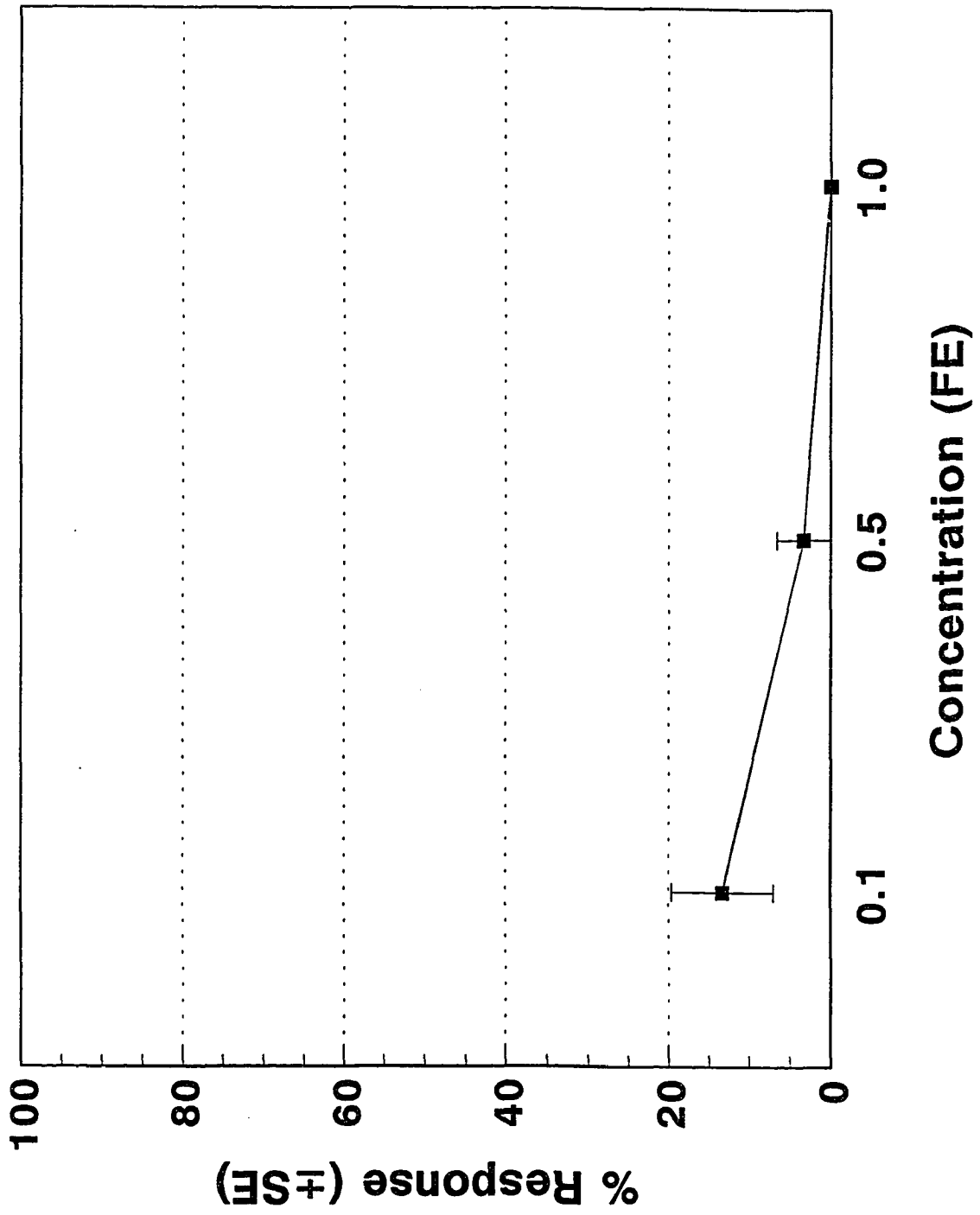


Table 13. Mounting results, in dose response bioassays, of *D. variabilis* male ticks to inanimate ("dummy" tick) objects after occluding the claw sensilla of the male's forelegs with gelatin. The inanimate object was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Concentration (FE)	Bioassay	χ^2 value
0.1	hexane/co	20.382†
0.1	co/gel	19.076†
0.5	hexane/co	20.382†
0.5	co/gel	20.382†
1.0	hexane/co	18.344†
1.0	co/gel	19.076†

* n = the number of males used in each bioassay. The same male tick was bioassayed three times (3x): (1) with a control inanimate object coated with hexane, (2) an inanimate object coated with one concentration of cholesteryl oleate and (3) the same inanimate object, with the claw sensilla of the first pair of legs of the male tick covered with gelatin.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = inanimate object control

co = inanimate object coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of the first pair of legs of the male tick

group was able to mount an inanimate object coated with 1.0 FE of cholesteryl oleate (Fig. 19).

In the dose response bioassays for post-mounting behaviors of *D. variabilis* males to inanimate objects, a significant difference ($p=0.001$) was exhibited, at all three FE concentrations, between the males' failure to complete the post-mounting behaviors on the controls (for the reasons previously stated) and the completion of the behaviors by the same males on inanimate objects coated with cholesteryl oleate. There was no significant difference, at all three FE concentrations, in the ability of the same male to complete the post-mounting behaviors on an inanimate object coated with cholesteryl oleate, regardless of whether legs 2-4 of the male were gelatin-coated (Table 14). Analysis of the number of males with legs 2-4 covered with gelatin that failed to complete the post-mounting behaviors on inanimate objects coated with cholesteryl oleate did not reveal a dose response relationship over the three concentrations of the pheromone (Fig. 20).

The transfer of stimulus bioassays demonstrated that the *D. variabilis* and *D. andersoni* male mounting response could be transferred to "cleaned" females and inanimate objects by moving the MSP stimulus (cholesteryl oleate) to these two objects. This confirms previous data that shows that the claw sensilla of the first pair of legs are responsible for

Figure 19. Mean mounting behavior responses (dose response bioassays) of *D. variabilis* male ticks with the claw sensilla of the first pair of legs covered with gelatin to inanimate ("dummy" ticks) objects, i.e., the number of male ticks that completed mounting. The inanimate objects were coated with either 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. Bars represent standard error of the mean. (n=30).

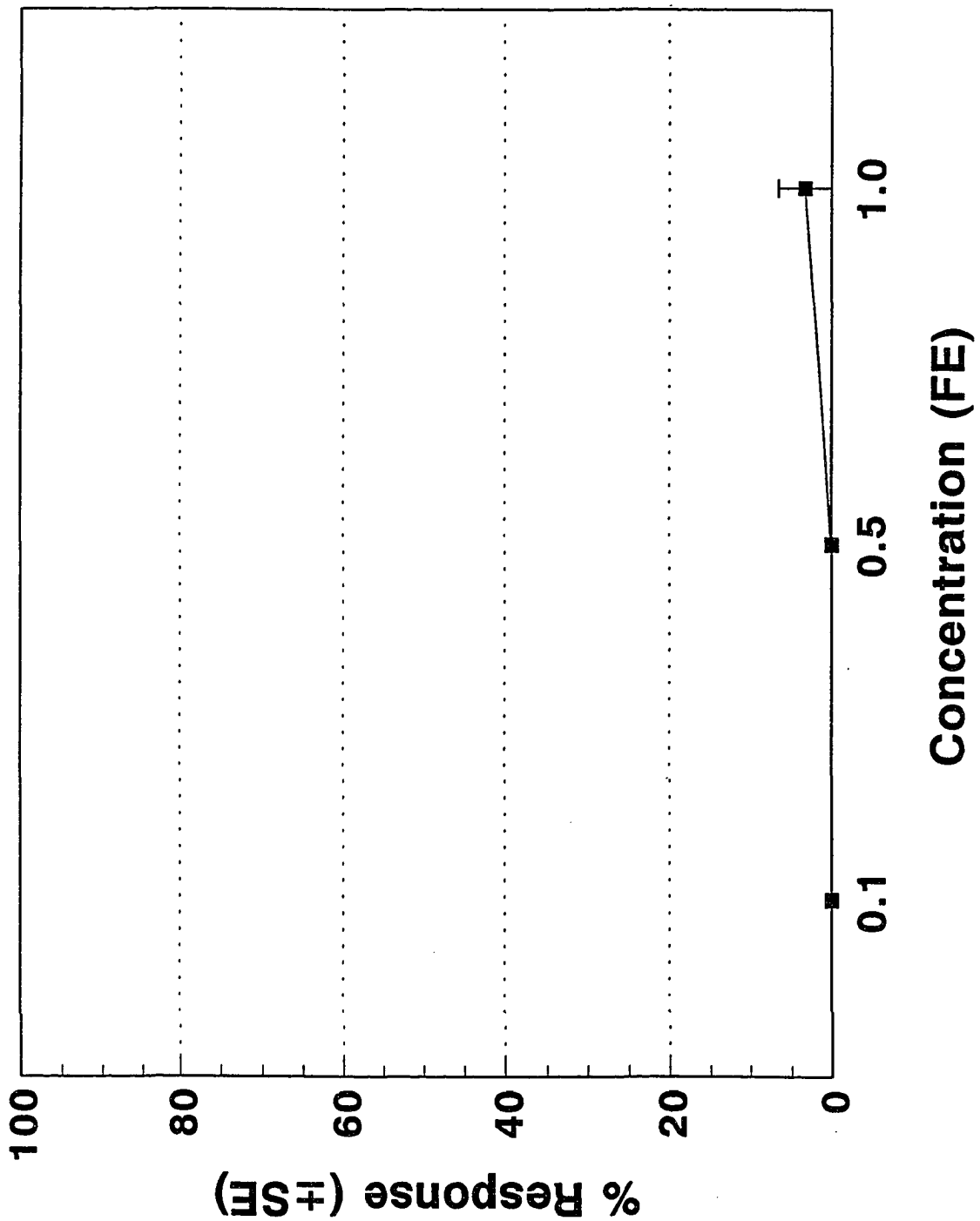


Table 14. Post-mounting results, in dose response bioassays, of *D. variabilis* male ticks to inanimate ("dummy" tick) objects after occluding the claw sensilla of the legs 2-4 of the male with gelatin. The inanimate object was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Concentration (FE)	Bioassay	χ^2 value
0.1	hexane/co	17.665†
0.1	co/gel	1.110‡
0.5	hexane/co	20.382†
0.5	co/gel	1.110‡
1.0	hexane/co	18.344†
1.0	co/gel	0.150‡

* n = the number of males used in each bioassay. The same male tick was bioassayed three times (3x): (1) with an inanimate object control tick coated with hexane, (2) an inanimate object coated with one concentration of cholesteryl oleate and (3) the same inanimate object, with the claw sensilla of legs 2-4 of the male tick covered with gelatin.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

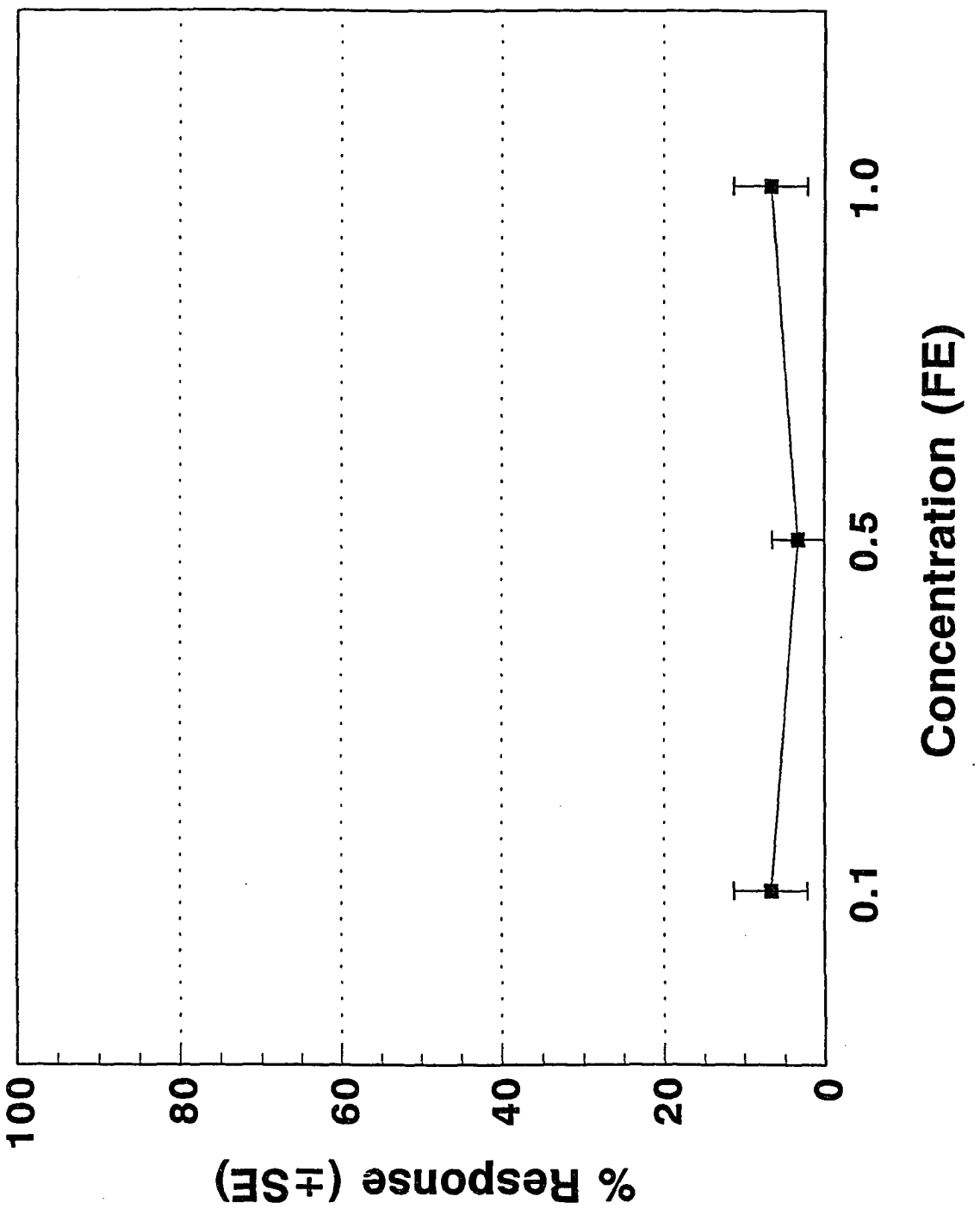
‡: Not significant. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = inanimate object control

co = inanimate object coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of legs 2-4 of the male tick

Figure 20. Mean post-mounting behavior responses (dose response bioassays) of *D. variabilis* male ticks with the claw sensilla of legs 2-4 covered with gelatin to inanimate ("dummy" ticks) objects, i.e., the number of male ticks unable to complete post-mounting behaviors. The inanimate objects were coated with either 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. Bars represent standard error of the mean. (n=30).



perceiving the MSP. In these bioassays the males responded only to the cholesteryl oleate that was used to coat the "cleaned" female and inanimate objects. The transfer of stimulus bioassays also demonstrated that the mounting behavior could be regulated by covering the claw sensilla of the first pair of legs with gelatin and likewise, removing the gelatin from the claw sensilla.

In the "cleaned" female transfer of stimulus bioassays, *D. variabilis* males failed to mount the hexane-coated controls; however, the same males mounted the "cleaned" females coated cholesteryl oleate. This resulted in a significant difference ($p=0.001$) between the two bioassays. Covering the claw sensilla of the male's forelegs with gelatin suppressed most mounting behavior; resulting in a significant difference ($p=0.001$) between the mounting behavior responses of the males with the claw sensilla intact and occluded by gelatin. Removal of the gelatin coating restored the response, i.e., most males were once again able to perceive the stimulus (Table 15).

The same significant results ($p=0.001$) also were obtained with males of both species, at all three FE concentrations, when the same mounting bioassays were conducted with inanimate objects. Most of the male ticks failed to mount hexane controls but would mount inanimate objects coated

Table 15. Mounting results, in transfer of stimulus bioassays, of *D. variabilis* and *D. andersoni* male ticks with the claw sensilla of the first pair of legs covered with gelatin and subsequent to the removal of the gelatin to "cleaned" (dead), conspecific females. The "cleaned" female tick was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Species	Concentration (FE)	Bioassay	χ^2 value
DV	0.1	hexane/co	19.024†
DV	0.1	co/gel	18.334†
DV	0.1	gel/off	18.334†
DA	0.1	hexane/co	19.024†
DA	0.1	co/gel	19.024†
DA	0.1	gel/off	19.024†
DV	0.5	hexane/co	19.076†
DV	0.5	co/gel	19.076†
DV	0.5	gel/off	19.076†
DA	0.5	hexane/co	20.382†
DA	0.5	co/gel	20.382†
DA	0.5	gel/off	20.382†
DV	1.0	hexane/co	19.076†
DV	1.0	co/gel	19.024†
DV	1.0	gel/off	19.024†
DA	1.0	hexane/co	20.382†
DA	1.0	co/gel	19.076†
DA	1.0	gel/off	19.076†

* n = the number of males used in each bioassay. The same male tick was bioassayed four times (4x): (1) with a "cleaned" female control tick coated with hexane, (2) a "cleaned" female tick coated with one concentration of cholesteryl oleate, (3) the same "cleaned" female tick at the same concentration of cholesteryl oleate, however, the claw sensilla of the first pair of legs of the male were covered with gelatin and (4) the same "cleaned" female tick at the same concentration of cholesteryl oleate, however, the gelatin was removed from the claw sensilla of the first pair of legs of the male.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = "cleaned" female control

co = "cleaned" female coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of first pair of legs of the male tick

off = gelatin removed from the claw sensilla of first pair of legs of the male tick

with cholesteryl oleate. The same males would not mount the same inanimate objects coated with cholesteryl oleate when their foreleg claw sensilla were covered with gelatin; however, when the gelatin was removed, mounting of the inanimate objects was restored (Table 16).

Table 16. Mounting results, in transfer of stimulus bioassays, of *D. variabilis* and *D. andersoni* male ticks with the claw sensilla of the first pair of legs covered with gelatin and subsequent to the removal of the gelatin to inanimate ("dummy" tick) objects. The inanimate object was coated with either hexane or 0.1, 0.5 or 1.0 female equivalent (FE) of cholesteryl oleate. n*=30.

Species	Concentration (FE)	Bioassay	χ^2 value
DV	0.1	hexane/co	19.076†
DV	0.1	co/gel	19.076†
DV	0.1	gel/off	19.076†
DA	0.1	hexane/co	19.076†
DA	0.1	co/gel	19.076†
DA	0.1	gel/off	19.076†
DV	0.5	hexane/co	20.382†
DV	0.5	co/gel	20.382†
DV	0.5	gel/off	20.382†
DA	0.5	hexane/co	20.382†
DA	0.5	co/gel	20.382†
DA	0.5	gel/off	20.382†
DV	1.0	hexane/co	18.334†
DV	1.0	co/gel	19.024†
DV	1.0	gel/off	19.024†
DA	1.0	hexane/co	19.076†
DA	1.0	co/gel	20.382†
DA	1.0	gel/off	20.382†

* n = the number of males used in each bioassay. The same male tick was bioassayed four times (4x): (1) with a control inanimate object tick coated with hexane, (2) an inanimate object coated with one concentration of cholesteryl oleate, (3) the same inanimate object at the same concentration of cholesteryl oleate, however, the claw sensilla of the first pair of legs of the male were covered with gelatin and (4) the same inanimate object at the same concentration of cholesteryl oleate, however, the gelatin was removed from the claw sensilla of the first pair of legs of the male.

†: Significant at p=0.001. McNemar Test for Significance of Changes with a Williams correction factor.

hexane = inanimate object control

co = inanimate object coated with cholesteryl oleate

gel = gelatin covering the claw sensilla of first pair of legs of the male tick

off = gelatin removed from the claw sensilla of first pair of legs of the male tick

Discussion

The results of this study clearly indicate that the foreleg claw sensilla of *D. variabilis, D. andersoni* and *A. americanum* male ticks are the receptors of the female sex pheromone, MSP. Of the six sensory setae present at this location, only the dorsal and middle pairs of claw sensilla are mechanogustatory, while the ventral pair are strictly mechanosensory. Morphological study of the dorsal and middle sensory setae reveals a single pore-like structure located at their tips; pores are absent along the sensillar shaft. These features are characteristic of gustatory or mechanogustatory sensilla, similar to those found on the palps that are believed to function as pheromone receptors (Foelix and Chu-Wang, 1972; Chu-Wang and Axtell, 1973; Balashov *et al.*, 1976; Ivanov and Leonovich, 1983; Hess and Vlimant, 1982, 1986). A detailed comparison of these setae will be discussed below. In contrast, the mechanosensory ventral setae taper to a sharply-pointed tip and lack pores, a structure characteristic of mechaonsensory sensilla found in numerous locations on the tick's appendages and body (Hess and Vlimant, 1982, 1986).

Experimental evidence described in this study demonstrates that only the male foreleg claw sensilla perceive the MSP. Male mounting behavior is suppressed when the claw sensilla are ablated or covered with gelatin, but this

behavior is restored when the gelatin is removed. Mounting behavior is unaffected when the ventral pair of foreleg claw sensilla are ablated in the *Dermacentor* species. These results provide behavioral confirmation that the ventral pair of claw sensilla are not essential for perception of the chemical stimulus and are solely mechanoreceptors. Furthermore, during the post-mounting behaviors, the male fails to reach the female's gonopore only when the foreleg claw sensilla are ablated or covered with gelatin. Bioassay results in this study demonstrate that the claw sensilla of legs 2-4 do not aid in MSP perception once the male has mounted the female, despite the fact that Hess and Vlimant (1982, 1986), using SEM and TEM, have determined that the unpaired seta of the claw sensilla of legs 2-4 is a mechano-gustatory receptor in the metastriate tick, *A. variegatum*.

Little has been done concerning the ultrastructure and function of tick claw sensilla. Moreover, the lack of a unified system of setal nomenclature (as well as of all tick setae) makes it difficult to compare the results of different studies. Various investigators have devised different systems of nomenclature for these setae (Elizarov, 1963; Zolotarev and Sinitzina, 1965; Chu-Wang and Axtell, 1973; Axtell, 1974; Waladde, 1976, 1977; Hess and Vlimant, 1982, 1983a, 1983b, 1986). Hess and Vlimant (1986) report that among the metastriate ixodid ticks there is a strong resemblance between species of the same and different genera in

the number, location, morphology, ultrastructure and function of the setae of tarsus I. For comparative purposes and because Hess and Vlimant's (1986) alphanumeric scheme was part of an overall plan for characterizing the structure and function of the leg setae, this system also was used in this study. The dorsal, middle and ventral pairs of claw sensilla are designated vItp/B, vItp/A and vInp/A, respectively (vI=first ventral group of setae; tp=terminal pore; np=no pore; A and B are setal types based on ultrastructure).

The claw sensilla of species of ticks in the genera *Amblyomma, Dermacentor* and *Boophilus* (and presumably all other metastriate ixodid genera) are composed of six setae arranged in three symmetrical pairs around the apotele of the claw in the same manner described for those of *D. variabilis, D. andersoni* and *A. americanum* in this study. Using light microscopy, SEM, TEM and electrophysiology, it has been determined that the dorsal and middle pairs of claw sensilla in metastriate ixodid ticks are mechanogustatory setae having either a terminal or subterminal pore or slit at the end of the shaft. In the dorsal pair of claw sensilla (tp/B), four chemoreceptive dendrites innervate the lumen of the shaft and two mechanoreceptive dendrites are inserted at the base. There are three to six chemoreceptive dendrites that innervate the lumen of the shaft of the

middle pair of claw sensilla (tp/A), as well as two mechanoreceptive dendrites inserted at the base. The ventral pair of claw sensilla are mechanoreceptors that function as touch or vibration receptors (Elizarov, 1963; Zolotarev and Sinitzina, 1965; Chu-Wang and Axtell, 1973; Axtell, 1974; Waladde, 1976, 1977; Waladde and Rice, 1982; Hess and Vlimant, 1982, 1986). The descriptions of the ultrastructure of the dorsal and middle pairs of foreleg claw sensilla are those of a typical contact chemoreceptor in arthropods (Slifer, 1970; Hodgson, 1974; Zacharuk, 1980).

The tp/A sensilla occur not only in the middle pair of claw sensilla but also on most articles of all of the legs (Hess and Vlimant, 1982, 1986) and identical or similar sensilla have been reported in the palps of *H. asiaticum* (Balashov *et al.*, 1976) and *A. americanum* (Foelix and Chu-Wang, 1972). The tp/B sensilla only occur in the dorsal pair of foreleg claw sensilla (Hess and Vlimant, 1982, 1986). Setae which are possibly the same type are reported to be located on the palpal organ in *H. asiaticum* (Balashov *et al.*, 1976) and *A. americanum* (Foelix and Chu-Wang, 1972). There are no known comprehensive reports concerning these terminal pore sensilla from the bodies of ticks.

The six claw sensilla of the foreleg of prostriate ixodid ticks also contain mechanogustatory setae. Using electrophysiology, Zolotarev and Elizarov (1964) demonstrated that

one pair of trichoid sensilla in the foreleg of *Ixodes persulcatus* and *I. ricinus* function as contact chemoreceptors. Further work by Zolotarev and Sinitzina (1965) corroborated these results. However, Hess and Vlimant (1986) report that the claw sensilla of *I. ricinus* are identical to those of other ixodid ticks; the dorsal and middle pairs of claw sensilla in this species also are mechanogustatory in function.

Argasid ticks exhibit varying numbers of foreleg claw sensilla both between individuals of the same species and different species (Chu-Wang and Axtell, 1973; Hess and Vlimant, 1986). *Argas (Persicargas) arboreus* usually has six pairs of setae arranged symmetrically on the lateral and medial sides of the apotele of the claw; however, the number of type 2 claw sensilla (=vI tp/A or middle pair of claw sensilla of ixodid ticks) varies from four to seven in different specimens. The first (dorsal) and last (ventral) pairs of claw sensilla are always type 1 (=vI tp/B or dorsal pair of claw sensilla in ixodid ticks) and mechanoreceptors, respectively. The type 2 sensilla also vary in the degree of innervation of the shaft (Chu-Wang and Axtell, 1973). The foreleg claw sensilla of *Ornithodoros moubata* consist of four paired setae all of which function as mechanogustatory setae (tp/A and tp/B) (Hess and Vlimant, 1986). Ixodid ticks do not exhibit this difference between specimens of the same species and between species of different genera;

homologous groups of setae consist of the same types of sensilla arranged in a similar or identical pattern (Hess and Vlimant, 1986).

In their study of the foreleg claw sensilla of *A. (P.) arboreus* and *A. americanum*, Chu-Wang and Axtell (1973) state that their results on the ultrastructure of the claw sensilla correlate well with the behavior of the ticks. The tip of the foreleg tarsi are used to probe the substrate when walking and feeding and the contact chemoreceptive claw sensilla are ideal for detecting the nature of the tick's surroundings. The authors also note, that like the claw sensilla, the contact chemoreceptors of the palps are brought into contact with the substrate during feeding and mating.

While the previous studies of the sensory function of the foreleg claw sensilla of ixodid ticks (above) confirm the results of the present investigation, some discrepancies do occur. Based on the SEM results of this report and that of Hess and Vlimant (1982, 1986), the structures of the terminal end of the setae and the pores of the tp/B and tp/A setae differ between the ticks in the different species. The terminal pore of the tp/B setae in Hess and Vlimant's (1982, 1986) studies of tarsus I setae of *A. variegatum, A. nuttali, D. marginatus* and *B. microplus* is starfish-shaped, but it is circular and located in a subterminal

depression at the end of the shaft in *D. variabilis*, *D. andersoni* and *A. americanum*. In *A. variegatum*, *A. nuttali*, *D. marginatus* and *B. microplus* the pore of the tp/A setae is a slit-like opening at the terminal end of the shaft, while in the ticks described in this study the pore is roughly circular at the terminal end of the setae and slits in the shaft near the terminal end are absent. In both studies, the presence of terminal pores attest to the gustatory function of these setae; thus, if homologous groups of setae are identical in type and arrangement (Hess and Vlimant, 1986), why is there a difference between the tp/B and tp/A setae in these ticks? Several factors may explain these differences: (1) artifacts due to SEM preparation, (2) the magnitude of resolution attained by SEM used by the different workers and (3) morphological differences in the setae between the American tick species and their Old World counterparts.

Few electrophysiological studies have been conducted to determine what types of chemical stimuli are perceived by the foreleg claw sensilla of ticks. Zolotarev and Elizarov (1964) and Zolotarev and Sinitzina (1965) demonstrated that one pair of foreleg claw sensilla in *I. persulcatus* and *I. ricinus* and two pairs of foreleg claw sensilla in *H. asiaticum* were sensitive to repellant chemicals. Waladde (1978) obtained electrophysiological responses from the distal dorsal pair of claw

sensilla; tp/B) of *B. microplus* to salt solutions, such as NaCl, KCl, LiCl, choline chloride and $MgCl_2$, suggesting that these sensilla are used by the tick to taste substances on the host's surface in preparation for feeding (Waladde and Rice, 1982). The distal lateral (middle pair of claw sensilla; tp/A) pair of claw sensilla in *B. microplus* are similar to the distal dorsal pair; however, the sensillar lymph areas of the distal lateral sensilla have very electron-dense staining properties and their dendrites are surrounded by a structure resembling the type 2 claw sensilla of *A. americanum* and *A. (P.) arboreus* (Waladde, 1976, 1977, 1978; Chu-Wang and Axtell, 1973). Waladde and Rice (1982) were unable to obtain electrophysiological responses from the distal lateral sensilla due to either the very high resistance at the tip of the sensillum or their responses may have been limited to chemical factors not tested.

It was previously noted that tp/A and tp/B setae are similar to setae found in the palps of *A. americanum* (Foelix and Chu-Wang, 1972) and *H. asiaticum* (Balashov *et al.*, 1976). The type 1 and type 2 claw sensilla (dorsal (tp/B) and middle (tp/A) pairs of claw sensilla, respectively) are reported to resemble the type B (short, thick) and type A (long, thin) sensilla, respectively on the palpal organ of *A. americanum* (Chu-Wang and Axtell, 1973; Foelix and Chu-Wang, 1972). However, electrophysiology was not performed

on either the setae of the claw sensilla or the palps in those studies.

Balashov *et al.* (1976), using electrophysiology, obtained responses from type A palpal sensilla to salt solutions, as well as glucose and sucrose solutions but could not obtain the same responses from the type B palpal sensilla. Ivanov and Leonovich (1983) conclude that palpal organ type B sensilla probably perceive pheromones. If the tp/A and tp/B setae of the claw sensilla are similar to the type A and B sensilla of the palps, respectively, then Balashov *et al.*'s results contradict Waladde's (1978) work with tick claw sensilla. Waladde (1978) was able to obtain responses from the tp/B setae (dorsal pair of claw sensilla) but not the tp/A setae (middle pair of claw sensilla), whereas, Balashov *et al.* (1976) were able to obtain responses only from the type A palpal sensilla (=tp/A claw sensilla).

Furthermore, during SEM studies of adult *B. microplus*, Waladde and Rice (1982) reported larger, prominent pores in the tips of the type A and B palpal organ sensilla as compared to the minute pores in those of *A. americanum*. They noted that an exudate was sometimes secreted from the pores which probably was produced from cellular projections of the enveloping cells of the lumen. They also stated that features of both the type A and B sensilla were characteristic

of mechanogustatory receptors. Using electrophysiology, they reported that some of the sensilla contain a neuron which responds to stimulation with NaCl solutions; however, which type of palpal sensilla contain this type of neuron is unknown. Previously, Waladde (1978) sectioned the palpal organ of *B. microplus* and found that it contained sixty-four chemoreceptor neurons. Electrophysiological recordings were made from very few of these neurons, perhaps because the previously noted exudate prevents the stimulating solutions from reaching the dendrites.

Leonovich and Dusbábek (1991), using an ion-etching technique, reported a third type of sensilla in the palps of the argasid tick *A. (P.) persicus*, as well as palpal sensilla that correspond to type A and B in ixodid ticks. This third type of sensilla is similar to type B but lacks mechanoreceptors. The authors state that the sensilla were noted by Foelix and Chu-Wang (1972) in their study of *A. americanum* but were not regarded as a separate type.

Further electrophysiological studies need to be conducted in conjunction with behavioral bioassays on both the tp/A and tp/B claw sensilla and the type A and B palpal sensilla to authenticate these results and to determine if the tp/A and tp/B setae of the claw sensilla and the type A and B palpal sensilla are functionally, as well as structurally similar.

The only known mechanogustatory receptor structures of ticks that detect non-volatile (contact) pheromones are the palps and the cheliceral digits. The function of the palps is two-fold, possibly due to the setal types involved. Behavioral experiments have shown that the palps are important in locating the place of attachment for feeding on a host; when the palps are ablated, most ticks fail to attach (El-Ziady, 1958).

The palps also perceive contact pheromones. Experiments by Leahy *et al.* (1975) demonstrated that the palps are responsible for the perception of an assembly pheromone in *O. moubata*. Ablating or coating the first tarsi of the tick reduces the assembly response, however, removal of the palps completely eliminates the behavior. The palps also may be responsible for the perception of an assembly pheromone in other species of *Ornithodoros*, as well as several species of the genus *Argas* (Leahy, 1979). Gothe and Kraiss (1982) believe that the palps are the receptors of the assembly pheromone of *Argas (Persicargas) walkerae*. Graf (1975) reported palpal perception of the assembly/sex attractant pheromone of the ixodid tick, *I. ricinus*. Feldman-Muhsam and Borut (1971) stated that male ixodid ticks would not copulate if the palps were ablated, but Sonenshine *et al.* (1984) reported that the ixodid tick, *D. variabilis* copulated readily when one palp was removed; however, if both palps were ablated, copulations decreased.

Taylor (1989), using electrophysiological techniques, noted that the palps of *D. variabilis* and *D. andersoni* male ticks were not sensitive to crude GSP extract or ecdysteroids that partially comprise the GSP of these species.

Like the palps, the cheliceral digits exhibit a dual function. For years, the cheliceral digits were believed only to be uninnervated cutting structures, until Waladde and Rice (1977) demonstrated the existence of contact chemosensilla and mechanosensilla in the appendages. Electrophysiological studies of the gustatory pore sensilla of the cheliceral digits of *B. microplus* determined that the sensilla respond to normal plasma components of the host's blood (Waladde and Rice, 1982). *D. variabilis* also exhibit these gustatory pore sensilla, as well as a large placoid sensilla located at the base of the inner digit and innervated by a small nerve (Sonenshine *et al.* 1984).

In addition to their role in feeding, the chelicerae also function in contact sex pheromone perception. In the courtship ritual of *D. variabilis*, *D. andersoni* and *A. americanum*, copulation does not occur until the contact GSP, in the female's anterior reproductive tract, is perceived by the chemosensory receptors on the male's cheliceral digits (Sonenshine *et al.*, 1984, 1985; Allan *et al.*, 1988, 1991). Ablating the cheliceral digits of male

ticks not only eliminates copulation but also the release of spermatophores (Sonenshine *et al.*, 1984).

Like the palps and cheliceral digits, the dorsal (tp/B) and middle (tp/A) pairs of setae of the foreleg claw sensilla are mechanogustatory receptors. Waladde (1978) obtained electrophysiological responses from the dorsal pair of claw sensilla to chemicals similar to those secreted on the host's surface in *B. microplus;* however, he was unable to obtain electrophysiological responses from the middle pair of claw sensilla, perhaps because the correct chemical stimulants were not administered. Waladde and Rice (1982) assumed that the dorsal pair of claw sensilla are used to taste host substances in preparation for feeding. The forelegs of ticks are analogous to insect antennae because of the abundance of sensory setae located there and the manner in which ticks use them to receive information concerning the surrounding environment.

Until this report, the foreleg claw sensilla of the ticks were not known to function as contact sex pheromone receptors. The results of this study demonstrated that only the tips on the male's foreleg tarsi initially contact the sexually active female during the courtship ritual and remain in continual contact throughout the post-mounting behaviors. Previously, in *D. variabilis* and *D. andersoni*, the male's palps were believed to contact the female's

cuticle throughout the courtship ritual. However, *D. variabilis* males rarely palpate the female during the behaviors for which the MSP is reported to be responsible, although approximately 50% of *D. andersoni* males palpate the female. At initial contact with the female only the male's foreleg claw sensilla are responsible for perceiving the contact sex pheromone, MSP.

Additional evidence in support of the hypothesis that the foreleg claw sensilla are pheromone receptors is found in the results of the occlusion studies reported here. During the post-mounting behaviors, males failed to reach the female's gonopore only when the foreleg claw sensilla were ablated or covered with gelatin. While mounting behavior is due exclusively to the detection of the MSP by the male's foreleg claw sensilla, preliminary evidence (Phillips, unpublished data), as well as some bioassay results in this report, suggests that the male is guided through the post-mounting behaviors not only by the MSP but also a concentration gradient of the ASP, 2,6 DCP, secreted from the foveal glands (when the male reaches the foveae dorsales, it becomes momentarily akinetic before proceeding to the female's venter). The two pheromones, the non-volatile MSP and the volatile ASP, may function in a synergistic manner to enable the male to reach the female's gonopore. Perhaps the volatile sex pheromone enhances the effect of the contact sex pheromone just as vision,

tactile stimuli or interactive behavior patterns enhance the contact sex pheromone perception in some insects (Carlson *et al.*, 1978; Matthews and Matthews, 1978; Langley *et al.*, 1982). The mechanogustatory function of the foreleg claw sensilla is similar to that of the palps and cheliceral digits. The sensilla on all three appendages function (1) as taste receptors for host-feeding and (2) as receptors of contact (sex) pheromones. The function of the foreleg claw sensilla as receptors of the cuticular contact sex pheromone, MSP, should not be surprising, because most tick chemosensory structures are located on the distal portions of their body where there is easy access to important stimuli from their environment.

It is not known which of the mechanogustatory claw sensilla detect the MSP. Do the dorsal (tp/B) and middle (tp/A) pairs of setae of male ticks detect both host secretions and pheromones or does one pair of claw sensilla detect host secretions while the other perceives the MSP? Occasionally, one finds that only one or two sensilla in a complex sensory field perceive a specific chemical stimulus. Thus, Waladde (1982), using electrophysiology, demonstrated that the olfactory Ap_1 sensillum in the Haller's organ of male *R. appendiculatus* and *A. variegatum* responds to both ammonia (host secretions) and to the pheromone 2,6 DCP (although an electrophysiological study by Haggart and Davis (1981) of the Ap_1 sensillum in *A. americanum* males shows

that the receptor is specific for 2,6 DCP). Waladde (1978) obtained electrophysiological responses from the dorsal pair of claw sensilla of *B. microplus* to chemicals similar to substances on the host's surface. In contrast, he did not obtain responses from the middle pair of claw sensilla suggesting a high degree of compound specificity in their perceptive roles. Might the tp/A setae of the middle pair of claw sensilla be used to detect pheromones in the sexually active males?

The claw sensilla complex develops to full maturity only in the adult stage, a pattern characteristic of other gustatory sensilla. In *A. variegatum*, only the dorsal (tp/B) and ventral (np/A) pairs of claw sensilla occur in the larval and nymphal stages. The number of tp/A gustatory setae increase greatly in the adult stage, while the number of olfactory setae remains at the nymphal level. This increase in the number of tp/A sensilla suggests that taste plays an important role in the sexual behavior of *A. variegatum* (Hess and Vlimant, 1983b). Thermoreception also is increased in adults because some thermosensitive units are associated with tp/A sensilla (Waladde *et al.*, 1981); however, none have been observed to occur in the claw sensilla. In this study, *D. variabilis, D. andersoni* and *A. americanum* nymphs also lack the middle pair of claw sensilla; it only occurs in the adults of these three species. One might speculate then that the dorsal (tp/B) pair of claw sensilla are used

exclusively to taste substances secreted onto the host's skin; the electrophysiological data of Waladde (1978) indicates this. As larvae and nymphs, the ticks need only to secure a host on which to feed; mating is not involved at this stage and the middle (tp/A) pair of claw sensilla which detect pheromones would be superfluous. Additionally, the middle pair of claw sensilla are developed along with the increased numbers of tp/A sensilla at the adult stage and may be used exclusively by sexually active males to detect the contact pheromones for mating. The same claw sensilla would function as additional gustatory receptors for host secretions in the adult females. In order to verify this hypothesis, electrophysiological studies need to be conducted in conjunction with behavioral bioassays. Unfortunately, selective depilation of individual setae is difficult to perform and such studies were beyond the scope of this investigation.

That both the male and female ticks possess the same claw sensilla does not affect the hypothesis that the male uses the middle pair (tp/A) of setae to detect pheromones, while the female utilizes them in an unknown manner. Ample evidence is available from studies of other sensory organs which are morphologically similar in both sexes but perceive different chemical signals, e.g., Haller's organ. The olfactory Ap_1 sensillum is found in the Haller's organ of both sexes, but only that of the male recognizes the

female-produced attractant sex pheromone, 2,6 DCP. Strong electrophysiological responses have been obtained from the males to the pheromone, whereas only weak responses were elicited by the females (Haggart and Davis, 1981). The females may use the Ap_1 sensillum to detect host odors, such as ammonia (Waladde, 1982). Likewise, while morphologically similar chemosensory receptors occur in the cheliceral digits in both sexes, only those of the male are used to detect the GSP in the female's genital tract (Sonenshine *et al.*, 1984).

One way in which sex pheromones can be classified is by their relative volatility (Bradshaw *et al.*, 1983). Volatile sex pheromones, which enable individuals to locate mates over relatively large distances, and their olfactory receptors have been studied extensively in arthropods, especially in the Insecta (Jacobson, 1972; Matthews and Matthews, 1978; Zacharuk, 1985; Steinbrecht, 1987; Schneider, 1992). Conversely, the role of contact sex pheromones in coordinating arthropod mating activities has been recognized relatively recently, even though they are not uncommon in the Insecta. These pheromones, located predominantly on the cuticular surface of the organism, are non-volatile or of low volatility; thus the chemosensitivity of the seta that detects the pheromone is through direct contact with the chemical(s) in solution (Zacharuk, 1985). However, a few gustatory setae also have been shown to respond to olfactory stimuli in

Phormia (Dethier, 1972) and larvae of *Manduca sexta* (Stadler and Hanson, 1975), although the responses are so close to the source that they verge on being contact in character. Only the non-volatile compound(s) that is detected when the gustatory seta is in direct, physical contact with the cuticle can be correctly termed a cuticular contact sex pheromone. This pattern was observed in the response of the males when their claw sensilla were in contact with the MSP in this study.

Contact sex pheromones that provide mate recognition (and stimulate copulation) have been demonstrated in the Acari, as well as other arthropod groups. However, less is known about the receptors that perceive these pheromones as compared to the Insecta. A contact sex pheromone presumably similar to the MSP of metastriate ixodid ticks is secreted in the coxal fluid of the argasid ticks *O. erraticus* and *O. savignyi*. Like the MSP, this pheromone is a byproduct of female blood-feeding. Argasid males respond to the pheromone by mounting the female's dorsum and palpating the regions of the coxal gland orifices on either side of the body. Like the MSP, this pheromone is not species specific; heterospecific coxal fluid from *Ornithodoros* species induces the same behaviors. Active, ambulatory females also are essential to the mating behavior; the male mating response is not evoked if the female is immobilized. However, if the female is freed, the normal mating behavior is restored

(Schlein and Gunders, 1981; Mohamed *et al.*, 1990). The pheromone is probably non-volatile or of very low volatility and can be classified as a contact sex pheromone. It would not be surprising if, once the behavioral pattern is stimulated by movement, perception of this pheromone is accomplished by the claw sensilla. *Ornithodoros* species have two pairs of mechanogustatory claw sensilla on each foreleg; the pair of mechanoreceptive claw sensilla, found in the ixodids, is absent (Hess and Vlimant, 1986).

Experiments by Sonenshine *et al.* (1991) indicated that the MSP, cholesteryl oleate, and/or other cholesteryl esters, occur not only in *D. variabilis*, *D. andersoni* and *A. americanum* but also in *A. maculatum* and possibly *R. sanguineus*. Because these species conform to the *Dermacentor* courtship behavioral model, it is probable that these species also use the foreleg claw sensilla to recognize potential mating partners.

In the order Araneae, vibration and vision play as active a role in most courtship behaviors as do mechanogustatory and olfactory perception (Foelix, 1982). Although they may vary in their external appearance, the chemosensory sensilla of arachnids are very similar to those of the other arthropod groups (Foelix, 1985). Suter *et al.* (1987) demonstrated the presence of an unidentified cuticular contact sex pheromone in *Frontinella pyrimitela* which enables the male spider

to recognize a conspecific mating partner. The receptor of the pheromone is unknown. Male wolf spiders use the contact chemoreceptors on their tarsi and palps to recognize the silk of conspecific females. Draglines of female wolf spiders, with a contact sex pheromone secreted on them, induce "following behavior" and courtship in the male (Tietjen, 1979; Tietjen and Rovner, 1980).

A number of studies have been conducted in the identification of the cuticular contact sex pheromones and their receptors in the African tsetse flies because they are the vectors of African sleeping sickness. Langley *et al.* (1975) first described the cuticular contact sex pheromone of *Glossina (morsitans) morsitans* as 15, 19, 23-trimethylheptatriacontane; later, Carlson *et al.* (1978) isolated, identified and synthesized the compound along with non-stimulatory components of the pheromone. Carlson *et al.* (1978), Huyton *et al.* (1980), Langley *et al.* (1982) and Langley and Hall (1984) also noted that visual and tactile stimuli are important in the mating behavior of tsetse flies. Males do not contact non-moving females. Males also are acutely sensitive to differences in the size of the female. In pseudofly bioassays, in which the decoy is coated with the female-produced contact sex pheromone, males can detect differences as small as 0.5 mm in the radius of the pseudofly to that of a sexually active female and do not continue the mating behavior. The non-volatile contact sex

pheromone is important in reinforcing mate selection and releasing mating activity only after the male is attracted to the female by visual and tactile stimuli. It has been suggested that methylalkanes, found in other species of *Glossina*, have contact sex pheromone activity (Offor *et al.*, 1981; Langley *et al.*, 1982; Nelson *et al.*, 1988).

Schlein *et al.* (1981) studied the receptors of the contact sex pheromone in *G. (m.) morsitans*. The authors covered regions of the legs that contained gustatory setae, including the upper part of the tibia and the adjacent area, the tarsi and the lower part of the tibia and femur, with paraffin wax. Only when the proximal tibial area was covered with wax was mating behavior eliminated; control flies in which the distal part of the tibia or femur were coated with wax behaved normally. SEM revealed receptors arranged in two pairs on the inner and outer sides of the proximal ends of the legs. They concluded that these receptors function in cuticular contact sex pheromone perception.

Contact sex pheromones also have been shown to occur in a number of other species of *Diptera*. Species specific olefins are responsible for female mate recognition in *Fannia* species (Uebel *et al.*, 1977, 1978). In the face fly, *Musca autumnalis*, straight-chain monoalkenes were determined to play the same role (Sonnet *et al.*, 1975; Uebel *et al.*,

1975), while in the stable fly, *Stomoxys calcitrans*, unsaturated hydrocarbons having C_{31} and C_{33} carbon chains were the most active components in stimulating mating behavior (Muhammed *et al.*, 1975; Uebel *et al.*, 1975). All of these compounds are of very low volatility and induce mating strikes on the females; once contact is made the male recognizes the female as a mating partner (Tobin and Stoffolano, 1973). Although the receptors of these pheromones have not been identified, the gustatory tibial receptors used by other species for the same and similar behavioral responses (Schlein *et al.*, 1981) are possible sites for this role.

Mosquitoes use visual, tactile and sound stimuli as well as mechanogustatory and olfactory perception in their mating behaviors (Matthews and Matthews, 1978). Nijhout and Craig (1971) found that in various species of *Aedes* mosquitoes belonging to the subgenus *Stegomyia*, females use flight sound to initially attract males; however, female mate recognition only occurs after the male contacts the female and detects a species specific contact sex pheromone. The chemical composition of the pheromone is unknown, but the receptors are believed to be tarsal sense organs. In *Culiseta inornata*, Lang and Foster (1976) noted that after contacting the females, male mosquitoes were stimulated by male-female leg contact. They suggested that a leg-associated contact sex pheromone plays a role in mate recognition and the release of male copulatory attempts. The authors

further noted that visual and sound stimuli play a very small part in the mating behavior of *C. inornata*. Using chemical and behavioral bioassays, Lang (1977) demonstrated that a contact sex pheromone on the female leg surfaces plays a major role in male recognition of conspecific females; physical components on the female leg surface also may play a minor role. The chemical composition of the pheromone is unknown. It is believed that gustatory setae on the legs of the male detect the pheromone; however, this has not been proven.

In metastriate ixodid ticks, the non-volatile MSP is the second pheromone in a hierarchy of compounds involved in the mating behavior. As each pheromone is detected it releases an increasingly more specific series of mating behaviors. This behavioral system is different from that of most other arthropods in that it is entirely chemically-mediated and relies on perception of the pheromones solely through olfaction and gustation. In other groups of arthropods, non-chemical stimuli, such as size, shape, sound, vibration, movement and interactive behavior patterns, are as important as olfactory and gustatory perception in courtship behavior; this is best demonstrated in the Insecta. The metastriate ixodid ticks even differ from their argasid counterparts in that the latter also appear to be initially stimulated by movement. The behavioral mating system of these ticks,

involving multiple pheromones and different, discrete receptors for the detection of each compound, may be unique among arthropods.

Ticks are major vectors of diseases that are of worldwide medical and veterinary importance. Determining a sensory receptor, studying and correlating its ultrastructure, neurophysiology and response to specific chemical cues can lead to an understanding of host-finding, feeding and mating behaviors in ticks. Comparisons of receptors of closely related species or different genera and families may lead to insights into habitat and host factors, as well as evolutionary relationships. An understanding of tick pheromones and the receptors that detect them also can be used in more effective integrated pest management programs that are less harmful to the overall environment. Since the behavior of the target pest species must be fully understood before such strategies can be exploited, the chemicals to which the ticks respond must be defined, their origins determined, the receptors that detect them identified and the sensitivity of the recipient evaluated. The chemicals can then be incorporated with acaricides into a delivery system for more efficient, practical application in the field for population suppression.

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AUTOBIOGRAPHICAL STATEMENT

James S. Phillips was born on September 9, 1959 in Scranton, Pennsylvania. He received his B.S. in Biology Research from the University of Scranton in 1981 and earned his M.S. in Biological Science from Bloomsburg University of Pennsylvania in 1984 with a thesis entitled, "The Socio-biology of *Hymenolepis diminuta* (Cestoda) and *Moniliformis moniliformis* (Acanthocephala) in Single, Crowded and Concurrent Infections in Laboratory Rats."

He has co-authored the following articles:

Taylor, D., J.S. Phillips, D.E. Sonenshine and F.E. Hanson. 1991. Ecdysteroids as a component of the genital sex pheromone in two species of hard ticks, *Dermacentor variabilis* and *D. andersoni* (Acari: Ixodidae). Exp. and Appl. Acarology 12:275-296.

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He has received the following awards:

Student Membership Award in American Ornithologists Union (AOU) 1981.

Special Doctoral Research Assistantship (SDRA), Old Dominion University 1985-1988.

He is a member of the following professional and honor societies:

Entomological Society of America
American Ornithologists Union
Virginia Academy of Sciences
Beta Beta Beta Biological Honor Society
Honor Society of Phi Kappa Phi
Sigma XI, The Scientific Research Society