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Semiochemical Receptors in Steatoda Grossa

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Abstract-Web building spiders are known to have poorly developed eyes, and acoustic organ for communication. Thus the only mechanism possible for mate identification is chemical communication. In nature female spider build a web and male wander around until find a terrestrial pheromones through female silk. Our preliminary experiments have confirmed absence of air borne pheromones. To ensure existence of semiochemicals series of experiment were carried out on a Theridiidae spider *Steatoda grossa* between combination of mature male with mature and immature female *Steatoda*. Male *Steatoda* when introduced on web of female showed vibration and female also showed vigorous vibration in response to male vibrations. Male responded to female web as soon as he landed on web confirming existence of some kind of identification factor on web which can be semiochemicals. The semiochemicals receptor have been detected on leg of *Steatoda grossa* through SEM. The receptor are located on the hair arranged in rows concentrating mainly on tarsal segment both the short and long hairs act as receptor for receiving stimulus.

Key words: *Steatoda* , Semiochemicals , Receptor

INTRODUCTION

Communication in spider is an interesting aspect to study in depth. Though spider generally live a solitary life , Communication plays an important role throughout their life because possibility of finding mating partner is very low. Spider communicates by chemical, tactile, acoustic and visual signals (Huber, 2005). Web building spider have evolved to rely heavily on chemical signal as they are long lasting, work over long distance and required in small quantities. Chemical signals are likely the oldest means of communication evolved in nature (Wyatt, 2003; Gaskett, 2007; Suter and Hirscheimer, 1986) but are highly understudied in spider. The molecules that are web attached and spread information among individual are known as Semiochemicals (Schulz, 2004a). In spiders, studies have given strong indication for presence of contact semiochemicals (Huber, 2005; Dondale and Hegdekar, 1973)

Accurate mate identification is an important aspect in spiders communication due to the small size and less lifespan. Non- web building spiders make use of the well developed eyes they have but for web builders eyes are rudimentary and the major role is of chemical communication (Suter *et al.*, 1987).

In vicinity of female male perceives information on coming in contact with female silk ,excreta or pheromones (chemical substance) secreted by females body (Gaskett, 2007; Rovner, 1981; Montraveta and Bellido, 2000; Rypstra *et al.,* 2003; Stoltz *et al*., 2007; Clark and Jackson, 1995a; Clark and Jackson, 1995b). Pheromones are highly informative and help in species identification (Roberts and Uetz, 2004b; Roberts and Uetz, 2005). These pheromones play major role in mate selection in invertebrates specially insects (Papke *et al*., 2001; Chinta *et al*., 2010; Aiesenberg,*et.al*, 2010; Kohl et al., 2009).

Earlier studies have detected presence of chemoreceptor on third pair of leg of some spider (Ross and Smith, 1979). When Male pedipalp come in contact with female dragline silk initiate courtship behavior that results in reciprocal vibratory interaction by receptive female (Rovner and Barthe, 1981; Barthe ,1993).

MATERIAL AND METHOD

Spiders:

Steatoda grossa was collected from Bagoda (22.5:75.9) District Indore; M.P in September and November 2011.These spiders were then reared in laboratory condition in plastic vials and aquarium and feed were given according to their size. The feed included fruit flies, dragon flies, damselflies, grasshopper and cockroach. Mature and immature female *Steatoda* were taken for experiments. While mature male *Steatoda* was taken for all the experimental setup.

Chemicals Used

Absolute alcohol was used to remove all web based pheromones . The alcohol was sprayed for web treatment and kept for two days to ensure complete evaporation of alcohol.

Apparatus

Vials were used for rearing spiders and in preliminary experiments while aquarium are used for interaction and to observe courtship and mating.

Electron Microscopy(S.E.M)

Scanning electron microscopy of *S.grossa* legs were performed to find receptors for mate recognization g. Specimens who died (naturally) were preserved in 90% alcohol and were further used in performing S.E.M (Scanning Electron Microscopy).SEM was performed at Electron microscopy and microanalysis facility in IISc Banglore .

The stub was introduced in Gold Coating Sputter coater. It was introduced in it for 25 sec and a thickness of 6.5 nm of gold was applied.

The instrument used for SEM was FEI Quanta ESEM - 200.This microscope has magnification power of 10000X. The working distance of specimen was 8.8 mm to 30.3 mm and the voltage maintained was 2Kv. The detector used for observation here was Secondary Electron Detector.

Statistical Analysis

Analysis of variance or ANNOVA was performed for the available data.

Experimental Design

Three parameter were taken for experiment which are response of Male *Steatoda* with mature and immature female *Steatoda*, Response of mature and immature female *Steatoda* with male *Steatoda* and response of male *Steatoda* on alcohol treated web of mature female *Steatoda*. For each set of experiment 5 male and 3 female were taken .In each experiment mature male of *Steatoda* (reared in vials) were left on web of mature or immature female spider in aquarium. Each female was observed for response with 5 males one by one. Males were replaced only if they died during the course of experiment. For each parameter 15 experiments were performed. After each experiment web of female was destroyed and a fresh female was given a time of 2 days for reconstruction. It was ensured that female was properly fed before destruction of web so that re construction would be easy. Each of the specimen were feed properly with grass hoppers, dragon fly, damsel flies and house flies which were caught from various places. Alcohol was sprayed in vial or aquarium after all experiments. Spraying was done each time after previous experiment to ensure absence of any false result.

RESULTS

As mentioned in Graph D-1 and D-2 when a mature male *S.grossa* was introduced on web of immature female no prominent movement was observed and in only 13.33 % cases did the male react initially by vibration produced by female .When mature male was introduced on web of mature female *S.grossa* in 86.66% cases did the male react. In 80%case female lay quiescent , in 6.66% case female moved towards male and in remaining 13.33% case female moved away from male.

When male *S.grossa* were introduced on web of mature female *Steatoda* it was observed that in 86.66% cases male vibrated and moved towards female and in 13.33% cases male showed no vibrations .In 20% case female lay quiescent in 73.33% cases female showed movement towards male and in 6.66% cases away from the male.

As mentioned in graph D-3 when Male was introduce on web of *S.grossa* treated by alcohol and air dried in all cases there was no movement observed by Male. Male lay quiescent at point of entry in 66.66% cases and lay quiescent at the corner in 33.33% of cases; on the other hand when introduced on an untreated web of female *Steatoda* in her absence in all cases male started vibrating and later cutting the web.

Figure D-1 (**Response of male *Steatoda* with female *Steatoda***)

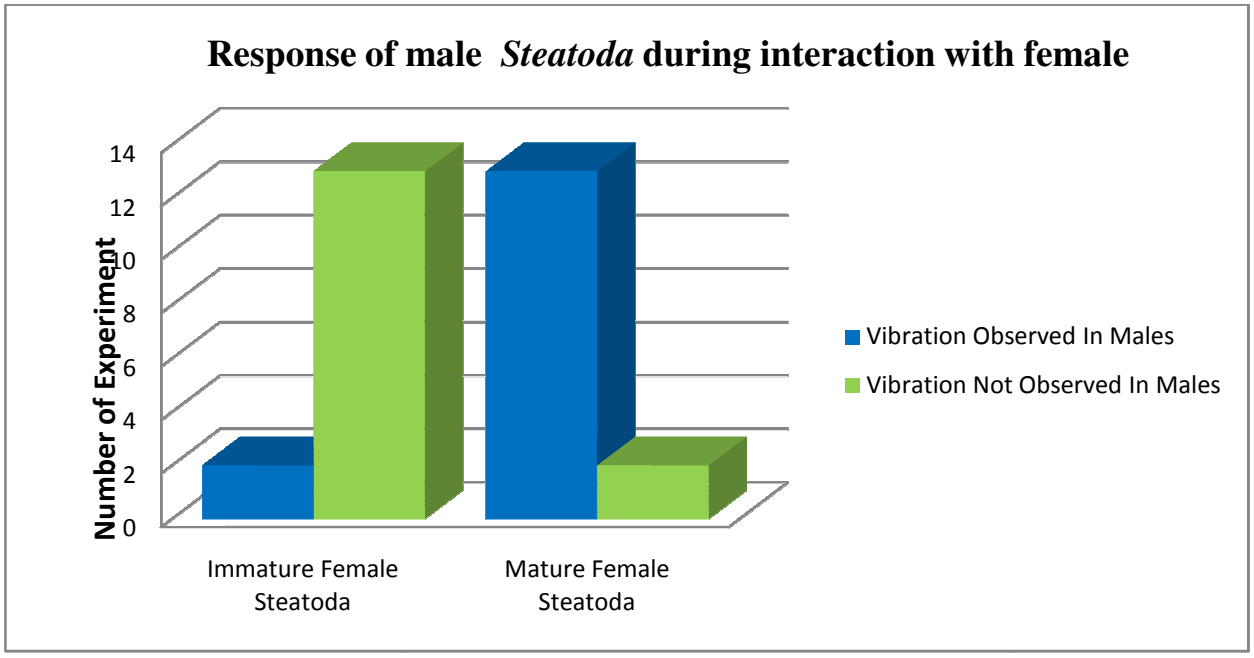


Figure D-2 (**Response of female *Steatoda* with male *Steatoda***)

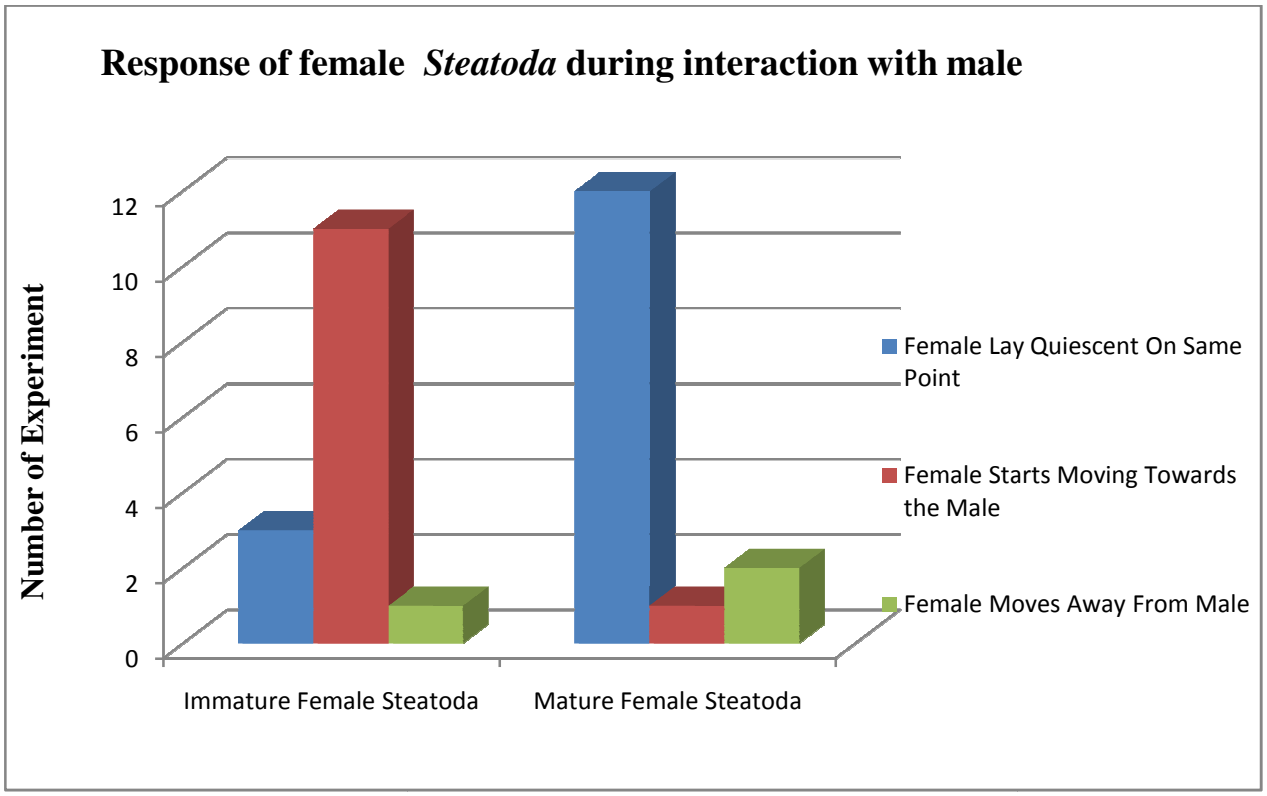
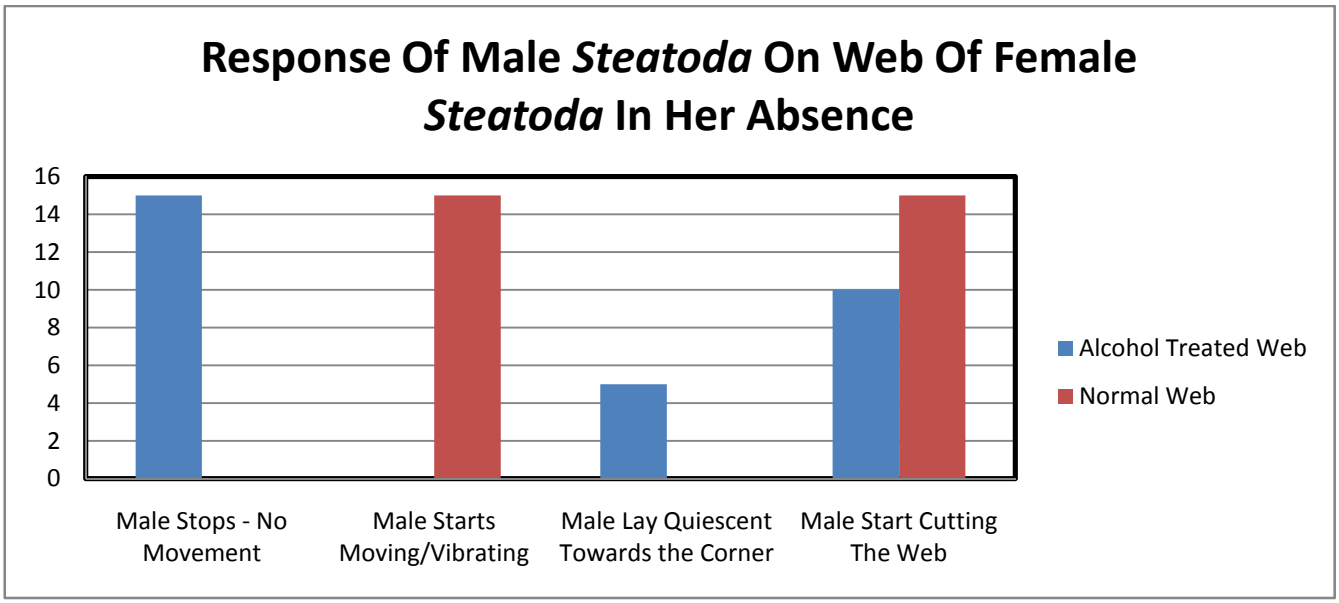


Figure D-3 (**Response of Male *Steatoda* On Web Of Female *Steatoda* In Her Absence**)



DISCUSSION

On analyzing above result it's clear that male showed positive response more towards mature female as compared to the immature *S.grossa* ; considering a fact that the animals never came in direct contact. There was no direct contact between male and female at initial level so it can be considered that the web played a major role in communication between the spiders. The pheromones on web of mature female activated male. In vicinity of female, male perceives information on coming in contact with female silk ,excreta or pheromones (chemical substance) secreted by females body (Gaskett 2007, Tietjan Rovner, 1982 Fernandez Montraveta and Ruano Bellido 2000, Rypstra *et al.*, 2003, Stoltz *et al.*, 2008)

Our prelimary experiment has reveled absence of air pheromones in this animal because when open vial of male and female *S.grossa* were kept in mouth facing position for half an hour never did the spider move towards each other or showed the sign of recognization. Initially movement was observed to maintain position on web.

Above experiment suggested that chemicals involved in communication between opposite sex are semiochemicals and not airborne chemicals.

When vial of male and female were kept on one another (mouth of vial facing each other) on each other initially movement was seen as spider try to make adjustment in changed posture. No other movement was observed; if existence of air borne pheromones was there male would have approached female.

When mature male *Steatoda* was introduced on web of mature female *Steatoda* (in her absence: female was removed 2 hrs prior to experiment) in all such cases male performed courtship vibration supporting the fact that web of mature female spider had some web attached pheromones enough to stimulate male for response.

The male after few minutes of courtship vibration around the superficial part of web starts cutting web of female spider. The reason of such behavior can be absence of response from side of female. As female fails to respond towards male activity; male starts cutting her web.

When male was introduced on alcohol treated and air dried web of mature female *Steatoda* (in her absence) in all the cases no vibration was seen by male spider and male lay quiescent towards the corner. The alcohol treatment removed all pheromones and thus the male could not identify it as web of its own female (same species).

All above mentioned result support the fact that there is existence of some substance on web of mature female spider . The web building spiders have evolved to hang on web. The part of spider always come first in contact with web is leg of male spider. It's known that the leg of spider specially the last segment i.e tarsus bear tuft of hairs which

are known to act as receptors. To confirm the presence of these receptors Electron Microscopy was performed focusing receptors on legs of both male and female.

SEM studies in earlier experiment (Ross and Smith, 1973) showed that the tarsal segment of spider posses a series of hair working as receptors and that the receptors present on third leg are responsible for sexual stimulus. Presence of such receptors on other segment of leg is observed. Moreover the structural similarity between long and short hairs((EM-1,2,5,6) on the legs prove that both hair work as semiochemicals receptor.

Figure EM-1 (Tarsus Of Second Leg Of Female)

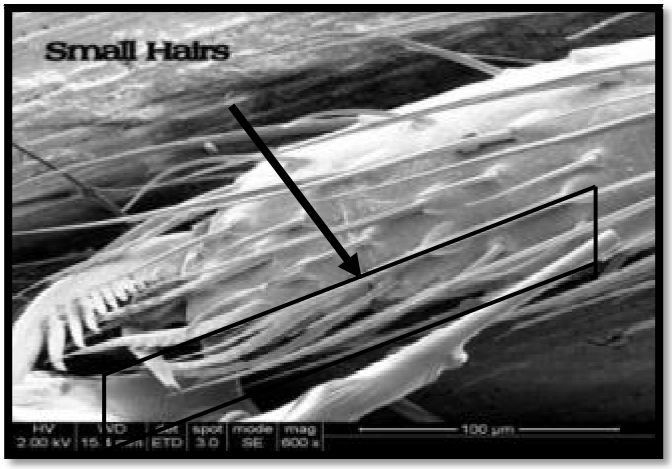


Figure Em-2 (Tarsus Of Third Leg Of Male)

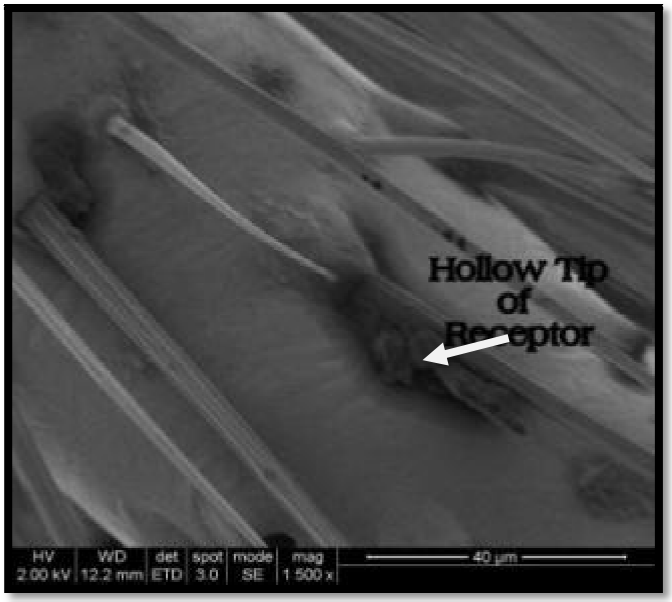


Figure Em-3 (Hair On Tarsus Of Second Leg Of Male)

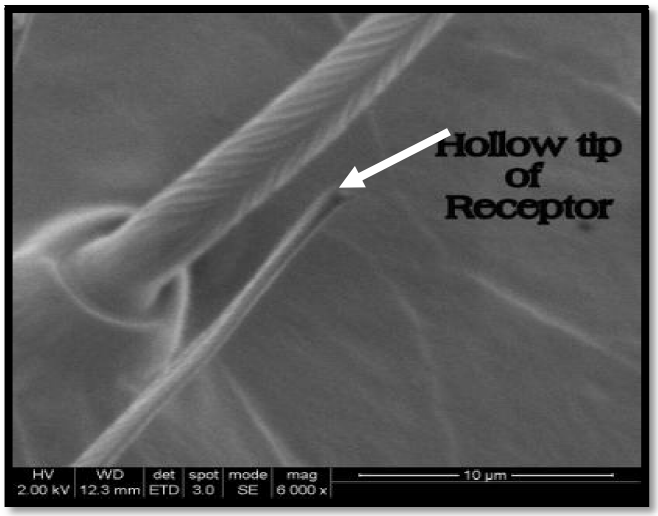


Figure Em-4 (Metatarsus Of Second Leg Of Male)

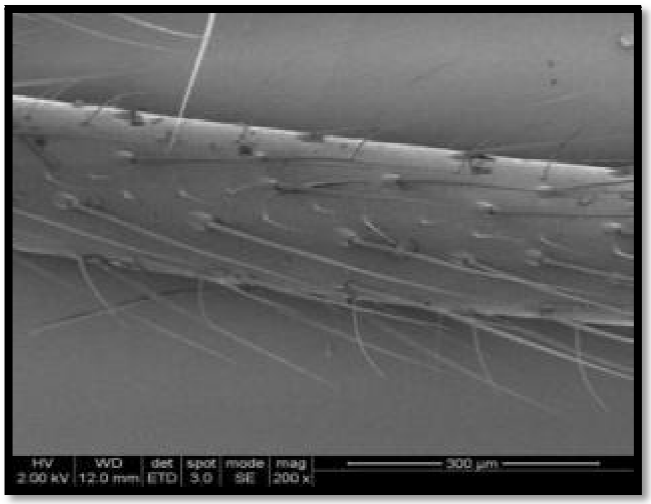


Figure Em-5 (Tarsus Of Fourth Leg Of Female)

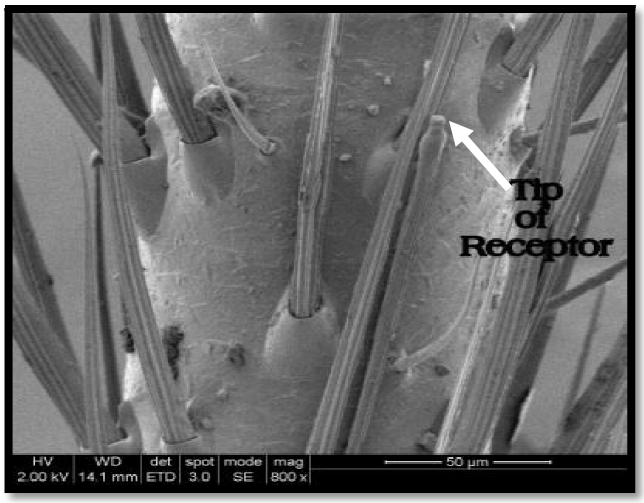
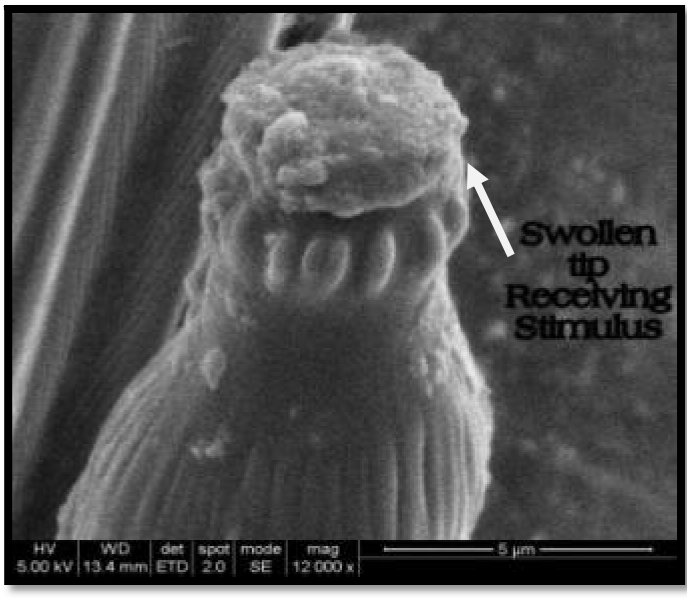


Figure Em-6 (Tip Of Hair Of Tarsus Of Fourth Leg Of Female)



In *Figure* EM-1 dense group of two differenr hairs are clearly visible on segment of leg image presented here is of Tarsal segment of III pair of leg of female spider. A series of long hair together with a series of small hair .

In *Figure* EM-2 *Figure* EM-3 are magnified image of small hair with a wide and hollow tip.

In *Figure* EM-4 series of long and short hairs can be observed in metatarsus of second leg of male. .

In *Figure* EM-5 a particle is clearly visible at the tip of hair of leg whereas in *Figure* EM-6 a highly magnified image of the tip can be seen receiving stimuls from a foreign particle.

Concluding that the reception is not only performed by long hairs in spider but also by the small hairs present on the leg.

CONCLUSION

Reaction of male is clearly more toward the mature female then towards the immature female considering the fact that there was never a direct contact between male and female though female vibrated when male came in contact with the web of female.

Web alone was enough to stimulate male positively towards female confirming existence of web bounded chemicals (semiochemicals).There is special role of semiochemicals secreted by spider and this chemical attract male towards it partner leading to copulation.

The receptors for receiving the chemical stimulus lie on leg and these receptors are present on all legs of both male and female spider. Receptors are hair like structures with blunt ends which when magnified appear to be hollow.

The existence of receptor is clearly visible on Tibia, Tarsus and Femur segment of spider in the images of legs of spider (S.E.M image).

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SCOPE OF FURTHER RESEARCH

Spider plays a key role in maintenance of ecosystem as it is an important part of many trophic levels. They act as both prey and predator for many species thus helping in regulation of a stable ecosystem they are top predators of Arthropods and keep population of insects in check. They also act as prey for both Vertebrate and invertebrate predator .Spiders would be ideal animals to study the evolution of female dominance and sexual size dimorphism. There silk has a great tensile strength and can be used for making space suits and bullet proof jacket. Characteristics that make spiders promising study animals in the context of the evolution of mating systems are the existence of low male mating success in some species, which is related to low variation, adaptations and constraints in male and female reproductive morphology and the variation in female reproductive strategies. My study on communication of spider will help in learning more about its behavior which will help us in there rearing.

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