BVG Trust

J. Sci. Trans. Environ. Technov. 2011, 5(2) :77-81

Scientific Transactions in Environment and Technovation

# Structure, construction and protein profile of the web and egg case, of *Argiope pulchella* (Araneae: Araneidae)

**A. Mary Agnes[1]*and A.Sen[2]**

[1]Department of Zoology, Auxilium College, Gandhinagar, Vellore - 632 006, Tamil Nadu, India.
[1]Unit of Entomology, National Chemical Laboratory, Pune - 411 008, India.

## Abstract

The web structure, construction and protein profile of web and egg case of *Argiope pulchella* are described. The number of radii and spirals in the web are more in the field condition when compared to lab condition. The chromatographic analyses suggest the presence of glycine, serine, threonine, valine and tyrosine in the web while tyrosine is absent in the egg case. The protein profiles of the web and egg case showed different polypeptide profiles.

**Keywords:** *Argiope*, egg case, protein profile, spider web, web construction, web structure

## INTRODUCTION

Spiders are dominant arachnids, known to all men as the spinner of silk threads that are often used to make elaborate ingenious and beautiful webs (Savory, 1977). They are found throughout the surface of earth right from arctic to dry desert region. They are particularly found in areas of rich vegetation and have occupied all possible ecological niches on land (Turnbull, 1973). Their biology, occurrence and distribution was studied by Levi (1968) and Foelix (1982). Spiders are divided into two groups *viz.*, the wandering spiders and sedentary web spiders.

Spiders belonging to the family Agelenidae are funnel weavers, Lynyphidae are sheet web weavers, Theridiidae and Pholicidae build some irregular webs and Araenidae build the most impressive webs the orb webs. Bradoo (1973) had described the web patterns of various spiders. Witt (1967) had given an account of web building spiders and the method of web spinning.

The thread from which the web is constructed is only several microns thick. The fine structures of the thread where they connect to the attachment disc possess remarkable strength (Dewilde, 1943). An orb web contains 1000-1500 connection points most of which occur between the radii and the spiral threads (Jackson, 1971). In *Araneus diadematus*, usually 25-30 radial threads forming fairly angles of 12-15°. Webs of young spiders often have many more radii than adults (Witt *etal.*, 1971, 1973). Kaston (1964) explained the remarkable ability of spider to use several forms of silk. Bristowe (1941) had reviewed spider's spinning skill, their magical abilities to move through space in invisible silken lines and their construction.

The silk is produced in silk glands and released by the spinnerets. The spider was found to regulate the release, mixing and the kind of the silk (Katson, 1964). Silk is a proteinaceous secretory product and scleroprotein of unknown structure. The molecular weight of the fibroin of orb weaver *Nephilia* is 30,000 (Braunitzer 1955).The molecule of the polypeptide change their orientation from alpha configuration to beta which changes the water soluble silk to insoluble thread.

Anderson (1971) had analysed amino acids in spider web and cocoon. The pioneering studies of Fischer (1907) revealed the amino acid content of the spider silk. Recent research on the properties of the silk has unearthed more fascinating facts that make ideal for its function (Vollrath, 1972). This paper describes the web structure, construction and protein profile of the web and egg case of *Argiope pulchella*.

## MATERIALS AND METHODS

The spiders were collected in polythene bags and transferred to the lab without any damage or injury. Later, they were allowed to grow in individual plastic containers of height 19.5cm and breadth 11cm. The containers used were provided with holes in the lid for aeration/ventilation. Spiders were provided with grasshopper, drosophila and small flies every day. The containers were not kept at direct sun light in order to maintain room temperature. Small cotton soaked in water was placed to have 70-85% humidity. Spiders get acclimated to these conditions in 2-3 days.

### The following observations were made

***Web construction*:** The old web in the site was destroyed and the new web built by the spider was observed and

*Corresponding Author
email: ***agnesanthony14@gmail.com***

the following were recorded both in the field and lab conditions.

i. The time taken for the construction.

ii. Number of radii and spirals.

iii. Partial destruction of the web and the time taken for repair and the repairing ability.

iv. Size and shape of the web.

v. Egg case construction.

**Mechanical Property**

i. Effect of water on the web: The spider was removed and the web was kept under running water for 3-5min.

ii. Effect of vibration on the web: The web was disturbed by vibration and the spider behaviour was observed.

**Chemical properties**

**1.Analysis of the amino acid content of the web and the egg case**

The samples of the web and the egg case were subjected to Thin Layer Chromatography (TLC) to study the amino acid content.

One hundred mg of the web egg case was taken in a dry test tube and to it was added 10ml of 6 N HCL. Air was removed and the test tube was sealed and heated for 24 hrs at 110-150° in oil bath and the content was taken in a petridish. The tube was washed with 2ml of water. Then the solution was evaporated in a water bath till the residue was formed. Then it was dissolved in 10ml of 10% isopropanol, filtered and refrigerated.

The samples are spotted on the plate and suspended in the chamber and then the solvent front was marked. The plate was air dried and sprayed with Ninhydrin and the Rf values were calculated.

**2.Protein profile study of the egg case and the web**

The total soluble protein from the web and the egg case samples were extracted using 50mM Tris – HCL buffer, pH 7.2 containing 20% Sucrose, 0.2% Cysteine Hydrochloride and 2% ascorbic acid. The homogenate was centrifuged at 12,000rpm for 15min at 4°C in a centrifuge. The supernatant was preserved for Electrophoresis.

The protein profile was studied by using SDS-PAGE and following the method of Laemmli (1970) and the gel was scanned to measure the quantity of individual polypeptides using a Laser Densitometer Scanner.

The samples were subjected to run in a mini slab gel for 4hrs, under a constant voltage of 60V for stacking gel and 100V for separating gel. The gel was stained with Coomassie Brilliant Blue R-250 and was destained sufficiently to visualize clear polypeptide bands and scanned using a Laser Densitometer Scanner.

## RESULTS AND DISCUSSION

**i.Web construction:** At first the spider selects a suitable place to start with and takes a head down position. Then it secrets the adhesive disc or the attachment disc at which time feeding was not seen and many mooring threads were fixed. After that the radial threads were established with the help of front legs. Then the spiral threads were drawn by connecting the radial threads. After that the circular strengthening zone and the hub were constructed. The final step was the formation of the spirals that are equal in distance and according to the will of the spider the sticky and non sticky spirals are laid. The time taken for the construction was 25-30minutes. Then in the next day the X shaped stabilimentum was completed. (Fig.1)

**ii. Number of radials and spirals:** The number of radii in the field was more than in the lab condition. The number of radii and spirals in the field were 33-36 and 52-56, respectively. On the other hand in the lab the number of radii and the spirals were 24-28 and 35-43, respectively.

**iii. Partial destruction of the web:** When the webs were destructed to a little extent, they were repaired. But if the web was destructed often / more than 3-4 times, the spider moved to a new position.

**iv. Size and shape of the web:** The web in the field had a diameter of 30-45cm, while in the lab it was only 11-12.5cm in diameter.

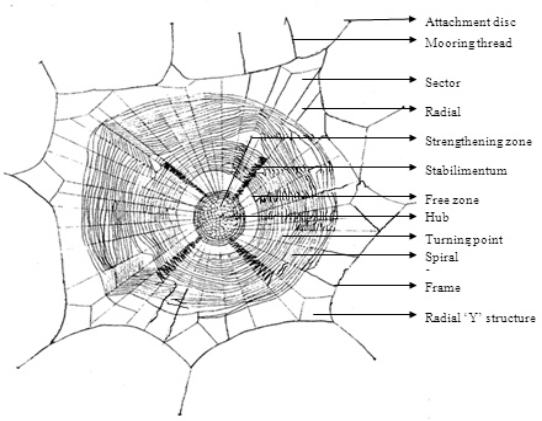
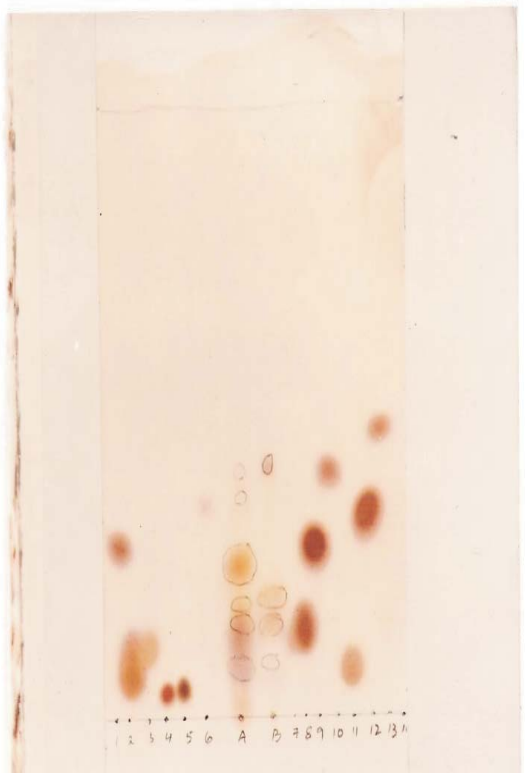



**Fig- 1. Web of *Argiope pulchella***

**v. Construction of egg case:** The case was constructed in the early hours. At first the base was constructed by repeated to and fro movements of the abdomen. The time taken for the construction was 15min. Then the eggs were laid in the centre of the mat which took 3-4min. Later the spider covered the eggs with another layer of silk thread mat. The entire construction took 40min.

**Mechanical property: i. Effect of water on the web:** When the web was exposed to water flow, the

**Plate-1.** Amino acid profile of web and egg case of *A.pulchella* obtained from Thin Layer Chromatography (TLC).



A-WEB, B- EGG CASE and Standards 1 to 13

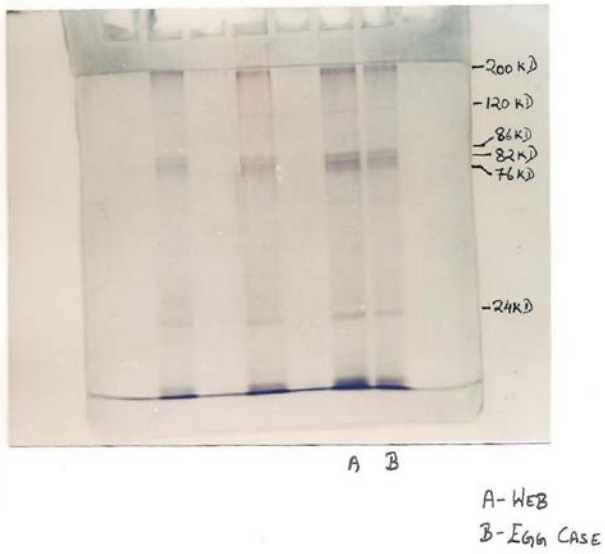
**STANDARDS:**

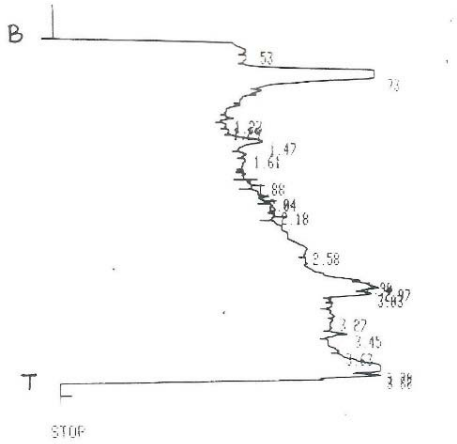
1. CYSTINE
2. GLYCINE
3. HYDROXY-PROLINE
4. LYSINE
5. HISTIDINE
6. TYROSINE
7. SERINE
8. THREONINE
9. VALINE
10. ISO-LEUCINE
11. ASPARGINE
12. METHIONINE
13. TRYPTOPHAN

**Table 1.** Rf Values of amino acids in the web and egg case of *Argiope pulchella*

| Amino acids | Rf Values | | |
|---|---|---|---|
| | Standard | Web | Egg case |
| Glycine | 0.138 | 0.111 | 0.102 |
| Serine | 0.155 | 0.120 | 0.129 |
| Threonine | 0.195 | 0.203 | 0.185 |
| Valine | 0.324 | 0.287 | 0.277 |
| Tyrosine | 0.361 | 0.352 | - |
| ISO-Leucine | 0.379 | 0.388 | 0.388 |

**Plate-2.** Protein profile of web and egg case of *A. pulchella* obtained from SDS-PAGE




RUN # 2

AREA%

| RT | AREA | TYPE | AR/HT | AREA% |
|---|---|---|---|---|
| 0.62 | 4.4756E+07 | SHH | 0.157 | 7.789 |
| 1.09 | 6.7482E+07 | SHH | 0.245 | 11.747 |
| 1.23 | 2.1218E+07 | DSHH | 0.080 | 3.692 |
| 1.36 | 3.2535E+07 | SHH | 0.126 | 5.662 |
| 1.47 | 4.8537E+07 | SHH | 0.159 | 8.446 |
| 1.88 | 82295 | DSPB | 0.012 | 0.014 |
| 2.04 | 357250 | PB | 0.025 | 0.062 |
| 2.18 | 698130 | BV | 0.079 | 0.122 |
| 2.58 | 2.2508E+07 | BV | 0.214 | 3.917 |
| 2.90 | 4.0660E+07 | VV | 0.169 | 7.076 |
| 2.97 | 2.5470E+07 ↑ | VV | 0.090 | 4.432 |
| 3.03 | 1.8585E+07 | VV | 0.066 | 3.234 |
| 3.27 | 5.9132E+07 | VV | 0.219 | 10.290 |
| 3.45 | 2.6839E+07 | VV | 0.084 | 4.671 |
| 3.63 | 4.8317E+07 | VV | 0.142 | 8.408 |
| 3.79 | 8.3565E+07 ↑ | VV | 0.193 | 14.542 |
| 3.88 | 3.3901E+07 ↑ | VB | 0.076 | 5.899 |

TOTAL AREA= 5.7464E+08
MUL FACTOR= 1.0000E+00

**Figure 2.** Protein profile scan of egg case of *A.pulchella*.

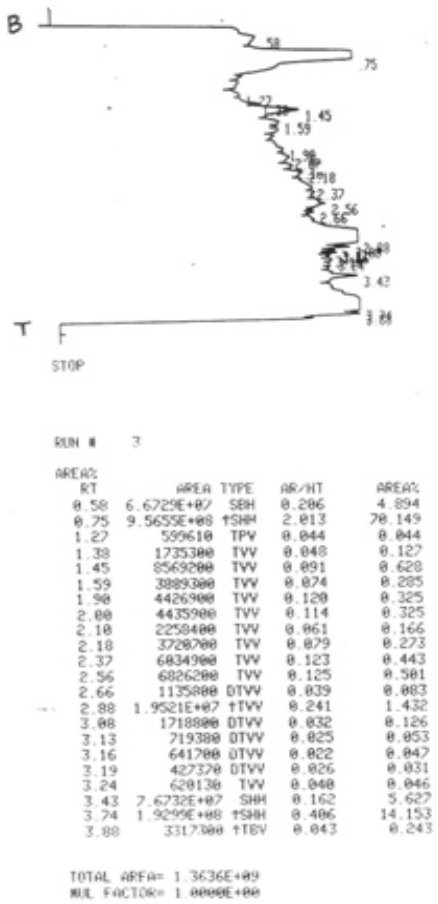



**Figure 3.** Protein profile scan of web of *A. Pulchella*

attachment disc stayed in its position. Small air bubbles were seen in between the network of the web. After 2-3minutes of continuous exposure the spirals got loosened.

**ii. Effect of vibration on the web:** When the web was vibrated, the spider showed rocking movement for about 2-3minute. If there was a prolonged vibration, then it dropped like a dead individual and stayed without moving for about 20minutes.

**Chemical property: i. Amino acid content of the web and the egg case:** The Chromatographic analyse of the samples suggest the presence of amino acids *viz.*, Glycine, Serine, Threonine, Valine, Tyrosine and Iso-Leucine in the web and in the egg case Tyrosine was absent.(Plate-1 & Table 1)

**ii. Protein profile**: The protein profile showed several polypeptide bands in the gel ranging from 15KD to 200KD, both in the web and in the egg case samples. The prominent bands were at 200 KD, 120 KD, 86 KD, 82 KD, 80 KD, 76 KD and 24KD. (Plate-2, Figs. 2 and 3).

Web spinning is an innate mechanism. Even though it is innate the selection of the site, size, structure and texture of the web depend upon the space available which are perceived by the leg muscle and hence it can adapt to the given situation.

The radial threads help in communication and provide quick access routes to the spider. The spirals help to capture the prey once when the prey is caught it tries to free itself which makes it to get entangled more. The arrangement of the sticky and non-sticky spirals and the distance between them is altered every time a new web is constructed.

The common feature of the web which is the X shaped stabilimentum help the web to avoid looping and removal of water during rainy season. This also helps to support the body against gravitational force and it also acts as a shield against heat radiation.

Recent research on the properties of the spider silk has unearthed more fascinating facets that make ideal for its function. Study of web design had led to engineering marvels which resulted in buildings constructed with minimum raw materials, without much pillars inside to support, extensive use of gravitational force etc. Apart from these uses, spider silk is used in medical field as well. It is used as suture threads, in burn treatments and the medical use is traced to Ayurvedic preparation also. Current fields like Robotics, use the spiders as models for constructing robots. As such, future studies on these lines are suggested.

## ACKNOWLEDGEMENT

I express my heart felt thanks to Dr.S.Sivaraman Retd. Professor, PG & Research Department of Zoology, Loyola College, Chennai, for he is the one who had introduced me to the field of Arachnology.

I am for ever indebted to Dr.Alok Sen, Entomology unit, National Chemical Laboratory, Pune, for his magnanimity in offering the necessary facilities for the study and his availability for academic discussion .

## REFERENCES

Anderson, S.D.1971. Amino acid composition of spider silk. *Comp.Biol.Phy.*, 35:705-711.

Braunizer, G. and Wolf, D. 1955. Vergleichende chemische untersuchungen uberdie Fibroine von Bombyx mori and Nephila mada gascariensis. Z. Nuturforschg., 10b : 404 - 408.

Bristowe,W.S.1939-1941. The Comity of spiders Vol.1.1-228-560, Ray Society, London.

Bradoo, B.L.1973. Spiders and Webs, *Science Reporter* C.S.I.R.Delhi.10(8):374-376.

Dewilde, J. 1943. Some Physical properties of the spring threads of *Araneus diadematus. Arch. Neuro. Phy.*, 27:188-192.

Fischer, E. 1907. Veber Spinnenenseide 2. *Phy. Chem.*, 53:126-139

Foelix.1982. *Biology of Spiders*. Harward University Press, Cambridge.

Jackson.1970. The structure of thread connection in the orb web of *Araneus diadematus*. *Psyche.,* 78:12.

Kaston, B. J. 1964. The evolution of spider web. *Am. Zool.,* 4:191-207.

Levi.1968. The spider genera and argiope in America.(Aranea: Araneidae). *Bull. Mus. Comp. Zool. Harv.,* 1361:319-352.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227: 680-685.

Savory, Theodore, M. A. 1977. *Arachnida*. 2nd edition. Academic press. London.

Turnbull, A.L.1973.Ecology of true spiders (Araeneomorphae) *Ann. Rev. Ento.,* 18:305.

Vollrath, Fritz .1972. The Silken Snare of the spider. *Proc. Royal Soc.* London. Series B. May22.

Whitt, P.N.1971.Instruction for working with web building spiders in the laboratory. *Bioscience*., 21:23-25.

Witt *et.al.,*1968. A Spider's Web. Springer , Berlin.