MAJOR AND MINOR AMPULLATE SILK GLAND PROTEINS OF NEPHILA PILIPES FROM KARNATAKA

*Deepa B. M.¹ and Jaya Prakash²

¹Department of Applied Genetics, Indian Academy Research and Post graduate studies, Bangalore-43, India
²Department of Zoology, Bangalore University, Bangalore-56, India

*Author for Correspondence

ABSTRACT
Spider silk is predominantly composed of structural protein called spider fibroins or spidroins. Spiders spin high performance silks through the expression and assembly of tissue restricted fibroin proteins. Spider silks are composite protein biopolymers that have complex microstructures. Protein concentration and estimation of molecular weight was carried out for major and minor ampullate silk gland of Nephila pilipes by Lowry’s method and SDS-PAGE analysis. The Lowry method revealed that protein concentration was more in the major ampullate gland (33.5924 µg) compared to minor ampullate gland (29.8084 µg). SDS-PAGE revealed similar bands in the range of 35 to 40 KDa in weight.

Key Words: Nephila, Major Ampullate Silk Gland, Minor Ampullate Silk Gland, SDS-Page.

INTRODUCTION
Spiders spin high performance fibers with diverse biological functions and mechanical properties. Spider silk is an intriguing biomaterial that is light weight, extremely strong and elastic and exhibits mechanical properties comparable to the best synthetic fibers produced by modern technology (Hinman et al., 2000). Silk fibers are protein based biopolymer filaments secreted by specialized abdominal glands connected the spinnerets, ducts or spigots and are used in different combination to produce structures for prey capture, reproduction and locomotion (Gosline et al., 1986). Spider silk is spun near ambient temperatures and pressure using water as the solvent, which gives rise to an environmentally biodegradable material (Asakura, 1994). The different silk types are protein based polymers that are members of the spider silk protein super family and display restricted expression in seven morphologically distinct silk glands. These distinct abdominal glands are thought to have evolved from a single type of gland, and have subsequently diverged in their anatomy, luminal contents and morphology (Vollrath, 1992).

Based upon the differentiated amino acid composition of the luminal contents, the silk proteins within each gland are proposed to be assembled to create specific fibers with particular functions. To date most research has focused on the major ampullate gland which manufactures dragline silk constituents. Dragline silk is well known for its combination of high tensile strength and elasticity, which leads to a fiber with extraordinary toughness (Gosline, 1986). Spiders use dragline silk to create web anchors, as well as safety lines for survival. The minor ampullate gland, which shows morphological similarity to the major ampullate synthesizes web radii filaments and temporary capture silk. The present study is on the Indian spider Nehila, a preliminary study intended to compare the protein in the major and minor ampullate silk glands by Lowry’s method and SDS-PAGE analysis.

MATERIALS AND METHODS
Spider Collection and Handling
Spiders were collected from different regions of Karnataka in India. Collection was done early in the morning from January 2012 to October 2012 and identified up to species. A sample size, n=10/ were utilized for the present analysis.
Spider Dissection
Spiders were euthanized with ethyl acetate. The abdomen (opisthoma) was separated from the cephalothorax and submerged and dissected in spider ringer solution 1x Ringer: 13g NaCl, 0.5 KCl, 0.89g CaC\(_{12}\), 1.04g MgC\(_{12}\).6H\(_2\)O buffered with tris base (100 nM, pH=7.2) under a stereomicroscope (Dicko et al., 2005). The tissues of the alimentary canal and blood vascular system were removed to make the glands clear.

Protein Estimation and Molecular Weight by SDS PAGE
The major and minor ampullate gland proteins of Nephila were separated by SDS following Yigit et al., (2004). Protein concentration was measured by Lowry method (1951) using bovine serum albumin as standard at 660nm. Electrophoretic separation of proteins was performed using 12% acrylamide gel with 1 mm thickness following dissociating and discontinuous buffer system. Protein bands were visualized by Commessei blue staining protocol.

RESULTS
There was a significant difference in the protein concentration of the two glands of Nephila pilipes. Major gland the concentration was 33.5924 and the concentration in the minor gland was 29.8084.
SDS PAGE showed two prominent bands for both the major and minor ampullate silk glands of Nephila pilipes. The molecular weight was in the range of 35kdaltons.

Table 1: Protein content in two different glands of Nephila pilipes

<table>
<thead>
<tr>
<th>S.No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Major ampullate gland</th>
<th>Minor Ampullate Gland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc. µg/tube</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>40</td>
<td>80</td>
<td>120</td>
<td>160</td>
<td>33.5924</td>
<td>29.8084</td>
</tr>
<tr>
<td>O.D @ 660nm</td>
<td>0.000</td>
<td>0.115</td>
<td>0.2</td>
<td>0.39</td>
<td>0.55</td>
<td>0.68</td>
<td>0.9</td>
<td>0.286</td>
<td>0.276</td>
</tr>
</tbody>
</table>

![Figure 1: Standard Graph](image-url)
DISCUSSION
In this study the difference between the concentration of major and minor ampullate silk protein of Nephila pilipes species of Karnataka have been done. The major ampullate silk gland protein of Nephila was more than that of minor ampullate silk gland protein (major 33.5924µg/tube and minor 29.8084 µg /tube). The band in the SDS-PAGE for major and minor silk glands showed a band of molecular weight in the range of 35KD. Major and minor silk glands though have the same protein their function is different The concentration of protein is more in major ampullate gland and less in minor ampullate gland throwing insight in their functional variation.

REFERENCES