CHAPTER FIVE

INDUCTION AND SIGNIFICANCE OF CYCLOMORPHOSIS IN BRACHIONUS CALYCIFLORUS IN LABORATORY

This chapter deals with the laboratory study on the induction of spines in Brachionus calyciflorus by its predator, Asplanchna brightwelli. It is found that non-spined B. calyciflorus responds to the presence of A. brightwelli by growing defensive spines, thus, making itself less vulnerable to the size selective planktivory.
INTRODUCTION

Appearance of morphological variations in zooplankton has been related with certain environmental factors such as temperature, turbulence, food quality and quantity and other abiotic factors (Gallagher, 1957; Green, 1963) or has been associated with soluble chemical substances released by their dominant predators (Gilbert, 1967; Havel, 1987). This type of cyclomorphosis has been referred to as chemomorphosis (Hebert and Grewe, 1985). The chemical substances released by predators direct the parthenogenetic female eggs of zooplankton to develop into individuals having exuberant morphological forms which are often better protected than the basic phenotype against the predators that induce them (Gilbert, 1980; Stemberger and Gilbert, 1984).

According to Repka and Pihlajamaa (1996), predation is an important selective agent, affecting both the evolution of the prey and the predator. Defences against the predation can be either constitutive or inducible. These inducible defences seem to be common in aquatic clonal invertebrates and they are especially well-documented in freshwater rotifers and cladocerans (Havel, 1987). Induced antipredator morphologies were first recognized in rotifers by De Beauchamp (1952) and then by Gilbert (1966). Since then many such defenses have been found in a wide range of prey among the rotifers (Stemberger and Gilbert, 1984; Gilbert and Stemberger, 1987) and cladocerans (Grant and Bayly, 1981; Krueger and Dodson, 1981; Hebert and Grewe, 1985; Havel, 1987).
Present laboratory study is, therefore, an attempt in order to determine, (1) whether the course of spine length variation of *B. calyciflorus* is related with the presence of its predator, *Asplanchna brightwelli*, which is known to be the causative factor behind phenotypic plasticities of several prey rotifers including *B. calyciflorus* (Gilbert and Waage, 1967; Green and Lan, 1974; Stemberger and Gilbert, 1987), and (2) are the spined forms better protected against *A. brightwelli* predation?

Laboratory cultures of both *B. calyciflorus* and *A. brightwelli* were maintained and induction as well as predation experiments were run under the controlled conditions to test the selective force that causes phenotypic variation besides the adaptive value of this variation in reducing predation.

**MATERIALS AND METHODS**

**Algal cultures**-

Chlorophyte, *Chlorella sp.* was cultured in synthetic culture medium of Kuhl and Lorenzen (1964). 50 ml of Kuhl and Lorenzen's medium mixed with 0.75 mg Agar was autoclaved at 15 lb/sq inch pressure for 15 minutes and solidified in sterilized Petri dish. A diluted drop of pond water containing *Chlorella* cells was put on the nutrient-Agar and spread evenly. Petri-dish was incubated at room temperature under natural day light. After one week, small algal colonies developed on the surface of nutrient-Agar. *Chlorella* colonies were picked up by inoculation wire and transferred in autoclaved conical flasks containing 100 ml culture medium. Flasks were incubated at room temperature (20 ± 2° C) under natural light for two weeks, after which cultures turned green and were harvested during their exponential phase of growth. Sub-cultures were maintained in the same way to ensure vitality and motility of algal cells in culture.
**Paramecium culture** -

*Paramecia* were used to feed predatory rotifer, *Asplanchna sp.* Cultures were maintained in a 100 ml beaker containing 50 ml of distilled water, cooked hay and wheat grains. Inoculation was done by adding a drop of natural pond water in the beaker containing *Paramecia*. Culture was kept at room temperature near the window under indirect light. Small quantities of cooked hay and wheat grains were added each week to maintain the *Paramecium* culture.

**Asplanchna culture** -

Predatory rotifer, *Asplanchna brightwelli* was cultured in small Petri dishes at 20±2°C temperature under diffused light in the culture medium consisting of one parts of grass extract and nine parts of dechlorinated tap water. Individual organisms were isolated from the natural population with the help of glass dropper under a stereoscopic microscope and transferred to Petri dishes after washing thrice in distilled water. Culture medium was renewed after every 48 hours to restrict ageing. *A. brightwelli* was fed with *Paramecia*.

**Brachionus calyciflorus culture** -

Clonal cultures of *B. calyciflorus* were maintained in pond water filtered through 10 µm mesh size netting and enriched with *Chlorella* cells (5 x 10⁶ cells/ml) at 22 ± 2°C with 12 hours dark: 12 hours light period in 100 ml glass beakers. Initiation and maintenance of clonal rotifer cultures is simple and techniques are similar to those used in microbiology.

**Induction Experiment** -

Non-spined females of *B. calyciflorus* were isolated from the clonal stock culture and were acclimatized to the experimental temperature and
food level for at least 10 days prior to the commencement of the experiment. Induction experiments were run in 15 ml capacity glass cavity blocks each having 5 ml of filtered pond water which was made Asplanchna-sbstance free by keeping it at 60° C for 12 hrs prior to use. At the time of the collection of pond water, in the month of October, Asplanchna density was very low and perhaps there was no Asplanchna-substance activity in the water as almost all the individuals of B. calyciflorus collected at that time had no postero-lateral spines. Twelve test vials were inoculated with 20 individuals each of non-spined form of B. calyciflorus from the acclimated population and were supplied with Chlorella at a density of 5x10⁶ cells/ml. Two sets, each having six vials, were kept separately in 12 beakers as in life table experiments at two experimental temperatures (20° and 30° C) containing Asplanchna-substance free water. Now the three beakers each of both the sets were inoculated with A. brightwelli from the stock culture at a density of 3 individuals per ml which served as treated sets. While no A. brightwelli was introduced in the other six beakers (3 each at both temperatures). Experimental animals were transferred to fresh medium at every 24 hrs with the help of 55 μm mesh size netting. Experiment was terminated after 10-12 days, after which all experimental as well as control population of B. calyciflorus were fixed in 5% formaldehyde solution. Number of individuals were counted and spination noted. Chi square (X²) statistic was used to test the significance of the frequencies of spined and unspined phenotypes in control and experimental sets.

Predation Experiment -

For the determination of survivorship of typical spineless and spined
phenotypes against *Asplanchna* predation, this test was conducted. 10 non-spined and 10 spined individuals of *B. calyciflorus* were placed in each of the six Petri dishes containing 5 ml of culture medium. Four overnight starved *Asplanchna* were also placed in three Petri-dishes which served as experimental set while the other three Petri dishes, having no *Asplanchna* and served as control. Experiment was terminated after 6 hrs and immediately after the termination, all experimental and control populations were fixed in 5% formaldehyde solution. The final number of each morphotype was determined in Sedgwick-Rafter cell under the inverted microscope. Experiment was repeated thrice. The proportions of postero-lateral spined and non-postero-lateral spined phenotypes of *B. calyciflorus* ingested during the experiment were tested by constructing two by two contingency table and computing Chi square ($X^2$) statistic.

**RESULTS**

**Induction experiment** -

Presence of *Asplanchna* induced a significant response to develop postero-lateral spine in *B. calyciflorus* at $20^\circ$ C. Mean and percentage values of typical and exuberant forms developed in both experimental and control sets at $20^\circ$C are given in Table-7. The percentage of postero-lateral phenotypes was found to be much more in experimental set as compared to their values in control set at this temperature. Difference in percentage of occurrence of non-spined and spined forms in experimental set was found to be highly significant at $P<0.001$. At $30^\circ$ C, however, no induction was noted in the experimental population of *B. calyciflorus* and only few specimens with very short postero-lateral spines were found to develop in experimental set (Table 8).
Predation Experiment -

Results of predation experiments are presented in Table-9. The postero-lateral spined form was ingested to a much lesser extent than that of the non-spined form. Percentage of ingestion of the two forms is also given in Table-9. The difference in the percentage ingestion of two types of prey *B. calyciflorus* was highly significant at $P<0.001$.

**DISCUSSION**

De Beauchamp (1952) first of all pointed out the relationship between occurrence of spined form of *B. calyciflorus* and presence of *Asplanchna* sp. Gilbert and Waage (1967) have also concluded that variation in the length of postero-lateral spines of *B. calyciflorus* is correlated with *A. brightwelli* density and controlled by the concentration of *Asplanchna*-substance in the medium. They further suggested that *Asplanchna*-substance probably induces postero-lateral spine development in very low concentrations. Present study also shows that the presence of *Asplanchna* significantly induces the development of postero-lateral spines in *B. calyciflorus* at $20^\circ$ C (Table-7). It is, therefore, quite probable that *B. calyciflorus* responds to this substance in nature and that *Asplanchna* induced spine production is not simply a laboratory phenomenon created by abnormal conditions. Now, it has been established and well evidenced fact that populations of some freshwater zooplankton including *B. calyciflorus* undergo morphological changes in response to water soluble chemicals released by their predators like *Asplanchna* (Pourriot, 1964; Gilbert, 1967; Havel, 1985; Stemberger and Gilbert, 1984).

Nothing is clear about the role of other variables which may affect the stability of the *Asplancha*-substance. Low temperature usually has
been related with the occurrence of postero-lateral spined forms of \textit{B. calyciflorus}. However, the role of temperature is suggested to be important in sustaining the activity of \textit{Asplanchna}-substance, which being proteinaceous in nature, lose its activity at higher temperature, rather than to affect the spine formation directly (Gilbert and Waage, 1967). Laboratory studies of Gilbert (1966, 1967) showed that xenic \textit{Asplanchna}-conditioned media gradually lose activity at 25°C, becoming inactive in slightly less than a week. This loss of activity is more rapid when live \textit{B. calyciflorus} are present. It has been further suggested that the \textit{Asplanchna}-substance, although fairly stable at 25°C, is probably decomposed by bacteria and may be actively incorporated and metabolized by \textit{B. calyciflorus} (Gilbert and Waage, 1967). In this study, no significant induction of spines was noticed at 30°C which confirms the above findings and shows that at higher temperature, the \textit{Asplanchna}-substance is inactivated.

Concerning the adaptive value of morphological shift, it is quite easy to understand the meaning of predator induced spine development in the rotifers. Gilbert (1966) showed that short spined forms suffer greater predation than those of the long spined forms. Pejler (1980) also pointed out that the spines may be partly regarded as an adaptation for escaping predation. Our experiments show that \textit{Asplanchna brightwelli} always ingested more non-spined phenotypes (60-90\%) in comparison to spined phenotypes of \textit{B. calyciflorus} as they were less susceptible to ingestion (10-50\%, Table-9). Exuberant phenotypes of other zooplankton like \textit{Daphnia spp.} and \textit{Keratella spp.} are also known to be better protected against their predators (Grant and Bayly, 1981; Havel and Dodson, 1984;
Stemberger and Gilbert 1987).

It is evident from the present study that seasonal morphological shift in certain zooplankton including *B. calyciflorus* is directly related with the presence of its predator, *Asplanchna brightwelli*, and that the long spined form of this rotifer could significantly reduce the predation pressure by *Asplanchna*. It is the evolutionary achievement of a phenotypic, often developmental, plasticity which makes possible the antipredator morphology. Like this, many, if not all, such adaptive responses in freshwater ecosystem are mediated by a semio-chemical cue ("kairomone") which signals the presence of predator. In no case have the kairomone been identified and in future, elucidation of the cellular and molecular mechanisms of the morphological responses using radiolabelled or photoaffinity labelled kairomone also awaits its identification (Parejko and Dodson, 1990). However, role of other environmental factors can not be denied completely which may modify the phenomenon of phenotypic plasticity among zooplankton.
Table 7: Summary of induction experiment at 20°C to show the effects of *Asplanchna*-substance on postero-lateral spine development in *Brachionus calyciflorus*

<table>
<thead>
<tr>
<th>N</th>
<th>INITIAL NO. OF TYPICAL B. CALYCI FLORUS</th>
<th>GROWTH PERIOD (DAYS)</th>
<th>FINAL NO. OF B. CALYCI FLORUS TYPICAL</th>
<th>FINAL NO. OF B. CALYCI FLORUS SPINED</th>
<th>FINAL PERCENTAGE OF B. CALYCI FLORUS TYPICAL</th>
<th>FINAL PERCENTAGE OF B. CALYCI FLORUS SPINED</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>E 20</td>
<td>10</td>
<td>51</td>
<td>127</td>
<td>28.65%</td>
<td>71.35%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>10</td>
<td>187</td>
<td>16</td>
<td>92.12%</td>
<td>7.88%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>2-</td>
<td>E 20</td>
<td>10</td>
<td>71</td>
<td>121</td>
<td>36.98%</td>
<td>63.02%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>10</td>
<td>168</td>
<td>19</td>
<td>89.84%</td>
<td>10.16%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>3-</td>
<td>E 20</td>
<td>12</td>
<td>29</td>
<td>181</td>
<td>13.81%</td>
<td>86.19%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>12</td>
<td>185</td>
<td>13</td>
<td>93.43%</td>
<td>6.57%</td>
<td>&lt; 0.001</td>
</tr>
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</table>

E = Experimental Set  
C = Control  
N = Number of Replicate  
* = Comparison by Chi square Test
Table 8: Summary of induction experiment at 30°C to show the effects of *Asplanchna*- substance on postero-lateral spine development in *Brachionus calyciflorus*

<table>
<thead>
<tr>
<th>N</th>
<th>INITIAL NO. OF TYPICAL B.CALYCIORUS</th>
<th>GROWTH PERIOD (DAYS)</th>
<th>FINAL NO. OF B.CALYCIORUS</th>
<th>FINAL PERCENTAGE OF B. CALYCIORUS</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TYPICAL</td>
<td>SPINED</td>
<td>TYPICAL</td>
</tr>
<tr>
<td>1-</td>
<td>E 20</td>
<td>10</td>
<td>215</td>
<td>12</td>
<td>94.71%</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>10</td>
<td>243</td>
<td>17</td>
<td>93.46%</td>
</tr>
<tr>
<td>2-</td>
<td>E 20</td>
<td>10</td>
<td>191</td>
<td>6</td>
<td>96.95%</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>10</td>
<td>218</td>
<td>11</td>
<td>95.20%</td>
</tr>
<tr>
<td>3-</td>
<td>E 20</td>
<td>12</td>
<td>238</td>
<td>14</td>
<td>94.44%</td>
</tr>
<tr>
<td></td>
<td>C 20</td>
<td>12</td>
<td>224</td>
<td>9</td>
<td>96.14%</td>
</tr>
</tbody>
</table>

E = Experimental Set
C = Control
N = Number of Replicate
* = Comparison by Chi square Test
Table 9: Summary of predation experiments to show the relative predation on non-spined and spined morphos of *Brachionus calyciflorus* by *Asplanchna brightwelli*

<table>
<thead>
<tr>
<th>TIME HRS.</th>
<th>NO. OF A SEIBOLDI</th>
<th>INITIAL NO. OF B CALYCIFLORUS</th>
<th>FINAL NO. OF B CALYCIFLORUS</th>
<th>PERCENTAGE OF B CALYCIFLORUS INGESTED</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E 5</td>
<td>7</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>C 5</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>E 5</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>C 5</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>E 6</td>
<td>3</td>
<td>10</td>
<td>10</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>C 6</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

E = Experimental Set  
C = Control  
N = Number of Replicate  
* = Comparison by Chi square Test