Morphogenetic derangements in the reproductive system of *Bracon hebetor*, a beneficial parasitoid, bred on juvenoid treated host (*Corcyra cephalonica*) larvae

Sudipta Chanda & Sanjib Chakravorty*
Department of Zoology, University of Kalyani, Kalyani 741235, India.

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Effect of juvenoids (hydroprene and methoprene) on the ecto-parasite *B. hebetor* was investigated by rearing them upon the juvenoid treated ultimate instar host larvae of *C. cephalonica*. Emerged adultoid wasps of either sexes obtained from treated series showed anatomical deformities in the reproductive systems. Ill-developed ovaries with reduced length, terminally free ovarioles and abnormal testicular growth showing non-fusion of lobes were the important abnormal features. Data on measurements of male reproductive system, e.g., width (transverse axis) of testis, length of common vas deferens plus ejaculatory duct and length of accessory gland showed significant difference (P<0.05) from control.

Juvenoids constitute the most promising group of hormonal insecticides that can bring about the death of insects in several ways. The use of these chemicals as insect control agents demands an exploration on their role in host parasite relationship because some ichneumonid parasitoids need to feed on their hosts for egg maturation. The ecto-parasite *Bracon hebetor* Say (Hymenoptera: Braconidae) is reared in the laboratory using the larvae of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) as its host. Before oviposition the parasitoid injects a paralyzing toxin by its ovipositor into the body of the mature larvae of *Corcyra*. The larvae of the parasitoid develop at the expense of the host’s body fluid and undergo pupation. Eggs of the parasitoid laid on host’s integument, get contact with juvenoid from the body fluid of the host, exuded during injection of paralyzing toxin to the host larvae and also during feeding of the host’s body fluid by the parasitoid. Moreover, the ovipositor of the parasitoid gets contaminated with juvenoids while injecting the paralyzing toxin to the juvenoid treated host larvae and subsequently the eggs, laid through the ovipositor, also get contaminated with the juvenoids. The present paper reports the findings on the reproductive system of the deformed and ill-developed *Bracon* wasps obtained from the juvenoid contaminated eggs and larvae.

Materials and Methods

Hydroprene (ethyl 3, 7, 11- trimethyl- 2, 4-dodecadienoate) and methoprene (isopropyl 11-methoxy- 3, 7, 11- trimethyltrideca- 2, 4- dienolate) were applied topically upon the ultimate instar larvae of *Corcyra cephalonica* in 1μl solution of acetone delivering 50, 100 and 200μg of a juvenoid per individual. Control larvae received 1μl acetone per individual. The procedure of treatment upon the host larvae followed the earlier methods.

A population of *B. hebetor* was maintained by rearing them in the larval stage of the host *C. cephalonica* which was maintained in the laboratory at 29±1°C, 80-90% RH and 14:10 hr light (semi-dark)-dark cycle. After 2 hr of juvenoid treatment, the treated and control host larvae were exposed to infection by three different categories of *Bracon* wasps (Fig. 1). The egg population per host larva was maintained at 10 for better development of the parasitoid. The metamorphosed forms of the parasitoid were collected from the culture and preserved in 70% alcohol for microscopic study. Dissection of freshly killed adultoid wasps from experimental series and normal wasps from Control-II series were made in insect Ringer’s solution under dissecting microscope. Whole mounts were also prepared for anatomical study.

Results

The following types of morphs of *B. hebetor* were obtained: (i) prolonged pupae- these were obtained after the expiry of +4 days of pupal duration in control; (ii) non-emerged adultoids- these were
Experiment - I

Development of JH - contacted wasps

1st Category of wasps = Mated O+ wasps

Paralysation & Oviposition

- Wasp Direct JH Contacted
  - JH - treated Corcyra larvae
  - Skin JH - Contaminated

- Control Corcyra larvae Control - I

- Wasp Indirect JH Contacted

Oviposition (Contaminated egg)

Ovipositor of wasp JH - Contaminated

Experiment - II

Development of wasps JH - contacted/ no JH - contact

2nd Category of wasps = Mated O+ wasps

Only for Paralysation (Egg laying not allowed on this paralysed host)

- Group A wasps
  - JH - treated Corcyra larvae
  - Skin JH - Contaminated

- Untreated Corcyra larvae Control - II

- No JH Contact

Wasp, egg & larva JH - Contacted

No JH - Contact

Fig. 1—Design of treatment
Table 1—Occurrence of abnormalities in the female and male reproductive systems of *B. hebetor* obtained from juvenoid (H= hydroprene; M= methoprene) treated hosts (n= 20 for each sex).

<table>
<thead>
<tr>
<th>Characters</th>
<th>Doses of JH (µg/individual)</th>
<th>H-50</th>
<th>H-100</th>
<th>H-200</th>
<th>M-50</th>
<th>M-100</th>
<th>M-200</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ill-developed ovary</td>
<td></td>
<td>9-13d</td>
<td>9-12d</td>
<td>9-13d</td>
<td>9-13d</td>
<td>9-15d</td>
<td>9-15d</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(35.0)</td>
<td>(25.0)</td>
<td>(40.0)</td>
<td>(20.0)</td>
<td>(45.0)</td>
<td>(45.0)</td>
<td>Nil</td>
</tr>
<tr>
<td>Reduced ovariole number</td>
<td></td>
<td>Nil</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(15.0)</td>
<td>(5.0)</td>
<td>(20.0)</td>
<td>(15.0)</td>
<td></td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Ovarioles terminate freely</td>
<td></td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10.0)</td>
<td>(15.0)</td>
<td>(10.0)</td>
<td>(5.0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compound egg chamber</td>
<td></td>
<td>Nil</td>
<td>1</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal structure of ovary</td>
<td></td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(55.0)</td>
<td>(55.0)</td>
<td>(45.0)</td>
<td>(55.0)</td>
<td>(55.0)</td>
<td>(55.0)</td>
<td>(100.0)</td>
</tr>
<tr>
<td>Non-fusion of testicular lobe</td>
<td></td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>nil</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(10.0)</td>
<td>(15.0)</td>
<td>(15.0)</td>
<td>(10.0)</td>
<td>(15.0)</td>
<td>(15.0)</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parentheses are % of occurrence. d = Egg to adult/adultoid duration in days.

Table 2—Measurements (mm) of male reproductive system of *B. hebetor* obtained from juvenoid treated host.

<table>
<thead>
<tr>
<th>Characters</th>
<th>Doses of JH (µg/individual)</th>
<th>H-50</th>
<th>H-100</th>
<th>H-200</th>
<th>M-50</th>
<th>M-100</th>
<th>M-200</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width (transverse axis) of testis</td>
<td></td>
<td>1.21±0.20 b c</td>
<td>1.17±0.07 b c</td>
<td>1.28±0.10 b c</td>
<td>1.36±0.13 a</td>
<td>1.30±0.14 a b</td>
<td>1.15±0.08 c</td>
<td>1.27±0.21 c b</td>
</tr>
<tr>
<td>Length (antero-posterior axis) of testis</td>
<td></td>
<td>0.78±0.13</td>
<td>0.77±0.06</td>
<td>0.80±0.07</td>
<td>0.86±0.15</td>
<td>0.80±0.11</td>
<td>0.73±0.07</td>
<td>0.77±0.11</td>
</tr>
<tr>
<td>Length of vas deferens</td>
<td></td>
<td>1.12±0.10</td>
<td>1.09±0.12</td>
<td>1.18±0.08</td>
<td>1.09±0.25</td>
<td>1.07±0.13</td>
<td>1.10±0.10</td>
<td>1.16±0.12</td>
</tr>
<tr>
<td>Length of common vas deferens plus ejaculatory duct</td>
<td></td>
<td>1.63±0.15 b</td>
<td>1.68±0.19 b b</td>
<td>1.88±0.13 a</td>
<td>1.70±0.28 ab</td>
<td>1.58±0.17 b b</td>
<td>1.87±0.34 a b</td>
<td>1.76±0.14 b b</td>
</tr>
<tr>
<td>Length of accessory gland</td>
<td></td>
<td>1.39±0.17 b c</td>
<td>1.57±0.06 a b</td>
<td>1.51±0.12 ab</td>
<td>1.43±0.21 ab c</td>
<td>1.35±0.16 c</td>
<td>1.56±0.19 a</td>
<td>1.49±0.14 ab c</td>
</tr>
</tbody>
</table>

H= hydroprene; M= methoprene. Means followed by a common letter are not significantly different at 5% level by DMRT. Means without letter are non significant.

Fig. 2—Female reproductive system of a control parasitoid ([pg= Poison gland, pr= Poison reservoir, spt= Spermatheca, Ig= Lubricating gland, st= Sting, vu= Valvula]).
Fig. 3—Female reproductive system of a parasitoid obtained from 50μg hydroprene treated host. [Single ovariole in one side. Scale bar 0.2mm]; Fig. 4—Female reproductive system of a parasitoid obtained from 100μg hydroprene treated host. [Ovarioles terminate freely in one side. Scale bar 0.2mm]; Fig. 5—Female reproductive system of a parasitoid obtained from 100μg methoprene treated host. [Both ovaries are ill-developed with single ovariole in one side. Scale bar 0.2mm]; Fig. 6—Female reproductive system of a parasitoid obtained from 200μg methoprene treated host. [Deformities in ovaries. Scale bar 0.2mm]; Fig. 7—Male reproductive system of a control parasitoid. [tl= Testis (fused), v= Vasa deferentia, cc= Common vas deferens, ej= Ejaculatory duct, ac= Accessory gland. Scale bar 0.5mm]; Fig. 8—Male reproductive system of a parasitoid obtained from 200μg methoprene treated host. [Non-fused testicular lobes. Scale bar 0.5mm].
collected by removing the pupal exuviae; (iii) deformed adults - emerged wasps with deformed wings, and (iv) externally normal adults - emerged wasps were similar to normal ones. Adultoid wasps showed anatomical deformities in the reproductive systems. Normally the female reproductive system was comprised ovaries, spermatheca, spermathecal glands, lubricating gland, poison gland and the sting (Fig. 2) . Each ovary consisted of two ovarioles, each possessing a few relatively large eggs. The ovarioles of the treated individuals showed marked reduction in their length. In a number of cases the ovarioles of one or both the ovaries were ill-developed having indistinct oocysts. Besides these, altered ovariole number and free terminal ends of ovarioles in one or both the ovary of an individual were some other abnormal features (Table 1)(Figs. 3-6).

In male reproductive system the two testicular lobes of a control individual were enclosed in a sac from which extended two vasa deferentia which united posteriorly to form common vas deferens (Fig. 7). The treated individuals showed non-fusion of the testicular lobes (Fig. 8)(Table 1). Measurements of male reproductive system showed significant differences in width (transverse axis) of testis, length of common vas deferens plus ejaculatory duct and accessory gland (P <0.05)(Table 2).

Discussion

The eggs and larvae of B. hebetor got contact with the juvenoids from exuded body fluid through the punctures made by the parasitoid during injection of paralyzing toxin and also during feeding of body fluid of the treated host larvae. This contact with juvenoids resulted into various abnormalities in the internal systems. Such abnormalities have earlier been reported in other insects after topical application on their immature stages.

The effects on parasites caused due to contact of juvenoid through host have also been reported in different parasitic insects . Topical application of juvenoids to B. hebetor reduced fecundity and caused embryonic mortality in eggs . The anatomical derangements, reported in the present work, thus, suggest the physiological basis of the production of different functional derangements. It is, thus, obvious that the population growth of the parasitoids will be affected via their hosts as has been suggested for some endo-parasites .

Acknowledgement

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References