Chapter 2

Feeding and Reproduction

2.1. Introduction

Feeding is an important factor which alters or affects the total biology of living organisms. Feeding directly not only affects the biomass or body size, but also the overall behaviour pattern. Diet plays an important part in egg production by female insects (Johansson, 1964). In Lepidopterans which do not feed as adults, nutrients are stored by the larvae and the larval diet is important in egg production. In the majority of insects, however, fecundity is largely related to adult nutrition, although food reserves derived from the larva may have some importance (Chapman, 1982).

The pulse beetle, *C. maculatus* (Coleopteran - holometabolic) is one of the several cosmopolitan and polyvoltine insects that infest a number of legume seeds in storage (Bhattacharya and Banerjee, 2002). Pupae and adults of *Callosobruchus* sp. do not eat (Messina, 1991a and Bhattacharya and Banerjee, 2000), but depend for their survival on organic materials (mostly lipids) stored in the tissues by the larval instars. They are facultatively aphagous, requiring neither food nor water in the adult stage (Fox et al., 2004).

Messina (1991a) and Messina et al. (1991) reported that females reared on mung beans (*Vigna radiata* (L.) R. Wilcz.) with competition (larval density of two per bean) tended to have lowered emergence weight than those reared without competition. Females of Asian strains which had highly competitive larvae, uniformly
distributed egg on grains and showed stronger preferences for egg-free seeds than do females from strains with less competitive larvae.

In this study, the adult female beetles were allowed to lay eggs on splintered grains; by no choice experiments and their reproductive potential was calculated. The splintered grains could provide only partial nutrition and clearly indicate the role of wholesome nutrition on insect development.

The feed type seems to affect the behaviour of insect pests of stored grains. The reproductive behaviour is altered by the type of food consumed by insects. This is due to the differences in the nutritional quality of each grain type. Grains with phytostimulants are likely to be the ideal feed types resulting in the production of insects with high reproductive rate and fitness. Studies in this line have already been done by Giga (1982), Credland et al. (1986) and Vamosi (2005). In the present study chronology of various events associated with mating have been studied in *C. maculatus* reared exclusively.

A study was done to observe whether the adult *C. maculatus* could feed or not because researchers had doubts about the biology of *C. maculatus* in natural environment during various seasons. Longevity also varied among populations based on the quantity and quality of nutrition received by the insects during their larval stages and also due to environmental forces like temperature and humidity. Larval host plants of most of the bruchids remain unknown, partly because collections of these beetles were usually made by net-sweeping of adults, which often fed on non-larval host plants which had a source of nectar (Tuda *et al.*, 2005). In this study the adult *C. maculatus* were fed with sugar syrup and their longevity and fecundity were studied.
2.2. Materials and Methods

2.2.1. Feeding behaviour of grubs

The feeding behaviour of grubs of *C. maculatus* was studied in four different pulses – cowpea, black gram, green gram and chickpea. The stadial periods of *C. maculatus* was studied by providing each grain in three different forms except chickpea, which was provided in four forms. The three forms of grains were full, half and quarter grains. These forms were placed in separate containers and young healthy mated females were introduced into each of the experimental containers. The four different larval instars were carefully followed in each feed form and stadial periods recorded. The pupal period and the total developmental period were also calculated. The percentage of mortality before reaching adulthood was recorded in each separate group and adult female longevity and fecundity were also recorded. In the case of chickpea, full, half, quarter grains and 1/8th size grains were used in this study. The objective of this study was to understand the impact of feeding, especially the quantity of food consumed on the reproductive success of *C. maculatus*.

2.2.2. Feeding and longevity

*C. maculatus* adults are opportunistic feeders. Experiments were conducted to find out the impact of adult feeding on the longevity of *C. maculatus*. The control beetles were placed in containers where pulses were not provided. In another set of control beetles, pulses were provided to the beetles at the rate of 100g/100 beetles. Still another set of beetles were provided with sucrose crystals at the rate of 2g/100 beetles. In another experiment, 100g of *V. radiata* were coated with sucrose syrup prepared by dissolving
2.5g of sucrose in 5ml of water. The longevity was followed by checking the number of live insects at 5/10 day intervals to a maximum of 70 days.

2.2.3. Mating behaviour

The mating behaviour of *C. inaculatus* was studied in young beetles raised on *V. radiata, V. ungulata* and *V. mungo*. Grains with pupae were taken in separate containers. Just before eclosion, each grain was placed inside an individual container. As soon as the adults eclosed they were brought together as mating pairs. The container with a mating pair consisted of a young male and female that had eclosed about 15 minutes earlier. Such mating pairs started mating as soon as they were left together. The mating phenomenon was compartmentalized into precisely aimed sequential events. The timing of these events and the pattern were recorded in flowcharts.

Larval competition was studied by allowing gravid *C. maculatus* females to lay eggs on grains that already had 5, 10 and 15 day old grubs developing in them. 5 females were allowed into a set of 300 grains, all of them invariably containing 5, 10 or 15 day old grubs developing within them. These grains were specially selected by allowing *C. maculatus* for 24h to lay eggs in surplus grains so that the females normally laid only one egg per grain. Such grains were transferred to containers and allowed to incubate. Development of grubs started soon after hatching.

Thus, grains containing 5 day old larvae were those grains on which eggs were deposited 11 days earlier, taking into account the time taken for incubation (6 days). The grains were further incubated and emergence of adults was followed. If adult emergence time exceeded the normal time of development of the first egg it was evident that the old grub had been cannibalised by the newly developing grub. Developmental failure percentage was calculated.
2.3. Results

Significant variations were seen in the biology of *C. maculatus* raised in the splintered cotyledons (full, half, quarter and 1/8th) of the four different pulses. Females from pulses splintered into quarter or 1/8th were significantly smaller than those reared in full grains (Table 2.1). Larval mortality was very high in 1/4th and 1/8th pieces. In cowpea, the percentage of adult emergence was 81.70 ±3.37, 63.56 ±5.83 and 20.3 ±1.86 for full, half and quarter grains respectively. In black gram, the percentage of adult emergence was 53.54 ±4.12, 38.94 ±0.82 and 9.14 ±1.08 for full, half and quarter grains respectively. 5.12 ±1.03% of adults only successfully emerged in 1/8th piece of gram. Total developmental period was also comparatively high in 1/4th and 1/8th pieces of grains than full grains. The total developmental period of *C. maculatus* developed from full grain of cowpea was 26 days while it took 35 days when it was developed on 1/4th piece of cowpea (Table 2.1, 2.2).

Females outlived males when reared in all sizes of grains. The beetles that emerged from 1/8th pieces lived longer than the beetles emerged from the full grain. The longevity of female *C. maculatus* emerged from full grain of cowpea was 7.5 ±1.20 days, while it was 14.31 ±1.31 days when reared on quarter grains. Females that emerged from the quarter grains of black gram showed higher longevity of 18.3 ±1.81 days. Fecundity of *C. maculatus* reared on the full grain of cowpea was 91.3 and it was 12.32 when it was reared on the quarter grain of black gram.

When the adult *C. maculatus* was fed with sucrose, longevity increased up to 70 days. The normal longevity of adults emerging from green gram was 10 to 20 days (in the absence of oviposition stimulus). When the control adults were supplied with
grains for oviposition their longevity decreased to a maximum of 10 days. Adults supplied with grains and sucrose could live a maximum of 40 days with a mean fecundity of 134.5 ±10.16 eggs while the fecundity of control was 78.5 ±7.76 (Table 2.3).

Male *C. maculatus* raised in *V. unguiculata* took about 3.1 minutes to spot a female and get excited. The female responded in about 20 sec. Total duration of copulation was 16.6 ±1.1, 14.4 ±1.2, 11.8 ±1.1 and 14.3 ±1.2 for *V. unguiculata*, *V. radiata*, *V. mungo* and *C. arietinum* respectively. Time taken for the ejection of the spermatophore capsule was 52.6 ±2.8 and 82.6 ±7.2 for *V. unguiculata* and *V. mungo* respectively (Table 2.4).

The flow charts for the four grains (Chart 2.1 - 2.4) indicated the sequence of events associated with mating and the precise timing of each such event.

### 2.3.1. Larval cannibalism

The 5, 10, 15 or 20 day old grubs developing within *V. radiata* grains failed to eclose and eclosion was observed after 26-50 days (Table 2.5). The respective controls eclosed conforming to the normal developmental schedule (Table 2.5).

In control, 99.4 ±0.5% adults emerged within 26-30 days. But it is 48.33 ±2.73% when the grain carrying eggs oviposited by gravid females again after 10 days for incubation. 64.49 ±6.45% adults emerged after 41-45 days when the infested grains were again oviposited by *C. maculatus* after 15 days of incubation. The developmental failure percentage reached 78.41±6.84 when the grains were allowed for oviposition for every 5 days interval during first 20 days of incubation.
Table 2.1. Incubation and stadial period of *C. maculatus* raised on different sizes of grains (full and splintered)

<table>
<thead>
<tr>
<th>Grains</th>
<th>Incubation period</th>
<th>Larval period</th>
<th>Pupal period</th>
<th>Total developmental period</th>
<th>% Adult emergence</th>
<th>Immature mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt;</td>
<td>3.6 ± 0.48</td>
<td>4.6 ± 0.25</td>
<td>4.1 ± 0.21</td>
<td>3.8 ± 0.17</td>
<td>3.5 ± 0.22</td>
<td>6.4 ± 0.38</td>
</tr>
<tr>
<td>A&lt;sub&gt;2&lt;/sub&gt; (A&lt;sub&gt;1&lt;/sub&gt; vs A&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>3.6 ± 0.46</td>
<td>5.2 ± 0.43</td>
<td>4.9 ± 0.37</td>
<td>4.5 ± 0.39</td>
<td>4.1 ± 0.28</td>
<td>6.7 ± 0.47</td>
</tr>
<tr>
<td>A&lt;sub&gt;3&lt;/sub&gt; (A&lt;sub&gt;1&lt;/sub&gt; vs A&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>3.8 ± 0.38 (5.56)</td>
<td>5.6 ± 0.45</td>
<td>5.3 ± 0.38</td>
<td>5.2 ± 0.39</td>
<td>4.9 ± 0.35</td>
<td>7.2 ± 0.58</td>
</tr>
<tr>
<td>B&lt;sub&gt;1&lt;/sub&gt; (A&lt;sub&gt;1&lt;/sub&gt; vs B&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>3.9 ± 0.62 (7.69)</td>
<td>4.9 ± 0.42</td>
<td>4.4 ± 0.37</td>
<td>4.2 ± 0.36</td>
<td>3.8 ± 0.31</td>
<td>6.8 ± 0.62</td>
</tr>
<tr>
<td>B&lt;sub&gt;2&lt;/sub&gt; (A&lt;sub&gt;2&lt;/sub&gt; vs B&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>4.7 ± 0.45 (30.56)</td>
<td>5.1 ± 0.48</td>
<td>4.9 ± 0.44</td>
<td>4.6 ± 0.41</td>
<td>4.4 ± 0.39</td>
<td>6.9 ± 0.63</td>
</tr>
<tr>
<td>B&lt;sub&gt;3&lt;/sub&gt; (A&lt;sub&gt;3&lt;/sub&gt; vs B&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>4.15 ± 0.39 (9.21)</td>
<td>5.2 ± 0.45</td>
<td>6.5 ± 0.55</td>
<td>6.1 ± 0.54</td>
<td>4.8 ± 0.45</td>
<td>7.3 ± 0.71</td>
</tr>
<tr>
<td>C&lt;sub&gt;1&lt;/sub&gt; (A&lt;sub&gt;1&lt;/sub&gt; vs C&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>5.1 ± 0.73 (41.67)</td>
<td>5.5 ± 0.45</td>
<td>5.2 ± 0.38</td>
<td>5.1 ± 0.43</td>
<td>4.7 ± 0.35</td>
<td>9.3 ± 0.79</td>
</tr>
<tr>
<td>C&lt;sub&gt;2&lt;/sub&gt; (A&lt;sub&gt;2&lt;/sub&gt; vs C&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>5.2 ± 0.61 (44.44)</td>
<td>6.5 ± 0.51</td>
<td>5.9 ± 0.55</td>
<td>5.4 ± 0.52</td>
<td>4.2 ± 0.38</td>
<td>9.8 ± 0.87</td>
</tr>
<tr>
<td>C&lt;sub&gt;3&lt;/sub&gt; (A&lt;sub&gt;3&lt;/sub&gt; vs C&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>5.1 ± 0.52 (34.21)</td>
<td>6.4 ± 0.53</td>
<td>7.2 ± 0.61</td>
<td>7.2 ± 0.61</td>
<td>5.2 ± 0.43</td>
<td>10.01 ± 0.97</td>
</tr>
<tr>
<td>D&lt;sub&gt;1&lt;/sub&gt; (A&lt;sub&gt;1&lt;/sub&gt; vs D&lt;sub&gt;1&lt;/sub&gt;)</td>
<td>3.8 ± 0.67 (5.56)</td>
<td>5.3 ± 0.49</td>
<td>4.9 ± 0.41</td>
<td>4.7 ± 0.42</td>
<td>4.5 ± 0.41</td>
<td>7.2 ± 0.68</td>
</tr>
<tr>
<td>D&lt;sub&gt;2&lt;/sub&gt; (A&lt;sub&gt;2&lt;/sub&gt; vs D&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>3.9 ± 0.56 (7.69)</td>
<td>5.4 ± 0.48</td>
<td>5.1 ± 0.45</td>
<td>4.9 ± 0.43</td>
<td>4.6 ± 0.41</td>
<td>7.3 ± 0.68</td>
</tr>
<tr>
<td>D&lt;sub&gt;3&lt;/sub&gt; (A&lt;sub&gt;3&lt;/sub&gt; vs D&lt;sub&gt;3&lt;/sub&gt;)</td>
<td>4.1 ± 0.51 (7.89)</td>
<td>5.4 ± 0.51</td>
<td>6.2 ± 0.55</td>
<td>5.3 ± 0.45</td>
<td>4.6 ± 0.43</td>
<td>7.5 ± 0.64</td>
</tr>
<tr>
<td>D&lt;sub&gt;4&lt;/sub&gt;</td>
<td>4.21 ± 0.48</td>
<td>5.5 ± 0.53</td>
<td>7.5 ± 0.65</td>
<td>5.4 ± 0.45</td>
<td>4.6 ± 0.38</td>
<td>7.8 ± 0.65</td>
</tr>
</tbody>
</table>

A = *V. unguiculata*  
B = *V. radiata*  
C = *V. mungo*  
D = *C. arietinum*  
1 = Full grain  
2 = Grains broken into two  
3 = Grains broken into four  
4 = Grains broken to eight
Table 2.2. Longevity, biomass and reproduction of *C. maculatus* raised on different sizes of grains (full and splintered)

<table>
<thead>
<tr>
<th>Grains</th>
<th>Longevity (days)</th>
<th>Biomass (mg)</th>
<th>Feecundity (mg)</th>
<th>Reproductive potential (rm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>A_1</td>
<td>6.1 ± 1.14</td>
<td>7.5 ± 1.20</td>
<td>2.84 ± 0.23</td>
<td>3.56 ± 0.21</td>
</tr>
<tr>
<td>A_2</td>
<td>7.3 ± 1.13</td>
<td>8.9 ± 1.42</td>
<td>2.82 ± 0.18</td>
<td>3.48 ± 0.17</td>
</tr>
<tr>
<td>A_3</td>
<td>12.3 ± 1.21*</td>
<td>14.31 ± 1.31*</td>
<td>2.08 ± 0.11</td>
<td>2.34 ± 0.14</td>
</tr>
<tr>
<td>B_1</td>
<td>8.0 ± 0.89</td>
<td>11.9 ± 1.7</td>
<td>2.13 ± 0.1</td>
<td>2.89 ± 0.18</td>
</tr>
<tr>
<td>B_2</td>
<td>9.3 ± 0.73</td>
<td>12.38 ± 0.98</td>
<td>1.89 ± 0.08</td>
<td>2.21 ± 0.13</td>
</tr>
<tr>
<td>B_3</td>
<td>11.3 ± 1.01*</td>
<td>13.13 ± 0.83*</td>
<td>1.68 ± 0.11</td>
<td>1.79 ± 0.13</td>
</tr>
<tr>
<td>C_1</td>
<td>13.1 ± 1.3</td>
<td>16.8 ± 2.74</td>
<td>2.31 ± 0.1</td>
<td>3.18 ± 0.25</td>
</tr>
<tr>
<td>C_2</td>
<td>13.31 ± 1.21*</td>
<td>16.82 ± 1.89*</td>
<td>2.14 ± 0.15</td>
<td>2.75 ± 0.18</td>
</tr>
<tr>
<td>C_3</td>
<td>16.21 ± 1.83*</td>
<td>18.31 ± 1.92*</td>
<td>1.84 ± 0.12</td>
<td>2.23 ± 0.17</td>
</tr>
<tr>
<td>D_1</td>
<td>7.4 ± 1.01</td>
<td>9.1 ± 1.37</td>
<td>2.34 ± 0.18</td>
<td>3.2 ± 0.24</td>
</tr>
<tr>
<td>D_2</td>
<td>8.12 ± 0.68</td>
<td>10.73 ± 0.78</td>
<td>2.31 ± 0.17</td>
<td>2.82 ± 0.21</td>
</tr>
<tr>
<td>D_3</td>
<td>10.57 ± 1.30</td>
<td>14.39 ± 1.34*</td>
<td>1.99 ± 0.13</td>
<td>2.64 ± 0.18</td>
</tr>
<tr>
<td>D_4</td>
<td>12.3 ± 1.28*</td>
<td>16.45 ± 1.08*</td>
<td>1.72 ± 0.11</td>
<td>1.92 ± 0.13</td>
</tr>
</tbody>
</table>

A = *V. unguiculata*  
B = *V. radiata*  
C = *V. mungo*  
D = *C. arietinum*  

I = Full grain  
2 = Grains broken into two  
3 = Grains broken into four  
4 = Grains broken to eight  

(*) = flight morphs
Table 2.3. Adult longevity of *C. maculatus* - days after treatment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number of insects alive – days after treatment (replicates = 3, n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>M</td>
</tr>
<tr>
<td>A</td>
<td>48.5</td>
</tr>
<tr>
<td>B</td>
<td>44.5</td>
</tr>
<tr>
<td>C</td>
<td>49.5</td>
</tr>
<tr>
<td>D</td>
<td>49.5</td>
</tr>
</tbody>
</table>

M - Male; F – Female; (-) No oviposition option

A - Control-1 (Beetles without grains); B – Control -1 [Beetles with grains (100g)]; C - Beetles fed sugar (2g) Without grains; D - Beetles in sugar coated grains (100g)

(2.5g sugar + 5ml water)
Table 2.4. Chronology of mating in *C. maculatus* raised in different feed types

<table>
<thead>
<tr>
<th>Feed type</th>
<th>Arousal and approach (in min.)</th>
<th>Mounting and riding over time (in min.)</th>
<th>Anti-directional orientation time (in min.)</th>
<th>Number of intromissions</th>
<th>Total duration of copulation</th>
<th>Time taken for ejection of spermatophore capsule</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>V. unguiculata</em></td>
<td>3.1 ± 0.42</td>
<td>18.4 ± 1.32</td>
<td>13.2 ± 1.2</td>
<td>14.0 ± 1.8</td>
<td>16.6 ± 1.1</td>
<td>52.6 ± 2.8</td>
</tr>
<tr>
<td><em>V. radiata</em></td>
<td>6.6 ± 0.85</td>
<td>15.1 ± 1.16</td>
<td>11.6 ± 1.04</td>
<td>10.7 ± 1.12</td>
<td>14.4 ± 1.2 ± 4.1</td>
<td>68.2 ± 4.1 (29.66)</td>
</tr>
<tr>
<td><em>V. mungo</em></td>
<td>8.4 ± 0.68 (170.97)</td>
<td>13.7 ± 1.11</td>
<td>9.5 ± 0.84</td>
<td>9.6 ± 0.87</td>
<td>11.8 ± 1.1</td>
<td>82.6 ± 7.2 (57.03)</td>
</tr>
<tr>
<td><em>C. arietinum</em></td>
<td>6.4 ± 0.71 (106.45)</td>
<td>15.5 ± 1.28</td>
<td>12.1 ± 1.09</td>
<td>11.8 ± 0.99</td>
<td>14.3 ± 1.2</td>
<td>65.7 ± 6.56</td>
</tr>
</tbody>
</table>

Percent change over *V. unguiculata* in parentheses
Table 2.5. Larval cannibalism of *C. maculatus*

<table>
<thead>
<tr>
<th>Initial oviposition (no. of females)</th>
<th>Number of gravid females</th>
<th>Day-wise adult emergence percentage</th>
<th>Developmental failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5  10  15  20</td>
<td>26-30  31-35  36-40  41-45  46-50</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>-  -  -  -</td>
<td>99.4 ± 0.5  0.6 ± 0.021  -  -  -</td>
<td>17.63 ± 1.03</td>
</tr>
<tr>
<td>5</td>
<td>5  -  -  -</td>
<td>70.27 ± 5.55  27.72 ± 2.13  2.01 ± 0.07  -  -</td>
<td>20.31 ± 1.85</td>
</tr>
<tr>
<td>5</td>
<td>-  5  -  -</td>
<td>48.33 ± 2.73  2.45 ± 0.15  42.32 ± 3.89  6.9 ± 0.38  -</td>
<td>25.41 ± 2.31</td>
</tr>
<tr>
<td>5</td>
<td>-  -  5  -</td>
<td>28.21 ± 0.98  1.96 ± 0.06  -  -  -  -  -  -</td>
<td>-64.49 ± 5.34 ± 23.21 ± 1.99</td>
</tr>
<tr>
<td>5</td>
<td>-  -  -  5</td>
<td>98.73 ± 1.21  1.27 ± 0.05  -  -  -  -</td>
<td>19.21 ± 1.05</td>
</tr>
<tr>
<td>5</td>
<td>5  5  5  5</td>
<td>21.3 ± 1.84  26.4 ± 2.4  28.91 ± 2.39  23.39 ± 2.53  -</td>
<td>78.41 ± 6.84</td>
</tr>
</tbody>
</table>
Chart 2.1. *C. maculatus*: Flowchart of mating behaviour (Timings in minutes except otherwise indicated on *V. unguiculata* (cowpea))
Chart 2.2. *C. maculatus*: Flowchart of mating behaviour (Timings in minutes except otherwise indicated on *V. radiata* (green gram))

- Sex starved
- Mating pre-disposition
- Aroused 6.1
- Submission
- Percepcion (vicinity)
- Mating pre-disposition
- Aroused and approach 6.6
- Mounting and riding over 15.1
- Embraced
- Intromission-male genitalia extension and connection 34 sec
- Copula (anti-directional orientation) 11.6
- Copula male in mounted position 2.8
- Ejection of spermatophore capsule 68.2
- Post copulatory behaviour 5.2
- Termination of copulation
- Post copulatory behaviour 6.8
Chart 2.3. *C. maculatus*: Flowchart of mating behaviour (Timings in minutes except otherwise indicated on *V. mungo* (black gram))

- **Sex starved**
- **Mating pre-disposition**
  - **Perception (vicinity)**
  - **Aroused and approach 8.3**
- **Aroused 8.3**
- **Submission**
  - **Mounting and riding over 13.7**
  - **Embraced**
- **Intromission-male genitalia extension and connection 38 sec**
  - **Copula male in mounted position 2.3**
  - **Copula (anti-directional orientation) 9.5**
- **Ejection of spermatophore capsule 82.6**
- **Post copulatory behaviour 6.3**
- **Termination of copulation**
  - **Post copulatory behaviour 7.2**
Chart 2.4. *C. maculatus*: Flowchart of mating behaviour (Timings in minutes except otherwise indicated on *C. arietinum* (chick pea))

- **Sex starved**
- **Mating pre-disposition**
- **Aroused 6.2**
- **Submission**
- **Perception (vicinity)**
- **Aroused and approach 6.4**

- **Mounting and riding over 15.5**
- **Embraced**
- **Intromission-male genitalia extension and connection 33 sec**
- **Copula male in mounted position 2.2**
- **Copula (anti-directional orientation) 12.1**
- **Ejection of spermatophore capsule 65.7**
- **Post copulatory behaviour 5.3**
- **Termination of copulation**
- **Post copulatory behaviour 6.4**
Fig. 2.1 Probability diagram of the biology of C. maculatus raised on different sizes of grains (full and splintered)

A. *V. unguiculata*

B. *V. radiata*

C. *V. mungo*

D. *C. cressinum*

- Developmental period (days)
- % adult emergence
- Male longevity (days)
- Female longevity
- Biomass - male (mg)
- Biomass - female
- Fecundity
- Reproductive potential
Fig. 2.2 Adult longevity of *C. maculatus* - days after treatment

A. Beetles only

B. Beetles in grains

C. Beetles and sugar only

D. Beetles with sugar coated grains
2.4. Discussion

Feeding and nutrition modified the biology of organisms and this is quite common in insects (Chapman, 1982; Bhattacharya and Banerjee, 2000 and 2002). *V. unguiculata*, the common cowpea seemed to be the ideal food for *C. maculatus* since the stadial periods were significantly lower when raised in this grain with a high level of adult emergence (81.7%) and a high fecundity of 91.3 ±10.81. The other grains also supported the development of *C. maculatus* and they could be considered as substitute feed materials in the event of the non-availability of the ideal meal.

Most Bruchidae feed on the seeds of legumes and all of the species that are important pests of stored products are legume feeders. As a member of Bruchidae *C. maculatus* attack a wide range of stored pulses including beans (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*), pigeon peas (*Cajanus cajan*), chick peas (*Cicer arietinum*), grams (*Vigna radiata, V. mungo* etc) and other legumes (*Pisum canavalia, Dolichos lablab, Glycine* sp.). Some of these are used less frequently than others or are resorted to only under stress conditions (Haines, 1991).

When splintered grains were used for rearing *C. maculatus*, there was an increase in the percentage of adult emergence. This was associated with higher life stage mortality. The fecundity significantly decreased indicating that the food material available in a splintered grain was not sufficient to support normal development of *C. maculatus*. Even though, the development to adulthood was achieved, there seemed to be a deficiency associated with storage proteins needed for normal egg production.

A common assumption in studies of intra- and inter-specific competition is that developmental conditions affect adult fitness only indirectly through their effect
on body mass (Carnov and Skinner, 1985; Godfray, 1987; Wilson, 1994; Ellers et al., 1998 and Hirschberger, 1999). Fecundity of *C. maculatus* was reduced when the size of the grains reduced. A recent study by Vamosi (2005) reported that there was no effect of larval competition treatments on mass at emergence of *C. chinensis* females reared in adzuki or mung beans. On controlling of body mass, there was a significant effect of competition treatment on a number of eggs laid by *C. chinensis* females that had been reared on mung beans, with those reared with a competitor laying significantly fewer eggs than females that had been reared in the absence of competition. Competition had a highly significant effect on mass at emergence of *C. maculatus* females reared in mung beans. In the absence of a significant effect of competition on mean mass at emergence in adzuki beans, females reared with two competitors laid significantly fewer eggs than did females reared with either one or no competitors.

The present experiment was almost equal to the experiment conducted by Vamosi (2005). But in this study the effect of quantity of grains on the biology of *C. maculatus* was elucidated. The South Indian population showed severe larval competition as a result only one adult emerged at a time especially in *Vigna* spp. *C. arietinum* could support more than one *C. maculatus* at a time. Strains of Brazil and Nigeria could produce more than twelve adults from a cowpea seed bearing numerous eggs, whereas a strain of The Yemen Republic rarely produced more than three (Credland et al., 1986). Larval competition was very high in the strain used in this study and only one larvae of *C. maculatus* successfully developed in to adult. The larva hatched later had more chance to become an adult.

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In the study the adult females were allowed to lay eggs on the grains that already had 5, 10, 15 and 20 day old larvae of *C. maculatus*. In all the experiments the larvae that hatched later preyed upon the larvae that were already found in the grains. This indicated the stiff competition among the developing grubs of *C. maculatus* and conditions that could develop in the event of non-availability of grains for laying eggs. The experiments revealed the complex inter-relationship in *C. maculatus* between adult size, fecundity, type and the quantity (size) of the host, all of which was investigated in a single species of the genus for the first time; previous data on one or more of the inter-relationships was obtained for a species other than the one used for the study (Smith and Lessels, 1985; Credland *et al.*, 1986 and Vamosi, 2005).

The results that have been obtained can be used to try and predict how females would distribute their eggs among available hosts and also try to understand why egg fecundity was suppressed to a high level among the four different hosts with varying quantity. In attempting to answer both the questions, it is imperative to define an objective which would be referable to recognise evolutionary processes. Nothing is known in detail about the reproductive capacity of male *C. maculatus* but each male can mate successfully with more than one female (Credland *et al.*, 1986) and at present, the assumption is made that male offspring production did not impose overriding selective pressures on the ovipositional strategies of female beetles. It should, however, be noted that in the related bruchid *Acanthoscelides obtectus* Say there was some evidence that the reproductive capacities of the males did not change in the course of their lives (Labegrie, 1996). There is currently no evidence that females can control the sex of their offspring and any influence that such a factor may have on their ovipositional behaviour has not been considered further. The primary
concept is that each adult female beetle attempts to maximize her fitness by
distributing her eggs in such a way that she will produce not simply the maximum
number of reproductive offspring but which will as a group produce the maximum
number of eggs. Egg production by females was positively correlated with their
weight at the time of mating, shortly after emergence (Credland, 1986).

In full grains, the adults developing from black gram showed very less
biomass (2.89 ±0.18mg) and comparatively less fecundity (44.4 ±4.92 eggs) and high
longevity (16.82 ±1.89 days) and low adult emergence (53.54). So the fecundity of *C.
maculatus* was positively correlated with biomass and negatively correlated with
longevity. Dongre et al. (1996) found out that the larval resistance (*C. maculatus*) in a
wild progenitor of black grain *V. mungo* var. *silvestris*. They observed that only
17.3% of the larvae only completed their larval stages and successfully entered into
adulthood, rest of the larvae died because of larval antibiosis in the black gram.

The adults of *C. maculatus* are polymorphic with two morphs, a flightless and
a flight morph that differ in their morphological, physiological and behavioural
characteristics (Utida, 1954). The adults of the flight morph represented a dispersal
morph which colonized the cowpea field during the rainy season (Prevett, 1967;
Southgate, 1979; Huignard et al., 1985). The females laid their eggs on the maturing
pods and the larvae completed their development in the seeds. They gave rise to
adults of the flightless morphs which emerged one month later in the fields or in the
cowpea granaries after pod harvesting. Adults of this morph were sexually active;
they reproduced on the stored seeds and four to five of the flight morphs began to
emerge after six months of storage and their population progressively increased
throughout successive generations (Ouedraogo et al. 1996 and Zannou et al., 2003).
The adult polymorphism was induced during post-embryonic development, the increase in temperature, seed water content and larval density encouraged the emergence of a high number of flight morph adults (Sano, 1967; Fujii, 1980 and 1984 and Ouedraogo et al., 1991). In West Africa, the flight morph adults left the poorly closed granaries and were able to survive in the tropical ecosystem until the fructification of the cowpea in August (Prevett, 1967; Southgate, 1979). The survival conditions of the adults of these morphs and their reproductive activity during the host plants colonization period remains unknown. Messina and Renwick (1985) suggested that the adults of the flight morphs could be in reproductive diapause as observed in Bruchidius atrolineatus (Huignard et al., 1985) or in the active morph of C. subinnotatus.

In this study, the flight morphs developed especially from black gram (the adults developing from quarter grains of V. mungo and V. radiata and 1/8th piece of C. arietinum) and showed higher longevity (20 days) than the adults developing from other pulses. Its fecundity was also less when compared to the other pulses. The fecundity of C. maculatus developing from full grains of cowpea was 91.3 ±10.81 while it was 44.4 ±4.92 for the adults developing from the full grains of black gram.

Utida (1972) and Lextrait et al. (1995) reported that the flight morph females of C. maculatus were in reproductive quiescence only at the beginning of their imaginal life and began to reproduce after 10 to 15 days latency period in the absence of external stimulation. The duration of their imaginal life did not exceed 20-30 days and thus they were unlikely to survive until host plant fructification under normal conditions.
Other than food quality and quantity, fecundity of *C. maculatus* was also affected by mating and complex genetic architecture. Multiple mating resulted in elevated lifetime fecundities in *C. maculatus*. When oviposition resource was plentiful the elevation was apparent after a second mating. But when oviposition sites were limited, fecundity increased after third mating only (Wilson *et al.*, 1999). Fox *et al.* (2004) studied the genetic architecture of differences in lifespan and shapes of mortality curves between two populations of the seed beetle, *C. maculatus* (South India and Burkina Faso populations). These populations differ in various traits (such as body size and adult lifespan) that had likely evolved through host-specific selection. It was found that the genetic architecture of lifespan differences between populations differed substantially between males and females; there was a large maternal effect on male lifespan (but not on female lifespan), and substantial dominance of long-life alleles in females (but not males). The large maternal effect in males was genetically based (there was no significant cytoplasmic effect) likely due to population differences in maternal effects genes that influenced lifespan of progeny. Rearing host did not affect the genetic architecture of lifespan and there was no evidence that genes on the Y-chromosome influenced the population differences in lifespan.

In this study, carrying host affected the lifespan but it might have not affected the genetic architecture of lifespan because when the affected females found a suitable host, they oviposited on it and the progeny produced would be without any effect on lifespan and fecundity.

Digestion, absorption and nutrition play an important role in the metabolism of all living organisms. Especially, in holometabolous insects (which do not grow after
they had metamorphosed into adult), the body mass is determined by the nutritional status of the larva. Bruchids were among the ones which do not feed as adults. So the larval diet was crucial for the normal biology of *C. maculatus* adults. If the diet was adequate qualitatively, but only available in limited amounts the resulting adults would be reduced in size (Chapman, 1982). *Ephestia kuehniella* Zeller for instance, needed about 0.13g of wholemeal flour for normal development. On smaller amounts, even as little as 0.04g, the moths emerging were normal, but smaller (Norris, 1933). The proportions of the wings relative to the body could also be altered by the diet. In this experiment, the adults emerging from the quarter and 1/8th pieces of cotyledons of the four pulses showed reduced biomass (1.92mg) than the adults emerged from full grains (3.2mg).

Differences in diet could lead to polymorphism. *Thecalaid melittobia* is parasitic on the wasp, *Trypoxylon* sp. and the first 12-20 larvae to develop within a host did so rapidly. The resultant adults emerged with short crumpled wings and proceeded to mate and oviposit in the same host. Later larvae developed more slowly and gave rise to fully winged adults which left the host. These differences were due to changes in the food supply from the host (Chapman, 1982).

Diet plays an important part in egg production by female insects (Johansson, 1964). Subramanian (2000) reported that the black and green grams and chickpeas had almost equal amounts of protein and carbohydrates. Fat content was high in chickpeas; calcium and iron were high in black gram. The calorific value was 347, 451 and 372 joules for black and green gram and chickpeas respectively. Decreased calorific value of black gram could be the reason for poor fecundity of adults developed on black gram. In this experiment, minimum developmental period was
reported in bruchids that were reared on full grains of cowpea (26 days) and the
maximum developmental period was reported in full grains of black gram (35 days).

Moisture content plays an important but major role in the feeding and
developmental success of *C. maculatus*. When the moisture content increased the
larval development period decreased. Chandrakantha *et al.* (1987) reported that the
food consumption of *C. maculatus* larvae decreased with increased temperature.
When the temperature increased the moisture content of seeds would automatically
decrease. The moisture content of cowpea was the highest (13.4) followed by black
gram (10.5), green gram (10.4) and chickpeas (9.8).