Wandering Behavior as a Response to Crowding is Coupled with the Mechanism Controlling Development Rate in *Plodia interpunctella* (Lepidoptera: Phycitidae)

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Behavioral and developmental responses to crowding were investigated in the two lines of *Plodia interpunctella* selected for fast (FDL) and slow (SDL) development rates. The two lines were reared at three density conditions, i. e., 100, 400 and 1000 eggs.

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/30 g rice bran. The development time increased in both the lines as density level increased. However, the FDL developed significantly faster than the SDL under all the conditions examined. The SDL produced heavier larvae upon wandering than the FDL, especially at 400 eggs /30 g rice bran. The FDL produced heaviest adults under the least crowded condition of the three rearing densities examined while in the SDL reduction of weight at high densities was less apparent than in the FDL. Survival was higher in the SDL than in the FDL under less crowded conditions. The selection also affected the behavior of the larvae. Some larvae left rice bran when they reached maturity (wandering larvae) while the others stayed in the bran to spin cocoon (non-wandering larvae). Wandering was less prominent in the FDL than in the SDL but the selection for fast development further lowered the rate of wandering, although no selection was imposed on this particular trait. The selection for slow development, on the other hand, did not increase the rate of wandering. The present result showed that the mechanisms controlling developmental rate are somehow coupled with the mechanisms that control other developmental and behavioral traits, notably wandering behavior. Some pleiotropic or epigenetic interplay seems to help avoid a mass extinction upon starvation and cannibalism while maximize the reproductive rate under favorable conditions.

**Key words:** *Plodia interpunctella*, Artificial selection, Development rate, Crowding

**Introduction**

Density-dependent regulation of life cycles is common in insects (Uvarov, 1921; Utida, 1972). Population density influences insect life cycles in various ways, altering the wing morph, body size, developmental fate and rate, fecundity, behaviors, pigmentation and other life cycle parameters (Peters and Barbosa, 1977). In the housefly, *Musca domestica* L., an increase in larval density decreased the development rate and adult body weight and different strains differed in the duration of larval development under crowded conditions (Sullivan and Sokal, 1963). In *Ephestia kuehniella*, crowding decelerated the larval development and decreased the adult body weight (Smith, 1969). In the black carpet beetle, *Attagenus elongatus*, increased levels of larval crowding resulted in slow larval development and delayed pupation (Barak and Burkholder, 1977). In this species, selection for early emergence reduced the sensitivity to larval crowding in only six generations (Barak and Burkholder, 1977). Crowding delayed pupation also in *Tribolium freemani* (Nakakita, 1983; Kotaki and Fuji, 1995). Tsuji (1963) investigated the effect of crowding on diapause determination in *Plodia interpunctella*, and demonstrated that high larval density induced diapause in the last larval instar, though this diapause is averted at high temperatures. He has reported three different types of diapause induced by three different cues: short photoperiod, temperature fall and crowding. Each type has a distinctive sensitive stage to a specific cue. He also showed that inbreeding and continuous rearing of larvae under constant conditions in the laboratory resulted in modification of develop-
ment characteristics including a reduction in the rate of density-dependent diapause.

By selecting fast and slow developing individuals from a population of *P. interpunctella*, two selection lines were established in our laboratory at 25 °C under LD 16:8. Subsequent experiments using these selection lines revealed that artificial selection not only altered development rate and metabolic rate but also responses to photoperiod and temperature (Naeemullah and Takeda, 1998). The selection, however, had no or very little effect on the cold-hardiness (Naeemullah et al., 1999).

This paper investigates the effect of artificial selection for the response to crowding. The regulation of development rate is a metabolic regulation while behaviors are regulated by the nervous system. Ultimate goal for this study is to identify a pleiotropic mechanism and the consequence of selection to alter the steady-state point of canalized condition, if such exists (Schlichting and Pigliucci, 1998).

**Materials and Methods**

**Procedure of selection**

A stock culture of *P. interpunctella* was established on rice bran from approx. 200 adults of mixed age at 25 °C under LD 16:8 with 50% R. H. from a population that had naturally colonized on wheat bran. From this culture two selection lines were established: the FDL for fast development and SDL for slow development. The detailed procedure of selection has been published elsewhere (Naeemullah and Takeda, 1998). Briefly, adults that had emerged on the first day (day 34) were selected for the FDL and those which emerged at the tail of emergence period (day 62) were selected as the SDL. From these adults, 100 eggs were placed under controlled conditions on 30 g of diet; rice bran and glycerol mixed at 10:3 by weight. To ensure high survival, eggs laid for the second 24 hrs of adult life were used for experiments, since the majority of the first laid eggs were infertile (Sardesai, 1968). The same procedure was repeated in the following generations.

**Effects of selection for fast and late development on developmental responses to crowding**

Groups of 100, 400, and 1000 eggs from both the selection lines were established on 30 g diet in glass jars measuring 9 x 10 cm and reared at 25.6 ± 0.5 °C, under LD 16:8 and R. H. 62 ± 2.8%. Under optimum rearing conditions, most larvae, when full grown, come out (wander) of the food and spin cocoons or remain as larvae under sheltered places or on the walls of container. Corrugated cardboard was introduced into the jars for pupation / hibernation sites. The jars were checked daily to count the larvae which entered the corrugated sheets or were wandering. These larvae were transferred to new jars. The number of adults both from the individuals that pupated in the corrugated sheets and those which remained in the food were counted and weighed within 24 hours after emergence, separately for males and females. The experiment was replicated thrice.

**Effects of the selection on the weight of wandering larvae**

Another set of glass jars containing 100, 400 and 1000 eggs in 30 g of diet was set up for both the selection lines. These jars were checked daily from the time the first larva started wandering until when no larvae were found on the food. Larvae were weighed in groups without checking the sex on the assumptions that the sex ratio should not be too biased and transferred to new jars.

**Effects of the selection on the proportion of larvae wandering out of food**

Eggs of generations 18 (FDL) and 19 (SDL), were incubated at densities of 100, 400 and 1000 as described above. At the wandering stage, larvae
were removed and counted daily. Generations 39 (FDL) and 32 (SDL) were compared for any change(s) during selection in wandering behavior.

Results

Development

Fig. 1 shows the adult emergence patterns of the two selection lines at three density levels. Crowding affected the larval development in both lines. The higher the density, the later occurred the adult emergence. The FDL, developed faster at all densities showing mean development periods (±SD) of 31.6 ± 1.47, 32.4 ± 1.64, and 33.3 ± 1.45 days at densities 100, 400, and 1000, respectively. The mean development periods in the SDL were 37.3 ± 2.26, 38.8 ± 2.18, and 39.5 ± 2.68 days at densities 100, 400, and 1000, respectively. Regression analysis showed a positive relationship between larval development periods (Y) and the densities (X) with coefficients of correlation (r) of 0.98 and 0.90 for the FDL and SDL, respectively. The differences in development periods between the three density levels were statistically significant by post hoc (Fisher’s PLSD 5%) test (p<0.0001). Statistical analysis also showed a significant difference between the two lines, the FDL developing faster than the SDL, at all the density levels.

Although, development was prolonged at higher densities (Fig. 1), adults produced from both the wandering and non-wandering groups showed similar emergence patterns with no differences in development time in either behavioral group (Fig. 2).

Adult weight

An anticipated consequence of reduced food supply and living space is a reduction in body size as reflected in the adult weight. Data on adult body weight were analyzed for males and females separately and compared within and between the selection lines by post hoc (Fisher’s PLSD) test at 5%. Females were twice heavier than males at all densities (p<0.0001, Table 1).

The two selection lines showed different responses to density in adult body weight. In the FDL, the differences between the different densities were significant (p<0.0001), adults produced at the lowest density (100) were the heaviest and those at the highest density (1000) were the lightest while those at density 400 were intermediate between them. In the SDL, in contrast, the difference was not significant between densities 100 and 400 (p =
Selection in Developmental Rate Affected Responses to Crowling in Plodia

Fig. 2 Adult emergence pattern at different densities from wandering (W, closed circles) and non-wandering (NW, open circles) larvae in the FDL and SDL of P. interpunctella. Rearing conditions were as the same as given in Fig. 1.

Fig. 3 Survival rates (%) of the two selection lines of P. interpunctella at different densities. Rearing conditions were the same as given in Fig. 1.

400 were significantly heavier than those from the SDL (\( p < 0.0001 \) and \( p < 0.01 \), respectively). However, females from the two lines showed no significant difference in their body weight at density 1000 (\( p = 0.6938 \)). Adults from wandering larvae were significantly lighter than those from non-wandering larvae except for SDL males. SDL adults produced at high density were heavier than FDL adults produced at high density.

**Survival to adult stage**

Fig. 3 shows the rates of survival to adult stage. The results were compared between the three densities and between the selection lines by ANOVA and Fisher's PLSD test at 5% after arcsine square root transformation. In the FDL, survival rates at three densities, 100, 400 and 1000 were not significantly different (\( p = 0.58 \)), being 52, 56.3 and 49.8 percent, respectively. In the SDL, survival rates at low (100) and intermediate (400) densities were 77.7 and 69 percent, respectively, and the difference was not significant statistically (\( p = 0.146 \)). A significant reduction in survival was, however, observed at the highest density, 1000 (49.43%) (\( p < 0.001 \) and \( p < 0.01 \) in comparison with densities 100 and 400, respectively). The survival rates in the SDL were significantly higher than in the FDL at density 100.
Table 1 Comparison between the weight of adults (mg) which emerged from wandering (W) and non-wandering (NW) larvae in the two selection lines (FDL and SDL) of *P. interpunctella* at three density levels

<table>
<thead>
<tr>
<th>No. eggs in 30 g rice bran</th>
<th>Behavior (W / NW)</th>
<th>Adult weight (mean ± SD)</th>
<th>FDL vs. SDL p value</th>
<th>FDL vs. SDL p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FDL</td>
<td>SDL</td>
<td>FDL vs. SDL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mean ± SD)</td>
<td>(mean ± SD)</td>
<td>p value</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>9.70 ± 0.81 (50)</td>
<td>9.34 ± 1.03 (46)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>9.96 ± 0.83 (55)</td>
<td>9.34 ± 0.97 (45)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>NW</td>
<td>9.08 ± 0.96 (45)</td>
<td>9.47 ± 0.99 (95)</td>
<td>0.05</td>
</tr>
<tr>
<td>400</td>
<td></td>
<td>9.52 ± 1.26 (71)</td>
<td>9.79 ± 1.17 (30)</td>
<td>0.303</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>7.28 ± 1.26 (91)</td>
<td>7.84 ± 0.97 (146)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>NW</td>
<td>8.74 ± 1.48 (59)</td>
<td>9.08 ± 1.09 (34)</td>
<td>0.249</td>
</tr>
</tbody>
</table>

In each column, within each density level and between wandering and non-wandering individuals, means with the same letter are not significantly different by post hoc (Fisher’s PLSD) test at 5% level. In parentheses is given the number of samples.

(p < 0.01). The differences in survival between FDL and SDL were not significant statistically at densities 400 (p = 0.12) and 1000 (p = 0.923).

Wandering behavior

Number and weight of wandering larvae

Wandering upon maturation is a general characteristic of larvae of *P. interpunctella*. The number of larvae leaving the food increased considerably as the larval density increased in identical space and amount of food (Table 2, column B). Within the selection line, the differences in the number of wandering larvae at densities of 100, 400 and 1000 were statistically significant by post hoc (Fisher’s PLSD) test at 5% (p < 0.0001). The FDL and SDL showed

Table 2 Effect of artificial selection on frequencies of wandering (WL) and non-wandering larvae (NW) under different rearing densities

<table>
<thead>
<tr>
<th>No. larvae / 30 g diet</th>
<th>A #</th>
<th>B †</th>
<th>Comparison A vs. B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td>NWL</td>
<td>WL</td>
<td>WL</td>
</tr>
<tr>
<td></td>
<td>NWL</td>
<td>WL</td>
<td>WL</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FDL</td>
<td>35</td>
<td>34</td>
<td>49.3</td>
</tr>
<tr>
<td>SDL</td>
<td>10</td>
<td>54</td>
<td>84.4</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>144</td>
<td>59.8</td>
</tr>
<tr>
<td>400</td>
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<td></td>
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<tr>
<td>FDL</td>
<td>63</td>
<td>177</td>
<td>73.8</td>
</tr>
<tr>
<td>SDL</td>
<td>19</td>
<td>208</td>
<td>91.6</td>
</tr>
<tr>
<td></td>
<td>394</td>
<td>290</td>
<td>42.4</td>
</tr>
<tr>
<td></td>
<td>145</td>
<td>769</td>
<td>84.1</td>
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<tr>
<td>1000</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FDL</td>
<td>34</td>
<td>515</td>
<td>93.8</td>
</tr>
<tr>
<td>SDL</td>
<td>25</td>
<td>456</td>
<td>94.8</td>
</tr>
<tr>
<td></td>
<td>348</td>
<td>1574</td>
<td>81.9</td>
</tr>
<tr>
<td></td>
<td>172</td>
<td>1668</td>
<td>90.7</td>
</tr>
</tbody>
</table>

Within each column and with each density level, values with different letters indicate significant difference between two selection lines and vice versa; # represents data on wandering larvae at generation 18 and 19 (A) and at generation 39 and 32 (B) for FDL and SDL, respectively. † Sum of three replicates. †† Number emerged from the diet. ** p < 0.01, *** p < 0.001.
Table 3  Weight (mg, mean ± SD) of wandering larvae under different density conditions in the FDL and SDL of P. interpunctella. Eggs were incubated at 25.6 ± 0.5 °C, LD 16:8 and R. H. 62 ± 2.8%. Statistical analysis shows comparison between the two selected lines by ANOVA, one way (p values)

<table>
<thead>
<tr>
<th>Eggs/30g diet</th>
<th>FDL</th>
<th>SDL</th>
<th>FDL vs. SDL (p-values)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>19.20 ± 2.71 (63)</td>
<td>21.65b ± 1.53 (27)</td>
<td>0.0334</td>
</tr>
<tr>
<td>400</td>
<td>16.72 ± 3.53 (87)</td>
<td>22.89 ± 1.90 (56)</td>
<td>0.5037</td>
</tr>
<tr>
<td>1000</td>
<td></td>
<td>18.03 ± 3.69 (120)</td>
<td></td>
</tr>
</tbody>
</table>

Within each column means with the same letters are not significantly different by post hoc (Fisher’s PLSD) test at 5% level. □ no larva wandered out of the food. Given in parenthesis are the numbers of larvae that wandered out of the food.

significant differences in the proportion of wandering larvae at density 100 (p < 0.001) and 400 (p < 0.001) but not at density 1000 (p = 0.445).

Table 3 shows the effects of density on weight of wandering larvae. In the FDL, mean weight of wandering larvae was higher at the density of 400 than at the density of 1000 though not significantly different. SDL larvae produced at the density of 400 were significantly heavier than those produced at the density of 1000 but the difference was not significant at the density of 100. Further, statistical analysis showed no significant difference in weight of wandering larvae between the densities of 100 and 1000. Since no wandering larvae were produced at the density of 100 in the FDL, data were compared between the FDL and SDL at the densities of 400 and 1000 using ANOVA (one way). At the density of 400, SDL larvae were significantly heavier than FDL larvae. At the density of 1000, SDL larvae were heavier than FDL larvae, though the difference was not significant statistically.

Effect of artificial selection and inbreeding on wandering behavior

Table 2 summarizes the effect of artificial selection on wandering behavior clearly showing that selection and inbreeding affected the proportion of wandering larvae. In the FDL, the effect of selection was more pronounced at the lower and intermediate densities (100 and 400), showing a significant reduction in frequency of wandering larvae (p < 0.0001) but less so at the high density (1000). On the other hand, in the SDL, the effect of selection for 13 generations was less significant.

Discussion

These results clearly demonstrate that crowding affects the life cycle traits in P. interpunctella. The FDL developed faster than that of the SDL at all the densities examined. Fig. 1 shows a progressive delay in development with increasing larval crowding. Tschinkel and Willson (1971) reported a similar effect of crowding with several tenebrionid species and postulated that tactile stimulation might lead to neuronal stimulation of juvenile hormone (JH) synthesis which prevents pupation. Oral application of synthetic and natural JHs to larvae decreased the pupation rate in Trogoderma glabrum (Herbest) (Beck, 1972). Nakakita (1982, 1990) reported that physical contact between larvae prohibited pupation under crowded conditions in T. freemani, which he attributed to the stimulated JH synthesis. In this species, application of synthetic JH to larvae from crowded cultures increased the larval period even in isolation, a condition otherwise prompting pupation (Kotaki et al., 1993). Tsuji (1963) has demonstrated that young larvae of P.
*P. interpunctella* are sensitive to a certain density-dependent stimulus immediately after hatching to switch the physiological mechanism which later promotes diapause. However, crowded larvae developed normally after isolation, indicating the non-persistent nature of the crowding effect. The present investigation revealed that under all the density conditions examined crowding imposed similar effect on larval development in both wandering and non-wandering individuals (Fig. 2).

The effect of crowding was more pronounced in the SDL where larvae showed more delayed development than in the FDL (Fig. 1). The significance of this is obvious for insects such as *P. interpunctella* with polyphagous nature feeding on a variety of stored products. Under optimum conditions when sufficient food is available, fast developing individuals in a population will reproduce and disperse rapidly. Slow developing individuals sensitive to crowding could delay development and eventually enter diapause when food becomes scarce and subdue their activities until the periods when sufficient food is available.

Different trends were observed in adult body weight, when the wandering and non-wandering individuals were combined, between the two selection lines. FDL adults were the heaviest at a low density (100) and followed by those at densities 400 and 1000 (Table 1). This may be due to a general effect of crowding since similar trends have been reported in other stored product insects such as *Ephestia cautella* (Takahashi, 1955), *E. kuehniella* (Smith, 1969) and *T. molitor* (Weaver and McFarlane, 1990). SDL adults at densities of 100 and 400 had similar weight, but heavier than those at a higher density of 1000. Silhacek and Miller (1972) have reported that at 30±0.5°C and relative humidity 70±5 %, a quarter of larval weight in *P. interpunctella* is lost between the cessation of feeding and pupation. They attributed this loss to gut purge, water loss, increased utilization of food reserves and spinning. The differences in adult body weight between different densities may reflect the differences in loss of weight prior to pupation. Weaver and McFarlane (1990) ascribed decreased weight at higher densities in *T. molitor* pupae to less feeding time prior till pupation owing to more frequent interferences by other individuals. In *P. interpunctella*, larvae at the high density (1000) consumed all food within 15 days after hatching while ample amounts were available at densities 100 and 400 during this period. The less food available for individuals at the high density may have resulted in decreased adult body weight. However, the most important feature in this investigation is that at the low density the FDL produced heavier adults than the SDL, whereas at higher density the SDL produced heavier adults than the FDL and at least the difference was significant in wandering larvae.

In the FDL, the survival rate ranged from 50 to 56 % at all the density levels examined, showing no significant differences between the densities (Fig. 3). In the SDL, the difference in survival between the low and intermediate densities was not significant. However, survival was significantly higher at the densities 100 and 400 than at 1000. In both selection lines, cannibalism was observed on prepupae and pupae by slow developing larvae, since the heads and thoraces were left behind (unpublished). This has also been reported in *T. molitor* where large larvae may feed upon other large larvae and pupae (Weaver and McFarlane, 1990). Cannibalism should thus contribute to the mortality. Other possibilities are either that at the high density (1000) food shortage may lead to starvation and death in slow growing individuals or that fast developing individuals feed upon the delayed ones. As a consequence, in these cases, it is more adaptive
that high mortality should result at early stages.

Many insects leave the birthplace at a particular stage of their life cycle in response to both predictable (seasonal) and unpredictable (aseasonal) changes of the environment (Andrewartha and Birch, 1954). In both cases, insects must avoid extinction owing to overcrowding or habitat deterioration. In *Plodia interpunctella*, under normal rearing conditions, full-grown diapause larvae leave the food and find a suitable place to spin thick and closed hibernacula for overwintering in response to a decrease in photoperiod and temperature (Naemullah and Takeda, 1998). Larvae also migrate from the food in response to high population density (Tsuji, 1963). Table 2 shows that the number of wandering larvae increased as density increased. The two selection lines differed significantly in wandering behavior. At the low density (100), all larvae in the FDL remained and pupated within the food without wandering but 59.8% of the larvae wandered out in the SDL. The SDL also gave considerably higher frequencies of wandering larvae than the FDL at two other density conditions (400 and 1000). This suggests that the SDL is more responsive to crowding than the FDL. This study also showed that at the intermediate density SDL larvae fed, grew and gained weight faster than FDL larvae (Table 3).

Continuous selection for fast and slow development also modified wandering behavior in larvae of the *P. interpunctella* (Table 2). We observed that selection for fast developing individuals for 17 generations not only resulted in complete elimination of wandering at the lowest density but also the proportion of wandering larvae was greatly reduced under crowded conditions. Selection for slow developing individuals did not significantly decrease the proportion of wandering larvae. The results show that selection more readily affected the wandering behavior in the FDL than in the SDL at all the density levels, since $\chi^2$-values were much larger in the FDL.

The results lead to the conclusion that populations of *P. interpunctella* maintain a high flexibility in their life cycle characteristics, and the development time and wandering behavior are physiologically interdependent or genetically linked. Diapause tendency, growth rate, adult size, and wandering behavior may constitute a set of trade-off characters. The great variability in these characters in *P. interpunctella* must have an ecological significance in storage environment.

References


