Developmental Irregularity and Abnormal Elytra Formation in the Oriental Wood Borer Induced by Physical Disturbance

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ABSTRACT

Teratological deformity in arthropods may arise spontaneously or be induced by chemicals, mutagens, diseases, and physical disturbance. Coleopterans are known to be sensitive to physical disturbance, but detailed studies of how physical disturbance affects the juvenile development and causes deformities are limited. In this study, larval oriental wood borers Heterobostrychus aequalis (Waterhouse) in three weight categories (2 to <10 mg, 10 to <26 mg, and 26 to 36 mg) were used to examine the development of immature beetles when exposed to daily physical disturbance (i.e., handling). Morphological changes induced in the immature and adult stages were observed for disturbed individuals. Handled larvae were highly susceptible to physical disturbance, as none of them survived to the end of the experiment, regardless of their initial weight group. Unhandled larvae molted twice before turning into pupae, whereas handled larvae took longer to develop and underwent irregular molting. Handled larvae also exhibited various levels of morphological abnormality in the pupal stage; in particular, the elytra were deformed, the abdomen and head capsule were broader than normal. It is speculated that the irregularity and deformity observed in the handled larvae might be due to hormone alteration resulting from physical disturbance. In addition, the extensively exposed abdominal parts of deformed beetles might have increased the risk of dehydration and disease attack, which would have resulted in premature mortality.

Key words: Bostrichidae, deformity, ecdysteroid, juvenile hormone, octopamine

Morphological deformity is widely reported in many arthropods. It can arise spontaneously (idiopathic) due to cell division failure, and it also can be induced by chemicals (Martinez et al. 2001, Watson et al. 2014, Barbosa et al. 2015), mutagens (Hiyama et al. 2012, Akimoto 2014), diseases (Neoh and Lee 2011, Rocío et al. 2011), and physical disturbance (Spence et al. 1996). Excessive physical disturbance due to handling (e.g., pouring and rotation) of wheat has decreased adult emergence of several stored product pests (Bailey 1969, Loschiavo 1978). For example, mortality of the immature (i.e., prepupa and pupae) grain weevil Sitophilus granarius (L.) increased with increasing pouring height and speed. Bailey (1969) suggested that this premature mortality may be attributable to cuticle rupture that eventually caused water loss in the larvae, excessive secretion of neurosecretory materials that paralyzed the larvae, and lack of oxygen consumption by the disturbed larvae. Mertz and Robertson (1970) reported that daily sifting of Tribolium castaneum (Herbst; Coleoptera: Tenebrionidae) from wheat flour prolonged the larval incubation period and caused high pre-adult mortality. Based on these published records, two common phenomena occur during disturbance: a prolonged pre-adult developmental period and premature mortality. However, to date no study has focused on how physical disturbance affects the development of immature beetles and causes deformities, which eventually lead to low adult emergence.

Bostrichidae are among the most destructive wood-attacking beetles in forestry and lumber industries (Peters et al. 2002). Bong (2015) reported that 65% of the Bostrichidae infestations in Japan were caused by species of Lyctus. Although few cases involving Heterobostrychus (i.e., Heterobostrychus hamatipennis Lesne and Heterobostrychus aequalis [Waterhouse]) were reported, the prevalence of the attacks is gradually increasing in Japan (Bong 2015). Heterobostrychus species are not host specific and attack a wide range of timber types. Due to their cryptic nature of residing...
inside wood, direct assessment of the beetle life stage development is impossible.

In this study, we aimed to examine the development of *H. aequalis* exposed to physical disturbance and the relationship between frequency of physical disturbance and pupation rate and morphological deformity. We also provide a detailed biometric descriptor of deformed pupae.

**Materials and Methods**

Adult *H. aequalis* were reared in a walk-in environmental chamber at 26 ± 2°C and 65 ± 10% relative humidity located at the Research Institute for Sustainable Humanosphere, Kyoto University, Japan. The beetles were provided with an artificial diet block (8.0 cm length × 4.0 cm width × 2.0 cm depth) consisting of a mixture of 24% dried yeast (Asahi Food and Health Care, Tokyo, Japan), 50% starch (Nacalai Tesque, Kyoto, Japan), and 26% lauan wood sawdust (*Shorea* spp.) (Kartika and Yoshimura 2013). Three weight groups of larval *H. aequalis* (based on weight: 2 to <10 mg, 10 to <26 mg, and 26 to 36 mg) were used in this study. Twelve to seventeen larvae of each weight group were used. Each test larva was kept individually with an artificial diet block (2.5 cm length × 2.5 cm width × 0.5 cm depth) as harborage and food source. A hole measuring 2.5 mm diameter × 1.0 mm depth was drilled in the center of the artificial diet and the larva was placed in the hole. The block was stacked with another piece of diet block and tied with a rubber band. To induce physical disturbance, the larva was picked up using fine forceps and then returned to the diet block (referred to as handled hereafter). The block was checked for any exuviae left by the larva during molting. The handling was repeated on weekly basis. The experiment was terminated soon after the handled larvae died or emerged into adults. The dead pupae were preserved in 80% alcohol for further biometric analysis. Control larvae were left untouched until the adult emerged (referred as unhandled hereafter). We determined the number of moltings that occurred based on the exuviae found at the end of the experiment (until adult emergence).

A total of 21 pupae, representing all the pupae derived from handled larvae (across all weight groups), and five pupae from unhandled larvae were subjected to biometric analysis. The following parts of each specimen were observed using a BX51 stereomicroscope (Olympus, Tokyo, Japan) and measured using ImageJ Software: 1) length of head to tip of abdomen, 2) maximum width of abdomen, 3) length of head, 4) width of head, 5) length of abdomen, 6) length of elytra, and 7) width of elytra. Each specimen also was scored from 0 (absent) to 3 (severe) based on the degree of elytra deformities and the presence of black spots (Fig. 1).

![Fig. 1. Types of pupal teratology.](image-url)

(A–B) Ventral and dorsal views of a normal pupa, which was scored as 0. (C–D) Deformed pupa with only wrinkled elytra (we) (score 1) and a minimal spot on the dorsal abdomen, indicated with an arrow (score 1). (E–F) Deformed pupa with divergent elytra (di) and short left elytra (score 2). Minimal spot on the ventral abdomen (score 1). (G–H) Pupa with multiple deformities of the elytra. The left elytra was short and wrinkled, whereas the right elytra was diverted and wrinkled (score 3). Spots were present on the ventral abdomen and left elytra (score 2). (I–J) Pupa with multiple deformities, including wrinkled elytra and distorted left elytra (dt) that curled inside (score 3). High density of spots was present on the ventral and dorsal abdomen and elytra (score 3). Scale bar, 1 mm.
We used the head width, length of elytra, and degree of elytra deformity, which combined explained 91.6% of the total variance (see discriminant function analysis), in subsequent multivariate analyses. A morphological variation index (MVI) based on the differences of the head width and length of elytra between each handled and unhandled larva-derived pupa was generated (Warwick 1985). The values of MVI were summed for each individual (Supplementary Appendix S1).

The molting numbers between handled and unhandled individual was compared using Mann-Whitney U. The morphological variations between handled and unhandled larva-derived pupae were compared using Student’s t-test. We also performed discriminant function analysis to identify the morphological parameters that discriminate between deformed and non-deformed pupae (Neoh and Lee 2011).

Results and Discussion

In the control group (unhandled larvae), approximately 70.00 ± 3.33% of the larvae from each weight group emerged into adults after 12–15 wk. Every individual had two molts prior to pupations. For the handled larvae, the success rate of larval emergence to pupal stage increased with increasing larval weight group (46.7%, 66.7%, and 71.4%, respectively). However, no significant correlation between pupation rate and larval weight group was detected (Table 1). Almost all of these larvae were dead at the end of the experiment (with only an individual emerged to adult) regardless of initial larval weight group, suggested their high susceptibility to physical disturbance. The molting frequency of handled and unhandled larvae from weight group of 2 to <10 mg and 10 to <26 mg were significantly different (Table 2). Across larval weight groups, approximately 36.19 ± 3.81% of the larvae in the handled group experienced an irregular molting process consisting of at least two molting events before emerging as pupae (Fig. 3A–C). For example, in larval weight group of 2 to <10 mg, one beetle molted five times, whereas another only molted once before emerging as a pupa. However, the result should be viewed with caution owing to the small sample size in group weight of 26 to 36 mg and the frequency of handling on each individual is not consistent as we failed to standardize the age of the test larvae.

Most pupae from the handled groups also showed various morphological abnormalities. Of the 21 handled larva-derived pupae, five appeared to be normal in gross appearance. However, the head (3.10 ± 0.84 cm) was significantly broader compared to those in the unhandled group (2.33 ± 0.29 cm) ($t = 5.731; df = 4.681; P = 0.003$).

![Fig. 2. The morphological variation index decreased with increasing larval weight.](image)

Table 1. Results of generalized linear models testing whether the pupation rate, degree of elytra deformity, and morphological variation index differed among larval weight group

| Fixed effect                  | Response                        | Statistical values | df  | P    | $R^2$ |
|-------------------------------|---------------------------------|--------------------|-----|------|-------|
| Weight of larvae              | Pupation rate                   | $z = 1.414$        | 35  | 0.157| 0.08  |
|                               | Degree of elytra deformity      | $z = 1.208$        | 19  | 0.227| 0.03  |
|                               | Morphological variation index   | $t = -2.586$       | 19  | 0.018| 0.25* |

*Statistically significant difference among larval weight group at $\alpha = 0.05$.

Table 2. Molting frequency of handled and unhandled larvae across different weight group of larvae before pupation

| Group         | Molting frequency (median, range) | Test statistic               |
|---------------|----------------------------------|-----------------------------|
|               | Handled                          | Unhandled                   |
| 2 to <10 mg   | 2, 1–5                           | 1, 1–2                      | $U = 14.500; z = -3.063; P = 0.002^*$ |
| 10 to <26 mg  | 2, 1–4                           | 1, 1–2                      | $U = 49.500; z = -1.864; P = 0.062$ |
| 26 to 36 mg   | 1, 1–2                           | 1                           | $U = 7.500; z = -1.500; P = 0.134$ |

*Statistically significant difference between two groups at $\alpha = 0.05$. 

$H. aequalis$. We assumed that younger larvae were subjected to longer duration of physical disturbance throughout their development. We also used the weight of larvae as a surrogate for larval instar stage and included this parameter as a predictor. We used generalized linear models (GLMs) with a binomial error distribution (logit link), for which the response variable was larval pupation; a Gaussian error distribution, for which the response variable was MVI; and a Poisson error distribution (log link), for which the response variable was the score values for the degree of elytra deformities. All analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL) at $\alpha = 0.05$. 

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Fig. 3. Development process of handled larvae in the different weight groups: (A) 2 to < 10 mg, (B) 10 to < 26 mg, and (C) 26 to 36 mg. D, dead; M1–M5, one molt–five molts; P, pupa; A, adult; (d), deformed.

Fig. 4. Types of adult teratology. (A) Ventral view of normal adult. (B–C) Deformed adult with divergent (di) and wrinkled (we) elytra. Scale bar, 1 mm.
They also possessed longer elytra (left: 5.28 ± 0.45 cm; right: 5.24 ± 0.52 cm), but was not significantly different from the unhandled group (left: 4.63 ± 0.52 cm; right: 4.63 ± 0.86 cm). Only one pupa from larval weight group of 2 to <10 mg successfully turned into an adult at week 19, but it appeared to be morphologically abnormal (Figs. 3A and 4). The first discriminant variables (head width, length of elytra, and deformity of elytra) accounting for 91.6% of the total variance (eigenvalue: 14.714) separated handled larva-derived pupae from unhandled larva-derived pupae. The second discriminant variables (width of abdomen and width of elytra) accounting for 8.4% of the total variance (eigenvalue: 1.355) distinguished deformed pupae and non-deformed pupae from the handled group (Fig. 5).

The handled larva-derived pupae showed varying degree of elytra deformities and malformations (Fig. 1). Widths of the thorax and head capsule were significantly higher in handled pupae than in unhandled pupae (P < 0.05) (Table 3). In particular, larval groups subjected to physical disturbance during early stages of development were significantly more deformed compared to unhandled larvae (Fig. 2 and Table 1). Dark spots were present on all handled larva-derived pupae.

We cannot explain precisely the reason for the morphological anomalies observed in H. aequalis, but increased molting frequency can be stressful and unfavorable for their development. The molting process is regulated by the production of ecdysone (20E, active molting hormone 20-hydroxyecdysone) in the prothoracic gland and juvenile hormone (JH) in the corpora allata. Insect JH level remains high during larval–larval metamorphosis but decreases drastically at initiation of larval–pupal metamorphosis with excessive secretion of ecdysteroids in insects (Sparks and Hammock 1979, Sieber and Benz 1980, Newitt and Hammock 1986). The phenomena of morphological anomalies and developmental irregularity was evinced in several hormonal manipulation studies in insects. For instance, derivatives of JH inhibited metamorphosis of the cockroaches Blattella germanica (L.) and Dysdercus koenigi (F.) and resulted in various malformations in the adults (O’Farrell and Stock 1964, Saxena and Williams 1966). Application of JH esterase inhibitor to the last instar larvae resulted in delayed secretion of ecdysteroid, which in turn delayed pupal formation. Application of nonsteroidal ecdysteroid agonist to the last larval instar of various lepidopterans and the coleopteran: Leptinotarsa decemlineata (Say) had detrimental effects on pupation (Tateishi et al. 1993, Smaggle and Degheele 1994).

In our study, the level of JH and ecdysteroid imbalance in the handled larvae could be tightly linked to the interplay of metabolism of JH and ecdysteroids and the neurohormonal stress response. Physical disturbance by daily handling (i.e., stress) may have induced production of the biogenic amines octopamine (OA) and dopamine (DA). The response of Drosophila to unfavorable hot temperature was to increase the levels of 20E, DA, and OA and decrease JH degradation (Rauschenbach et al. 1993, Gruntenko and Rauschenbach 2008). To date, however, whether levels of these hormones change in the oriental wood borer due to physical disturbance is unknown.

The handled larvae had equal chance to develop into pupae regardless of the frequency of handling throughout the immature development period. These handled larva-derived pupae died before reaching the adult stage. One possible reason for the premature mortality is that the divergent elytra caused dehydration due to the extensively exposed abdomens. This phenomenon was documented in the yellow mealworm beetle Tenebrio molitor Linnaeus (Steinhaus and Zeikus 1968). In addition, mortality may have been increased by fungal attack, as indicated by the presence of dark spots on handled pupae. All in all, the biometrics developed in this study could be used to quantify sublethal deformities or pathology for existing pesticide trials or future nonchemical pest control studies for this important wood products beetle pest of Japan.

**Table 3. Measurements (mean ± SE) of handled and unhandled larva-derived pupae of H. aequalis**

| Morphological parameter (cm) | Handled larva-derived pupa (n = 20) | Unhandled larva-derived pupa (n = 5) | Test statistic |
|-----------------------------|------------------------------------|-------------------------------------|---------------|
| Length of tip of mouth to tip of abdominal | 8.19 ± 0.16 | 8.43 ± 0.50 | t = –0.60, df = 24, P = 0.554 |
| Width of thorax | 3.62 ± 0.09 | 2.97 ± 0.18 | t = 3.32, df = 24, P = 0.003* |
| Length of head | 1.93 ± 0.07 | 1.86 ± 0.12 | t = 0.42, df = 24, P = 0.676 |
| Width of head | 2.96 ± 0.06 | 2.33 ± 0.13 | t = 4.63, df = 24, P < 0.001* |
| Length of abdomen | 4.09 ± 0.16 | 4.62 ± 0.31 | t = 1.50, df = 24, P = 0.148 |
| Length of left elytra | 5.10 ± 0.12 | 4.63 ± 0.23 | t = 1.70, df = 24, P = 0.102 |
| Length of right elytra | 1.37 ± 0.04 | 1.44 ± 0.19 | t = 0.34, df = 24, P = 0.749 |
| Width of left elytra | 5.10 ± 0.11 | 4.63 ± 0.39 | t = 1.60, df = 24, P = 0.123 |
| Width of right elytra | 1.45 ± 0.06 | 1.25 ± 0.14 | t = 1.44, df = 24, P = 0.163 |

*Statistically significant difference between two groups at α = 0.05.
Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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