Larval growth and diapause in a tropical moth, Omphisa fuscidentalis hampson

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Larval Growth and Diapause in a Tropical Moth, *Omphisa fuscidentalis* Hampson

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ABSTRACT—The bamboo borer, *Omphisa fuscidentalis*, is a moth found in northern Thailand, Lao and Myanmar and its larvae feed on the inner pulp of bamboo shoots. In a tropical highland (about 500 m sea level) forest at 19°N near Chiang Mai, Thailand, the larvae feed on at least 5 bamboo species. Nucleotide sequence analysis of the region of mitochondrial cytochrome C oxidase subunit 1 gene amplified by the polymerase chain reaction (PCR) verified that larvae collected from different bamboos belong to the same species. Adults appeared in early August and laid clusters of eggs on the newly grown bamboo shoot. The newly hatched larvae bore a hole in the shoot, enter an internode of the shoot and feed on the inner pulp. After maturation in September, the larvae remain in an internodal cavity of bamboo for up to 9 months, from September to the following June. Number of larval instars was estimated by measuring the width of head capsules remained in internodes of bamboo shoots. The growth curve of the width fitted to Dyar's law and the mature larvae were estimated to be 5th instar. Mature larvae were collected in the field each month and their body weight, head capsule width, protein and fat contents and hemolymph ecdysteroid titer were measured. Body weight continuously decreased during the 9 months whereas head capsule width remained constant. Fat content fluctuated during this period while protein level remained at a similar level until March, after which it significantly increased. During this period, hemolymph ecdysteroid concentrations remained low. Current results show that the bamboo borer larvae enter diapause at the end of feeding period of the fifth (last) larval instar and the larval diapause lasts until June.

INTRODUCTION

The bamboo borer, *Omphisa fuscidentalis* Hampson (Pyralidae, Lepidoptera) is found in northern Thailand, Loa and Myanmar. The mature larvae are a favored food for mountain tribe people, and they have recently become popular in urban areas as well. They are sold in markets almost throughout the year, except for the months of July-September, indicating that there are no larvae in the field in those months.

The food plant of bamboo borer larvae is young bamboo shoot. In the Chiang Mai area, bamboo shoots initiate growth from late July through August. The adult lays an egg cluster on a bamboo shoot in early August. The newly hatched larvae bore a hole through the internodal wall so that all of the larvae from one egg cluster move into the internode and feed on the inner pulp. Within the plant, larvae bore a hole through the septum and move upward from internode to internode to obtain fresh inner pulp as food. When larvae become mature in middle-late September, they migrate down along the inner culm to the original internode with the entrance hole or the internode immediately above the original one in which they pupate in the middle of the following June. Adult eclosion takes place inside the internode and the newly eclosed moths escape from the entrance hole (Wiwatwittaya, 1992; Leksawasdi, 1994). The larval period thus lasts from mid-August until early the following June.

The latitude of Chiang Mai where larvae were collected is 18° 47'N, and the lowest temperature (monthly mean value) is above 20°C. *O. fuscidentalis* is thus a tropical insect. Diapause, a period of developmental arrest, is an adaptation to survive seasonally recurring adverse conditions and is common in the temperate zone as well as the tropics (Denlinger, 1986). Although it is difficult to distinguish between diapause and a simple quiescence in some tropical insects because the information is limited (Denlinger, 1986), there are several tropical insects in which a diapause period is a component of the life cycle. The stalk stemborers, *Chilo* (Scheltes, 1978) and the maize stemborer, *Busseola* (Usua, 1970), enter a larval diapause and tropical flesh flies enter a pupal diapause (Denlinger, 1979). An obligatory diapause for tropical insects has rarely been reported, but adults of the endomychid beetle, *Stenotarsus rotundus* enter diapause that lasts up to 10 months (Wolda and Denlinger, 1984). In the bamboo borer, preliminary observations suggested that the larvae were in diapause

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for 9 months but this observation has not been confirmed. Along with the unique habitat of larvae as in bamboo shoots, it was of interest to confirm the long larval diapause of this tropical insect.

Identification of experimental animals is prerequisite for physiological investigations of wild insects. Robinson *et al.* (1994) reported that there are more than 11 species in the genus *Omphisa*, two of which are distributed in South East Asia, but there are no report of distribution in Thailand nor description for their larval morphology. Preliminary observations showed that larvae in Chiang Mai Province feed on at least 5 different bamboo species, *Dendrocalamus membranaceus* Munro, *D. hamitonii* Nees & Arn, *Bambusa nutans* Wall. ex Munro, *B. blumeana* Schult and *Gigantechloa albociliata* Kurz. Moths collected in Chiang Mai were identified as *Omphisa fuscidentalis* but it was not known whether or not the larvae found from different bamboo species belong to the same species, *O. fuscidentalis* although we found no morphological differences among larvae from the different bamboo. Accordingly, it was prerequisite to verify whether or not larvae from 5 bamboo species belong to the same species before starting the present study. We addressed this issue by comparing the nucleotide sequences of the cytochrome C oxidase subunit 1 (COI) region of mitochondrial DNA in larvae collected from the five different bamboo species.

It is important to establish the life history of insects when developmental events such as diapause are studied. The bamboo borer possesses a long larval period in its life cycle as briefly described above but their precise life cycle, especially the larval growth was not known because of their unique habitat. To estimate the stage at which the larvae enter diapause, we estimated the number of larval instars using the head capsules remained in bamboo internodes. In order to confirm the larval diapause, we collected larvae from the field monthly and measured changes in body weight, nutrient contents and hemolymph ecdysteroid levels. The present paper describes the results of these measurements and discusses the implications for larval diapause in this tropical species.

MATERIALS AND METHODS

Animals

The moth of the bamboo borer we used was identified as *O. fuscidentalis* by two taxonomists, Dr. M. Shaffer of Natural History Museum, London and Dr. H. Banzinger of Chiang Mai University. Animals were obtained from bamboo shoots in a forest in Amphur Maewang, Chiang Mai Province, Thailand. Bamboo borer larvae were collected on the 15 or 16th day of each month from September, 1995 to May, 1996 and pupae were collected in June and July, 1996 and larvae used for DNA analysis were collected in January, 1998. Bamboo shoots for collecting head capsules were obtained in September and October, 1998.

Measurement of head capsule width

Bamboo shoots were cut in half and the residue inside the internodes were raked out into a container. The residue was suspended in water and the precipitated materials were rinsed again with fresh water and the head capsules were collected from the sediments with forceps under dissection microscope. After dried in air, the capsule width was measured under microscope with micrometer or using vernier calipers. The head capsule width of mature larvae was directly measured with vernier calipers. The sex of matured larvae was determined according to the presence of ovary or testis after measuring the head capsule width.

Measurement of gut contents

Larvae were anaestetized with ether and cut along the dorsal midline. Hemolymph was removed with rinsing the body well with Ringer's solution, foregut and hind gut were ligated with cotton thread, and the portions anterior to the ligature in foregut and posterior in hindgut were cut to remove the midgut. After the isolated gut was washed well and excess water was removed with paper, it was weighed. Then the gut was cut and washed well with Ringer's solution in order to removed the contents, and then the gut was weighed after removing excess water with paper. The weight difference was regarded as the weight of gut contents.

Quantification of the nutrient contents

Total protein was measured by the Kjeldahl method. Three larvae were boiled in 15 ml conc. H_2SO_4 with about 2 g selenium for 1 hr. The mixture was cooled to room temperature, diluted with 20 ml distilled water, neutralized with 50 ml 30% NaOH and then distilled using a distillation unit (Model 315, Buchi). The distilled solution was added to 50 ml 4% boric acid with a few drops of thashiro (mix-indicator) and the mixture was titrated with 0.1-N HCl. The amount of total nitrogen was calculated from the titration data according to Robert (1984). For measurement of total fat, larvae were placed in a fat extraction thimble, which was connected to a Soxhlet fat extractor using chloroform as the extraction solvent. The fat extractor was run at 15 rounds/hr for 6–8 hr. After incubation at 100°C for 2 hr to remove the solvent, the extracted fat was weighed.

Measurement of the hemolymph ecdysteroid concentration

Hemolymph was collected from each larva through an incision in the prolegs. The hemolymph (30 μl) was added to 270 μl methanol and centrifuged at 10,000×g for 5 min. The supernatant was transferred to a small test tube and dried *in vacuo* at room temperature. The residue was dissolved in water, and an aliquot of the aqueous solution was subjected to ecdysteroid radioimmunoassay (RIA) (Sakurai *et al.*, 1998). The cross-reactivity of the antibody to ecdysone and 20-hydroxyecdysone (20E) is 1:5 (Yokoyama *et al.*, 1996).

DNA extraction and sequencing of COI genes

Larvae were collected from 5 different bamboo species. The thoracic epidermis from 2 larvae from each bamboo species was separately dissected and cleared of all fat body. Each tissue was homogenized in 400 μl homogenizing buffer (10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 10 mM EDTA-NaOH (pH 8.0), 0.1% SDS) and DNA was extracted with phenol /chloroform (1:1 v/v) followed ethanol precipitation. A region of mitochondrial cytochrome *c* oxidase subunit 1 gene (COI) was amplified by polymerase chain reaction (PCR) with two primers, 5'-GA(G/T)C(A/T)CCW(A/T)GA(C/T)ATAGC(A/T)TT(C/T)CC-3' and 5'-C(A/C/T)GGTAAAATTAAAATATAAACTTC-3', designated according to Simon *et al.* (1994). The 20 μl reaction mixture consisted of 120 mM Tris-HCl (pH 8.0), 10 mM KCl, 2 mM $MgCl_2$, 6 mM $(NH_4)_2SO_4$, 0.001% BSA, 0.1% Triton X-100, 0.2 mM each of dNTPs, 0.2 μg DNA extracts, 0.2 μM of each of the above primers and 0.5 units of DNA polymerase (KOD Dash, Toyobo, Tokyo). The thermal profile was 94°C for 15 sec, 50°C for 2 sec and 74°C for 30 sec for 30 cycles controlled by DNA Engine (MJ Research, Watertown, MA). The PCR products were bluntended with KOD (Toyobo, Tokyo), separated on 2% agarose gel, and slices of agarose containing its bands of interest were excised, purified using Geneclean II (BIO 101, Vista, CA) and cloned into a pUC19 vector. The plasmid DNA from *E. coli* culture was purified using FlexiPrep (Amersham Pharmacia Biotech, Uppsala). DNA products were sequenced using DNA

sequencer (Hitachi 5500M, Hitachi), and sequences were analyzed with DNASIS (Hitachi Software Engineering, Tokyo).

Phylogenetic analysis

Sequence region corresponding to the amplification primer at both the 5' and 3' ends of the gene were removed prior to construction of phylogenetic tree. Protein-coding sequences were translated to amino acids using DNASIS for confirmation of alignment. The COI regions of other lepidopterans that corresponds to the amplified COI sequence of *O. fuscidentalis* were obtained from GEN Bank database. The COI sequences were analyzed using neighbor-joining (NJ) method. Stability of NJ tree was assessed via bootstrapping over 1000 replicates. Tree Vies PPC (Aladdin Systems, Watsonville, CA) was used to construct NJ tree.

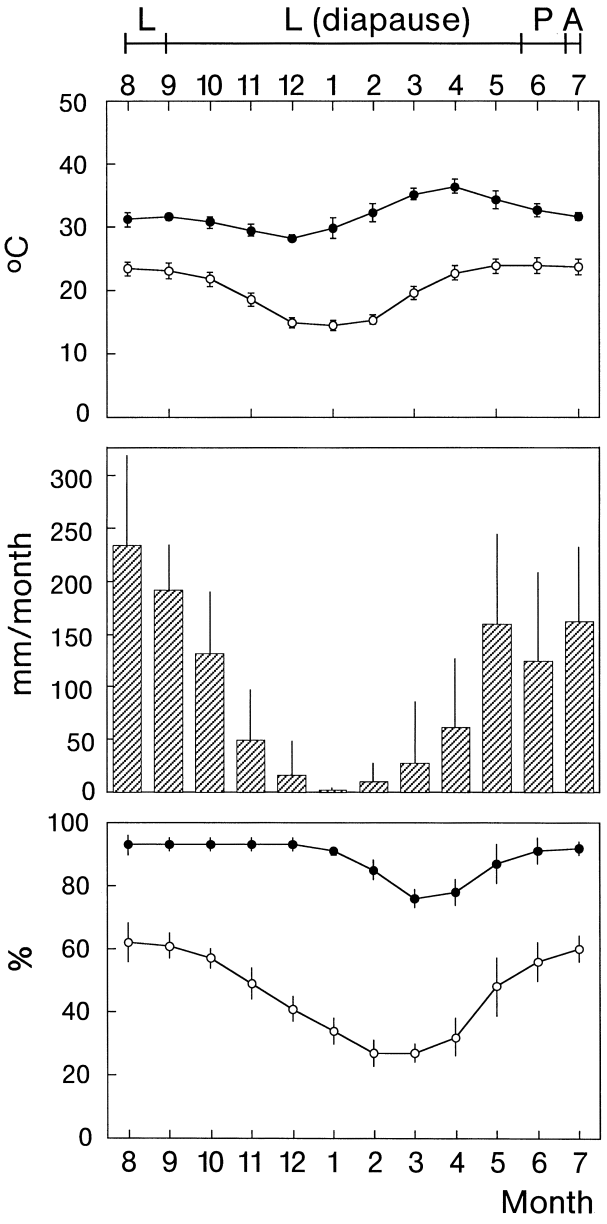

Fig. 1. Meteorological data from Chiang Mai, Thailand at 19°N. Top, highest and lowest temperature; middle, precipitation; bottom, highest and lowest relative humidity. Each datum point is a mean with SD of the statistical data for 9 years from 1988 to 1996, provided by Meteorological Observatory, Chiang Mai, Thailand.

Statistical analysis

Data were statistically analyzed with 2-way ANOVA. Analysis of head capsule width was performed using JMP (ver. 3.02; SAS Institute, Inc.).

Annual changes in climate in Chiang Mai area

The meteorological records of 9 years from 1988 to 1996 were provided by Meteorological Observatory, Chiang Mai, Thailand. Monthly precipitation of less than 100 mm is considered to be the threshold of drought in the tropical region (Whitmore, 1984). According to these criteria, the monthly changes in precipitation in Chiang Mai (Fig. 1) clearly show a wet-dry cycle: a dry season from November through April and a rainy season from June through October. The dry season is divided into two seasons according to temperature, a cool season (winter) from November to February and a hot season (summer) from March to June. Accordingly, there are three seasons, wet season followed by dry winter and dry summer.

RESULTS

Identification of *Omphisa fuscidentalis*

The nucleotide sequence of the COI region amplified by PCR (Fig. 2A) showed that there was only one nucleotide difference, A or G of 438 nucleotides determined: at position 312 where the nucleotide was A in larvae from the bamboos, *D. Hamitonii*, *B. blumeana* and *G. albociliata*, or G in those from *D. membranaceus* and *B. nutans*. This single replacement of nucleotide did not affect on the amino acid residue. The deduced amino acid sequences of the amplified COI region were identical among larvae collected from 5 different bamboo species. Accordingly, we concluded that the larvae found on different bamboo species belong to the same species.

To confirm that the amplified DNA is a region of COI, we compared the gene sequence and the deduced amino acid sequence with those of *Manduca* COI (Frohlich *et al.*, 1996). As seen in Figure 2B, The homology of the amplified region between *O. fuscidentalis* and *M. sexta* was 83% for the nucleotide sequence and 93% for the deduced amino acid sequence.

The phylogenetic tree (Fig. 3) was constructed with the present data and the sequences of the same region in other lepidopteran species. The NJ tree indicated that *O. fuscidentalis* fell in the same cluster as *Spodoptera*, *Manduca* and *Antheraea* since bootstrap value was 76%.

Estimation of number of larval instar

Fig. 4 shows frequency distribution of width of the head capsules which were collected from 17 bamboo shoots. The first and second peaks were clearly observed but the peaks were not clear in three clusters ranged between 1–1.5, 1.5–2.4 and 2.4–3.3 mm. In addition, we measured the width at different intervals according to the width: the width was measured at every 0.0263 mm if it was less than 2.6 mm and at every 0.05 mm for the width not less than 2.6 mm (Fig. 4). Thus, we standardized the distribution in common logarithm, converted each peak to a normal distribution and calculated the mean value for each peak (Table 1). Then the values of

A

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CGA ATA AAT AAT ATA AGA TTT TGA TTA TTA CCC CCA TCT CTA ACT CTT TTA ATT TCA 57
 R   M   N   N   M   S   F   W   L   L   T   P   S   L   T   L   L   I   S
AGA AGA ATT GTT GAA AAT GGA GTA GGA ACT GGA TGA ACT GTC TAC CCC CCC CTT TCA 114
 S   S   I   V   E   N   G   V   G   T   G   W   T   V   Y   P   P   L   S
TCC AAT ATT GCT CAC AGA GGA AGT TCT GTT GAT TTA GCA ATT TTT TCC TTA CAT TTA 171
 S   N   I   A   H   S   G   S   S   V   D   L   A   I   F   S   L   H   L
GCT GGA ATT TCT TCT ATT TTA GGA GCA ATT AAT TTT ATT ACA ACT ATT ATT AAC ATA 228
 A   G   I   S   S   I   L   G   A   I   N   F   I   T   T   I   I   N   M
CGT ATT AAT GGT CTA CTA TTT GAT CAA ATA CCA TTA TTC GTC TGA TCA GTA GGA ATT 285
 R   I   N   G   L   L   F   D   Q   M   P   L   F   V   W   S   V   G   I
                                   *
ACA GCT CTA TTA TTA CTT TTT TCT TTA CCT GTT CTA GCG GGA GCC ATT ACT ATA CTC 342
 T   A   L   L   L   L   F   S   L   P   V   L   A   G   A   I   T   M   L
TTA ACT GAT CGA AAC TTA AAT ACA TCC TTT TTT GAA ACT GCG GGA GGA GGA GAT CCA 399
 L   T   D   R   N   L   N   T   S   F   F   E   T   A   G   G   G   D   P
ATC CTT TAT CAA CAT TTA TTT TGA TTT TTT GGA CAT CCA 438
 I   L   Y   Q   H   L   F   W   F   F   G   H   P
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B

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                                  *                   *
Manduca  97 RMNNMSFWLL PPSLMLLISS SIVENGAGTG WTVYPPLSSN 136
Omphisa   1 RMNNMSFWLL PPSLTLLISS SIVENGVGTG WTVYPPLSSN 40

                                                       *
          137 IAHSGSSVDL AIFSLHLAGI SSILGAINFI TTIINMRINN 176
           41 IAHSGSSVDL AIFSLHLAGI SSILGAINFI TTIINMRING 80

              **           *     *     *
          177 MSFDQMPLFV WAVGITAFLL LLSLPVLAGA ITMLLTDRNL 216
           81 LLFDQMPLFV WSVGITALLL LFSLPVLAGA ITMLLTDRNL 120

                    **
          217 NTSFFDPAGG GDPILYQHLF WFFGHP 242
          121 NTSFFGTAGG GDPILYQHLF WFFGHP 146
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Fig. 2. DNA sequence and the deduced amino acid sequence of the mitochondrial cytochrome C oxidase subunit 1 (COI) region from the bamboo borer larvae collected from 5 different bamboo species (**A**) and a comparison of the amino acid sequence of the amplified region of COI with that of *Manduca sexta* (B) (Frohlich *et al.*, 1996). The nucleotide at position 312 as indicated with an asterisk in (**A**) was A in larvae from the bamboos, *D. Hamitonii*, *B. blumeana* and *G. albociliata*, or G in those from *D. membranaceus* and *B. nutans*. Asterisks in (B) indicate the different amino acids between *Omphisa* and *Manduca*.

head capsule width in common logarithm was plotted against the putative number of instars (Fig. 5). The curve gave a good correlation coefficient (r^2=0.998) and well fitted to Dyar's law and therefore we concluded that number of *Omphisa* larval instars is five. The ratio was calculated to be 1.51 from the curve gradient of 0.180 in Fig. 5.

In Fig. 4, the peaks for third, fourth and fifth instars appeared to be broad. Such broad peak indicated a sexual dimorphism in the larval size and thus we tried to plot the values of first and second peaks for the third through fifth instars after converting the values in common logarithm. Since a single peak was observed for each of first and second instars, these peak values were directly used for depicting two lines in Fig. 6. Each peak values were on a straight line and each line gave a good correlation coefficient (r^2) of 0.997 for upper line (larger width) and 0.999 for lower line (narrower width).

In order to confirm the sexual dimorphism, we determined the sex of 100 diapause larvae by dissecting and confirming the existence of ovary or testis and measured the head capsule width of the same larvae. Fig. 7 shows the frequency distribution of head capsule width of male and female larvae. The head capsule width in females was significantly larger than in males (p=0.0000), showing the sexual dimorphism in the head capsule width in the mature larvae. Mann-Whitney U-test showed that the population of 100 larvae is not significantly different from that of 5th instars used for depicting Fig. 4 (p=0.84). The mean values for male ($\log_{10}$ 0.421) and female ($\log_{10}$ 0.467) were therefore replaced with those for the 5th instar in Fig. 6. The newly depicted curves gave a correlation coefficient (r^2) of 0.996 and 0.999 for male and female, respectively. This clearly showed that the larger peaks as indicated with open triangles in Fig. 4 were for females' capsules while smaller ones (filled triangles for third, fourth and fifth instars) are males' capsules.

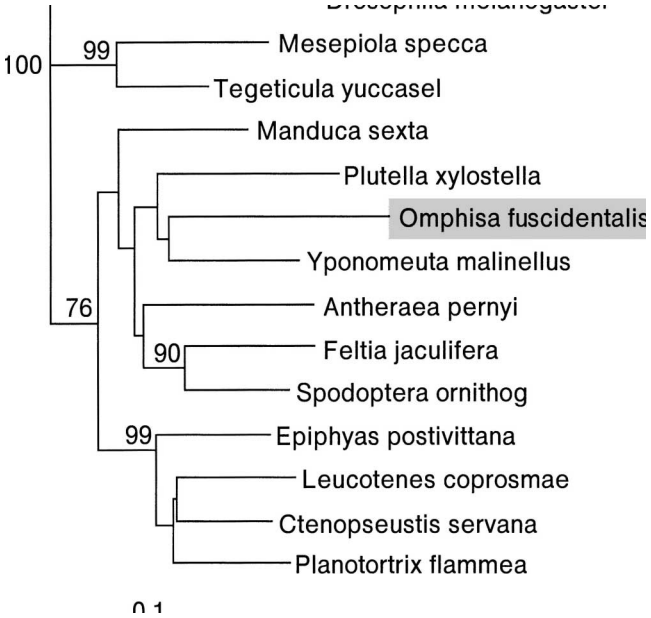

Fig. 3. Neghbor-joining tree calculated from an alignment of COI region sequences of 13 selected lepidopteran species. *Drosophila melanogaster* was used as an outgroup. Numbers at a node indicate percentage bootstrap values higher than 75%.

Body weight and head capsule width

Fig. 8 shows the changes in the wet body weight of larvae and head capsule width after maturation. Mean body weight was 0.61 g soon after larval maturation in October and then decreased continuously until February ($y=-0.045x+0.703$, $r=0.988$, Oct vs Dec, $p<0.0001$; Dec vs Feb, $p<0.002$). Body weight increased in March ($p<0.01$) and then again sharply decreased in May ($p<0.0001$). From September to May, larvae lost approximately 47% of their wet weight. Head capsule width did not change significantly during 9 months ($p>0.1$). There was considerable variation both in body weight and head capsule width among individuals in every month (Fig. 9). Head capsule width varied from 2.5 to 3.0 mm. However, no correlation was found between the head capsule width and body weight ($r=0.179$). This may indicate that the variation in head capsule width occurred within the same larval instar.

Gut observation

Gut contents were observed for larvae in diapause. In larvae collected in February, there was no solid material in their midgut. Wet weight of gut content was 21±1 mg (n=3) which was 3.5±0.3 % of larval body weight (595±23 mg). In the gut of the bamboo borer larvae, there was no solid materials except in anus where a mass of fibrous materials was found to shape a plug in anus. To estimate the loss of gut contents during the diapause, larvae collected in January was kept at 25°C under high humidity for one month and the gut contents was measured. In those larvae, gut contents was 9.3±3.7 mg (n=3) which was 1.9±0.8 % of larval body weight (504±34 mg).

For comparison, we measured the gut contents of the silkworm, *Bombyx mori* at the time after cessation of feeding but before purging gut contents. The aqueous contents was 14.2±2.4% of fresh body weight (3.71±0.12 g; n=6). At this time, no solid piece of artificial diet was found in fore- and mid-gut. Only a piece of frosty feces was observed at the anus.

Changes in protein and fat contents during larval diapause

Larval body weight in May was about half the initial weight. It was therefore interest to determine the changes in fat and protein content throughout the diapause period. As shown in

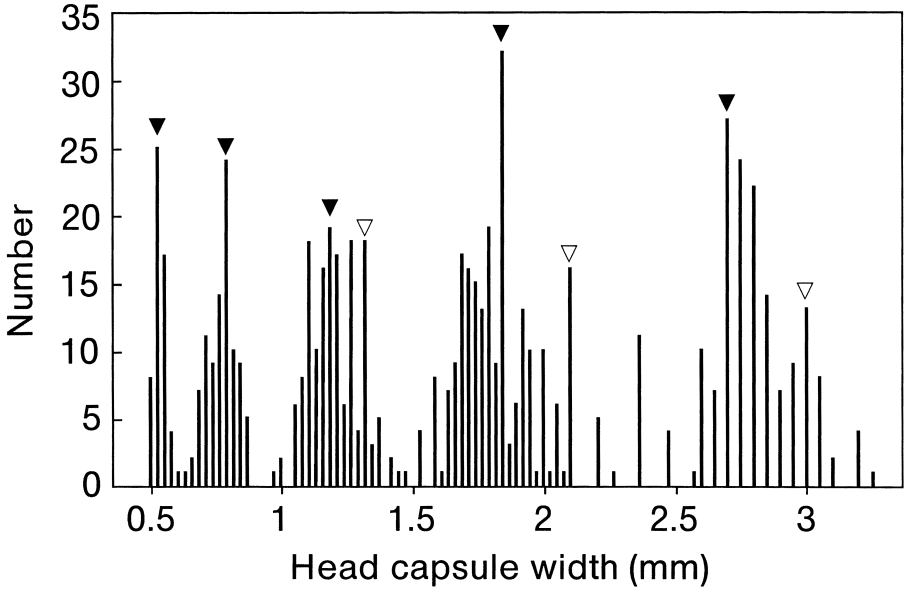

Fig. 4. Frequency distribution of the head capsule width in *O. fuscidentalis* larvae. Head capsule were collected from 17 bamboo shoots in September and October, 1998. Solid and open triangles indicate the peak values for dipicting Fig. 6. See text for details.

Table 1. Various moments for each peak of head capsule width.

Moment	Instar 1	2	3	4	5
Number	55	91	154	223	164
Mean value (mm)	0.533	0.768	1.199	1.820	2.774
Standard deviation	0.026	0.056	0.098	0.161	0.184
Upper 95% mean value	0.541	0.779	1.214	1.841	2.803
Lower 95% mean value	0.529	0.756	1.214	1.184	2.745

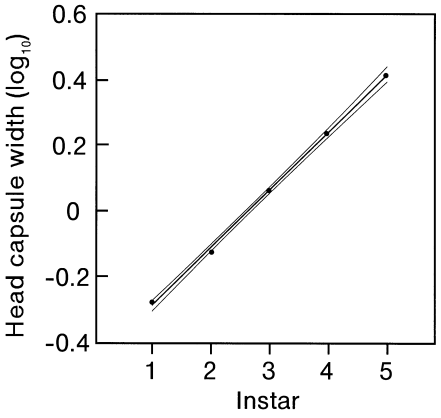

Fig. 5. Correlation of the head capsule width and number of larval instars in *O. fuscidentalis*. Head capsule width is expressed in common logarithm. y=0.180x–0.462; r^2 = 0.999. Thin lines indicate the upper and lower 95% mean value, respectively.

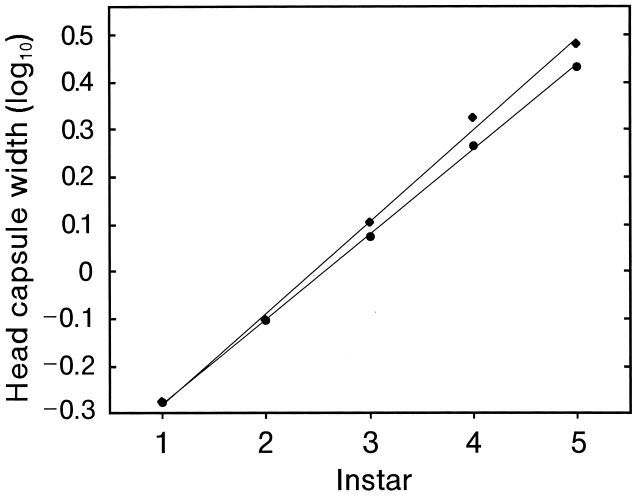

Fig. 6. Indication of sexual dimorphism in the larval growth. Values of head capsule width as indicated with filled and open triangles in Fig. 4 was converted to common logarithm and plotted against the number of instars. The curves for filled and open triangles in Fig. 4 are indicated with diamonds and circles, respectively. The correlation coefficient (r^2) for diamonds and circles are 0.997 (y=0.194x–0.459) and 0.999 (y=0.179x–0.478), respectively.

Fig. 10, the proportional fat content fluctuated largely month by month: the highest level was found in October (0.201) and the lowest in March (0.048). By contrast, the protein level did not change significantly until January (p>0.1, ANOVA), after

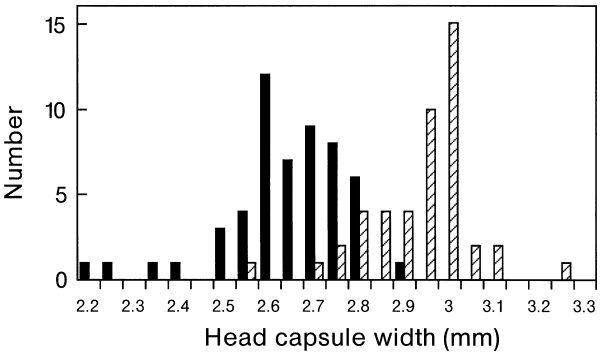

Fig. 7. Sexual dimorphism in the head capsule width. Head capsule width was measured and then the larval sex was determined by confirming the presence of testis or ovary. The mean values for females (filled column, n=46) and males (hatched column, n=54) were 2.64±0.13 and 2.94±0.12 mm, respectively. p=0.0000 (Student's *t*-test).

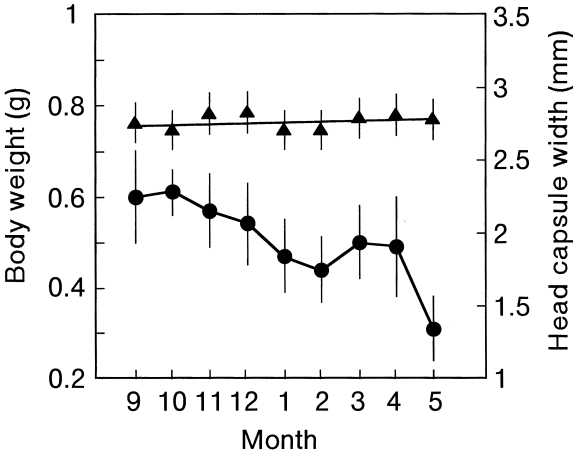

Fig. 8. Changes in wet body weight (circles) and head capsule width (triangles) of *O. fuscidentalis* larvae from September to May. Each datum point is a mean of 20 mature larvae with SD. The correlation coefficient (r) for head capsule width is 0.186 (y=0.009x+2.71).

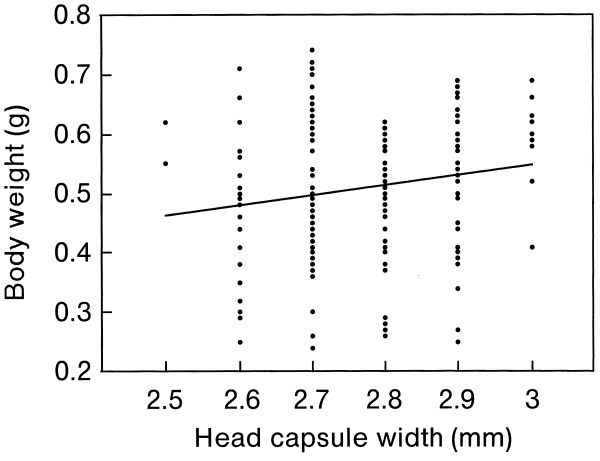

Fig. 9. Correlation between the head capsule width and wet body weight of mature larvae. The correlation coefficient (r) is 0.179 (y=0.17x+0.041).

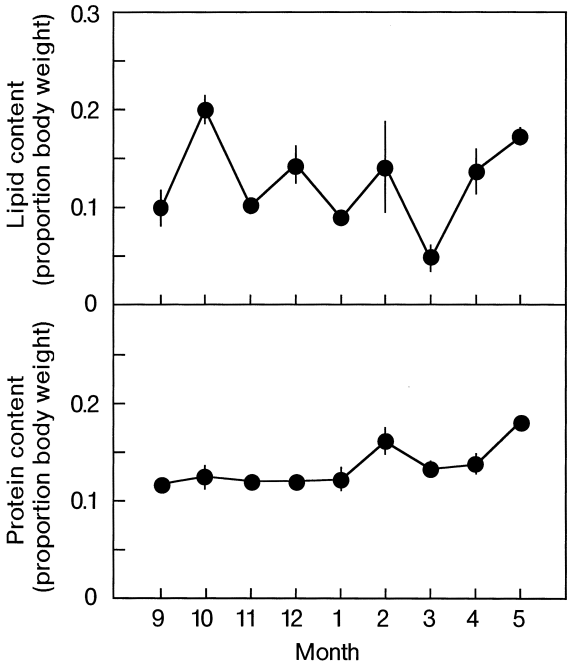

Fig. 10. Monthly changes of the proportional protein and fat contents of mature larvae of *O. fuscidentalis* from September to May. The ordinate indicates fat or protein content proportional to g wet body weight. Each datum point is a mean with SD of 3 different determinations.

which it appeared to increase in February, decrease in March and then increase again in May.

Changes in the hemolymph ecdysteroid levels

Hemolymph ecdysteroid titer was determined for larvae collected in September through May and for pupae in June and July (Fig. 11). The titer was low during the larval period but fluctuates in a range from 3 to 22 ng/ml. For the first 3 months, the titer was less than 3 ng/ml and increased to a peak value of 22 ng/ml in December after which it declined until March. A small peak was observed in April and then decreased again in May. The hemolymph ecdysteroid titer thus showed significant fluctuations but did not increase to more than 22 ng/ml. Accordingly, the titer was maintained at low levels up to May. During the pupal period, the titer increased to approximately 1,700 ng/ml in June and decreased to 770 ng/ml in July, shortly before adult eclosion.

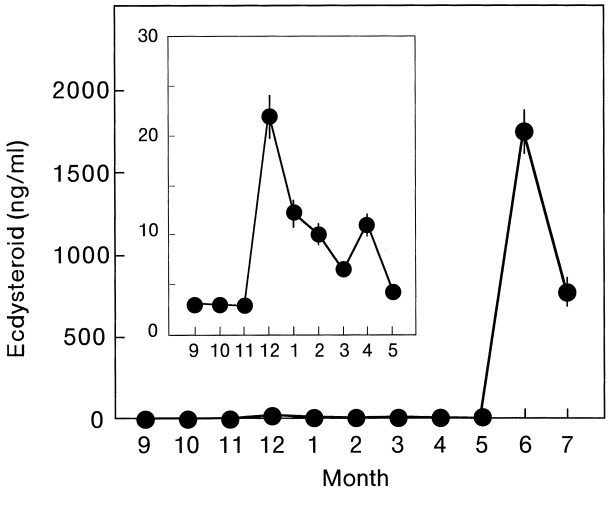

Fig. 11. Changes in hemolymph ecdysteroid concentration of *O. fuscidentalis* from September to May and pupal stage in June and July. Insert is an enlargement of the data from September to May. Each datum point is a mean with SD of 20 different determinations. Datum point with no SD bar indicates that SD was smaller than the mark size.

DISCUSSION

Identification of the bamboo borer larvae

For the present study, we monthly collected bamboo borer larvae from natural habitat. The moth of the bamboo borer we used was identified as *O. fuscidentalis* by two taxonomists, Dr. M. Shaffer of Natural History Museum, London and Dr. H. Banzinger of Chiang Mai University. In Chiang Mai area, we found the larvae from more than 5 different bamboo species, *D. membranaceus*, *D. hamitoni*, *B. nutan*, *B. blumeana* and *G. albociliata*. Robinson *et al.,* (1994) described the distribution of two species of genus *Omphisa* distribute in South-East Asia, and therefore it was possible that the bamboo borer larvae from different bamboos in the same area belong to different species. The nucleotide sequence and the deduced amino acid sequences, however, strongly suggested that all the larvae collected from 5 different bamboos belong to the same species. The NJ tree constructed from the nucleotide sequences of COI region amplified by PCR indicated that *Omphisa* share a cluster with *M. sexta* and *Spodoptera ornithog*. This is, however, only a matter of indication because the bootstrapping values were less that 75% inside the cluster.

Larval growth and diapause

Dyar's law shows that the head capsule of caterpillars grows in geometrical progression, increasing in width at each molt by a ratio which is constant for a given species (Wigglesworth, 1972). When this law is applicable, it is possible to deduce the actual number of ecdysis from the cast head capsules. The growth curve for the head capsule width of the bamboo borer showed a very good correlation coefficient with the ratio of growth of 1.51, showing that growth of the bamboo borer fits Dyar's law . The ratio of 1.51 is similar to that for other lepidopteran larvae, *Samia cynthia ricini* (r=1.52) and *Crambus mutabilis* (r=1.44). Accordingly, the number of larval instars was concluded to be five and the matured larvae found from the bamboo shoot in September and thereafter must be the 5th instar.

Head capsule width of insects increases at every ecdysis but not during an intermolt period (Nijhout, 1975; Chippendale and Yin, 1976, Suzuki and Nishimura, 1997). In *O. fuscidentalis*, head capsule width of larvae did not change throughout the 9 month period from September to the follow-

ing May, showing that the bamboo borer larvae did not undergo an additional larval ecdysis until pupation in the field, and therefore *O. fuscidentalis* possesses a very long period of larval diapause. During the diapause, larval body weight decreased. When larvae were disturbed, they actively moved. There was no solid material in fore- and mid-gut while a mass of fibrous material was placed like a plug at anus. Such plug-like mass is usually observed at anus before gut purge. Prior to gut purge, such mass which is usually frosty is excreted as a last piece of feces. In addition, their body length shortened prior to pupation in June (unpublished observations), which commonly occurs at the onset of the pharate pupal period in lepidopteran larvae. Accordingly, all the circumstantial evidences suggest that the larvae entered diapause at the end of the phagoperiod in the last larval instar. The long larval diapause was also confirmed by very low hemolymph ecdysteroid concentrations throughout the diapause period.

Loss of body weight is common in diapause larvae. Pharate pupal of the Mediterranean tiger moth, *Cymbalophora pudica* spend summer in a summer diapause (aestivation) and lose up to 50% of their body weight for 3 months. The dry weight of *C. pudica* larvae in aestivation remained stable (Kostal *et al*., 1998), an indication that the loss may mainly due to desiccation. Larval body weight in *O. fuscidentalis* decreased to about half of the initial weight over 9 months. The small decrease compared with *C. pudica* may be due to the special habitat of the larvae, i.e. inside the bamboo internode where the relative humidity is more than 90% (unpublished observation) and desiccation is less likely. Nevertheless, desiccation may be partly involved in the loss of body weight. Wet weight of gut contents of February larvae was 3.5% of wet body weight. Though we did not weigh the gut contents for September larvae, the weight may be more than 10% of wet body weight because it was about 14% in *Bombyx* mature larvae immediately before gut purge. If this can be the case in *Omphisa*, water of gut contents must be lost during the diapause. In addition, the loss of gut contents was also observed when larvae were kept in an incubator though the container of larvae was kept under high humidity. Accordingly, aqueous gut content could partly cover such desiccation during the long larval period in diapause in *Omphisa*.

Protein content proportional to wet body weight remained at the initial level for the first 7 months except in February. This means that the total amount of protein of individual larvae decreased during this period because wet body weight decreased rapidly in the diapause period. The proportional fat content greatly fluctuated month by month. Although the monthly fluctuation was statistically significant, it is not obvious whether such fluctuations actually occur in a single larva or possesses physiological meanings. The proportional fat content appeared to increase at the end of diapause, similar to the protein content.

An increase in both of proportional protein and fat contents was observed in May, concurrent with a decrease in body weight. May was one month prior to pupation. It is a common feature that the diapause is actually terminated long before developmental events become overt, such as pupation after larval diapause (Yin and Chippendale, 1973), adult development after pupal diapause (Bowers and Williams, 1964) and egg maturation after adult diapause (Tanaka *et al*., 1988). Accordingly, the profile of protein and fat levels in April and May may indicate that termination of larval diapause might be initiated in or before April.

Larval diapause in tropical insects

The increase in plant growth stimulated by the rains provides a wealth of new food resources for many phytophagous insects and the availability of food may be influenced profoundly by seasonal rhythms (Denlinger, 1986). The long diapause is, therefore, important in maintaining synchrony between the insect life cycle and the phenology of its host plants in the tropics: diapause is adaptive as a seasonal trait as the depletion of food supply (Tauber *et al*., 1986). Such a situation may be produced in the Chiang Mai area with dry-wet season cycle by the regular occurrence of droughts (see Fig. 1) that deplete the food supply in young bamboo shoots. The bamboo borer larvae feed on the soft inner pulp of the new bamboo shoots. The bamboo produces new shoots in the wet season and the shoots become hard by the end of the wet season. This indicates that larval diapause in the bamboo borer has evolved in response to the depletion of the food supply and thereby larvae survive seasonally recurring adverse conditions.

Diapause in tropical species is often variable and may occur only in a relatively small portion of the population (Tauber *et al*., 1986). The diapause in tropical insects is mostly facultative diapause in which diapause or non-diapause depends on environmental cues as reported for *Diatraea grandiosella* (Kikukawa and Chippendale, 1983), the flesh flies (Denlinger, 1979), *Chilo* species (Scheltes, 1978) and *Busseola fusca* (Usua, 1973). In *Omphisa*, however, the long larval diapause appears not to be facultative. *Omphisa* larvae are found in local markets in Northern Thailand through a year except 4 months from June through September. When the larvae collected in October to December were kept in an incubator for months at constant temperature under dark and high humidity, they remained as larvae. Accordingly adult may appear once a year and the larval diapause may be obligatory. It remains, however, to be determined whether the larval diapause is obligatory and what environmental and genetic factors influence diapause of bamboo borer.

Pupation of individual larvae of a single colony appears to occur synchronously since adult development in pupae in one internode was well-synchronized (unpublished observation). This indicates that the break of diapause must be environmentally regulated. The environmental factor that changes largely through a year is monthly precipitation and thus possibly humidity. We kept the larvae collected from the field in laboratory conditions with a high humidity at around 25°C, but larvae did not pupate for months if collected in October-December. Under the same conditions, the May and June

larvae occasionally pupated within a month, but synchronized pupation was not observed (unpublished observation). The habitat of the larvae is inside the internode of a bamboo culm where the humidity is rather constant. This indicated that the cue might not be humidity. Photoperiod is a common environmental cue to break diapause. The bamboo culm wall is more than 1 cm thick and it may be impossible that light would pass through the wall because the light permeability of the wall was about 1×10^{-19}/cm (unpublished observation). The remaining possible cue is temperature but changes in temperature is not a reliable cue for predicting seasonally recurring favorable conditions. The factor which is tightly involved in the break of larval diapause in *Omphisa* remains is obscure.

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