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Digestive enzyme secretion during metamorphosis in *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Abstract. Protease, amylase, lipase and trehalase are present in the larval and adult midguts of *Oryctes rhinoceros*; cellulase is absent. Invertase is present only in the adult. The presence of trehalase in the pupal midgut suggests that food digestion is not the normal function of gut trehalase. Quantitative studies reveal that protease and amylase in the third instar larvae reach very low levels as the larvae become older and consume little or no food. These two enzymes are not measurable in the non-feeding prepupa and pupa and reappear in the adult with commencement of feeding. Secretion of digestive enzymes is correlated with feeding.

Keywords. Coconut rhinoceros beetle; *Oryctes rhinoceros*; metamorphosis; digestive enzymes.

1. Introduction

Factors regulating the secretion of digestive enzymes in insects have recently been reviewed by Chapman (1985). In most of the insects studied, changes in digestive enzyme secretion occurred in relation to food intake (Dadd 1956; Baker 1976; Muraleedharan and Prabhu 1978). However, in a few instances digestive enzymes were noticed even in the absence of feeding (Dadd 1956; Langley 1967). Secretion of digestive enzymes also changed during metamorphosis reflecting their changing feeding habits (Spiro-kern 1974; Eguchi and Iwamoto 1975). The larva and the adult of *Oryctes rhinoceros* exhibit different feeding habits. While the larval food consists of cattle dung and decaying coconut stumps, the adult feeds on juice obtained from the tender parts of the coconut palm. Being a holometabolous insect, the animal possesses a non-feeding pupal stage in its life cycle. The animal also attains considerable size during the larval period, the third (final) instar gaining a weight of about 10 g and hence is a suitable insect for study. It was, therefore, thought worthwhile to investigate the changes in digestive enzyme secretion during metamorphosis of this animal.

2. Materials and methods

The larvae, prepupae and pupae of *O. rhinoceros* used for the study were taken from the stock colony reared on sterilised cowdung as described by Mini and Prabhu (1986). Adults were maintained on slices of ripe banana (Kannan and Prabhu 1985).

2.1 *Preparation of the enzyme extract*

The method of Applebaum *et al* (1964) was followed for the preparation of the

midgut extract. The concentration of the homogenate was adjusted to 1 midgut/5 ml distilled water.

2.2 *Qualitative study of digestive enzymes*

The presence or absence of amylase, invertase, trehalase and cellulase was studied by the method of Noelting and Bernfeld (1948) with some modifications. The reaction mixture contained 0·2 ml Tris-HCl buffer (pH 8·2), 0·2 ml enzyme extract and 0·4 ml 1% substrate solution (table 1) and it was incubated for 2 h. Lipase activity was studied by the method of Rockstein and Kamal (1954) using a reaction mixture containing 0·2 ml coconut oil, 0·2 ml buffer (pH 7) and 0·4 ml enzyme extract. Incubation was carried out for 4 h.

2.3 *Quantitative estimation of digestive enzymes*

Amylase and protease were studied quantitatively with a spectrophotometer. Amylase activity was estimated using the method of Noelting and Bernfeld (1948) limiting the incubation time to 20 min. The procedure of Birk *et al* (1962) was followed for estimating protease activity. The reaction mixture had a composition as described for amylase. Glycine-NaOH buffer (pH 9) was used and incubation period was 30 min.

2.4 *Control*

Enzymes denatured by heating the midgut extract at 100 °C for 10 min served as control in all experiments.

2.5 *Measurement of food taken by larva*

Weight of larva together with excreta after feeding for 24 h minus weight of larva before feeding was taken as the amount of food consumed by the larva per day (Langley 1966).

3. Results

Data on qualitative studies on digestive enzymes are summarised in table 1. The per day consumption of food by the third instar larva increased with age. The larvae as

Table 1. Digestive enzyme activity during metamorphosis in *O. rhinoceros*.

Enzyme	Substrate used	Larva	Pupa	Adult
Protease	Casein	+ + +	−	+ + +
Amylase	Starch	+ + +	−	+ + +
Lipase	Coconut oil	+ +	−	+ +
Invertase	Sucrose	−	−	+
Cellulase	Carboxymethyl cellulose	−	−	−
Trehalase	Trehalose	+ +	+ +	+ +

+ + +, High activity; + +, medium activity; +, low activity; −, no activity.

they became older consumed little or no food and started losing weight. In about 20–30 days time, they became considerably reduced in size and weight. Evacuation of most of the midgut contents occurred at this stage and the midgut contained no fresh food material. The midguts of the prepupa and pupa were completely devoid of any food material. Results of quantitative studies on digestive enzymes are given in table 2. Secretion of protease and amylase reached a maximum in the 14–16 week old larva and then declined to low levels. These two enzymes were absent in the prepupa and the pupa and reappeared in the adult with commencement of feeding.

4. Discussion

Digestive enzymes were studied in the larva of *Oryctes nasicornis* using only qualitative methods (Bayon 1980). Reported here are the results of both qualitative and quantitative studies on metamorphic changes in digestive enzyme secretion in *O. rhinoceros*. The results of qualitative studies revealed that the larva and the adult of this insect possess same digestive enzymes except invertase which is present only in the adult. This is due to the fact that unlike the larva, the adult feeds on fresh plant material which contains a good amount of sugars (Dadd 1985). Although cellulase was not observed in any of the stages in *O. rhinoceros*, the possibility of cellulose digestion in the larva by gut micro-organisms cannot be ruled out since the food of scarabaeidae larvae contains cellulose which forms an important source of energy (Bayon 1980). In the larva of *O. nasicornis* no cellulase activity was demonstrated in gut contents or epithelium (Bayon 1980); but detection of carbohydrate fermentation products such as volatile fatty acids and methane provided evidence for microbial cellulolysis (Bayon 1980; Bayon and Mathelin 1980). The pupa of *O. rhinoceros* being a non-feeding stage, has lost its digestive capacity as evidenced by the closure of the

Table 2. Body weight, food consumed and secretion of protease and amylase at various stages of *O. rhinoceros*.

Stage	Body weight (g)	Food consumed (g)	Protease units[a]/ midgut	Amylase units[b]/ midgut
Third instar 3–4 weeks old	8·61 ± 0·39 (9)	2·54 ± 0·32 (9)	95·62 ± 3·38 (7)	260·47 ± 8·89 (7)
Third instar 7–9 week old	10·86 ± 0·49 (10)	4·51 ± 0·23 (10)	122·66 ± 8·95 (8)	454·51 ± 11·85 (8)
Third instar 14–16 week old	13·87 ± 0·38 (9)	4·61 ± 0·46 (9)	140·82 ± 11·66 (9)	482·32 ± 9·66 (9)
Third instar older larva	10·67 ± 0·45 (8)	NC	20·69 ± 8·46 (8)	45·99 ± 24·11 (8)
Prepupa	NC	Non-feeding	Not measurable	Not measurable
Pupa	NC	Non-feeding	Not measurable	Not measurable
Adult, female	NC	NC	73·03 ± 25·79 (7)	385·48 ± 72·38 (7)
Adult, male	NC	NC	56·82 ± 32·24 (8)	237·65 ± 37·13 (8)

[a] One unit = the amount of enzyme required to liberate one μg of tyrosine per min.
[b] One unit = the amount of enzyme required to liberate one μg of maltose equivalents per min.
NC, Not calculated.
Number of determinations given in brackets.

mouth and anal openings and the progressive histomorphological transformations of the gut during the pupal period (unpublished results). Therefore, the presence of trehalase in the pupal midgut of *O. rhinoceros* as in *Bombyx mori* (Sumida and Yamashita 1977) suggests that food digestion is not the normal function of gut trehalase. The results suggest that qualitative changes in digestive enzyme secretion during metamorphosis in *O. rhinoceros* are in accordance with different feeding habits of the larva and the adult. The lepidopterans are a good example in this respect (House 1974).

Feeding is found to influence the secretion of digestive enzymes in *Dysdercus cingulatus* (Muraleedharan and Prabhu 1978). In the older larvae of *Attagenus megatoma* a decline in protease secretion was noticed which was attributed to a decreased intake of food (Baker 1976). Even feeding damp cellulose powder or water resulted in increased secretion of protease in adults of *Tenebrio molitor* (Dadd 1956). Furthermore, protease activity was not observed in the midgut of prepupa (Dadd 1956) and pupa (Langley 1967; Baker 1976). In the present study maximum protease and amylase secretions were observed in the 14–16 week old larva of *O. rhinoceros* which consumed the maximum amount of food. The midguts of older larvae contained no fresh food material. Secretion of protease and amylase reached very low levels at this stage. The absence of protease and amylase in the non-feeding prepupa and pupa is correlated with lack of feeding. These two enzymes reappeared in the adult with commencement of feeding. It may be seen that secretion of protease and amylase in different stages of *O. rhinoceros* shows considerable changes in relation to the state of feeding. Thus, from this study it appears that qualitative changes in digestive enzyme secretion during metamorphosis in *O. rhinoceros* occur as a result of transition from larval feeding habit to the adult type. Regarding the quantity of digestive enzymes present in various stages of development, secretion of digestive enzymes is correlated with feeding.

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