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EVOLUTION OF HOLOMETABOLA INSECT DIGESTIVE SYSTEMS: PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS

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This review takes into account primarily the work done in our laboratory with insects from the major Holometabola orders. Only the most significant data for each insect will be presented and a proposal on the evolution of Holometabola insect digestive systems will be advanced.

Neoptera insects are usually classified among three major groups: Polyneoptera, Paraneoptera and Holometabola (Boudreaux, 1979; Kristensen, 1981). The major Polyneoptera order is Orthoptera. Orthoptera amounts to about 2.3% of the total insect species. Among Paraneoptera, the major order is Hemiptera (7.1%), whereas among Holometabola the major orders are Coleoptera (35%), Hymenoptera (25.6%); Diptera (12.2%) and Lepidoptera (13%). Diptera, Lepidoptera and Trichoptera are grouped as the Panorpoid orders, and the Panorpoid orders together with Hymenoptera will be referred to as "higher Holometabola".

Coleoptera, the major insect order, display in common with Polyneoptera and Paraneoptera orders several characteristics which are in contrast to the higher Holometabola.

Thus, Coleoptera insects occupy, together with Polyneoptera and Paraneoptera insects, the relatively safe surface and subsurface niches. The evolution of the higher Holometabola occurred through the occupation of ephemeral and mainly of exposed niches. The occupation of less safe niches (or ephemeral ones) led to the appearance of several adaptations to assure insect survival (see discussion in Sehnal, 1985). Among these adaptations, the most effective probably is the reduction in life cycle, which make possible the development of more generations in a fixed time, thus assuring the survival of numerous individuals, even if large mortality occurs in each generation. Related to this decrease in life span, one would expect to find larger growth and food consumption rates in higher Holometabola than in Coleoptera, Polyneoptera and Paraneoptera. This is actually observed, although comprehensive data on Paraneoptera are lacking (review: Slansky & Scriber, 1985). It is possible that the remarkable increase, in relation to Coleoptera, of the growth rate (which depends on the food consumption rate) of higher Holometabola be related to changes in the digestive physiology of these insects and even in the morphology of their guts. Otherwise, the fact that both the growth and food consumption rates of Polyneoptera (exemplified by Orthoptera) are similar to those of Coleoptera suggest that significant differences may not be found among their digestive physiology and gut morphology. Of course, these and the previous considerations, refer to only the more generalized members of each group; specialized members may differ widely from the basic pattern of the group.

In this review, the results obtained in our laboratory with insects from the major Holometabola orders will be presented, and a proposal on the evolution of Holometabola insect digestive systems will be advanced. Only the most significant data for each insect will be presented.

SPATIAL ORGANIZATION OF DIGESTION IN HOLOMETABOLA INSECTS

Enzymatic determinations performed in the midgut cells and in the luminal spaces outside the peritrophic membrane of *Rhynchosciara americana* (Diptera: Sciaridae) larvae (Terra et al., 1979) showed that alpha-amylase, cellulase and proteinase are secreted into the ectoperitrophic space and passed into the endoperitrophic space, whereas aminopeptidase and trehalase are secreted into the ectoperitrophic space but are unable to cross the peritrophic membrane. The disaccharidases (except trehalase), dipeptidase and phosphatases are restricted to midgut cells.

The intracellular distribution of digestive enzymes in midgut caeca and anterior and pos-

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terior ventricular cells were studied by differential centrifugation of homogenates prepared in mild and vigorous conditions, as well as by calcium differential precipitation. This tecnique was developed by Schmitz et al. (1973) to prepare microvilli from human enterocytes. It consists in the centrifugation at low gravity values of the tissue homogenate in the presence of calcium ions. In these conditions, mitochondria, baso-lateral membranes and part of the endoplasmic reticulum is pelleted. Microvilli remain in the supernatant and are pelleted at higher gravity values. The results (Ferreira & Terra, 1980) showed that the disaccharidases (except trehalase) occur in midgut caeca cell microvilli and, in minor amounts, also in posterior ventricular cell microvilli. Aminopeptidase was found both in cell microvilli (caeca and posterior ventriculus) and in the cellular soluble fraction (Ferreira & Terra, 1984). In the light of research done in Lepidoptera larvae (see references below), it is possible that *R. americana* cellular soluble aminopeptidase is really an aminopeptidase trapped in the cell glycocalyx from which it is set free on homogenizing. The results discussed above support the conclusion that digestion takes place in three spatially organized steps. The first one occurs inside the peritrophic membrane under the action of depolymerases (amylase, cellulase and proteinase) and comprises the dispersion and/or decrease in molecular weight of the food molecules, which then diffuse out of the peritrophic membrane. The second phase of digestion occurs in the ectoperitrophic fluid (mainly in the caeca) and it consists of the hydrolysis of the polymeric food molecules to dimers and/or small oligomers. Finally, the major part of the terminal digestion occurs in the cell microvilli of midgut caeca and the minor part in the cell microvilli of the posterior ventriculus.

The molecular weights and the Stokes radii corresponding to *R. americana* soluble enzymes were determined by electrophoresis on polyacrylamide gels of different concentrations and by ultracentrifugation on glycerol linear gradients (Terra & Ferreira, 1983). The enzymes which penetrate the endoperitrophic space display diameters smaller than 7.0 nm, whereas the enzymes which do not pass through the peritrophic membrane have diameters larger than 8.0 nm. These results suggest than the peritrophic membrane pores of *R. americana* have diameters of 7-8 nm, and support the assertion that what determines whether a luminal hydrolase is going to cross or not the peritrophic is its size. The excretion rates of *R. americana* digestive enzymes (Terra & Ferreira, 1981) are surprisingly low, suggesting that at least 80% of the enzymes present in the endoperitrophic space are removed from the food bolus before it is expelled by the animal. A hypothetical endo-ectoperitrophic circulation of digestive enzymes may explain these results. According to this model (Terra & Ferreira, 1981), food flows inside the peritrophic membrane from the anterior midgut to the hind midgut, whereas outside the peritrophic membrane water flows from the hind midgut to the caeca. Due to these fluxes, as soon as the polymeric molecules of food become sufficiently small to pass through the peritrophic membrane (with the accompanying depolymerases), they are displaced towards the caeca, where intermediary and final digestion occur. Thus, the anterior position of midgut caeca may lead to the existence of an endo-ectoperitrophic circulation of digestive enzymes resulting in the conservation of digestive enzymes. Ultrastructural data on *R. americana* larval midgut (Ferreira et al., 1981) lent support to the above mentioned model.

Since houseflies are very amenable to experimentation, a more detailed investigation of enzyme recycling was carried out with these larvae than with *R. americana* larvae. Espinoza-Fuentes & Terra (1987) injected congo red dye in the blood space of *Musca domestica* larvae and, after a while, they dissected out their midguts. They found that the fore- and hind-midgut (i.e. the anterior and posterior midguts) were bright red, whereas the mid-midgut showed no color. This suggested that the fore- and hind-midgut are water-secreting and that the mid-midgut is water-absorbing. This was confirmed after feeding larvae with the dye Evans blue in a starch paste and observing that the dye becomes concentrated in mid-midgut and diluted elsewhere in the midgut. The existence of an endo-ectoperitrophic circulation of digestive enzymes, which might be driven by the fluid fluxes observed in the *Musca domestica* larval hind-midgut, was suggested by the finding that an increase in the dietetic protein fed to the larvae led to both a decrease in the trypsin gradient along their hind-midguts and to an increase in the trypsin excretory rate. Furthermore, ultrastructural data support the claim that *M. domestica* larval mid-midgut is water-absorbing, whereas the other midgut regions (mainly the posterior region of the hind-midgut) are water-secreting (Terra et al., 1988).

Techniques similar to those described above were used to study the digestive process in the

following insects: *Erinnyis ello* (Lepidoptera: Sphingidae) and *Tenebrio molitor* (Coleoptera: Tenebrionidae). Based on these studies, a proposal on the evolution of Holometabola insect digestive systems was advanced (Terra et al., 1985; Espinoza-Fuentes & Terra, 1987). This proposal will be discussed in the next section.

EVOLUTION OF HOLOMETABOLA INSECT DIGESTIVE SYSTEMS

The Holometabola ancestors are supposed to display, in relation to their digestive physiology, the following characteristics. (a) Endo-ectoperitrophic circulation of digestive enzymes caused by the secretion of fluid in the posterior midgut and its absorption in the anteriorly placed midgut caeca. (b) Oligomer and dimer hydrolases are free and small (less than 7.5 nm of diameter), and able for this reason to pass through the peritrophic membrane. Coleoptera larvae seem to have retained the majority of these characteristics. Derived characters are supposed to be: (a) anterior midgut carrying out water absorption in place of the midgut caeca which were lost; (b) the aminopeptidase became membrane-bound (for details see Terra et al., 1985).

Panorpoid ancestors are supposed to differ from Holometabola ancestors only in the molecular size of the oligomer and dimer hydrolases, which are more than 7.5nm in diameter and are not able to pass through the pores of the peritrophic membrane. As a consequence, the initial digestion in these insects takes place in the endoperitrophic space and the intermediate and final digestion in the ectoperitrophic space. By restricting to the ectoperitrophic fluid the enzymes which participate only in the intermediary digestion of food, the excretion of these enzymes due to absorption onto undigested food is avoided. Also, and probably the more important aspect, monomers are produced (from oligomers and dimers) only close to the surface of midgut cells, which may facilitate their absorption by the cells. From a molecular point of view, the change in size of the hydrolases may be a consequence of few mutations affecting their molecules so that they may assemble themselves into oligomeric hydrolases composed of a few identical subunits. The recent finding that several *E. ello* midgut enzymes which do not penetrate the endoperitrophic display subunits (Santos & Terra, 1986 a,b) lends support to the above proposal.

Diptera ancestors are supposed to display the following characteristics derived from the hypothetical Panorpoid ancestors. The oligomer hydrolases are free in the ectoperitrophic fluid (mainly in the large caeca), whereas the dimer hydrolases are plasma membrane integral proteins of the midgut cell microvilli. The occurrence of dimer hydrolases in the plasma membrane of midgut cells may be seen as a device to release monomers close to the transport sites responsible for their absorption. Sciaridae (Diptera Nematocera) flies seem to have retained these characteristics (for details see: Ferreira & Terra, 1980, 1983, 1984, 1985, 1986; Ferreira et al., 1981; Terra & Ferreira, 1981, 1983).

Cyclorrhaphous ancestors are supposed to differ from the Diptera ancestors in the following aspects (derived chacarters): (a) presence in mid-midgut of specialized cells responsible for water absorption and for buffering the luminal contents in the acidic zone; (b) presence of lysozyme and pepsin active in mid-midgut lumen; (c) endo-ectoperitrophic circulation of digestive enzymes in the hind-midgut caused by the secretion of fluid in the posterior hind-midgut and its absorption in the mid-midgut, in place of the midgut caeca which were greatly reduced and which lost their absorptive capacity; (d) oligomer hydrolases are microvillar enzymes, probably a consequence of the reduction of the ectoperitrophic space associated with the decrease in size of the caeca. The derived characters displayed by Cyclorrhaphous ancestors seem to be adaptations to a diet consisting mainly of bacteria. The mid-midgut cells are thought to have an embryological origin different from the cells of the fore- and hind-midgut (Poulson & Waterhouse, 1960). This is in agreement with the hypothesis put forward above that these cells have originated late in Diptera evolution (for details see: Espinoza-Fuentes & Terra, 1987; Espinoza-Fuentes et al., 1987; Terra et al; 1988).

Lepidoptera ancestors display the following characteristics derived from the hypothetical Panorpoid ancestors. (a) Anterior midgut carries out water absorption in place of the midgut caeca which were lost. (b) The oligomer and dimer hydrolases are trapped in the intermicrovillar glycocalyx space (except aminopeptidase, which is membrane bound), probably a consequence of the reduction of the ectoperitrophic space associated with the lose of the caeca. (c) Presence of long-neck goblet cells and stalked goblet cells respectivelly in the anterior and posterior midgut. These cells excrete K^+ (review: Harvey, 1982) absorbed from the leaves which are voraciously ingested by the larvae,

and they seem to assist the anterior columnar cells in water absorption and posterior columnar cells in water secretion (for details see: Santos et al., 1983, 1984, 1986; Santos & Terra 1984, 1986a).

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