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ENZYMATIC AND PROTEIN PATTERNS AT DIFFERENT DEVELOPMENTAL STAGES IN THE CHERRY FRUIT FLY *RHAGOLETIS CERASI* L.

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Abstract—Protein patterns and two isoenzyme systems (leucine aminopeptidase, LAP, and alanine aminopeptidase, AAP) were examined in the cherry fruit fly *Rhagoletis cerasi* L. at different stages of ontogeny LAP activity was quantitatively determined.

Differences were observed in protein patterns for puparia in diapause, post-diapause and at adult emergence. Some LAP and AAP isoenzymes appear in post-diapause which had not been observed during diapause. It is suggested that there is a direct link between variations in protein patterns and proteolytic activity.

Key Word Index: *Rhagoletis cerasi*, diapause, electrophoresis, isoenzymes, proteins

INTRODUCTION

The cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae), is a serious pest of cherries throughout Europe. It may also attack other species of *Prunus* and *Lonicera* spp. The flies overwinter in the soil as puparia and adult emergence is preceded by both diapause and post-diapause development (Vankirk and Aliniazee, 1982). During diapause, according to many authors (Wigglesworth, 1972; Tauber *et al.*, 1982), morphogenesis is interrupted while physiogenesis proceeds. Under favourable conditions, once autumnal–hibernal diapause is completed, post-diapause development will start up.

A number of studies have shown that changes occur in haemolymph protein concentrations and in electrophoretic patterns during insect development (Steinhauer and Stephen, 1959; McCormick and Scott, 1966; Agrell, 1964) particularly during metamorphosis in holometabolous insects (Chen and Levenbook, 1966; Lensky, 1971). These changes, in general, reflect the radical processes of histolysis, histogenesis and the morphogenetic events of ontogenesis. In addition to having these complex changes, many insects such as Tephritidae, have evolved a dormancy adaptation which enables them to survive unfavourable environmental conditions and to synchronize maturation with that of the fruit of the host plant.

Since biochemical changes during this period are insufficiently documented, our studies have focused on the enzymatic and protein patterns during diapause and post-diapause development of the European cherry fruit fly.

A possible correlation between activity of proteolytic enzymes and modifications in protein patterns has also been investigated.

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MATERIALS AND METHODS

Over 2000 *Rhagoletis cerasi* puparia, collected from a *Lonicera xylosteum* experimental field, were provided by the Fruit Fly Laboratory, Waedenswil (Switzerland) in November 1983. Diapausing puparia were placed in holed plastic containers and kept in a dark thermostatic cold chamber at +4°C (as suggested by Haisch, 1975), and 80 ± 5% r.h., for 7 months. During this period puparia were sampled every fortnight. Since during preliminary studies high variability in protein patterns of individual puparia was found (Turchetto *et al.*, 1984), we used samples of at least 60 individuals in order to be satisfied that any variations observed were due to different physiological stages and not to individual variation.

In May 1984 the remaining puparia were placed in small emergence cages with damp sand at room temperature and at natural photoperiod (13:11 hr, light:dark). Each day 30 puparia were removed. By pooling the puparia collected on two consecutive days, pools of 60 were obtained. After 22 days the first adults began to emerge. The adults, collected daily, were divided into pools of 60. Pools both of puparia and adults were stored at −20°C. When emergence ceased, the remaining puparia were put back into containers and stored at +4°C.

Preparation of protein extracts

Pools of both puparia and adults were treated with 50 mM Tris–citrate buffer (pH 7.5) containing 17% saccharose in a ratio of 25 μl buffer/mg. Contents were then homogenized with micro-Potter and shaken on a Vortex for 15 sec. Extracts were cleared by centrifugation at 8000 *g* for 10 min and then stored at −20°C until required. The samples used in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) were reduced, at 37°C overnight, after adding 50 μl of Tris (25 mM)–glycine (192 mM) buffer containing 7.5% (w/v) sodium dodecyl sulfate and 7.5% (v/v) 2-mercaptoethanol to 100 μl of the extract.

Electrophoretic systems

SDS–PAGE with acrylamide concentration at 10% was carried out using Laemmli's method (1970). The same method was also used to separate the enzymes but without the detergent and with monomer concentration at 7.5%. The Biorad Mini Vertical Slab Cell was used. Samples

end of emergence. In only two cases was a subuni
observed whose molecular weight was approx
100 kda (marked by an asterisk in Fig. 1).

Figure 2 shows protein patterns of two pools o
puparia in diapause (slots 2 and 3), of five pools i
post-diapause (slots 4–8, i.e. on the 2nd, 12th, 26th
28th and 30th day from the beginning of the post
diapause) and lastly of a pool of 1-day-old adult
(slot 9). As can be seen, the transition from diapaus
through post-diapause to adulthood entail
modifications in protein patterns. In particular, sub
units with a molecular weight from 66 to 92 kd
(indicated in Fig. 2 by the letter A), become les
strongly stained when passing from diapause to post
diapause, and the drop in stain intensity is especially
evident in adults. Decrease in stain intensity indicate
that the relative quantities of protein sub-units unde

marized in Fig. 4 from two pools of puparia in diapause (slots 1 and 2), from five pools of puparia in post-diapause (i.e. on the 2nd, 12th, 26th, 28th and 30th day, slots 3–7), and finally from a pool of 1-day-old adults (slot 8). The patterns from all pools of diapausing puparia examined were identical to those reported in Fig. 4 (slots 1 and 2). In post-diapause the pattern remained the same until the 12th day (slot 4th). On the 14th day the isoenzyme marked B (Fig. 4) began to appear and on the 24th day the isoenzyme marked A (Fig. 4) appeared. These isoenzymes were also observed in the remaining pools. In the two pools of puparia collected at the end of emergence, isoenzymes A and B were no longer

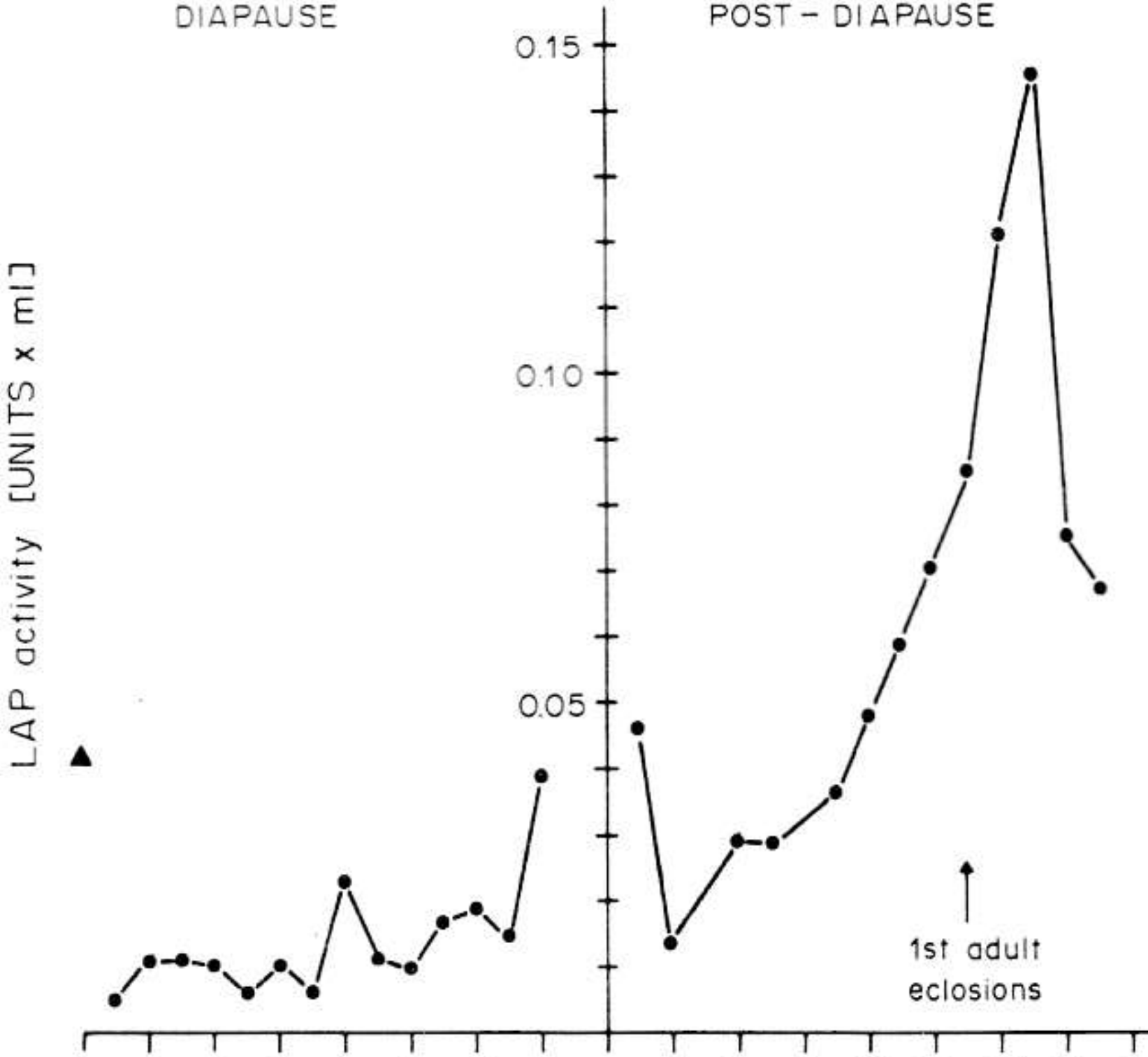

Fig. 3. Leucine amino peptidase activity. (●) Puparia sampled during diapaus
Puparia before being stored at +4°C. (■) Puparia at the end of emergence

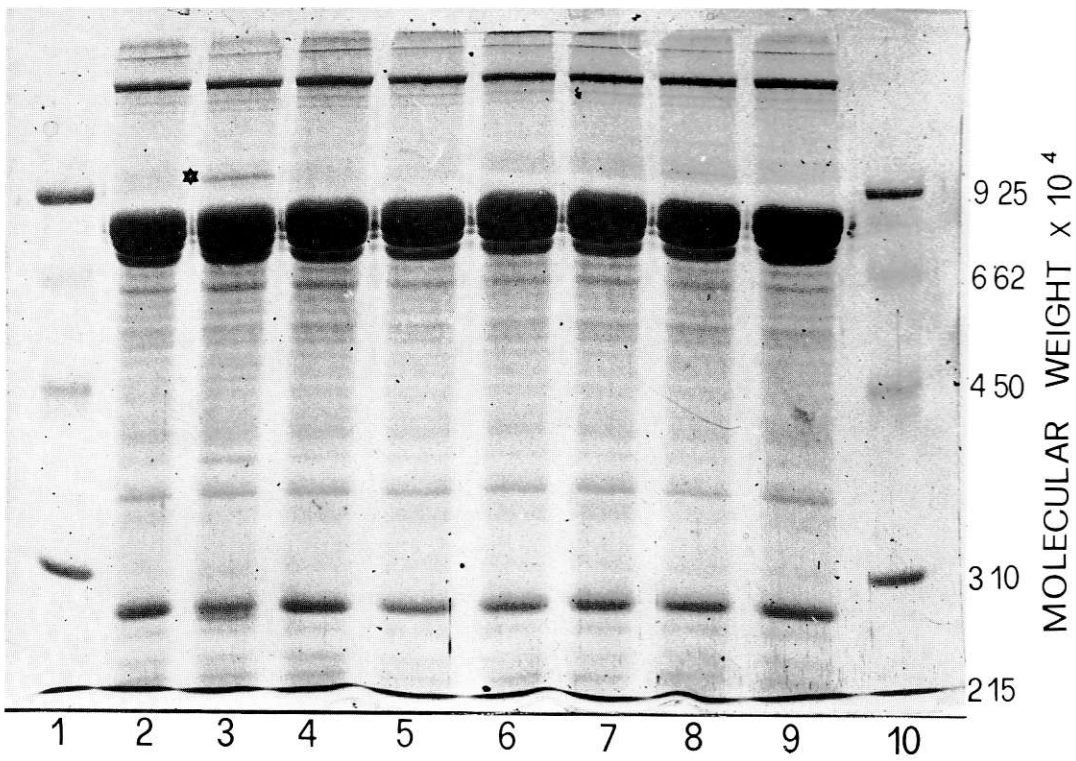

Fig. 1. SDS–PAGE protein patterns of eight pools of diapausing puparia. Standard proteins (slot 1 and 10): from top to bottom; phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor. * Indicates a subunit (molecular weight ~100 kda) observed in only two cases.

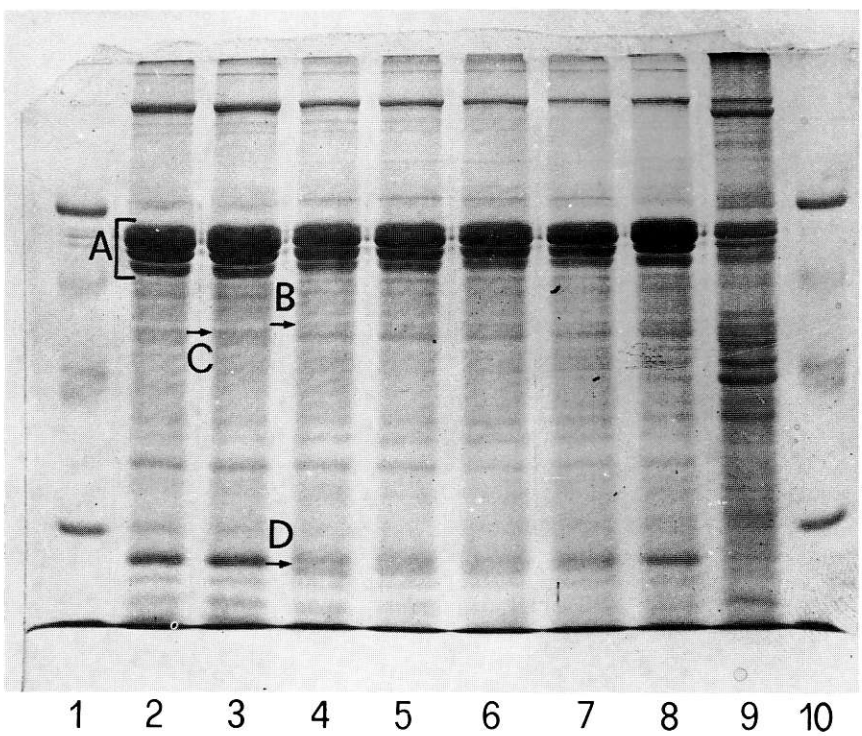

Fig. 2. SDS–PAGE protein patterns of diapausing (slots 2 and 3), post-diapausing puparia (slots 4–8) and adults (slot 9). For molecular weight standards (slots 1 and 10) see Fig. 1. Capital letters indicate the main differences in diapausing and post-diapausing puparia (see text).

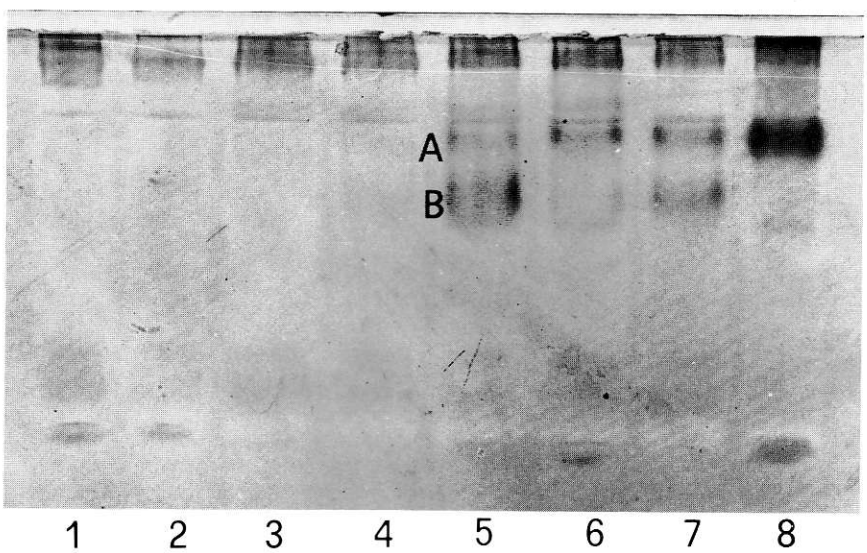

Fig. 4. Isoenzymatic patterns of leucine amino peptidase of puparia in diapause (slots 1 and 2), in post-diapause (slots 3–7) and of adults (slot 8). A—isoenzyme appearing at 24th day. B—isoenzyme appearing at 14th day.

visible. All the pools of adults yielded the same isoenzymatic pattern (slot 8). It differs from that of the puparia in that isoenzyme B is certainly missing. The same results were observed using alanine as substrate.

DISCUSSION

From these results it may be concluded that during diapause the protein pattern of *Rhagoletis cerasi* puparia remains virtually unaltered. LAP activity remains low throughout the whole period, even though in the last few months there is a tendency to increase. In the seventh month of diapause LAP activity can be observed to approach values similar to those present before being stored at +4°C (Fig. 3). During the post-diapause phase, protein patterns differ from those found during diapause. Nevertheless they are fairly constant throughout the whole period, even after emergence of the adults. LAP activity, on the other hand, varies considerably. On the 26th day of the post-diapause LAP activity is three times greater than its initial value. The drop in enzymatic activity, which can be observed from the 28th day onwards is probably due to the percentage increase in the pools of puparia which will not emerge. This hypothesis is supported by the observation that the unemerged puparia showed enzyme activity at the same level as that recorded in the last two pools in which emergence was in progress. It is interesting to note how the new enzymatic forms of LAP and AAP appear just before the beginning of emergence, as well as how they show up more strongly during that period and finally disappear in the puparia at the end of emergence.

The variations which can be seen in the pupae protein patterns occur at the same time as the appearance of the new isoenzymatic forms, which is why one may conclude that the two occurrences are closely related. With regard to this question Katzenellenbogen and Kafatos (1971a,b) showed that in the moulting fluid of *Antheraea* a number of inactive proteases are activated only just before emergence.

From these results, it may be suggested that the transition from pupae to adults in *Rhagoletis cerasi* entails a redistribution of pre-existing proteic subunits in the puparia rather than a completely new synthesis and that this re-distribution is mediated by proteolytic enzymes. This hypothesis is in accordance with the conclusions drawn by Loughton and West (1965) and Chen and Levenbook (1966) from the study on the variations in protein concentration in the haemolymph of holometabolous insects during metamorphosis. They observed that proteins already present are carried by haemolymph and serve as the main constituent for the construction of adult tissue proteins.

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