

ACID AND ALKALINE PHOSPHATASE ACTIVITY  
DURING EMBRYONIC DEVELOPMENT OF  
*CHILOMENES SEXMACULATA* FABR.  
(COL., COCCINELLIDAE)

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ABSTRACT. — SIDHU, D.S., DHILLON, S.S. & S.K.J.K. SANDHU, Dep. of Zool., Punjabi Univ., India. — Acid and Alkaline Phosphatase Activity During Embryonic Development of *Chilomenes sexmaculata* Fabr. (Col. Coccinellidae). — Acta entomol. Jugosl., 1984, 20 (1-2): 89-94 (Engl., Croat. Summ.).

Acid and alkaline phosphatase activity in relation to the various morphogenetic events occurring during the embryogenesis of *Chilomenes sexmaculata* Fabr. was determined. The presence of both the phosphatases was noted right at the time of oviposition. The activity of acid phosphatase was found to be higher immediately after oviposition corresponding to the period of active yolk protein lysis. The level of acid phosphatase activity was considerably higher than that of alkaline phosphatase throughout the period of embryogenesis as noted from the acid/alkaline phosphatase ratio.

**Coleoptera—Coccinellidae, *Chilomenes sexmaculata*, embryonic development, metabolism, enzymes, phosphatases.**

### Introduction

Phosphatases are the enzymes with multiple molecular forms and are useful for determining the time at which genes, controlling specific protein products are expressed. Alkaline phosphatase is known to cause the breakdown of the cytoplasmic inclusion during embryogenesis and at the same time the products of this process transform in some way the morphogenetic property of the cleavage energids which later become included in the primordial cells (Seidel, 1936, 60). The role of acid phosphatase in protein synthesis (Pearse, 1968) and of alkaline phosphatase in carbohydrate metabolism (Rosenthal et al., 1960), differentiation, formation of mucopolysaccharides and passage of metabolites and ions across the cell membrane (Fernelley, 1971) has been well recognized up to now.

Though Fitzgerald reported on the phosphatases of insect egg as early as 1949, yet there is a dearth of reports about phosphatase patterns during embryonic development of insects. Some other reports come from the study of Yao (1950, a, b), Tawfik (1957), Chino (1961), Indira (1963), Sridhara and Bhat (1962), Sawicki and MacIntyre (1977), Khan and Khan (1978) and Prakash and Reddy (1979). In the present study, an effort has been made to correlate the changes in phosphatase activity during the embryonic development of a coleopteran, *Chilomenes sexmaculata* to the morphogenetic changes going on in the embryo (Mukesh et al., 1980).

### Material and methods

Adults of *Chilomenes sexmaculata* were collected around Patiala (India) from a wildy growing plant *Calotropis procera* and a cultivated vegetable *Solanum melongena* during day time. The beetles were reared in glass jars, kept at a temp. of 22°C, R.H. 65 percent and a photophase of 10 hours. Each jar was covered with a muslin cloth, so as to prevent the beetles from escaping, and at the same time allowing fresh air to enter the jars.

The insects were fed twice daily on aphids, *Aphis nerii*, laid on leaves of *Calotropis procera*. Copulation took place freely in the laboratory and the females laid eggs in batches attached to dorsal and ventral sides of the leaves. These batches of eggs were removed at once and incubated on moist filter papers at the above mentioned conditions. Equal number of fresh eggs of different developmental periods were used for the estimation of acid and alkaline phosphatase activity. The method of Pant and Lacy (1969) was employed for the determination of both acid and alkaline phosphatases. The enzyme activity has been expressed as  $\mu$  moles of product formed (100 eggs) minute at 37°C.

### Results and discussion

The observations on the acid phosphatase activity has been recorded in Table 1 and represented through Figure 1 & 2. It is indicative from the present observations that the activity of Acid phosphatase is significantly higher than that of Alkaline phosphatase during the course of embryonic development. A similar trend has also been observed by Yasbin et al. (1977) in *Drosophila melanogaster* (Meigen). Khan and Khan (1978) report the acid phosphatase activity to be very much higher than alkaline phosphatase activity in freshly laid eggs, and it remained so during the remaining course of embryonic development. Further he observed the activity of both enzymes to increase during embryogenesis but decline after hatching. Similarly Dhand and Rastogi (1975) have observed the dominance of acid phosphatase activity over that of alkaline phosphatase throughout life in relation to egg laying and aging in coleopteran: *Callosobruchus analis* F. Contrary to these reports Dikshith and Vasuki (1969) have observed that both; rate of activity and quantity of alkaline phosphatase was higher than that of acid phosphatase in *Dactylopius confusus*.

Besides this both of the phosphatases are present from the beginning of egg

development of *Chilomenes*. The presence of acid phosphatase and alkaline phosphatase in the freshly oviposited eggs indicates that transcription of these enzymes takes place right during the period of egg maturation.

Table 1: Acid and Alkaline phosphatase activity during embryogenesis of *Chilomenes sexmaculata* Fabr.

Incubation period, Hours,	Acid Phosphatase activity $\pm$ S.D.	Alkaline Phosphatase activity $\pm$ S.D.	Acid/Alkaline Phosphatase activity ratio.
0	0.488 $\pm$ 0,0113	0.352 $\pm$ 0,0	1.39 : 1
12	0.572 $\pm$ 0.0282	0.328 $\pm$ 0.0339	1.74 : 1
24	0.572 $\pm$ 0.0283	0.384 $\pm$ 0.0226	1.49 : 1
36	0.508 $\pm$ 0.0169	0.376 $\pm$ 0.0113	1.35 : 1
48	0.488 $\pm$ 0.0113	0.384 $\pm$ 0.0452	1.27 : 1
60	0.416 $\pm$ 0.0	0.328 $\pm$ 0.0113	1.26 : 1
72	0.488 $\pm$ 0,0113	0.320 $\pm$ 0.0226	1.52 : 1
76	0.452 $\pm$ 0,0056	0.296 $\pm$ 0,0113	1.53 : 1
N.H.L.	0.468 $\pm$ 0.0169	0.336 $\pm$ 0.0226	1.39 : 1

N.H.L: Newly Hatched Larvae.

S.D.: Denotes standard deviation.

All the readings are an average of 4 determinations at least.

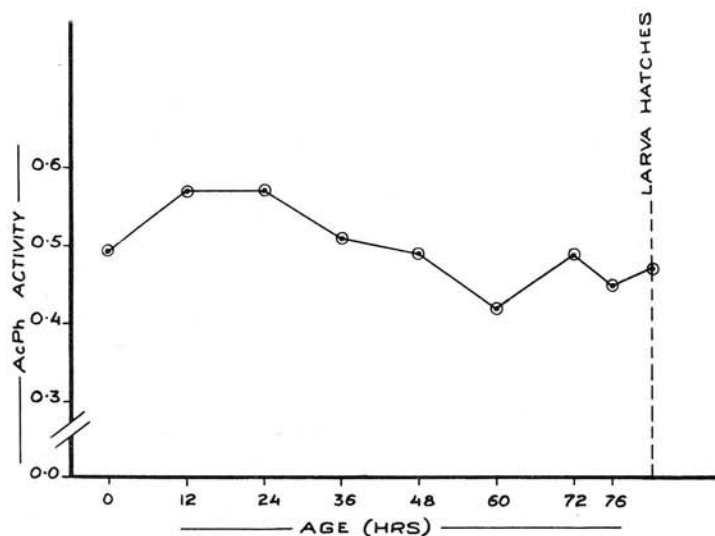


Fig. 1 The variations in acid phosphatase (AcPh) activity during the embryogenesis of *Chilomenes sexmaculata* Fabr.

The acid phosphatase activity is observed to increase during first 12 hours of embryogenesis which corresponds to a period of active yolk protein lysis as is evident from an increased level of free amino acids as described by Mukesh et al. (1980) and a decline in acidic protein fraction of proteins (S and hu, 1984).

Thus it is quite evident that acid phosphatase is involved in the process of yolk degradation. The presence of acid phosphatase from the initial stages of development is also reported by Yao (1950 a, b), Prakash and Reddy (1979) in *Drosophila*. Similarly reports coming from the workers of Borner (1970), Prakash and Reddy (1979), support the presence of alkaline phosphatase from the beginning. But Chino (1961); Sridhara and Bhatta (1963) did not detect it in early stages of embryogenesis. Probably this enzyme was present in undetectable quantity in their respective experimental insects.

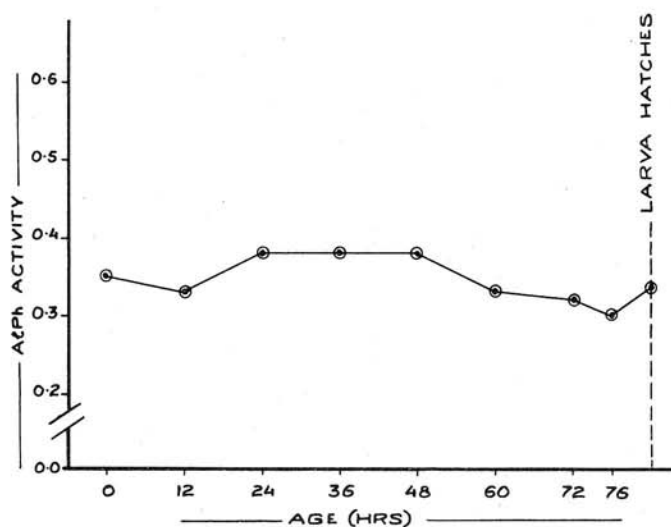


Fig. 2 The variations in alkaline phosphatase (AlPh) activity during the embryonic development of *Chilomenes sexmaculata* Fabr.

Alkaline phosphatase activity decreases from its initial level of 0.352/100 eggs at 0 hour to 0.328/100 eggs by 12 hours. Going through morphogenetic changes occurring during this particular time, it is noticed that this is the time for active movement of cleavage energids (Mukesh et al., 1980). Since the decrease in alkaline phosphatase activity coincides with migratory movement of cleavage energids, the assumption that the activity of alkaline phosphatase causes the breakdown of cytoplasmic inclusion and at the same time the products of this process transform in some way the morphogenetic property of the cleavage nuclei which later become included in the primordial germ cells by Tawfik (1957) seems to have some weight in it.

Acid phosphatases generally are involved in degradative processes (De Duve & Wattiaux, 1966) protein synthesis (Pearse, 1968) hence it is only rational that they show higher activity throughout the embryonic development as also seen in the present investigations. Sharp increase in the activity of acid phosphatases just after fertilization, in the present study, supports the hypothesis of a possible role of lysosomes in this process. Further higher activity of acid phosphatases during, mid and late stage embryogenesis may be related with the process of cellular multiplication and differentiation during this phase of development (Mukesh et al. 1980). A similar trend of variations is reported by Yasbin et al. (1977) also.

The ratio AcPh/AlPh activity remains above unity throughout the embryogenesis as well as in newly hatched larva supporting higher acid phosphatase activity during the development of *Chilomenes* eggs (Table-1). It shows two peaks, one during early embryogenesis and the second during late phase of embryogenesis. This indicates that acid phosphatase activity is more than alkaline phosphatase comparatively during early and late phase of embryonic development of *Chilomenes sexmaculata*.

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#### Sažetak

#### AKTIVNOST KISELE I ALKALNE FOSFATAZE ZA VRIJEME EMBRIONALNOG RAZVOJA *CHILOMENES SEXMACULATA* Fabr. (COL., COCCINELLIDAE)

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Određivana je aktivnost kisele i alkalne fosfataze u odnosu na različita morfogenetska zbivanja za vrijeme embriogeneze *Chilomenes sexmaculata* Fabr. Utvrđena je pojava obiju fosfataza za vrijeme ovipozicije. Aktivnost kisele fosfataze je veća neposredno nakon ovipozicije, što odgovara razdoblju aktivnog razgrađivanja žumanjka. Stupanj aktivnosti kisele fosfataze je znatno veći od alkalne za cijelo vrijeme embriogeneze, što se zaključuje iz omjera obiju fosfataza.

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