ONTOGENETIC DIFFERENTIATION OF ALPHA AND BETA ESTERASES IN UNIVOLTINE, BIVOLTINE AND TRIMOULTER RACES OF SILKWORM *Bombyx mori* L.

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ABSTRACT

Formation of an adult organism from a single fertilized cell is the result of selective differential gene expression, operating on a constant pool of genetic information. The differential gene action can be viewed by the differential synthesis and activity of certain enzymes. The variation patterns in two enzyme systems of alpha and beta esterases have been studied in a univoltine race I-380, bivoltine NB_4D_2 and in a trimoulter race of silkworm *Bombyx mori* L. In total, 10 different development stages of each of the races were analysed. The number of bands and their intensity varied during different developmental stages indicating differential gene action. The pattern of variation of enzyme bands and their significance are herein discussed.

Ann. Trop. Res. 17:45-53

KEY WORDS: *Bombyx mori*. Esterases. Isozymes. Ontogeny.

INTRODUCTION

During the course of ontogeny and tissue differentiation, understanding the isozyme systems are of immense biological significance (Markert, 1963). The studies on isozymes during different developmental stages pave the way to know differential gene action and to compare homologous enzyme systems in related species or groups. The ontogenetic changes in different enzyme systems of silkworm *Bombyx mori* have been studied by various workers (Hegde and Krishnamurthy, 1980; Subramanya and Reddy, 1982; Kai, *et al.*, 1986; Abraham, *et al.*, 1992). In silkworm, Morishima (1975) has studied the developmental changes in cyclic AMP and cyclic GMP phosphodiesterases. However, there is a need to study the variability of

alpha and beta esterases in different voltine groups and in the race which has less than four molts in its larval period, in view of its shorter life span than others. Therefore, the present investigation involving a univoltine race, bivoltine race and non-diapausing trimoulter of*Bombyx mori* in two enzymes are undertaken to compare the enzyme systems of the three distinctly different races and to understand the differential gene action among them.

MATERIALS AND METHODS

Sample preparation

Three races of silkworm *Bombyx mori* viz., univoltine I-380, bivoltine NB_4D_2 and a non-diapausing trimoulter race used in the present experiment were maintained in the laboratory following appropriate rearing technology. Individual samples of each of the ten developmental stages were prepared by homogenizing the whole individuals. To obtain comparable data for different developmental stages, enzyme assay was carried out by drawing an aliquot of 0.2 ml of the homogenate from each sample having a concentration of 0.1% of the tissue. Samples of egg, I instar to V instar larvae, male and female pupae, male and female moths were prepared during the course of rearing.

Enzyme assay

The polyacrylamide gel electrophoretic technique of Davis (1964) was employed for the enzyme assay. The gel tubes were fixed to electrophoresis chamber containing boric acid and sodium hydroxide buffer (0.3, pH 8.65). The samples of the homogenate were mixed with 0.2 ml of 40% sucrose solution and a drop of bromophenol blue was layered over the large pore gel. Electrophoresis was carried out at 4°C at 80 volts, for two hours. The gels were incubated in the staining solution at 37°C. The staining procedure of Ayala, *et al.* (1972) was employed with slight modification involving fast blue RR salt as the dye coupler. After the enzyme bands appeared, the gels were fixed in 7% acetic acid.

Stain preparation

The stain for alpha-esterase was prepared using 25 mg of alpha-naphthyl acetate which was dissolved in 1 ml acetone and 1 ml of water and was added to 12.5 ml of 0.1 M phosphate buffer of pH 5.9 to which 25 mg of

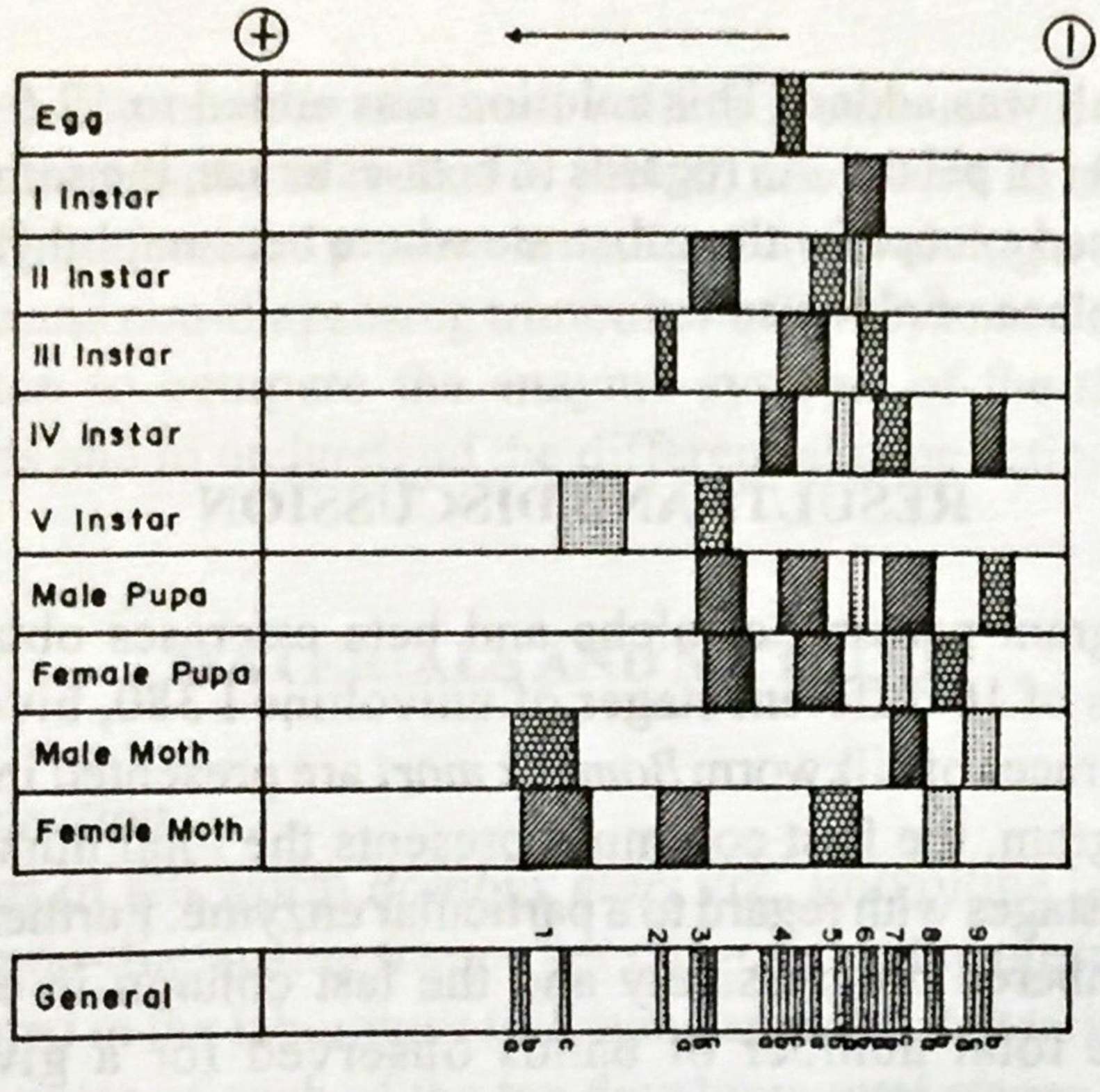
fast blue RR salt was added. This solution was added to 12.5 ml of 0.1 M phosphate buffer of pH 6.5. As regards to beta-esterase, the same incubating medium was used except for the substrate where beta-naphthyl acetate was substituted in place of alpha salt.

RESULTS AND DISCUSSION

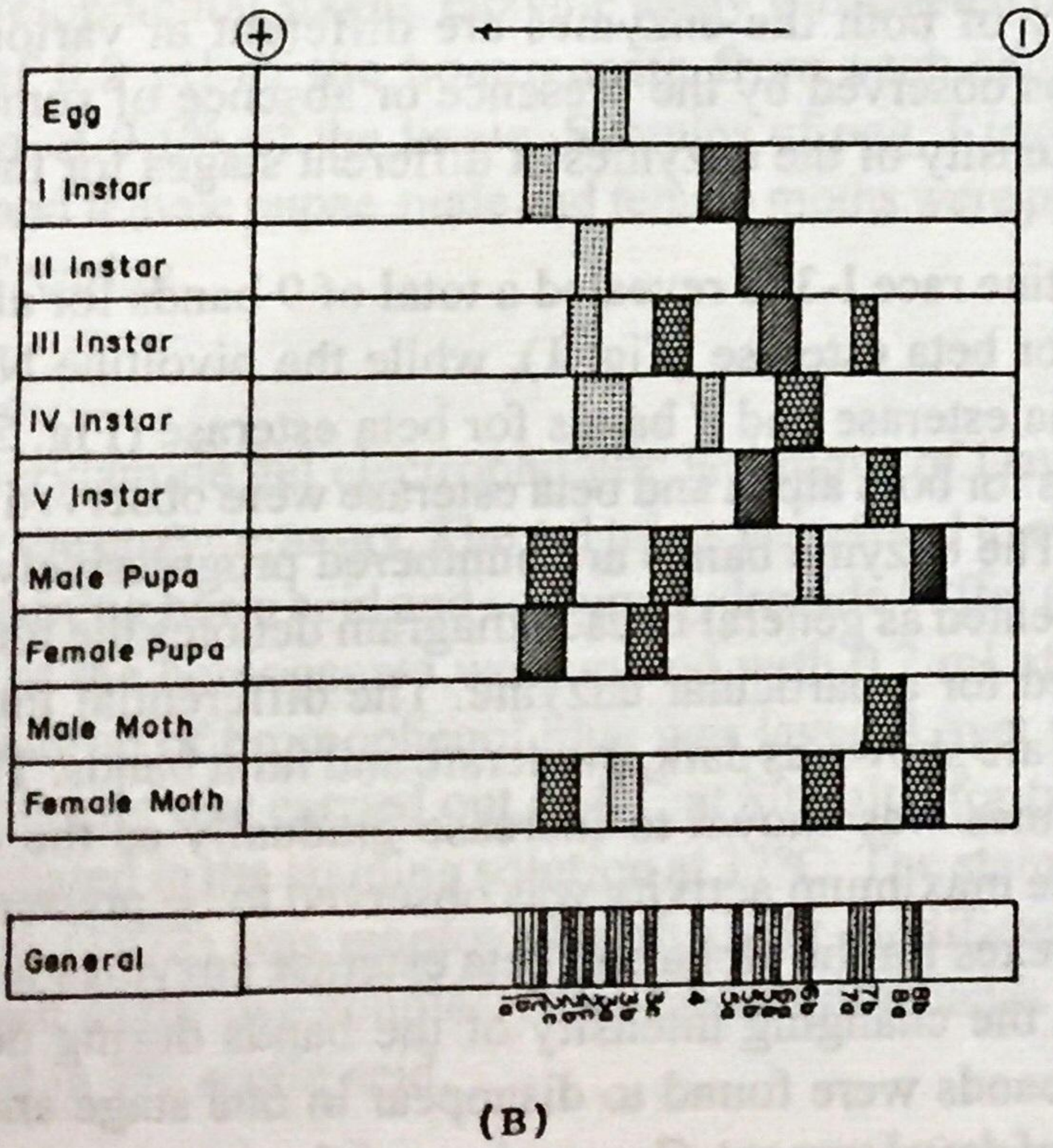
The Zymogram patterns of alpha and beta esterases obtained by gel electrophoresis of 10 different stages of univoltine I 380, bivoltine NB_4D_2 and trimoulter races of silkworm *Bombyx mori* are presented in Figures 1 to 3. In each diagram, the first column represents the total number of bands observed at all stages with regard to a particular enzyme. Further, the enzyme bands are numbered progressively and the last column in each diagram represents the total number of bands observed for a given enzyme. Differential activity of these enzymes at various stages of development is shown as faint, moderate and dark bands.

The activity of both the enzymes are different at various stages of development as observed by the presence or absence of some bands and differential intensity of the enzymes at different stages for the three races under study.

The univoltine race I-380 revealed a total of 9 bands for alpha esterase and 8 bands for beta esterase (Fig. 1), while the bivoltine NB_4D_2 has 11 bands for alpha esterase and 9 bands for beta esterase (Fig. 2). Further, a total of 9 bands for both alpha and beta esterase were observed in trimoulter race (Fig. 3). The enzyme bands are numbered progressively and the last column represented as general in each diagram denotes the total number of bands observed for a particular enzyme. The differential intensity of the enzyme bands are shown as dark, moderate and faint bands. The activity of both the enzymes was shown to increase gradually as the development proceeded. The maximum activity was observed to be present in the pupal stage in both sexes for the alpha and beta esterase enzymes in all the races. In addition to the changing intensity of the bands during developmental stages, some bands were found to disappear in one stage and reappear in another stage of development. Comparison of the zymogram patterns of the three races under study revealed marked differences between the three races not only in the number of bands but also in the intensity of the bands. In addition, the developmental stage and localization of the activity of alpha and beta esterase are quite variable.



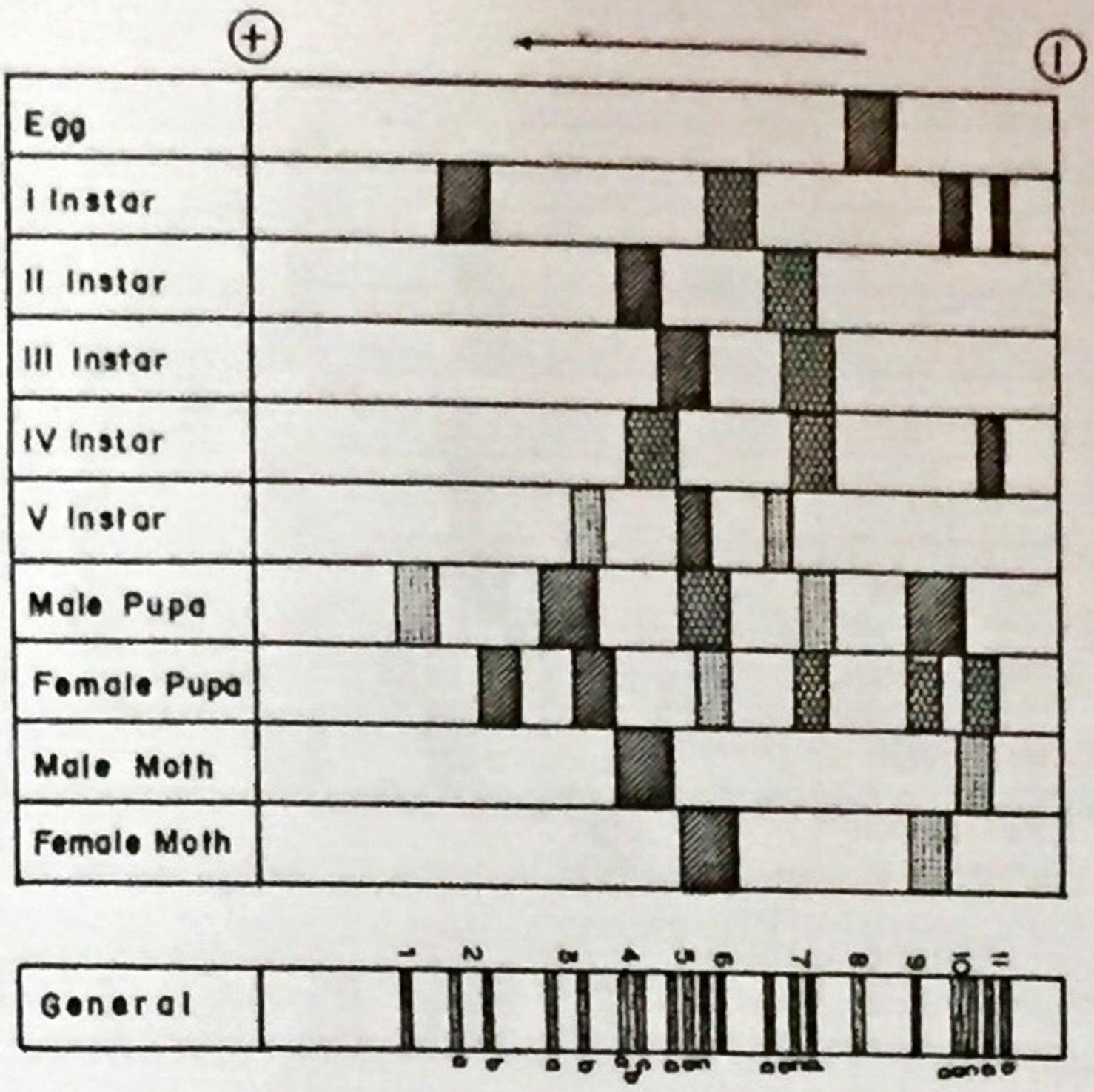
(A)



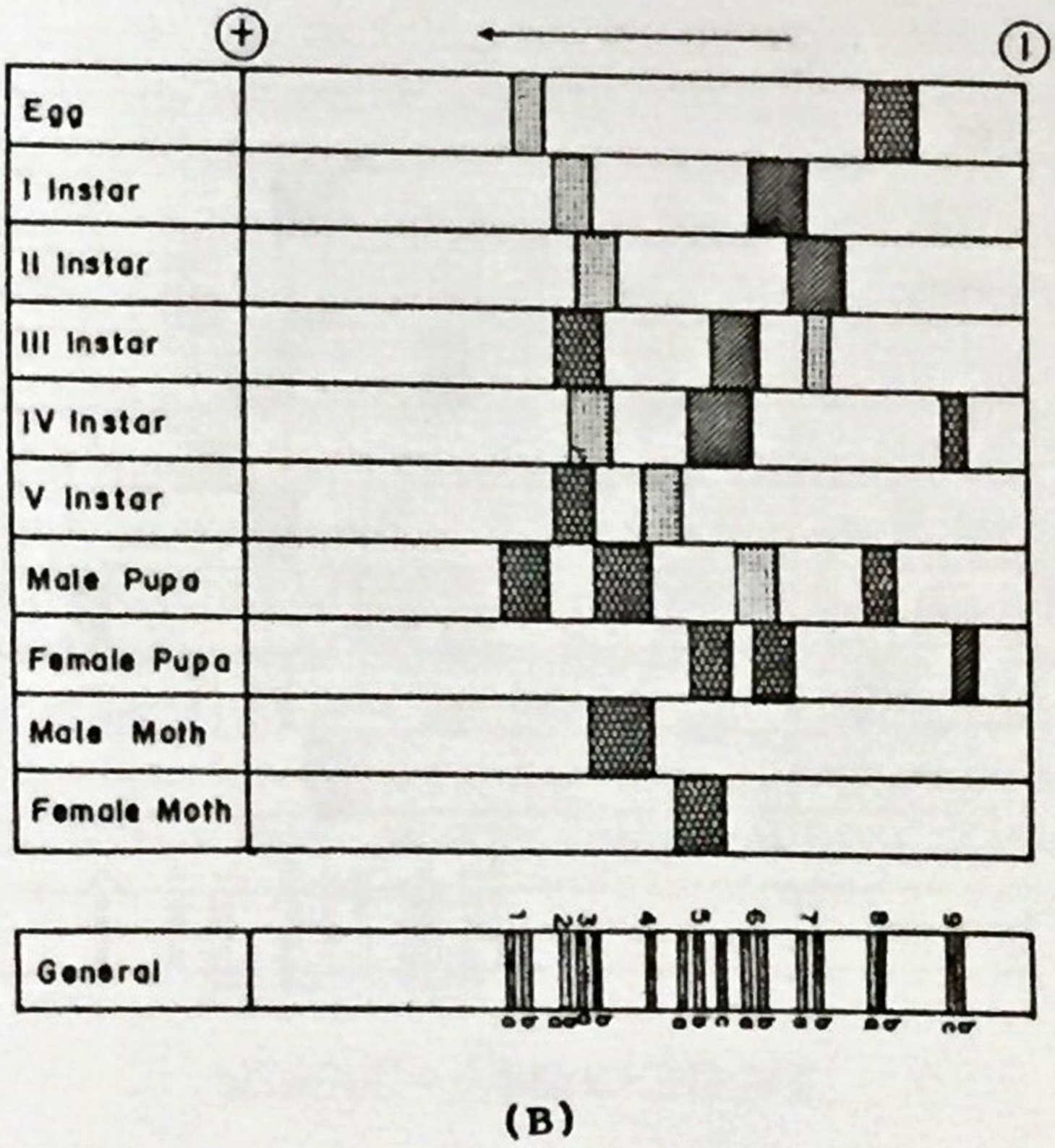
(B)

DARK MODERATE FAINT

Figure 1. Zymogram of alpha esterase activity (A) and beta esterase activity (B) in the ontogeny of univoltine race I-380 of *Bombyx mori* L.



(A)



(B)

DARK MODERATE FAINT

Figure 2. Zymogram of alpha esterase activity (A) and beta esterase activity (B) in the ontogeny of bivoltine race NB_4D_2 of *Bombyx mori* L.

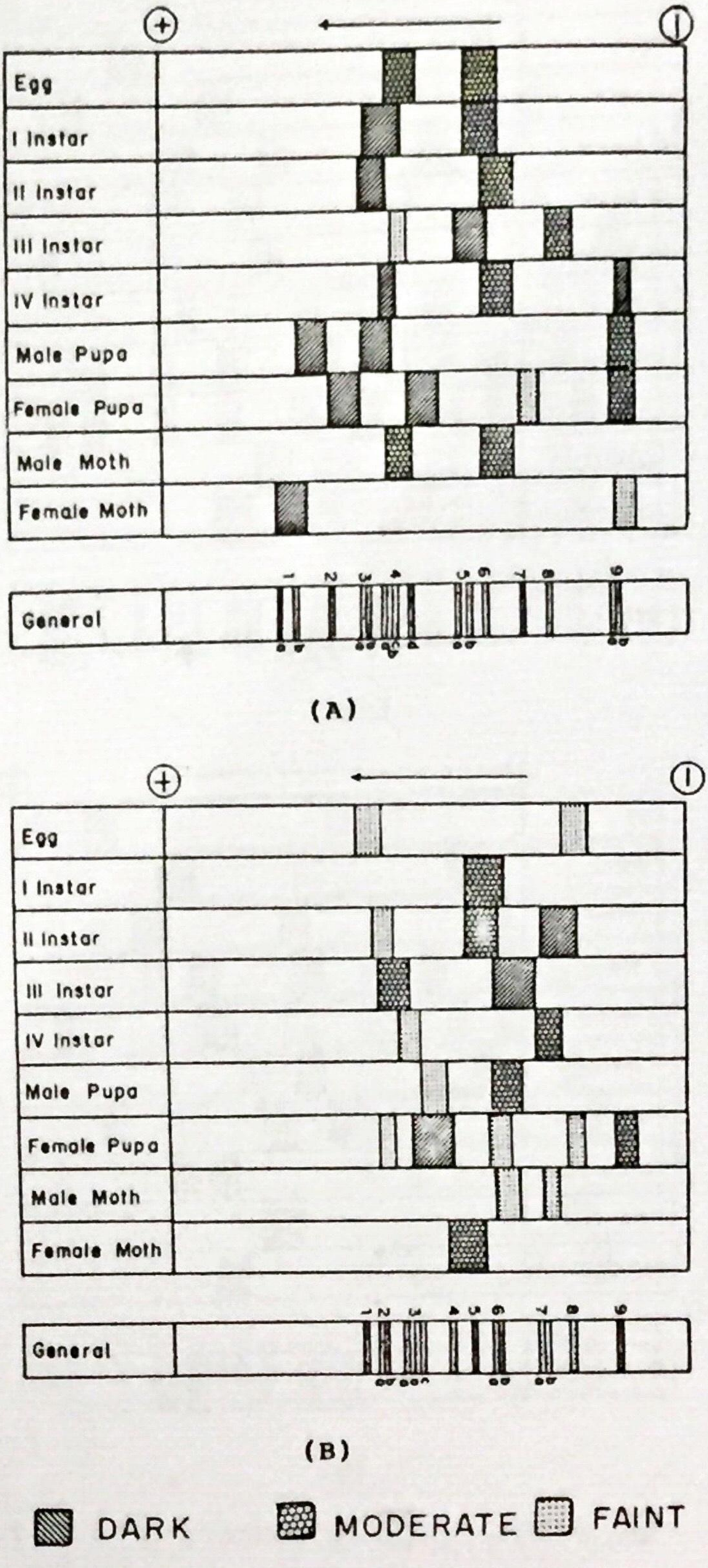


Figure 3. Zymogram of alpha esterase activity (A) and beta esterase activity (B) in the ontogeny of Trimoulter race of *Bombyx mori* L.

Enzymatic variations have been extensively studied in various biological systems through electrophoretic technique and ontogenetic variations in the isozyme patterns have been well documented in Drosophila and mammals. In silkworms, the biochemical differences between diapausing and non-diapausing strains are not well understood although such differences do exist (Gamo, 1983). Ontogeny of enzymes demonstrates the basic change occurring during differentiation (Masters and Holmes, 1972). This biochemical index provides a means to understand the differential gene action during different stages of life cycle of an organism as well as to compare molecular differentiation that occurs within and between species. Further, the enzyme variations in different species form the basis to understand the phylogeny and to establish the genetic basis of taxonomic relationships among species. In addition, enzyme polymorphisms are common and easy to locate in most species of Drosophila (Ayala, *et al.*, 1974). Furthermore, allelic variations in enzymes such as electrophoretic variants have provided population geneticists with a much needed tool for measuring the genetic variability in populations (Johnson,*et al.*, 1966).

The variable expression of the enzyme bands (Figs. 1-3) during the developmental stages of the three races with different voltinism reveals the differential gene action in the production of enzymes. Moreover, the marked differences in the development profiles of these two enzymes among the three races under study can be attributed to the genetic differences among them. This corroborates the findings of Keller and Glassman (1964) who demonstrated the wide range of quantitative differences in xanthine dehydrogenase during extensive stock survey of Drosophila. Thus, these differences in the enzyme activity of different voltine groups of silkworm *Bombyx mori* may be due to racial difference that might have taken place during the development of a race and may open up new vistas in understanding the number of genes involved although it is rather difficult to explore the possibility of their detection in polygenic systems. However, the genetic differences among the three voltine groups can be established by the variation in the enzyme profiles during ontogeny as revealed by the present study.

ACKNOWLEDGEMENT

The authors are grateful to the University of Mysore, Mysore for the facilities provided and the Central Silk Board, Government of India, Bangalore for the financial support provided through the National Sericulture Project.

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