RACIAL DIFFERENTIATION OF α- AND β-ESTERASE ISOZYMES IN SELECTED RACES OF SILKWORM *Bombyx mori* L.

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ABSTRACT

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Isozymes of α– and β–esterases were analyzed in four selected races of univoltine (B38P), bivoltine (NB_{18} and SF19) and multivoltine (C. nichi) by employing polyacrylamide gel electrophoretic technique (PAGE). Racial differences as a reflection of ontogenic differentiation of the multi-banded system in the races under study were identified, although the trend of differential activity of the esterase(s) follows a specific pattern in all the races. Certain differences are also known between α– and β–esterases where β–esterase is more prominent and exhibits higher activity.

KEY WORDS: *Bombyx mori*. Esterases. Isozymes. Ontogeny. PAGE. Voltinism. Zymogram.

INTRODUCTION

Isoenzymes serve as efficient biochemical markers either of a cell or tissue phenotype in terms of molecular composition. This in turn could be used for differentiating any given isozyme pattern between different tissues or same tissue in different races, species or sub-species. Since there are bound to be differences in the intensity of multiple band system as much as their electrophoretic mobilities, comparative studies on the tissue-specific isozyme patterns in the adults could provide information to highlight differential gene expression generated from a single precursor pattern from one cell stage of zygote through intricate series of isozyme changes during ontogeny or development.

To understand the program of selective gene expression and its differential manifestation during ontogeny, this study on the isozymes of α– and β–esterases in four different races of silkworm *Bombyx mori* L. was undertaken. This will provide not only the molecular basis of differentiation in terms of isozyme profiles as specific patterns to understand racial differences, but also the degree of activity of the said isozymes among different races.

MATERIALS AND METHODS

Four races of *Bombyx mori* were employed for the study, including a univoltine (B38P), two bivoltines (NB_{18} and SF19) and a multivoltine (C. nichi). B38P was procured from the germplasm bank of Central Sericulture Research and Training Institute, Pampore (Jammu and Kashmir); SF19 from Dehradun (CSB Grainage); and the other two from the germplasm bank of the Department of Sericulture, University of Mysore. These races were primarily reared under standard laboratory conditions at a temperature of 25 ± 1°C with a relative humidity of 75-90%. The four major developmental stages of the silkworm were further divided into 14 different stages, each of which was subjected to polyacrylamide gel electrophoretic technique (PAGE) to analyze α– and β–esterases. The 14 stages include the Egg (blue egg) stage, I-V instar larvae, Early (one day old) and Late (six days old) pupae of both sexes, and Early (5 h old) and Late (24 h old) adults/moths of both sexes. The multi-banded system for each of the races was plotted in the form of zymograms.

Sample preparation

Samples for each stage of the races under study were prepared by homogenizing the whole individual in double-distilled water, centrifuged at 3000 rpm for 3 min and the supernatant temporarily stored at -4°C. The samples were subjected to PAGE (7.5% native disc/tube gel type) following the procedure of Davis (1964) with boric acid-sodium hydroxide buffer as electrode buffer during the electrophoresis run and the sample homogenate was mixed with 0.2 mL of 40% sucrose solution and a drop of bromophenol blue per mL of sample. Electrophoresis was carried out at 4°C with 80 v for

a duration of 2-2.5 h until the tracking/marker dye reached the 8-cm mark. The staining procedure of Ayala et al (1972) was employed with slight modification using Fast Blue RR salt as dye coupler and 1-naphthyl acetate for α–esterase and 2-naphthyl acetate for β–esterase were used as respective substrates. Based on the R_m values obtained after calculation, the zymograms were plotted to correlate the isozyme activity.

RESULTS AND DISCUSSION

The zymogram patterns for the α– and β–esterases along with their general zymograms in B38P, NB_{18}, SF19 and C. nichi are presented in Figures 1-4. As per intensity of the bands observed, three types are identified as Dark, Moderate and Faint.

Based on the occurrence, differential appearance and disappearance of bands, they are classified into three major types (Raje Urs, 1988): (1) consistent bands, expressed in all stages of the pure races and hybrids; (2) transient bands, expressed in one stage, disappear in the next and reappear in subsequent stages in one or the other races or hybrids; and (3) stage-specific bands, exclusively confined to a single stage of a given race or hybrid.

In addition, the authors have recognized two more types of bands, namely: (1) race-specific, specific to only one of the races; and (2) sex-specific (sex dimorphic), found in one of the sexes of either pupa or moth in one or more races.

The following is the account of α– and β–esterase activity for each of the races.

α- and β-esterase activity in B38P

The zymogram patterns of the ontogenic differentiation of α– and β–esterases are depicted in Figures 1a-b. Perusal of Figure 1a for α–esterase in B38P revealed stage-wise differences in intensity as well as the molecular species. There are 26 generalized bands which differ in number and intensities according to the different stages under study. In the egg stage are a dark and a moderate band, which increased to two dark and one moderate band in I instar larva. In the II instar larva, one dark and two moderate bands were seen which further reduced to one dark and one moderate band in III instar

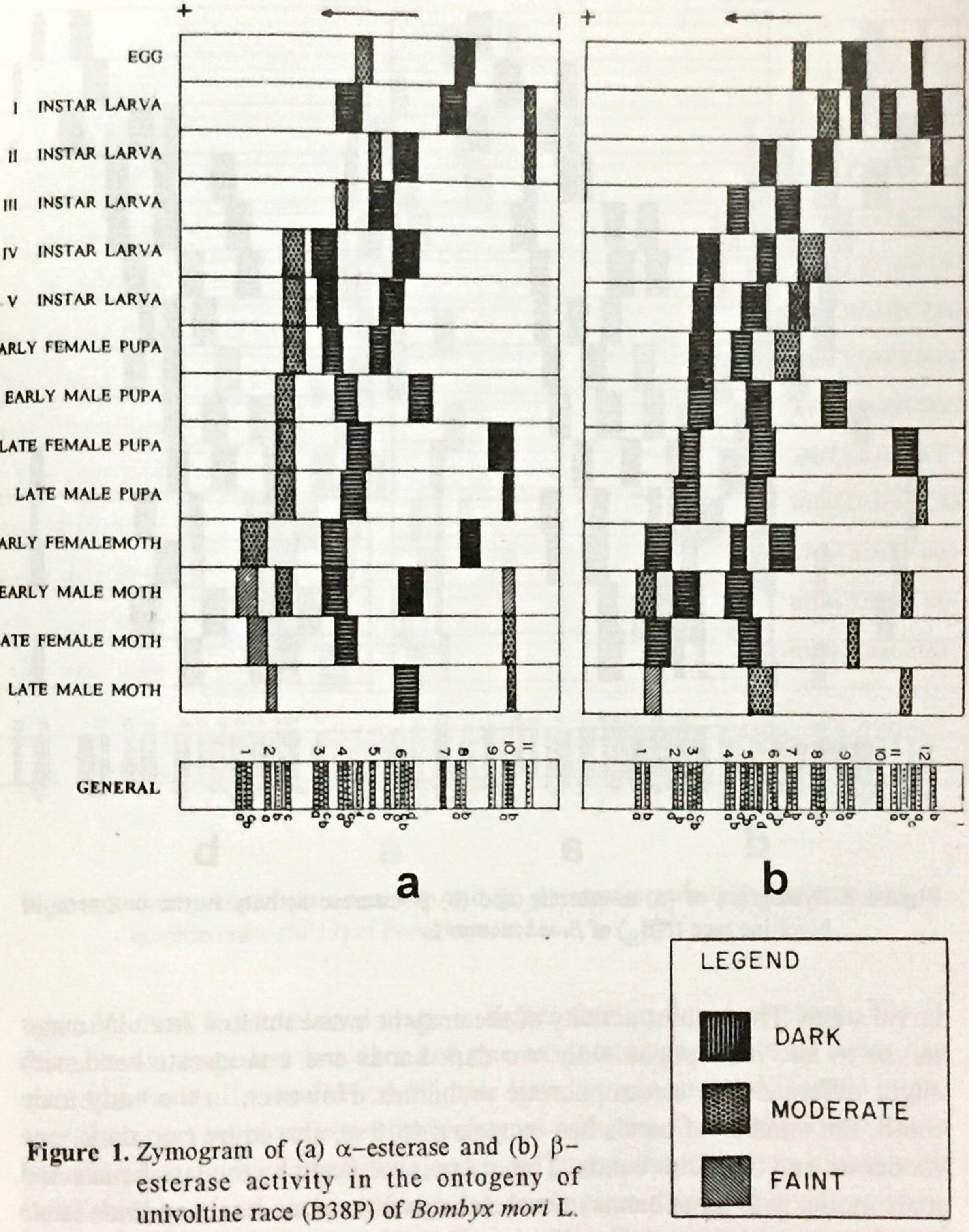

Figure 1. Zymogram of (a) α–esterase and (b) β–esterase activity in the ontogeny of univoltine race (B38P) of *Bombyx mori* L.

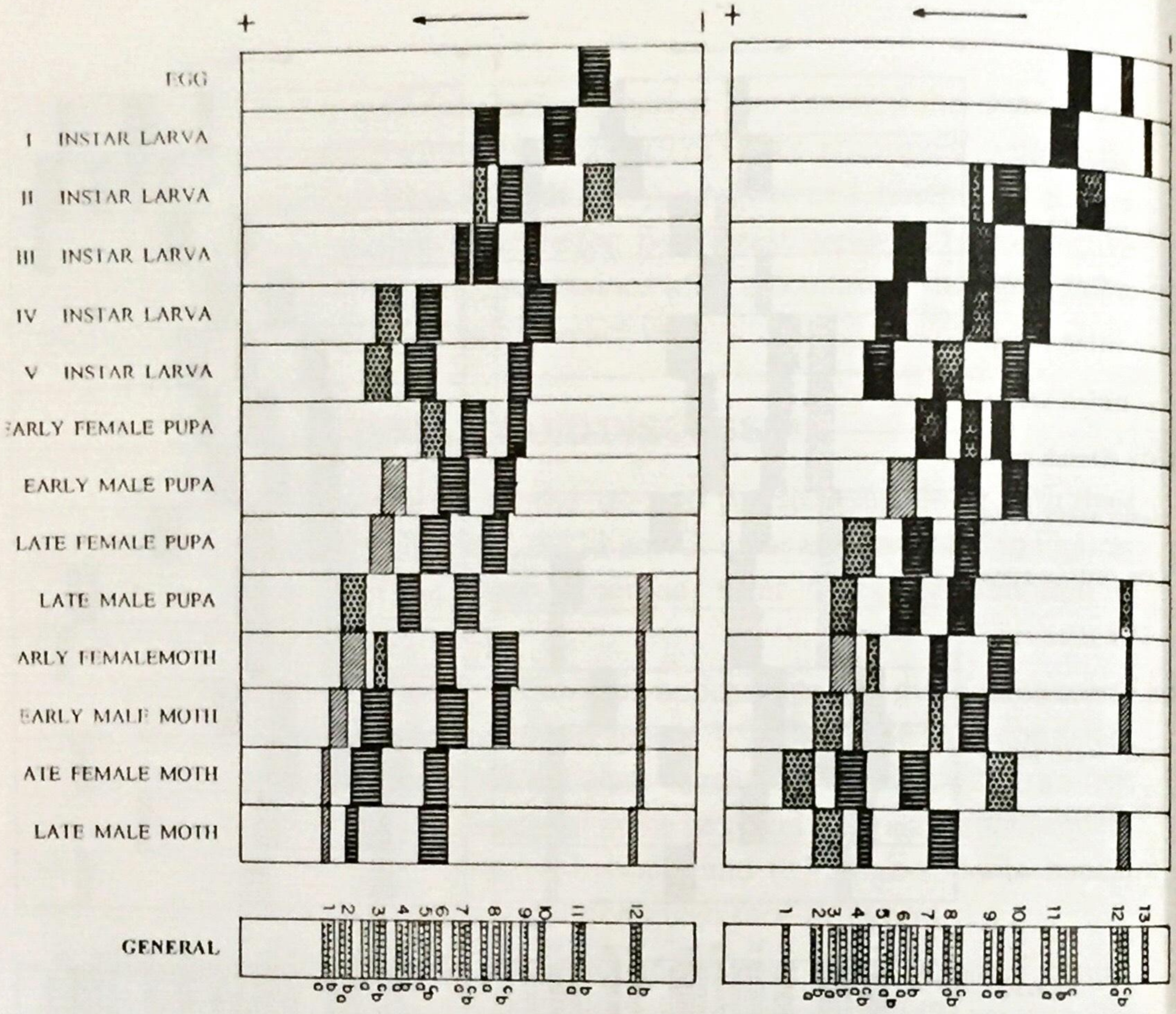

Figure 2. Zymogram of (a) α–esterase and (b) β–esterase activity in the ontogeny of bivoltine race (NB_{18}) of *Bombyx mori* L.

larval stage. The highest activity of the enzyme was exhibited from IV instar larvae to late male pupae with two dark bands and a moderate band with slight differences in electrophoretic mobilities. However, in the early male moth, the number of bands has increased to five, shared by two dark, one moderate and two faint bands. This stage is followed by the late female and male moths with three bands in each (one dark, one moderate and one faint) which vary in their mobility. Compared to the α–esterase, the β–esterase activity in B38P (Figure 1b) is slightly different both in regard to number of dark, moderate and faint bands, and in the total activity. The activity in the

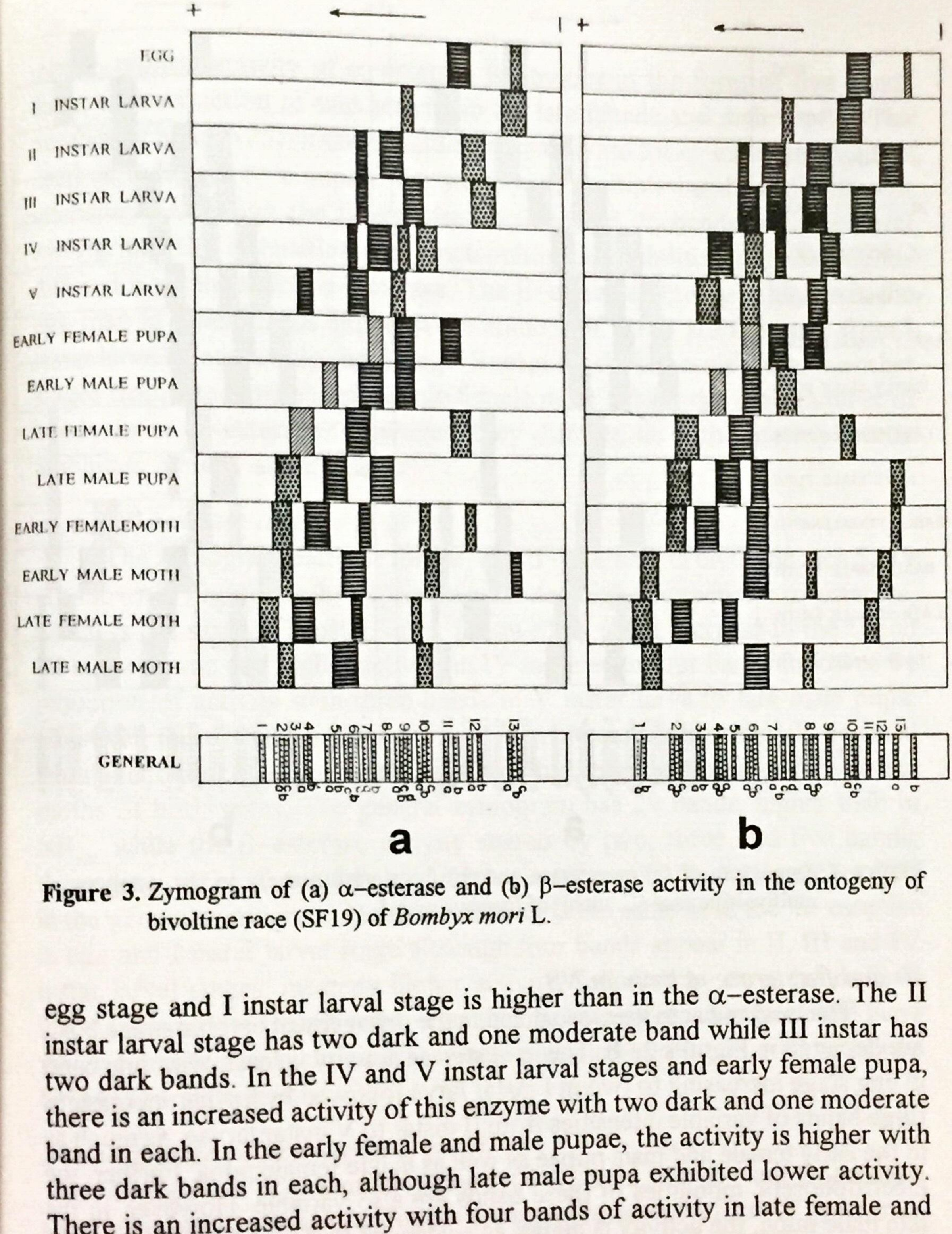

Figure 3. Zymogram of (a) α–esterase and (b) β–esterase activity in the ontogeny of bivoltine race (SF19) of *Bombyx mori* L.

egg stage and I instar larval stage is higher than in the α–esterase. The II instar larval stage has two dark and one moderate band while III instar has two dark bands. In the IV and V instar larval stages and early female pupa, there is an increased activity of this enzyme with two dark and one moderate band in each. In the early female and male pupae, the activity is higher with three dark bands in each, although late male pupa exhibited lower activity. There is an increased activity with four bands of activity in late female and male moths.

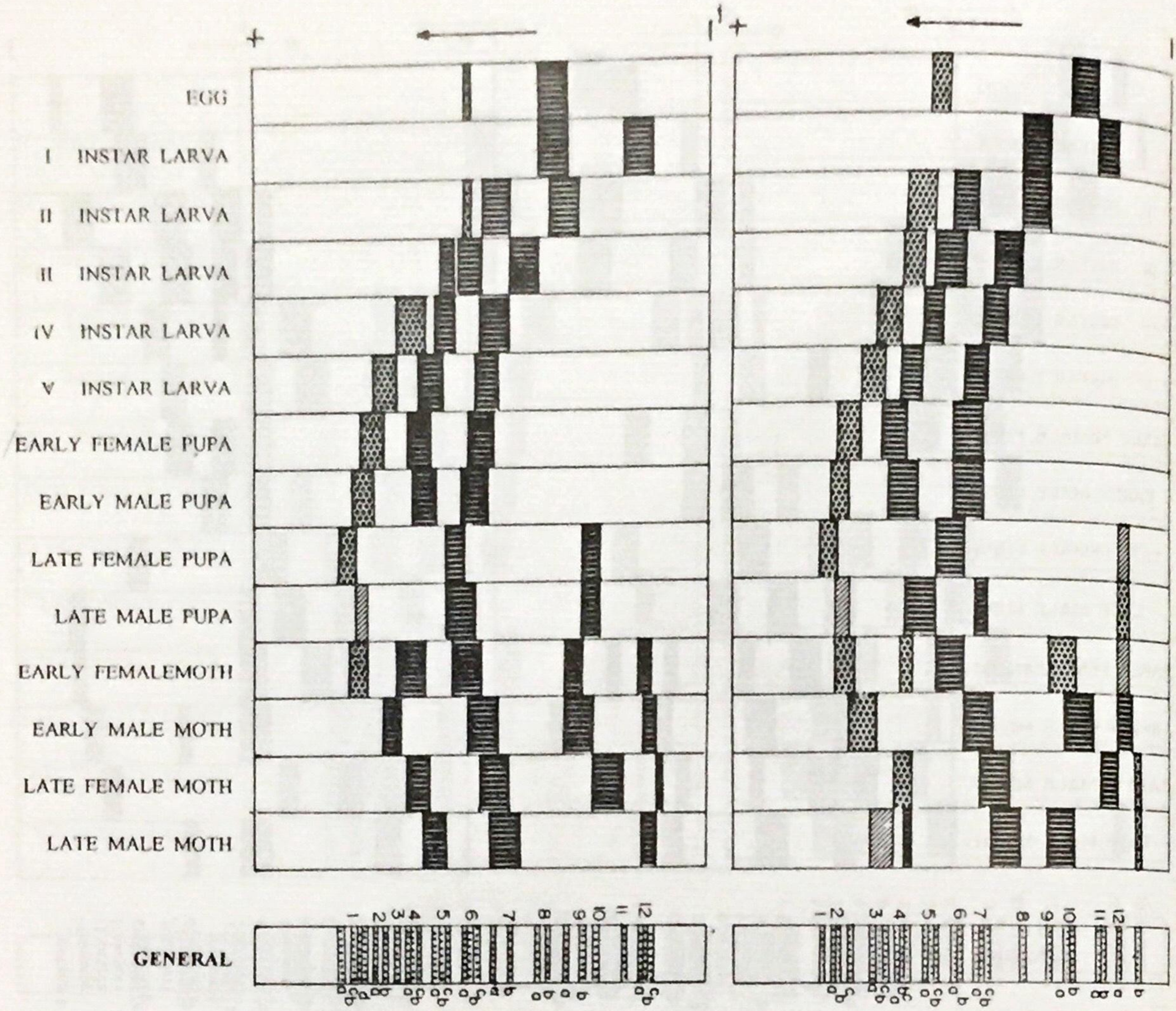

Figure 4. Zymogram of (a) α–esterase and (b) β–esterase activity in the ontogeny of multivoltine race (C. nichi) of *Bombyx mori* L.

α- and β-esterase activity in NB_{18}

The isozyme activities visualized in the ontogeny of bivoltine race NB_{18} are depicted in Figures 2a-b. The α–esterase activity is least, with one band in egg stage increasing to two in I instar larva followed by further increase to three bands of variable intensities from II instar to V instar larvae, as much as in the early female and male pupae as well as in late female pupa. Further, the electrophoretic mobilities of these bands are also variable. However, in the late male pupa, the activity is higher as evidenced by four bands represented by two dark, one moderate and one faint band. In the early female and male

moths, highest activity of α–esterase is obvious in the form of five bands each, with reduction of said activity in the late female and male moths. The number of bands (25) in the generalized zymogram for α–esterase could be used as indicator to compare the stage-wise multiple bands with different intensities. Although the β–esterase also showed 26 bands in the general zymogram, the intensities and electrophoretic mobilities of these bands differed from those for α–esterase. The β–esterase activity is higher in the egg than in other stages although the number of bands are the same from I instar larva to late female pupal stage. Except for the late male moth, the other stages from late female pupa to late female moth exhibit the same number of bands as in α–esterase. However, they differed in both electrophoretic mobilities as well as intensities.

α- and β-esterase activity in SF19

The zymogram patterns for α– and β–esterases in bivoltine race SF19 are shown in Figures 3a-b. Egg stage exhibited higher activity for α–esterase, which is the same in I instar larva, followed by slight increase in the II and III instar larvae and higher activity in IV instar with four bands followed by reduction of activity with three bands in V instar larva to late male pupa. However, there is higher activity in early female moth which slightly declined from five to four bands in later stages, with further reduction in activity in late moths of both sexes. The general zymogram has 29 bands, higher than in NB_{18}, while the β–esterase activity shared by two, three and five bands, depending on the stage of development, contributes to the total number (29) in the general zymogram. Here, the activity is the same as in the α–esterase in egg and I instar larval stage although four bands appear in II, III and IV instar larval stages, meaning higher activity, followed by three bands in V instar larva to late female pupa. The activity has increased in late male pupa which remained as such in early female and male moths, as well as in late female and male moths.

α- and β-esterase activity in C. nichi

In the case of the multivoltine race C. nichi, the α– and β–esterase activity in various stages of ontogeny are represented in the form of zymogram in Figures 4a-b. The general zymogram for α–esterase has 27 bands. In the egg and I instar larva, the activity is low with two bands while the three-

banded manifestation is clearly visible from II instar larva to late male and female pupal stages. The electrophoretic mobilities of these are quite variable with the highest migration towards anode and cathode in late pupal stages. The early female moth exhibited highest activity with five bands, while the early male moth and late female moth exhibited four each, but the activity declined in late male moth. There are a total of 26 β–esterase bands in the generalized zymogram of C. nichi. The activity for β–esterase is the same in the egg and I instar larva with an increased activity from II instar larva to late female pupa, with three bands in each, although their electrophoretic mobility differed from each other. However, in the late male pupa, the activity has increased to four bands followed by highest number of five bands in early female pupa. There is a slight decrease in activity in all three stages of early male, female moths and late male moth.

Analysis of isozymes through gel electrophoresis in recent times has been extensively employed to understand the molecular basis of differentiation as a biochemical manifestation of genome which was previously inaccessible. By such studies, it has been possible to understand the genetic relationship between different species, sub-species and races of plants and animals and at the same time to localize biochemical genetic markers in time and space during ontogeny. The present investigations undertaken to analyze the α– and β–esterase isozymes in four different races of silkworm *Bombyx mori* L. have revealed interesting information. In the univoltine race (B38P), the α–esterase activity which is initiated in the egg stage gradually increased in I instar to II instar larva, followed by a decrease in III instar larva, again increased in IV and V instar larvae up to the pupal stage. Later, it decreased in the female moth although increase was evident in the male moth. Compared to α–esterase, β–esterase activity in the egg stage of the univoltine race was higher, which slightly increased and again decreased in I and II instar larvae, decreased in III and IV instar larvae but increased in pupal to early moth stage. However, the activity was reduced in late moth stages. The same trend was manifested in α–esterase in the bivoltine race NB_{18} where the activity was highest in the early moth stage which declined in the late moth stage. Although the β–esterase activity in NB_{18} followed the same trend, the activity was lower in pupa and moth than α–esterase. In another bivoltine (SF19), although the trend was similar to NB_{18}, the α–esterase activity was

found to increase in IV instar larva, decline in V instar larva and again increase in early and late pupal stages and early moth stage. However, it decreased in late moth stage. β–esterase activity in SF19, although similar to that of α–esterase, exhibited the highest activity in V instar larva, followed by an increase in pupal stages irrespective of the sex which extended through the early moths to late moths of both sexes. In the multivoltine race (C. nichi), the overall activity in the egg stage was lower, compared to the univoltines and bivoltines. From II instar larval stage onwards, the activity increased up to late pupal stage. Further higher activity than the previous stages was evident with the five bands in early female moth, although the activity decreased in the late female and male moths. The β–esterase activity in C. nichi followed the same trend as α–esterase, with slight deviation in early moths where drastic change in activity was obvious. In all races, irrespective of α– or β–esterase, activity declined in the late moth stages. It is well known that esterases are transferred by male to female during mating which ensure higher fecundity. The higher the esterase activity, the higher is the fecundity. If the moths are not utilized for mating within 6-8 h following emergence, esterase activity is reduced. Hence, any later matings (stored moths) will obviously reduce fecundity. This is supported by the work of Aigaki et al (1987, 1988). Thus, the overall picture revealed that β–esterase activity is more variable with higher activity than the α–esterase in all the races studied. β–esterase activity was lower and less variable in some of the species of *Drosophila nasuta* group analyzed by Ramesh and Rajasekarasetty (1980). In this respect, the races of silkworm *Bombyx mori* studied exhibited higher β–esterase activity than α–esterase is, thus differing from *Drosophila*.

The above picture shows that each stage of development has different intensities and number of bands. This supports the fact that there is a differential expression of genes during different stages of development and that some genes are active in some stages while certain others are active in other stages of development. Moreover, all genes are not active at all times (Ramesh and Rajasekarasetty, 1980). The present findings of the authors also support the fact that different genes are switched on and off during different stages of development in time and space, depending on the needs of the developmental program.

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