Chapter 4: Discussion
Discussion

Racial diversity in silk producing insects has been considered as important parameters. The findings from the morphological observations, isozymes of esterase and amylase, haemolymph protein as well as certain element profile at certain larval stages of *Philosamia ricini*, collected from different localities of the NE region of India strongly advocate the existence of racial diversity. Morphological characters like colour of the wing, wing venation or the number of the segments in the antenna have been applied for racial identity and the data (Plate-I) indicate the presence of white plain as dominant character in *P. ricini* and is common in five groups viz. Bordour (25°17'N latitude and 91°38'E longitude), Dhanubhanga (25°37'N latitude and 90°16'E longitude), Khanapara (26°32'N latitude and 92°13'E longitude), Nangpoh (25°70'N latitude and 92°80'E longitude) and Titabor (27°3'N latitude and 94°5'E longitude) followed by blue plain in four groups viz. Bordour, Mendipathar (25°30'N latitude and 90°08'E longitude), Sillie (27°50'N latitude and 96°53'E longitude) and Titabor. The colouration white zebra was found in two groups in Bordour and Titabor and blue zebra in two groups in Bordour and Mendipathar (Table-1). The red eye colour as dominant was recorded in 8 (eight) groups viz. BWp, BBp, BWz, KWP, MBp, NWp, SBp and TWp, followed by brown eye colour in 5 (five) groups viz. BBz, DWp, MBz, TBp and TWz. Variations observed in wing colour suggest dark brown as dominant character, found in nine groups viz. BWp,
BBp, BWz, BBz, DWp, SBp, TWp, TBp and TWz followed by reddish brown (in three races) viz. MBp, MBz and NWp and golden brown in Khanapara white plain (Plate-II-IV). However, larval weight, cocoon assessment, wing venation and their measurement could not support any racial variation in this investigation. The racial observation obtained in *P. ricini* (*Samia cynthia ricini*) possibly could not be establishing the diversity as such, since many of the characters are environment dependent. Tikader et al. (2000) based on morphological variation suggests a number of variables in mulberry plants and view expressed by Harini et al. (2000) has been quite logical. This group of workers further emphasized that morphological descriptions are mainly taxonomical in nature and supposed to be heritable and helps in the identification of species.

The amylase from the silkworm have been the point of attention of sericulturists since 1934 (Matsumura, 1934) and a number of studies concerning the properties, activities and polymorphism of amylase isozymes have since been advocated (Hara et al., 1984; Asakawa and Hanano, 1989). The enzyme helps in the digestion of starch in the food plant; however, most of the studies are confined to the strain (*B. mori*) reared in the temperate zone and the oriental strains (Abraham et al., 1992; Chatterjee et al., 1992).
The electrophoretic pattern of digestive amylase with various pixel intensity in different groups confirms the presence of ecoraces. The anodic digestive and haemolymph amylases, although very low in activity, were ubiquitous in all the groups (Table-4). Earlier, Abraham et al. (1992) demonstrated the cathodic digestive haemolymph amylase, which was detected only in the geographically distinct populations of non-diapausing strains of B. mori, categorically supports the present findings. The isolated amylase has been purified from the silkworm and found to have subunits with molecular weight of 55kDa (Kanakatsu, 1978). The cathodal amylase-banding pattern observed in the non-diapausing strain resembles that of Drosophila hydei (Daone et al., 1975) Chicken (Lerner and Malacinski, 1975) and in human (Karn and Rosenblum, 1975). This group of author has convincingly demonstrated amylase heterogeneity in human subject, is due to deamidation. It is possible that the multimeric form of the cathodal amylase isozyme observed in the digestive fluid of non-diapausing strain may be the result of posttranslational modifications such as deamidation or could be multimeric form of the same polypeptide.

The increased activity of amylase and its diversity i.e. multimeric expression in terms of pixel intensity observed in the investigation may have adaptive significance and known to survive better under sub-tropical condition also recorded by Murakami (1994). The effectiveness of amylase in survival has been reported in insect species such as Tenebrio molitor (Buonocore et al.,
1976) indirectly supports the existence of racial diversity under different ecological conditions.

A total number of 4 anodic bands of amylase were detected in the 2nd and 3rd instar developmental stages except in Mendipathar group and only 3 bands in Titabor group (Table-4; Plate: IV.1 & IV.2). In the 4th instar 5 bands were seen except in Mendipathar and Nangpoh, where 4 bands were observed (Plate: VI.3) while in the 5th instar again 4 bands were observed with the exception in Mendipathar blue plain and in Titabor races 3 bands were observed while in Mendipathar blue zebra and Nangpoh 5 bands were recorded. The moving anodic bands indicate that the intensity of some concentrated bands changes during the larval period (Plate- IV.4). The pixel intensity recorded during this investigation demonstrated a general trend of increased tendency up to 3rd instar, with a decline in 4th stage, while increasing during the 5th instar larval period. The mid gut PAGE of amylase of *P. ricini* has demonstrated a clear signal of variation in all the 4 bands (Table-4; Plate-IV). Promboon et al. (1993) were able to find out 24 strain of polyvoltine silkworm based on the amylase properties and activities in different larval stages. The strains with high amylase activity detected are valuable for future breeding programme (Chatterjee et al., 1992). The highest activity of the gut amylase was detected at an alkaline pH (9.8) in the BWp, BBp, BWz and BBz and the lowest was MBp and SBp (Table 11). It has been logically concluded that amylase in polyvoltine may contribute
to the hardy character of the race. The significance of amylase isozyme variation in *P. sativum* in terms of ungerminated seeds (Przybyiska et al., 1985) and wheat differences (Nishikawa et al., 1988) extends the validity of this method in the process of determination of racial variation in eri silk.

Matsuo et al. (1999) recorded the relationship between the net change of molecules and their mobility on electrophoresis in *Drosophila* alpha-amylase. The amylase isozymes in *D. melanogaster* classified into 3 groups, I (AMY 1, AMY 2 Amy 3-A), II (AMY 3-B and AMY 4) and III (AMY 5, AMY 6-A and AMY 6-B) based on the differences in the reference sites. These results were confirmed by the analysis of 38 amino acid sites with charge difference in *Drosophila*. Although such analysis could not be performed in this experiment, yet the records of pixel intensity of amylase isozymes favour the distinctive variation in ascertaining the development/presence of ecoraces within the species collected from different places of the North Eastern region of India.

The apparent association of the activity of amylase in the digestive juice with cocoon characters induced a good deal of studies and its genetic variability (Hirata, 1974; Kanakatsu, 1978; Hara et al., 1984, 1986; He et al., 1998). Hirata (1974) analysed the relationship between amylase activity in the larval digestive juice and several quantitative characters using bivoltine strains with high and low digestive amylase activity. His results showed higher cocoon weight,
cocoon shell weight, shell percentage, and cocoon shell productivity per day in the 5th instar and survival rate for larva with a high amylase activity. Further, the significant differences were more apparent when larvae were fed with hardened or artificial diets. Genetic variation of regulatory genes with respect to the amylase activity in different developmental stages (developmental inducibility) as well as stage inducibility has been reported in ten isozymic strains in *Drosophila melanogaster*. Genetic variability of the activities of the amylase indicates that two types of regulatory gene polymorphism behaves differently in nature and that the regulatory pattern of the enzymes found in different environmental stages under normal environment have little to do with the adaptability (Tsuneyuti, 1986). However, the relationship between the ecoraces and the environmental parameters (Table-M1) is a clear signal for the ecorace formation.

Studies on the amylase isozymes of *D. melanogaster* have uncovered evidence that the duplication phenomenon may indeed result in the production of different isozymes (Kikkawa, 1964 and Done, 1969a; 1969b). Seven major amylase, have been distinguished, all of which apparently are coded for by one gene for amylase located in the 2nd chromosome. Eight different genotypes have been detected, one of which in amylase gives only one band in gel, but the others all extends two. Heterozygotes formed by crossing those strains producing
different isozyme patterns combine both patterns and do not show hybrid enzyme formation (Wagner and Selander, 1974).

When other strains of *D. melanogaster* and 42 other species of *Drosophila* were examined for amylase content, it was found that all had but one major band. In the *melanogaster* strains, this band corresponds to band 1 present in the amylase strains. This may be the product of the ancestral gene before gene duplication and mutation occurred to produce two band strains with the appearance of two closely linked amylase genes (Wagner et al., 1974) and this view may be extended to the amylase variability observed in different groups of *P. ricini*.

Enzyme electrophoresis and interspecific hybridizations, its adequate estimation in lower taxa opens a scope in *Lepidopteron* at subspecies and species level (Lorkovic, 1985). The intra- and inter-specific relationship of tetraploid ryegrass cultivars based on isozyme variation on electrophoresis was studied by Taiji (1986) and established the distinctive differences between the allele frequencies of *Lolium* species. Badino et al. (1988) had shown that esterase allozyme variation through electrophoresis helps in determining the variation in *Apis mellifera* and the same could be extended in support of racial variation of *P. ricini*. 
A total number of 3 moving anodic band of esterase detected in the different developmental stages in all the 13 ecoracial groups of *P. ricini* except in the Nangpoh group with only two bands (Table-5 i, ii, iii). The moving anodic bands indicated that the intensity of some concentrated bands changes during larval development. The pixel intensity of the 3 different band (I, II and III) has been observed to maintain identical trend from 2\textsuperscript{nd} to 5\textsuperscript{th} stages in all the groups except Nangpoh white plain (NWp), where only two bands were recorded. The trend of increased pixel intensity from 2\textsuperscript{nd} to 4\textsuperscript{th} and there after (in the 5\textsuperscript{th} instar) their decline suggests the involvement of this non-specific esterase in the fibroin and sericin synthesis. However, it is not known which one of the three bands infact used to enter in the synthesis of silk from the early part of the 5\textsuperscript{th} instar. The NWp group has been maintaining distinctive racial identity only with two bands (Plate-V.1-V.4). It is also clear that the pixel intensity for different groups are also not identical in all the groups which might be due to the variation of the expression of the esterase gene influenced by the environmental factor as well as geographical location. The characterization of the genetic expression needs further elaboration.

The possibility of the influence of climatic variation (abiotic factor) in determining the protein variation within the individuals of same species of an insect cannot be ruled out. Rockwood-Sluss et al. (1973) examined the correlation of principal component of variation from genetic loci with a measure of climatic variability
and with various aspects of the chemical composition of the cactus. Only acid phosphatase variation was correlated with climate, but variation at this locus and at two esterase loci was correlated with the sterol level in the cactus, suggesting a direct allozyme genotype-environment interaction (Wagner & Selander, 1974).

Application of the zymogram technique to taxonomic problem confirmed classifications based on morphological, cytological, behavioral and other more conventional characters. Allozymes may prove to be essentially valuable in the analysis of kin selection and other genetic aspect of sociality in insects (Hamilton, 1972). Gui-Qing and Huang (1988) demonstrated the protein patterns and esterase zymograms of one isolate of the known, monotypic species of the genus *Synecephalastrum* after PAGE. Marker differences of the form taxa and similarities of different isolates in the same taxon were shown in both protein patterns and esterase zymograms. The pattern of expression of esterase isozyme of carps were compared with red tigerheaded goldfish in order to shed light on the population relation to establish the variation and thus helps in establishing the esterase as tool (Wang and Wang, 1988). These results were helpful in confirming the establishment of new varieties as well as their inclusion in the same species. Esterase isozymic analysis and other isozymic examinations might have the potentiality to be applicable for the
Esterases, many of which have unusually broad substrate specification are the most highly polymorphic enzymes in insects and other organism. Over a dozen alleles have been identified at esterase loci in local populations of butterflies and the olive fruit fly. In these insects and in many species of *Drosophila*, silent or null allele occurs in high frequencies. But in population of cricket *Gryllus integris*, 24 alleles, none of which are silent, were detected at an esterase locus. Six-esterase isozyme during the developmental stages of *Anopheles darlingi* using PAGE showed the variation in their electrophoretic mobility (Joselita et al., 1986). Protein and non-specific esterase activities were also tested in pherate adult and emerged adult ovaries and in mature eggs (Kai et al. 1972). Recent studies on the racial divergence at cytological level has recently been detected in the *nasuta-albomicans* complex of *Drosophila*. Esterase analysis in six races revealed the presence of nine alleles of which only 1-Est$^{1.0 (d)}$ and 1-Est$^{1.25}$ were showed, (Tanuja et al., 2003; Aruna and Ranganath, 2004) although such interracial hybridization has not been extended in this investigation. Therefore, the possibilities of the esterase allozyme variation in the determination of ecoraces of *P. ricini* may be accepted.
Esterase isozyme, its significance in variation in *P. sativum* in terms of ungerminated seeds extends the validity of this method in the process of determination of racial variation in eri silk can be argued logically (Hanna et al. 1985). Crude mosquito homogenates were electrophoresed on gradient and on polyacrylamide gels and stained for esterase to demonstrate the high resolving power of the polyacrylamide gradient gel system (Novak et al. 1985). Electrophoretic mobility of esterase and its genetic variation in 107 inbreed strains of rat was distinguished by the use of electrophoresis technique (Von et al., 1988). The use of esterase isozymes in *O. spontanea, O. glaberrima* and *O. sativa* through electrophoresis had well established the similarities and dissimilarities in zymogram and has emphasized the esterase as markers of morphogenesis (Maheswaran et al., 1988).

Insect haemolymph protein (HP) contains many different proteins with a variety of functions. The total quantity of proteins are classified by function, are the storage protein, lipid transport proteins, vitellogenins, enzymes, proteinase inhibitors, chromoproteins and a range of various proteins probably involved in the immune responses of insects. The main class of storage proteins in Lepidoptera is the arylophorins in which phenylalamine and tyrosine comprise 18-26% of the total, in addition to 8% methionine rich proteins (Wheeler and Buck, 1995). Laufer (1960, 1981) was successful to demonstrate that much haemolymph protein in lepidopteran insects act as specific enzymes including
esterase, phosphatase, carbohydrases sulphatase, tyrosinase, chymotrypsin and dehydrogenases. The occurrence of tyrosinase in *Bombyx* (Ito, 1954; Valivittan et al., 1998) and trehalase in *Phormia* (Friedman, 1968) has been recorded. Thus the variation of pixel intensity observed during this investigation is only an affirmation of the above views, which possibly involved in different enzyme substrate reaction. It is known that haemolymph proteins are generally synthesized by the fat body (Shigematsu, 1960) and some of these proteins are abundant and exhibit a developmental stage specific protein and cessation of feeding with a rise in ecdysteroid titer. In this aspect Bosquet et al., (1989) was successful to demonstrate the effects of juvenile hormone on the major haemolymph protein with the suggestion that different regulation process are involved. In *Dysdercus koenigii*, protein band 3-5, found in all the stages of larval development, but absent in adult could be regarded as larval-specific proteins and bands 1 and 2 persisting in the adult, absent in the larval stages, as adult-specific proteins (Bhola, 1992). The band 7 and 8 has been considered as vitellogenins since they fulfill the criteria laid down for such protein. In *D. koenigii* bands 3-5 found in all the stages of the larva but specific proteins band 7 present in the IV and V instars and bands 11, 12 only in the 5th instar as instar-specific protein and bands 1,2,13, 14 appearing and persisting throughout the adult life, as adult-specific protein (Bhola et al., 1986). The massive accumulation of protein in the haemolymph of this stage is very important for the overall developmental economy of the insect, since it serves
as a major protein reserve for the adult differentiation. These proteins may be highly utilized in the preparative stage of development and some part may be stored in fat body in the form of protein granules (Tajo et al., 1980).

Electrophoreograms of the HP in the larval stage of *P. ricini* from 2nd to 5th instar suggest racial variation among the thirteen groups. Of these thirteen groups the eight groups viz. BWp, BBz, BBp, BWp, DWp, TWp, TBp, TWz demonstrated 27 numbers of band in their haemolymph (Plate-VI.4), while KWp, SBp with 23 bands (Plate-VI.4) and NWp with 26 bands (Plate-VI.4) whereas MBp and MBz presented 18 electrophoreogram (Plate-VI.4). The recorded electrophoretic pattern with definite and specific protein bands of various developmental phases could be argued with the earlier observation of Wyatt (1961). These variations are certain to take place during the developmental stages from 2nd to 5th instar (KWp-24; MBp-20; MBz-20; SBp, NWp-25) and these findings could be supported with the report of *Bombyx* and *Galleria* (Denuce, 1958). Each protein component appears at a definite stage and demonstrates its own pattern of change in concentration advocates that the synthetic process is under the genetic control (Duke and Pentelouris, 1963). Munoz-Parra et al. (1988) by use of SDS-PAGE technique tried to separate the distinct protein bands and its characterization in male and female *Bovicola carpaee*. Therefore, it is quite common to have variation in the haemolymph protein during the developmental stage of *P. ricini*. 
Investigation in the HP of this insect during larval development shows quantitative variation as in other insect (Table-10). This change has been attributed to histogenesis and histolysis of various tissues during metamorphosis involving extensive breakdown and replacement (histolysis and histogenesis) of the larval tissues, there is need for a much greater variety of building materials including the silk protein since these proteins must be synthesized and stored before the onset of need (Laufer, 1960; Chen and Levenbook, 1966; Chippendale, 1970). Generally most of the earlier observations indicate progressive increase of protein concentration from early larval development, attained maximum at the last larval stage and dropped in the pre-pupal stage. The variation in the HP concentration during the development of several lepidopteron is essentially identical (Chefurka, 1953 on H. cecropia; Ludwing, 1954 on P. japonica; Wyatt et al., 1956 on B. mori and Laufer, 1960 on Samia Cynthia ricini). It has been ascertained that the blood protein rose abruptly from the 3rd instar to a maximum in the 5th instar there being little or no change during spinning and in the first part of pupal period. The sharp increase in HP concentration in eri silkworm is attributable to intense feeding during late larval stages (Wyatt et al., 1956; Laufer, 1960); similar trend also reported in P. brassicae by Chippendale and Kilby (1969). Earlier Hill and Goldsworth (1968) opined that blood protein level merely reflects the nutritional state rather than the physiological process occurring in the larval growth. Although there is no substantial evidence to support this view yet another
logical point is that the silk gland differentiation is started from the 3rd instar stage and certainly some of the HP are used in the synthesis of silk.

The number of protein bands in different species for example 11 in *Drosophila melanogaster* (Duke, 1966), 17 in the *Prodenia litura* (Srivastava and Pareek, 1977), 22 in the *Dendrolimus spectabilis* (Yoo and Lee, 1974), 18 in the *Ephestia kuhniella* (Yoo and Lee, 1973) and 14 in the *Samia Cynthia* (Zaman and Chellapah, 1967) and a drastic change in the HP pattern takes place during the larval development. Thus the larval development marks the appearance of a large number of protein bands and variation in the HP banding pattern is a clear message for the presence of ecoraces.

The 27-moving band of protein detected in the haemolymph of different stages in all the 13 groups of *P. ricini* collected from different places of NE region indicate that the intensity of some protein bands change during the larval development. It is quite indicative that the rise and fall in different quantitative protein concentration is not related to general increase or decrease in all the protein, rather in some individual components (Telfer et. al. 1953; Chen, 1971). The variation in the pixel intensity(s) of the total number of 18, 23, 26 and 27 band during their life cycle irrespective of season, strongly favours the formation of ecoraces. Further characterization of the consolidated four groups with 18, 23, 26, and 27 numbers of protein bands in the identification of ecoraces finds a
favourable recommendation. Evidence in favour of the use of protein band with the help of pixel intensity in the identification of ecorace has been reported for the first time. However, it may be possible to identify, four distinct groups within 13 ecoraces may be a useful tool in sorting out the high yielding variety. The marker assisted racial distinction is being applied in understanding the molecular basis of defense mechanism and development of transgenesis for the transfer of genes.

Comparison between the overall HP concentration and the amylase activity suggest a positive correlation throughout the larval period of *P. ricini* (Fig-D1; Table-A1). The functions of haemolymph and gut amylase might be a part of major HP (27 band) is not fully understood, although Wyatt (1967) suggested its possible involvement in the degradation of fat body glycogen. The presence of this enzyme in abundance during larval development in diapausing and non-diapausing insect imply that this enzyme have some important physiological role (Wyatt, 1967). A significant correlation has been obtained between the 3rd band of esterase and the 2nd, 3rd and 4th band of amylase during this investigation (Table-A2).

Significant relationship was also recorded between amylase 1st, 2nd, 3rd and 4th band of amylase and protein (Table A7) whereas, a positive correlation was observed between the 3rd band of esterase and the amylase and protein
quantity and a negative correlation was recorded between the 2nd band of esterase and the amylase and protein quantity (Table- A3). Thus this analysis may possibly be applied for the possible occurrence of ecoraces in *P. ricini*.

![Protein - Amylase](image)

**Fig-D1: Comparison between the haemolymph protein concentration and the amylase activity**

Atomic absorption spectrophotometry, is one of the most advanced methods for quantitative determination of low concentration of element in biological materials. The significant variation of element profile viz. Cu, Zn, Mg, Fe, Se in different groups of population of larval silk gland of *P. ricini* were recorded and favours the existence/formation of ecoraces within the species and such an argument has been made for the first time in this investigation. The result of certain element qualification shows that Mg is dominating in 7 eco-groups (BWp, BBp, BWz, DWp, KWp, MBp, NWp) followed by Zn in 5 eco-groups (MBz, BBz, SBp, TWp, TBp) and Se in one eco-groups (TWp, Table-D1).
Investigation of mineral requirement in insect is probably the most neglected area of research with respect to nutrition. Minerals are not synthesized within insects although they are essential element and affect various metabolic processes (House, 1967).

Table D1: Quantification of element in ascending order.

<table>
<thead>
<tr>
<th>Group</th>
<th>Element</th>
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<tbody>
<tr>
<td>BWp</td>
<td>Cu (1.19) Fe (3.27) Se (18.23) Zn (51.72) Mg (169.43)</td>
</tr>
<tr>
<td>BBp</td>
<td>Cu (1.72) Fe (3.73) Zn (176.72) Se (188.43) Mg (200.02)</td>
</tr>
<tr>
<td>BWz</td>
<td>Cu (2.20) Fe (7.28) Zn (100.27) Se (163.35) Mg (390.53)</td>
</tr>
<tr>
<td>BBz</td>
<td>Cu (2.19) Fe (21) Se (74.42) Mg (171.08) Zn (191.09)</td>
</tr>
<tr>
<td>DWp</td>
<td>Cu (4.26) Fe (20.80) Se (24.95) Zn (106.95) Mg (159.81)</td>
</tr>
<tr>
<td>KWP</td>
<td>Cu (3.01) Fe (3.64) Se (30.40) Zn (58.05) Mg (165.57)</td>
</tr>
<tr>
<td>MBp</td>
<td>Cu (3.20) Fe (3.50) Se (33.80) Zn (107.82) Mg (176.41)</td>
</tr>
<tr>
<td>MBz</td>
<td>Cu (2.61) Fe (2.70) Se (98) Mg (165.99) Zn (432.74)</td>
</tr>
<tr>
<td>NWp</td>
<td>Fe (0.40) Cu (1.50) Se (2.16) Zn (19.65) Mg (22.43)</td>
</tr>
<tr>
<td>SBp</td>
<td>Cu (2.49) Fe (3.23) Se (109.28) Mg (166.26) Zn (215.99)</td>
</tr>
<tr>
<td>TWp</td>
<td>Cu (0.29) Fe (3.02) Se (69.94) Mg (195.97) Zn (241.40)</td>
</tr>
<tr>
<td>TBp</td>
<td>Fe (2.16) Cu (2.66) Se (70.23) Mg (80.92) Zn (114.95)</td>
</tr>
<tr>
<td>TWz</td>
<td>Cu (3.61) Fe (4.21) Mg (35.14) Zn (80.38) Se (98.53)</td>
</tr>
</tbody>
</table>

House (1974) recorded the requirements of minerals in *Bombyx mori* yet it was only Subburathinan & Krishnan (1992) who suggested for the first time the
change in two elemental composition in disease infected mulberry leaves and the economic characters of cocoons (Radha et al., 1988). However, no information is available till to date on the elemental analysis on *P. ricini*. Thus the present study has been able to elucidate only a little aspect on the non-mulberry elemental composition.

Variation in the elemental concentration observed might be due to the soil nutrient, but the distinction of BBz from other Bordour group could not be explained with the help of the available literature. Fe was recorded in between 0.40-21.00µg/gi; and the highest quantity (21.00µg/gi) was observed in Bordour blue zebra. The Fe quantum was noted to be lower in the Mendipathar blue zebra. Ito & Nlimura (1966) and Horie et al. (1967) reported that Fe and Zn played a significant role in the growth of silkworm and improves the cocoon weight, cocoon size and rearing percentage (Viswanath and Krishnamurty, 1982). Winzerling and Law (1997) stated the importance of Fe and Cu in insect nutrition. Iron as trace element occupies the second position in the ascending order in 11 groups while it occupies the first position in ascending order in NWp and TWz. (Table-D1) The relationship between Fe and amylase also suggests an interesting behavior in DWp and KWp (Table-A3). The importance of Fe in metabolism has been reviewed by Helen et al. (2002). The relation between the trace element (Fe, Cu, Zn) and physiological significance were discussed in *Diphyllobothrium macroovatum* and *Diphyllobothrium balaenopterae* (Yamane
et. al., 1986). Even Fe deficiency alters GABA and glutamate metabolism in rat brain (Shukla et. al., 1989) though such direct evidence are scanty in insect.

Figure D2 shows homogenous distribution of Fe quantity in all the groups except in BBz and DWp group. The significant role of Fe in insect life cycle has been described by Winzerling (2002), however, its reduced value in BBz and DWp races could not be explained.

Recently, Kling (2003) discussed in detail about the Fe metabolism and its sequential effect at intracellular level along with the sequencing of transferrins. However, the precise role played by transferrins in insect physiology is not known. The correlation between the behavior of gut amylase and Fe content could not be understood well.

![Protein - Fe](image)

**Fig-D2: Comparison between the haemolymph protein concentration and iron**
Shymala and Bhat (1968) in their technical report suggested that Mg is essential for the silkworm diet, where they indicated that the efficiency of Mg utilization was up to 20-25% and thus, the utilization by larvae has resulted in better cocoon weight and cocoon yield. Viswanath (1979) found good response to Mg and Mn in silkworm economic characters. According to Loaknath and Shivshankar (1985) foliar spray of Mg (1.25 kg/ha), Fe (2.50 kg/ha) favourably influenced the cocoon yield and shell percentage. Thangavelu & Bania (1990) reported that Mg reduces the larval duration and increase the oviposition rate in the adult and silk content in the cocoon of B. mori. The role of Mg in Lepidopteran physiology is immense since it improves phosphorus uptake necessary for enzyme action helps in the synthesis of fibroin protein (McNamara et al., 2002). Mg was detected in between 22-391 μg/g. The Mg level in the 5th instar larva was recorded as 75.6 μg/ml in the B. mori against 391 μg/ml of silk gland of P. ricini and 64.6 μg/ml in Barathra barassicaea (Naoumoff & Jeunioux, 1970).

Zn in the silk gland is regularly present (19-433 μg/g). Zn is required for the protein and RNA synthesis and for the activation of as many as 80 enzymes. The pupal weight and length of the filament increase in B. mori after foliar spray of Zn (Loaknath and Shivshankar, 1985). Zn is an essential element in reproduction and hence they are useful in the seed production. The pupal weight and filament length of B. mori were increased when foliar spray of Zn
was employed with mulberry (Viswanath, 1982). Figure D3 demonstrates a significantly low Zn and moderately higher quantity of protein in NWp. Characteristics of amylase activity and the Zn quantity in MBz and NWp extends favourable negative relationship (Fig-D4).

Cu concentration in the silk gland was determined in between 0.29-4.27μg/g. The Cu is essential for insect physiology (Devi, 1993). Cu deficiency inhibits cytochrome oxidase in the hemolymph and provides catalytic oxidation in the formation of melanin pigment of insect cuticles.
Figure D5 demonstrates a low Cu with higher HP in TWp and higher protein and higher Cu in TWz whose relationship could not be argued with the help of presently available literature. Higher amount of Cu and low amylase activity has been obtained in KWp, MBp, MBz, NWp, BBp and TWp (Fig-D6, Table-A4)
Figure D7 thus demonstrates the protein quantity and Mg concentration except in NWp and TWz, extends a message possibly for poor quality thread, however, needs characterization. Further, the first band of amylase and total amylase quantity with Mg suggest a positive correlation (Table-A4 & A7). Significant relationship between the amylase 1st band and Mg suggest, the involvement of the Mg in the protein synthesis. The peculiar observations made during the investigation are negative correlation between esterase 1st band and Mg (Table A5), positive correlation between Mg and Se (Table A6) and between amylase and Mg (Table A7).
It has been recorded that highest Mg and amylase in BBz while the reverse result has been seen in SBp. Such diverse finding could not be explained from the physiological point of view, yet it shows the race variability (Fig D8).

Se was detected in the silk gland of all the races. The highest quantity of Se was detected in the Bordour blue plain (186.43±2.44μg/g). Se is necessary for growth and fertility in animals and for the prevention of many diseases. Se exert antioxidant effects and because of the known nutritional interrelationship between Se and vitamin E it is fair to assume the sole metabolic function of Se is that of a non specific antioxidant which gives protection against peroxidation in tissue and membranes. Se has the capability of replacing the vitamin E (Diplock, 1973). Although the direct application of Se on the host plant nutrition, yet the spider mites fed with nitrogen and phosphorous has been proved to be vulnerable in terms of malathion susceptibility, indicates the significant importance of trace element in nutrition (Henneberry, 1964). Figure D9 of this investigation shows once again low quantity of Se in relation to protein in BWp and NWp.

Se and total amylase of the haemolymph suggest peculiar phenomenon (Fig-D10). The DWp, KWp, MBp and NWp have demonstrated the decline of amylase activity with the fall of Se concentration. The BWp has been seen to be
negatively correlated while the other groups like BBp, BWz, BBz, TWp, TBp and TWz showed definite positive correlation.

![Protein - Se graph](image1.png)

**Fig-D9** Comparison between the haemolymph protein concentration and selenium

![Amylase-Se graph](image2.png)

**Fig-D10** Comparison between the amylase concentration and selenium

It is very hard to extend a possible explanation to such type of behaviour observed during this investigation. A good number of publication on the role of Se as an essential element has been published, but with little to do with the role of Se in insect biology. Recently, Trumble (2002) described one impact of Se on plant-insect metabolism. The property that allows Se to be active at
physiological and synthetic level at dietary level is less than 1ppm (Mass, 2003).

Locations of elements were analysed by EDX (JFM-35CF, JEOL), which showed variation of the 4 groups (Group-I Bordour, Group-II Dhanubhanga, Group-III Khanapara, Nangpoh, Mendipathar and Group-IV Sillie and Titabor) in the distribution of certain element. (Fig. D11)

Thus it is reported for the first time that even the element distribution extends the occurrence of racial diversity in P. ricini. Therefore, it would be possible to find out the distinct races for genetic improvement. Thus the various characters observed in this investigation reflect the lack of correlation for diversification in a
multidimensional process with drift, possibly playing a significant role. Hence a precise formulation for these events could not be designed in this investigation. It is arguably true that this relationship may suggest racial diversity, needs full classification, however, a new approach has been emerged from this investigation.