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Studies on the ontogenetic changes in the isozymes in *Drosophila nasuta nasuta* and *Drosophila sulfurigaster neonasuta*

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Abstract. Ontogenetic manifestations of four isozymes namely, acid phosphatase, alkaline phosphatase, α-esterase and β-esterase have been analysed in two closely related species—*D. nasuta nasuta* and *D. sulfurigaster neonasuta*. By adopting polyacrylamide gel electrophoretic technique 15 different developmental stages have been assayed. Electrophoretically homologous and distinct enzyme phenotypes for each enzyme system have been recognised in the two species under study. The implications of these findings are discussed.

Keywords. *Drosophila*; Isozymes; Ontogeny; *Drosophila nasuta nasuta*; *Drosophila sulfurigaster neonasuta.*

1. Introduction

Nasuta subgroup of the *immigrans* species group of *Drosophila* is an assemblage of morphologically almost identical forms and has been proved to be a very useful group to study many facets of population genetics (Ranganath 1978). Extensive analysis on reproductive isolation, chromosomal and enzyme polymorphism, have been made on the members of this group (Wilson *et al* 1969; Kanapi and Wheeler 1970; Nirmala 1973; Ranganath 1975; Rajasekarasetty *et al* 1976; Ramesh and Rajasekarasetty 1976; Lambert 1975; Spieth 1969; Clyde 1977). But, the developmental studies on the manifestation of isozymes is totally wanting for the members of this subgroup. Further, Johnson (1966) has opined that "even without clear examples of altered developmental patterns within a species, some attempts are possible with closely related species which show developmental differences in homologous enzyme systems". Hence the present project has been undertaken to study developmental changes in the manifestation of 4 hydrolases, namely, acid phosphatase, alkaline phosphatase, α-esterase and β-esterase in two closely related species of the *nasuta* subgroup—*D. nasuta nasuta* and *D. sulfurigaster neonasuta*. The aims of this experiment are—

(i) to analyse the expression of these isozymes during different phases of development in two closely related species of *Drosophila*.

(ii) to compare the developmental manifestations of homologous enzymes in closely related species of *Drosophila.*

2. Materials and methods

Chromosomally monomorphic strains (Coorg, India) of *D. nasuta nasuta* and *D. sulfurigaster neonasuta* have been used in the present experiment. The eggs of these species were collected by adopting the modified procedure of Delcour (Ranganath and Krishnamurthy 1974). Eggs were allowed to develop in petri-dishes containing wheat cream agar medium seeded with yeast at 22° C.

In all, 15 different developmental stages have been assayed for acid phosphatase, alkaline phosphatase, α-esterase and β-esterase. They are:

D.n. nasuta	*D.s. neonasuta*
Eggs	Eggs
Larval phase	
(48, 72, 96, 120 and 130 hours)	(48, 72, 96, 120, 144, 168 and 192 hours)
Pupal phase	
(144, 154, 168, 192 and 216 hours)	(216, 240 and 264 hours)
Adult phase	
Just emerged flies, 2, 10 and 20 day-old flies.	Just emerged flies, 2, 10 and 20 day-old flies.

The egg-to-adult rate of development of *D.n. nasuta* is faster than *D.s. neonasuta* to the extent of 48 hr. In order to maintain uniformity in the quantum of material for different developmental stages, 5 mg in fresh weight of each were homogenised with 0·7 ml of distilled water. The polyacrylamide gel electrophoretic technique of Davis (1964) was employed for enzyme analysis.

The stain for alkaline phosphatase constituted sodium 1 naphthyl phosphate (100 mg), Polyvinyl pyrrolidone (500 mg), Fast blue RR salt (100 mg), $MnCl_2$ (60 mg), $MgCl_2$ (60 mg) and 2 gms of sodium chloride dissolved in 100 ml of 0·05 M Tris HCl buffer at pH 8·5. For acid phosphatase the above ingredients were dissolved in 0·125 M acetate buffer at pH 5·0 instead of tris HCl buffer.

The stain for α-esterase is alpha naphthyl acetate (100 mg) dissolved in 4 ml of acetone plus 4 ml of water and then added 50 ml of 0·1 M phosphate buffer, pH 5·9. To this 100 mg of fast blue RR salt was added. This entire solution is added to 50 ml of 0·1 M phosphate buffer of pH 6·5. For β-esterase assay, beta naphthyl acetate is used instead of alpha naphthyl acetate and the remaining ingredients are same as above.

Finally after the appearance of bands on the gels, they were fixed in 7% acetic acid.

The enzyme bands on the gels are named by numerical numbers, depending on their rate of mobility. Band 1 represents the fastest one and the others follow it.

In each figure, on the right side, a 'general' zymogram is given and it includes all the different bands which have been exposed at different stages of development or a particular enzyme in question.

3. Observations

3.1. *D.s. neonasuta*

3.1a. *Acid phosphatase* (figure 1). Eight different enzyme bands were recognised (1 to 8). Egg stage is the only one where total negative reaction was seen for all the eight bands. At any one of the other stages, the maximum number of bands recorded was six and it was in 48 hr and 72 hr larval stages. On the other hand the least number namely one band was seen in 216 hr and 240 hr old pupal stages. Out of the eight bands only the bands 1, 4 and 5 were seen in all the three phases, namely, larval, pupal and adult. The bands 3 and 8 appeared in larval and adult phases while the band 7 in larval and pupal phases; on the other hand the band 6 was confined to larval and the band 2 to the adult phase only.

3.1b. *Alkaline phosphatase* (figure 2). A total of eleven electrophoretically distinct alkaline phosphatase bands were recognised. The egg and the 240 hr pupal stage revealed the absence of all these bands. The 48 hr larval stage possessed the maximum number of bands namely, eight; while the 216 hr pupal

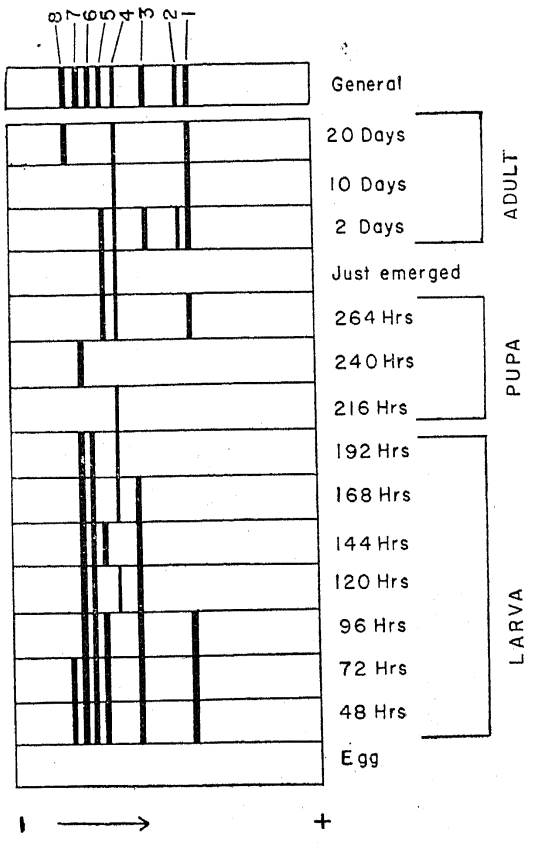

Figure 1. Ontogenetic manifestation of acid phosphatase in *D. sulfurigaster neonasuta.*

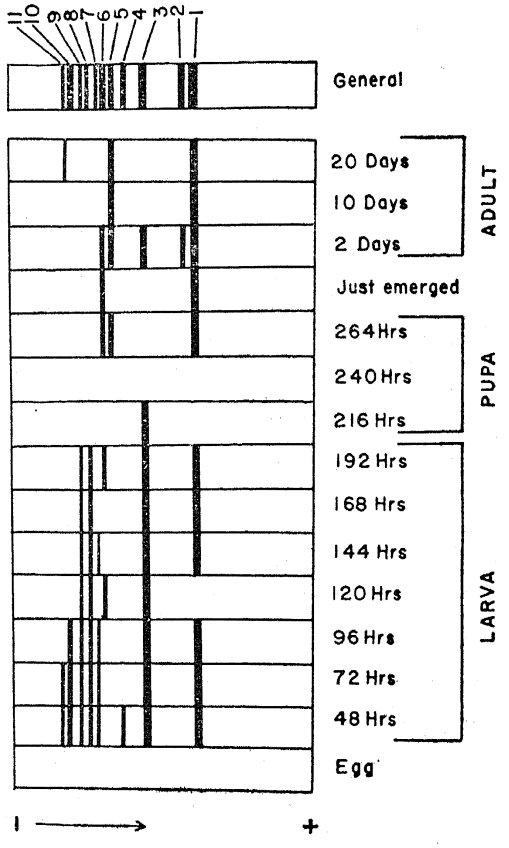

Figure 2. Ontogenetic manifestation of alkaline phosphatase in *D. sulfurigaster neonasuta.*

stage revealed only one band. The bands 1, 3 and 6 were common to larval, pupal and adult phases. Only the pupal and adult phases possessed the band 5, similarly the band 11 was located only in larval and adult phases. The larval phase is the only phase which had the bands 4, 7, 8, 9 and 10, while the band 2 was confined to the adult phase only.

3.1c. *α-esterase* (figure 3). Six different bands of activity were seen in the gels of α-esterase. The eggs revealed the lack of activity for all these bands. 48 hr larval stage housed the highest number of five bands, whereas only one band was noticed in 144, 168 and 192 hr larval stages. Bands 4 and 5 are represented in larval, pupal and adult phases while the band 1 in larval and adult phases and the bands 2, 3 and 6 were restricted to larval phase only.

3.1d. *β-esterase* (*figure* 4). Six variants of β-esterase were recognised. The highest number of bands namely, five was realised in 48 hrs larval stage while the 120, 144, 168, 192 hrs larval and 216 hrs pupal stages revealed the presence of only one band. Bands 4 and 5 were common to larval, pupal and adult phases. The band 1 was seen in larval and adult phases whereas the band 2 was only in adult and the bands 3 and 6 only in larval phases.

3.2. *D.n. nasuta*

3.2a. *Acid phosphatase* (figure 5). There were six electrophoretic variants for acid phosphatase in *D.n. nasuta.* Out of the fifteen different developmental stages

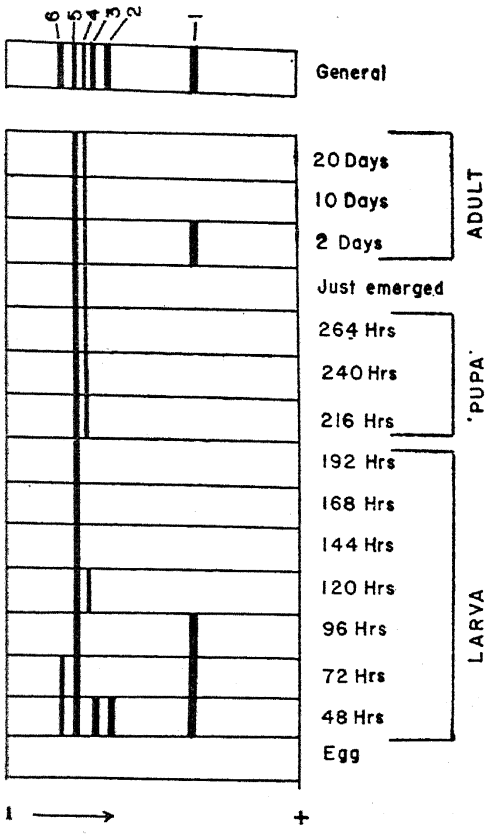

Figure 3. Ontogenetic manifestation of α-esterase in *D. sulfurigaster neonasuta.*

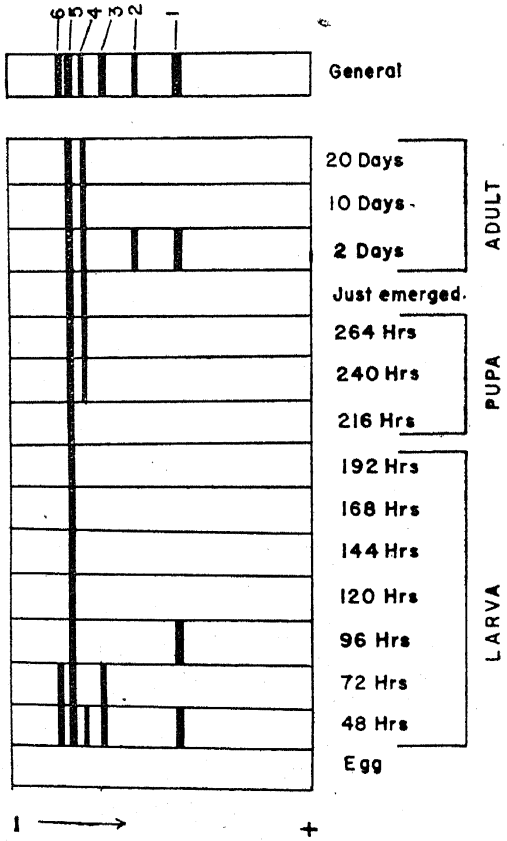

Figure 4. Ontogenetic manifestation of β-esterase in *D. sulfurigaster neonasuta,*

analysed, the egg, 48 hr larval and 216 hr pupal stages possessed none of these enzyme bands. Band 1 appeared in all the remaining stages with the exception of 168 hr pupal stage. Similarly the band 6 was located in all except in the just emerged flies. Maximum number of variants for acid phosphatase were seen at 72, 96 and 130 hr larval stages and in 20 day-old flies, while the least number of bands, of two only, was found at 168 hr pupal and in just emerged flies. Bands 1, 2, 3 and 6 are represented in larval, pupal and adult phases. Band 4 appeared in adult and larval phases and the band 5 was exclusively seen in larval and pupal phases.

3.2b. *Alkaline phosphatase* (figure 6). The zymograms of alkaline phosphatase for *D.n. nasuta* revealed the occurrence of eight different enzyme bands. None of these bands was seen in the egg and 216 hr pupal stages. In the remaining stages under study, band 1 appeared in all with the exception of 168 hr pupal stage. A maximum number of six bands was observed at 72 hr larval stage while the 48 hr larval stage possessed only one band. Bands 1, 4 and 6 were represented both in larval, pupal and adult phases. Bands 2 and 5 were absent in pupal phase and band 8 was absent in the adult phase. On the other hand, band 7 was confined to larval phase, while band 3 was restricted to adult phase only.

3.2c. *α-esterase* (figure 7). It exhibited five different electrophoretic bands in *D.n. nasuta.* Egg, 48 hr larval and 216 hr pupal stages showed negative reaction for all these bands. Maximum bands of three were seen at 120 hr larval and in 2-day old flies, while the 96 hr larval and 192 hr pupal stages revealed only one band. Bands 1, 4 and 5 are common in larval, pupal and adult phases while bands 2 and 3 were restricted to larval phase only.

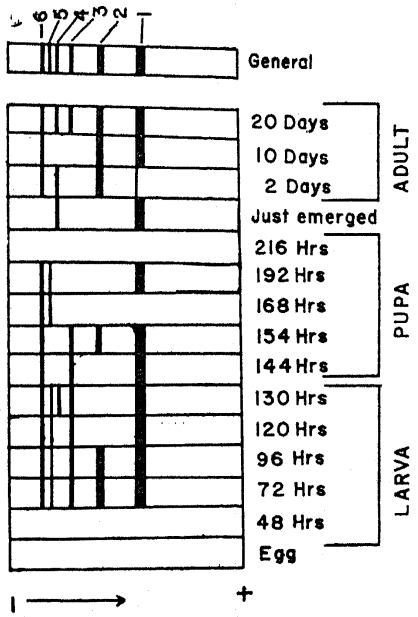

Figure 5. Ontogenetic manifestation of acid phosphatase in *D. nasuta nasuta.*

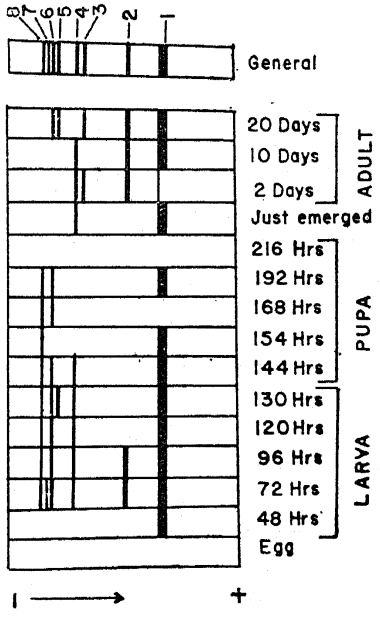

Figure 6. Ontogenetic manifestation of alkaline phosphatase in *D. nasuta nasuta.*

3.2d. *β-esterase* (figure 8). A total of five different electrophoretically distinct bands were seen for β-esterase in *D.n. nasuta*. Of these, band 2 appeared in the larval phase only while band 5 revealed in larval and pupal phases. On the other hand the remaining bands 1, 3 and 4 are frequent in larval, pupal and adult phases.

3.3. *Comparison of the zymograms in the two species*

Figure 9 presents a comparative picture of the 'general' zymograms of the four isozymes obtained for *D.n. nasuta* and *D.s. neonasuta*. Each general zymogram of the concerned enzyme expressed an overall picture of its various electrophoretically different bands encountered during the different stages of development. It can also be viewed as a reflection of the totality of the genetic system involved in the synthesis of these isozymes. Table 1 lists the electrophoretically homologous and distinct bands for each enzyme in the two species under study.

Acid phosphatase revealed six and eight different enzyme bands in *D.n. nasuta* and *D.s. neonasuta* respectively. Of these, only four bands occupied similar positions in the zymogram. So *D.n. nasuta* had two and *D.s. neonasuta* had four distinct acid phosphatase bands (table 1). Similarly, with regard to alkaline phosphatase, *D.n. nasuta* had two and *D.s. neonasuta* had five distinct enzyme bands while both of them share six common bands. With regard to α-esterase, *D.n. nasuta* had three and *D.s. neonasuta* had four altogether different bands and they

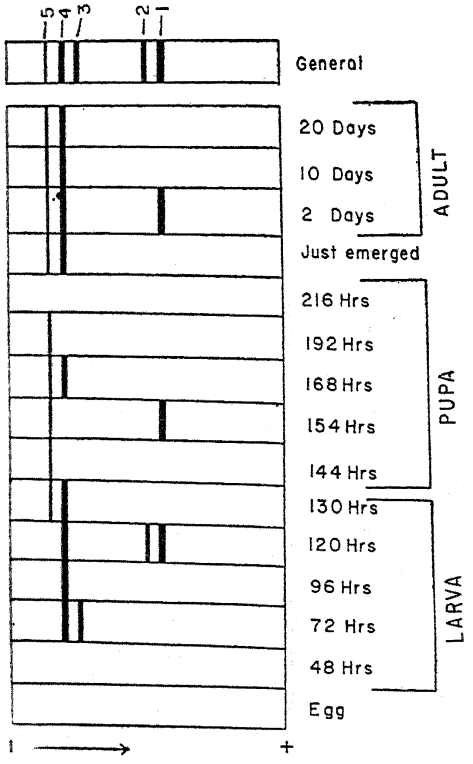

Figure 7. Ontogenetic manifestation of α-esterase in *D. nasuta nasuta*.

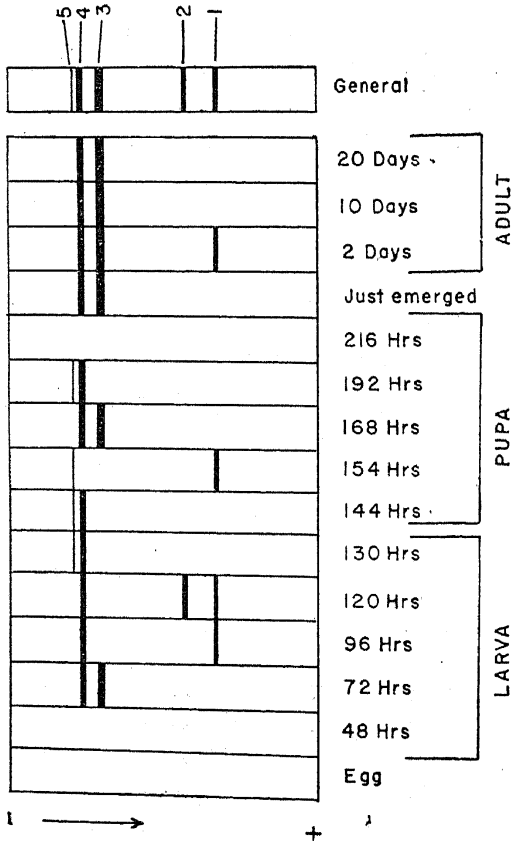

Figure 8. Ontogenetic manifestation of β-esterase in *D. nasuta nasuta*.

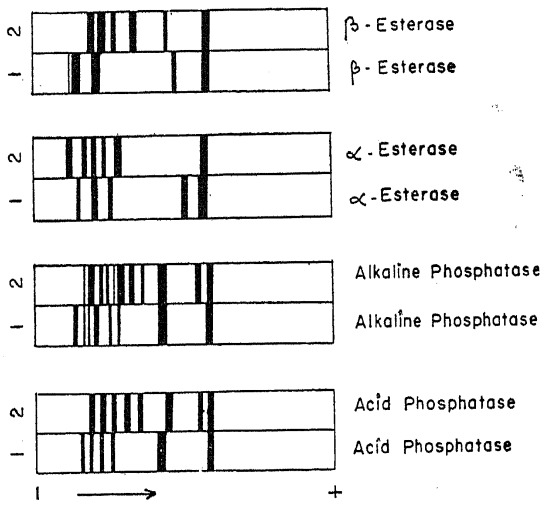

Figure 9. Comparative picture of the general zymograms of each enzyme in *D. nasuta nasuta* and *D. sulfurigaster neonasuta.*

Table 1. Comparison of the different enzyme bands as they are noticed in their respective zymograms to know the electrophoretically homologous and distinct bands for each enzyme in *D. nasuta nasuta* and *D. sulfurigaster neonasuta.*

Enzymes	Alkaline phosphatase		Acid phosphatase		α-Esterase		β-Esterase	
Species	*D.s.n.**	*D.n.n.***	*D.s.n.*	*D.n.n.*	*D.s.n.*	*D.n.n.*	*D.s.n.*	*D.n.n.*
Total number of bands	Eleven	Eight	Eight	Six	Six	Five	Six	Five
Matching of the bands	..	8	..	6	6	..	..	5
	11	7	8	5	..	5	..	4
	10	6	7	4	5	..	6	..
	9	..	6	3	4	4	..	3
	..	5	5	..	3	..	5	..
	8	..	4	..	..	3	4	..
	7	4	..	2	2	..	3	..
	6	3	3	..	..	2	2	..
	5	..	2	..	1	1	..	2
	4	..	1	1	..	..	1	1
	3	2	..	..	..	..	..	..
	2	..	..	..	..	..	..	..
	1	1	..	..	..	..	..	..

* *D.s.n.* = *D. sulfurigaster neonasuta.*
** *D.n.n.* = *D. nasuta nasuta.*

had two common enzyme bands. In the β-esterase system, *D.n. nasuta* had four while *D.s. neonasuta* had five distinct enzyme variants.

4. Discussion

Ontogeny of enzymes provides a sensitive indication of the basic changes occurring during differentiation (Masters and Holmes 1972). This biochemical index can be employed as one of the dependable parameters to compare the developmental events occurring in closely related species and thus one can estimate the genetic relationships between them. We have analysed the developmental manifestations of four hydrolases in two closely related species of *Drosophila*, namely, *D. nasuta nasuta* and *D. sulfurigaster neonasuta*.

Perusal of the literature reveals that there is very little information with regard to the developmental changes of acid phosphatase in *Drosophila* (cf. Dickinson and Sullivan 1975). Esposito and Ulrich (1966) reported that various bands of acid phosphatase appear and disappear as a function of a stage but no specifics are given. In *D. pseudoobscura* there is some degree of tissue specificity among the four acid phosphatase bands during development (Pasteur and Kastritsis 1971). Recently Triantaphyllids and Kastritsis (1976) reported the differential behaviour of three acid phosphatase band phenotypes in *D. auraria*. In the present study in both *D.n. nasuta* and *D.s. neonasuta*, it is interesting to note that none of the fifteen stages screened possess all the acid phosphatase enzyme bands together. Scrutiny of the zymograms (figures 1 and 5) revealed significant non-uniformity in the expression of acid phosphatase phenotypes during different stages of development. In *D.s. neonasuta* under study the biochemical phenotype of the larval phase has seven different bands whereas there are four and six different bands in pupal and adult phases respectively. Further, in *D.s. neonasuta* there exists phase specific bands, for instance, the acid phosphatase band 6 is revealed only in the larval phase while the band 2 in adult phase only. On the other hand, in *D.n. nasuta* there are six acid phosphatase bands in the larval phase while the pupal and adult phases have five different bands. In contrast to *D.s. neonasuta, D.n. nasuta* lacks any phase specific bands. Further, it is very interesting to note that in *D.n. nasuta*, the larval phase possesses the full complement of acid phosphatase bands while in *D.s. neonasuta* none of the three phases reveal the occurrence of all enzyme bands together.

The developmental manifestations of alkaline phosphatase in *D. melanogaster* were detected by Beckman and Johnson (1964a, b). In this species one of the bands was faintly visible at all stages and another was visible from early stages through second instar stage. Similarly Schneiderman *et al* (1966) detected in the same species a greater variety of bands and studied them in more detail. Of the seven bands they detected, band 1 was faintly visible at all stages while bands 2, 3 and 4 appear strongly in 18–20 hr embryos. In the present analysis of alkaline phosphatase, in *D.s. neonasuta* the larval phase has accommodated the highest number of types of bands, namely, nine out of eleven electrophoretic variants; while the pupal phase has four and the adult phase has six different bands. Bands 4, 7, 8, 9 and 10 are confined to the larval phase of *D.s. neonasuta* and are as such absent in its pupal and adult phases. Similarly in *D.n. nasuta*, out of the eight alkaline phosphatase bands, the larval phase has all the bands except the band 3. There are two bands which are phase specific. In *D.n. nasuta* band 7 is larval specific while band 3 is adult phase-specific.

Substantial work has been done with regard to the ontogenetic manifestations of esterases. Ogita and Kasai (1965) showed the differences in the expression of esterases at different developmental stages of *Musca domestica*. Pantelouris and Downer (1969) detected the changes in the esterase zymograms during metamorphosis in *D. immigrans*. Kambysellis *et al* (1968) examined developmental changes and tissue distribution in *D. aldrichi* and *D. mulleri*. They noted that there were changes related to aging in adults. Pasteur and Kastritsis (1971) examined the developmental changes of α and β-esterases in *D. pseudoobscura* and they found marked developmental variation in α-esterases but no variation in β-esterases. Changes during development and tissue or organ specificities of α and β-esterases of *D. virilis* were analysed by Sasaki (1974). Recently, Prakash and Reddy (1978a) studied the differential expression of α and β-esterases in *D. rajasekari* and showed that none of the bands was present throughout the developmental stages. In the present assay, *D.s. neonasuta* has six and *D.n. nasuta* has five different enzyme bands for the enzyme α-esterase. In both the species, the larval phases reveal all the noted variables of α-esterase. In *D.n. nasuta* the pupal and adult phases are devoid of the α-esterase bands 2 and 3. Similarly in *D.s. neonasuta* the pupal phase has just the bands 4 and 5 while its adult phase possesses the bands 4, 5 and 1. The enzyme β-esterase has five variants in *D.n. nasuta* and all these are expressed during the larval phase while its pupal phase is devoid of the band 2 and the adult phase lack the bands 2 and 5. On the other hand in *D.s. neonasuta*, which has six electrophoretically different bands for β-esterase, none of the three developmental phases under study possess all the bands together. In the larval phase band 2 is missing. The pupal phase has just bands 4 and 5 while the adult phase lacks bands 3 and 6.

Thus, the differential expression of acid phosphatase, alkaline phosphatase, α-esterase and β-esterase in two closely related species of *Drosophila* is striking. A critical scrutiny of the zymograms of the four enzyme systems in the two species of *Drosophila* understudy highlights the following interesting points:

(i) Of the three developmental phases, namely, larval, pupal and adult phases, the larval phase has housed most of the variants of the enzyme in question. Thus, qualitatively it is richer than the pupal and the adult phases. Of the latter two, in each case, the adult phase has more number of enzyme bands than the pupal phase.

(ii) Some of the enzyme bands are seen in all the three phases of development under study, while some are confined to one or the other phase. Based on their extent of appearance during different phases of development, the authors have categorised the enzyme bands into two types. They are:

  (a) Obligatory enzyme bands, these are the ones which are represented in all the three phases of development. For instance, in alkaline phosphatase system, bands 1, 3 and 6 in *D.s. neonasuta* and bands 1, 4 and 6 in *D.n. nasuta* are common in three phases of development understudy.

  (b) Stage specific enzyme bands, which are restricted to one of the developmental phases. For example, in *D.s. neonasuta* the alkaline phosphatase bands 4, 7, 8, 9 and 10 are restricted to larval while band 2 is located in the adult phase only.

(iii) Yet another significant observation involved in the present study is that in both *D.s. neonasuta* and *D.n. nasuta* there are no bands specific for pupal phase only. Wherever phase specific bands are noticed they are either for the larval or the adult phases.

Further, in all the zymograms of the enzymes understudy, there is marked appearance, disappearance and reappearance of enzyme bands during different stages of development. For instance, in acid phosphatase system of *D.n. nasuta*, band 3 appears at 72 hr stage and continues upto 154 hr and then disappears; further it appears in 20 day-old flies. Similarly in alkaline phosphatase, band 6 appears at 72 hr stage, continues upto 144 hr and then disappears, later it reappears at 168 hr and extends upto 192 hr. Once again it disappears to make its presence in 20 day-old flies. Such a differential expression of different enzyme bands during different stages of egg-to-adult development can be seen for most of the enzyme bands in acid phosphatase, alkaline phosphatase, α-esterase and β-esterase enzymes in both the species understudy (figures 1–8).

Similar observations have been reported by Prakash and Reddy (1978 a, b), Pasteur and Kastritsis (1971), Beckman and Johnson (1964 a, b) and a host of others (cf. Dickinson and Sullivan, 1975). In all the four enzyme systems under study, no two stages exhibit a similar phenotype. Each stage in development appears to be unique in itself having different groups of bands. This can be viewed as an added evidence for the differential expression of genes during development and it is in consonance with the idea that not all genes are active at all times and that they are switched on and off depending on the needs of the developmental programme.

Perusal of the literature reveals that very few workers have extended the analysis of ontogenic manifestations of isozymes for species comparisons. Johnson (1966) has done some comparative studies on the developmental expression of alkaline phosphatase in *D. melanogaster* and *D. ananassae*. Mcreynolds (1967) identified three zones of esterase activity in *D. virilis*, *D. americana* and *D. novamericana* that seemed to be homologous by position and specificity. Similarly Johnson *et al* (1968) proposed homology between most bands found in *D. aldrichi* and *D. mulleri*. The authors have made a detailed study of the manifestation of isozymes in 15 different developmental stages. The zymograms show the possible homologous and distinct bands for each enzyme understudy between *D.n. nasuta* and *D.s. neonasuta* (figure 9, table 1). These species share four enzyme bands in acid phosphatase system, while in alkaline phosphatase they have six common enzyme bands. Similarly there are two common α-esterase bands and there is only one band in β-esterase system. The manifestation of these homologous bands during different phases of development in the two species under study is therefore very interesting. For instance, the acid phosphatase band 1 of *D.s. neonasuta* and its homologous band in *D.n. nasuta* which is also band 1, are found in all the three phases, namely larval, pupal and adult phases of development. On the other hand, band 6 of acid phosphatase in *D.s. neonasuta* is restricted to the larval phase while its corresponding band 3 in *D.n. nasuta* is seen in all the three phases of development. Further, the acid phosphatase band 7 in *D.s. neonasuta* is absent in the adult phase while its homologous band 4 in *D.n. nasuta* is lacking in the pupal phase. Similarly in alkaline phosphatase, one of the homologous bands, band 1 in each species

is represented in all three phases of development. Further, the alkaline phosphatase band 2 in *D.n. nasuta* was seen in larval and adult phases while its corresponding band 3 of *D.s. neonasuta* was detected in all the three phases. Band 6 is located in all the three phases in *D.s. neonasuta* while its parallel band 3 of *D.n. nasuta* is confined to its adult phase. Similarly the other homologous bands of alkaline phosphatase system namely, bands 7, 10 and 11, of *D.s. neonasuta* are similar to the bands 4, 6 and 7 respectively of *D.n. nasuta* and there lies differences in their expression during development in their respective species. With regard to the α-esterase, band 1 of *D.s. neonasuta* is absent in pupal phase while its corresponding band in *D.n. nasuta*, band 1 appeared in all the three phases. Further, the α-esterase band 4 in each species is shown to be homologous and both of them have expressed in all the three phases of development. With regard to the β-esterase, there is only one homologous band, i.e., band 1 of each species, in *D.n. nasuta* it is found in all the 3 phases while in *D.s. neonasuta* the pupal phase is devoid of this band.

Thus the present data embody evidences for the differential behaviour of similar enzymes in a pair of cytogenetically/phylogenetically closely related species *Drosophila*. Thus, the two species under study for the four enzymes have some electrophoretically unique bands and also share some similar bands. In addition, there exists both similarities and differences in the manifestation of electrophoretically similar bands during different phases of development in the two species under study.

Population geneticists have extensively exploited the allelic frequencies of isozymes to construct phylogenies and to establish the genetic basis for the cytotaxonomic relationship between species (Nair *et al* 1971; Yang *et al* 1972; Ayala *et al* 1972 a, b; 1974 a, b; Kanapi and Wheeler, 1970; Hegde and Krishnamurthy 1976 a, b; Rajasekarasetty *et al* 1976). Further, most of these workers have confined to some specific stages, in most cases to the adult phase of development only. For instance, Kanapi and Wheeler (1970) have used twenty day-old flies for enzyme assay. Further, whenever, natural populations are screened, males collected from nature are assayed first and then the F_1 progeny of wild females are used for enzyme studies (Ayala *et al* 1972 a, b; 1974 a, b).

The present study involving a large number of stages has exposed very interestingly that no two stage in development reveals a similar phenotype. Further, there are some developmental phase specific bands being restricted either to larval or adult phases only. So the absence of an enzyme band at any one stage does not indicate the virtual absence of the respective gene in that individual. The authors' analysis show that in each figure (figures 1 to 8), that the general zymogram of each enzyme presents a better picture as to the extent of electrophoretically distinct enzyme bands present in species than at any one stage during development. The general zymogram includes all the enzyme bands for a particular enzyme appeared during different phases of development and thus it reflects the overall genetic involvement in the production of the enzyme in question.

In view of this, we feel strongly that if we restrict to one or two stages, it will not give a reliable picture of the totality of the genes involved in the synthesis of the enzymes. Hence, it is the considered opinion of the authors that to get a complete picture of the genetic set-up for the enzyme(s) in question, one has to study

the eniire developmental sequence of the species. The present study is the testimony for this opinion. For species comparisons and phylogenetic considerations one must have a thorough knowledge of the ontogenetic programme of the enzymes in each species which in turn reflects the totality of the genes underlying the production of the enzymes. The present study has amply proved it.

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