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Genetic and Developmental Analysis of Phosphatases in *Drosophila* Species Hybrids

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Two acid phosphatase (Acph) and four alkaline phosphatase (Aph) zones revealed differential expression in various tissues as well as in developmental stages. Data have been presented on the inheritance of electrophoretic variants of Acph and Aph. Phosphatase isoenzymes have a dimeric quarternary structure so that homozygotes depict single isozyme while heterozygotes show two homodimers and an intermediate heterodimer. Developmental shifts in Aph activity profiles have been observed at larval-pupal as well as at pupal-adult transitions whereas Acph activity shows a diphasic response with maximum activity in prepupal and adult stages. Similarities in isoenzyme characteristics on the basis of thermal inactivation and inhibitor specificity indicate structural relatedness of species specific allelic & non allelic phosphatases. The synchronous-codominant expression and the occurrence of multimerisation of species specific electrophoretic variants during ontogeny as well as in different tissues indicate homologies of loci coding phosphatases in hybridising *Drosophila* species. The data are consistent with the proposal that allelic asynchrony occurs less frequently in hybrids derived from closely related species.

Key Words: Gel electrophoresis, Acid and alkaline phosphatases, Thermostability, Inhibitor response, Gene homology, Ontogenetic profiles, *D. malerkotliana, D. bipectinata*

Introduction

Allelic as well as non-allelic isoenzymes constitute ideal biochemical markers to investigate temporal and spatial patterns of gene-expression during development and differentiation (Whitt et al. 1977, Yasbin el al. 1978). Analysis of interspecific hybrids has provided substantial insight into the biochemical basis of heterosis as well as evolutionary relationships among species (Manwell & Baker 1970, Ohno 1970, Korochkin 1978, Kankel & Hall 1976). Although phosphatases have been isolated and characterised from several animal as well as plant sources, the genetic and developmental studies have been limited (Dickinson & Sullivan 1975,

Yasbin et al. 1978). We have attempted at analysing the genetic control as well as homologies, developmental expression, and biochemical characteristics of ontogenetic isoenzyme-activity profiles of acid and alkaline phosphatases in interspecific hybrids derived from the crosses of *D. bipectinata* and *D. malerkotliana*.

Materials and Methods

The life spans of *D. bipectinata*, *D. malerkotliana* and their interspecific hybrids were found to be 30±3, 26±2; and 15±1 days respectively at 25±1°C. The synchronisation and timings of different developmental stages of hybridising *Drosophila* species was achieved by the method of Stocker and Jackson (1971). The stage-specific homogenates (100 mg/10ml of 0.5% triton-X) were centrifuged at 12000 × g for 30 min at −4°C in K-70 refrigerated centrifuge (Janetzki) and the supernatants were employed for quantitative and qualitative analyses. Genetic control of phosphatases was studied using zymograms of parents and of F_1, F_2 and/or backcross progeny of several single pair matings involving intra-and interspecific *Drosophila* hybrids.

Electrophoresis of horizontal starch gel (12%) was done using discontinuous buffer system adopted from Brewer (1970). Homogenates of single larva/pupa/adult as well as larval/adult organs were absorbed in rectangular wicks (6 × 4 mm) of Whatman filter paper and were inserted into the slits cut in the gel. The starch gel measured 19 × 10 × 2 cm and could accommodate 12 to 15 samples at a constant current of 25 mA and 250 volts for 3 hr at 6 ± 2°C. The gel slices were stained for acid and/or alkaline phosphatase activity by 1 hr incubation at 37°C in the staining solutions which included 1 g polyvinyl pyrrolidone, 0.2 g sodium-α-naphthyl phosphate, 0.2 g fast blue BB salt, 1 ml each of $Mgcl_2$ and $Mncl_2$ (12%) per 200 ml sodium acetate buffer (*p*H 5.0) in case of Acph activity; and tris-citrate buffer (*p*H 9.0) for Aph activity. Gels were subsequently washed in tap water and kept for 30 min in freshly prepared fixing solution (methanol: acetic acid: water, 5:5:1). The zones of acid (Acph) & alkaline (Aph) phosphatases have been numbered according to their electrophoretic mobility in a cathodal to anodal sequence.

The stage-specific homogenates were treated at 50 & 60°C for 10 & 20 min; and were also treated in a 1:1 mixture with different inhibitors (EDTA, KH_2PO_4 & urea for Aph; sodium fluoride & sodium oxalate for Acph) for 20 min at 30°C and were analysed electrophoretically alongwith control samples. The stage-specific activity profiles of Acph & Aph were determined quantitatively by following the procedures suggested by MacIntyre (1971). The protein content of different developmental stages of hybridising *Drosophila* species was determined according to the method of Lowry et al. (1951) using bovine serum albumin as the standard. The thermostability experiments were carried out by preincubating the stage specific extracts for 20 min at specified temperatures (35, 45 & 55°C for Acph; 50, 60 & 70°C for Aph); and at 55°C or 65°C for different time intervals and assayed the residual enzyme activity. To determine the extent of inhibition, the stage-specific extracts were preincubated for 20 min in a 1:1 mixture with various inhibitors of Acph & Aph activity and the residual enzyme activity was determined.

Results

Ontogeny of phosphatases: The ontogenetic profiles of phosphatases comprise

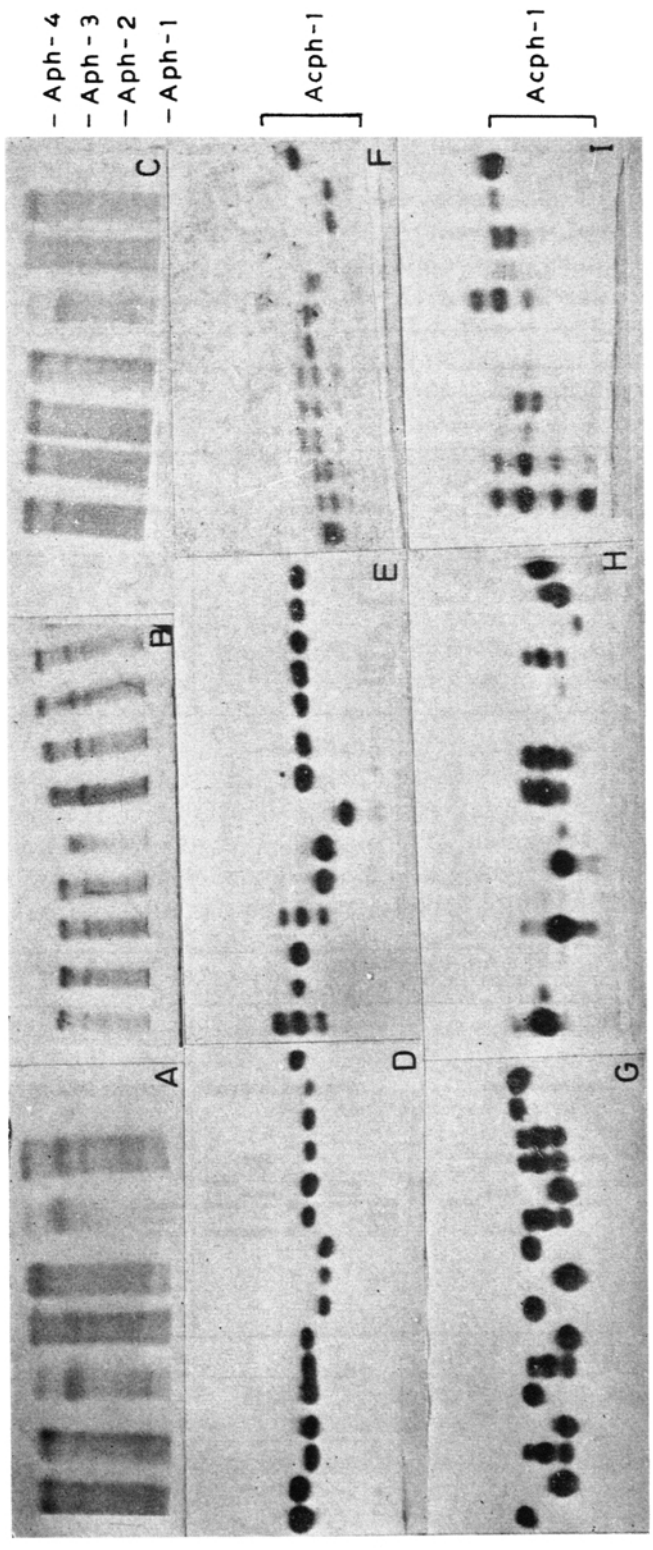

Figure 1 Starch gel electrophoresis of alkaline and acid phosphatases in *Drosophila* species hybrids. **A**, Ontogeny of alkaline phosphatases; **B**, thermostability of Aph isoenzymes; **C**, Tissue distribution of Aph isoenzymes; **D-E**, homozygous stocks of species specific allelic acid phosphatases; **F**, Acph patterns of *D. bipectinata* and *D. malerkotliana*; **G-H**, segregation of Acph variants in F_2; **I**, Acph patterns of interspecific hybrids

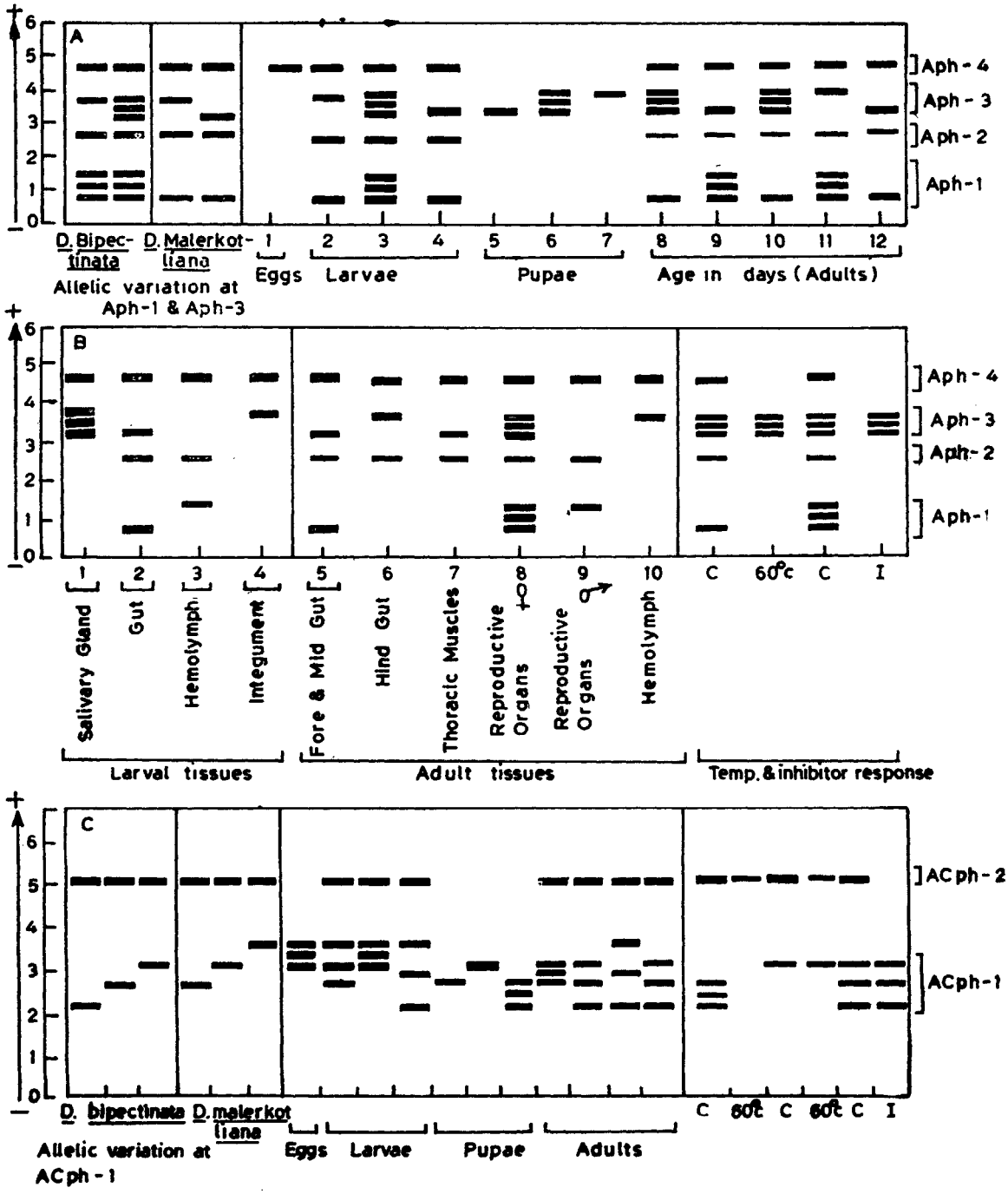

Figure 2 Ontogeny, tissue specificity, thermostability, and inhibitor response of acid phosphatase (Acph) and alkaline phosphatase (Aph) isoenzymes

of two Acph and four Aph zones in *Drosophila* species and their interspecific hybrids (figures 1-2). The eggs and pupal stages are characterised by single zone of enzyme activity i.e., Aph-4 in the eggs; Aph-3 in the pupal stages and Acph-1 in eggs and pupal stages. However, larval and adult stages have revealed two Acph and four Aph zones. The Acph-1 and Aph-3 zones persist through all stages of development in the hybridising *Drosophila* species. The Acph-2, Aph-2 and Aph-4 zones depict only single band phenotypes and are monomorphic. However Acph-1, Aph-1 and Aph-3 zones are polymorphic with individuals showing either distinct single fast (F)/medium (M) and/or slow (S) band or triple band patterns. The mobility values of the two single band Acph-1 variants are identical to both *Drosophila* species while third variant is species specific; and such variants have resulted in triple-banded patterns of variable mobilities in interspecific hybrids (figures 1-2).

Tissue specificity: The acid and alkaline phosphatase isozymes have revealed differential tissue specificity but the patterns are almost identical for larval and adult tissues. The localisation of maximum Acph & Aph isoenzyme profiles occur in gut, reproductive organs, integument and hemolymph (figure 2). The distribution of Aph zones comprises of four Aph zones in female reproductive organs and various gut regions; three zones (Aph-2 to 4) in muscles and in hind gut; Aph-1, -2 and 4 in larval hemolymph and male reproductive organs while Aph-3 and Aph-4 occur in integument, salivary glands and adult hemolymph. Two Acph zones have been localised in the larval and adult gut as well as hemolymph while Acph-1 zone occurs in salivary gland, integument and reproductive organs (figure 2).

Genetic basis of phosphatases: The data on occurrence of segregating patterns of single band variants and triple band patterns at polymorphic zones (Acph-1, Aph-1 & Aph-3) in parents as well as F_1, F_2 and/or backcross progeny of several single pair matings both within and between *Drosophila* species are in agreement with monogenic inheritance (tables 1–3). Thus Acph-1, Aph-1 & Aph-3 zones are coded by three distinct genes. The stage-specific Acph and Aph zones do not reveal any segregation patterns with respect to

Table 1 *Genetic control of electrophoretic variants at polymorphic acid phosphatase (Acph) zone in* D. malerkotliana *and* D. bipectinata

Acph zone	Parental Acph phenotypes	Acph phenotypes of progeny*						No. of individuals analysed
		FF	MM	SS	FM	MS	FS	
D. bipectinata Acph-1	FS×MS	—	—	13	11	15	17	56
	MM×FS	—	—	—	19	22	—	41
	FM×FM	23	29	—	52	—	—	104
D malerkotliana	FS×FS	31	—	37	—	—	62	130
	MS×FM	—	19	—	23	27	25	94
	FM×FM	21	17	—	42	—	—	80

F, M, S denote fast, medium and slow electrophoretic variants
*Chi-square values insignificant at 5 % level

Table 2 *Genetic control of electrophoretic variants at polymorphic Acph-I zone in* Drosophila *species hybrids* (D. bipectinata × D. malerkotliana)

Parental Acph phenotypes	Acph phenotypes of progeny*									No. of individuals analysed
	FF'	MM'	SS'	FM'	FS'	F'M	S'M	F'S	M'S	
FS×F'S'	10	—	13	—	9	—	—	8	—	40
FM×M'S'	—	9	—	7	6	—	7	—	—	29
FS×M'S'	—	—	9	11	14	—	—	—	12	46
MS×F'S'	—	—	8	—	—	5	7	6	—	26

F', M', S' indicate fast, medium and slow variants in *D. malerkotliana*
F, M, S indicate fast, medium and solw variants in *D. bipectinata*
*Chi-square values insignificant at 5 % level

Table 3 *Genetic control of electrophoretic variants at polymorphic zones of alkaline phosphatases (Aph) in* Drosophila *species and their hybrids*

Zone	Parental phenotypes	Enzyme phenotypes of progeny*			No. of individuals analysed
		FF	FS	SS	
D. bipectinata					
Aph-1	FS×FF	16	18	—	34
	SS×FF	—	12	—	12
	FS×SS	—	14	13	27
Aph-3	FF×SS	—	16	—	16
	FS×FS	14	30	12	56
	SS×FS	—	15	17	32
D. malerkotliana					
Aph-1	FS×FF	19	18	—	37
	FS×FF	12	10	—	22
Aph-3	FS×SS	—	17	16	33
	FS×FS	12	20	10	42
D. bipectinata × *D. malerkotliana*					
Aph-1	FS×SS	—	13	16	29
	FF×SS	—	18	—	18
	FS×SS	—	14	12	26
Aph-3	FF×FF	14	—	—	14
	FS×SS	—	23	28	51
	FF×FS	12	16	—	28
	FF×SS	—	14	—	14

*Chi-square values insignificant at 5 % level

each other and may be controlled by distinct genetic loci. The presence of two and/or three distinct alternating single band variants at Acph-1, Aph-1 and Aph-3 zones represent the occurrence of 2 or 3 alleles of the respective loci. Thus single band and triple band patterns represent homozygote and heterozygote phenotypes respectively. The presence of an intermediate band in the heterozygotes reveals that Acph-1, Aph-1 and Aph-3 code for dimeric phosphatases both in the parental species and hybrids. The synthesis of both parental allelic isozymes, with distinct electrophoretic mobilities, in the species hybrids have revealed that both parental alleles are functioning.

Isoenzyme characteristics: Thermal inactivation as well as inhibitor response of Acph and Aph isozymes has revealed identical patterns in hybridising species. The six species-specific Acph-1 allelic variants comprise of two thermostable (F and F') and four thermolabile allelic isozymes (allozymes) while Acph-2 zone has shown partial thermal inactivation (figure 2). Likewise, Aph zones have revealed differences in resistance to heat as well as urea denaturation. The activity of Aph-1, Aph-2 and Aph-4 zones gets eliminated after thermal treatment while Aph-3 retains complete activity. EDTA and KH_2PO_4 have no effect on Aph-3 zone whereas Aph-1, Aph-2 and Aph-4 zones show complete inactivation (figures 1-2). The activity of Aph-1, Aph-2 and Aph-4 zones is eliminated within 7 to 10 min of incubation with inorganic phosphate (KH_2PO_4) whereas 20 min incubation is required with EDTA. However, there is complete inhibition of Acph-2 zone by sodium fluoride & sodium oxalate whereas Acph-1 zone is partially inhibited. (figure 2).

Phosphatase activity profiles: The data on Acph and Aph activity have been expressed in terms of specific activity i.e., on the basis of ontogenetic protein profiles. Although there are species-specific variations in Acph and Aph specific activity profiles, the overall patterns are nearly the same for the hybridising *Drosophila* species (figure 3). There are developmental shifts in Aph activity at larval-pupal as well as at pupal-adult transitions resulting in U-shaped profile. Variations in Acph activity have shown a diphasic response with maximum activity both in the prepupal and adult stages (figure 3). The larval stages show maximum Aph activity but Acph activity is low. The pupal stages are characterised by significantly low Aph activity in contrast to peak values of Acph activity. There is quick build up of Aph activity in early adult stages whereas there is delayed synthesis of Acph. Thus, the occurrence of ontogenetic maximum activity is different for both phosphatase enzyme systems.

Thermostability: The slopes of decay of Acph and Aph activity as a function of time; and effect of various temperature profiles have been illustrated in figure 4. The stage specific Acph & Aph activities are significantly inactivated at 55° and 65°C respectively. The pupal % residual Aph activity is about 75, 45 and 30 at 45°C, 55°C and 65°C respectively. However, the extent of % residual Aph activity is 55, 15 and 8 at 45°C, 55°C 65°C in case of larval and adult specific Aph activity (figure 4). Thus, the thermal inactivation profiles are different for stage specific Aph activity. The pupal activity is relatively thermostable as compared to larval and adult Aph activity. On the contrary, the pupal-specific Acph activity is significantly thermolabile than that of larval and adult specific activity (figure 4).

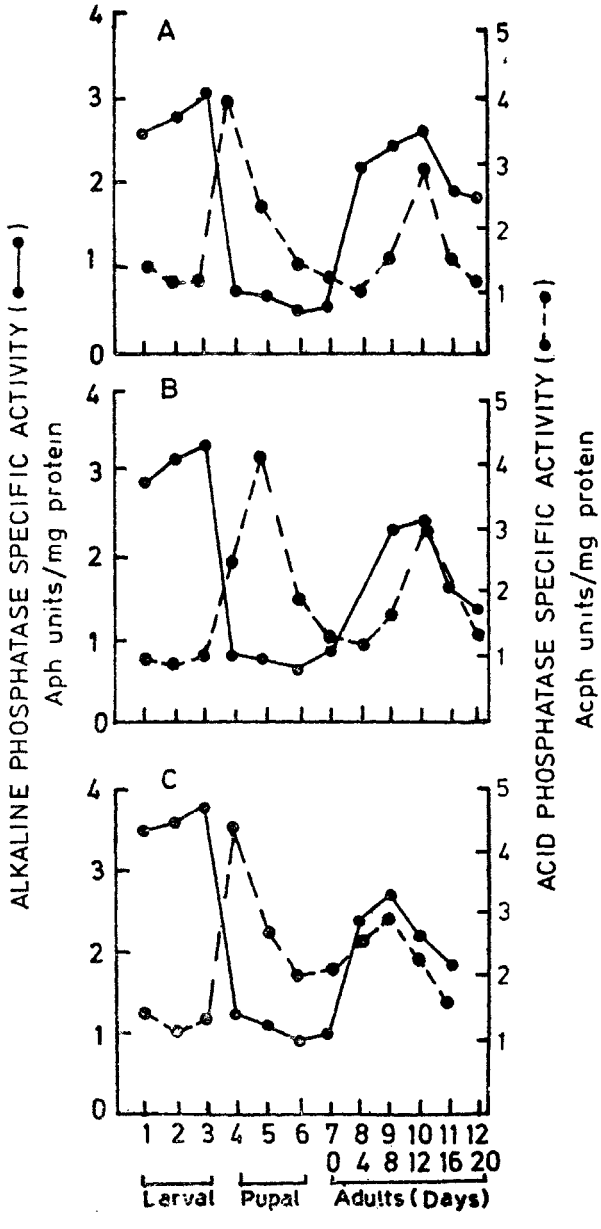

Figure 3 Ontogenic activity profiles of acid phosphatase and alkaline phosphatase in *D. bipectinata* (A); *D. malerkotliana* (B); and interspecific hybrids (C)

Inhibitor response: The patterns of inhibition of stage-specific Acph and Aph activity are different with various inhibitors (figure 4). The pupal Acph activity is significantly less inhibited as compared to that of larval and adult specific activity. However, the pupal specific Aph activity has depicted resistance towards EDTA and KH_2PO_4 whereas both larval and adult specific activities are significantly inhibited (figure 4).

Discussion

In the present studies, electrophoretic analyses of *Drosophila* species hybrids have provided data on monogenic control of electrophoretic variants of Acph and Aph; assembly of species specific polypeptide subunits into homodimeric and heterodimeric phosphatase isoenzymes; absence of allelic repression; similarities of allelic and nonallelic isoenzymes on the basis of thermostability and inhibitor response; and co-expression of species-specific alleles in differentiated tissues. Thus, the data are consistent with synchronous activation and co-dominant expression and/or lack of any species dominance in the preferential expression of paternally or maternally derived allelic phosphatases. On the basis of developmental analysis of interspecific as well as intergeneric hybrids among fishes, it has been suggested that greater the degree of synchrony observed in allelic activation during ontogeny of species hybrids, the greater the similarities of the parental alleles (Subtelny 1974, Ohno 1970). According to this criterion, the Acph and Aphloci are homologous in the hybridising *Drosophila* species. The data in the present studies are consistent with the proposal that allelic asynchrony occurs less frequently in hybrids derived from closely related species (Champion & Whitt 1976,

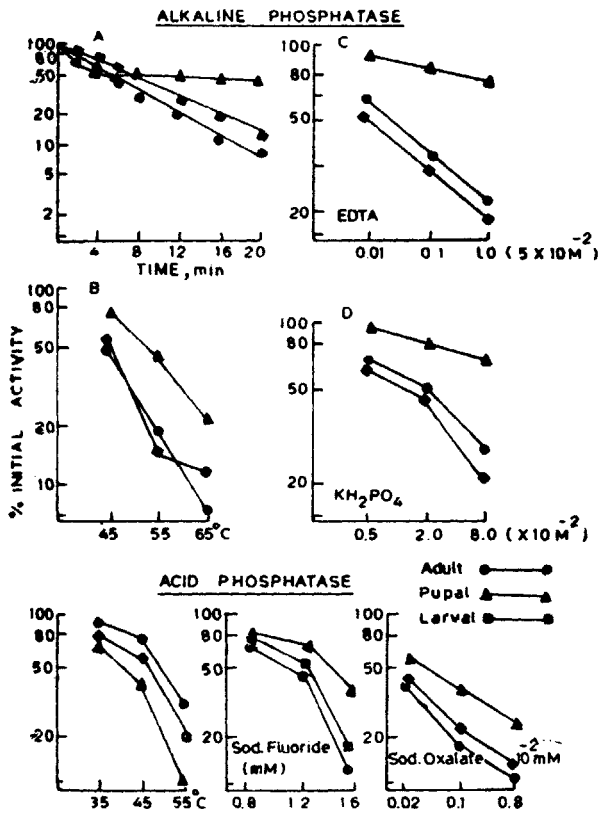

Figure 4 Thermostability and inhibitor specificity of stage-specific alkaline phosphatase and acid phosphatae activity in *Drosophila* species hybrids

Meyerhof & Haley 1975, Korochkin & Matveeva 1974, Wall & Blackler 1974).

The Acph and Aph gene expressions have also been examined during ontogeny at the level of tissue-specificity of isoenzymes as well as enzyme activity profiles. The occurrence of peak Acph activity during metamorphosis (pupal stages) suggests their possible involvement in histolysis. The significantly low Aph activity in pupal stages seems to be correlated with the expression of single zone of activity (Aph-3). The peak Aph activity during larval growth period and the occurrence of maximum Aph isoenzyme profiles in gut and reproductive organs indicate their possible involvement in transport of various metabolites among tissues. The present observations on the activity profiles as well as tissue specificity of Acph and Aph find support from earlier reports on histochemical and biochemical analysis of phosphatases in various organisms (Pasteur & Kastritsis 1971, Whitmore & Goldberg 1972, Sawicki & MacIntyre 1978). It may be suggested that larval integument specific Acph and Aph isoenzymes may cleave phosphate esters and channalise the availability of metabolites essential for puparium formation (sclerotisation). Thus, data on differential patterns of isoenzyme/activity profiles reflect regulation of phosphatase activity so as to meet different metabolic requirements.

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