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Ontogeny of Acetylcholinesterases in Hybridising *Drosophila* species

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Five acetylcholinesterase (AChE) zones revealed differential expression in different tissues as well as at various developmental stages of *Drosophila* species hybrids (*D. bipectinata*×*D. malerkotliana*). Genetic basis of AChE zones was resolved through electrophoretic analysis of the parents and the progeny of single pair matings involving intra- as well as inter-specific hybrids. DDVP (2, 2-Dichlorovinyldimethyl phosphate) significantly inhibited the adult-specific AChE activity and showed least effect on the larval and pupal activity-profiles. Similarities in isoenzyme characteristics on the basis of thermostability and inhibitor specificity (DDVP, eserine sulphate and ISO-OMPA) revealed structural relatedness of species-specific acetylcholinesterases. The synchronous and co-dominant expression of AChE zones during ontogeny as well as in tissues of *Drosophila* hybrids revealed homologies of species-specific AChE zones.

Key Words: Gel electrophoresis, Acetylcholinesterases, Isoenzymes, Ontogenetic profiles, Thermostability, Inhibitor response

Introduction

Analysis of isoenzyme profiles of hybridising species constitutes a potential tool for investigating patterns of gene expression during development (Whitt et al. 1977). Acetylcholinesterases have been isolated and characterised from vertebrates such as chick and rat (Varela 1973, McIntosh & Plummer 1973, Tennyson et al. 1973, Wade & Timiras 1980) but information on genetic control, developmental regulation and correlation of qualitative and quantitative aspects of acetylcholinesterases (AChE) is still lacking (Dickinson & Sullivan 1975). Thus, AChE gene-enzyme system has been employed as an ontogenetic marker in hybridising *Drosophila* species. The present investigation reports the findings on genetic control, tissue-specificity, developmental expression and biochemical characteristics of AChE isoenzyme/activity profiles in the hybridising *Drosophila* species. (*D. bipectinata* & *D. malerkotliana*).

Material and Methods

The synchronization and timing of different developmental stages of *D. bipectinata*, *D. malerkotliana* and their hybrids were done according to Stocker

and Jackson (1971). The homogenates (100 mg/10 ml of 0.5% Triton-X) of tissues at different developmental stages were centrifuged at 12,000 Xg for 30 min at — 4°C and the supernatents were assayed for qualitative and quantitative estimation of AChE activity. The samples were subjected to slab starch-gel electrophoresis (250 V, 25mA. The gels were stained for AChE activity according to the procedure followed by Hall (1973). The AChE bands were numbered in the cathodal to anodal sequence. The stage-specific homogenates were maintained at 50 and 60°C for 15 min; and were also pre-incubated with different inhibitors (10 and 20mM Eserine sulphate; 0.2 & 0.4% DDVP and 5mM ISO-OMPA) for 15 min at 30°C and were analysed electrophoretically alongwith the controls.

AChE activity was determined according to Ellman et al. (1961). The acetylthiocholine iodide was used as the substrate and O. D. was measured at 412 nm with Spectronic 20. The absorbance units were converted into μ moles of substrate by compairing with the glutathione standard curve. Thermostability of acetylcholinesterase from stage-specific homogenates was determined by pre-incubating the extracts at 45°C, 55°C and 65°C for 15 min followed by enzyme activity determination by standard assay method. The extracts were pre-incubated at 60°C for different time intervals so as to distinguish between thermostable and thermolabile properties and to analyse the patterns of denaturation of AChE activity. To determine the extent of inhibition, the stage-specific extracts were pre-incubated with different inhibitors (10 and 20mM Eserine sulphate; 5mM ISO-OMPA (Tetra isopropylpyrophosphoramide); and 0.2 and 0.4% of DDVP and the residual AChE activity was determined.

Results

The developmental expression as well as biochemical characteristics of AChE isozymes in *Drosophila* species and in their hybrids are represented in figures 1 and 2.

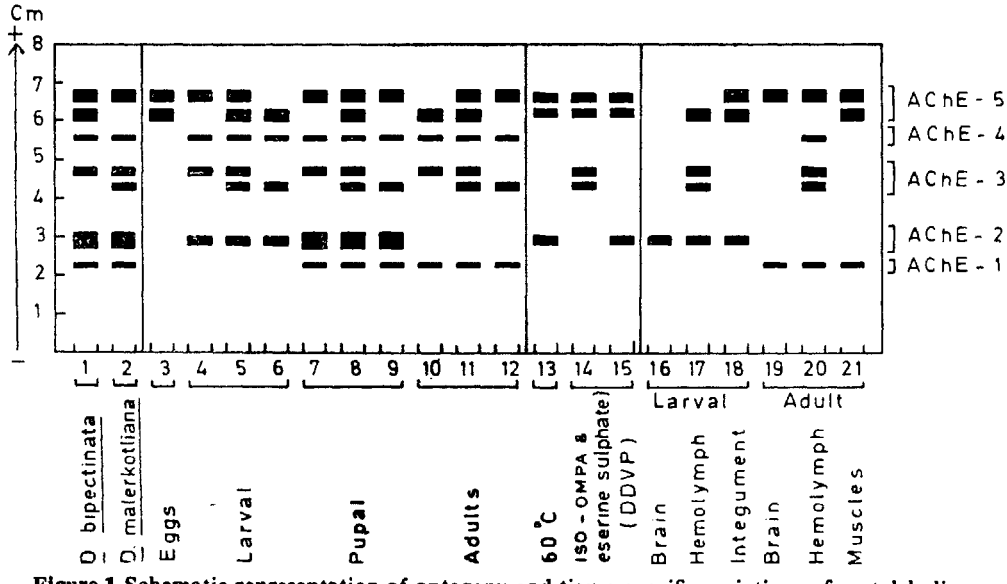

Figure 1 Schematic representation of ontogeny and tissue-specific variations of acetylcholinesterase isoenzymes in ***D. bipectinata, D. malerkotliana*** and their hybrids

There was only one AChE zone in the eggs; four in the larval and the adult stages; and five in the pupal stages. AChE-2 zone was confined to the larval and the pupal stages only while the AChE-1 zone expressed itself during the subsequent developmental stages too. AChE-3 to AChE-5 zones occurred throughout ontogeny and they showed intensity differences (figures 1 & 2). AChE-3 and AChE-5 zones were polymorphic while other zones were monomorphic. The interspecific hybrids revealed composite patterns of allelic and non-allelic AChE isoenzymes and expression of species-specific AChE isoenzymes was codominant as well as synchronous. The tissue-specific patterns of AChE-isozymes showed AChE-2 in the brain, AChE-2 and 5 in the integument; and AChE-2, 3 & 5 in the hemolymph. AChE-1 & 5 were localised in adult brain and muscles while all the four adult specific isozymes occurred in the adult hemolymph (figures 1 & 2).

Genetic basis : The zymograms of parents and progeny of several single pair

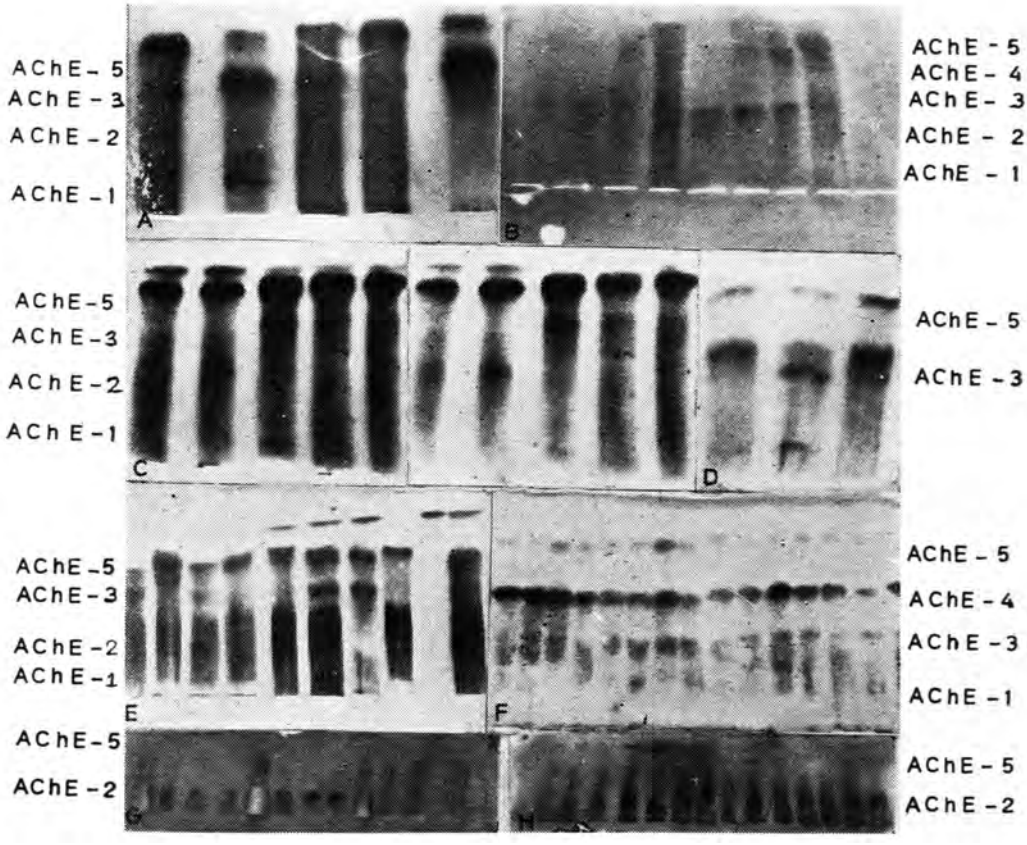

Figure 2A–H Electrophoretic profiles of AChE-isoenzymes of hybridising *Drosophila* species. **A**, *D. bipectinata*; **B**, *D. malerkotliana*; **C**, interspecific hybrids; **D**, inhibitor response (ISO-OMPA and eserine sulphate); **E**, tissue specificity; **F**, adult hemolymph; **G**, thermostability; **H**, effect of DDVP on AChE isoenzymes

Table 1 *Genetic control of electrophoretic variants at polymorphic AChE loci in* Drosophila *species hybrids* (D. bipectinata × D. malerkotliana)

AChE locus	Parental phenotypes	Phenotypes of Progeny*			No. of individuals analysed
		FF	FS	SS	
AChE-3	FS × FS	14	27	15	56
	FF × SS	—	11	—	11
	FF × FS	7	9	—	16
	FS × SS	—	10	13	23
AChE-5	FS × FS	5	12	7	24
	FS × SS	—	8	10	18
	FF × SS	—	11	—	11
	FS × FS	6	14	8	28
	SS × FS	—	8	9	17

* Chi-square values insignificant at 5% level; F & S, fast and slow electrophoretic variants respectively; —, absence of enzyme phenotypes

matings involving intra- and inter-specific hybrids were compared to reveal genetic control of AChE isoenzymes. The polymorphic zones were represented by any of the two alternating single band variants (fast or slow) or by the composite two band patterns (fast and slow). The seggregating AChE phenotypes of progeny of various single pair matings depicted either back cross ratio of 1:1 or F_2 ratio of 1:2:1 indicating monogenic control of the polymorphic zones (table 1). However, AChE-1 and AChE-2 zones may be coded by distinct loci or may be related epigenetically. Electrophoretic variation at AChE-3 and AChE-5 zones had no effect on the expression of AChE-1, 2 and 4 zones indicating that AChE zones may be under distinct genetic control.

AChE isozyme characteristics: The AChE zones showed differential thermal inactivation. AChE-2 & 5 zones are thermostable, while AChE-1, 3 & 4 zones showed complete inhibition at 50°C & 60°C (figures 1 & 2). Eserine sulphate and ISO-OMPA caused differential inhibition of AChE isozymes, i.e. AChE-1, 2 & 4 were completely inhibited whereas AChE-3 & 5 were resistant to inhibitors. DDVP (0.2 & 0.4%) caused complete inhibition (AChE-1, 3 & 4) or partial inhibition (AChE-2 & 5). The adult-specific AChE-1 was susceptible to all the three inhibitors whereas larval-specific AChE-2 was so to eserine sulphate and ISO-OMPA only.

AChE-activity profiles: The AChE-level was low in larval stages followed by a rapid build up of the enzyme activity in pupal stages (figure 3). However, after eclosion, there was further increase in AChE activity during early-adult life followed by a gradual but slight decrease in the enzyme activity with the advancement of age upto 20 days. The species-specific variations in AChE activity

profiles were observed but the overall pattern was nearly the same for the hybridising *Drosophila* species (figure 3).

Thermostability: The stage-specific residual activity was about 65% at 45°C, 35% at 55°C and 15% at 65°C and depicted almost identical trends for different developmental stages and for hybridising *Drosophila* species. The slope of the decay of AChE activity at 60°C as a function of incubation time was compared on the semilogarithmic plot (figure 4). The loss of stage-specific activity of extracts can be attributed to the presence of two components, the major being slightly more heat-labile while a minor being relatively stable (figure 4).

Inhibitor response: The extent of inhibition with 10mM eserine sulphate was 50% in the larval, 70% in the pupal and 80% in the adult stages, whereas there was significant inhibition (85±5%) at 20mM concentration (figure 3). The effect of ISO-OMPA (5mM) was not dependent on stage-specific enzyme activity and the residual AChE-activity was about 75±5% in the parental *Drosophila* species as compared to 55±5% in the interspecific hybrids. DDVP (0.2 and 0.4%) revealed 85±5% residual activity in the larval and the pupal stages, and 21±5% and 6±2% respectively in the adults (figure 3).

Discussion

The occurrence of maximum AChE activity/isozyme profiles in the pupal stages may be due to maturation and elaboration

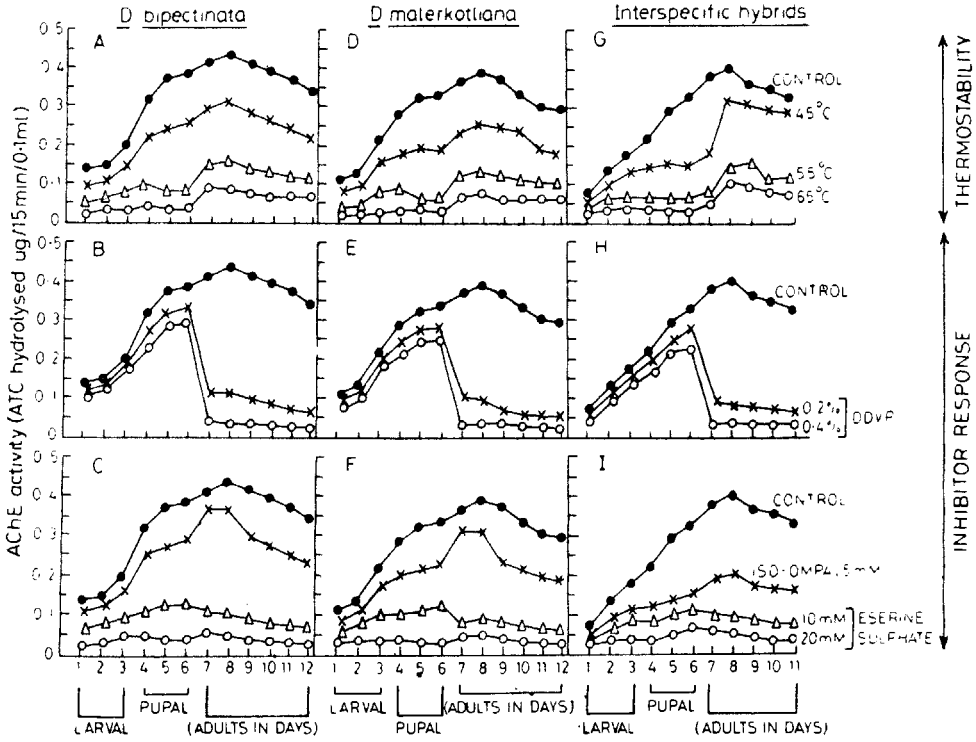

Figure 3 Ontogenetic AChE activity profiles and their characterisation on the basis of thermostability and inhibitor response in *Drosophila* species and interspecific hybrids

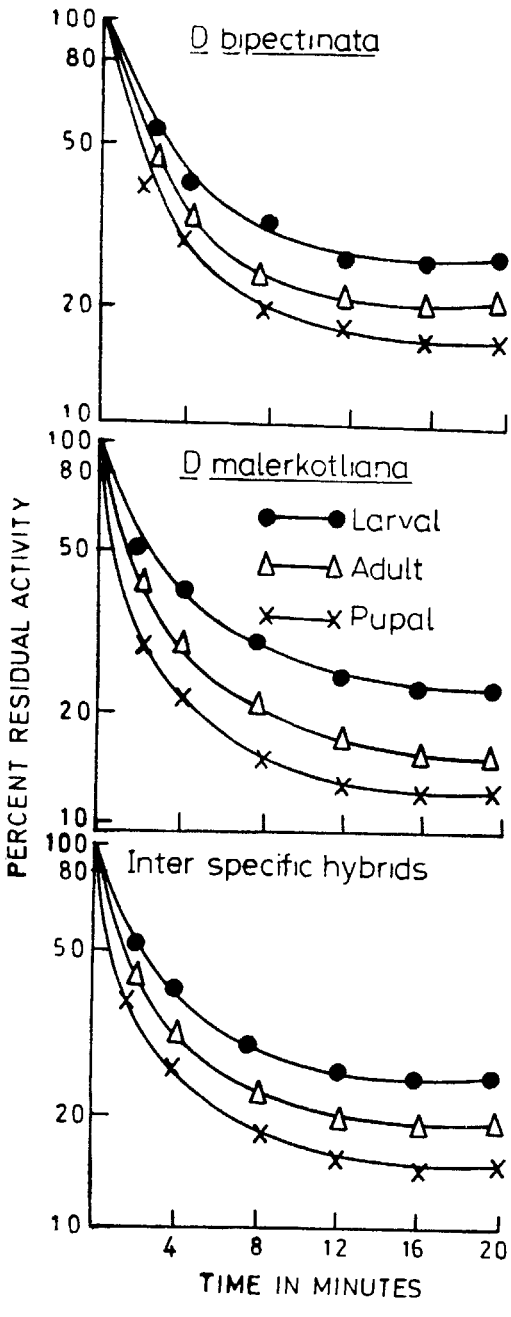

Figure 4 Thermal inactivation profiles of stage-specific acetylcholinesterases at 60°C in *Drosophila* species and their hybrids

of the nervous system during this period. The gradual build up of AChE-activity during larval-pupal-adult transition points out temporal regulation of AChE gene activity at the transcription and/or translation level. The similarities in the AChE isozyme characteristics on the basis of thermostability and inhibitor response suggests structural relatedness of the species-specific AChE-isoenzymes. The insignificant effect of DDVP on the larval and the pupal-specific total AChE-activity profiles can be due to the occurrence of AChE-2 zone which confers resistance to organophosphate (DDVP). The brain- and muscle-specific AChE-isozymes suggest their involvement in the regulation of synaptic transmission. However, the occurrence of AChE isozymes in non-neural tissues (hemolymph and integument) suggests their role in general metabolism and cell functions not concerned with the synaptic activity. It is well established that AChE hydrolyses acetylcholine and releases acetyl as well as choline moieties (Wilson 1960). The role of integument- and hemolymph-specific isoenzymes may be to channelise the utilization of choline for lipid metabolism. The present observations on qualitative and quantitative aspects of ontogenetic AChE activity find support from previous studies on histochemical localisation of AChE-activity in non-nervous tissues i.e. cellular organelles, cytosol, red blood cells and eggs (Silver 1974, Ryberg 1973, and Bridges 1972); biochemical analysis of stage-specific activity profiles in various insects (Silver 1974); and biochemical differences among four or five muscles and/or brain-specific AChE-isozymes in rat as well as in Chick (Wade & Timiras 1980, Tennyson et al. 1973, McIntosh & Plummer 1973, Varela 1973, Tripathi et al. 1973, Juul 1968 and Dudai 1972).

The data on electrophoretic analysis of interspecific *Drosophila* hybrids are consistent with synchronous activation and co-dominant expression of species-specific AChE-isoenzymes during ontogeny as well as in differentiated tissues. The zymograms revealed lack of preferential expression of paternally and/or maternally-derived allelic acetylcholinesterases. It has been pointed out that greater the degree of synchrony observed in allelic activation during ontogeny of species hybrids, the greater is the similarity of the parental alleles (Subtelny 1974, and Ohno 1970). According to this criterion, the polymorphic AChE loci are homologous in the hybridizing *Drosophila* species. Present data is consistent with the view that allelic asynchrony occurs less frequently in hybrids derived from closely related species (Champion & Whitt 1976, Korochkin & Matveeva 1974).

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References

Bridges R G 1972 Choline metabolism in insects; in *Advances in Insect Physiology* vol **9** pp 51–91 eds JWL Beament, J E Treherne and V B Wigglesworth (New York : Academic Press)

Champion M J and Whitt G S 1976 Differential gene expression in multilocus isozyme systems of the developing green sunfish; *J. expl. Zool.* **196** 263–282

Dickinson W J and Sullivan D T 1975 in *Gene-enzyme-systems in Drosophila* (Berlin: Springer-Verlag)

Dudai Y, Silman I, Kalderon N and Blumberg S 1972 Purification by affinity chromatography of acetylcholinesterase from electric organ tissue of the electric eel subsequent to tryptic treatment; *Biochim. Biophys. Acta* **268** 138–157

Ellman G L, Courtney K D, Andres V and Featherstone R M 1961 A new and rapid colorimetric determination of acetylcholinesterase activity; *Biochem. Pharmacol.* **7** 88–95

Hall Z W 1973 Multiple forms of acetylcholinesterase and their distribution in endplate and non-endplate regions of rat diaphragm muscle; *J. Neurobiol.* **4** 343–361

Juul P 1968 Human plasma cholinesterase isozymes; *Clin. Chim. Acta* **19** 205–213

Korochkin L I and Matveeva N M 1974 Genetics of esterases in *Drosophila* of the virilis group II. Sequential expression of paternal and maternal esterases in ontogenesis; *Biochem. Genet.* **12** 1–7

McIntosh C H S and Plummer D T 1973 Multiple forms of acetylcholinesterase from pig brain; *Biochem. J.* **133** 655–665

Ohno S 1970 in *Evolution by Gene Duplication* (Berlin & New York: Springer-Verlag.)

Ryberg E 1973 The localisation of cholinesterases and non-specific esterases in echinopluteus; *Zool. Scri.* **2** 163–170

Silver A 1974 The biology of cholinesterases; in *Frontiers of Biology* **36** pp 596 (Amsterdam: North-Holland)

Stocker A J and Jackson J 1971 A technique for the synchronization of *Drosophila* for developmental studies; *Dros. Inf. Ser.* **46** 141

Subtelny S 1974 Nucleo-cytoplasmic interactions in development of amphibian hybrids; *Int. Rev. Cytol.* **39** 35–88

Tennyson V M, Brzin M and Kremzner L T 1973 Acetylcholinesterase activity in the myotube and muscle satellite cell of the fetal rabbit: An electron microscopic, cytochemical and biochemical study; *J. Histochem. Cytochem.* **21** 634–652

Tripathi R K, Chiu Y C and O'Brien R D 1973 Reactivity in vitro toward substrate and inhibitors of acetylcholinesterase isozymes from electric eel, electroplax and housefly brain; *Pestic. Biochem. Physiol.* **3** 55–60

Varela J M 1973 Kinetic characterization of acetylcholinesterase of muscle and rat brain; *Experientia* **29** 1347–1349

Wade P D and Timiras P S 1980 A regional study of the molecular forms of acetylcholinesterase in the brain of developing and adult rats; *Dev. Neurosci.* **3** 101–108

Whitt G S, Philipp D P and Childers W F 1977 Aberrant gene expression during the development of hybrid sunfishes (Perciformes, Teleostei); *Differentiation* **9** 97–109

Wilson I B 1960 Acetylcholinesterase; in *The Enzymes* (2nd edn) vol **4** pp 501–520 eds P D Boyer, Z Hardy and K Myrback (New York: Academic Press)