BIOCHEMICAL GENETICS OF TWO HIGHLY POLYMORPHIC ESTERASES IN *DROSOPHILA BUZZATII*

by

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PREFACE

The work presented in this thesis is original, and was carried out under the supervision of Professor J.S.F. Barker in the Department of Animal Science, University of New England, Armidale.

I certify that all results reported herein have not, and are not currently being submitted for any other degree, and that all sources of information have been duly acknowledged.

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ABSTRACT

This thesis presents the results of a series of experiments which were undertaken in an attempt to describe some basic biochemical and physiological features of the two predominant adult esterase isozymes of the cactophilic species *Drosophila buzzatii*.

The two isozymes ESTERASE-1 and ESTERASE-2 were first characterised with respect to their ontogenic and tissue distributions. ESTERASE-1 was found to be located predominantly in the haemolymph, and although present in all stages of development showed highest titres in late third instar larvae and metamorphosis and again in young adults immediately after eclosion. In addition another esterase activity, EST-J was described during late larval and pupal development, and this enzyme appears to be related to the EST-1 isozyme. The EST-2 enzyme was found to be distributed primarily in the alimentary tract, and it also was found to be present in all life stages. However, EST-2 showed multiple peaks of activity corresponding approximately to the mid-late second and third larval instars, mid-pupal development and a broad peak of activity over the first week of adult life.

Biochemical comparisons of the EST-1 and EST-2 isozymes revealed that these two enzymes differed for every character examined. ESTERASE-1 was apparently a dimer of molecular weight 128,000 daltons. It had a pH optimum in the vicinity of 8.0 to 9.0, was relatively stable at 50^{o}C and showed a marked substrate preference for formate esters, and esters of propionic acid. By contrast, EST-2 was a monomer of molecular weight 54,000 daltons. It had a pH optimum around 7.0 to 7.5, was unstable at 50^{o}C and showed a preference for esters of butyric acid. These two isozymes also differed in their response to inhibitors. These biochemical and physiological results permitted the establishment of fairly convincing homologies between the *Est-1* and *Est-2* loci of *D. buzzatii*, and the major β- and α-*Est* loci of other *Drosophila*

species in the *virilis-repleta* radiation.

Some biochemical comparisons were made of allozymic variants produced by the *Est-1* and *Est-2* loci. Significant differences were detected between two variants of the *Est-1* locus, but these were never large, and one allozyme was almost invariably catalytically superior to the other. This was not the case for four allozyme variants of the *Est-2* locus, where there was evidence of thermostability and substrate specificity differences. Further kinetic analyses of the *Est-2* allozymes confirmed that they were not biochemically equivalent with respect to thermodynamic and substrate specificity parameters. It remains to be determined whether these biochemical differences are manifested as physiological differences *in vivo*.

Finally, a preliminary analysis of electrophoretically cryptic variation was undertaken for two electromorph classes of the *Est-2* locus. The results indicated the existence of a large number of electrophoretically cryptic variants, at least some of which appeared to behave as allelic variation at the *Est-2* locus. The data suggested that resolution of these cryptic variants may profoundly affect the nature of gametic disequilibrium between the *Est-2* locus and a polymorphic second chromosome inversion in *D. buzzatii*.

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