romethylketone exhibited noncompetitive inhibition with an estimated K_I-value of 5.9×10^{-3} M.

Earlier studies with Tos-Lys-CH_2Cl and Tos-Phe-CH_2Cl have demonstrated the utility of these agents as active site inhibitors of trypsin and chymotrypsin respectively[10,11]. However, proteases like subtilisin and plasma kallikrein, which possess in common, the esterase activity on lysine derivatives exhibited by trypsin, are not affected by Tos-Lys-CH_2Cl[6]. The synthetic Ac-Phe-Lys-CH_2Cl described here should prove very useful in such studies with subtilisin, in view of the fact that the peptide structure derived from the acetyl aminoacid and lysine affords better structural similarity to its physiological substrate.

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Genotype-isopropanol interaction in the *Adh* locus of *Drosophila buzzatii*[1]

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Summary. Drosophila buzzatii, when reared in a medium with isopropanol, shows a significant band interconversion in ADH zymograms and a general lowering of ADH activity. Changes in activity are greater in Adh^F homozygotes than in Adh^S homozygotes and generate a significant genotype-isopropanol interaction. These mechanisms are relevant to an explanation of the high natural *Adh* polymorphism of this species.

In recent years much effort has been devoted to demonstrating selection in individual allozyme loci. The alcohol dehydrogenase *(Adh)* locus has been the one used most successfully in this search, since now a general agreement exists that this locus seems to be adaptive in at least 2 species of *Drosophila*[2-9]. Here we show evidence that the *Adh* locus of *Drosophila buzzatii* may be also a likely candidate for the operation of natural selection.

We have sampled 16 different natural populations of *D. buzzatii* from a large area which includes localities in the Mediterranean (Egypt, Balearic islands, and Iberian peninsula) and in the Atlantic regions (Canary islands, Madeira island and Cotonou). The *Adh* polymorphism has been found to be high in all but 1 of the sampled populations, with 2 electromorphs in high frequencies (Adh^F and Adh^S) and 1 electromorph very rare and found only in 1 population. Figure 1 shows the standard electrophoretic bands of the 2 common alleles. Adh^F has frequencies ranging from 0.35 to 1.00. We have performed segregation tests (F_2 and backcrosses) which showed that Adh^F and Adh^S allozyme variants segregated in a Mendelian way. All this information is in accordance with data reported by Barker and Mulley[10] for Australian populations.

We observed that the intensity of the electrophoretic bands was highly variable and faded away during a time of culturing in the laboratory. In order to understand this variability in activity, we tested changes in staining intensity when larvae were reared on media containing several kinds of alcohols, since we know that these are likely substrates for the ADH enzyme. Tests were performed with 3 short chain alcohols (methanol, ethanol and isopropanol), which were added to the culture medium at initial concentrations of 1 and 2% in volume. Staining of zymograms was carried out with each of 4 alcohol substrates individually: methanol, ethanol, isopropanol and isobutanol, and revealed that only the medium with isopropanol induces significant changes in the standard pattern of bands. These changes are of 2 kinds: 1st, the most electropositive bands disappear or lose much of their activity; and 2nd, there is an increase of staining in the electronegative bands. This enhancement of activity, although general, is much stronger when the substrate is isopropanol, in which case new, more electronegative, bands may appear (figure 2).

This qualitative experiment showed that important changes in ADH activity may be induced by isopropanol in *D. buzzatii.* However, we wanted to know how these changes may be quantified and assigned to individual *Adh* genotypes. For this purpose, we isolated 5 independent homozygous strains for each of the 2 alleles Adh^F and Adh^S. These strains were cultured separately in standard laboratory medium either with 1% isopropanol or without alcohol, and their 'in vitro' specific activity (SA) measured spectrophotometrically. Previous experiments indicated that activity is highest in early pupae, so we chose this stage for the experimental check.

Table 1 shows that isopropanol induces an overall significant decrease of SA. This decrease is much stronger for the Adh^F homozygote than for the Adh^S homozygote. The analysis of variance of table 2 shows a significant genotype-isopropanol interaction, which confirms the non-proportional changes of activity between genotypes. The analysis of variance depicts also significant differences between genotypes, which are due exclusively to the differential effect of isopropanol on the genotypes. Thus, differences in SA between genotypes are only statistically significant when isopropanol is added to the medium ($t = 7.25$; $p < 0.001$).

David et al.[11] have advanced the hypothesis that the high toxicity shown by secondary alcohols on *Drosophila* may be explained not as a direct toxic effect of the alcohol, but as

an accumulation of an intermediate metabolite (e.g. an aldehyde or a ketone) highly toxic to the cell. Then, any regulatory mechanism that decreases the ADH activity would be highly adaptive in that environment. Precisely this decrease in activity is observed in *D. buzzatii* when flies are cultured in an isopropanol environment. It is not by chance that these changes in SA are observed in conjunction with band interconversion. This interconversion has been known in *D. melanogaster* for some time[12–14]. Schwartz and Sofer[15] demonstrated that ketones are able to induce interconversion by fixing 0, 1 or 2 molecules to the different electrophoretic bands. In a recent study McDonald and collaborators[16] suggested that in *D. melanogaster* electronegative forms are much less biochemically efficient in ADH-F than in ADH-S allozymes. All this information is relevant here and may explain why isopropanol induces in *D. buzzatii* genotype-dependent SA changes through band interconversion.

These changes in SA are likely to operate in nature.

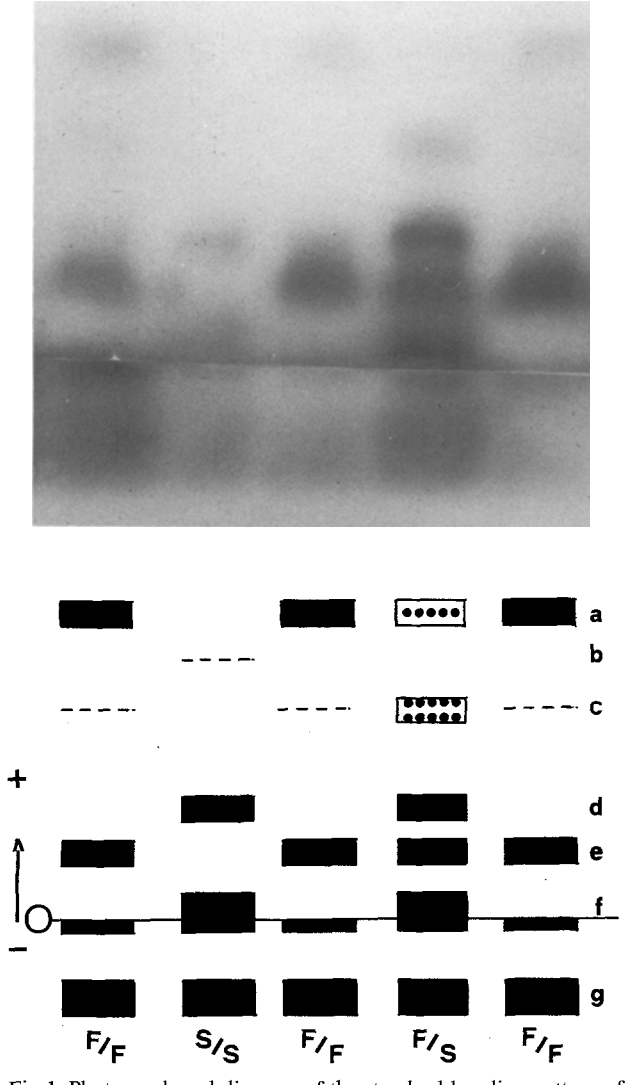

Fig. 1. Photograph and diagram of the standard banding pattern of Adh^F homozygotes (F/F), Adh^S homozygotes (S/S) and heterozygotes (F/S). O = origin. Intensity of staining is indicated by degrees of shading. Bands are designated from a to g in order to compare with bands in figure 2. The starch electrophoresis technique was carried out as in Fontdevila et al.[17] using early pupae. The substrate used was isopropanol, with NAD as coenzyme, phenacine metasulphate as electron transporter and nitroblue tetrazolium as stain.

Table 1. Mean specific activities (SA) with standard errors as nmoles of NAD^+ reduced/ml/min/mg live weight of *D. buzzatii* *Adh* genotypes

Genotype	Type of culture No isopropanol	With isopropanol
Adh^F/Adh^F	3.42±0.08	1.60±0.15
Adh^S/Adh^S	3.53±0.32	2.89±0.03

Values of SA are the average of means of 6 replicated assays for each of 5 independent strains by genotype and culture type, except in the case of Adh^S homozygote cultured in isopropanol medium, where only 4 strains were used. Individual assays of SA's were initiated by centrifuging at 1100 G for 45′ homogenates of 10 mg of early pupae in 1 ml of deionized water. The supernatants were assayed for ADH activity in a Pye Unicam SP 1800 UV Spectrophotometer by following the increase in absorbance at 340 nmoles after addition of the enzyme extract to a solution containing nicotinamide adenine dinucleotide (NAD) and isopropanol.

Table 2. Analysis of variance for values of SA

Source of variation	Degrees of freedom	Mean square	F-value
Genotypes (G)	1	10.127	8.84*
Isopropanol (I) concentration	1	31.855	27.80***
Interaction (G×I)	1	10.494	9.16*
Strains (S)	12	1.146	5.33***
Within cells	80	0.215	

* $p<0.05$; *** $p<0.001$. The analysis follows a model with 2 main factors (G and I) and a nested factor (S), within G and I[20]. In order to perform a balanced analysis we have used only 4 strains of each genotype.

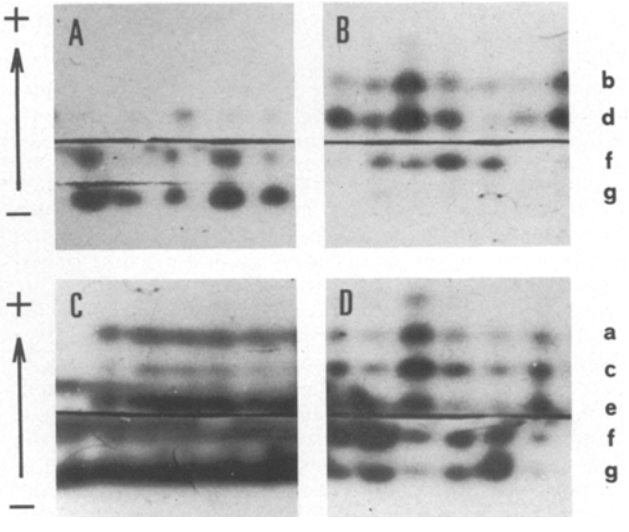

Fig. 2. 4 partial close-ups of a single starch gel which shows the comparative banding patterns of *Adh* zymograms when no isopropanol (A, C) and 1% isopropanol (B, D) is added to the larval growth medium. When Adh^S homozygotes are treated with isopropanol (B) they show a band interconversion which inactivates the most electropositive band (g) and activates the most electronegative band (b) of the zymogram of non-treated flies (A). In Adh^F homozygotes, comparison between zymograms of non-treated (C) and treated (D) flies shows a similar band interconversion as reflected by changes in activity of bands g and c. The starch electrophoresis technique was carried out as in Ursprung and Leone[19] using early pupae. The substrate used was isopropanol, with NAD as coenzyme, phenacine metasulphate as electron transporter and nitroblue tetrazolium as stain. Arrows indicate migration towards anode.

D. buzzatii is a cactophilic species known to be highly associated with prickly pear (*Opuntia* sp.)[17]. We have observed (unpublished work) that among all *Drosophila* species which coexist in the *Opuntia* areas of the Old World, only *D. buzzatii* can efficiently exploit rotted *Opuntia*. We have also analyzed (unpublished results) alcohol content in rotted *Opuntia* from 5 different localities and found that isopropanol is the most abundant; the mean isopropanol content is of the order of the concentrations tested in our experiment. However, there is a large variability in the isopropanol concentration in samples of rotted *Opuntia*.

In a natural population, rotted material with a high isopropanol content would favour the Adh^F genotypes, whereas those with a low content would not. Other selective factors should be found to operate in order to explain satisfactorily the maintenance of this polymorphism in nature, but the posttranslational mechanism here suggested would support the notion of an adaptive significance of *Adh* polymorphism in *D. buzzatii*.

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Cytotaxonomy of the Seychelles tree frog, *Megalixalus seychellensis* (Duméril and Bibron) (Amphibia: Hyperoliidae)[1]

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Summary. The Seychelles tree frog, *Megalixalus seychellensis* has 2n = 24 chromosomes of gradually decreasing length. Pairs 2, 3, and 4 are submetacentric, and the remaining pairs are metacentric. The karyotype affirms hyperoliid assignment of this species, and indicates a link between the Seychellean fauna and the African-Madagascan faunas.

Ranoid frogs may be divided into 2 major groups based on the presence or absence of an intercalary cartilage between the ultimate and penultimate phalanges. Those without intercalary cartilages are generally ground-dwelling or terrestrial forms, whereas those with intercalary cartilages are usually arboreal in habit. The relationships among the genera of arboreal ranoids are poorly understood, and their classification has been highly unstable[2,3]. Liem's[3] study of ranoid frogs led him to conclude that the arboreal genera form 2 monophyletic assemblages evolved from 2 separate, terrestrial ranoid groups. 1 group of arboreal genera, the Hyperoliidae, is thought to have evolved from a group of terrestrial African ranids, and the 2nd arboreal group, the Rhacophoridae, is believed to be derived from terrestrial, Asiatic ranids.

The Hyperoliidae and Rhacophoridae have overlapping ranges and complementary east-west diversity gradients. Hyperoliid diversity decreases in an easterly direction. There are 11 genera and 63 species of hyperoliids in Africa, 1 genus and 2 species in Madagascar, and a single, monotypic genus *(Megalixalus)* in the Seychelles Archipelago. By contrast, rhacophorid diversity increases in an easterly direction: 1 genus and 4 species in Africa, 6 genera and 44 species in Madagascar, and 8 genera and 66 species in Asia. The presence of the hyperoliid, *Megalixalus seychellensis*, in the Seychelles seems anomalous since the Seychelles Archipelago lies between the 2 major centers of rhacophorid diversity (Madagascar and Asia) and far (1600 km) from the major center of hyperoliid diversity (Africa). Furthermore, the ancient elements of the endemic flora and fauna of the Seychelles indicate a closer affinity to the Oriental than to the Ethiopian region[4]. Given these facts of distribution, one would have predicted, a priori, a rhacophorid relationship for arboreal, Seychellean ranoids. However, as I will show, comparison of the karyotype of *M. seychellensis* to the karyotypes of other arboreal ranoids supports the current assignment of *M. seychellensis* to the Hyperoliidae.

Specimens were collected 26 August 1977, at 400 m along the Grand Bois River, Mahé, Republic of Seychelles. Meiotic and mitotic karyotypes were obtained from 3 adult males which are now in the collections of the Museum of Zoology, The University of Michigan (UMMZ 146624-6). A squash technique, described elsewhere[5], was used to obtain the karyotypes. *M. seychellensis* has a karyotype