

Alcohol Dehydrogenase Polymorphism of *Drosophila melanogaster*: Aspects of Alcohol and Temperature Variation in the Larval Environment

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Abstract

Some natural habitats of *D. melanogaster* larvae were examined for short-chain alcohols by gas-liquid chromatography. All habitats contained a mixture of such alcohols, although ethanol predominated and was found at concentrations ranging from 0.02 to 4.0% (v/v). Normal propanol, propan-2-ol, n-butanol and methanol were nearly always present. Larval-to-adult survival was determined at three temperatures on laboratory media supplemented with alcohol mixtures similar to those found in nature, and using strains which differed in alcohol dehydrogenase genotype. The basic medium was one in which strains compete for limited amounts of dead yeast. Significant genotype-alcohol and genotype-temperature interactions highlighted the sensitivity of this technique for detecting larval fitness variation. The alcohol effect on survival to the adult stage was strongly dependent on temperature—a result which may be of value in our understanding of the ecology of this species.

Extra keywords: genetic polymorphism.

Introduction

Considerable information is available about the alcohol dehydrogenase locus (*Adh*) in *Drosophila melanogaster*—a locus polymorphic in this species throughout the world (see references and discussions in van Delden *et al.* 1978; MacDonald *et al.* 1980 and Oakeshott *et al.* 1981). However, the nature of the balancing selective forces which maintain the polymorphism in natural populations is still not understood. In this study the levels of some naturally occurring environmental alcohols were estimated and the possible influence of these, and of larval development temperature, on survival and genetic variation at the locus were examined.

A major function of alcohol dehydrogenase (ADH) is to metabolize environmental alcohols. A number of laboratory studies have demonstrated the potential of ethanol to select at the locus (see, for example, Gibson 1970; Oakeshott 1976; and Cavener and Clegg 1981). However, it is difficult to extrapolate from these studies to possible selective effects in the field since little is known about the type or levels of alcohols present in natural habitats. ADH catalyses *in vitro* the oxidation of a number of other short-chain primary and secondary alcohols (Dickinson and Sullivan 1975) and these alcohols have also been implicated in fitness determination at the locus (Morgan 1975; Ainsley and Kitto 1975; van Delden *et al.* 1975). If they occur in nature these alcohols may select at the *Adh* locus.

Both field and laboratory studies have implicated environmental temperature as a second factor selecting at the locus (see, for example, Johnson and Powell 1974; McKenzie and McKechnie 1981). Under constant-temperature conditions the life cycle of this species can only be completed within a restricted temperature range (from about 15 to 30°C—see McKenzie 1978). Although short exposures to temperatures outside this range may be tolerated (Parsons 1977; Schenfeld and McKechnie 1979) most natural populations presumably develop at variable temperatures within this range. If ADH plays a significant role in developmental metabolism, and this is indicated by its high levels of activity in larval fat body (Ursprung *et al.* 1970), these permissive temperatures would seem appropriate to use in larval tests for selection at the locus.

Gas-liquid chromatography (GLC) was used to detect short-chain alcohols in some larval habitats of *D. melanogaster* (mainly decomposing fruit), and to give an approximation of the level at which they occurred. Then, in laboratory medium at three temperatures, two different mixtures of alcohols, similar to mixtures found in nature, were tested for possible survival and selective effects during larval development.

Materials and Methods

Estimation of Environmental Alcohols

Seven larval habitats were sampled. Five of these were damaged fruit placed in separate heaps under tree shade in suburban Melbourne at the peak of the *D. melanogaster* season (March). These were sampled for alcohol content after about 8 days of 'maturation' as a larval habitat (when third-instar larvae could be readily detected). Small pieces of rotting fruit were taken from larval sites and either squeezed for juice or centrifuged (without homogenization) to obtain a clear supernatant. These aqueous samples were sealed into a small test tube with Parafilm and deep frozen. Evaporation of alcohols was minimized by keeping tubes in an ice-bath before analysis and unsealing only for short periods. A sixth sample was taken from a pile of grape skin and stalk residues at the Tahbilk Winery, approximately 100 km north of Melbourne. This site, about 200 m south of the Tahbilk cellar, attracts large numbers of *D. melanogaster* and is a rich larval habitat (McKenzie and McKechnie 1979). The samples were from the drier, post-fermentation stage of the decomposing pile. To extract juice from skins a minimum volume of distilled water was added and the pulp squeezed. Finally, red wine seepage was taken from the base of an oak cask in the Tahbilk cellar. For analysis, 5- μ l aliquots were injected into a Hewlett-Packard research chromatograph, model 5750, with dual flame ionization detectors. Routinely, two 1.8 m (3 mm o.d.) stainless-steel columns packed with Porapak Q (120–150 mesh) (Waters Assoc. Inc., Mass., U.S.A.) were used (column temperature 130–240°C, programmed at 6°C/min; carrier gas N₂; flow rate 16 ml/min; injection port and flame detector temperature both 270°C). To distinguish n-butanol from propionic acid a column of 5% (w/w) free fatty acid phase on acid-washed dimethylchlorosilane (Chromosorb W, 80–100) was used. Peak 'spiking' was carried out when necessary. Estimates of alcohol concentration were made by peak height comparison of mixtures of known alcohols made fresh each day and injected at the same attenuations as unknowns. Each sample was injected in duplicate, once before and once after the standard mixture, and the mean peak height compared to its standard. In no duplicate comparison was the taller peak more than 14% higher than the shorter.

The Flies

Two large populations, one homozygous *Adh^S* and the other homozygous *Adh^F*, were established at the same time by pooling, for each, the progeny of 20 pair-matings derived entirely by outbreeding from a population polymorphic at the *Adh* locus. This population was initiated several generations earlier from large numbers of flies caught at Tahbilk (>200♀). The aim was (1) to obtain a large random sample of background genes across the entire genome and (2) to randomize the sample of