

## Genetic Variability of *Drosophila melanogaster* at the Alcohol Dehydrogenase (ADH) locus

In nature, larvae and adults of *Drosophila melanogaster* feed on fermenting plant materials. Fermentation is generally due to yeasts, which release ethanol and higher alcohol that may partly inhibit the growth of competing organisms. Fruit flies thrive in this otherwise poisonous ecological niche, in part because they have evolved metabolic enzymes that confer tolerance to the toxic effects of alcohols.

More than 90% of the alcohol ingested by *D. melanogaster* is degraded by a biochemical pathway in which the first step involves the enzyme Alcohol Dehydrogenase (ADH - **NB: non-italics for a protein; italics or underlined for a gene or alleles**). ADH activity occurs in the fat body, midgut, and Malpighian tubules (= kidneys) of larvae and adults and in the reproductive organs of adults. ADH converts ethanol to acetaldehyde and higher alcohols to higher aldehydes, which are rapidly converted to acetate, which enters central metabolism. Ethanol exerts most of its toxic effects in flies, as in mammals, by altering the activity of membrane-bound ion channels important in neurotransmission and neuromodulation. Aldehydes are extremely toxic, but other enzymes normally prevent their accumulation to harmful levels.

The molecular biology of the *Drosophila ADH* gene has been well studied and the DNA sequence is known for the gene and its control regions in many strains of this and related species. This enables us to relate the evolution of the *ADH* gene to its function. Virtually all natural populations of *D. melanogaster* are polymorphic for two alleles *AdhF* (Fast) and *AdhS* (Slow) that code for proteins that differ by a single amino acid, threonine (neutral charge) versus lysine (positively charged) at position 192 (There are 255 amino acids in the whole ADH protein.). Because of this amino acid substitution, the proteins can be separated by electrophoresis, one being "fast", the other "slow". Out of many sequenced *ADH* genes sampled from widely separated wild populations, this same *AdhF/S* polymorphism was the only DNA difference that changed the amino acid coding properties of the gene. The frequencies of the *AdhF/S* alleles vary from place to place and from time to time. For example, there is an allele frequency cline from North to South down North America, with the *AdhS* allele being at its highest frequency in the South. This can be replicated in a laboratory study: Van Delden (1984) kept polymorphic fly populations for several generations at different temperatures and found that the frequency of the *AdhS* allele rose to 0.45 at 29.5 C, but dropped to 0.15 at 20 C and 25 C. These observations indicate that the genotype at the *ADH* locus is under differential selection by temperature, but the exact selective advantage of the *AdhS* allele at higher temperatures remains unclear.

### **Aims: This laboratory will give you experience in:**

- loading samples of homogenized tissues from individual *Drosophila* flies into an electrophoresis gel
- running the electrophoretic gel
- staining the gel, after electrophoresis, for the activity of the enzyme Alcohol dehydrogenase (ADH)
- with the aid of standards, interpreting the resulting allozyme phenotypes (pattern of bands on the gel) in terms of the alleles present in the genotypes of individual flies
- calculating
  1. observed population allele frequencies (autosomal co-dominant, dominant and recessive alleles),
  2. genotype frequencies expected under Hardy-Weinberg Equilibrium,
  3. expected frequencies of homozygotes and heterozygotes given allele frequencies (assuming Hardy-Weinberg Equilibrium conditions),

**Learning objectives: This laboratory will help you to learn and understand:**

- the relationship between gene, protein and band in gel for allozyme polymorphisms, and to be able to interpret band patterns in terms of homozygous and heterozygous genotypes
- the biochemical basis for the selective staining of a specific enzyme activity in a gel after electrophoresis
- how to calculate from allozyme phenotype frequencies the frequencies of the different autosomal dominant and recessive alleles present in a population and how to carry out calculations to test for agreement with or departure from Hardy-Weinberg genetic equilibrium
- how to predict the frequencies of homozygous and heterozygous genotypes given allele frequencies, and the assumptions required to make these predictions
- **and (most importantly)** make sure that you relate the experiences gained in this laboratory to what you learn elsewhere about
  - molecular diversity in populations,
  - molecular ecology, and
  - evolutionary population genetics.

**Experimental Protocol: You need to work quickly because:**

- (i) enzyme activity will decay during the loading process
- (ii) the electrophoresis needs over an hour to be completed.

1. Get ice bucket/ice
2. Obtain samples of homogenized *Drosophila* flies from Nancy. The samples will be frozen and will look slightly opaque. Place them on the lab bench for the ***minimum*** time needed to thaw --- watch carefully and as soon as the samples become slightly translucent, cool them on ice.
3. Load the cellulose gel: Nancy will demonstrate this to you.
4. As soon as your gel is loaded, get Nancy to help connect and switch on the electrophoresis power supply.
5. The gel needs to run for 45 mins and stain for 15 mins.
6. When it is time to stain the gels, ask Nancy to help disconnect and switch off the electrophoresis power-pack.
7. Use the staining mixture to detect the ADH enzyme activity in the gel.
8. Record your group's results in the table provided later in this packet.
9. Transfer your group's allele frequency results (once only!) to the Class Results Table and copy the class results to your schedule. Examine the Class results for agreement or departure from Hardy-Weinberg equilibrium.