How-To-Do-It

Teaching a Mendelian Codominant *Drosophila Melanogaster* Trait with Alcohol Dehydrogenase Allozyme Polymorphisms

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The purpose of this article is to show how *Drosophila* can be used in the teaching genetics laboratory to correlate the principles of heredity with the modern understanding of enzymes. There is probably no better organism than *Drosophila* in which to study inheritance of characters and the mechanism of heredity. *Drosophila* has been used in hereditary studies beginning with those of T. H. Morgan (1910) on sex inheritance. Study of the biochemical basis of genetics of *Drosophila* began in the 1930s with the work of Beadle, Tatum and Ephrussi on eye pigment biosynthesis using imaginal disk transplantations. This work had an important role in the development of the one gene: one enzyme hypothesis (Beadle & Tatum 1941). Following the improvement of gel electrophoresis by Smithies (1955), Wright (1963) performed the first electrophoretic detection of *Drosophila* enzyme variants using esterases. Despite this brilliant history, *Drosophila* has been absent from enzyme experiments in the genetics teaching laboratory.

Studies of enzymes can be traced back to Louis Pasteur. When juices of grapes are extracted and stored under anaerobic conditions, naturally occurring anaerobic yeast cells on the grape skins convert the sugar in the juice to ethyl alcohol, producing wine. The formation of alcohol from sugar is called fermentation. Louis Pasteur knew from his research that yeasts changed grape juice into wine and recognized the association of yeast and fruits to alcohol formation. The agent capable of producing fermentation was called a chemical ferment (now called an enzyme), and the process of fermentation was the first enzymatic pathway to be extensively studied (deKruif 1953).

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The common fruit fly, *Drosophila melanogaster*, is a frequent inhabitant of the fermentation environment of wineries throughout the world. This species produces alcohol dehydrogenase (ADH), an NAD+ requiring enzyme which catalyzes the oxidation of alcohols to aldehydes or ketones. Both primary and secondary alcohols can serve as substrates (Grell et al. 1968), however the natural substrate is probably ethanol.

The alcohol dehydrogenase genetic variation in *D. melanogaster* was first described by Johnson and Denniston (1964). Their observation of the variation was based on the enzyme exhibiting differential mobility when extracts of natural populations of flies were subjected to starch-gel electrophoresis. Two major electrophoretic bands, one moving faster than the other, were observed. They named the two allelic genes that produced the ADH Fast and ADH Slow polypeptides Adh^F (fast) and Adh^S (slow), respectively. When homozygous Adh^F/Adh^F flies were mated to homozygous Adh^S/Adh^S flies, the resulting heterozygotes (Adh^F/Adh^S) possessed the parental ADH bands (FF,SS) and, in addition, a hybrid band of intermediate mobility (FS). When the same enzyme, in this case alcohol dehydrogenase, shows multiple forms, the enzyme is referred to as an isoenzyme or isozyme. Since the enzyme variation is due to an alternative allele, the isozyme is often referred to as an alleloenzyme or allozyme (Prakash et al. 1969).

Ursprung and Leone (1965) concluded that the genes were autosomal and consistent with a mono-factorial inheritance pattern. They also assumed the enzyme to be a dimer, being comprised of two subunits. Grell et al. (1965) concluded that the hybrid bands were formed by the association of one fast and one slow subunit. Retzios and Thatcher (1979) found the Adh^S to be a point substitution of Adh^F at amino acid position 192, where lysine is substituted for treonine. Thatcher (1980) determined the complete amino acid sequence of the ADH^F and ADH^S allozymes and found that each subunit of the dimeric allozyme had 254 amino acids and a molecular weight of 27,400. Evolution and selection theories regarding ADH allozyme polymorphisms have been reviewed (Van Delden 1982).

Materials & Methods

Several strains of *D. melanogaster* were obtained from the National Drosophila Species Resource Center at Bowling Green State University (Bowling Green, OH, 43403-0212). One strain, 14021-0231.0 from Oahu, Hawaii, was found to be homozygous for the Adh^S allele and one strain, 14021-0231.9 from Oregon was found to be homozygous for the Adh^F allele. The flies were cultured with Blue Formula 4-24 from Carolina Biological Supply (2700 York Rd, Burlington, NC 27215). Three drops of yeast suspension were added for maintenance of the strains and for the genetic cross.

Horizontal starch-gel electrophoresis was performed on single flies. The gel was prepared by mixing 37 g of hydrolyzed starch (Connaught Laboratories, Ltd. ordered from Fisher Scientific, 585 Alpha Dr., Pittsburgh,

PA 15238) with 100 mL of 0.005 M histidine (adjusted to pH 8.0 with 2 N NaOH) in a 1000 mL flask with a side tubulation and a magnetic stir bar. Another 250 mL of buffer was heated to boiling and added to the unheated 100 mL/starch suspension and brought back to a boil while swirling the flask over a Bunsen burner. The starch suspension was degassed for 30 seconds by water vacuum and poured into a 13 cm × 31 cm × 0.6 cm Plexiglas mold. The mold can be made by placing Plexiglas strips on a thick glass plate and holding them with large paper clamps (May 1975). The starch is easier to handle if covered with plastic wrap and refrigerated overnight.

Individual flies were crushed directly onto a cut strip of Whatman chromatography paper with a glass rod. The size of the paper depends on the depth and width of the gel. The gel was cut widthwise. The paper holding the crushed flies was placed between the cut halves. A 10 minute run at 200 volts (15 amps) was done after which the paper was removed. The cut halves were pushed together and a run was made for three hours at 200 volts (15 amps), 6°C using 0.41 M sodium citrate bridge buffer (adjusted to pH 8.0 with 0.41 M citric acid) (Brewer 1970).

The gel was then placed on a flat surface and Plexiglas strips one-half the thickness of the gel were placed on either side to serve as a guide while pulling a tightly held nylon thread through the gel. The top section was discarded and the bottom section was placed in a tray for staining.

The stain for ADH consisted of 90 mL 0.05 M Tris-HC1 pH 8.5, 4 mL NAD nicotinamide adenine dinucleotide (10 mg NAD/mL), 4 mL phenazine methosulfate (2 mg PMS/mL), 2 mL nitro blue tetrazolium (10 mg NBT/mL) and 0.75 mL of 95 percent ethanol (Grell et al. 1965). The gel and stain were incubated at 37°C for 45 minutes and then fixed with a solution of acetic acid (30 mL), methanol (75 mL) and water (75 mL) and refrigerated. (Chemicals for stain can be purchased from Sigma Chemical Company, P.O. Box 14508, St. Louis, MO 63178.)

Results

Two strains that exhibited differential mobility and a single band phenotype for the ADH allozyme were selected for the experiment. Figure 1 illustrates the cross of the ADH allozyme phenotypic variations of the "fast" and "slow" allelic *Adh* locus. A female of strain stock number 14021-0231.9 homozygous (Adh^F/Adh^F) and a male of strain stock number 14021-0231.0 homozygous (Adh^S/Adh^S) were parents of the F_1 generation and came from true-breeding strains. The phenotypic difference between the parental ADH zymograms showed the ADH-SS allozyme traveled a greater distance from the origin towards the cathode than the ADH-FF allozyme. However, the F_1 hybrids (Figure 1), both female and male, were polymorphic and had three ADH allozymes (ADH-FF, ADH-SS and ADH-FS). The ADH-FF allozyme corresponded to the Adh^F/Adh^F parent, ADH-SS allozyme corresponded to the Adh^S/Adh^S parent and ADH-FS allozyme was intermediate between the FF and SS allozymes.

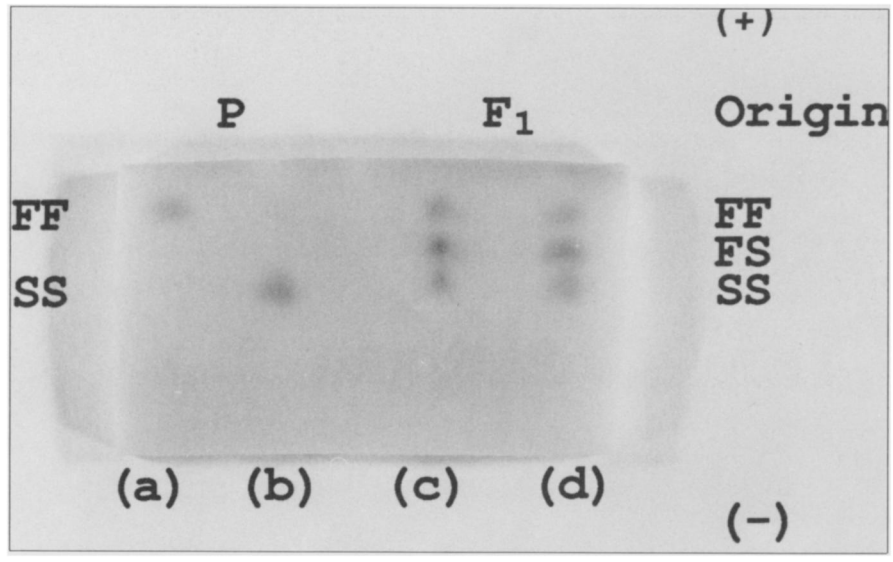

Figure 1. Electrophoretic zymograms of parental (P) and first filial generation (F_1) phenotypes of alcohol dehydrogenase allozymes in *Drosophila melanogaster*. (a) Adh^F/Adh^F female, (b) Adh^S/Adh^S male, (c) Adh^F/Adh^S female, (d) Adh^F/Adh^Smale.

Three phenotypes were observed in the F_2 generation (Figure 2); 15 flies had the ADH-FF, 19 had ADH-SS and 46 had ADH-FS phenotypes. Assuming the three bands are the result of the activity of two codominant alleles at a single locus, the phenotypic ratio should be a 1:2:1 or 1 ADH-FF:2 ADH-FS:1 ADH-SS. Testing for this hypothesis, the chi-square value was 2.20. With two degrees of freedom the probability (P) was >0.30. The hypothesis of the 1:2:1 ratio was accepted.

Discussion

Allozyme Activity

Our hypothesis assumed the functional ADH enzyme to be a dimer resulting from two active alleles which after transcription and translation would produce two polypeptides F and S. The model would be $(F + S)^2$, or 1 FF + 2 FS + 1 SS = 1, and would also assume a random association of the two different subunits, F and S. This model predicts twice as much hybrid enzyme (FS) as either of the homodimers, FF or SS. Thus, if there are twice as many heterodimers or hybrid enzyme molecules, one should expect twice the enzyme activity. One can directly make a quantitative determination of the three dimer forms by noting the staining intensity of the FS heterozygotes.

Generally, the heterozygotes exhibited the following activity: FS>FF>SS. The FF and SS homodimers should have the same activity level, however the SS homodimer frequently had less staining intensity. This is probably due to *in vitro* allozymic differences, such as heat stability and kinetic parameters (Jacobson 1968, Day & Needham 1974).

Most zymograms also exhibit several faint staining bands other than the three major *in vivo* bands. These bands can be ignored in this experiment since they also represent *in vitro* changes. Thatcher (1980) suggested that these extra bands are the product of some labile amino acid residues in the primary polypeptide sequence by deamination.

Biochemistry of the Variants

The zymogram showing the migration pattern of the "fast" and "slow"

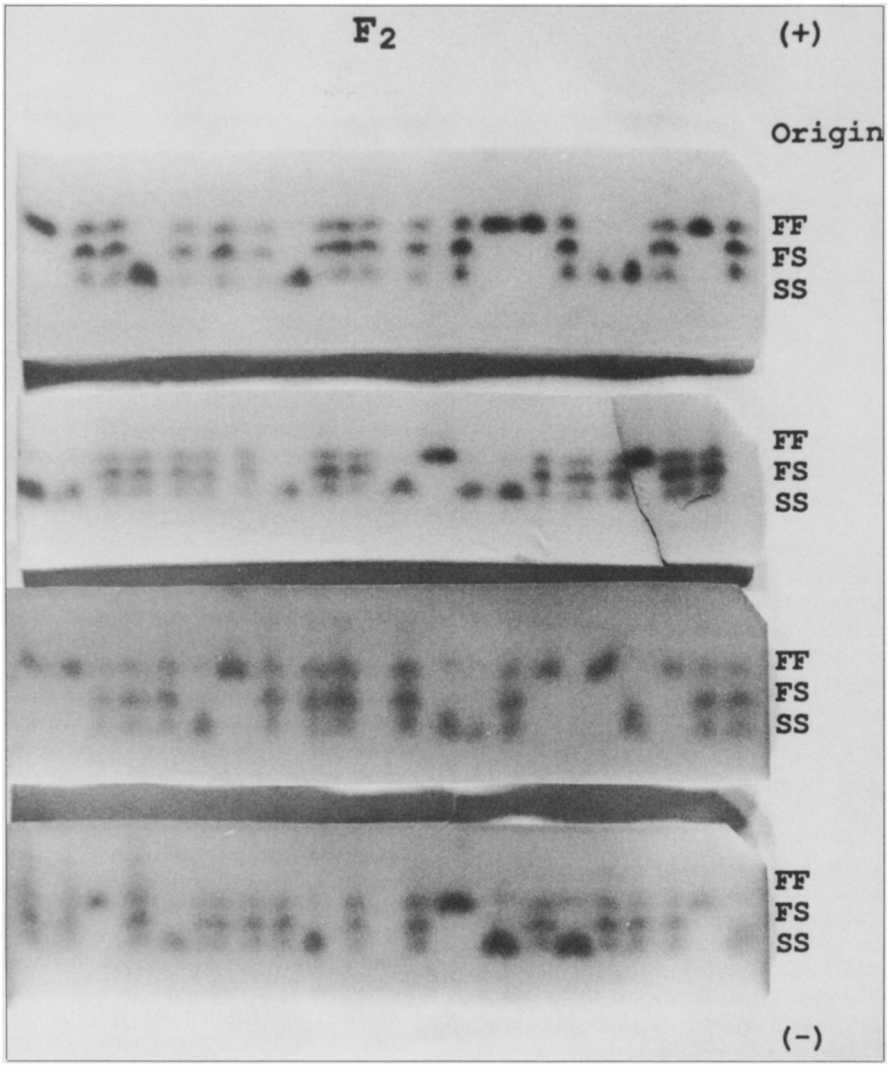

Figure 2. Electrophoretic zymograms of the second filial generation (F_2) phenotypes of alcohol dehydrogenase allozymes in *Drosophila melanogaster*. Homozygous flies have only FF or SS allozymes. Heterozygous flies have FF, FS and SS allozymes.

allozyme variants could be interpreted as involving missense base substitutions of the structural gene causing an alternative amino acid to be incorporated into the polypeptide. Although the primary protein sequence of the variants has not been determined for these particular populations, an amino acid substitution similar to Thatcher's (1980) sequence analysis of three ADH allelic variants in isogenic strains of *D. melanogaster* is assumed.

Both allozymic ADH homodimers migrate cathodically with the discontinuous buffer system used in this experiment. With the present buffer, the "fast" homodimer travels a shorter distance toward the cathode than does the "slow" homodimer. The different distances traveled by the allozymes depend on the net overall charge of the molecule. The proportion of the carboxyl (COO^-) and amino groups (NH_3^+) on the side chains of the amino acids making up the polypeptides determines the net overall charge. Therefore, one amino acid substitution could change the proportion of basic (+) or acid (−) amino acids resulting in a more or less positive charge on the allozyme. This in turn would cause a differential attraction of the allozymes to the electrodes during electrophoresis. Different buffer systems also enable one to manipulate the ionizations occurring on the primary amino acid subunits, thereby causing directional change in the migrational pattern.

Polymorphism & Allelic Frequencies

From the ratios of phenotypes in the F_2 polymorphic population, the allelic gene frequencies can be calculated. For example, the 80 F_2 phenotypes reflect the three genotypes FF, FS and SS in a ratio of 15:46:19. The allelic frequency of the F gene is:

$$F = [(2 \cdot 15) + 46] / (2 \cdot 80) = 0.48.$$

Because there are only two alleles, the allelic frequency of the S gene is:

$$1 - F \text{ or } 1 - 0.48 = 0.52.$$

The F_1 parents were FS heterozygotes with F = 0.5 and S = 0.5, so one can observe that some genetic drift occurred after only one generation, probably due to the small population size.

Conclusion

Mendel's first law, the Principle of Segregation, states: In the formation of gametes, the paired hereditary determinants separate (segregate) such that each gamete is equally likely to contain either one. Mendel experimented with peas, in which all traits had clear dominant-recessive patterns, producing F_2 generations in the phenotypic proportions of 3 dominant:1 recessive.

The fruit fly replaced peas as the organism of choice for heredity laboratory studies in the 20th century, but typically the dominant-recessive pattern is still used in teaching laboratory experiments. The experiment presented by this paper offers a codominant hereditary example to illustrate Mendel's first law. Since codominance is the full expression of two alternative alleles in a heterozygote, which results in a phenotype expressing the presence of both alleles, a phenotypic F_2 generation expresses a 1:2:1 ratio.

Since some skill in preparation of the starch gel is required, the instructor could make the gel the day before the lab and have the students crush the flies on the paper. After the gel has been run, fixed and refrigerated for 24 hours, it will be hardened and can be removed from the fixer and wrapped in plastic wrap. It is at this point that the gel can be handled by the students. Counts of the phenotypes can be made and the students can do their own calculations for chi-square and allelic frequencies.

The instructor can use this experiment to explain point mutation and the transcription differences of alleles with resulting polypeptide differences at the translational level of enzyme synthesis. Finally, explanations and definitions of enzymes, isozymes and allozymes can be made.

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