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Cellulose acetate measurement of *Adh* allele frequencies is a simple exercise in population genetics.

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In 1990, we began teaching a course on "Molecular Techniques for Field Biology" at the University of Oklahoma Biological Station on Lake Texoma, Oklahoma. This field station provides an excellent environment to gain extensive hands-on experience with molecular techniques and explore their application to population biology questions. One simple exercise can easily be carried out on *Drosophila* sampled from any natural population. Single males are screened to confirm they are *D. melanogaster*, rather than the sibling species *D. simulans* that has distinctive amber-colored clam-shaped claspers on the genitalia easily seen from the side. Homogenates from single flies are run on cellulose acetate plates and stained for ADH to determine *Adh* genotypes. Cellulose acetate electrophoresis is rapid (less than 30 minutes), and the equipment is simple. The resulting data can then be tested for fit to Hardy-Weinberg equilibrium frequencies. Our data for the last four years are given in Table 1.

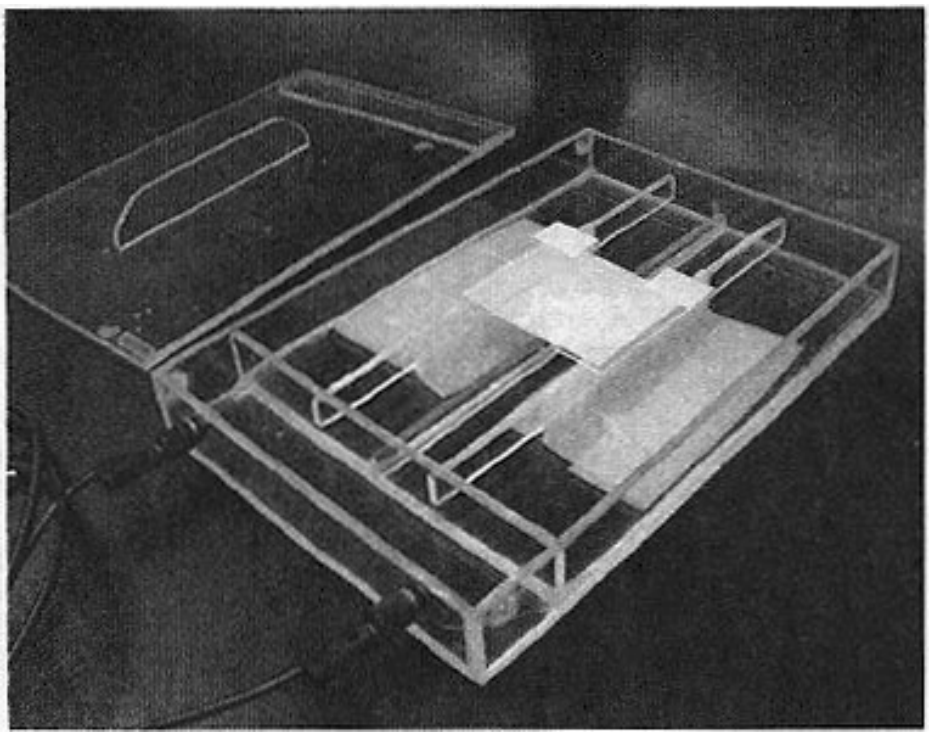

Figure 1. Electrophoresis unit constructed from plexiglas, with support bars to hold the cellulose acetate plate. Microscope slides at the ends of the plate hold it firmly against the filter paper wicks.

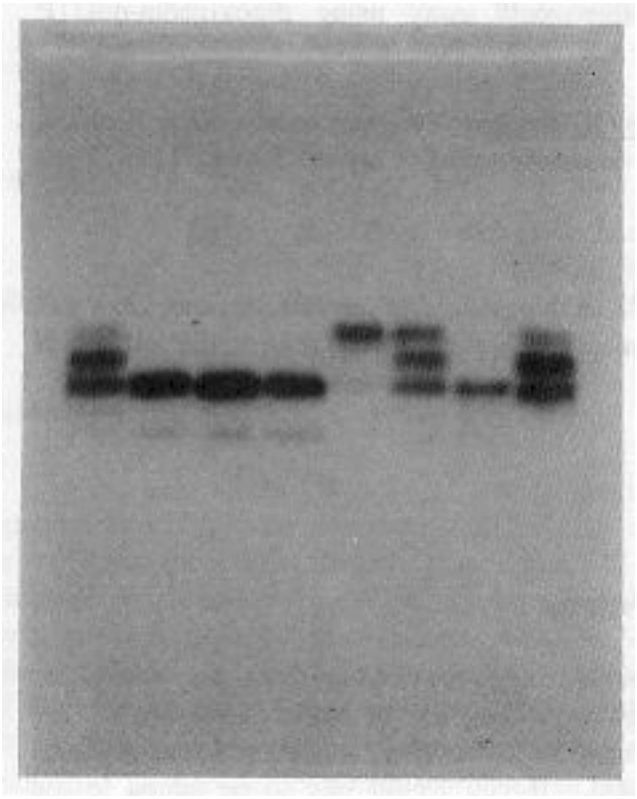

Figure 2. Representative *Adh* genotypes.

Cellulose acetate plates (Helena Laboratories, PO Box 752, Beaumont, Texas, 77704; telephone 800-231-5663) are simple to use and provide very good results with a number of different enzymes. Although plates are somewhat expensive, they have a long shelf life and we have successfully used them over a period of two or more years. Methods for cellulose acetate electrophoresis are described in a manual prepared by Hebert and Beaton (1993), which can be obtained from Helena Laboratories along with a pricelist for other equipment. Methods are also outlined briefly here, and we can provide additional details on request.

We constructed electrophoresis units from plexiglas (Figure 1), but existing electrophoresis units can probably be reversibly-modified to accommodate the plates by placing long square bars parallel to each other to serve as bridges to support the cellulose acetate plates (we use 60 mm × 76 mm plates that accommodate eight samples). Plates are connected to the two electrode buffer chambers by wet filter paper wicks. The electrode buffer is Tris Glycine (TG: 30g Trizma Base, 144g glycine made up to 1 liter; this stock solution is diluted 1:9 for use).

Table 1. *Adh*^{F} and *Adh*^{S}-allele frequencies at the University of Oklahoma Biological Station, Lake Texoma, Oklahoma.

Year	ADH Genotypes						
	FF	FS	SS	Total	p	q	χ^2
1997	2	14	27	43	0.209	0.791	<0.01
1998	18	16	9	43	0.605	0.395	2.15
1999	3	11	18	32	0.266	0.734	0.87
2000	8	14	13	35	0.429	0.571	1.04
	31	55	67	153	0.382	0.618	8.74**

** 0.01 > P > 0.001

Individual adult flies are homogenized in microfuge tubes using plastic rods rounded on a grinding wheel. Flies are ground in 20 µl of TG buffer and tubes are then spun briefly on a microfuge to separate cuticle pieces that could interfere with the homogenate transfer. Ten µl of each homogenate is transferred to a depression on a Sample Well Plate, which is supplied with the Super Z Applicator Kit (Helena Laboratories, catalog number 4088). The Applicator picks up fluid between closely-spaced parallel wires that, therefore, transfer consistent samples to the cellulose acetate plate; the plates are carefully hydrated before use. A second application is sometimes useful for increasing the concentration of enzyme applied to the plates.

There are several potential ways to reduce costs of the procedure. Although we have not tried it recently, we believe that reasonable results could be obtained by transferring samples using capillary tubes. We also constructed our own hydration chamber for the cellulose acetate plates (details available on request).

For ADH, we run the plates at 200V for 25 minutes. The plates are then stained by pouring on a solution containing hot 1.6% bacto-agar (ADH stain: 0.6 ml Tris HCl, pH 7.0; 1.5 ml NAD stock

solution [2-10 mg/ml]; 250 µl MTT stock solution [10-20 mg/ml]; 150 µl ethanol or isopropanol; 250 µl PMS stock solution [2-10 mg/ml]; 2 ml of melted agar; we often use amounts of NAD, MTT, and PMS at the high end of these ranges for best results). This stain mixture forms a gel over the plate as the agar cools. The stain is gently rinsed off of the plate when bands are clearly visible (usually within 10 minutes).

In class, the students carry out their first assay of ADH using strains of known genotypes: for example, *sepia hairy* (Adh^F/Adh^F); L-2-29, a line we isolated from a natural population, (Adh^S/Adh^S); *Cy*/*Pm*;*D*/*Sb* (balanced hetero-zygote, Adh^F/Adh^S); Adh^{n1} (a null allele). Another interesting comparison is to include other species in the sample. In *D. hydei*, for example, ADH runs as an ultra-Slow. The class then runs samples collected from fruit traps to estimate allele frequencies in the local population (*e.g.*, Figure 2). Note that with this electrophoretic procedure, the Adh^S allele runs faster than the Adh^F allele due to the buffer in the system, but this does not significantly complicate interpre-tation. In our assays, with sample sizes of approximately 40 flies, the allele frequencies were not significantly different from Hardy-Weinberg expectations, except when all samples were pooled. This provides a good chance to talk about various population genetic phenomena, such as the deficiency of heterozygotes that can result from pooling genetically different subpopulations.

References: Hebert, P.D.N., and M.J. Beaton 1993, *Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis: A Practical Handbook*. Helena Laboratories (technical manual), 32 pp.