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Survey Guidelines and Techniques Workshop
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Where to sample? How to sample? How do you apply the data? What does it all mean???? These are questions everyone who samples freshwater mussels has had to answer. The FMCS 2020 Guidelines and Techniques Workshop has been designed to provide you with those answers. Whether you are a beginning biologist or an experienced malacologist, we all need to collect mussels, and interpret and apply the data.

This Workshop will include two levels of content: for introductory/intermediate field workers, and for those with more experience, but there will be overlap depending on participant interest. The content for the introductory and intermediate group will cover equipment and design for sampling mussels, including

Fine Scale Genetic Variation in a Population of Freshwater Snails

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Studies of fine-scale allelic frequency variance in populations of land snails were influential in the development of the "Ecological Genetics" movement of the 1950s and 1960s (Cain & Curry 1963) and in some of the more important population genetic studies of the 1970s (e.g. Selander & Kauffman 1975). The phenomenon is surprisingly understudied in freshwater gastropod populations, however. Dillon (1988a) documented significant allele frequency discontinuity in a population of *Pleurocera proxima* inhabiting a 1,500 m section of Naked Creek, a tributary of the Yadkin River in Wilkes County, in northwestern North Carolina. This discordance was traceable to the waterfall undercutting a metal culvert, apparently constituting a significant barrier to dispersal.

The Naked Creek data set published by Dillon in 1988 was collected at a single allozyme-encoding locus (octopine dehydrogenase, Odh) from a series of four sample sites over a six-year period (1979 – 1985). The complete 1979 survey, however, included 16 loci, sampled from two additional sites extending another 2 km downstream in Naked Creek, corresponding to no obvious barriers, plus samples from sites in three small nearby tributaries. Although published in the Ph.D. dissertation of Dillon (1982), these data have not been widely available.

A complete map of the 1979 sample sites (including Sites 1 – 4 reported previously) is shown in Figure 1 with lat/long coordinates. Thirty snails from each of these nine sites were assayed for variance at the following allozyme-encoding loci: Aspartate aminotransferase, Acid phosphatase, Fumarase, Glucose-6-phosphate dehydrogenase, Glucose phosphate isomerase, Hexanol dehydrogenase, Isocitrate dehydrogenase, Leucine aminopeptidase, Malic Enzyme, Mannose phosphate isomerase, Octopine dehydrogenase, 6-phosphogluconate dehydrogenase, Phosphoglucomutase, Sorbitol dehydrogenase, Superoxide dismutase, and Xanthine dehydrogenase. All methods employed, together with recipes for all buffers and enzyme stains, are detailed in Dillon (1982).

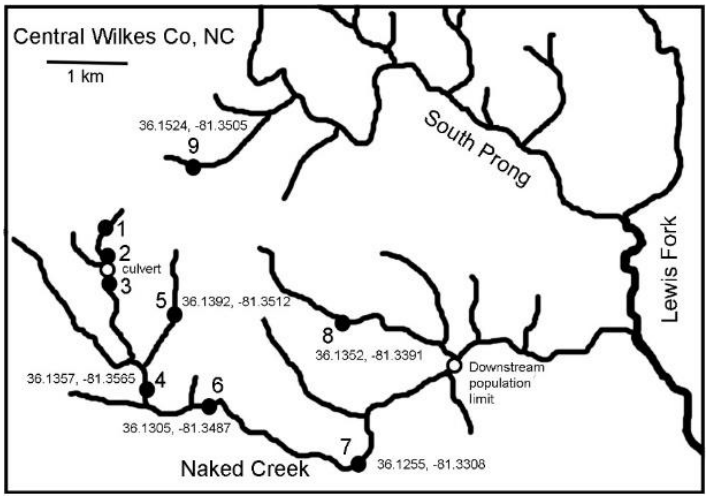

Figure 1. Sample sites for the 1979 population genetic study of *Pleurocera proxima* published by Dillon (1982).

In addition to the Odh locus, polymorphism was discovered at a second locus, Mannose-phosphate isomerase (Mpi). Results for (previously-reported) Site 4, plus unreported Sites 5 – 9, are shown in Table 1.

Within Naked Creek, Odh allele frequencies at downstream Site 7 were significantly different from all other sites, with chi-squares between Sites 4 and 7 = 18.72, between Sites 5 and 7 = 27.19, and between Sites 6 and 7 = 6.00, all with two degrees of freedom. The difference between Sites 5 and 6 was also significant, chi square = 9.07. All these differences are attributable to isolation by distance (Wright 1943).

Table 1. Gene frequencies at two allozyme-encoding loci (Odh & Mpi) in six 1979 samples of *Pleurocera proxima* collected from Naked Creek and environs by Dillon (1982).

Site	4	5	6	7	8	9
Odh 106	0.592	0.557	0.565	0.512	0.741	0.573
109F	0.099	0.094	0.178	0.270	0.259	0.427
111	0.020	0.047	0.023	0.028	0.0	0.0
113F	0.289	0.302	0.234	0.189	0.0	0.0
Mpi 95	0.831	0.827	0.818	0.825	0.883	1.00
100	0.169	0.173	0.182	0.175	0.117	0.0
No.	76	96	107	124	29	109

The inverse relationship between *P. proxima* population density and stream size has been well documented (Foin & Stiven 1970). In Naked Creek, mean population densities reached over 500/m^2 at the three uppermost sites, decreasing exponentially from 293/m^2 at Site 4 to 1.8/m^2 at Site 7, disappearing entirely thereafter.

At Site 8, back up a side branch, allele frequencies at the Odh locus were strikingly different, apparently missing alleles 111 and 113F entirely. Assuming the mean frequency of 24% demonstrated by allele 113F in Naked Creek, the binomial probability of drawing no individuals bearing Odh 113F in 29 attempts, as in Site 8, is 5×10^{-6}. This difference is not attributable to any obvious dispersal barrier such as the culvert between Sites 2 and 3 previously documented. Rather, we suggest that the striking allele frequency discordance between Site 8 and Sites 4 – 7 is a consequence of net population dispersal in an upstream direction.

No significant allele frequency differences were discovered at the Mpi locus at Sites 4 – 8. But at Site 9, approximately 1.5 km further downstream and another 6 km back up the South Prong of Lewis Fork, the minority allele Mpi 100 was missing entirely. Odh alleles 111 and 113f were also entirely missing at Site 9, making the Lewis Prong site both the most geographically distant and the most genetically divergent of the sample sites. The general correlation between distance and divergence *in P. proxima* was documented at a much larger scale by Dillon (1984).

Significant heterozygote deficiencies were common, even within sites. This phenomenon may result from migration rates that are not insignificant, but insufficient to maintain panmixia. Generally, the sexually mature snails, aged two years and over, were sampled for allozyme analysis. Perhaps the admixture that occurs in the *P. proxima* population over the two years between conception and insemination (Dillon 1988b) yields a Wahlund Effect in time, rather than in space.

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