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The Influence of Minor Human Disturbance on Biochemical Variation in a Population of Freshwater Snails

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ABSTRACT

A 1979 survey of gene frequencies at the octopine dehydrogenase locus in a population of the snail Goniobasis proxima *uncovered significant heterogeneity within a 500 m stretch of stream, apparently due to blockage of gene flow by a culvert. Minor earthwork and local siltation in 1980 resulted in homogenisation of gene frequencies, but the 1985 significant differences had been restored. Thus apparently minor environmental alterations seem to have influenced the evolution of a natural population.*

INTRODUCTION

Evolution is most broadly defined as 'a change in gene frequencies'. The emerging field of 'conservation genetics' is concerned with the extent to which humans have influenced evolution in natural populations (Bishop & Cook, 1981; Frankel & Soulé, 1981; Schonewald-Cox *et al.*, 1983). Particularly famous examples include the rapid spread of melanism in populations of moths and other organisms in industrial England (Kettlewell, 1973; Bishop & Cook, 1980), the evolution of heavy metal tolerance in plants (Antonovics *et al.*, 1971), and the evolution of resistance to poisons and antibiotics in microorganisms, insects, and rats (Bishop & Cook, 1981). Man has also influenced gene frequencies in natural populations of snails

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(Cameron *et al.*, 1980; Owen & Reid, 1986), fish (Avise & Smith, 1974; Smith *et al.*, 1983), and *Drosophila* (McKenzie & Parsons, 1974). In almost all of these examples, selection caused by human modification of the environment has resulted in the evolution of a wild population.

Reports of other disturbances, such as artificial gene flow restriction, having an influence on evolution in natural populations are rare. One case has been described where isozyme frequencies in *Helix aspersa*, a land snail introduced to the US, varied widely over a two-block area in Bryan, Texas (Selander & Kaufman, 1975). Significant differences seemed to correspond to barriers to dispersal, such as roads and buildings. Selander & Kaufman suggested that observed patterns were due to genetic drift among artificially isolated colonies, although selection could not be ruled out. In this paper, I report a second case where artificial gene flow restriction may have influenced evolution in a snail population. In this instance, however, a species native to the US is involved.

Goniobasis proxima (Say) is a freshwater prosobranch snail common in streams of the Appalachian Mountains and foothills from Virginia to Georgia. For reasons that remain obscure, maximum population densities are reached in very small tributaries, usually no more than a few metres wide. At any time, the majority of individuals in typical *G. proxima* populations are crawling upstream, and this tendency seems to more than counterbalance the occasional individuals who must lose their grip and wash downstream. Maximum rates of gene flow have been estimated at 15–20 m year^{-1} upstream but only 5–10 m year^{-1} downstream (Dillon, to be published).

Goniobasis proxima is rarely found in large rivers. The ready availability of scores of isolated populations, each inhabiting a small tributary, has made *G. proxima* the subject of a number of evolutionary studies (Dillon & Davis, 1980; Dillon, 1982, 1984*a*,*b*, 1985, 1986). Briefly, the amount of genetic divergence between *G. proxima* populations is much greater than one normally expects between conspecific populations, and the heterozygosity much lower. Evidence has been gathered that genetic drift, selection, and gene flow restriction have all contributed to population divergence, with gene flow restriction—the amount of time since populations shared a common ancestor—perhaps the most important. Chambers (1980) has collected similar data on *Goniobasis* from Florida.

METHODS

The initial objective of my research was simply to determine whether current rates of gene flow are sufficient to maintain panmixia within a typical *G.*

proxima population. For study, I selected a population inhabiting Naked Creek, a tributary of the Yadkin River, 20 km west of Wilkesboro, North Carolina (population YAD1 of Dillon, 1984*a*). This particular population shows an unusual degree of polymorphism at the octopine dehydrogenase locus.

Octopine dehydrogenase (*odh*, EC 1.5.1.11) catalyses the terminal step of anaerobic glycolysis in a variety of invertebrates (Storey & Dando, 1982). Four *odh* alleles are segregating in the YAD1 population, *odh106, odh109F, odh111* and *odh113F*, each encoding a unique isozyme (Dillon & Davis, 1980; Dillon, 1984*a*). Alleles are codominant and inherited in simple Mendelian fashion (Dillon, 1986).

On 1 and 2 May 1979, approximately 100 adult snails were sampled from each of nine sites spread down the 5 km length of the study population. The results of only the three most upstream sites will be reported here. Snail densities at these sites were sufficient to employ a Surber sampler, with about 1 m^2 sampled at each site. For reasons that will become clear presently, the three 1979 sites will be designated 1, 3 and 4, from upstream to downstream.

Whole individual snails were ground in a tissue buffer and the resulting supernatant blotted onto paper wicks. These wicks were transferred to gels of 14% hydrolysed starch, buffered at pH 6·0 with *tris* (hydroxymethyl) aminomethane and citric acid. Details of the electrophoretic procedure used to resolve the isozymes, as well as recipes for buffers and stains employed, are available elsewhere (Dillon & Davis, 1980; Dillon, 1982, 1985).

Allele frequencies at the sites were compared using chi-square tests for two independent samples. Because of very low expected frequencies, the allele *odh111* was omitted for the purposes of these tests.

The results of the 1979 study, together with subsequent events at the study area, prompted more concentrated surveys of *odh* allele frequencies in 1980 and 1985. Methods were similar, except that a fourth site was added (number 2) between sites 1 and 3, and collections were made by hand.

RESULTS

Numerous striking differences among the nine sites were discovered in 1979. Particularly significant was an increase in the frequency of the allele *odh113F* moving 500 m downstream from site 1 to site 3, with a corresponding decrease in *odh109F* (Table 1). The value of chi-square comparing sites 1 and 3 was significant at the 0·05 level (Table 2). There were no obvious differences in the environment between these two sites, although the stream passed under a road through a corrugated metal pipe just upstream from site 3. Since stream bed erodes faster than metal pipe, the lip of the culvert had been

TABLE 1
Octopine Dehydrogenase Gene Frequencies at Four Sites over Three Sampling Periods in the Study Population of *Goniobasis proxima*

Year	*Site no.*	*Number of individuals*	*Allelic frequencies (%)*			
			106	*109F*	*111*	*113F*
1979	1	80	60·0	16·9	1·2	21·9
	3	87	62·1	8·0	0·6	29·3
	4	76	59·2	9·9	2·0	28·9
1980	1	95	64·7	8·4	3·2	23·7
	2	66	65·9	10·6	3·8	19·7
	3	92	67·4	13·0	3·3	16·3
	4	93	52·2	17·7	2·7	27·4
1985	1	107	68·2	15·9	1·4	14·5
	2	105	70·9	8·6	3·8	16·7
	3	100	60·0	14·5	2·0	23·5
	4	99	63·6	11·1	2·0	23·2

undercut, and a small waterfall had formed. Judging by the increased population density below this culvert, it appeared that upwardly-migrating snails were unable to surmount this obstacle. (Although the population density was approximately $54\,m^{-2}$ at site 3, density was much too low to estimate with a Surber sampler just upstream.) Thus in 1979 it was theorised that the culvert was responsible for the significant genetic differences observed between sites 1 and 3. Other barriers in the intervening 500 m, isolation by simple distance, or selection, could not be ruled out, however.

Several months after the 1979 sample was made, bulldozing and clearing

TABLE 2
Values of Chi-square (with 2 degrees of freedom) Comparing All Pairs of Sites Sampled Each Year

Sites	*1979*	*1980*	*1985*
1 and 2	—	1·09	5·00
1 and 3	7·14*	4·35	6·35*
1 and 4	4·39	9·51**	6·24*
2 and 3	—	1·16	7·15*
2 and 4	—	7·09*	3·36
3 and 4	0·40	10·2**	0·87

* Significant at the 0·05 level.
** Significant at the 0·01 level.

operations were begun on one or two hectares of brushy land immediately upstream from the culvert. Subsequent rains eroded topsoil into the stream. Silt covered the rock-and-cobble bottom, making it more difficult for snails to cling to the substrate and adversely affecting the periphyton upon which they feed. Population densities upstream from the culvert decreased, either because snails were physically dislodged, or because they withdrew into their shells and were washed downstream to a more favourable environment. In addition, silt filled the pool directly below the culvert, raising the stream bottom to the point where it met the lip of the pipe. Any barrier to upstream migration was removed.

Although the bulldozing activities continued for well over a year, siltation had slowed considerably by May 1980. The silt had cleared from the pool below the culvert, so that the pipe once again constituted a significant barrier. I then resampled about 100 individual snails from the three sites of 1979 and added the fourth site between sites 1 and 3, directly upstream from the culvert. Population densities at sites 2 and 3, those most affected by the bulldozing and siltation, remained so low that many metres of stream were thoroughly searched to obtain the sample. Gene frequencies at site 3 in 1980 were much different from those of 1979 (Table 3). No significant differences

TABLE 3
Values of Chi-square (with 2 degrees of freedom) Comparing 1979 Allele Frequencies to Those of Subsequent Years

Site	*1980*	*1985*
1	5·24	4·19
3	9·23**	4·48
4	4·93	1·54

** Significant at the 0·01 level.

in *odh* gene frequency were detected among any of the three disturbed sites upstream (Table 2). It would appear that increased rates of migration were sufficient to homogenise gene frequencies over the 500 m where significant differences had existed one year previously. Sites 1–3 remained significantly different from site 4, 500 m downstream from the disturbance and apparently unaffected by siltation.

The newly cleared land was reseeded with grass in 1981, and no further disturbance has occurred. Thus in May, 1985, I returned for a third time, sampled about 100 individuals from the four sites, and re-examined *odh* gene frequencies (Table 1). Allele frequencies in 1985 were not significantly

different from those observed in 1979 (Table 3). Once again I found the frequency of *odh113F* significantly depressed downstream from the culvert (Table 2).

DISCUSSION

Over the 6 years encompassed by this study, *odh106* remained the most common allele, with a frequency usually about 60–70%. The frequency of *odh111* was similarly stable in the range of 0·6–3·8%. The greatest variation occurred in the relative frequencies of the remaining two alleles, *odh109F* and *odh113F*. Thus, Fig. 1, the frequency of *odh113F* over the three sample periods, can be cautiously viewed as a summary of the results of this study.

It seems clear that the culvert between sites 2 and 3 exerts some influence upon the genetics of this *G. proxima* population. Figure 1 suggests that the frequency of *odh113F* is much greater downstream, and that of *odh109F* much less, so that a 'bulge' of *odh113F* tends to build downstream from the culvert. (More extensive surveys in 1979 and 1980 support this idea.) This disruption of allele frequencies may have been occurring for years, as the district engineer estimates that this particular culvert was installed 'in the early 1950s'.

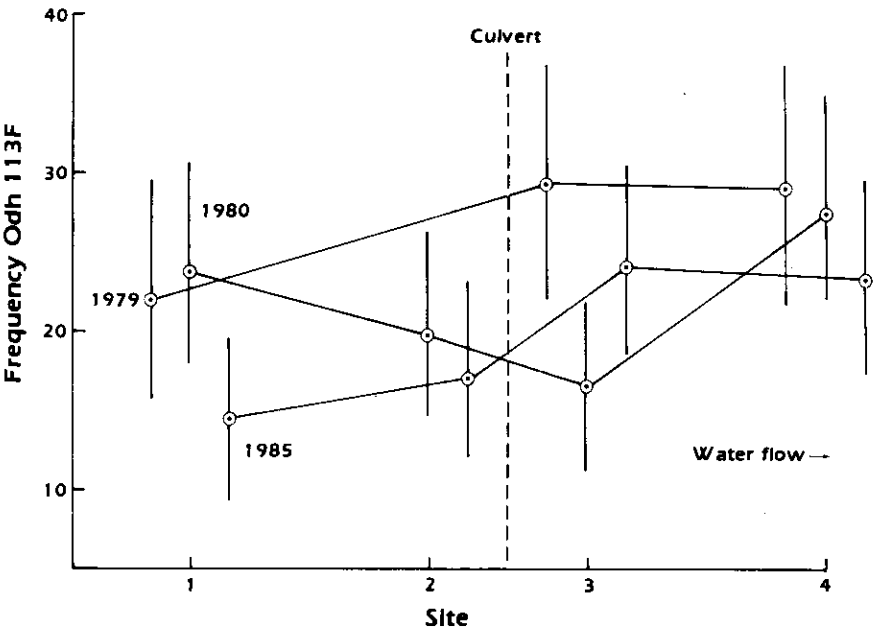

Fig. 1. The frequency of *odh113F* over the three sampling periods. Vertical bars represent binomial 95% confidence limits. Only the culvert, indicated by a dashed line, separates sites 2 and 3. Site 1 is approximately 500 m upstream, and site 4 is 500 m downstream.

It also seems apparent that the land-clearing activities of 1979–80 altered gene frequencies in the population of snails inhabiting the nearby stream, if only temporarily. Here an artificial impact on gene flow seems clearly responsible. It is not certain what role, if any, artificially-induced selection has played in these phenomena. Because *odh* gene frequencies returned to their prior levels after perturbation, one cannot rule out the possibility of selective differences over the few metres the stream passes through the culvert.

It should be emphasised that *odh* gene frequencies in this population of snails seem to be naturally quite dynamic. Gene flow does in fact occur both upstream and down, but apparently not at a rate sufficient to homogenise allele frequencies. For example, *odh106* is not the most common allele at a site up a side branch 2 km distant by water. Even samples taken from a single square metre generally show heterozygote frequencies significantly less than Hardy–Weinberg expectation (Dillon, 1982). This may be due to the fact that the individuals sampled are generally two or three years old, and females may store sperm for life. Hence adults sampled at a single site may have been conceived five years previously, in areas of much different gene frequency. Thus the imposition of a culvert and a bulldozer may not have been of any lasting genetic consequence. But the observations reported here are important as an illustration of how apparently minor environmental alterations can affect the evolution of a natural population.

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