The *Goniobasis* (“*Elimia*”) of southwest Virginia,

II. Shell morphological variation in *G. clavaeformis*

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ABSTRACT

The population of pleurocerid snails inhabiting Indian Creek at its junction with the Powell River in Claiborne County, Tennessee, includes individuals bearing shells with the phenotypes of *Goniobasis clavaeformis*, its junior synonym *G. acutocarinata*, and *Pleurocera unciale*, as well as intermediate forms. Gene frequencies at two highly polymorphic allozyme-encoding loci were not significantly different among samples representing these three nominal forms, and genotype frequencies matched Hardy-Weinberg expectation when snails bearing shells of all types were pooled and tested together. These results suggest that *Pleurocera unciale* is a junior synonym of *Goniobasis clavaeformis*, the heavier shell of the former being a consequence of stronger water currents, differential predation pressure, or some other aspect of the large-river environment.

INTRODUCTION

Intraspecific variation in the shell morphology of American pleurocerid snails has been the object of research interest for many years (Adams 1915, Wiebe 1926). The phenomenon has best been documented by Calvin Goodrich, who published a series of eight papers on the subject between 1934 and 1941. Goodrich showed that populations of pleurocerids often vary in shell shape and sculpture in a downstream direction, from slender in smaller tributaries to “obese” in larger rivers (1934, 1935, 1937). In many of the situations documented by Goodrich, earlier taxonomists had described the morphological variants as distinct species, which Goodrich subsequently synonymized.

Among the many species whose intrapopulation variation was examined by Goodrich was *Goniobasis clavaeformis* (Lea 1841), a common inhabitant of streams and rivers in east Tennessee and southwest Virginia. Although the body whorls of the shells borne by adult *G. clavaeformis* are typically rounded, Goodrich (1940) recognized that populations can be variably carinate, forms with more pronounced carination having previously been described as "*Goniobasis acutocarinata*." The carinate forms more commonly inhabit small streams, and snails bearing heavier, more rounded shells increase in frequency in mid-sized tributaries. We have recently surveyed 33 populations of *G. clavaeformis* inhabiting tributaries of the Tennessee River in southwest Virginia and east Tennessee, as part of a larger study of genetic divergence among pleurocerid populations in the region (Dillon & Robinson 2007). Shell variation of the type reported by Goodrich was widespread.

Larger streams in southwest Virginia, such as the Clinch, Powell, and Holston Rivers, are inhabited by pleurocerid populations that have been identified by Goodrich (1940) as *Pleurocera unciale* (= *uncialis*, Haldeman 1841). These populations bear shells that are thicker and heavier than typical *Goniobasis* populations, their whorls typically marked with prominent anterior angulation. However, at one site we sampled during our recent survey, where a large creek joins the main Powell River, individual snails were discovered bearing shells intermediate between *Pleurocera unciale* and *Goniobasis clavaeformis*. The purpose of the present work is to test the hypothesis that populations of pleurocerids in major tributaries of the Tennessee River long identified as *Pleurocera unciale* might actually be heavily-shelled variants of *G. clavaeformis*, uniting slender, carinate forms in smaller streams to obese forms in the large rivers as documented by Goodrich.

METHODS

Our study area (site "VG052") was located at the mouth of Indian Creek, 4 km SE of Shawanee, in Claiborne County, Tennessee. On June 10, 2006 we made a qualitative sample of several hundred individual snails from this site. We sorted our sample into five categories by shell morphology, as follows. Type U snails bore heavy shells with anteriorly angular whorls, matching the description and figures of *Pleurocera unciale* (Tryon 1873, Burch 1989). Type T snails bore rounded whorls, as is typical of *Goniobasis clavaeformis*. The shells of Type C snails bore strong mid-whorl carination, matching the description and figures of *G. acutocarinata* (Tryon 1873, Burch 1989). Category U/T snails were intermediate between types U and T, and category T/C snails were intermediate between types T and C. Example shells representing these five types are shown in Figure 1.

We selected 10 individuals each from types U, T, and C and screened for allozyme polymorphism at the same 11 loci analyzed in Part I of this series, using electrophoretic techniques and methods as described by Dillon & Robinson (2007). Samples of *G. clavaeformis* from population C1, located in Indian Creek 25 km upstream from the present study area, served as controls. This initial sample of 30 snails was monomorphic at the following loci: GPI, MPI, EST1, PGM, IDHF, IDHS, XDH, and SDH, and weakly polymorphic at OLDH, showing in all cases the same alleles recorded in control population C1.

Our initial sample of 30 individuals showed, however, high levels of polymorphism at two enzyme loci - 6PGD and OPDH. We then analyzed 36 additional individuals of type U, 21 additional individuals of type T, and 26 additional individuals of type C to estimate allele frequencies at these two loci. The Tris Cit 6 buffer (XIII of Shaw & Prasad 1970) and the Poulik (1957) buffer were used to resolve both 6PGD and OLDH enzyme activity, giving us two opportunities to examine the gene products of both loci. Bands were identified by their mobility relative to controls from population C1, which we had previously calibrated with standards from *G. simplex* type locality S5 (Dillon & Robinson 2007). Details regarding all our electrophoretic techniques, including recipes for all buffers and enzyme stains employed, are available in Dillon (1992).

Chi-square tests for independence were employed to examine the hypothesis that the three samples of snails (U, T, and C) might have been drawn from one homogeneous population, pooling rare alleles as necessary. Then the entire 113-individual sample was combined and tested for conformance to Hardy-Weinberg expectation as a single randomly breeding population. Goodness-of-fit chi-square tests were employed for this purpose, combining homozygous and heterozygous classes, with one degree of freedom.

RESULTS

Gene frequencies and observed heterozygosities at the 6PGD and OPDH loci in our total samples of N = 46 type U snails, N = 31 type T snails, and N = 36 type C snails are shown in Table 1. The value of chi-square from the 3x2 test for independence at the 6PGD locus was 0.693, not significant with 2 degrees of freedom. Combining the two rarest allelic classes at the OPDH locus (OPDH 115 & OPDH 110), the value of chi-square from the 3x3 test for independence was 1.999, again not significant with 4 degrees of freedom. Pooling allele frequencies over the entire sample of 113 individuals as shown in the right column of Table 1, conformance to Hardy-Weinberg expectation was excellent. Values of chi-square from goodness-of-fit tests were 0.025 for the 6PGD locus and 0.092 for the OPDH locus, both nonsignificant with one degree of freedom.

DISCUSSION

Our data suggest strongly that the three samples of snails we initially sorted from the mouth of Indian Creek in Claiborne County, Tennessee, comprise a single randomly breeding population. Thus, it would appear that the shell form long attributed to *Pleurocera unciale* is one of the many variants taken by *Goniobasis clavaeformis*, and that the nomen “*unciale*” (Haldeman, October 1841) is a junior synonym of *clavaeformis* (Lea, February 1841). Also subsumed under *clavaeformis* would be the 10 synonyms of *unciale* previously listed by Goodrich (1940), as well as the subspecies *hastatum* and the "transition forms" *exarata*, *gradatum*, *aratum* and *curatum*.

Our field observations suggest that individuals with the type U or "*unciale*" shell morphology are most common in the major tributaries of the Tennessee River - the Clinch, Powell, and Holston Rivers. Individuals bearing typical type T shells prevail in mid-sized creeks and smaller rivers, and snails bearing the thinner, more carinate type C shells reach greatest abundance in small streams and spring-fed brooks. We suggest that shell morphology in *G. clavaeformis* demonstrates variation of the sort documented by Goodrich (1934, 1935, 1937) to a greater extent than Goodrich himself recognized in his own works.

Goodrich (1934) attributed the greater frequency of heavier, more obese shells borne by pleurocerids in downstream populations to physical disturbance or water current. He cited the work of Weibe (1926) who suggested that the "relatively great obesity" of *Goniobasis livescens* populations on exposed shores of Lake Erie originated from the adaptive value of a large foot in areas of heavy wave action. The broader shell would be a secondary consequence of a larger foot. Goodrich suggested that this phenomenon might generalize to rivers and streams, saying "an ecological analogy would appear to exist between the exposed situations of Lake Erie, inhabited by obese *G. livescens*, and the rapid and sometimes tumultuous southern creeks and river headwaters where other obese pleurocerids have their habitats." Urabe (1998) reported a similar phenomenon in a population of the Japanese pleurocerid *Semisulcospira reiniana* - snails with larger body whorls and lower spires being more common in areas of rapid current.

More recently, considerable research interest has been directed towards the effects of predation on freshwater gastropod shell morphology (DeWitt 1998, DeWitt et al. 2000, Holomuzki & Biggs 2006). Krist (2002) reared *G. livescens* in effluent from crayfish feeding on conspecific snails and documented a significant narrowing of the body whorl when compared to controls. Rivers in our study region are indeed inhabited by populations of crayfish attaining large adult sizes. We have no direct data on crayfish distribution in the streams inhabited by *G.*

clavaeformis, however, nor the incidence of predation.

The extent to which variance in pleurocerid shell morphology may be under direct, additive genetic control is also an open question. *Goniobasis* populations are not panmictic (Dillon 1988). The gene frequencies we here report at the 6PGD and OPDH loci in the *G. clavaeformis* population at the mouth of Indian Creek were both strikingly different from those we reported from site C1, just 25 kilometers upstream (Dillon & Robinson 2007). And in fact, our sample of type U individuals carried a rare allele (OPDH 110) not seen in samples T or C, perhaps reflecting additional genetic divergence in the main Powell River from which they may have emigrated. It is certainly possible that the variance in shell morphology displayed by the Indian Creek population of *G. clavaeformis* from headwater to mouth reflects heritable genetic divergence. But laboratory experiments have also documented a great deal of phenotypic plasticity in the morphology of freshwater gastropod shells (Urabe 1998, 2000, Krist 2002). Such questions would seem fertile ground for future studies.

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Table 1. Gene frequencies and observed heterozygosities at the two polymorphic allozyme loci in three samples of *Goniobasis* from the mouth of Indian Creek. Shell "type" refers to morphology as shown in Figure 1.

	Type U (46)	Type T (31)	Type C (36)	Combined (113)
6PGD				
103	.435	.419	.486	.447
100	.565	.581	.514	.553
H_o	.435	.516	.528	.487
OPDH				
118	.109	.177	.153	.142
116	.739	.661	.722	.712
115	.098	.161	.125	.124
110	.054	.000	.000	.022
H_o	.457	.484	.389	.442

Figure 1. Example shells of the five types borne by the *G. clavaeformis* population inhabiting the mouth of Indian Creek.