Verification of the Specific Status of the Endangered Anthony's River Snail, *Athearnia anthonyi*, Using Allozyme Electrophoresis

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ABSTRACT

Although nominally the single surviving representative of a unique pleurocerid taxon, *Athearnia anthonyi* (Budd, in Redfield, 1854) is so rarely collected that even its specific status has been uncertain. We used allozyme electrophoresis to compare a population of *A. anthonyi* to the similar pleurocerid snail, *Leptoxis praerosa* (Say, 1821), co-occurring with it in the Sequatchie River of Tennessee, and to a second population of *L. praerosa* collected approximately 500 km distant. Observed levels of heterozygosity offered no evidence of inbreeding or unusually severe population bottlenecking in any of these populations. Strikingly different allele frequencies at five of the eleven enzyme loci examined, together with differences in shell morphology especially apparent in young individuals, confirmed that *A. anthonyi* and *L. praerosa* are distinct species. Their similarity at the six loci remaining supports previous suggestions that *Athearnia* may be a subgenus of *Leptoxis*.

Key words: *Athearnia*, *Leptoxis*, Pleuroceridae, freshwater gastropods, proteins, enzymes, Tennessee.

INTRODUCTION

The pleurocerid genus *Athearnia* was proposed by Morrison (1971) to include the two species, *Anculosa anthonyi* (Redfield, 1854) and *Anculosa crassa* (Haldeman, 1842), previously but incorrectly included in the genus *Eurycaelon* (Goodrich, 1931). Some authors (Davis, 1974; Burch, 1982) have subsequently considered *Athearnia* to be a subgenus of the widespread genus *Leptoxis*, but we follow Bogan and Parmalee (1983), Garner (1992), and the Federal Register in retaining its generic rank. The distinction between the two nominal *Athearnia* species, *A. anthonyi* and *A. crassa*, has been confused for some years. In any case, since *A. crassa* is generally considered to be extinct (Bogan & Parmalee, 1983), *A. anthonyi* appears to be the sole existing representative of this distinctive pleurocerid taxon.

Historically, populations of *Athearnia* have been recorded from the Tennessee River and its larger tributaries upstream from Muscle Shoals, Alabama, including the Clinch, Powell, Nolichucky, Little Tennessee, French Broad, Sequatchie, and Elk Rivers (Goodrich, 1940). However, the impoundment of the Tennessee drainage that began in the 1930's, improvement of the stream bed for navigation, and general habitat degradation due to farming, mining, and industry eliminated *Athearnia* from the great majority of its former habitat. Bogan and Parmalee (1983) feared that *Athearnia* "may be extinct throughout its range except for a possible relic population."

More recently, it has become clear that small but apparently viable populations of *A. anthonyi* still inhabit lower regions of the Sequatchie River in Marion County, Tennessee, and Limestone Creek in Limestone County, Alabama (Garner, 1992). These snails also survive in at least one channel of the main Tennessee River downstream from the mouth of the Sequatchie (Jenkinson, 1994; Garner, 1994). In early 1994, *A. anthonyi* became the first pleurocerid gastropod formally listed as "endangered" by the United States government (Federal Register 59:17994–17998). In addition to protecting extant populations, the *A. anthonyi* recovery plan (U.S. Fish & Wildlife Service, 1996) calls for the future reestablishment of at least a few populations into portions of the snail's historic range.

Although great interest has focused on the distribution and abundance of *A. anthonyi*, questions remain regarding its taxonomic status. Adult *A. anthonyi* may be confused with large, senescent *Leptoxis praerosa* (Say, 1821), a much more common pleurocerid with which *Athearnia* often occurs. Juveniles of *A. anthonyi* have not often been described. Given that trematode infection may induce extreme and anomalous growth in gastropod hosts (Sturrock, 1966; Hodasi, 1972), it has seemed possible that *Athearnia* may be parasitically castrated, gigantic, *L. praerosa*.

Allozyme electrophoresis has become established as an

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important tool for measuring genetic diversity both within and among populations of pleurocerid snails (Chambers, 1978; 1980; Stiven & Kreiser, 1994). Published work to date has, however, concentrated exclusively on the diverse genus *Goniobasis*, primarily an inhabitant of smaller streams. Genetic variation seems to be unusually low within *Goniobasis* populations, but unusually high between them (Dillon & Davis, 1980). The levels of both intra- and interpopulation gene flow also seem to be unusually low (Dillon, 1988a). Significant gene frequency differences have been reported between samples of *Goniobasis* taken at distances as short as 500 m (Dillon, 1988b). Geographically distant *Goniobasis proxima* (Say, 1825) populations may share no alleles at as many as six enzyme loci of seven studied, yet show no evidence of reproductive isolation (Dillon, 1984; 1986; 1988a). Distinct species of *Goniobasis* generally share alleles at very few enzyme loci (Chambers, 1980; Dillon & Davis, 1980). So although no data have been published on divergences in other pleurocerid genera to this date, there is reason to expect that if *A. anthonyi* is indeed different from *L. praerosa*, differences in allozyme frequencies will be apparent.

In the present work, we use allozyme electrophoresis to compare a population of *A. anthonyi* from the Sequatchie River to a co-occurring population of *L. praerosa* and to a population of *L. praerosa* from the Duck River 500 river kilometers distant (approximately 350 km downstream, then 150 km upstream). In addition to verifying the specific status of *A. anthonyi*, our data also bear on two ancillary questions: the systematic placement of the genus *Athearnia* and the degree to which an important population of *A. anthonyi* may be inbred or bottlenecked.

MATERIALS & METHODS

Athearnia anthonyi and *Leptoxis praerosa* were collected from a site on the Sequatchie River in Marion County, Tennessee. *Leptoxis praerosa* were most common in riffle areas, while *A. anthonyi* were more common on larger submerged objects (rocks, snags, etc.) in the transition areas between riffles and pools. A second sample of *L. praerosa* was collected from the Duck River at Lillard Mill, Marshall County, Tennessee. Voucher specimens of *A. anthonyi* have been deposited in the Ohio State University Museum of Biological Diversity (catalog number 19820). Individuals were transported alive to Charleston, S.C., where the shells were cracked and the digestive glands examined for parasites. Tissues were then frozen in a tris-phosphate buffer at −70°C and examined electrophoretically within one week.

Horizontal starch gel protein electrophoresis was performed on whole animal homogenates as has been previously described (Dillon, 1985; 1992). We initially screened 15 *A. anthonyi* and 14 Sequatchie River *L. praerosa* for variation in 10 enzyme systems: aspartate aminotransferase (AAT), esterases (EST α-napthyl acetate as substrate), glucose phosphate isomerase (GPI), alcohol dehydrogenase (ADH, hexanol as substrate), mannose phosphate isomerase (MPI), octopine dehydrogenase (ODH), phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH), and superoxide dismutase (SOD). Simple Mendelian inheritance of codominant alleles has been demonstrated at the 6PGD and PGM loci in *Goniobasis floridensis* (Reeve, 1860) by Chambers (1980) and at the GPI, ODH, and EST1 loci in *G. proxima* by Dillon (1986). It should be noted that a great many esterase allozymes are generally detectable in pleurocerids, but that to date only the strong, slowly-migrating products of the "EST1" locus are genetically interpretable.

An effort was made to examine allozyme phenotypes in as wide a range of buffers as possible. The following gel buffers were employed: Tris-Cit 6.0 (for PGM, GPI, ODH, and MPI), AP 6.0 (for 6PGD), TEB 8.0 (for EST and 6PGD), Poulik (for SDH, ODH, and GPI), and TEB 9.0 (for SOD, ADH, AAT, PGM, and SDH). Recipes for most of these buffers and stains were obtained from Shaw and Prasad (1970) or Harris and Hopkinson (1976), modified for agar overlay in many cases.

Also included in the initial survey for allozyme variation were *G. proxima* standards (population SUGR of Dillon, 1984). *Leptoxis* and *Athearnia* bands were labeled by their mobilities in millimeters relative to this population in a standard buffer. As was the case in 1984, some "hidden" variation was detected at the ODH locus - bands not resolved by the standard Tris-Cit 6.0 buffer were detected with a Poulik buffer. These isozyme classes were labeled "S", "F", and "VF" for their migration in Poulik gels.

After the initial screening, we concentrated our investigations on the five putative enzyme loci apparently varying in our comparison of *A. anthonyi* and *L. praerosa*. Ultimately we examined 37 *A. anthonyi*, 29 *L. praerosa* from the Sequatchie River, and 25 individual *L. praerosa* from the Duck River. Gene frequencies and observed heterozygosities were calculated using BIOSYS version 1.7 (Swofford & Selander, 1981).

RESULTS

Figure 1 compares representative shells from Sequatchie populations of *A. anthonyi* and *L. praerosa*. *Athearnia anthonyi* is distinguishable by the higher shoulder of its whorls, beginning as a pronounced keel (or "carina") but becoming less distinct with age. The oldest *A. anthonyi* are quite smooth, and come to resemble large *L. praerosa*.

Very little evidence of parasitism was detected in either Sequatchie pleurocerid population. Only 2 of 29 *L. praerosa* showed obvious trematode infections, while none of the 37 *A. anthonyi* appeared to be infected. Thus the large sizes attained by individual *A. anthonyi* do not appear to be due to parasitic gigantism.

Figure 1. Growth series for *Athearnia anthonyi* (above—five specimens) and *Leptoxis praerosa* (below) from the Sequatchie River, TN.

No variation was detected between populations of *A. anthonyi* and *L. praerosa* at AAT, ADH, 6PGD, SDH, SOD, or at a faster-migrating PGM locus. Divergence was striking, however, at the five loci listed in Table 1. The two sympatric populations share no alleles at EST1, GPI, MPI, or at the slower PGM locus, "PGMS". And at the ODH locus, *A. anthonyi* appears to be fixed for an allele present at a frequency of only 0.345 in Sequatchie *L. praerosa*.

Although spatially separated by over 500 km, the two *L. praerosa* populations seem to have remained rather similar genetically. Table 1 shows that the differences in allozyme frequency at the GPI, MPI, and ODH loci are significant, but not fixed. The Sequatchie population appears substantially more polymorphic.

Rather low levels of genetic variation were detected within these three populations of pleurocerids, as has been commonly reported in the past. Judging by the 95% criterion, only four of the 15 loci shown in Table 1 could be considered polymorphic. The fit to Hardy-Weinberg expectation at these four loci was very good, however.

DISCUSSION

It is clear that previous misgivings about the specific status of *A. anthonyi* were unfounded. Although not as divergent as typical species of *Goniobasis*, the data presented in Table 1 show clearly that *A. anthonyi* and *L. praerosa* are reproductively isolated in the Sequatchie River. Their shell morphologies are distinct. The greater size attained by *A. anthonyi* appears to be a natural

Table 1. Allele frequencies and observed heterozygosities (H) at variable loci in populations of *Athearnia anthonyi* from the Sequatchie River, *Leptoxis praerosa* from the Sequatchie River, and *L. praerosa* from the Duck River. Sample sizes 37, 29, and 25, respectively.

		Sequatchie R.		Duck R.
Locus	Allele	*A. anthonyi*	*L. praerosa*	*L. praerosa*
EST1	105	0.0	1.00	1.00
	102	1.00	0.0	0.0
	H	0.0	0.0	0.0
GPI	102	0.392	0.0	0.0
	97	0.0	0.586	1.0
	95	0.608	0.0	0.0
	90	0.0	0.414	0.0
	H	0.622	0.552	0.0
MPI	100	0.0	0.034	0.0
	95	0.0	0.966	1.0
	90	1.0	0.0	0.0
	H	0.0	0.069	0.0
ODH	115	0.0	0.086	0.460
	113VF	0.0	0.310	0.0
	113F	1.0	0.345	0.520
	113S	0.0	0.034	0.0
	107	0.0	0.224	0.020
	H	0.0	0.670	0.440
PGMS	103	0.0	1.0	1.0
	90	1.0	0.0	0.0
	H	0.0	0.0	0.0

feature of development, not a consequence of parasitic gigantism.

The level of intrapopulation variation in *A. anthonyi* is low compared to that of most other organisms, but comparable to that seen in *L. praerosa* and in other pleurocerids. We did discover one highly polymorphic locus in *A. anthonyi*, GPI, showing genotype frequencies not significantly different from expectation under Hardy-Weinberg equilibrium (Yates-corrected $\chi^2 = 2.53$). Thus we are unable to detect evidence of inbreeding or an unusually severe population bottleneck.

Previous electrophoretic investigations of *Goniobasis* populations have generally detected higher levels of divergence than we report here between Sequatchie River and Duck River populations of *L. praerosa*. Multiple fixed differences are often observed between *G. proxima* populations isolated in small creeks at distances as great as 500 km. The less dramatic differences between conspecific *Leptoxis* populations reflected in Table 1 may be a consequence of their adaptation to larger rivers. Populations of *Leptoxis* may have inhabited occasional rocky shoals down the length of the Tennessee River before its impoundment, connecting such tributary populations as those of the Sequatchie and Duck Rivers in stepping-stone fashion.

Previous studies have shown greater divergence between pleurocerid species than uncovered here. Dillon and Davis (1980) and Chambers (1980) reported that typical species of *Goniobasis* rarely share any similarity at any allozyme locus. However, *L. praerosa* and *A. anthonyi* were indistinguishable at six of the eleven loci initially screened, and seem to share one fairly common allele at a seventh locus (ODH). This constitutes some support for the nomenclature of Burch (1982) and Dillon (1989) in which *Athearnia* is placed as a subgenus of *Leptoxis*. Regardless of whether they represent a unique genus or a unique subgenus, the populations of *A. anthonyi* now restricted to just a few rivers of central Tennessee are a valuable resource for evolutionary study, and warrant protection at the highest levels.

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