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To the FMCS Community:

I hope each and every one of you are staying safe and mentally engaged during these times of uncertainty. I know that restrictions can be taxing as you juggle professional objectives with parental duties and personal sanity. Personally, my wife and I, with our three children (3rd grade, 6th grade, and 9th grade), have had some interesting mornings. The first time we both had a simultaneous conference video, we did not hesitate to approve when our youngest asked if she could make a peanut butter and M&M sandwich for breakfast.

Population Genetic Survey of *Lithasia geniculata* in the Duck River, Tennessee

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Among Calvin Goodrich's greatest contributions to science was his 1934 discovery that populations of *Lithasia* previously referred to the nominal taxa *pinguis* (Lea 1852), *fuliginosa* (Lea 1841), and *geniculata* (Haldeman 1840) were shell variants of a single biological species clinally arrayed down the length of the Duck River in Middle Tennessee (Goodrich 1934). This observation presaged our understanding of cryptic phenotypic plasticity (CPP) in the North American Pleuroceridae (Dillon 2011, 2014; Dillon et al. 2013) by 80 years. Here I report on a 2002 survey of genetic variation at three polymorphic allozyme-encoding loci across seven Duck River populations of *Lithasia geniculata* that reinforce and augment Goodrich's 1934 insight.

The samples analyzed here were collected incidentally during a 2000-02 survey of the Duck River unionid mussel fauna by Ahlstedt and colleagues (2017), transmitted to us by P. D. Johnson, whose assistance is gratefully acknowledged. Two populations of *L. geniculata pinguis* were sampled: pinA from the Little Duck River in Manchester (TNC84, 35.4836, -86.0808) and pinB in the main Duck River at Old Fort State Park (TNC101, 35.4842, -86.1089). They also sampled two populations of *L. geniculata fuliginosa*: fulC from the Duck adjacent to US41A (TNC70, 35.4629, -86.3574) and fulG from the Buffalo River at the Gilmer Bridge (35.7846, -87.7737). And they sampled three populations *of L. geniculata* from the main Duck River: genD from the Fountain Ck confluence (TNC94, 35.5695, -86.9682), genE from Wright Bend (TNC110, 35.8267, -87.6657), and genF at the Watered Hollow boat launch (35.9322, -87.7475). See Figure 1.

I initially screened ten individuals from pinA and ten individuals from genE for polymorphism in 17 enzyme systems (21 nominal loci) on horizontal starch gels with four buffer systems, using the methods of Dillon (1982, 1985, 1992). Allozyme variation interpretable as the product of codominant Mendelian inheritance was discovered at just three loci: mannose phosphate isomerase (Mpi) on buffers TrisCit6 and TEB8, octopine dehydrogenase (Odh) on buffers TrisCit6 and Poulik, and hexanol dehydrogenase (Hexdh) on buffers TEB8 and Poulik. Genetic variation was subsequently assessed at these three loci only for the remainder of the populations and individuals.

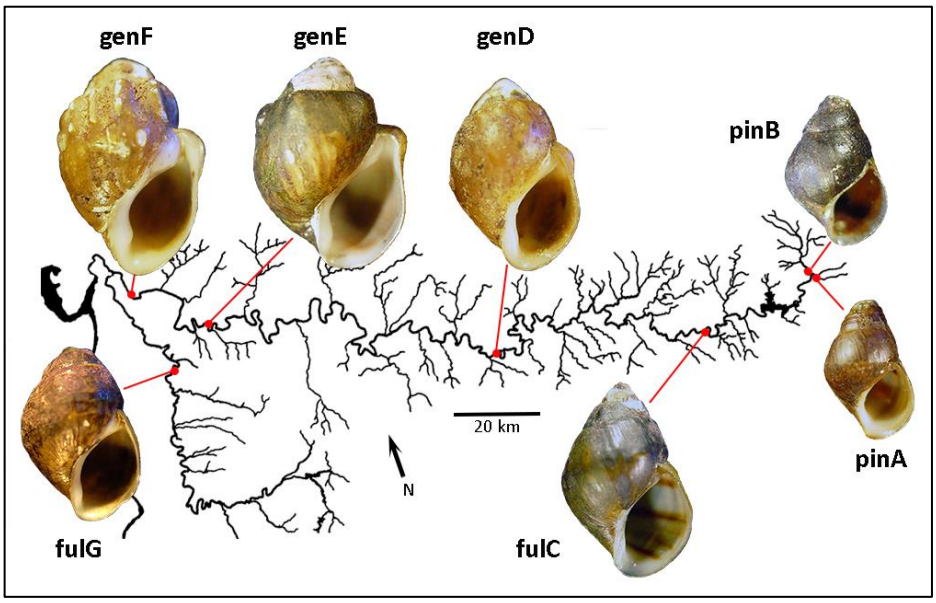

Figure 1. The Duck River drainage of Middle Tennessee, showing the sample sites for *Lithasia geniculata* collected in 2000-02. Shell length of pinA is 14.7 mm; the other shells are presented to scale.

Table 1 shows an apparently fixed difference at the Mpi locus and a nearly-fixed difference at the Odh locus between populations pinA and pinB, a morphologically-indistinguishable pair of samples separated by just four river kilometers. This section of the river includes a series of dramatic waterfalls, currently preserved in Old Fort State Park. Dillon (1988) reported a similar (although much less striking) discontinuity in gene frequencies at a 25 cm cascade created by a culvert in a population *of Pleurocera proxima* inhabiting Naked Creek, Wilkes County, North Carolina.

Table 1. Gene frequencies at three allozyme-encoding loci in seven populations of *Lithasia geniculata* sampled from the Duck River.

	Duck River Basin Populations						
Loci	**pinA**	**pinB**	**fulC**	**genD**	**genE**	**genF**	**fulG**
Odh (N)	(41)	(28)	(36)	(32)	(49)	(33)	(35)
112	1.0	0.054	0.264	0.547	0.582	0.561	0.914
109	0.0	0.946	0.736	0.453	0.418	0.439	0.086
Mpi (N)	(41)	(28)	(36)	(32)	(49)	(33)	(35)
97	0.0	0.0	0.0	0.0	0.0	0.015	0.0
94	0.0	1.0	1.0	1.0	0.867	0.667	1.0
91	1.0	0.0	0.0	0.0	0.133	0.318	0.0
Hexdh (N)	(34)	(28)	(36)	(25)	(41)	(33)	(35)
99	0.015	0.161	0.042	0.0	0.012	0.015	0.0
93	0.985	0.839	0.958	1.0	0.988	0.985	1.0

Significant gene frequency differences were also detected at the Odh locus between samples pinB, fulC, and genD. The values of contingency chi-square for these comparisons were 9.8**, 11.3*** and 33.6*** for pinB/fulC, fulC/genD, and pinB/genD, respectively, with one degree of freedom (df), where two asterisks indicate significance at the p = 0.01 level, and three asterisks indicate significance at the p =

0.001 level. Samples genD, genE, and genF were not different at the Odh locus, but demonstrated significant differences at the Mpi locus. Values of contingency chi-square were 9.2**, 8.6** and 24.7*** genD/genE, genE/genF, and genD/genF comparisons, respectively, with 1 df, lumping rare alleles. Sample fulG differed statistically from genF at both the Odh and Mpi loci (chi-squares = 22.2*** and 27.8***, respectively, with 1 df, lumping rare alleles). Differences on these scales are clearly attributable to isolation by distance, analogous to the situation in *P. proxima* through the remainder of Naked Creek as detailed by Dillon (2020).

Whelan et al. (2019) have suggested that the absence of a correlation between shell shape differences and genetic patterns in pleurocerid populations should constitute evidence of ecophenotypic plasticity in the former. Our observation that the maximum pairwise genetic divergence in the Duck River *Lithasia* population corresponds to the smallest degree of shell morphological difference would appear to satisfy the criterion of Whelan and colleagues.

Minton and Lydeard (2003) uncovered no CO1 sequence divergence among any of their Duck River *Lithasia* samples, regardless of subspecific designation, across six populations (16 individuals). They did uncover a 4.3% difference between the common Duck River sequence and an individual *L. geniculata fuliginosa* sampled from Garrison Fork (an upper tributary of the Duck) and a 2.8% sequence difference between the Duck sequence and two *fuliginosa* individuals sampled from the Buffalo River. This latter observation prompted Minton (2013) to describe the Buffalo *Lithasia* as a new species, *L. bubala*. The allozyme data presented here do not support that hypothesis.

References:

Ahlstedt, S. A., J. R. Powell, R. S. Butler, M. T. Fagg, D. W. Hubbs, S. F. Novak, S. R. Palmer and P. D. Johnson. 2017. Historical and current examination of freshwater mussels (Bivalvia: Margaritiferidae: Unionidae) in the Duck River basin Tennessee, USA. *Malacological Review* 45:1-163.

Dillon, R. T., Jr. 1982. *The correlates of divergence in isolated populations of the freshwater snail, Goniobasis proxima* (Say). Ph.D. Dissertation, The University of Pennsylvania.

Dillon, R. T., Jr. 1985. Correspondence between the buffer systems suitable for electrophoretic resolution of bivalve and gastropod isozymes. *Comparative Biochemistry and Physiology* 82B: 643-645.

Dillon, R.T., Jr. 1988. The influence of minor human disturbance on biochemical variation in a population of freshwater snails. *Biological Conservation* 43:137-144.

Dillon, R. T., Jr. 1992. Electrophoresis IV, nuts and bolts. *World Aquaculture* 23(2):48-51.

Dillon, R. T., Jr. 2011. Robust shell phenotype is a local response to stream size in the genus *Pleurocera*. *Malacologia* 53:265-277.

Dillon, R. T., Jr. 2014. Cryptic phenotypic plasticity in populations of the North American freshwater gastropod, *Pleurocera semicarinata*. *Zoological Studies* 53:31.

Dillon, R. T., Jr. 2020. Fine scale genetic variation in a population of freshwater snails. *Ellipsaria* 22(1): 24-25.

Dillon, R. T., Jr., S. J. Jacquemin and M. Pyron. 2013. Cryptic phenotypic plasticity in populations of the freshwater prosobranch snail, *Pleurocera canaliculata*. *Hydrobiologia* 709:117-127.

Goodrich, C. 1934. Studies of the gastropod family Pleuroceridae - I. *Occasional Papers of the Museum of Zoology, University of Michigan* 286:1-17.

Minton, R. L. 2013. A new species of *Lithasia* (Gastropoda: Pleuroceridae) from the Buffalo River, Tennessee, USA. *The Nautilus* 127:119-124.

Minton, R. L. and C. Lydeard. 2003. Phylogeny, taxonomy, genetics, and global heritage ranks of an imperiled, freshwater snail genus *Lithasia* (Pleuroceridae). *Molecular Ecology* 12:75-87.

Whelan, N. V, M. P. Galaska, B. N. Sipley, J. M. Weber, P. D. Johnson, K. M. Halanych and B. S. Helms. 2019. Riverscape genetic variation, migration patterns, and morphological variation of the threatened Round Rocksnail, *Leptoxis ampla*. *Molecular Ecology* 28:1593-1610.