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PRIMARY RESEARCH PAPER

Cryptic phenotypic plasticity in populations of the freshwater prosobranch snail, *Pleurocera canaliculata*

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Abstract We sampled four populations of the robustly shelled *Pleurocera canaliculata* from large rivers and five pleurocerid populations bearing more fusiform shells (nominally *P. acuta* and *P. pyrenellum*) from smaller streams in a study area extending from upstate New York to northern Alabama, USA. Gene frequencies at 9 allozyme-encoding loci revealed that each population of *P. acuta* or *P. pyrenellum* was more genetically similar to the *P. canaliculata* population inhabiting the larger river immediately downstream than to any nominal conspecific. Thus, the extensive intraspecific variation in shell robustness displayed by these nine populations has apparently been rendered cryptic by taxonomic confusion. We then employed geometric morphometrics to explore a gradient in shell morphology from the *acuta* form to the typical *canaliculata* form in 18 historic samples collected down the length of Indiana's Wabash River. The shell forms appeared generally distinctive on the major axes yielded by relative warp analysis (increasing robustness and decreasing spire elongation), although some overlap was apparent. MANCOVA returned a significant relationship between multivariate shape variation and stream size, as measured by drainage area. Possible drivers for this phenomenon include an environmental cline in the risk of dislodgement due to hydrodynamic drag and shifts in the community of predators.

Keywords Gastropoda · Inducible defenses · Shell morphology · Allozyme electrophoresis · Geometric morphometrics · Predation

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Introduction

Among the earliest demonstrations of adaptive phenotypic plasticity, to be recognized as such, were the experiments conducted by Woltereck (1909) on *Daphnia*. And, freshwater invertebrates continued to serve as useful model organisms for such research throughout the twentieth century (e.g., Gilbert, 1966). Among the more important recent additions to the list of model organisms for studies of phenotypic plasticity have been the freshwater snails (Brönmark et al., 2011, 2012).

The gastropod shell offers an easily measured and permanent record of the environment in which its owner lived. Much attention has focused on the effects of predation on shell phenotype and especially upon

situations where different classes of predators induce different responses in shell size, thickness, and coiling (DeWitt et al., 2000; Lakowitz et al., 2008; Hoverman & Relyea, 2009, 2011). Variation in water chemistry, temperature, current, and substrate can also induce significant variance in shell phenotype (Lam & Calow 1988; Rundle et al., 2004; Britton & McMahon, 2004; Dillon & Herman, 2009). The evolutionary significance of variance in phenotypic plasticity (broadly) has been the subject of extensive review (e.g., Scheiner, 1993; DeWitt et al., 1998).

Here, we introduce a new concept, "cryptic" phenotypic plasticity. We suggest that phenotypic plasticity may be considered "cryptic" when intrapopulation morphological variance is so extreme as to prompt a (erroneous) hypothesis of speciation. Then, if taxonomists attribute phenotypic variance observed in the field to reproductive barriers between populations, rather than to its true environmental basis, the existence of intrapopulation phenotypic plasticity becomes obscured or "cryptic."

There is evidence that cryptic phenotypic plasticity is a widespread phenomenon in freshwater mollusk populations. As early as 1920, A. E. Ortmann proposed a "law" that the shell of unionid mussels tends to develop a broader, heavier, and more robust phenotype in larger streams. This "law" was generalized to pleurocerid snails by Ortmann's protégé Calvin Goodrich (1937), who used it as a basis to synonymize upstream/downstream groups of nominal species differing only in their shell robustness throughout North America (e.g., Goodrich, 1940). Using gene frequencies at allozyme-encoding loci, Dillon (2011) showed that four East Tennessee populations of the pleurocerid genus *Goniobasis* (or *Elimia*) bearing elongated or fusiform shells were each more genetically similar to robust populations of *Pleurocera* inhabiting larger rivers immediately downstream than any of the nominally congeneric populations were to each other. Thus, the historic distinction between the generic nomena *Pleurocera*, *Goniobasis*, and *Elimia* would seem attributable to cryptic phenotypic plasticity.

Pleurocera acuta is among the best known of the North American freshwater gastropods, first described from Lake Erie by Rafinesque (in Blainville, 1824). Populations of *P. acuta* are widespread and common in streams, small rivers, and lake margins of the Mississippi and Great Lakes' drainages from Vermont west to Minnesota, south to Louisiana (Goodrich, 1939a, b, 1940), although (perhaps anomalously) absent from tributaries of the Tennessee River. The biology of *P. acuta* has been reviewed by Dazo (1965), with additional ecological notes by Houp (1970) and morphological observations by Strong (2005).

Populations of *Pleurocera canaliculata* inhabit the larger rivers downstream from many populations of *P. acuta*. Described by Thomas Say (1821) from the falls of the Ohio River, *P. canaliculata* ranges from the main stem of the Mississippi River at Omaha, Nebraska, upstream through the Ohio, Cumberland, and Tennessee Rivers (Goodrich, 1939b, 1940). The biology of *P. canaliculata* has been reviewed by Magruder (1935).

Qualitatively, *P. acuta* and *P. canaliculata* are similar in appearance, *P. canaliculata* typically bearing a larger, lower-spired, and more robust shell than *P. acuta*. Goodrich (1939a) observed that "the two species (or forms) merge in at least three streams of the Ohio River basin. In tributaries of the Cumberland and Duck Rivers of Tennessee are mollusks, clearly derived from heavier *Pleurocera* of the main streams, which are indistinguishable from *acuta*." Thus, the relationship between *P. acuta* and *P. canaliculata* across much of the Midwest would appear similar to that documented by Dillon (2011) in the *Pleurocera* populations of East Tennessee, shell robustness increasing with stream size.

If the basis for the distinction between *P. acuta* and *P. canaliculata* is indeed attributable to cryptic phenotypic plasticity correlated with stream size, the absence of *P. acuta* populations upstream from the *P. canaliculata* populations of the main Tennessee River would be anomalous. However, smaller tributaries of the Tennessee River system are inhabited by populations of *Pleurocera pyrenellum*, originally described by Conrad (1834) from "streams in North Alabama." Goodrich (1937) reported the same "merging" phenomenon between *P. pyrenellum* and *P. canaliculata* in Alabama as he observed between *P. acuta* and *P. canaliculata* in the Ohio River tributaries further north.

The purposes of the present research are twofold. First, we test the hypothesis that populations of pleurocerid snails previously assigned to the nominal species *Pleurocera acuta*, *P. canaliculata*, and *P. pyrenellum* are conspecific, the shell morphological variance among them apparently attributable to

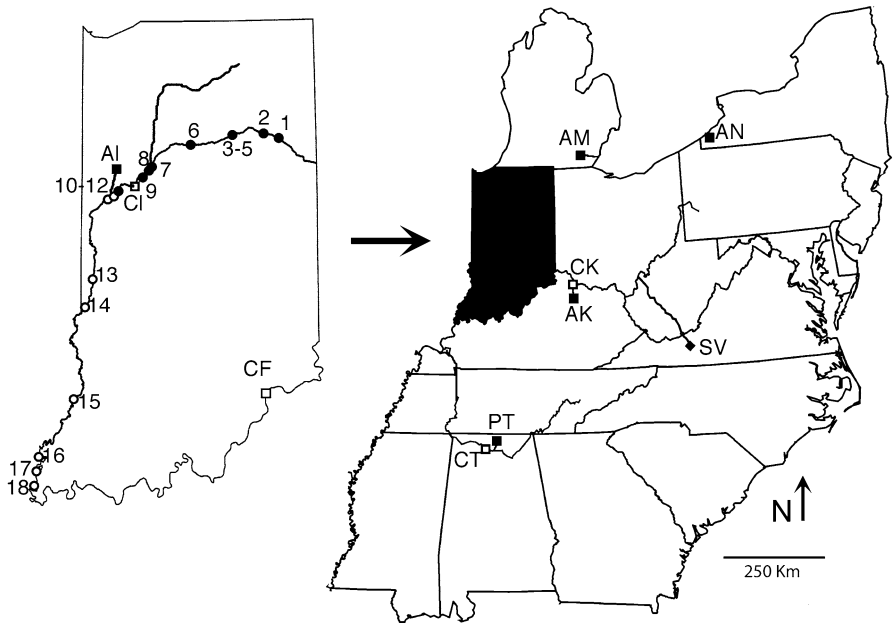


Fig. 1 Sites sampled for the morphometric study are mapped as *circles* (*open* for typical *canaliculata*, *closed* for *acuta*). Sites sampled for the allozyme study are shown as *squares* (*open* for typical *canaliculata*, *closed* for *acuta* and *pyrenellum*) or as a *diamond* (*semicarinata* standard)

phenotypic plasticity rendered cryptic by nineteenth century taxonomy. Our approach is patterned after that of Dillon (2011), using protein electrophoresis to survey a variation at 11 allozyme-encoding loci in 10 carefully chosen populations of varying shell robustness.

Then, after establishing that populations previously assigned to the three nomina are indeed conspecific, we focus on the (combined) *Pleurocera* population of the Wabash River, one of the streams specifically mentioned by Goodrich (1937) where *P. acuta* and *P. canaliculata* "merge." Goodrich and van der Schalie collected large samples of *Pleurocera* from the Wabash in connection with their (1944) "Revision of the Mollusca of Indiana." These, Goodrich identified as either *P. acuta* or *P. canaliculata*, depositing his collections in the University of Michigan Museum of Zoology.

Modern geometric morphometric techniques have recently found widespread application in the study of freshwater gastropod shell morphology (DeWitt et al., 1999; Langerhans & DeWitt, 2002; Minton et al., 2008, 2011). In contrast with more traditional linear morphometrics, geometric approaches do not rely on chord measurement series, better preserving the overall shape of the subject under study. Coordinate-based morphometrics permits the dissociation of scale and position from shape (Zelditch et al., 2004). So, here we apply geometric morphometrics to reexamine 18 lots of *Pleurocera* sampled down the length of the Wabash River in the early twentieth century, exploring the correlation between stream size and shell phenotype that led Goodrich (and indeed workers for almost 200 years) to recognize multiple species where only a single biologic species apparently exists.

Methods

Our study of allozyme variation involved three upstream–downstream pairs of *Pleurocera*, three reference populations, and one standard, for a total of ten pleurocerid populations (Fig. 1). Since there are no reliable characters by which *P. canaliculata*, *P. acuta*, and *P. pyrenellum* can be distinguished, we focused on type localities or populations previously identified by other authorities.

Population AI of *P. acuta* was sampled from Big Pine Creek, 7.5 km NW of Attica, Warren County, Indiana (40.3402N; 87.3139W). Population CI of *P. canaliculata* was sampled approximately 30 km distant, in the Wabash River at the S700W bridge, 13.5 km W of Lafayette, Tippecanoe County, Indiana (40.4125N; 87.03656W). Population AK of *P. acuta* was sampled from the South Fork Licking River at Cynthiana, Harrison County, Kentucky (38.3876N; 84.2987W). Population CK of *P. canaliculata* was sampled approximately 50 km downstream, in the

main Licking River at Butler, Pendleton County, Kentucky (38.7906N; 84.3750W). This was the population studied by Magruder (1935). Population PT was topotypic *P. pyrenellum*, sampled from Limestone Creek at Nick Davis Road, 2 km N of Capshaw, Limestone County, Alabama (34.8027N; 86.8163W). Population CT was *P. canaliculata* sampled approximately 25 km downstream in the Tennessee River at Decatur, Morgan County, Alabama (34.6279N; 86.9564W).

Although *P. canaliculata* populations seem to have become extinct from their type locality at the Falls of the Ohio, we were able to sample a near-topotypic population CF of *P. canaliculata* from the Ohio River approximately 60 km upstream from Louisville at the US 421 bridge, Milton, Trimble County, Kentucky (38.7259N; 85.3688W). Topotypic population AN of *P. acuta* was sampled from Silver Creek at the US 20 bridge, Chautauqua County, New York (42.5438N; 79.1650W). Population AM of *P. acuta* was sampled from Portage Creek at the Toma Road bridge, Washtenaw County, Michigan (42.4246N; 83.9448W). This is the well-studied Station 2 population of Dazo (1965) and Dillon (1991). Finally, to serve as a standard for the entire study, population SV of *Pleurocera semicarinata* was sampled from Little Pine Creek at the state route 100 bridge, Pulaski County, Virginia (36.9468N; 80.7898W). This was population "PINE" in the allozyme study of Dillon & Davis (1980) and population "Gs" in the mtDNA sequence study of Dillon & Frankis (2004). Voucher specimens have been deposited in the Academy of Natural Sciences of Philadelphia.

At least 30 individuals from each pleurocerid population were returned alive to the laboratory, where they were cracked and frozen in Tris tissue buffer for electrophoretic analysis. Techniques and apparatus for horizontal starch gel electrophoretic resolution of allozyme variation in homogenates of molluscan tissues are detailed in Dillon (1992), along with recipes for all the buffers and stains employed here. Allozyme bands interpretable as the products of codominant genes segregating in Mendelian fashion were resolved at 11 loci using 10 enzyme stains as in Dillon (2011).

The Tris Cit 6 buffer (buffer XIII of Shaw & Prasad, 1970) was used to resolve 6-phosphogluconate dehydrogenase (6PGD), octopine dehydrogenase (OPDH), and isocitrate dehydrogenase (2 loci, the cathodal IDHF and the anodal IDHS). A Poulik (1957) discontinuous buffer system was employed for glucose-phosphate isomerase (GPI), sorbitol dehydrogenase (SDH), and octopine dehydrogenase (a second time). The TEB8 buffer system (buffer III of Shaw & Prasad, 1970) was used to analyze phosphoglucomutase (PGM—the strong, fast locus only), xanthine dehydrogenase (XDH), and mannose phosphate isomerase (MPI). A TEB9.1 buffer (Dillon & Davis, 1980) was used for octanol dehydrogenase (OLDH), esterases (EST1—the strong, slow locus only), and xanthine dehydrogenase (a second time).

Putative allelic designations for each zone of allozyme activity were assigned by setting the most common band in standard population SV to "100" and naming all other alleles by the mobility of their allozymes (in millimeters) relative to this standard. Then, gene frequencies and mean direct-count heterozygosities (the unbiased estimate of Nei, 1978) were calculated using Biosys version 1.7 (Swofford & Selander, 1981). Because large numbers of alleles were resolved at some loci, sample sizes dictated that genotypes be pooled into three classes to test for Hardy–Weinberg equilibrium: homozygotes for the most common allele, common/rare heterozygotes, and rare homozygotes together with other heterozygotes. Yates-corrected Chi square statistics were then employed for this purpose. We calculated matrices of Nei's (1978) unbiased genetic identity and distance as well as the Cavalli-Sforza & Edwards (1967) chord distance. As chord distances are Pythagorean in Euclidean space (Wright, 1978), they were used to calculate a neighbor-joining tree with Phylip v3.65 program NEIGHBOR (Felsenstein, 2004).

We received on loan from the University of Michigan Museum of Zoology 18 dry lots of *Pleurocera* collected down the length of the Wabash River between 1916 and 1963 (Table 1; Fig. 1). Most of these shells were identified by Calvin Goodrich, if not collected by him personally (Goodrich, 1934; Goodrich & van der Schalie, 1944). We selected the shells of up to 15 mature individuals (shell length $\geq$1.5 cm) with intact, uneroded spires from each lot and photographed them at 12 pixels/mm against a projected grid background (serving as a scale reference) using a mounted Nikon D70 digital camera with an AF-S DX Macro Zoom Lens. Ultimately, our analysis included 178 shells: 110 of which had been identified as *P. acuta* and 68 of which were *P. canaliculata*.

Table 1 Samples of *Pleurocera* from the Wabash River (Indiana) analyzed in the morphometric study

Site	Drainage (km^2)	River (km)	Species	County	Years	UMMZ	*N*
1	535	697	PA	Wells	–	49261	8
2	1074	644	PA	Wells	–	39295	5
3	1138	633	PA	Miami	1920	39296	2
4	1138	633	PA	Miami	1923	30104	2
5	1138	633	PA	Miami	1923	30106	1
6	3812	558	PA	Cass	1916	39297	12
7	4400	524	PA	Carroll	–	150752	11
8	7489	477	PA	Tippecanoe	1941	150753	15
9	7489	477	PA	Tippecanoe	1963	227343	6
10	7630	471	PA	Tippecanoe	–	39298	6
11	7630	471	PC	Tippecanoe	1927	40585	15
12	7630	471	PC	Tippecanoe	–	40371	15
13	12263	348	PC	Parke	1928	44208	15
14	12681	304	PC	Vigo	1936	128759	15
15	16308	165	PC	Knox	–	242184	5
16	29150	68	PC	Posey	1927	44025	15
17	29200	61	PC	Posey	–	40372	15
18	29585	47	PC	Posey	1930	51403	15

Species PA, *P. acuta*; PC, *P. canaliculata*. River km is the distance upstream from confluence with the Ohio River, *N* is the sample size, UMMZ is the University of Michigan Museum of Zoology collection number

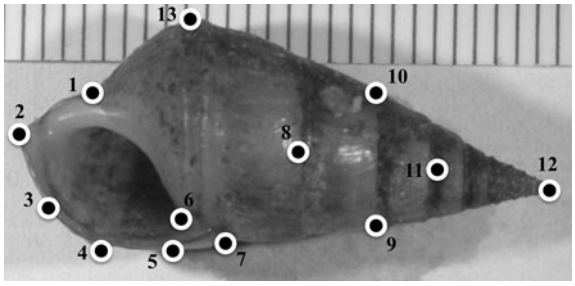


Fig. 2 Locations of the 13 landmarks digitized for geometric morphometry. Scale is in mm

Shell shape was quantified using tpsDIG2 (Rohlf, 2008) following the 12 landmarks of Dunithan et al. (2012) with one additional landmark to better encompass the spire variation in these populations (Fig. 2). Centroid size was calculated as an overall measure of body size for each of the 178 shells. Then, the Procrustes technique (least squares superimposition with orthogonal data projection) was applied to remove the effects of rotation, translation, and scaling discrepancies, with points along curves (landmarks 3, 5, and 13) treated as sliding semi-landmarks.

Shape variation was first visualized using principal components of aligned landmark coordinates (relative warp analysis) implemented in tpsRELW (Rohlf, 2007). This unconstrained ordination approach allowed us to identify the major shape gradients demonstrated across all individuals and compare the regions occupied by the two nominal taxa visually, using thin-plate spline methods (see Zelditch et al., 2004). We tested for differences in the scores of nominal *acuta* and *canaliculata* on the two major shape components we obtained using t-tests.

We estimated drainage area upstream from each of the 13 discrete sites represented by our 18 samples from Hoggatt (1975) as a measure of stream size. We then constrained all principal components explaining greater than 1% of the total shape variation in a MANCOVA model to test for a simple relationship between overall shell shape variation and drainage area. Our MANCOVA model was implemented in the "heplots" package (Fox et al., 2012) within the R statistical environment (R Development Core Team, 2011), evaluating the shape variation explained with Pillai's partial η^2 statistics (Langerhans & DeWitt, 2004). We also ran a second MANCOVA, adding shell size (multivariate allometry) to drainage area and their

interaction term. Overall shape variation as a function of drainage area was visualized by alignment in tpsRELW, ported into tpsREGR (Rohlf, 2011).

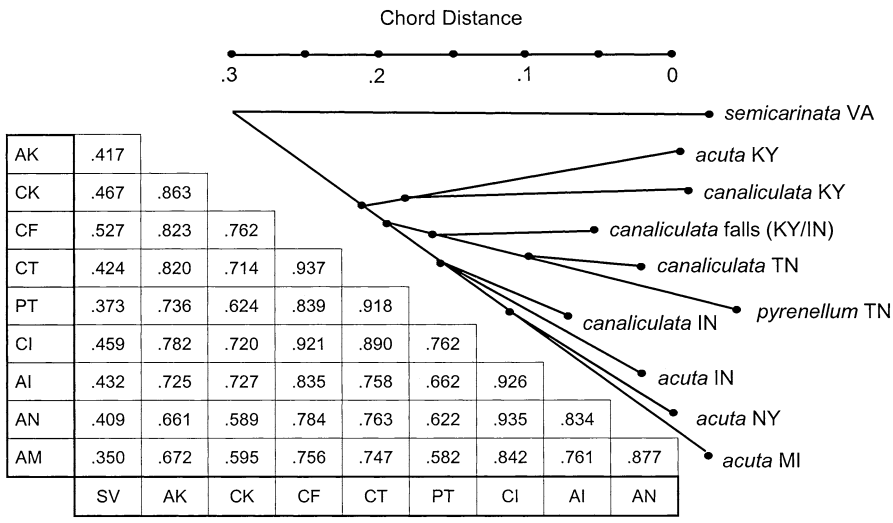
Results

Of the 11 allozyme-encoding loci surveyed, no variation was detected across all ten pleurocerid populations at either the SDH or the IDHS locus. Gene frequencies at the other nine loci are available as supplementary material, together with mean direct-count heterozygosities. Across the 9 loci × 10 populations at which any variation was detected, 46 loci were polymorphic by the 95% criterion. The genotype frequency observed at just one of these 46 loci was significantly different from the Hardy–Weinberg expectation (at the nominal 0.05 level) by Chi square tests (OPDH in population AN), a result which might be attributable to type I statistical error.

The matrix of pairwise Nei (1978) unbiased genetic identities among populations is shown in Fig. 3, together with the results of a neighbor-joining analysis based on Cavalli-Sforza and Edwards Chord distances. The SV population of *P. semicarinata* serving as a standard for this study was strikingly different from the other 9 populations, with genetic identities ranging from 0.350 to 0.527, as has typically been observed in interspecific comparisons (Chambers, 1980; Dillon & Reed, 2002; Dillon & Davis, 1980). The genetic identities among the other nine populations were more modest, ranging from 0.589 to 0.937, as has typically been reported in intraspecific comparisons (Dillon, 1984; Dillon & Robinson, 2011).

Within the study group of nine populations, clustering was entirely by region, independent of putative taxonomy. The Indiana population of nominal *P. acuta* (AI) was most genetically similar to the populations of *P. canaliculata* (CI) sampled immediately downstream in the Wabash River ($I = 0.926$, Fig. 2). The Kentucky population of nominal *P. acuta* (AK) was most genetically similar to the population of *P. canaliculata* (CK) immediately downstream in the main Licking River ($I = 0.863$). And, the Tennessee population of nominal *P. pyrenellum* (PT) was most genetically similar to the population of *P. canaliculata* (CT) sampled immediately downstream in the main Tennessee River ($I = 0.918$). The exact probability that all three of these upstream test populations might most closely match their downstream *P. canaliculata* analog by chance is $(1/9)^3 = 0.0014$.

Principal components' analysis of the aligned coordinates from our large set of historically collected shell samples from the main Wabash River yielded 12 axes accounting for greater than 1% of the variance, together explaining a total of 95% of the variation among individuals. The two major axes explained 59% of the total variation among individuals, shape component 1 explaining 33% and shape component 2 explaining 26% (Figs. 4, 5). The scores of the two nominal taxa differed significantly on both of these axes. Individuals identified as *P. acuta* by Goodrich scored higher on component 1 than those identified as *P. canaliculata*, the shell shape of the latter more



AK	.417								
CK	.467	.863							
CF	.527	.823	.762						
CT	.424	.820	.714	.937					
PT	.373	.736	.624	.839	.918				
CI	.459	.782	.720	.921	.890	.762			
AI	.432	.725	.727	.835	.758	.662	.926		
AN	.409	.661	.589	.784	.763	.622	.935	.834	
AM	.350	.672	.595	.756	.747	.582	.842	.761	.877
	SV	AK	CK	CF	CT	PT	CI	AI	AN

Fig. 3 Nei's (1978) unbiased genetic identities based on 11 allozyme loci resolved across ten populations of *Pleurocera* sampled from the eastern United States. The neighbor-joining tree is based on the Cavalli-Sforza and Edwards (1967) chord distances

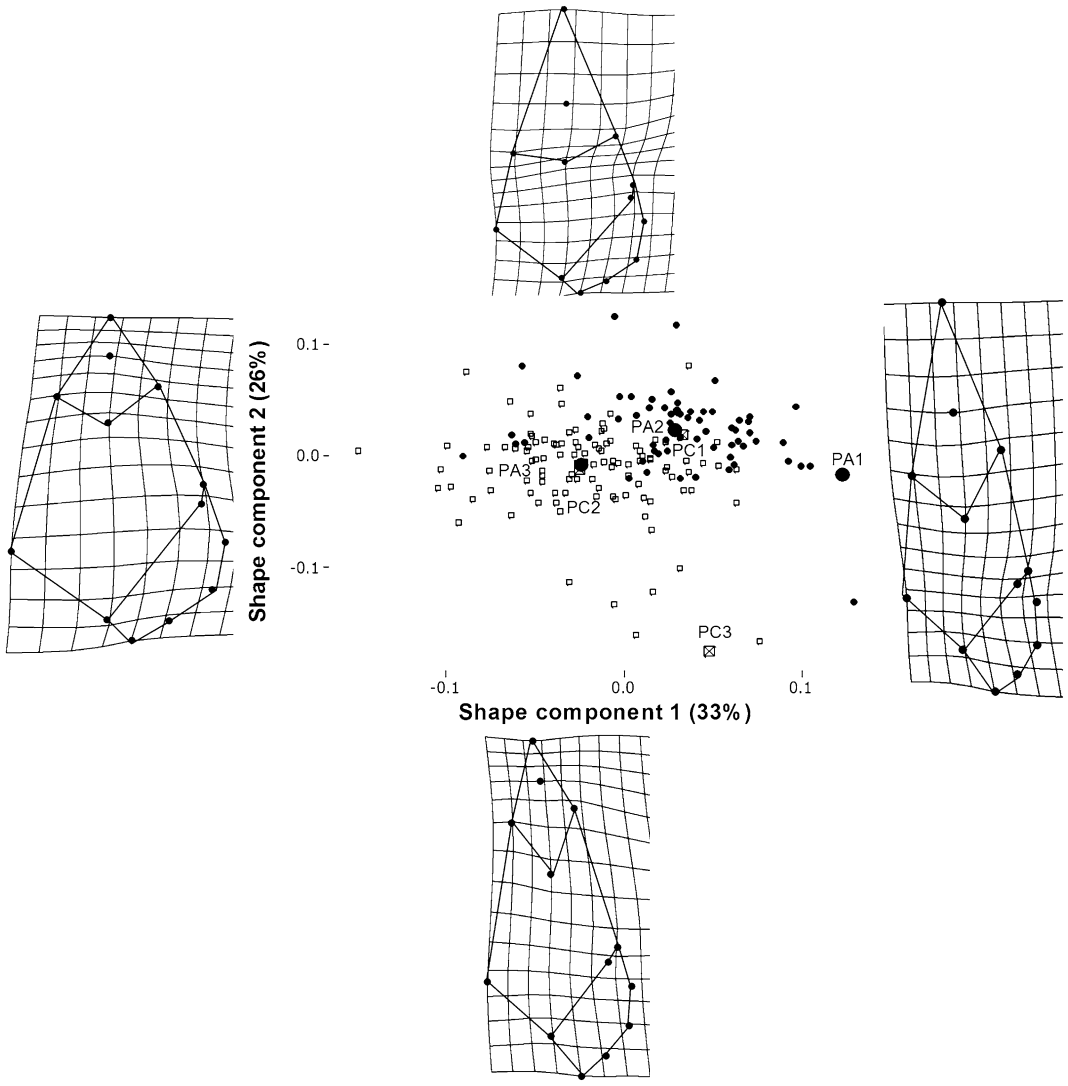


Fig. 4 Shape variation in the *Pleurocera* populations of the Wabash River. Closed circles represent shells identified by Goodrich as *P. acuta* and open squares *P. canaliculata.* Deformation grids represent extreme axis shape change relative to consensus. Refer to Fig. 5 for the six individual data labeled

robust and that of the former more fusiform ($t_{176} = 7.8$, $P < 0.001$). Nominal *acuta* also scored significantly higher on component 2, the *acuta* form demonstrating an elongated spire and typical *canaliculata* a shorter spire ($t_{176} = 6.3$, $P < 0.001$).

Our (one-factor) MANCOVA testing of the simple model that shape variance (over the 12 axes extracted) is a function of drainage area returned a highly significant Wilks statistic and partial variance explained $\eta^2 = 0.46$ (Table 2). Our two-factor MANCOVA revealed highly significant relationships between shell shape and shell size, as well as between shell shape and drainage area ($\eta^2 = 0.39$ and $\eta^2 = 0.40$, respectively). The interaction term was not significant.

Discussion

The allozyme results shown in Fig. 3 for *P. canaliculata* are strikingly similar to those reported by Dillon (2011) for *P. clavaeformis* in East Tennessee. Throughout the extensive range of the species, populations of *P. canaliculata* sampled from large rivers tend to demonstrate more genetic similarity with

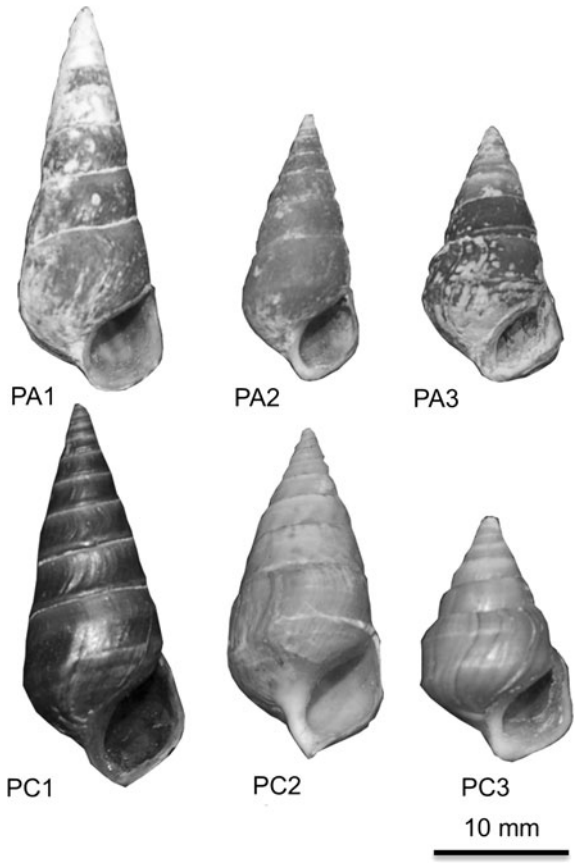


Fig. 5 Representative individuals displaying mean and extreme shape variation in nominal *Pleurocera acuta* and typical *Pleurocera canaliculata*. PA1, nominal *acuta* least similar to mean typical *canaliculata*; PA2, *acuta* form mean; PA3, *acuta* most similar to mean typical *canaliculata*; PC1, typical *canaliculata* most similar to mean nominal *acuta*; PC2, typical *canaliculata* mean; and PC3, typical *canaliculata* least similar to mean nominal *acuta*

Table 2 MANCOVA results testing for a relationship between shell shape variation in the Wabash River *Pleurocera* and stream size ("simple model") or stream size, shell size, and their interaction ("full model")

Model	*F*	df	*P*	η^2
Simple				
Drainage area	11.2	12, 165	<0.001	0.46
Full				
Drainage area	12.6	12, 163	<0.001	0.40
Shell size	8.70	12, 163	<0.001	0.39
Drainage area × shell size	1.58	12, 163	0.10	0.10

Pleurocera populations of other nominal species sampled from small tributaries nearby than they do with nominally conspecific populations sampled from more distant drainages. All nine of the populations shown in the main cluster of Fig. 3 would appear to be conspecific, the nomen *canaliculata* (Say, 1821) having priority over *acuta* (Rafinesque, 1824) and *pyrenellum* (Conrad, 1834) for the combined group.

In all three regions where the phenomenon was revealed, the shell morphology demonstrated by the upstream cognate (*acuta* in Indiana and Kentucky, *pyrenellum* in Tennessee) was less robust and more elongated than the typical *canaliculata* morphology immediately downstream. This is consistent with "Ortmann's Law" as generalized by Goodrich (1937) that "*Pleurocera* increases in relative diameter in a downstream direction." This "natural law" seems to prevail to a greater extent than Goodrich himself realized, across populations Goodrich (following nineteenth century authorities) identified as distinct *Pleurocera* species.

Focusing on the Wabash drainage of Indiana, Figs. 4 and 5 depict a large range of variation in the shell morphology of *P. canaliculata*, populations of the typical morphology (open circles) blending smoothly into populations that Goodrich identified as *P. acuta* (closed circles). Individual specimens bearing shells of the most extreme morphologies are distinctive: fusiform and high spired for the *acuta* form (e.g., individual PA1), short spired and robust for the typical *canaliculata* (e.g., individual PC3). But, Goodrich did identify some snails bearing rather fusiform shells as *P. canaliculata* (e.g., individual PC1) and some bearing robust shells as *P. acuta* (PA3), demonstrating his own uncertainty in the matter.

Our simple one-factor MANCOVA returned a significant relationship between overall shape (12 partial warp shape components extracted, 95% of the variance) and stream size, as estimated using drainage area (Table 2). Figure 6 illustrates that, as might have been predicted from "Ortmann's Law," *Pleurocera* populations in the headwaters of the Wabash River bear more fusiform shells with elongated spires, becoming more robust and shorter spired downstream. These trends extend from populations that Goodrich unambiguously identified as *P. acuta*, through mixed populations, to populations unambiguously displaying the typical *canaliculata* form.

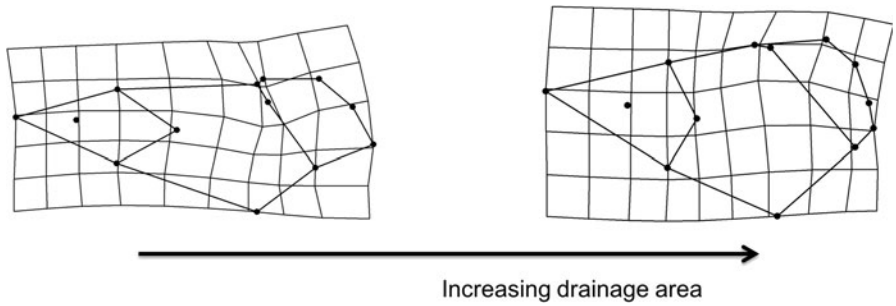


Fig. 6 *Pleurocera* shell morphology as a function of log drainage area. Thin-plate spline transformations (magnified ×2) were generated by tpsRELW, ported into tpsREGR (Rohlf, 2011)

Our two-factor MANCOVA returned a significant relationship between shell shape and shell size, as well as between shell shape and drainage area, the more robust snails tending to be larger. This result confirms that all three variables (stream size, shell size, and shell shape) are highly intercorrelated, the larger and more heavily shelled snails tending to be found in the larger rivers.

Taken together, the shell morphological variation displayed across all 178 individuals plotted in Fig. 4 is striking. The additive heritability of this variance is an open question, however. Controlled studies have returned evidence of strikingly plastic responses in the shell phenotypes of the pulmonate snails *Physa* (DeWitt, 1998; Langerhans & DeWitt, 2002), *Lymnaea* (Rundle et al., 2004; Lakowitz et al., 2008), *Helisoma* (Hoverman & Relyea, 2007), and *Ferrissia* (Dillon & Herman, 2009), as well as in the pleurocerids (Urabe, 1998; 2000; Krist, 2002) and the hydrobiids (Holomuzki & Biggs, 2006).

Goodrich (1934) correlated the robustness of pleurocerid shells with river flow, observing that a relatively broad shell would imply a relatively large foot and suggesting that a larger foot would be adaptive in the "rapid and sometimes tumultuous" currents of larger rivers. The field observations of Urabe (1998) indeed confirmed that individual pleurocerid snails with larger body whorls and lower spires tend to be found in more rapid currents, while his laboratory rearing studies suggested heritabilities for these traits not different from zero.

The most commonly manipulated independent variable in studies of ecophenotypic response in freshwater gastropod shell morphology has, however, been predation. Broader, more robust shells are more resistant to crushing, while fusiform or elongate shells offer more protection from predators that might attack a snail through its aperture (Dillon, 2000, pp. 309–314). The observation that freshwater snails reared in the effluent of crushing predators tend to develop broader shells with larger body whorls than control populations has been repeated in at least three independent laboratories (Langerhans & DeWitt, 2002; Auld & Relyea 2011; Brönmark et al., 2011, 2012). The experiments of Krist (2002) demonstrated an opposite effect from crayfish attack on *Pleurocera livescens.* When pleurocerid snails are reared in the effluent of crayfish, extracting conspecifics through their apertures, they develop narrower body whorls. So, if (for example) crayfish tend to be more common in the smaller tributaries of our study area, and crushing predators (such as drum and other large fish) tend to predominate in the larger rivers, shell morphological variance such as that displayed in Fig. 4 might be the consequence.

Any direct evaluation of the hypotheses outlined above for *Pleurocera* will await common garden experiments. Most of the research on phenotypic plasticity in freshwater gastropods conducted to date has focused on pulmonate taxa, which mature rapidly in static water culture at room temperature. *Pleurocera* typically require two years to reach maturity, and experimental manipulation of their cooler, lotic-water conditions will require specialized facilities.

Whatever environmental factor may be the basis for the distinction between the *acuta* form and the typical *canaliculata* form, however, it would seem triggered by the confluence of the Tippecanoe River and the main Wabash River at river km 518 in Lafayette. Table 1 and Fig. 1 show that the *acuta* form ranges from the headwaters of the Wabash as drainage areas gradually increase to just below its confluence with the Tippecanoe. At the junction of the Tippecanoe, the drainage area almost doubles, from 4,440 to 7,489 km^2. The *acuta* form was found mixed with the typical form in lots 10, 11, and 12 at river km 471 (7,630 km^2), but disappeared by km 348 (12,263 km^2), where lot 13 contained only the typical

form. And, only the typical form was found in samples taken from the river as drainage areas continue to gradually increase further downstream.

Rivers typically converge in a pairwise fashion. We suggest that this fundamentally binary character of river drainage systems, which overlies their broad tendency toward gradual augmentation, may explain a great deal of the confusion in pleurocerid taxonomy in North America. For example, the *Pleurocera* population of Big Pine Creek (from which our sample AI was taken for allozyme electrophoresis) bears high-spired, fusiform shells of the *acuta* form. Any adult snails emigrating from Big Pine Creek (drainage area approximately 500 km^2) directly into the main Wabash River will mix unconformably with the robustly shelled resident population at River km 465 (drainage area approximate 8,000 km^2), appearing to the taxonomist as a distinct species. Cryptic phenotypic plasticity such as this, accentuated where small streams meet large rivers throughout North America and reinforced by taxonomic inertia, may have misled freshwater biologists for two centuries.

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