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Distinct snail (*Physa*) morphotypes from different habitats converge in shell shape and size under common garden conditions

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ABSTRACT

Background: Aspects of snail shell morphology may be plastic or genetically fixed. Even within a single population, environmentally induced shell shape plasticity can lead to unclear species identifications as a result of extreme shape variation. Extrinsic factors, such as predation pressure and stream flow, tend to induce adaptive plastic changes in shell morphology, such as elongate shells with narrow apertures and short-spired shells with wide apertures, respectively. Snail populations from a local stream and adjacent wetland exhibit these distinct morphotypes.

Questions: Do the snail morphotypes represent a single cryptic species? Are the morphotypes environmentally induced and plastic, or epigenetic?

Organisms: We captured wild *Physa* snails from either a stream population (low predation, high flow site) or a nearby pond population (high predation, low flow site) in Stillwater, Oklahoma, USA.

Predictions: If distinct snail morphotypes represent a single cryptic species, their phenotypes may be plastic. In this case, raising snail offspring under similar conditions of predation and stream flow will result in one shell shape and size.

Methods: We reared and maintained snail offspring of both morphotypes in laboratory aquaria (low water flow, no predation). We measured the shell morphology of wild, of first-generation laboratory, and of second-generation laboratory snails using geometric morphometrics.

Results: Shell shape and size of wild snails from the two populations were significantly different. After a single generation, however, the shell shape of both populations resembled the wild snails from the pond site (elongate with narrow apertures). Shell size decreased in the first generation, but shell size in the two populations did not fully converge until the second generation.

Conclusions: The shape differences are plastic responses to environmental variation. Thus, the two morphotypes constitute a single snail species (*Physa acuta*). The single generation lag in size convergence suggests there is an epigenetic difference between generations within populations.

Keywords: geometric morphometrics, morphology, phenotypic plasticity, predation, water flow.

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INTRODUCTION

Phenotypic plasticity is a major focus of ecological and evolutionary research (West-Eberhard, 1989; DeWitt *et al.*, 1998; Pfennig *et al.*, 2010), especially with regard to its adaptive nature (Pigliucci, 2001). The ability of similar genetic backgrounds to produce wildly different phenotypes in different environments has been of notable interest across a diverse array of taxa (e.g. Bradshaw, 1965), including snails (Vermeij, 1995), which are becoming model organisms for the study of plasticity (Brönmark *et al.*, 2011).

Snail shell dimensions, the most easily quantifiable of morphological traits, have historically been considered phylogenetically informative in several publications regarding species identification (e.g. Burch, 1989). However, the ecological conditions of freshwater habitats have been suggested to induce plastic, sometimes adaptive, changes to shell morphology (Vermeij, 1995), and it is becoming increasingly evident that shell plasticity has led to historically unclear species identifications (Minton *et al.*, 2008; Perez and Minton, 2008). For example, snail species with extensive gene flow between populations can have dramatically different phenotypes within populations due to different habitat conditions, which has resulted in the misidentification of species based on morphology (Johnson and Black, 1999). Furthermore, local selection pressures can result in cryptic phenotypic plasticity within a single snail population when extreme intra-population morphological variation incorrectly suggests there are multiple species (Dillon *et al.*, 2013).

Historical studies suggest stream size and flow induce dramatic changes in mollusc shell shape (Ortmann, 1920; Ball, 1922; Wiebe, 1926). This hypothesis has been tested several times and, generally, as a plastic response to water current and potential dislodgement, snail foot size and shell aperture width increase (Kitching *et al.*, 1966; Trussell *et al.*, 1993). Although water current undoubtedly has a large impact on shell plasticity, reciprocal transplant experiments suggest water current is not the sole factor affecting snail shell morphology (Etter, 1988). Dillon (2011) notes that differences in water flow are associated with differences in water chemistry, temperature, productivity, and biotic communities. This includes predation, which has also been suggested as a major force affecting snail shell morphology (Palmer, 1979; Trussell, 1996; DeWitt *et al.*, 2000; Langerhans and DeWitt, 2002; Salice and Plautz, 2011). In general, these studies indicate predation cues induce snails to produce thick, elongate shells with narrow apertures.

Snails in the family Physidae are morphologically diverse and molecular data suggest morphologically based species descriptions have overestimated physid species diversity by 50% (Wethington and Lydeard, 2007). Although several studies have demonstrated shell plasticity in physid snails when exposed to different environmental conditions (DeWitt *et al.*, 2000; Langerhans and DeWitt, 2002; Britton and McMahon, 2004), to our knowledge, no study has assessed changes in shell shape and size when distinct morphotypes experience the same ecological conditions.

In this study, we hypothesize that, in general, physid morphotypes are not distinct genetically, but are plastic responses to their environment. To test this hypothesis, we use two distinct *Physa* morphotypes: one from a pond in Teal Ridge Municipal Park and one from an adjacent stream in Babcock Park, Stillwater, Oklahoma, USA. Stevison (2013) suggested that these two unique site-specific morphologies are a response to the high density of predatory crayfish at Teal Ridge (more than 40 crayfish per trap) and the low density of predatory crayfish at Babcock Park (0 crayfish per trap but visually observed at the site). However, in his study, Stevison did not consider the effect of water current or other ecological factors on snail morphology. Here, we provide the first experimental assessment of these two hypotheses by raising the two geographically adjacent, but distinct *Physa*

morphotypes in a common garden (with no predators and no water current), and quantifying their morphology for two generations. If predation is the main driver of snail shell divergence between these two populations, we would expect to see the shape of snails previously exposed to predation pressure to converge across generations towards a shape of snails experiencing low predation pressure (Stevison, 2013). In contrast, if water current is the main driver of snail shape, we would expect to see the opposite pattern. It is also possible, given the numerous differences between pond and stream environments that both processes and/or additional processes are occurring simultaneously, in which case, the shell shape of common garden offspring would be expected to diverge away from both wild population morphotypes and converge towards a novel morphotype.

MATERIALS AND METHODS

Specimen collection and culture

We collected *Physa* snails from a pond at Teal Ridge Municipal Park [a high predation/low flow (HP/LF) site] and a stream in Babcock Park [a low predation/high flow (LP/HF) site] on 31 May 2013 in Stillwater, Oklahoma. Teal Ridge (henceforth: HP/LF) is a restored wetland area characterized by ephemeral ponds that remain seasonally inundated most of the year. Babcock Park (henceforth: LP/HF), in contrast, is located along Stillwater Creek. The stream flows from an Army Core of Engineers Dam approximately 10 km upstream. Collection sites are approximately 600 m away. However, at its minimum, the pond is only 200 m away from the creek and during heavy rains, drains into Stillwater Creek through a man-made drainage. Approximately 100 snails of unknown ages were collected from each location and placed in one of two respective 5-gallon (22.73-litre) sterile buckets filled two-thirds full with dechlorinated well water. Ten algal pellets were placed in each bucket as a food source for snails, and the buckets were aerated in the laboratory overnight. To induce snails to lay eggs, porcelain, Styrofoam™, and plastic containers were placed in each bucket as egg-laying substrates. Egg masses were collected from each substrate the following day. Immediately after egg collection, we preserved 20 wild (generation F0) randomly selected snail individuals from each population in 95% ethanol.

Next, we prepared six 10-gallon (45.46-litre) tanks filled with 9.8 gallons (44.55 litres) of dechlorinated tap water and 5 cm of washed gravel for substrate. All tanks were aerated, room temperature was held constant at 25 ± 1°C, and fluorescent lights were set to a 14/10 h light/dark cycle. Five egg masses (containing 50–60 eggs each) produced by F0 snails from the HP/LF site were split into thirds and deposited evenly into three of the 10-gallon laboratory tanks. We then repeated this procedure for the other three tanks with eggs produced by F0 snails from the LP/HF site. In this way we controlled for the genetic structure in each tank, but attempted to maintain some of the variation from each population. Hatched first laboratory generation (F1) snails were fed algae pellets and frozen green leaf lettuce *ad libitum*. Consistent with previous reports (Wethington and Dillon, 1993), F1 snails began laying eggs within 6–7 weeks of hatching.

Seven weeks after hatching, we removed all F1 snails from each tank and left all snail egg masses produced by the F1 individuals intact. We preserved 20 randomly selected F1 snails from each tank in 95% ethanol. Similarly, after an additional 7 weeks, 20 randomly selected second-generation (F2) laboratory snails were preserved from each tank as previously described. To assess snail size and shell morphology, we photographed all preserved F0, F1,

and F2 snails. Briefly, fixed snails were individually placed ventral side up next to a ruler with the apex away from the individual taking the photograph (Fig. 1A). Digital images were taken with a Canon G11 Powershot digital camera attached to an Olympus SZ61 trihead dissecting microscope. All snails were deposited as voucher specimens in the Museum of Zoology: Mollusk Division, University of Michigan (UMMZ accession numbers: UMMZ304402–UMMZ304407).

Molecular and morphological species identification

We sequenced the cytochrome oxidase *c* subunit I (COI) mitochondrial gene for three individuals from each population. All DNA extractions were performed by grinding frozen (−80°F) soft tissue from snail bodies and using E.Z.N.A.® Insect DNA kits (Omega BioTek, Norcross, GA, USA). We used the following primer pair: LCO1490 (forward) 5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′ and HCO2198 (reverse) 5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′. We performed PCRs using an initial denaturing of 94°F for 3 min, followed by 35 cycles of denaturation at 94°F for 30 s, annealing at 47°F for 1 min, and extension at 72°F for 1 min. We used one final hold of 72°F for 7 min. PCR products were gel-purified and cleaned with the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Sequencing was performed in both directions for all sequences at the Core Facility of Oklahoma State University on an ABI7000 sequencing analyser. We reverse complemented and aligned sequences using CLC Main Workbench 6.8.2, then edited alignments by hand. Sequences were subsequently deposited in GenBank (accession numbers: KJ769123–KJ769128). DNA sequences were compared with other

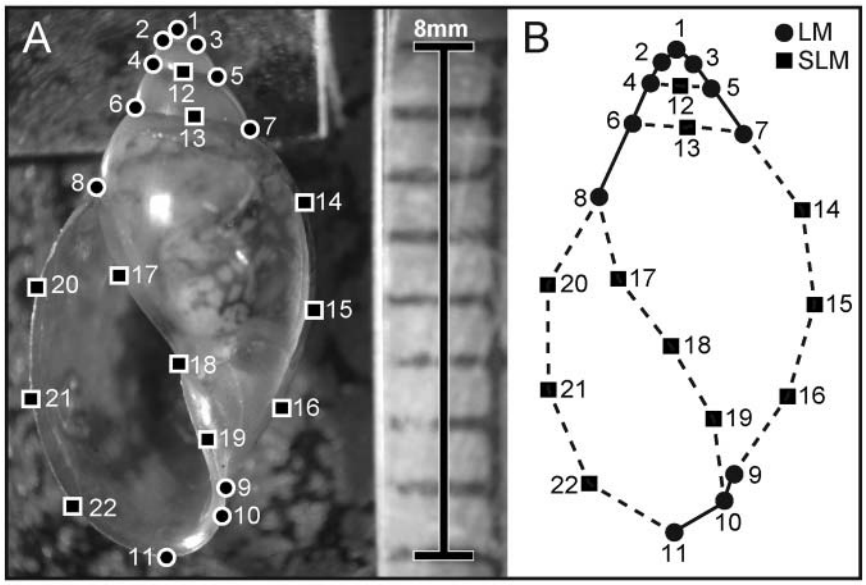

Fig. 1. Landmark locations for *Physa acuta* snails on (A) a typical photograph and (B) the consensus snail (i.e. the average shape of all snails). Homologous landmarks (LM 1–11) are represented by circles; sliding landmarks (SLM 12–22) are represented by squares. Dashed lines indicate connections with sliding landmarks and solid lines were added for visualization. Scale bar = 8.0 mm.

sequences in GenBank using BLAST (Morgulis *et al.*, 2008), which utilized program BLASTN 2.2.29 (Zhang *et al.*, 2000). In addition, snails were identified by penial complex according to Wethington (2004) and Wethington and Lydeard (2007).

Shape and size analyses

We used geometric morphometric methods to assess snail shell morphology (Rohlf and Marcus, 1993; Zelditch *et al.*, 2004). We digitized 22 landmarks (Fig. 1) on digital images of each specimen ($N = 260$) using tpsDig2 (Rohlf, 2013). Snails that lacked homologous landmarks (i.e. discrete and easily recognizable endpoints) or semi-landmarks (i.e. not a discrete endpoint; treated here as equidistant points along curves or whorls) because of broken shells (typically broken apertures and apexes) were removed from the analyses [for landmark discussion, see Zelditch *et al.* (2004)], resulting in 128 HP/LF snails (F0 = 20, F1 = 57, F2 = 51) and 128 LP/HF snails (F0 = 19, F1 = 55, F2 = 54). We used tpsRelw (Rohlf, 2010) to perform generalized least-squares Procrustes superimposition [i.e. rotation, translation, and scaling to remove positional effects (Bookstein, 1991)] to obtain shape variables (partial and relative warps) for further analyses.

Relative warp (RW) axes (i.e. axes that summarize the variation in shape among the specimens) were used to test population source and generation effects on snail shell morphology using a multivariate analysis of covariance (MANCOVA). In addition, we calculated a divergence vector (DV), according to Langerhans (2009), as a measure of directional differences in shape across full shape space (i.e. all relative warps). Briefly, we performed a principal components analysis (PCA) of the sums of squares and cross-products matrix of the generation term from the MANCOVA to derive an eigenvector of divergence (Langerhans, 2009). The divergence vector describes linear combinations of dependent variables exhibiting the greatest differences between generations (within populations) in Euclidean space and avoids distortion of shape space. An analysis of covariance (ANCOVA) was used to test for significant shape divergence between generations within and between source populations. Centroid size (i.e. the square-root of the sum of squared distances from the landmarks to the centroid of the landmarks) was used as the measure of snail size. To account for geometric morphometric allometry, centroid was included as a covariate in all shape analyses. Furthermore, an analysis of variance (ANOVA) of centroid size was used to test for significant generational differences in size within and between populations. To allow for easier interpretation, we also calculated snail shell length, shell width, aperture length, aperture width, spire length, and spire angle using tpsDig and assessed the correlation of these traditional snail shell metrics with RW axes, divergence vector, and centroid. We used tpsRegr (Rohlf, 2011) to visualize shape differences relative to DV scores.

RESULTS

Molecular and morphological species identification

The lengths of the six COI gene sequences ranged from 615 to 684 bp. For all six sequences, the BLAST search resulted in 72 hits for *Physella acuta* [≈ *Physa acuta* (Wethington and Lydeard, 2007)] and 2 hits for *Physella virgata* [recently synonymized with *Physa acuta* (Wethington and Lydeard, 2007)]. In addition, for all sequences, at least the first 20 BLAST results for each

sequence had a 98–99% identity score for *Physella acuta* and/or *Physella virgata*. The top BLAST results, in all cases, included the sequence AY651170.1 (*Physella virgata*) or KF737951.1 (*Physella acuta*) (mean maximum score ± S.D.: 1154.67 ± 37.55; mean maximum identity: 98.83 ± 0.41%; mean *E*-value: 0.0 ± 0). Analyses based on penial morphology were consistent with molecular sequence data. Each snail had the same overall penial complex indicative of *Physa acuta*, including a preputial gland and one-part, non-glandular, muscular, penial sheath (Wethington, 2004; Wethington and Lydeard, 2007).

Shape and size analyses

The relative warp (RW) analysis resulted in 9 RW axes, each accounting for more than 1% of the overall shape variation. Of those, the first 2 RW axes accounted for 70.11% of the shape variation (Fig. 2) and RW axes 1–4 (81.75% cumulative shape variation) were the only axes to each account for more than 5% of shape variation (Table 1). RW axis 1 was significantly and primarily related to measures of snail shape, including various spire and aperture width–length ratios and was weakly related to snail length and width measurements by themselves (Table 1). We interpret the range of RW axis 1 as a change in snail shell shape from shells with relatively wide apertures and relatively short spires to shells with relatively narrow aperture widths and relatively long spires. RW axis 2 was also significantly and primarily related to snail shape, including spire length ratios, aperture-length to shell-length ratio, and spire angle (Table 1). We interpret RW axis 2 as a change in snail shell shape from shells with relatively long apertures and spires to more compact shells (i.e. those with relatively short aperture and spire lengths). In addition, spire angle decreased with increasing values on both RW axes.

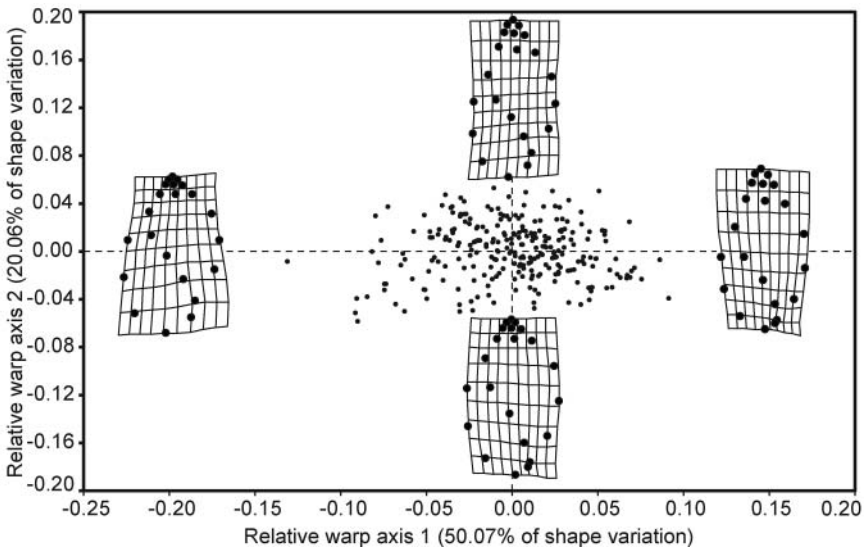

Fig. 2. Shape variation of all *Physa acuta* snails (i.e. wild and laboratory-reared) from HP/LF and LP/HF. Overlaid thin-plate spline deformation grids are models of snail phenotypes on the extreme ends of the relative warp axes. Values for the models along the x-axis (y value = 0) are –0.15 and 0.10. Values for the models along the y-axis (x value = 0) are 0.06 and –0.06.

Table 1. Significant ($P < 0.05$) Bonferroni-corrected Pearson's R^2 correlations for shape (RW: relative warp axes accounting for more than 5% of shape variance; DV: divergence vector) and size (centroid) with traditional snail morphological metrics

	Shape					Size
Variable	RW1	RW2	RW3	RW4	DV	Centroid
Shell length (SL)	—	—	—	0.07	—	**0.99**
Shell width (SW)	—	—	—	—	—	**0.97**
Aperture length (AL)	−0.05	—	—	0.06	—	**0.97**
Aperture width (AW)	−0.13	—	—	0.06	−0.07	**0.87**
Spire length (SPL)	—	0.15	—	—	—	**0.75**
Spire angle (SPA)	−0.25	−0.27	—	—	−0.27	−0.11
SW–SL ratio	−0.20	−0.27	−0.25	−0.04	−0.24	—
AL–SL ratio	−0.38	**−0.52**	—	—	−0.32	—
AW–SL ratio	**−0.63**	—	−0.19	—	−0.48	—
SW–AL ratio	—	—	−0.47	—	—	—
AW–SW ratio	−0.40	0.24	—	0.05	−0.24	—
AW–AL ratio	−0.31	0.26	−0.26	—	−0.22	—
SPL–SL	0.44	0.29	—	−0.08	0.26	—
SPL–SW	0.43	0.34	—	—	0.30	—
SPL–AL	0.45	0.37	—	—	0.29	—
SPL–AW	**0.69**	0.07	—	−0.06	0.46	—
SPL–SPA	0.08	0.18	—	—	0.08	**0.65**

Note: R^2 values > 0.5 are in **bold**. Non-significant correlations are not presented (—).

For snails reared in laboratory tanks (i.e. F1 and F2), nested ANOVA results indicate that tank, nested within source, did not have a significant effect on shape ($F_{4,210} = 0.494$, $P = 0.74$). Furthermore, there was no significant effect of tank when nested within source × generation ($F_{9,205} = 1.03$, $P = 0.41$). Thus, tank was dropped from the analysis and snails from the same source and same generation were pooled for further analyses. Size was significantly related to shape (Table 2), indicating the presence of geometric morphometric allometry. Thus, size was included as a covariate in subsequent analyses. MANCOVA results indicate that there were significant shape differences between source populations, generations, and a significant source × generation interaction (Table 2 and Fig. 3).

The interpretation of DV is similar to the interpretation of RW axis 1 where snail shells with negative DV scores had relatively wide apertures, relatively short spires, and wide spire angles; snail shells with high DV scores had relatively narrow apertures, long spires, and narrow spire angles (Table 1 and Fig. 4). However, compared with RW axis 1, DV had lower correlation coefficients with specific variables as a result of being a measure over full shape space (i.e. over all RW axes). ANCOVA results for DV were congruent with our MANCOVA results for RW axes (Table 2). However, the DV analysis made multiple comparisons readily apparent. Results of the ANCOVA indicated that wild (F0) stream (LP/HF) and pond (HP/LF) snails had significantly ($P < 0.001$) different shapes (Figs. 3 and 4). However, there were no significant shape differences between source populations within the F1 generation ($P = 0.65$) or the F2 generation ($P = 1.0$) (Figs. 3 and 4A). In a multiple-comparison of means, the LP/HF F0 generation of snails was the only group to be

Table 2. Results of MANCOVA examining shape variation (relative warps) and ANCOVA examining shape variation described by the divergence vector

Predictor variable	MANCOVA				ANCOVA		
	Wilks' λ	F	d.f.	P	F	d.f.	P
Centroid (size)	0.61	3	40, 210	<0.001	4.51	1, 250	0.035
Population	0.58	4	40, 210	<0.001	17.78	1, 250	<0.001
Generation	0.33	4	80, 420	<0.001	21.9	2, 250	<0.001
Population × Generation	0.3	4	80, 420	<0.001	13.35	2, 250	<0.001

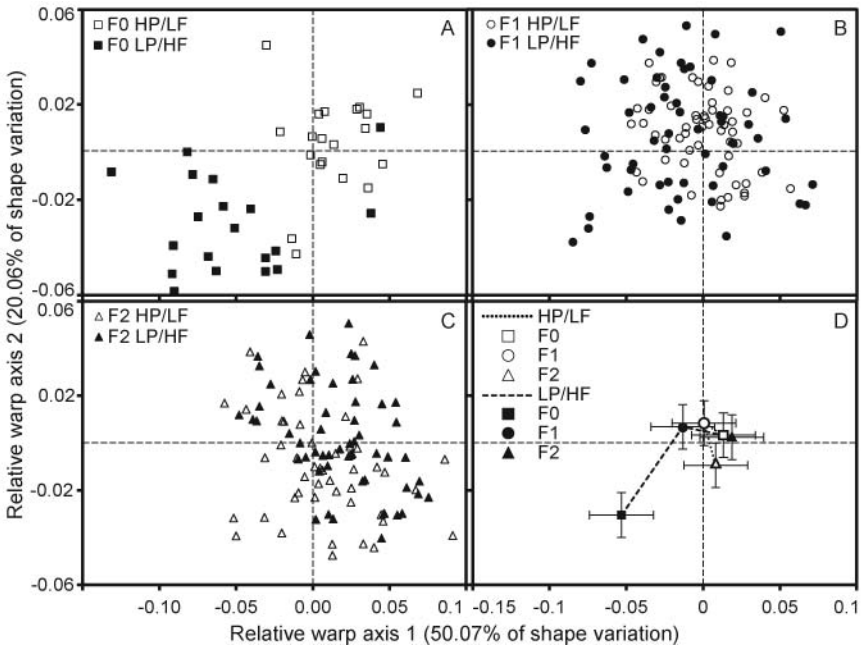

Fig. 3. Shape variation of (A) wild (F0) *Physa acuta* snails, (B) first-generation (F1) laboratory-reared snails, and (C) second-generation (F2) laboratory-reared snails from HP/LF and LP/HF. (D) Combined data with population centroids (± 95% confidence intervals) display generational shape changes for each source population.

significantly different from all the other groups (Fig. 4A) and was the only group with a centroid and 95% confidence intervals that did not cross the intercept of either major RW axis (Fig. 3D). After a single generation, the shape from both populations converged (Figs. 3B and 4A). Finally, the shape of both F2 generation snails converged in the opposite direction of the LP/HF F0 snails (Fig. 4A). The shell shapes of F2 generation snails from both populations were nearly identical to and not significantly different from HP/LF F0 snails (Figs. 3D and 4A). In addition, HP/LF snails (F0, F1, and F2) did not significantly change shape throughout the experiment (Fig. 4A). DV scores ranged from −13.14 to 8.73 and a gradient in shell shape (from snails with wide apertures, short spires, and wide spire

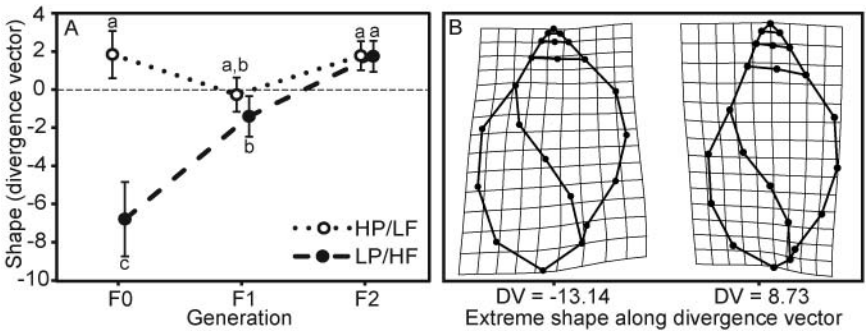

Fig. 4. Shape [divergence vector (DV)] convergence (A) of *Physa acuta* snails from HP/LF and LP/HF over full shape space. Generation F0: wild snails; F1: first-generation laboratory-reared snails; F2: second-generation laboratory-reared snails. Means with 95% confidence intervals are presented (letters indicate significantly homogenous groups based on Bonferroni *post-hoc* tests). Thin-plate spline deformation grids (B) represent extreme shape variation along the DV (i.e. y-axis of Fig. 4A) over the observed range. Lines between landmarks are displayed for visual purposes only.

angles to snails with narrow apertures, tall spires, and small spire angles) could be visualized over the observed range (Fig. 4B).

Size (centroid) showed similar patterns to shape where size was significantly related to generation ($F_{2,250} = 192.43$, $P < 0.001$), population source ($F_{1,250} = 159.69$, $P < 0.001$), and a generation × source interaction ($F_{2,250} = 54.24$, $P < 0.001$). All statistical relationships remained significant when the wild-caught F0 generation snails (of unknown age) were removed from the analysis. Overall, wild HP/LF snails were much larger than wild LP/HF snails (mean shell length ± S.D.: HP/LF F0, 10.38 ± 1.47 mm; LP/HF F0, 6.96 ± 1.17 mm). Within each population, size significantly decreased in the first generation (HP/LF F1: 7.64 ± 0.95 mm; LP/HF F1: 6.17 ± 0.72 mm) until the sizes of snails from both populations converged in the F2 generation (HP/LF F2: 5.46 ± 0.81 mm; LP/HF F2: 5.53 ± 0.75 mm) (Fig. 5). Centroid was significantly and strongly correlated to length and width variables (Table 1), all of which showed the same patterns as centroid (data not shown). Centroid was also negatively correlated to spire angle, suggesting larger individuals had smaller spire angles as indicated by the strong relationship with the spire-length to spire-angle ratio (Table 1).

DISCUSSION

Our work indicates that although offspring of wild snails from both populations became smaller, they both converged on a single phenotype. In addition to the molecular and penial data, the complete convergence of both size and shape in these two populations suggests that these two morphotypes are indeed the same species, *Physa acuta*, and the phenotypic differences are plastic responses to environmental variation. We suggest that this pattern may be common in freshwater snails and our study supports the overestimation of physid species based on shell characteristics alone (Wethington and Lydeard, 2007).

The most notable result of shape, which rapidly converged, is that both populations converged on the wild pond (HP/LF) morphotype. Although offspring of snails from the pond (HP/LF) dramatically decreased in size compared with offspring of snails from

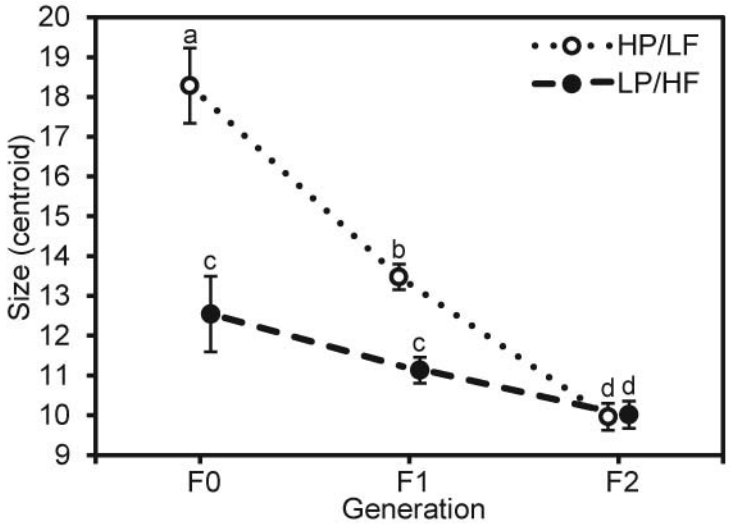

Fig. 5. Size (centroid) convergence of *Physa acuta* snails from HP/LF and LP/HF through multiple generations. Generation F0: wild snails; F1: first-generation laboratory-reared snails; F2: second-generation laboratory-reared snails. Means with 95% confidence intervals are presented (letters indicate significantly homogenous groups based on Bonferroni *post-hoc* tests).

the stream (LP/HF) habitat, it was the offspring of stream snails (LP/HF) that changed shape and ultimately converged with the shape of F0, F1, and F2 pond (HP/LF) snails. Several studies have demonstrated a plastic response of snail shell morphology to predation (Palmer, 1979; Trussell, 1996; DeWitt *et al.*, 2000; Langerhans and DeWitt, 2002; Salice and Plautz, 2011). For these two *P. acuta* populations specifically (elongate shells with narrow apertures vs. short-spired shells with wide apertures), Stevison (2013) hypothesized that the unique site-specific morphologies were a response to the high density of predatory crayfish at the Teal Ridge pond (HP/LF) and the low density of predatory crayfish at Stillwater Creek (LP/HF). This hypothesis predicts that the lack of predators in the laboratory would release any energetically costly predator-induced morphologies, resulting in a shell shape shift towards the stream (LP/HF) morphotype (short-spired shell with wide aperture) or resulting in a novel morphotype. This was not the case and, although there are many potentially different environmental characteristics between ponds and streams, these results are congruent with the hypothesis that water current is a major factor affecting molluscan shape. The water current in Stillwater Creek is relatively high compared with the standing water in the Teal Ridge pond, and as an induced plastic response, foot size and aperture width may increase in stream (LP/HF) snails to resist dislodgement (Kitching *et al.*, 1966; Etter, 1988; Trussell *et al.*, 1993). It is also possible that a predator-defended phenotype was retained throughout generations in the pond (HP/LF) snails, even in the absence of predatory cues. Similarly, in the absence of water flow, the offspring of stream (LP/HF) snails could have converted to a 'default' predator-defended phenotype. In other words, predation could be the predominant ultimate cause of shell morphology, whereas water current could simply be a proximate cause. However, the shape of the wild pond snails (HP/LF) may not have been a specific response to predation at all, and the resultant shape of wild pond snails (HP/LF) and laboratory

snails may simply be a 'default' developmental morphotype of *P. acuta* snails in standing-water environments.

Whereas shape appears to be plastic and converged on the pond morphotype (HP/LF) after a single generation, snail size did not converge until the F2 generation. We hypothesize that the reduction in snail size was not a response to selection or any specific genetic differences between populations and generations but a result of early maturation. For example, Clampitt (1970) observed dramatic size reductions and early maturation in two *Physa* species under laboratory conditions where snails tended to mature and reproduce well within 2 months. Similarly, Wethington and Dillon (1993) observed laboratory-reared *Physa* snails reproducing around 6–8 weeks after hatching. DeWitt (1955) suggested the lack of fluctuating environmental variables (i.e. temperature, water level, parasites, dissolved oxygen, and food) allows laboratory snails the opportunity to successfully develop and reproduce, whereas wild snails would be less likely to experience these conditions. Thus, maturation at a smaller size may simply be a result of optimal habitat conditions and the allotment of resources from somatic growth to reproduction. Although both populations became smaller, there was a single generation lag in size convergence that was not observed for shape. This single generation lag in size convergence may be a maternal effect or some other epigenetic difference between generations. An obvious difference between the early environments of the F1 and F2 generation snails was the egg sac itself. The F1 snails were reared from wild snails that produced eggs sacs that could potentially contain hormonal or nutritional differences depending on the nutritional, environmental, or developmental state of the functional mother. The other major difference between generations was the age and maturity of the bacterial community in each tank. The different sizes of snails between generations may be a result of F2 generation snails being exposed to an older and more stable microbe fauna; however, we could only speculate as to the mechanism for this possible relationship because it has not been studied.

In conclusion, we set out to assess whether the morphological variation between these two geographically adjacent but distinct morphotypes was a result of phenotypic plasticity or genetics. The pond (HP/LF) and stream (LP/HF) snails are a single species with a large amount of phenotypic plasticity in shell morphology. These snails converged with the shape of pond (HP/LF) snails after only a single generation. Although snails from both populations became smaller, there was a single generation lag in size convergence, suggesting either a maternal or other epigenetic difference between generations, but not a classical genetic difference between populations. Although we cannot rule out that other environmental factors were involved in the shell shapes expressed in our laboratory-reared snails, our results suggest that water current (or lack of current and/or associated correlates) has a large impact on snail shell morphology.

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