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Trophic niche drives the evolution of craniofacial shape in Trinidadian guppies

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Abstract

Diverse clades of fishes adapted to feeding on the benthos repeatedly converge on steep craniofacial profiles and shorter, wider heads. But in an incipient radiation, to what extent is this morphological evolution measurable and can we distinguish the relative genetic vs. plastic effects? We use the Trinidadian guppy (*Poecilia reticulata*) to test the repeatability of adaptation and the alignment of genetic and environmental effects shaping poecilid craniofacial morphology. We compare wild-caught and common garden lab-reared fish to quantify the genetic and plastic components of craniofacial morphology across 4 populations from 2 river drainage systems (*n* = 56 total). We first use micro-computed tomography to capture 3D morphology, then place both landmarks and semilandmarks to perform size-corrected 3D morphometrics and quantify shape space. We find a measurable, significant, and repeatable divergence in craniofacial shape between high-predation invertivore and low-predation detritivore populations. As predicted from previous examples of piscine adaptive trophic divergence, we find increases in head slope and craniofacial compression among the benthic detritivore foragers. Furthermore, the effects of environmental plasticity among benthic detritivores produce exaggerated craniofacial morphological change along a parallel axis to genetic morphological adaptation from invertivore ancestors. Overall, many of the major patterns of benthic-limnetic craniofacial evolution appear convergent among disparate groups of teleost fishes.

Keywords: convergence, craniofacial evolution, benthic, guppy, local adaptation, morphometrics

Introduction

Convergence, or the repeated evolution of similar phenotypes, highlights the power of natural selection to produce similar independent solutions in the context of parallel environmental problems (Albertson et al., 2003a; Blount et al., 2018; Cooper et al., 2010; Gould, 1990; McGee et al., 2013). This similarity in phenotype can occur due to genetically encoded developmental causes, or in some cases may simply reflect a tissue-level response to mechanical demands placed on the system. Such cases of environmentally regulated phenotypic plasticity are well documented (Cooper et al., 2010; Day & McPhail, 1996; Robinson & Parsons, 2002) and may act as a precursor to genetic evolution (Adams & Huntingford, 2004; Conith et al., 2020; Wund et al., 2008).

A classic idea in evolutionary biology, the Baldwin Effect, argues that plasticity determines the axis along which a population is most likely to evolve (Baldwin, 1902; Crispo, 2007; West-Eberhard, 2003). At the plastic level, it has been well-established that organisms are capable of producing phenotypic responses to their immediate environment at the behavioral, physiological, and anatomical levels that are non-hereditary but proximately beneficial (Simpson, 1953). For example, in response to variation in substrate type, the craniofacial apparatus of a winnowing cichlid remodels to match each novel environment (Gilbert et al., 2023). Although the Baldwin Effect would predict that such plastic

responses become genetically encoded, few studies examine the axes along which plastic and genetic components of adaptive processes occur. Here we use a model system for the study of adaptive evolution, the Trinidadian guppy (*Poecilia reticulata*), to quantify the relative contribution and direction of genetics and plasticity in population-level trophic convergence of craniofacial morphology.

Fishes frequently converge in craniofacial morphology when adapting to herbivorous benthic feeding from open-water (limnetic/pelagic) forms of resource acquisition (Albertson & Kocher, 2001; Kocher, 2004; Malmquist, 1992; Schluter, 1995). This evolutionary axis has been thoroughly studied in the trophic morphology of cichlids and this work has informed many of our expectations about the associated biomechanical optima in fish (Albertson et al., 2003a, 2003a; Cooper et al., 2010; McGee et al., 2013). Many cichlid species have evolved from a limnetic generalist ancestor into an herbivorous benthic specialist through modifications to individual bony elements as well as changes to overall head shape and jaw orientation. Specifically, herbivorous fish tend to assume more downturned, wider mouths as well as shorter heads through this niche shift while limnetic cichlids tend to have terminal mouths in conjunction with long narrow heads (Albertson & Kocher, 2001; Cooper et al., 2011; Powder et al., 2015). This adaptive morphological trend to shorter, wider, and more down-turned mouths among benthic or
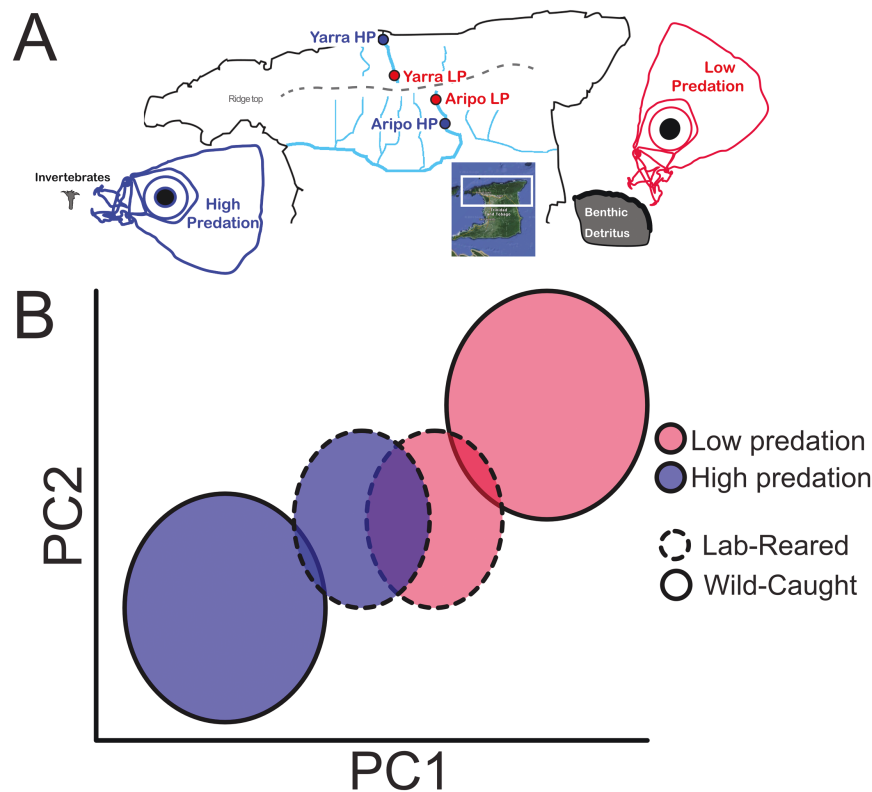
Figure 1. Ecology and expected craniofacial morphospace of high- and low-predation guppies adapted to alternate diet types from the Aripo and Yarra rivers in Trinidad. (A) A map of the Northern Range mountains of Trinidad showing the different drainages from which fish were collected as well as the expected ecology of different populations. Red represents low-predation (LP) populations that feed primarily on detritus encrusting the river bottom; blue represents high-predation (HP) populations that feed mainly on free-floating invertebrates. (B) Hypothesized craniofacial morphospace of the study populations. We predict that the primary axis of divergence will separate out low- and high-predation fish, with wild-caught fish being more diverged from each other than lab-reared fish. Part A shows that different river systems in northern Trinidad posess the same ecological divergence where upstream fish eat benthic detritus and downstream fish eat invertibrates. Part B shows a hypothesized principal component analysis where feeding ecology separates individuals on the x-axis and rearing conditions separate them on both the x-axis and y-axis.

substrate-scrapers converges across different old-world cich-lids inhabiting the Rift lakes of eastern Africa, new-world cich-lids, white fish, arctic char, and threespine stickleback (Adams et al., 1998; Ahi et al., 2014; B. Robinson, 2000; Fraser et al., 1998; Kristjánsson et al., 2002b, 2002a; Malmquist, 1992; Østbye et al., 2005; Schluter, 1995; Todd et al., 1981; Walker & Bell, 2000; Willacker et al., 2010). Functional differences often accompany this morphological evolution, with limnetic cichlids outperforming benthic cichlids at suction feeding (Bouton et al., 1998; Matthews & Albertson, 2017; Selz & Seehausen, 2019) and benthic fish more effectively removing epilithic algae from a substrate (Bouton et al., 1998; Selz & Seehausen, 2019). Here we ask to what extent are these mor-phological specializations occurring at the population level and to what extent does genetically based morphological divergence occur along the same axis as plastic effects?

Many authors have used the Trinidadian guppy (*Poecilia reticulata*) to study adaptive life history evolution in response to differential predation pressures (high predation vs. low pre-dation; Endler, 1978; Gilliam et al., 1993; Haskins et al., 1961; Houde & Endler, 1990; Reznick, 1982; Reznick et al., 1996; Seghers, 1973, 1974), but this system is also well-suited to address the question of convergence in craniofacial adaptation across environments given the diet specializations that occur among populations (Gibb et al., 2015; Zandonà et al., 2011). In Trinidad, this live-bearing fish occupies the riparian system

flowing from the Northern Range mountains at the large island's north end (Figure 1A). In mouths and large tributaries of the mainstem waterways, the fish is exposed to high-predation pressures from piscivorous heterospecifics, which limits guppy population size and exerts strong selection for faster life histories, cryptic coloration, and antipredator behaviors (Magurran, 2005). In response to relaxed predation selection and increased intraspecific competition within the headwaters, the guppies evolve slower life histories, brighter male color-ation, and more effective foraging strategies (Bashey, 2008; Kemp et al., 2018; Reznick et al., 1990). This happens both in response to relaxed selection from predators and from an accompanying increase in competition for limited resources within populations that have reached their carrying capacities (Bassar et al., 2013; Grether et al., 2001; Reznick et al., 2001). Concomitant with the infiltration and adaptation to low-predation environments is also observed a dietary shift from a largely invertebrate food source (high nitrogen to carbon ratio) in high-predation populations to one that is over 90% detritus and algae (low N:C) in low-predation populations (Bassar et al., 2010; Zandonà et al., 2011). Therefore, this system offers an opportunity to address the extent to which guppies have adapted to two highly divergent food acquisition strategies; high-predation guppies to the rapid motions required to cap-ture free-swimming prey; and low-predation guppies to the mechanical demands of scraping detritus off the substrate.

Trinidadian guppies also offer a highly tractable system for studying the extent to which early morphological specialization is driven by both environmental plasticity and genetic divergence. To answer this question, it is first necessary to quantify morphological divergence between ecologically distinct populations in situ. Any observed differences will be the product of both the genetic divergence between the populations and environmentally induced plasticity. Since guppies continue to be a popular model system for field studies, there are a number of well-established, ecologically divergent locations that can be sampled to conduct such an analysis. Then, to disentangle the relative contributions of genetics and plasticity the guppies from divergent habitats can then be raised for several generations in identical laboratory conditions to remove the effects of environmental plasticity. Morphological differences among lab-reared individuals reflect only the genetic differences among these populations. Again, this is readily achievable in this study system because guppies are easily raised and propagated in a lab environment.

In this study, we quantify 3D craniofacial morphology of adult female guppies from four populations, specifically low-predation (benthic detritivore) and high-predation (invertivore) populations within each of two separate drainage systems corresponding to the Aripo and Yarra rivers. Using individuals from both lab-reared, common garden groups (genetic determinants of morphology) and wild-caught groups (plastic and genetic determinants of morphology), we landmark homologous craniofacial points, correct for the effects of size, and perform 3D geometric morphometrics to specifically address shape differences between populations and the relative contributions of genetics and plasticity to this variation. We then consider the population's evolutionary trajectories through morphospace to investigate whether populations evolve in parallel or if they converge toward a common morphology. Finally, we examine the extent to which the skulls of benthic-feeding guppies in low-predation environments converge on morphologies observed in other fishes that occupy similar niches.

We make two sets of predictions, the first regarding the morphological divergence of craniofacial shape among populations and the second on the relative influence of genetic and plastic effects on these traits. First, we predict that the specimens will occupy distinct portions of morphospace from one another based on their native trophic ecology (Figure 1B, red vs. blue), and that these differences will repeat across the two drainages. Specifically, we predict that low-predation individuals possess wider, shorter craniofacial apparatuses and more downturned mouths, as has been observed in other species adapted to feed off of the benthos (Albertson & Kocher, 2001; Cooper et al., 2010, 2011). Secondly, if guppy populations have indeed adapted craniofacially, we predict that phenotypic plasticity in response to divergent trophic ecologies will enhance genetic divergence (measured in lab-reared fish) to drive these already divergent morphologies farther from the mean phenotype among wild-caught fish (Figure 1B, dashed vs solid lines).

Materials and methods

Collection and animal husbandry

We collected female and male guppies, *Poecilia reticulata*, from four distinct populations in the Northern Range mountains on the island of Trinidad in spring 2013 and spring 2014. Guppies were collected from two independent drainages: Aripo river (southern slope) and Yarra river (northern slope) (Figure 1A); these watersheds are completely isolated from one another, with no admixture occurring over the mountain divide (but some can occur within each drainage).

The specific populations collected were from benthic detritivore, or low predation, and invertivore, or high predation, sites within the Aripo and Yarra drainages (four populations total). Twenty females and five males were collected at each of the four sites ($n = 100$ total); the fish were transported in bottles filled halfway with freshstream water and treated with 2 mg tetracycline (1 mg/L) and StressCoat (according to manufacturer directions) to minimize infection during transportation. The fish were housed in an open-air field laboratory in Verdant Vale, Arima, Trinidad, within 2-L tanks each equipped with air stone at ($\sim$18 $\pm$ 3 °C) temperatures and exposed to ambient light, which maintained a light:dark schedule at approximately 12:12 hr. Fish were fed twice daily on algae flakes (TetraMin Tropical Flakes) in rations that amounted to 2 min of feeding time per meal (Arendt & Reznick, 2005; Reznick, 1982). A total of 30% water changes were performed weekly throughout the husbandry period.

We randomly selected 10 female guppies from each population to represent the "wild-caught" phenotype and euthanized them as quickly as possible after capture to minimize plastic response to laboratory settings. We did not record the exact duration that we had the fish in captivity, but it was often less than a week and never more than 30 days. These fish were euthanized via overdose of buffered 1 g/L tricaine methanesulfonate (Tricaine-S, Western Chemical Inc., Ferndale, WA, USA), fixed for 24 hr in 4% paraformaldehyde and preserved in 70% ethanol at –20 °C.

A subset of live female ($n = 10$) and male ($n = 5$) guppies from each of the four populations ($n = 60$ total) were exported from Trinidad with a permit issued by the Fisheries Division of the Ministry of Agriculture, Land and Fisheries, Republic of Trinidad and Tobago, and imported into the United States with a valid U.S. Fish and Wildlife Service Declaration of Importation permit (USFWS Form 3-177). These fish were bred to produce the "lab-reared" treatment of the study.

Lab-reared fish were housed in community aquaria, which are 38-L tanks with open stocks of fish. Population density was regulated to reduce overcrowding. Open stock temperature was maintained at 25–26 °C and fish were fed newly hatched Artemia, Repashy Community Crave (a gel that is prepared in small amounts every few days), and occasional Omega one flakes, Daro worms and Daphnia, which were cultured in the laboratory. This mixed diet was used to minimize strong directional plasticity. It should be noted that we did not conduct a reciprocal feeding experiment; fish from all four represented populations were fed the same food throughout their life in the lab, which provides a common garden environment from which to compare the genetic basis of craniofacial traits. Lab-reared fish were housed for a minimum of two generations to remove any epigenetic effects. We selected 5–10 individual lab-reared female guppies from each of the four populations. These lab-reared fish were euthanized, fixed, and preserved following the above procedure.

All research reported here followed strict ethical guidelines and complied with the US federal government's regulations. Procedures were approved by the Institutional Animal Care and Use Committee at Brown University (protocol: 1211035 to E. L. Brainerd), Harvard University (protocol: 20-03-2
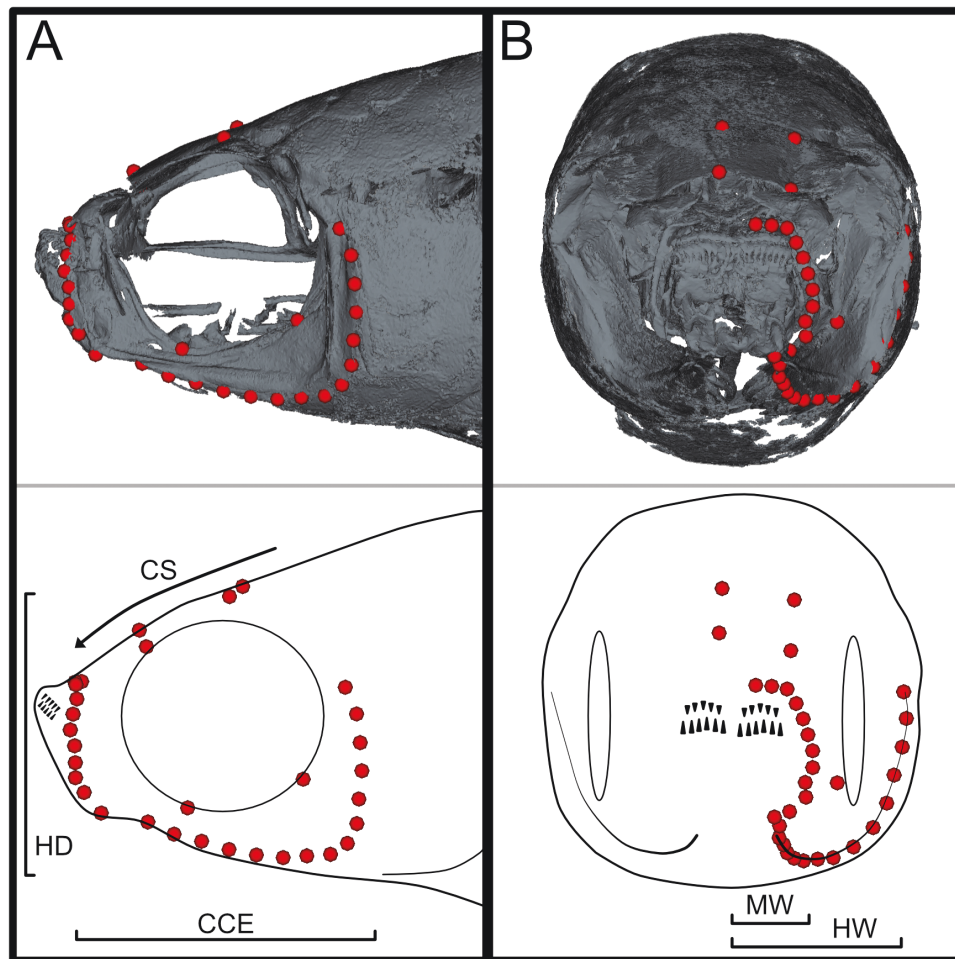
Figure 2. We placed a total of 11 landmarks and 21 semilandmarks across the left or right side of each skull to represent craniofacial shape, including craniofacial compression and expansion (CCE), head depth (HD), head width (HW), mouth width (MW), and craniofacial slope (CS). Points are displayed from a lateral (A) and anterior (B) perspective both with and without the 3D model present. Semilandmarks include eight points along the anterior margin of the right and left premaxilla as well as 13 points along the ventral margin of the right and left preopercular lateral line canal. A full list of landmark positions is given in Supplementary Figure S1. Side and front views of a CT scan of a guppy showing the landmark positions used in this study.

to G. V. Lauder), and University of California Riverside (#:20200003 to D. N. Reznick).

CT scanning and segmentation

We scanned all specimens using a micro-computed tomography (CT) scanner (Bruker SkyScan 1173, Kontich, Belgium). To scan a group of fish, we first wrapped one to seven individuals in Kimwipes and placed them within 5-ml transport tubes with a small amount of ethanol to keep the specimens moist and stabilized within the tube. We μ-CT scanned each tube using 0.5° increments over 180° with the x-ray source set at 38 kV and 190 μA. This resulted in image stacks with an isotropic resolution between 6.8 and 9.0 μm that were then reconstructed using NRecon (Bruker, Kontich, Belgium). We used Mimics (v. 22.0; Materialise NV, Leuven, Belgium) to segment individuals from each scan and isolate the prepectoral skeleton, then exported scans as 3D models for morphometric analysis. We removed any scans where the heads showed obvious distortion such as a lateral skew of the craniofacial morphology. Finally, we used MeshLab (v. 2020.07; Cignoni et al., 2008) to simplify our meshes (Quadratic Edge Collapse Decimation filter) to reduce necessary computing power for visualization. We used Python to batch process the meshes and reduced each scan to 1×10^6 faces.

3D geometric morphometric analysis

We placed 11 fixed landmarks and 21 sliding semilandmarks across the craniofacial apparatus to capture its three-dimensional shape (Figure 2; Supplementary Figure S1). We use these landmarks to capture elongation and compression across all three spatial axes, as well as the craniofacial slope, orientation of the mouth (including the articulation of the lower jaw and the quadrate), and shape of the suspensorium. While many past studies have included landmarks on the oral jaws, we exclude these points due to confounding variation induced by inconsistent positioning during fixation. However, each of the traits that we measure has been used to infer functional differences between populations or species of fish that have diverged along a trophic axis (Albertson et al., 2003b, 2005; Cooper et al., 2010, 2011). We restricted landmarks to the left side of the head and the midline to avoid capturing asymmetries within individuals. In cases where the left side of the head was damaged, we instead landmarked the right side and reflected the points about the × axis to correct the chirality. We collected sliding semilandmarks by first placing an arbitrary number of points along either the maxilla or preopercle to fully capture the curvature of the structure, then resampling these curves to a fixed number of equally spaced semilandmarks (8 on the maxilla, 13 on the preopercle)

bounded by two fixed landmarks on each structure. All land-marking was done in 3D Slicer (v. 4.11; Fedorov et al., 2012).

We created three datasets, one with all the data points, one with just the lab-reared fish, and one with just the wild-caught fish. Using Geomorph (v. 4.0.0; D. Adams et al., 2021), we conducted a geometric morphometric analysis on each data-set. First, we subjected each coordinate dataset to a generalized Procrustes analysis using bending energy. This method com-pares the shapes of each individual after accounting for size, rotation, and position. Next, we regressed the shape variable on log-transformed geometric centroid size for each of our data-sets. In all cases, there was a significant allometric effect, so we took the residuals of these regressions to attain an allometry-free measure of shape (Outomuro & Johansson, 2017). For each dataset, we then used the function procD.lm to run mul-tivariate regressions of allometry-corrected shape on feeding niche, drainage, and the interaction effect between the two. For the dataset containing both lab-reared and wild-caught fish, we also included the rearing condition as an explanatory variable. To visualize the distribution of each dataset in shape space, the shape data were subjected to a principal component analysis (PCA) using the gm.prcomp function and plotted in the morphospace defined by the first two principal compo-nent axes. Finally, we subjected the lab-reared and wild-caught datasets to a trajectory analysis (RRPP, V1.3.1)(Collyer & Adams, 2022) to determine whether the evolved path through morphospace from high-predation to low-predation popula-tions differed between river drainage systems. We plotted pop-ulation mean shapes and the linear trajectories between them to visualize their evolutionary paths.

The regression and PCA analyses on the full dataset revealed fish differed based on feeding niche and drainage, but that the rearing condition primarily affected the magni-tude of the divergence and not the direction of the divergence in shape space. Therefore, we only visualized the shape differ-ences between benthic detritivore and invertivore individuals in the wild-caught individuals since these further diverged than were the lab-reared fish. We did this by plotting the size-corrected and allometry-corrected mean position of each landmark for benthic detritivore and invertivore fish sepa-rately for each drainage. We placed lines between landmarks on the same bone and filled in the area between homologous lines to aid in visualization. All geometric morphometric anal-yses were run using Geomorph (v. 4.0.0) (Adams et al., 2021) in RStudio (v.1.3.1056).

Although these landmarks inform craniofacial elongation among these populations, they do not allow us to measure head length since this is typically measured from the oral jaws to the caudal end of the neurocranium. Instead, we infer head length based on the distance along the anterior-posterior axis from the dorsal-most point on the posterior end of the preopercle (Supplementary Figure S2) to the dorsal tip of the maxilla. To compare our results using this nontraditional metric to past studies on head length we additionally mea-sured the distance from the dorsal tip of the maxilla to the posteroventral margin of the basioccipital. We ran a linear regression between these two measurements to check if our metric of craniofacial elongation was positively correlated with head length. Similarly, we compared our measurement of head width, the distance from the widest point of the preoper-cle to the midline plane, to a more common measurement of width, the distance from the lateral-most point on the opercu-lum (Supplementary Figure 2) to the midline plane of the fish.

Results

Allometry

All three geometric morphometric datasets showed signifi-cant allometric scaling effects. Logarithmically transformed geometric centroid size explained 23.1% of the variation in shape across all females, lab-reared and wild-caught ($p < .001$). Within the dataset containing only lab-reared females, we found that size explained 32.1% of shape dif-ferences ($p < .001$). Among only the wild-caught females we found that size explained 12.8% of variation in shape ($p < .001$). Accordingly, all three datasets were corrected for allometric effects and all further results are obtained using these allometry-free data.

All females

Consistent with our hypotheses, we find that guppy cra-niofacial morphology repeatedly differs between niche (i.e., low-predation or benthic detritivore vs. high-predation or invertivore) ($R^2 = 0.066$, $p < .001$), and that environmental effects magnify the observed results, presumably through phenotypic plasticity. Among wild-caught guppies (Figure 3; triangles, solid lines) low- and high-predation populations separate in craniofacial morphology along a combination of PC1 and PC2, with low-predation individuals occupying the extreme lower left shape space and high-predation individu-als occupying the extreme upper right. Lab-reared fish (Figure 3; rings, dashed lines) also differ by ancestral niche, but not as much as in wild-caught fish. We also observed differences in the relative distribution of lab-reared and wild-caught individuals between low- and high-predation populations. Specifically, only fish from low-predation populations showed a greater divergence among wild-caught individuals com-pared to lab-reared fish (Figure 3, red circles vs red triangles). While high-predation populations did show more variation among wild-caught fish than lab-reared ones, most of this additional variation was perpendicular to the axis that sep-arates low-predation from high-predation populations. We also find a significant effect of drainage ($R^2 = 0.072$, $p < .001$), with fish collected in the Aripo river being distinct from those collected in the Yarra river (Table 1). Finally, we found no sig-nificant indication that the drainage affected the way in which fish from different niche spaces diverged ($p = .17$) (Table 1).

Lab-reared

The extent to which craniofacial patterning is genetically encoded can be observed among common garden lab-reared guppies (Figure 4). We find that shape differences in lab-reared guppies occur between niche type (low predation vs. high predation) ($R^2 = 0.072$, $p = .037$) and between drainage (Aripo vs. Yarra) ($R^2 = 0.084$, $p = .026$). Lab-reared Aripo fish separate modestly between niche types, where high-predation individuals are generally higher than low-predation ones on PC2. More strikingly, lab-reared Yarra females show a signif-icant genetic basis of craniofacial patterning when reared in a common garden environment. In particular, high-predation fish are generally higher on both PC1 and PC2 than low-predation fish from the Yarra river. The interaction between niche and drainage reflects a difference in the direction of the effect (Table 1; $R^2 = 0.065$, $p = .057$), also evidenced by a sig-nificant trajectory correlation (Table 2; 91.8°, $p = .004$), indi-cating that the difference between low- and high-predation fish depends on drainage. Together, these results indicate a
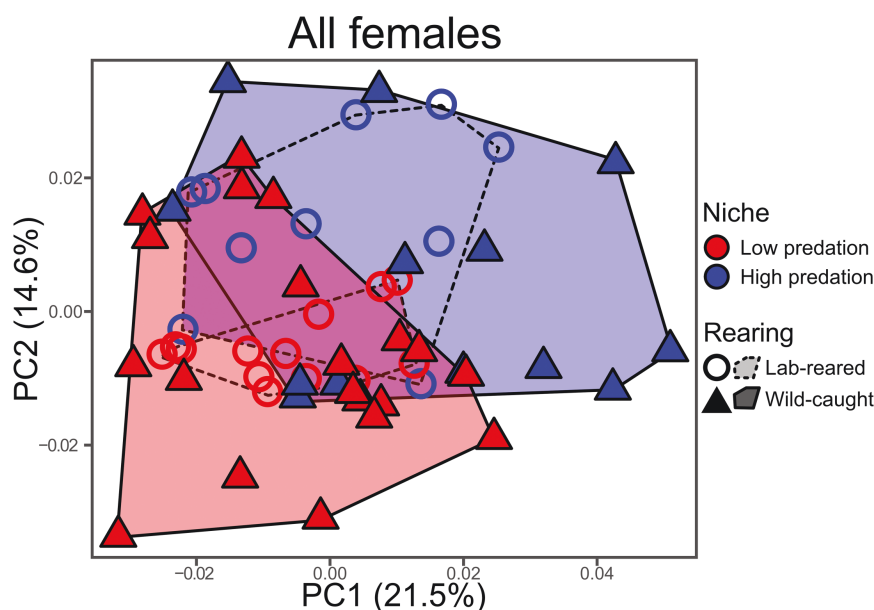
Figure 3. There is a significant divergence in morphospace between high- and low-predation fish that is captured by both PC1 and PC2 among all of the fish used in this study. Additionally, the wild-caught fish occupy a much broader swath of morphospace than lab-reared fish do. A principal component analysis plot shows that there are shape differences among all the fish used in this study that are best explained by differences in their trophic niche.

Table 1. Statistical results of size corrected linear models explaining craniofacial shape across different subsets of data.

	R^2	F-score	p value
All females ($n = 56$)			
Rearing condition	—	—	.16
Drainage	0.072	4.49	< .001
Niche	0.066	4.12	< .001
Drainage*Niche	—	—	.17
Lab-reared ($n = 23$)			
Drainage	0.084	2.05	.026
Niche	0.072	1.77	.037
Drainage*Niche	0.065	1.59	.057
Wild-caught ($n = 33$)			
Drainage	0.148	5.94	< .001
Niche	0.087	3.50	.003
Drainage*Niche	0.044	1.78	.040

genetic basis for niche-based craniofacial morphological divergence.

Wild-caught

Among wild-caught fish, both niche ($R^2 = 0.087$, $p = .003$) and drainage ($R^2 = 0.148$, $p < .001$) significantly explain variation in craniofacial shape (Figure 5). High-predation wild-caught fish for both Aripo and Yarra drainages show higher PC1 scores and lower PC2 scores compared to their low-predation counterparts. In addition, Aripo fish are higher on both PC1 and PC2 than Yarra fish from the equivalent niche. Although the trajectory analysis suggests that the evolutionary paths are not parallel to one another (Table 2; 71.3°, $p = .007$), the trajectories represented only in PC1 and PC2 appear to be parallel (Figure 5). This significant angle between trajectories is likely due to variation in other PC axes since shape space is comprised of many more planes than the one visualized

here. However, given that none of the other PC axes explain more than 2% of the variation while also being correlated with niche or drainage (Supplementary Table S1), we find that they are evolving in parallel among the ecologically relevant axes in shape space (PC1 and PC2). Therefore, environmental plastic effects alter craniofacial morphology in a repeated and consistent direction, but the ancestral state (high-predation morphotype) depends on the micro-environment and the genetic background of the fish.

The differences between low- and high-predation individuals are seen across the entirety of the skull, with low-predation fish from both the Aripo and Yarra rivers tending to have shorter and deeper craniofacial apparatuses, wider ventrolateral suspensoria, and narrower mouths (Figure 6). Furthermore, low-predation individuals collected from the Aripo and Yarra rivers display caudal retraction of the maxilla, ventral, and medial displacement of the anterior portion of the preopercle, and dorsal shifting of the frontal bone (see Supplementary Figure S1). Among these shared trends we find that changes in the frontal bone and maxilla appear greater in fish from the Yarra river. In addition, we find that Aripo low-predation fish have a more dorsally and medially oriented suborbital skeleton, while Yarra low-predation fish have an elongated and more ventrally oriented suborbital skeleton (Figure 6; landmarks 5 and 6, Supplementary Figure S1). Finally, we find a slight broadening of the posterior portion of the frontal bone among Yarra low-predation individuals.

Head length and width

We found that the distance along the anterior-posterior axis from the preopercle to the maxilla was highly correlated with head length, as measured to the caudal end of the neurocranium ($R^2 = 0.71$, $p < .001$). We therefore consider our measurement of head elongation to be comparable to past measurements of head length. We also found that the maximum width of the preopercle correlated significantly with the maximum width of the operculum ($R^2 = 0.36$, $p < .001$), and
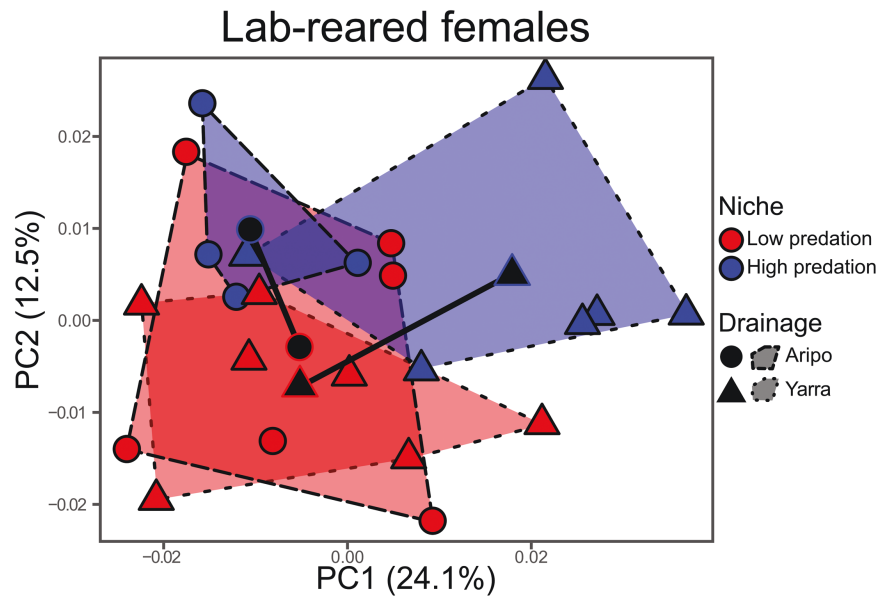
Figure 4. Geometric morphometrics of lab-reared females indicates a genetic basis to craniofacial shape among guppy populations. These results suggest that craniofacial shape has diverged between niche type, including both predation and trophic factors. Additionally, the trajectory of mean shape change within a drainage (black lines) indicate that there is convergence between the drainages as they evolve from high-predation to low-predation environments. This suggests that there is population-level adaptation to the different diet types encountered within high-predation vs. low-predation environments. Black points with colored outlines represent mean population shape. A principal component analysis plot shows that morphology is different between lab-raised fish based on their trophic niche and the drainage that they were collected from.

Table 2. Trajectory analysis reveals that evolutionary trajectories from high-predation to low-predation environments are not parallel between the Aripo and Yarra drainages. However, much of the divergent shape evolution among wild-caught individuals happens along morphological axes that are not correlated with trophic ecology (Supplementary Table S1).

	Angle	p value	Z score
Lab-reared	91.8°	0.004	2.44
Wild-caught	71.3°	0.007	2.45

again considered this to be a reasonable proxy measurement of whole head dimensions.

Discussion

Divergence in morphospace

In this study, we use the Trinidadian guppy, a well-known model for the study of local adaptation of life history traits (Reznick, 1982), to present findings on a measurable, significant, and repeatable divergence in craniofacial shape between high-predation and low-predation populations (Table 1, Figure 3). This finding supports the prediction that these Trinidadian guppy populations diverge in craniofacial morphology because of their divergent diet types wherein low-predation fish attain up to 90% of their diet from benthic sources (detritus and algae) (Zandonà et al., 2011) and are thought to be under strong selection for resource acquisition ability (Reznick & Travis, 2019). In contrast, high-predation populations downstream consume as much as 80% of their diet from invertebrates, a readily available food source in high-predation streams (Zandonà et al., 2011), instead experiencing strong selection from predation (Reznick & Endler, 1982). Interestingly, we found that low-predation guppies display several classically benthic traits, in particular cranial

compression along the anterior-posterior axis and expansion along the dorsoventral axis (Figure 6). The convergence of craniofacial shapes among guppy populations with similar ecologies, as well as with other benthic-limnetic divergences in fishes (Adams et al., 1998; Ahi et al., 2014; Albertson et al., 2003a; B. Robinson, 2000; Fraser et al., 1998; Kristjánsson et al., 2002a, 2002b; Malmquist, 1992; Østbye et al., 2005; Schluter, 1995; Todd et al., 1981; Walker & Bell, 2000; Willacker et al., 2010), indicates that diverse clades of fishes may evolve along a similar morphological axis.

Results from the common-garden experiment show that craniofacial morphology groups by population, implicating a genetic basis to the observed divergence among populations. Trajectory analysis on the lab-reared dataset suggests that the two low-predation populations converge toward a similar craniofacial morphology, despite their high-predation counterparts exhibiting distinct shapes (Table 2, Figure 4). Low-predation lab-reared individuals from both Aripo and Yarra drainages converge on shorter wider craniofacial morphology. This convergence of benthic morphology among populations is particularly notable because the primary selective pressure in the low-predation environment is likely competition for limited resources (Grether et al., 2001). In contrast, guppies from high-predation environments are selectively preying on abundant prey (Bassar et al., 2010), which could correspond to a decreased selection on feeding structures. The pattern of divergence from the high-predation ancestral populations and toward a convergent low-predation morphotype suggests that not only is there a genetic basis to population-level differences in craniofacial morphology, but that selection may be acting to specialize craniofacial shape in low-predation populations. However, it is important to note that our results do not rule out other selective pressures that may accompany the shift from high-predation to low-predation environment, for instance, head morphology may be shaped by selection
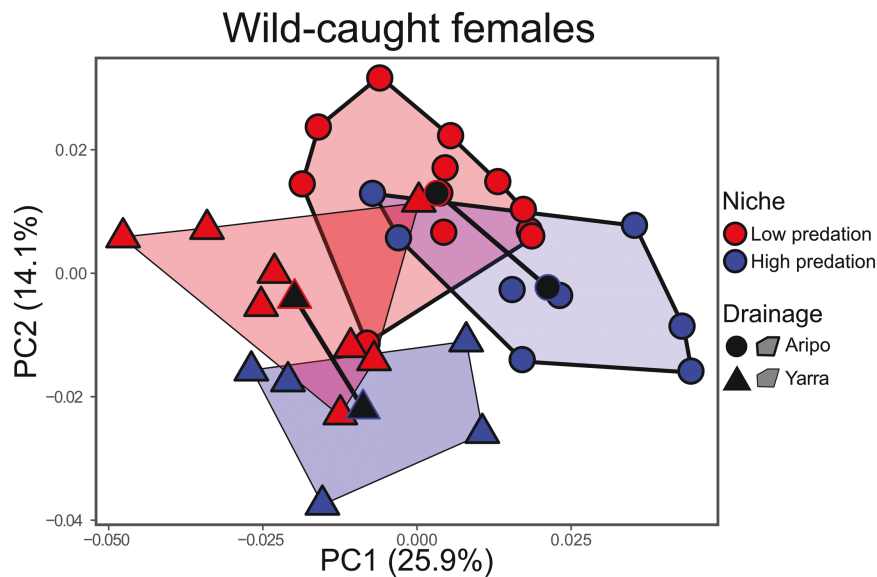
Figure 5. Morphometrics among wild-caught fish indicates a clear and consistent effect of predation environment, and therefore trophic regime, on craniofacial shape. This suggests that in addition to genetic effects, the environment induces divergent craniofacial shapes. Furthermore, the mean shape change trajectory (black lines) is parallel between drainages, suggesting that the plastic effects of the micro-environment alter craniofacial shape morphology. Black points with colored outlines represent mean population shape. A principal component analysis plot shows that morphology is different between wild-caught fish based on their trophic niche and the drainage that they were collected from.

on swimming performance (Ghalambor et al., 2004; Walker et al., 2005). Nevertheless, the observation that both low-predation populations, which are from two independent and unrelated drainages, converge on similar craniofacial profiles implies that low-predation fish are responding similarly to common features of their local environment.

Plasticity

Within the wild-caught individuals, we find little overlap in craniofacial morphospace between any of the sampled populations (Figure 5). This greater distinction between groups in the wild implies that the environment induces additional divergent morphological variation to the craniofacial skeleton beyond the genetic component (Figure 4). Specifically, we find that benthic detritivore low-predation fish tend to be lower on PC1 and higher on PC2 than their invertivore high-predation counterparts (Figure 5). These morphological shifts equate to a posterior retraction of the maxilla and the dorsal displacement of the frontal bone in both low-predation populations (Figure 6). This finding supports our second prediction—that plasticity would drive populations farther apart than those raised in a common garden environment—which suggests that although selection is acting on the genetic basis of craniofacial shape to create a more benthic morphotype, the micro-environment still has a significant role in determining craniofacial shape through phenotypic plasticity.

In the guppy system, the Baldwin effect predicts that as fish move from high-predation (predominantly invertebrate diet) to low-predation (predominantly detritus) environments, genetically based changes to morphology should occur along the same axis as plastic changes. Consistent with this, the results from this study show that wild-caught low-predation populations diverge further along the high-predation–low-predation axis compared to their lab-reared counterparts (Figure 3). In contrast, the additional variation due to plasticity observed in high-predation populations acts perpendicular to the high-predation–low-predation divide.

This implies that although local environmental influences affect all the fish, they only add to the trophic divergence within the derived low-predation individuals. Therefore, our second prediction, that the genetic basis of morphology will occur on the same axis as phenotypic plasticity, is only supported within low-predation populations, which makes sense given that these derived populations are likely under stronger selection for resource acquisition. However, plasticity driving further divergence in a novel population does not by itself prove selection; for this trait to be selected there must be adaptive significance to the axis along which plasticity drives divergence.

Functional implications in the context of diet

Previous studies have shown that low-predation individuals have an advantage in procuring limited benthic resources through differences in competitive abilities (Bashey, 2008) and oral jaw kinematics (Dial et al., 2017). Most of the craniofacial changes that we observe support this functional advantage in low-predation individuals and correspond with classical assumptions about the demands of substrate-based feeding (Albertson & Kocher, 2001; Bouton et al., 1998; Cooper et al., 2011; Wainwright & Richard, 1995). Specifically, we found more downturned, ventrally oriented oral jaws and shorter, wider heads. We did not specifically examine the shape of the oral jaw elements, but we observed a steeper frontal profile in the low-predation populations, consistent with more downturned jaws, which aligns with other benthic divergences that are ecologically similar to the low-predation guppies (Cooper et al., 2011; Hu & Albertson, 2021). A shorter head also aligns with previous work and could be associated with increased bite force (Cooper et al., 2011), though we did not specifically examine muscle insertion moment arms or the mechanics of the oral jaw apparatus.

Interestingly, morphological divergence along the mediolateral axis was generally opposite of these expectations.
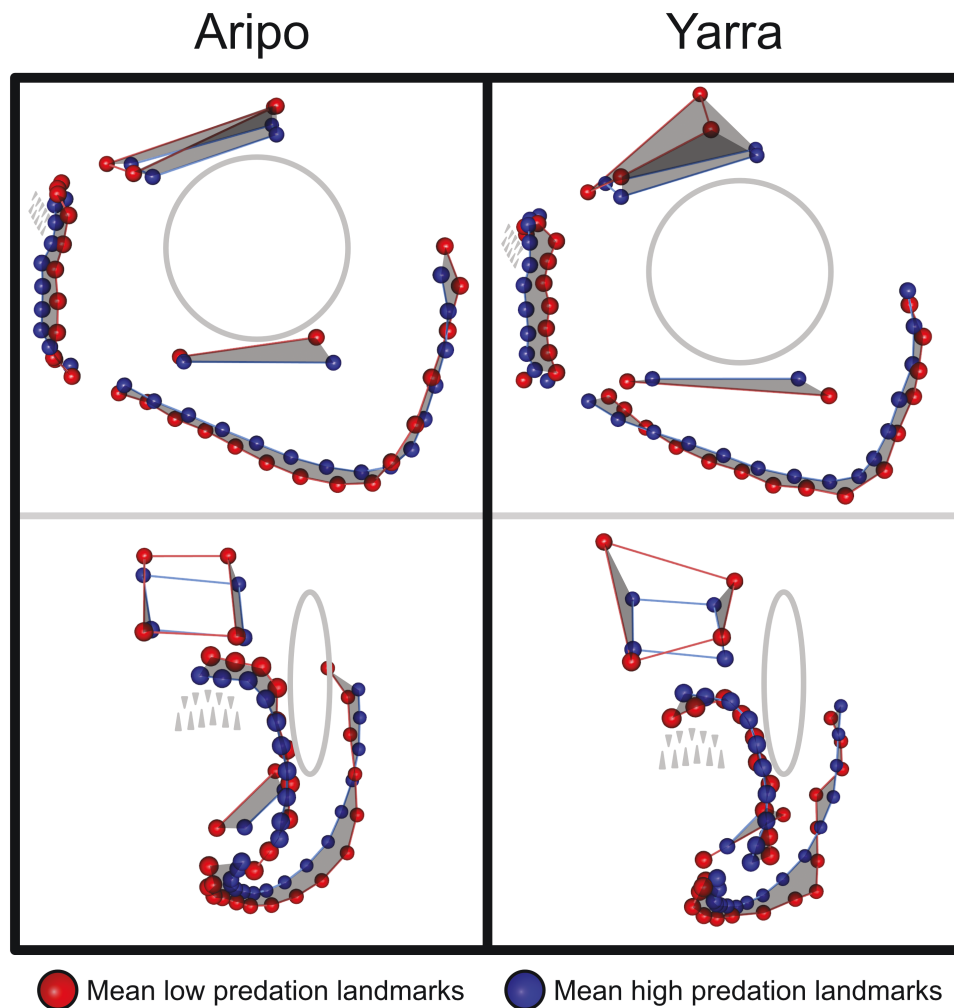
Figure 6. Mean population shapes of Aripo low predation versus Aripo high predation as well as Yarra low predation versus Yarra high predation, with differences magnified 5× show that low-predation fish tend to have shorter, taller, and wider craniofacial apparatuses. Yarra populations are slightly more divergent from one another than Aripo populations. Side and front views of the average landmark positions for low predation fish vs. high predation fish from each river drainage.

Specifically, we found that benthic guppies had narrower pre-orbital craniofacial skeletons, as evidenced by a more medially oriented preopercle and quadratomandibular joint (Figure 6). Narrowing of the oral aperture tends to be associated with better suction feeding, while wider mouths are favored in many benthic lineages. Specifically, narrow mouths create a more tube-like oral aperture and buccal chamber that helps fish to direct the flow and increase the forces produced during suction feeding (Higham, 2007; Holzman et al., 2007; Matthews & Albertson, 2017; Wainwright et al., 2007; Day et al., 2005). Alternatively, wide mouths allow benthic fish to take more food with each bite (Albertson & Kocher, 2001). The observation that benthic low-predation guppies possess narrower heads suggests a potential limitation to the amount of substrate procured with each bite, and therefore could be interpreted as a non-adaptive craniofacial alteration among low-predation populations. However, we note that our shape analysis corrects for and removes the effects of size and low-predation fish tend to be larger, which may compensate for their relatively narrow oral jaw aperture.

Regardless of size, low-predation individuals assume a shorter, deeper craniofacial apparatus with wider suspensoria than their high-predation counterparts (Figure 6). The observed low-predation morphology could allow for increased attachment area for the adductor mandibulae, the main jaw-closing muscle. The poecilid adductor mandibulae forms a complex of separate subunits, each attaching to a different oral jaw element: premaxilla, maxilla, and anguloarticular, all of which originate from the ventrolateral aspect of the suspensorium (Hernandez et al., 2008). Although we did not directly measure adductor mandibulae size, previous studies have shown that this muscle scales with positive allometry in Trinidadian guppies (Dial et al., 2017). Therefore, we expect that, due to their larger size and wider suspensoria, low-predation guppies should display increased muscle cross-sectional area and produce more force at the oral jaw to enhance substrate feeding.

Another measurement that would be needed to measure jaw mechanics would be the shape of the individual elements of the oral jaw. Unfortunately, we did not preserve the fish such that oral jaw posture was standardized, and were thus unable to place any landmarks on these important structures. However, the shape of the overall craniofacial apparatus and of subunits such as the suspensorium are still important to feeding function (Cooper et al., 2010, 2011; Ferry-Graham &

Konow, 2010). While our results allow us to suggest a functional correlation with the observed morphological evolution, this adaptive hypothesis can only be truly tested by studying divergence in the oral jaws.

Concluding remarks

We present data on a measurable, significant, and repeatable divergence in craniofacial shape between high-predation and low-predation populations of the Trinidadian guppy. Importantly, these morphological trends parallel population-level differences in diet type and selective pressures, providing evidence of a plastic and genetic basis for the observed adaptive response to ecological variation. Although this documented craniofacial shape variation among guppy populations is largely convergent with other benthic-limnetic systems, we recognize that there is much more to be done, particularly in the individual elements of the oral jaws (Dial et al., 2017; Gibb et al., 2015). Past studies on other systems have shown that there are measurable differences in single bones between ecologically divergent populations, for example, in the anguloarticular bone (Albertson, 2008; Albertson & Kocher, 2001). Therefore, the guppy provides a rich system in which future studies can examine how these same mechanical components would evolve differently contingent on the baseline biomechanics of the system in which they evolve.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All data used to obtain these results are available at https://doi.org/10.5061/dryad.95x69p8rw.

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