Many-to-one form-to-function mapping weakens parallel morphological evolution

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Evolutionary ecologists aim to explain and predict evolutionary change under different selective regimes. Theory suggests that such evolutionary prediction should be more difficult for biomechanical systems in which different trait combinations generate the same functional output: "many-to-one mapping." Many-to-one mapping of phenotype to function enables multiple morphological solutions to meet the same adaptive challenges. Therefore, many-to-one mapping should undermine parallel morphological evolution, and hence evolutionary predictability, even when selection pressures are shared among populations. Studying 16 replicate pairs of lake- and stream-adapted threespine stickleback (*Gasterosteus aculeatus*), we quantified three parts of the teleost feeding apparatus and used biomechanical models to calculate their expected functional outputs. The three feeding structures differed in their form-to-function relationship from one-to-one (lower jaw lever ratio) to increasingly many-to-one (buccal suction index, opercular 4-bar linkage). We tested for (1) weaker linear correlations between phenotype and calculated function, and (2) less parallel evolution across lake-stream pairs, in the many-to-one systems relative to the one-to-one system. We confirm both predictions, thus supporting the theoretical expectation that increasing many-to-one mapping undermines parallel evolution. Therefore, sole consideration of morphological variation within and among populations might not serve as a proxy for functional variation when multiple adaptive trait combinations exist.

KEY WORDS: Convergence, Form, Function, Many-to-one mapping, Transformation, Trophic.

Morphological traits enable organisms to perform certain functions (Lauder 1981; Wainwright 1996). These functions, in turn, affect the fitness of individuals. Consequently, natural selection acts on morphological traits indirectly through selection on the traits' function(s) (Arnold 1983; Irschick et al. 2008). The rate of morphological evolution will therefore depend on how strongly functional variation, the target of selection, is correlated with the underlying morphological variation (Walker 2007). Some morphological traits have a direct one-to-one correspondence of form to function. For example, the lower jaws of vertebrates act as simple levers whose force transmission properties are simple linear

functions of lever arm relative lengths (Wainwright and Shaw 1999). For such traits, selection on biomechanical function is effective at driving change in the underlying morphological structures (assuming they are heritable). However, most morphological structures have functional redundancy, in which many distinct morphological trait combinations can confer similar functional outcomes—a phenomenon known as many-to-one mapping (Wainwright 2005). This many-to-one mapping can arise when function depends on multivariate combinations of morphology and/or on certain kinds of non-linear form-to-function relationships (Fig. 1). When many-to-one mapping occurs,
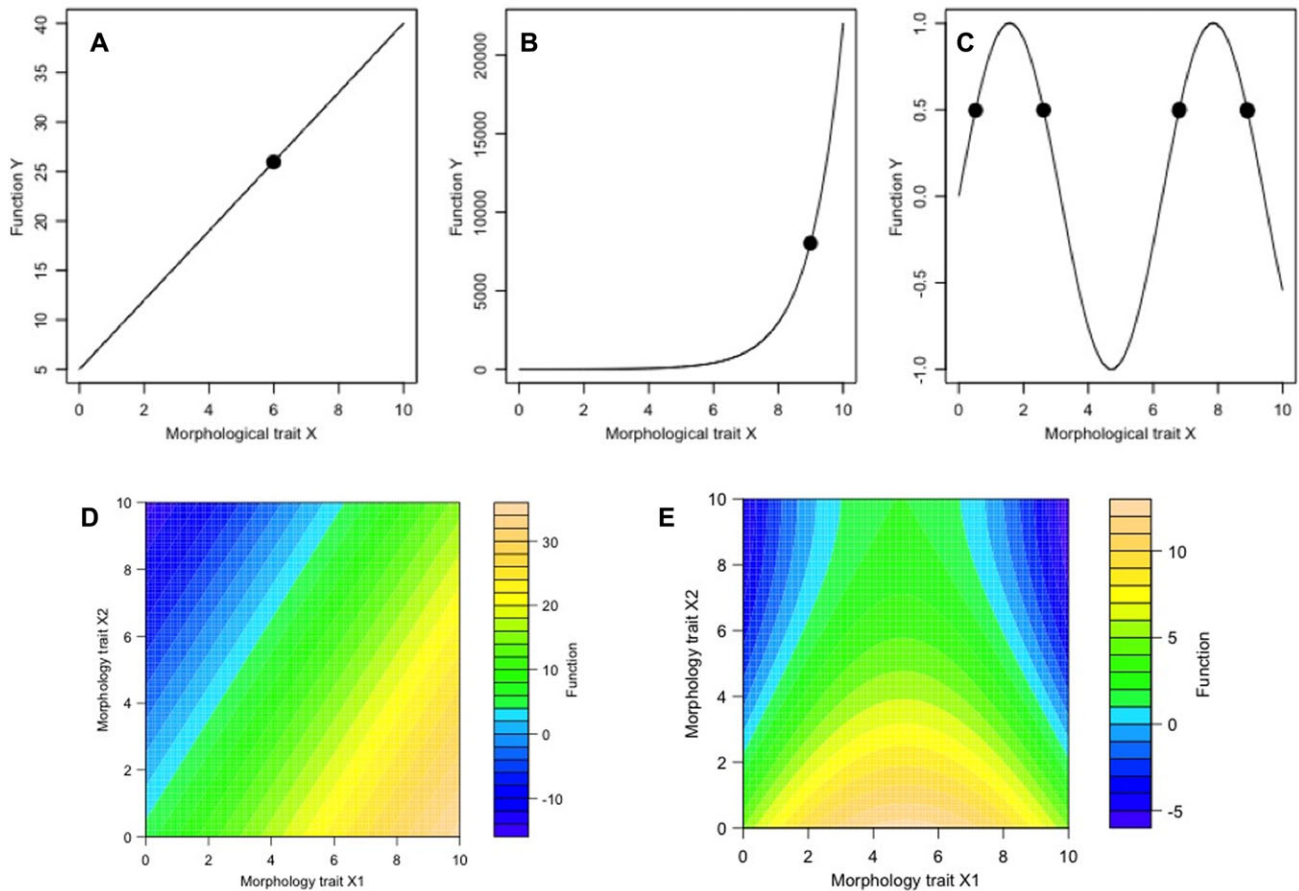
Figure 1. Examples of various kinds of form-to-function mapping. One-to-one mapping for (A) linear and (B) nonlinear form–function relationships. (C) Many-to-one mapping is possible for univariate traits, if function depends nonmonotonically on morphology (e.g., $Y = \sin(X)$), so that multiple values of X yield a given value of Y. (D) The simplest example of many-to-one mapping involves a function (Y) that is linearly dependent on the sum of two or more morphological traits (e.g., $Y = a + bX_1 + cX_2$). Function in this case can be graphed as a tilted plane over a 2-dimensional trait space, where height above the trait space corresponds to increasing function. Here we plot this plane as a topographic map. This is a many-to-one system because contour lines represent many combinations of morphology (X_1, X_2) for any given value of Y. (E) Many-to-one mapping is particularly acute when morphology is multi-variate and has nonlinear effects on function, here represented as a curved surface. Such curvature exaggerates the many-to-one aspect of form–function mapping that was already present with the plane described in (D). As an approximation, the extent of many-to-one mapping can be estimated by the coefficient of determination in a regression of function on morphology (R^2). However, we point out that there are 1:1 functions (e.g., panel B) that have trait-function correlations less than 1.

morphologically divergent organisms might or might not have different realized functional capability (the latter must be estimated using a biomechanical model or, preferably, empirical measurements of function itself). Consequently, morphological diversity at the individual and population levels can be a poor proxy for functional or ecological diversity (Hulsey and Wainwright 2002), even though there is a true, deterministic relationship between any one morphology and its function. Many-to-one mapping therefore reduces the covariance between morphological traits and their emergent function ((Wainwright 2005), though see (Cooper and Westneat 2009)). For such traits, selection on biomechanical function will be less effective at driving the evolution of the entire system.

Replicate populations that colonize similar environments might be subjected to similar selection pressures and therefore evolve similar functional adaptations, resulting in parallel or convergent evolution (Losos et al. 1998). However, reduced covariance between form and function generated by many-to-one mapping is expected to reduce the efficiency by which selection on biomechanical function can affect morphological evolution (Walker 2007). Drift, migration, and historical contingency may thus play a proportionally stronger role in the evolution of biomechanical systems with greater many-to-one mapping.

To evaluate the effect of many-to-one mapping on convergence across multiple populations, Alfaro et al. (2004, 2005) simulated stabilizing selection acting on the teleost maxillary 4-bar

lever system, which translates muscle force into forceful versus rapid jaw opening. Although the kinematic transmission properties (i.e., the estimated function calculated from a biomechanical model) of this multi-part lever converged as expected, the morphological structures underlying the function did not. Instead, replicate populations subject to the same selection pressures arrived at different morphological solutions with identical (calculated) function. Thus, many-to-one mapping of form to function should make evolution less predictable in empirical systems (Alfaro et al. 2004, 2005). In particular, we expect many-to-one systems to show less reliable parallel evolution than one-to-one systems (McGee and Schluter 2013).

Indeed, biologists frequently find incompletely parallel evolution of morphology where parallel evolution is expected, given replicate instances of seemingly similar habitats (Oke et al. 2017) (Stuart et al. 2017). The arguments presented above suggest that incompletely parallel morphological evolution might still be underlain by parallel selection, just on many-to-one-mapped biomechanical systems. Of course, alternative hypotheses are also plausible: incomplete parallel evolution may simply be a legacy of genetic drift or founder effects that add stochasticity to the process of adaptation; or, deviations from parallel evolution might reflect subtle environmental differences between habitats that, at first glance, appear replicated (Stuart et al. 2017).

Here, we empirically evaluate the effects of many-to-one mapping on trait-function correlations and on parallel evolution, by measuring the extent of parallel evolution of form and function for three biomechanical systems in threespine stickleback (*Gasterosteus aculeatus*). First, the morphology of the lower jaw has a one-to-one relationship with its lever ratio (LR), which measures trade-off between force and speed as a fish rotates its lower jaw downward during prey capture (Westneat 2004; McGee and Schluter 2013). A second system, the epaxial-buccal cavity, involves multiple traits that have a nonlinear and many-to-one (Fig. 1) relationship with fishes' abilities to generate negative pressure in the mouth during suction feeding, as estimated by the suction index, SI (Carroll 2004; Wainwright et al. 2007). Last, the opercular four-bar linkage is a multi-part lever whose kinematic transmission (KT) affects the force and speed of jaw opening and protrusion: changes in joint angles between four interconnected parts of the skull translate to rotation of skeletal elements that protrude the jaw during suction feeding (Westneat 2004; Holzman et al. 2008; McGee and Schluter 2013). The relationship between the four-bar morphology and KT is both multivariate and highly nonlinear and therefore also many-to-one.

We measured the morphology of the lower jaw, epaxial-buccal cavity, and opercular four-bar on wild-caught fish from 16 replicate pairs (32 populations) of lake and adjoining-stream populations of threespine stickleback. We used these morphological traits (hereafter, "component traits" sensu (McGee and Schluter 2013)) to directly calculate functional coefficients for LR, SI, and KT for each fish from biomechanical models established for each system. These data allow us to contrast the degree of parallel evolution in both morphology and its modeled function for a one-to-one system (LR) against two many-to-one systems (SI and KT). Furthermore, simulations and visual inspection (Supplementary Materials; Fig. 2) suggest that these biomechanical systems can be ordered along a continuum of many-to-one mapping, from one-to-one mapping (LR) to intermediate (SI) and high (KT) many-to-one mapping; hereafter, we present methods and findings in order of LR:SI:KT to help the reader recall the increasing order of many-to-one mapping. These three functional coefficients (LR, SI, KT) are unitless and therefore directly comparable.

We investigated two key relationships. First, we estimated linear correlation coefficients between observed morphology and calculated function to empirically confirm and validate our ranking of the biomechanical three systems in order of increasing many-to-one mapping. Second, we quantified lake stream divergence in modeled function and measured component traits to ask whether the one-to-one system (LR) shows lake-stream divergence that is more parallel across the 16 replicate lake-stream pairs than either SI or KT, the many-to-one systems.

We had a priori reason to expect at least some parallel lake-stream divergence of trophic morphology and function in our three biomechanical systems across our 16 lake-stream pairs. Independently evolved pairs of lake and stream stickleback show substantial parallel evolution for certain traits (Berner et al. 2008; Kaeuffer et al. 2012) despite ongoing gene flow (Weber et al. 2016), indicating that these ecotypes are subject to divergent selection. For example, trophic traits like gill raker number are consistently different between lake and stream stickleback, almost always in the same direction (Berner et al. 2008; Kaeuffer et al. 2012) (Stuart et al. 2017). This past result confirms that trophic function is a target of reasonably consistent divergent selection between lake and stream fish, presumably due to well-documented dietary differences (Berner et al. 2008; Kaeuffer et al. 2012).

Many-to-one mapping should reduce parallel responses to divergent trophic selection because multiple morphological solutions can satisfy a given functional adaptation (Alfaro et al. 2004). As a result, the outcome of many-to-one trait evolution is expected to be sensitive to initial conditions (e.g., different timing and diversity of initial colonization, ongoing gene flow, and idiosyncratic environmental events), which all might favor or require the coöption of different morphological routes to the same functional goal. Furthermore, for many other traits, lake-stream divergence is only partly parallel, or is even nonparallel (Hendry and Taylor 2004; Kaeuffer et al. 2012) (Stuart et al. 2017). Specifically, among-lake and among-stream
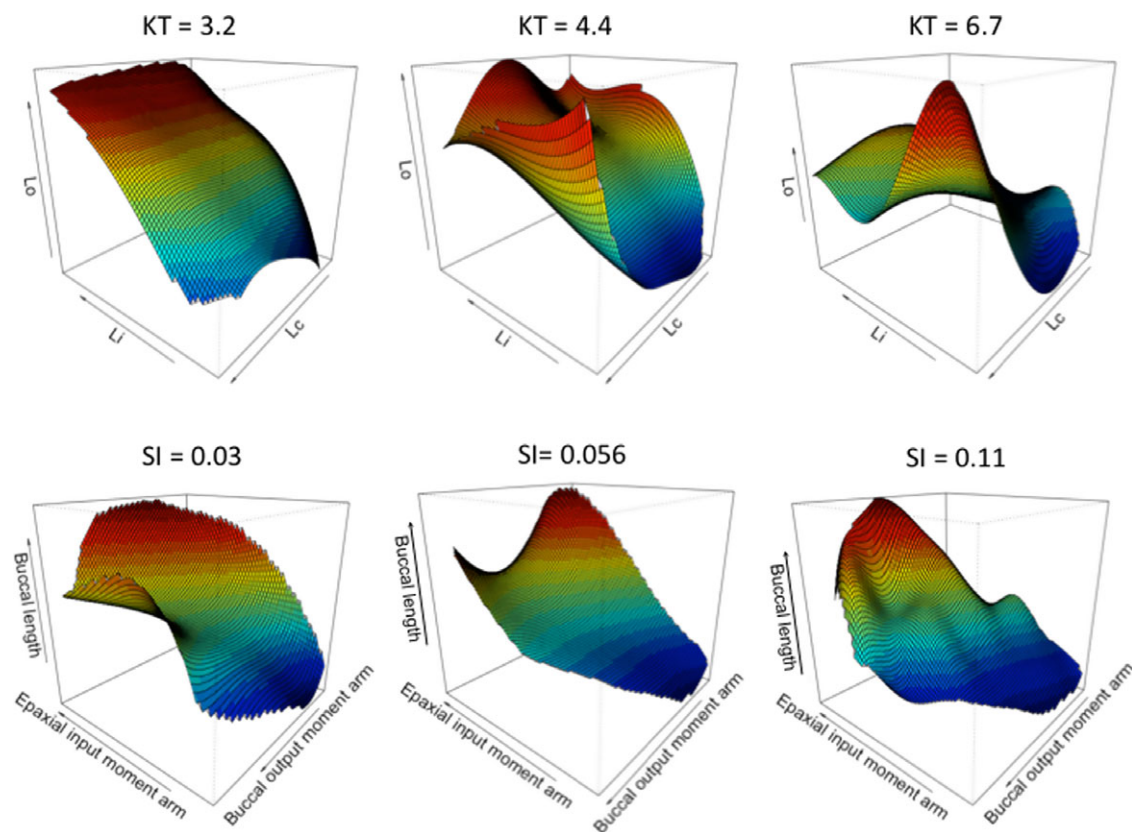
Figure 2. Isoplanes of identical function for KT (top row) and SI (bottom row), showing somewhat greater many-to-one mapping for KT. Each figure plots morphology as a 3-dimensional morphospace. Through this morphospace, we plot a surface representing various combinations of the three morphological traits that have identical function, chosen to represent the 20th, 50th, and 80th percentiles of KT and SI, from left to right. Movement along a given plane changes morphology but keeps function constant. Movement off the plane changes function. More convoluted surfaces have more surface area, indicating more possible trait combinations for a given functional value and therefore increased many-to-one mapping. (Color coding merely corresponds to variation in the vertical axis as a visual aid; it is not indicative of variation in functional score). In reality, each system has more dimensions (more than three morphological traits). To allow us to plot these functions, all traits were scaled to be relative to a reference trait (Fixed link for KT, epaxial cross-sectional area for SI), reducing the number of dimensions by one. Next, we arbitrarily dropped one dimension from the four-bar linkage (diagonal length) and one dimension from the suction system (gape width), to allow us to plot this in 3-dimensional space. Similar results hold when we use different combinations of traits. Functional scores were calculated as normal.

environmental variation contributes to apparently adaptive deviations from phenotypic and genetic parallelism (Stuart et al. 2017). Thus, deviations from parallel evolution in trophic phenotype might reflect environmental variation rather than many-to-one mapping. Fortunately, we can distinguish these alternative explanations by comparing the relative extent of parallel evolution among the three biomechanical traits, taking advantage of their differing levels of many-to-one mapping. We predicted that both morphology and function of the lower jaw would show more parallel lake-stream divergence, across replicate watersheds, for the same fish, than the two many-to-one systems.

Materials and Methods
COLLECTION

In May to June 2013, we collected adult threespine stickleback from 16 lakes and their adjoining streams (32 populations in total, Table S1). Lake-stream pairs were chosen from different watersheds to minimize the influence of among-pair gene flow and shared evolutionary history on lake-stream divergence. Genomic analyses of single nucleotide polymorphisms (SNPs) generated via ddRAD-seq (Peterson et al. 2012) support the mostly independent origin (from a common marine ancestor) of the lake-stream pairs in the different watersheds (Stuart et al. 2017). To

capture fish at each site, we set a transect of 50 unbaited minnow traps along a ~100 m stretch of shoreline (in the lake or stream). Captured fish were euthanized with neutral buffered Tricaine (MS-222) and stored in formalin. At the University of Texas at Austin (UT), the fish were stained with Alizarin Red and then stored in 40% isopropanol. Collections were conducted with approval from the British Columbia Ministry of Land, Environment, and Natural Resources (NA12-84189 and NA13-85697) and from the University of Texas at Austin Institutional Animal Use and Care Committee (AUP-2012-00065 and AUP-2014-00293).

TRAIT MEASUREMENTS

We pooled sexes in our analyses to increase statistical power. Sex ratios in each population were close to 1:1 (324 females and 319 males in total across all populations). We measured three sets of trophically important biomechanical traits (Table S2) on ~20 adult fish from each of the 32 populations (Table S1). These traits were measured on lateral and ventral photographs of the fish (Fig. 3, Fig. S1), except for lower jaw traits and epaxial muscle area. To measure jaw traits, we dissected the lower jaw from the skull by cutting behind the quadrate-articular joint and then photographed the jaw on a stage micrometer with a microscope-mounted camera. We then removed the head from the body using a razor blade, cutting along the coronal plane behind the supracleithrum/postemporal articulation (McGee and Schluter 2013), and photographed the epaxial muscle to get cross-sectional area. To measure trait lengths and areas from photographs, we used the Object-J plugin (https://sils.fnwi.uva.nl/bcb/objectj/index.html; v.1.03) for FIJI (Schindelin et al. 2012), an ImageJ-based digitization software program.

We pooled individuals across populations and inspected the component trait data for outliers. Using 3.5 standard deviations (SD) as a rough guide to data points far from their means, we visualized the data, finding that 13 individuals exceeded this threshold for epaxial muscle cross-sectional area, and two individuals exceeded the threshold for the length of the jaw-opening inlever. The extreme epaxial data were all from the Village Bay lake-stream pair, and the extreme inlever data were both from the Joe Lake pair, suggesting that these data were not the result of error but rather shared divergence in those populations. We therefore kept all data for the analyses.

Typically, studies use Principal Components Analysis (PCA) to summarize the relationships among traits, and then try to infer functional differences among populations from differences in PCA-rotated morphological variables among populations. We instead use ratio-standardized data to predict function. (In the Supplementary Materials, we compare PCA-based approaches to our ratio-based approach; Table S6.) Specifically, for each biomechanical system and for each individual fish, we standardized one component trait to a value of 1 by dividing it by itself, and cal-

culated ratios of the other traits to that one standardizing trait. For example, we chose the Fixed component of the opercular 4-bar system as our standardizing trait and so for each fish, we divided all component measurements (i.e., Fixed, Input, Output, Coupler, and Diagonal; Fig. 3) by the Fixed component. Thus, the Fixed component was standardized to one, and the other links were expressed as a ratio of the Fixed component. We took the same approach for the component traits of LR and SI, using each function's in-lever (L_{in1} and L_{in2}, respectively; Table S2) as the standardizing trait (i.e. the denominator of each ratio). We used the square root of the epaxial cross-sectional area to linearize it before standardization. These ratio-standardized traits are the component traits used to calculate the form–function correlations, described below.

CALCULATING FUNCTION

Using the morphological measurements described above (and in Table S2), we calculated three functional coefficients: kinematic transmission of the lower jaw (Alfaro et al. 2004; Westneat 2004), suction index (Wainwright et al. 2007), and kinematic transmission of the opercular 4-bar system (Westneat 2004; McGee and Schluter 2013). Here, and throughout the article, we emphasize that we are calculating a predicted function from a mathematical model of biomechanics, rather than directly measuring feeding function in the laboratory. The linear correlations we subsequently calculate between component traits and functional coefficients will be stronger than the linear correlations between component traits and actual function, because the latter contains additional sources of variation including fish behavioral variation (motivation, prior experience, etc.) and condition. We also note that these functional formulae do not incorporate error, except indirectly through measurement error on component traits. To calculate functional coefficients, we used raw values for traits, that is without any data transformations (Westneat 2004; Wainwright et al. 2007; McGee and Wainwright 2013).

Kinematic transmission of the lower jaw, or Lever Ratio (LR):

$$LR = L_{out1}/L_{in1},$$

where L_{out1} is the length of the jaw-opening outlever and L_{in1} is the length of the jaw-opening inlever (see Table S2 for measurement details). Note that unlike some workers (Westneat 2003), we did not include measurements of the jaw muscle attached to the inlever when calculating kinematic transmission of the lower jaw. The Lever Ratio is one-to-one mapped onto the kinematic transmission of the lower jaw (i.e., the function) (Alfaro et al. 2004), even though its component traits can evolve independently.

Suction Index (SI):

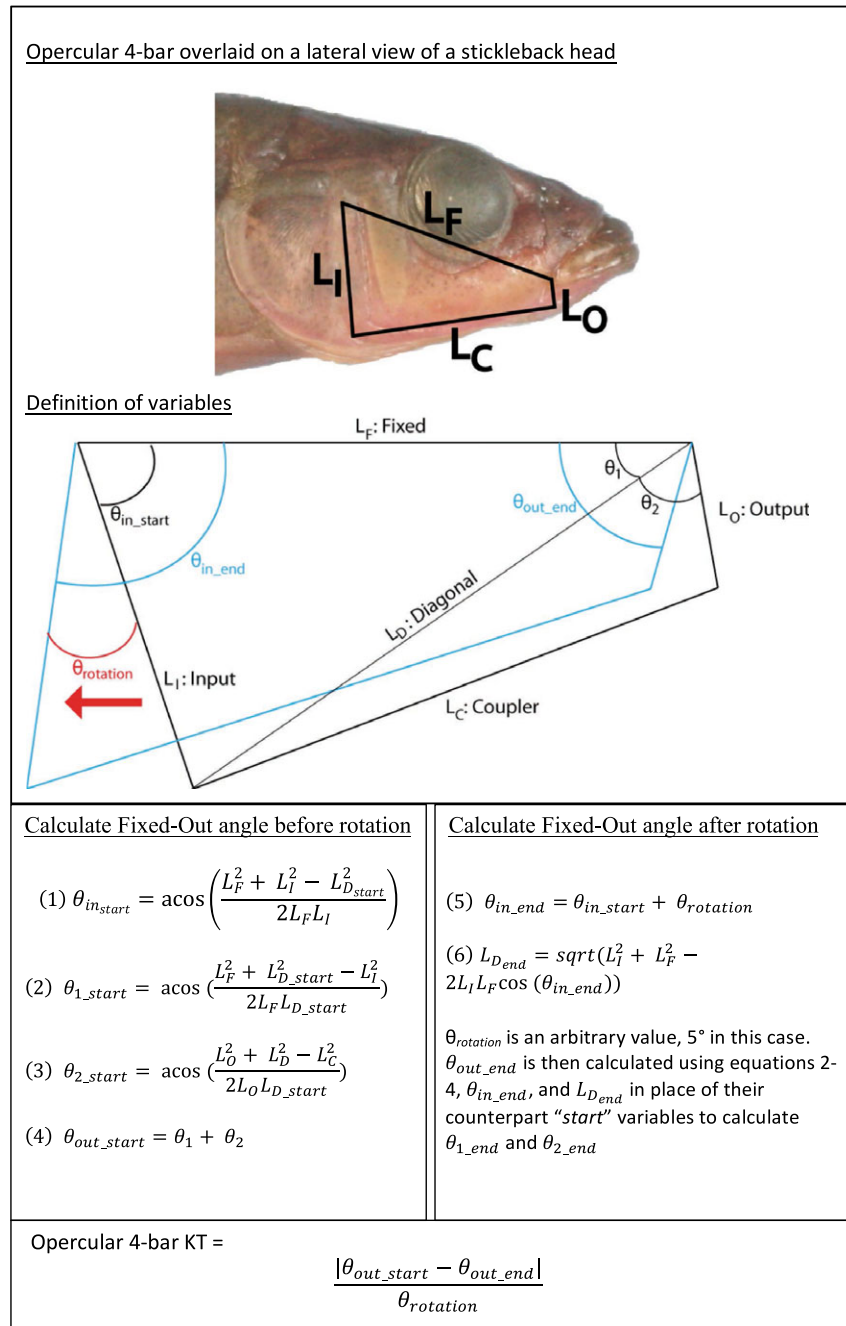$$SI = \frac{CSA_e * \left(\frac{L_{in2}}{L_{out2}}\right)}{gw * bl},$$

Opercular 4-bar overlaid on a lateral view of a stickleback head

Definition of variables

Calculate Fixed-Out angle before rotation

$$(1)\ \theta_{in_{start}} = \mathrm{acos}\left(\frac{L_F^2 + L_I^2 - L_{D_{start}}^2}{2L_F L_I}\right)$$

$$(2)\ \theta_{1\_start} = \mathrm{acos}\left(\frac{L_F^2 + L_{D\_start}^2 - L_I^2}{2L_F L_{D\_start}}\right)$$

$$(3)\ \theta_{2\_start} = \mathrm{acos}\left(\frac{L_O^2 + L_D^2 - L_C^2}{2L_O L_{D\_start}}\right)$$

$$(4)\ \theta_{out\_start} = \theta_1 + \theta_2$$

Calculate Fixed-Out angle after rotation

$$(5)\ \theta_{in\_end} = \theta_{in\_start} + \theta_{rotation}$$

$$(6)\ L_{D_{end}} = sqrt(L_I^2 + L_F^2 - 2L_I L_F \cos{(\theta_{in\_end})})$$

$\theta_{rotation}$ is an arbitrary value, 5° in this case. θ_{out_end} is then calculated using equations 2-4, θ_{in_end}, and $L_{D_{end}}$ in place of their counterpart "*start*" variables to calculate θ_{1_end} and θ_{2_end}

Opercular 4-bar KT =

$$\frac{|\theta_{out\_start} - \theta_{out\_end}|}{\theta_{rotation}}$$

Figure 3. Calculation of the kinematic transmission of the opercular 4-bar system.

where *bl* is buccal cavity length, *gw* is gape width, CSA_e is the cross-sectional area of the epaxial muscle, L_{in2} is the height of the epaxial muscle, and L_{out2} is the length of the neurocranium (see Table S2 for measurement details; Fig. S1).

Kinetic transmission of the Opercular 4-bar (KT):

Please see Figure 3. For a given input rotation of the Fixed-Input joint (in our case, $\theta_{input} = 5°$), we calculated the rotation (i.e. the kinematic transmission coefficient, KT) of the Fixed-Output joint (Westneat 2004; McGee and Wainwright 2013). We note that this metric takes into account angular transmission of the 4-bar system only, and does not include rotational transmission about the Fixed-Output joint, which might result in underestimating the total displacement of the 4-bar system (Olsen and Westneat 2016).

ANALYSIS

All analyses were done with the statistical software program R (version 3.3.1).

Question 1: Do correlations between component traits and calculated function diminish as many-to-one mapping increases?
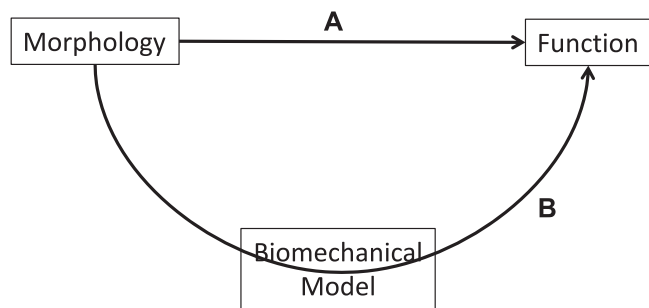
Figure 4. Morphology, predicted function, and empirical function. (A) Many studies measure morphology only and assume a linear relationship between form and function; that is, knowing something about variation in form among individuals or populations is taken to reveal something about variation in function. However, because of multidimensionality and/or nonlinearity, that form–function relationship will be many-to-one (Figs. 1 and 2), even for predicted function, and R^2 will be less than one for models assuming one-to-one mapping. Thus, R^2 can serve as an estimate of many-to-oneness (Fig. 1). This advocates for measuring function itself, or at least using a biomechanical model to estimate it from morphology (B). The biomechanical model is deterministic: knowing form predicts function exactly.

We first used ANOVA and MANOVA to test for variation among populations and pairs in calculated function and component trait morphology, respectively (Table S3; Table S4). Finding such variation (Table S3), we predicted that the correlation between component traits and estimated function should decay as many-to-one mapping increases (Fig. 4). We tested this prediction by regressing each functional measure (LR, SI, KT) on its component morphological traits (R: *lm*). Specifically, we pooled all individuals and built three linear models, one for each functional coefficient as the dependent variable and corresponding trait measurements as independent variables. We report just this simple model; models with population included as a random effect had nearly identical results. These linear models are ignorant of the biomechanical formulae used to estimate function in the three biomechanical systems. The R^2 of these models provides a measure of how effectively morphological variation (i.e., without processing through a biomechanical model) can be used as a proxy for functional variation. A linear model is justified here because it is a standard statistical approach that most investigators would use to analyze morphological data in the absence of a biomechanical model (Fig. 4). The performance of the linear model thus describes how well our standard analytical approaches can capture functional variation that is in truth more likely to be nonlinear. We compared measures of R^2 for the least (LR), intermediate (SI), and most (KT) many-to-one functions, to ask whether trait-function (or, rather, calculated function) correlations decrease as many-to-one mapping increases.

Question 2: Does many-to-one mapping erode parallel evolution?

We predicted that the LR biomechanical system, with one-to-one mapping of morphology to function, would show more parallel lake-stream divergence across the 16 replicate pairs than SI or KT. The extent of parallel lake-stream divergence across the 16 pairs can be quantified as the effect size of the habitat term in models that test functional coefficients or component traits against habitat, watershed, and the habitat-watershed interaction (e.g., Kaeuffer et al. 2012). That is, a larger habitat effect size suggests more consistent (i.e., parallel) lake-stream divergence across watersheds. A larger interaction effect size suggests that the focal-dependent variable is divergent between lake and stream stickleback but that the direction or magnitude of that divergence varies among watersheds. The interaction term thus provides an estimate of the strength of the deviation from parallel divergence. For calculated functional coefficients and their component traits, we used ANOVA and MANOVA, respectively, to estimate the effect sizes of habitat, watershed, and their interaction (using R commands: *aov, manova,* and *EtaSq*). This ANOVA-based approach of quantifying parallel evolution (and deviations from parallel) is widely used (Langerhans and DeWitt 2004).

Results
Question 1: Do correlations between component traits and calculated function diminish as many-to-one mapping increases?

All three sets of morphological component traits varied among populations and among individuals within populations (Table S3). As predicted, these component trait values varied in their strengths of correlation with calculated measures of functional output (Table 1). The kinematic transmission properties of the lower jaw opening lever (LR) is simply the ratio of the lengths of the input to output lever arms. Because the function of the opening lever has a one-to-one dependence on the underlying morphology, jaw morphology completely explained variation in LR (Adj. $R^2 = 1.000$). However, the form-function relationship was weaker for biomechanical systems with increasing many-to-oneness. For SI, the component traits strongly but incompletely explained variation in function (Adj. $R^2 = 0.855$). For KT, the relationship was weaker still (Adj. $R^2 = 0.792$). These empirically calculated form–function correlations closely resemble our simulated form–function correlations (Supplementary Material; $r = 0.996$ between adjusted R^2 from our data versus R^2 from our simulations, across the $N = 3$ biomechanical systems). This is not surprising, given that the same mathematical formulae were used to convert empirical morphology into calculated functional coefficients, as were used to convert simulated morphology into functional coefficients. But, the strong correlation lends further support to ordering these biomechanical systems from one-to-one

A. Parallelism in estimated function declines with increasing many-to-oneness.

Trophic function	Habitat	Watershed	Habitat × Watershed
Lever ratio	Eta^2 = 0.044	Eta^2 = 0.185	Eta^2 = 0.069
Suction index	Eta^2 = 0.017	Eta^2 = 0.221	Eta^2 = 0.285
4-bar KT	Eta^2 = 0.000^	Eta^2 = 0.153	Eta^2 = 0.073

B. Parallelism in component traits declines with increasing many-to-oneness.

Trophic components	Habitat	Watershed	Habitat × Watershed
Lever ratio	Eta^2 = 0.044	Eta^2 = 0.185	Eta^2 = 0.069
Suction index	Eta^2 = 0.035	Eta^2 = 0.267	Eta^2 = 0.066
4-bar KT	Eta^2 = 0.006	Eta^2 = 0.294	Eta^2 = 0.061

All effects in all models $P < 0.001$, except for: ^$P = 0.61$. Eta^2 = effect size.

(LR) to increasing many-to-one (SI, KT) (see also Fig. 2, Supplementary Material).

Question 2: Does many-to-one mapping erode parallel evolution?

For each functional set of component traits, MANOVAs revealed that lake stickleback were morphologically diverged from their adjoining stream stickleback in every watershed save one (Moore; Table S4). Most lake and stream populations also differed with respect to calculated functional coefficients, though this divergence was weaker and less consistent than for the component traits: SI was divergent for 13 of 16 lake-stream pairs, whereas LR and KT differed in only six and five pairs lake-stream pairs, respectively (Fig. 5; Table S5).

Habitat effect sizes (i.e., parallel divergence) and interaction effect sizes (i.e., nonparallel divergence) were strong in two of the three biomechanical systems for their calculated functions (Table 1A) and for their component traits (Table 1B). The exception was the 4-bar linkage KT, where the habitat effect was small for the component traits (Eta^2 = 0.006; Table 1B) and nonexistent for the calculated function (Eta^2 = 0.000; Table 1A). Instead, the habitat × watershed interaction was more important, indicating that these traits and functions did diverge between lake and stream stickleback—just not in a fashion that was consistent across watersheds (Table 1). In addition, the watershed effect was strong for many traits (Table 1), perhaps suggesting habitat-independent vagaries of colonization history and differential selection in the different watersheds. In comparison with 86 univariate traits measured in the same populations (described in Stuart et al. 2017), the

habitat effect sizes for LR and its component traits were relatively high (Fig. S2; 74th percentile of habitat effect sizes, substantially above a large set of near-zero-effect traits), again supporting the existence of strong habitat-related parallel lake-stream divergence in foraging-related traits and functions. SI component traits were also in the top third of habitat effects (71st percentile; 60th percentile for SI function). In contrast, KT traits and function are the least parallel of all traits measured in these populations (0th percentile for the habitat term, for both form and function).

As predicted, the effect size of habitat in our models declined with increasing many-to-oneness (Table 1). The habitat effect for the moderately many-to-one suction index (SI) was 38% of the one-to-one lower jaw opening lever ratio (LR), and the habitat effect for the 4-bar KT was effectively zero. The same trend holds for the component traits underlying these estimated functions: multivariate morphology for suction index (SI) has a habitat effect 80% of that for LR, whereas the habitat effect of 4-bar morphology is also effectively zero. Importantly, the negative correlation between habitat effect size (i.e., parallel lake stream divergence) and extent of many-to-one mapping does not arise merely because these many-to-one systems simply fail to diverge between populations. On the contrary, the many-to-one traits are frequently and strongly different between lake and stream pairs (Table S4). However, this divergence is inconsistent across replicate watersheds, resulting in the strong habitat × watershed interaction effects for component traits of all three biomechanical systems (Table 1). This tendency for increasingly many-to-one systems to be divergent but less parallel confirms our initial expectations.

Discussion

For some biomechanical systems, multiple morphological combinations can generate the same functional outcome. Consequently, even though any individual's morphological phenotype determines that individual's function, the difference or similarity between two morphologies among individuals (and populations) might not reflect the difference or similarity of their functional output. This many-to-one mapping means that convergent or stabilizing selection for one functional optimum might not lead to convergent evolution of one single morphology (Alfaro et al. 2005), but see (Cooper and Westneat 2009). We therefore expect that biomechanical systems with more pronounced many-to-one mapping will tend to exhibit less predictable, less repeatable, morphological evolution. To test this, we compared the extent of parallel evolution of three biomechanical systems ranging from one-to-one (LR), to modest many-to-one (SI), to strong many-to-one mapping (KT). Using morphological data from 16 stickleback lake-stream pairs, we asked whether systems with more many-to-one mapping showed less parallel evolution.
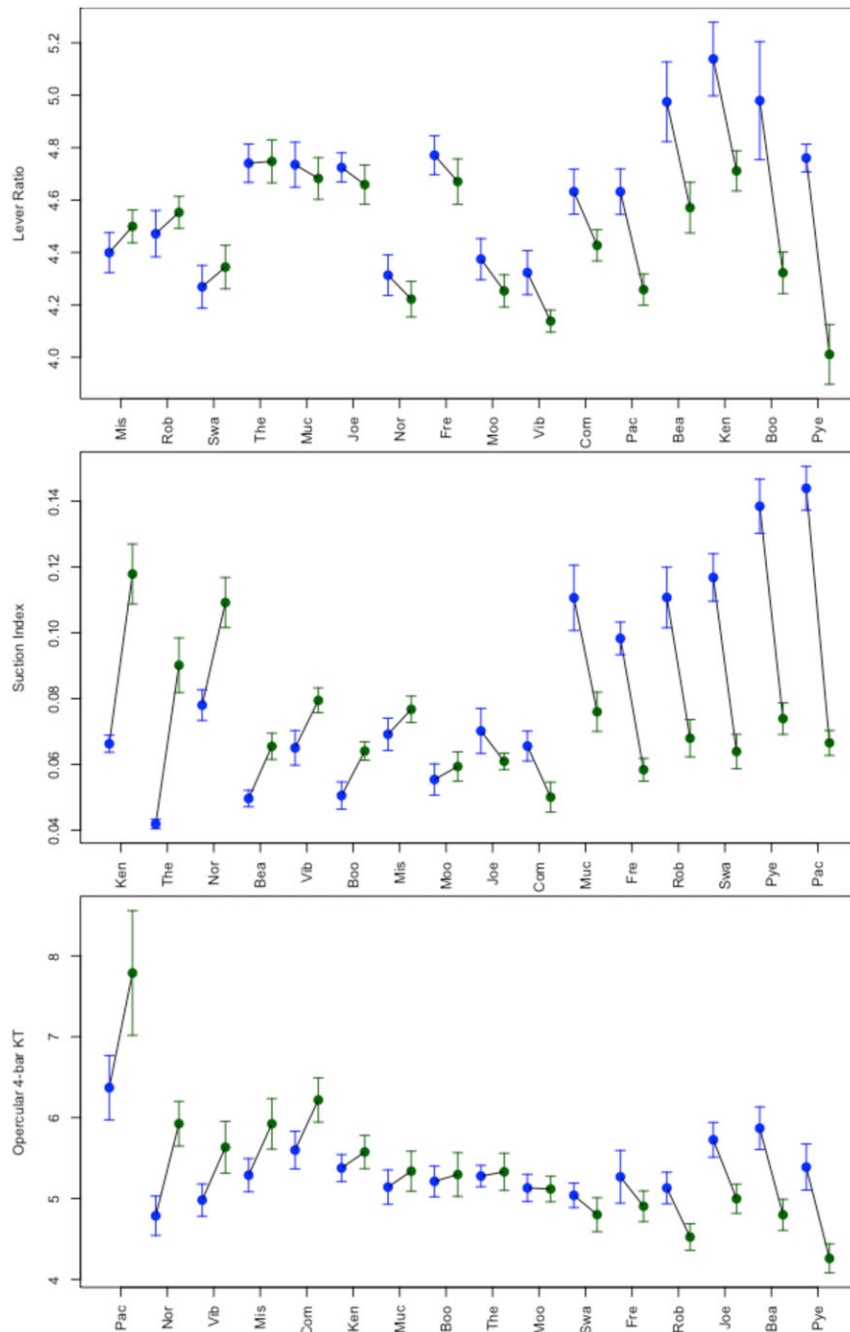
Figure 5. Lake-stream divergence in three estimated trophic functions varies in magnitude and direction. Each point is the lake (blue) or stream (green) mean. Error bars are standard errors of the means. Sample sizes (18–21 fish per population) are given in Table S1. Pairs are ordered from left to right by the magnitude of lake-stream divergence (note that the direction of divergence changes sign). Lever ratio = LR, Suction index = SI, Opercular 4bar KT = KT.

Several lines of evidence suggest that the order of increasing many-to-one mapping in our three biomechanical systems is LR < SI < KT. First, visual inspection of form-function relationships confirm that isoplanes in morphospace for a given SI function are typically less convoluted than the isoplanes for KT (Fig. 2), suggesting that the latter system has more many-to-one mapping. Quantitatively, the population-level correlation between observed component traits and their calculated function is strongest for LR and gets sequentially weaker for SI and KT (Question 1). Our simulations revealed an almost identical trend toward weaker form-function covariance for SI and particularly KT (Supplementary Materials). Notably, these form-function covariances decline even though function is deterministically calculated from morphology. However, R^2 alone is an imperfect measure of many-to-one

mapping because it may be below one even for one-to-one traits (e.g., Fig. 1B) or can approach 1.0 for many-to-one traits. We suggest that more work is needed to mine mathematical tools to quantify functional redundancy.

We detected a role of many-to-one mapping in shaping the parallelism of lake-stream divergence of biomechanical systems (Question 2). The habitat effect (i.e., evidence for parallel lake-stream divergence) is fairly substantial for LR (in the top quarter of all traits measured in Stuart et al. 2017; Fig. S2), confirming that parallel lake stream divergence in trophic biomechanical traits is present, at least for some pairs (Fig. 5). However, the habitat effect was weaker for component traits and functional estimates of SI, and weaker still for KT (Fig. S2). Thus, although each biomechanical system has significant deviations from parallel evolution among replicate lake-stream pairs (the significant interaction effect), these deviations are more pronounced for the systems with increasing many-to-oneness. This result confirms our initial prediction that many-to-one mapping undermines parallel evolution (Walker 2007).

Implications and Caveats

One implication of our empirical finding that trait-function relationships decay as many-to-one mapping increases is that morphology might not be a good proxy for inferring ecological interactions. This has long been an argument in favor of using direct measurements of true biomechanical function (Hulsey and Wainwright 2002; Irschick et al. 2008). For example, if multiple different trait combinations result in the same trophic function, then relying solely on phenotype to infer ecology will overestimate the number of trophic strategies under a naïve assumption of one-to-one mapping. This is a common problem, as many evolutionary ecology studies rely on morphology, implicitly assuming that morphological differences correspond linearly to similar functional divergence. In our system, for example, lake and stream stickleback exhibit repeatable differences in diet (Kaeuffer et al. 2012). These differences would lead us to expect repeatable (parallel) functional differences in feeding morphology and function. Instead, for LR, SI, and KT, both morphology and function show substantial deviations from parallel lake-stream divergence (Fig. 2; Table 1; Table S5). In nearly every case we see effects of habitat, watershed, and of habitat × watershed interactions (Table 1). The interaction, in particular, shows that the direction and magnitude of lake-stream divergence varies among watersheds, being shared (i.e., parallel) in some but not others.

These deviations from parallel lake-stream divergence could be due to several factors. First, any heritable lake-stream divergence could in principle be due to genetic drift or founder effects, which would undermine any parallel evolution. We consider this unlikely because most watersheds exhibit substantial lake-stream divergence despite gene flow, which implies that morphology and

function are evolving in response to selection. Second, deviations from parallel lake-stream divergence could reflect varying environments (and thus varying functional optima) among lakes, or among streams. Despite Kaeuffer et al (2012), Stuart et al. (2017) provide some support for this adaptive explanation because environmental heterogeneity among lakes, and among streams, co-varied with deviations from multivariate measures of lake-stream morphological divergence. Third, we do not here test whether the trait or functional differences among populations are heritable. The deviations from parallel lake-stream divergence could be a result of phenotypic plasticity. Oke et al. (2016) used a common-garden rearing experiment to show that much (but not all) of the phenotypic divergence between lakes and stream stickleback is heritable (in three lake-stream pairs also included in the present study). This was especially true for trophic traits like gill rakers. Accordingly, Arnegard et al. (2014) found a number of quantitative trait loci underlying benthic-limnetic divergence in component traits for LR and SI (they did not measure opercular KT). To the extent that plasticity is present, our trait-function correlation analyses are still valid because we are asking how current morphology corresponds to function, and trait-function patterns would be unchanged.

A second implication of our findings is the contradiction of prior theory in one key way. Models of the evolution of many-to-one systems (Alfaro et al. 2004) presume that function is subject to stabilizing selection and converges accordingly, whereas morphology of many-to-one systems does not. In contrast, our data suggest that systems with greater many-to-one mapping are less parallel for both form and function (Table 1). In fact, the functional output (KT) of the four-bar linkage shows no main effect of habitat at all. Clearly, function is not diverging in parallel in these complex systems, even though other trophic traits do exhibit some degree of parallel lake-stream divergence. One possible explanation is that the four-bar linkage might be especially sensitive to environmental variation within each habitat category. Alternatively, the weaker form-function relationship for the four-bar could make it harder for selection on KT (if any) to translate into heritable change (Walker 2007), which not only introduces noise to morphological evolution but can also reduce the speed and predictability of the evolution of KT function.

A crucial caveat to our work is that the three biomechanical systems we study are nested in a broader biological context that we do not incorporate. Each system has variation in muscles and connective tissue that we do not here account for. Each system is actuated by a complex neurological system. These additional factors will likely introduce additional layers of many-to-one mapping. Moreover, the three systems we examine are all involved in different subsets of the same overall task, feeding. Consequently, these systems are likely to interact with each other. Finally, we also note that in contrast to many-to-one mapping, mechanical

sensitivity (Anderson and Patek 2015) may strengthen the correlations between some component traits and calculated function, if, for example, one of the component traits in a biomechanical system has an outsize effect on the functional output, relative to the other components. For SI, all four component traits were significantly correlated with inferred function; for KT, only two of the four traits were significantly correlated with inferred function (Table S6), suggesting that KT is less many-to-one than might be expected for a 4-bar linkage.

Conclusion

Many biological systems entail a degree of redundancy. This is often discussed as a means of buffering organisms against costs of mutations, developmental change, or environmental stresses. But, this redundancy can also reduce the efficacy of natural selection and reduce the likelihood of parallel evolution. Our results support this notion because, of the three biomechanical systems involved in fish feeding, the system with one-to-one mapping of form to function showed the greatest degree of parallel lake-stream divergence across replicate lake-stream pairs.

AUTHOR CONTRIBUTIONS
C.J.T., Y.E.S., and D.I.B. planned the study. C.J.T., N.I.A., and Y.E.S. executed the study. C.J.T., Y.E.S., T.V., and D.I.B. analyzed the data. All authors participated in manuscript writing.

CONFLICT OF INTERESTS
The authors declare no conflicts of interest.

DATA ARCHIVING
Data and code are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.5b1k0

LITERATURE CITED
Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. Am. Nat. 165:E140–E154.

Alfaro, M. E., D. I. Bolnick, and P. C. Wainwright. 2004. Evolutionary dynamics of complex biomechanical systems: an example using the four-bar mechanism. Evolution 58:495–503.

Anderson, P. S. L., and S. N. Patek. 2015. Mechanical sensitivity reveals evolutionary dynamics of mechanical systems. Proc. Royal Soc. B 282:20143088.

Arnegard, M. E., M. D. McGee, B. Matthews, K. B. Marchinko, G. L. Conte, S. Kabir, N. Bedford, S. Bergek, Y. F. Chan, F. C. Jones, et al. 2014. Genetics of ecological divergence during speciation. Nature 511:307–311.

Arnold, S. J. 1983. Morphology, performance and fitness. Am. Zool. 23:347–361.

Berner, D., D. C. Adams, A.-C. Grandchamp, and A. P. Hendry. 2008. Natural selection drives patterns of lake–stream divergence in stickleback foraging morphology. J. Evol. Biol. 21:1653–1665.

Carroll, A. M. 2004. Morphology predicts suction feeding performance in centrarchid fishes. J. Exp. Biol. 207:3873–3881.

Cooper, W. J., and M. W. Westneat. 2009. Form and function of damselfish skulls: rapid and repeated evolution into a limited number of trophic niches. BMC Evol. Biol. 9:24.

Hendry, A. P., and E. B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319–2331.

Holzman, R., S. W. Day, R. S. Mehta, and P. C. Wainwright. 2008. Jaw protrusion enhances forces exerted on prey by suction feeding fishes. J. Royal Soc. Interface 5:1445–1457.

Hulsey, C. D., and P. C. Wainwright. 2002. Projecting mechanics into morphospace: disparity in the feeding system of labrid fishes. Proc. Biol. Sci. 269:317–326.

Irschick, D. J., J. J. Meyers, and J. F. Husak. 2008. How does selection operate on whole-organism functional performance capacities? A review and synthesis. Evol. Ecol. Research. 10:177–196.

Kaeuffer, R., C. L. Peichel, D. I. Bolnick, and A. P. Hendry. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66:402–218.

Langerhans, R. B., and T. J. DeWitt. 2004. Shared and unique features of evolutionary diversification. Am. Nat. 164:335–349.

Lauder, G. V. 1981. Form and function: structural analysis in evolutionary morphology. Paleobiology 7:430–442.

Losos, J. B., T. R. Jackman, A. Larson, K. de Queiroz, and L. Rodriguez Schettino. 1998. Contingency and determinism in replicated adaptive radiations of island lizards. Science 279:2115–2118.

McGee, M. D., and D. Schluter. 2013. Functional basis of ecological divergence in sympatric stickleback. BMC Evol. Biol. 13:277.

McGee, M. D., and P. C. Wainwright. 2013. Convergent evolution as a generator of phenotypic diversity in threespine stickleback. Evolution. 67:1204–1208.

Oke, K. B., M. Bukhari, R. Kaeuffer, G. Rolshausen, K. Rasanen, D. I. Bolnick, C. L. Peichel, and A. P. Hendry. 2016. Does plasticity enhance or dampen phenotypic parallelism? A test with three lake-stream stickleback pairs. J. Evol. Biol. 29:126–143.

Oke, K. B., G. Rolshausen, C. LeBlond, and A.P. Hendry. 2017. How parallel is parallel evolution? A comparative analysis in fishes. Am. Nat. 190:1–16.

Olsen, A. M., and M. W. Westneat. 2016. Linkage mechanisms in the vertebrate skull: structure and function of three-dimensional parallel transmission systems. J. Morphol. 277:1570–1583.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7:e37135.

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, et al. 2012. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9:676–682.

Stuart, Y. E., T. Veen, J. N. Weber, D. Hanson, M. Ravinet, B. K. Lohman, C. J. Thompson, T. Tasneem, A. Doggett, R. Izen, et al. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. Nature Ecology & Evolution, 1(6), 0158. http://doi.org/10.1006/jtbi.2000.2043.

Wainwright, P. C. 1996. Ecological explanation through functional morphology: the feeding biology of sunfishes. Ecology 77:1336–1343.

———. 2005. Many-to-one mapping of form to function: a general principle in organismal design? Integr. Comp. Biol. 45:256–262.

Wainwright, P., A. M. Carroll, D. C. Collar, S. W. Day, T. E. Higham, and R. A. Holzman. 2007. Suction feeding mechanics, performance, and diversity in fishes. Integr. Comp. Biol. 47:96–106.

Wainwright, P., and S. Shaw. 1999. Morphological basis of kinematic diversity in feeding sunfishes. J. Exp. Biol. 202(Pt 22):3101–3110.

Walker, J. A. 2007. A general model of functional constraints on phenotypic evolution. Am. Nat. 170:681–689.

Weber, J. N., G. S. Bradburd, Y. E. Stuart, W. E. Stutz, and D. I. Bolnick. 2016. Partitioning the effects of isolation by distance, environment, and physical barriers on genomic divergence between parapatric threespine stickleback. Evolution 71:342–356.

Westneat, M. W. 2003. A biomechanical model for analysis of muscle force, power output and lower jaw motion in fishes. J. Theoret. Biol. 223:269–281.

———. 2004. Evolution of levers and linkages in the feeding mechanisms of fishes. Integr. Comp. Biol. 44:378–389.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Depiction of the measurements used to estimate Suction Index from external photographs.

Figure S2. A comparison of the strength of parallel evolution, and non-parallel evolution, traits and functional coefficients studied here, relative to 86 univariate traits measured in BLINDED et al. 2017, from the same 16 lake-stream pairs in this study.

Table S1. Collection localities and sample sizes used in analyses.

Table S2. Description of component traits used to calculate trophic function.

Table S3. Populations differ from one another in their component traits and functional coefficients.

Table S4. Nearly every pair shows lake-stream divergence in component trait morphology.

Table S5. Lake-stream divergence in functional coefficients is less prominent, relative to component traits.

Table S6. Variation in trophic function explained by component trait data considered three ways: as ratios of a fixed trait, as PC axes 1 and 2 of raw data, and as PC axes 1 and 2 of size-corrected data.