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Context dependence in complex adaptive landscapes: frequency and trait-dependent selection surfaces within an adaptive radiation of Caribbean pupfishes

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The adaptive landscape provides the foundational bridge between micro- and macroevolution. One well-known caveat to this perspective is that fitness surfaces depend on ecological context, including competitor frequency, traits measured, and resource abundance. However, this view is based largely on intraspecific studies. It is still unknown how context-dependence affects the larger features of peaks and valleys on the landscape which ultimately drive speciation and adaptive radiation. Here, I explore this question using one of the most complex fitness landscapes measured in the wild in a sympatric pupfish radiation endemic to San Salvador Island, Bahamas by tracking survival and growth of laboratory-reared F2 hybrids. I present new analyses of the effects of competitor frequency, dietary isotopes, and trait subsets on this fitness landscape. Contrary to expectations, decreasing competitor frequency increased survival only among very common phenotypes, whereas less common phenotypes rarely survived despite few competitors, suggesting that performance, not competitor frequency, shapes large-scale features of the fitness landscape. Dietary isotopes were weakly correlated with phenotype and growth, but did not explain additional survival variation. Nonlinear fitness surfaces varied substantially among trait subsets, revealing one-, two-, and three-peak landscapes, demonstrating the complexity of selection in the wild, even among similar functional traits.

KEY WORDS: Adaptive landscape, adaptive radiation, diversification, ecological speciation, fitness landscape, fitness surface, frequency dependence, macroevolution, natural selection, trophic divergence.

The adaptive landscape is one of the most fundamental unifying concepts in evolutionary biology, integrating microevolutionary processes and macroevolutionary patterns of speciation, niche diversification, and novelty (Wright 1932; Simpson 1944; Arnold et al. 2001). As both a metaphor and a measurement of the complex association between fitness and phenotype or genotype (Lande 1979; Lande and Arnold 1983), the adaptive landscape (frequently a synonym for the individual fitness landscape [Fear and Price 1998]) frames many foundational problems in biology (Svensson and Calsbeek 2012). This includes the frequency and form of natural selection in the wild (Endler 1986; Kingsolver et al. 2001), the complexity of mate choice (Blows et al. 2003; Bentsen et al. 2006), macroevolutionary signatures of adaptive radiation (Hansen 1997; Losos and Mahler 2010; Uyeda and Harmon 2014), evolutionary trajectories (Poelwijk et al. 2007; Lozovsky et al. 2009), and the ruggedness and connectivity of fitness landscapes themselves (Kauffman and Levin 1987; Kauffman and Johnsen 1991; Gavrilets 1999; McCandlish et al. 2015). Even limiting discussion to phenotypic fitness landscapes (Simpson 1944), outstanding discoveries arising from this perspective in wild populations have shaped the field of evolutionary biology, including the paradox of widespread disruptive selection (Kingsolver et al. 2001, 2012; Martin 2012, 2013; Haller and Hendry 2014), lack of phenotypic evolution despite strong directional selection

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(Price and Schluter 1991; Morrissey et al. 2010), many-to-one mapping of morphology to performance to fitness (Arnold 1983; Wainwright et al. 2005; Langerhans 2009), and the extent of temporal and spatial variation in selection (Grant and Grant 2002; Losos et al. 2004; Siepielski et al. 2009, 2013; Kingsolver et al. 2012; Morrissey and Hadfield 2012).

The fitness landscape perspective also requires major caveats. First, it is widely recognized that there is no fixed landscape due to the context-dependence of fitness; a better metaphor might be the frothing surface of a stormy sea (Gavrilets 2004; Svensson and Calsbeek 2012). Resource abundance fluctuates, sometimes dramatically altering selective regimes (Grant and Grant 2002). However, even with a stable unimodal resource distribution, an increasing number of individuals with similar phenotypes competing for the most abundant resources can change a fitness peak to a fitness valley (Dieckmann and Doebeli 1999; Gavrilets 2004; Weissing et al. 2011). Thus, a third metaphor for the fitness landscape is the surface of a trampoline depressed by increasing weight of competitors (Arnold et al. 2001). This process, known as negative frequency-dependent disruptive selection, has been demonstrated in wild populations (Cresswell and Galen 1991; Hori 1993; Bolnick 2004; Koskella and Lively 2009) and is believed to arise from any competitive interaction for shared resources (Doebeli et al. 2005; Haller and Hendry 2014). However, beyond a certain phenotypic distance, competitor frequency should become less relevant to individual fitness. This has some empirical support (Pritchard and Schluter 2001) and is captured by the width of the competition function (kernel) within speciation models (Gavrilets 2004; Arau et al. 2009), but is still largely ignored in discussions of the prevalence of frequency-dependent selection due to a focus on intrapopulation dynamics (Haller and Hendry 2014).

A second major caveat is that organismal fitness reflects the performance of an interacting set of functional traits (Arnold 1983, 2003; Armbruster 1990; Armbruster et al. 2005; Calsbeek and Irschick 2007; Johnson et al. 2008; Langerhans 2009), yet most studies only measure a few morphological traits and neglect correlational selection (Kingsolver et al. 2001, 2012). Many-to-one mapping of morphology to performance (Wainwright et al. 2005) and the nonlinear interactions among functional traits contributing to organismal performance (Holzman et al. 2008, 2012) suggest that a focus on morphology alone may dilute estimates of selection. A few studies have examined selection on performance (Armbruster 1990; Schemske and Bradshaw 1999; Benkman 2003; Calsbeek and Irschick 2007) or dietary variation directly (Arnegard et al. 2014), but more work is needed to connect multivariate phenotypes with performance and fitness.

Third, multivariate selection studies nearly always first reduce the dimensionality of the dataset to two or three axes that best capture the overall phenotypic or fitness variation in the dataset through a variety of approaches, including geometric morphometrics (Adams et al. 2004), principal component analysis (which is ill-advised for selection analyses [Mitchell-Olds and Shaw 1987]), discriminant analysis (Martin and Wainwright 2013a), projection pursuit regression (Schluter and Nychka 1994), canonical rotation (Phillips and Arnold 1989; Blows et al. 2003), or tensor analysis (Calsbeek 2012). However, maximizing the strength of selection along a few multivariate axes may obscure the diversity of trait interactions with fitness. For example, on complex fitness surfaces with multiple fitness peaks, as in this study, multivariate axes can obscure how fitness peaks split and merge or how the form of nonlinear selection varies across different trait subsets corresponding to different functional modules. In part due to the curse of dimensionality and the rarity of multivariate studies of fitness with sufficient power to detect complex landscapes, few studies explore the diversity of nonlinear fitness interactions within their data (Blows et al. 2003; Rundle et al. 2008; Siepielski et al. 2009; Bank et al. 2014). Exploring a greater diversity of trait by fitness interactions provides a more comprehensive view of how high-dimensional fitness landscapes shape multivariate phenotypes and complements multivariate summaries of major selection axes. In turn, different subsets of this landscape can inform genetic, ecological, or biomechanical constraints on phenotypic diversification. For example, different trait interactions with fitness may (1) illuminate high-fitness paths between otherwise distinct fitness peaks (e.g., Gavrilets 1997; Whibley et al. 2006; Poelwijk et al. 2007), (2) illustrate alternative selective regimes acting on developmentally independent aspects of an organism's phenotype (Wagner et al. 2007), (3) enable visualization of selection on specific components of a functional system with known relevance to performance, such as suction-feeding biomechanics (e.g., Carroll et al. 2004; Holzman et al. 2012; Martin 2012), or (4) suggest how existing genetic or phenotypic variance–covariance structures may be modified by selective regimes (i.e., G matrix evolution [Schluter 1996; Brodie and McGlothlin 2007; Arnold et al. 2008; Shaw and Shaw 2014]).

These fundamental discoveries and caveats highlight a major gap in our understanding of phenotypic fitness landscapes in the wild: nearly all investigations estimate linear or quadratic selection surfaces within populations of one or two species. Very few studies use phenotypic manipulation of any kind (Schluter 1994; Hauser et al. 1998; Schemske and Bradshaw 1999; Schmitt et al. 1999; Svensson and Sinervo 2000; Stelkens et al. 2008; Huang et al. 2010; Keagy et al. 2015); measure more than a few traits (Blows et al. 2003; McBride and Singer 2010); or study the behavioral, performance, or dietary traits expected to be most directly shaped by selection (Armbruster 1990; Benkman 1993, 2003; Korves et al. 2007; Johnson et al. 2008; Langerhans 2009; Arnegard et al. 2014). Thus, despite substantial theoretical attention, we still have a very poor understanding of the structure of fitness landscapes at larger scales: among multiple species and across complex features of the landscape, such as the distance between fitness peaks, the shape of an adaptive ridge, or the width of a fitness valley. These concepts, which may affect speciation in important ways (e.g., Kramer and Donohue 2006; Martin and Feinstein 2014), cannot be described by the traditional categories of stabilizing or disruptive selection. Indeed, the entire machinery for measuring multivariate selection gradients is ill-equipped for dealing with the complex features that define any "landscape" and can provide systematically misleading estimates (Mitchell-Olds and Shaw 1987; Shaw and Geyer 2010). Furthermore, detecting these nonlinear features requires larger sample sizes (Hersch and Phillips 2004; Kingsolver and Pfennig 2007; Morrissey and Hadfield 2012) and the power to detect selection is reduced by measuring additional traits and increasing trait covariance (Mitchell-Olds and Shaw 1987). Thus, sufficient statistical power and the curse of dimensionality are two of the greatest barriers to future research. Although complex landscapes can be measured without phenotypic manipulation (Benkman 2003; Blows et al. 2003; Pfaender et al. 2016), to the best of my knowledge, only a single study has both manipulated phenotype distributions and examined individual fitness surfaces across more than two species (Martin and Wainwright 2013a), yet this approach is one of the most desirable for directly measuring the large-scale structure of fitness landscapes driving macroevolutionary processes, such as adaptive radiation.

The rarity of studies investigating large-scale fitness landscapes is due in part to the rarity of systems with closely related species amenable to phenotypic manipulation (such as hybrid crosses), large sample sizes, and fitness measurements in the wild. An adaptive radiation of pupfishes endemic to San Salvador Island, Bahamas is a nearly ideal system for investigating fitness landscapes due to the rapid evolution of three sympatric species, a generalist (*Cyprinodon variegatus*) and two novel specialist species: a scale-eater (*C. desquamator*) and a molluscivore (*C. brontotheroides*), in several hypersaline lakes on the island within the past 10,000 years (Holtmeier 2001; Turner et al. 2008; Martin and Wainwright 2011). These species have a four-month generation time, high fecundity, and can be crossed to generate fertile and viable hybrids, yet remain largely reproductively isolated in sympatry (Martin and Wainwright 2011, 2013c; Martin and Feinstein 2014; West and Kodric-Brown 2015), in contrast to most *Cyprinodon* species (Rosenfield and Kodric-Brown 2003; Tech 2006; Martin et al. 2016). Previous measurements of the growth and survival of F2 hybrids between all pairs of the three species demonstrated the presence of a complex fitness landscape driving rapid trait diversification, up to 51 times faster than other pupfishes (Martin and Wainwright 2011, 2013a), assuming that the current selective environment maintaining reproductive isolation also initiated divergence. Each hybrid was raised in a common garden laboratory environment, individually tagged and photographed, and placed in high- and low-density field enclosures in two of their natural lake environments for three months to measure growth and survival relative to phenotype. Intriguingly, hybrids most similar to the generalist phenotype resided on a local fitness peak surrounded by a fitness valley in all directions isolating this species from a significantly higher fitness peak corresponding to the phenotype of the molluscivore specialist (Fig. 1). In contrast, hybrids most similar to the scale-eater suffered reduced survival and growth rate in all four enclosures in both lakes, indicating the presence of a large fitness valley separating scale-eaters from generalist phenotypes (Fig. 1). This complex fitness landscape illustrates the classic problem of a population stranded on a local fitness optimum despite higher neighboring optima and suggests a possible explanation for the rare evolution of specialist pupfishes across the entire Caribbean (Martin and Wainwright 2013a).

Here I present new analyses of these fitness data to explore how three prevailing assumptions from within-population studies of natural selection scale up to a complex fitness landscape with multiple fitness peaks spanning a radiation of three species. (1) Is there evidence of negative frequency-dependent selection? Models and experimental results predict that extreme hybrid phenotypes at the edges of morphospace with few competitors should show the highest fitness. (2) Do stable isotope ratios, a measure of dietary carbon source and trophic position over the experimental period, better explain variation in fitness than the observed set of morphological traits? Direct selection on trophic niche, rather than the limited set of measured morphological traits, may be easier to detect due to selection on foraging performance and the many-to-one mapping of morphology to trophic niche. (3) How do complex fitness surfaces change across different sets of functional traits implicated in rapid diversification? Traditional analyses of only multivariate selection axes may obscure the diversity of complex trait interactions with fitness.

Methods

FIELD EXPERIMENT

The field experiment on San Salvador Island, Bahamas has been described previously (Martin and Wainwright 2013a). In brief, captive colonies of all three species from two different lake populations (Crescent Pond and Little Lake; interlake generalist × generalist $F_{st} = 0.08$; interlake generalist × scale-eater $F_{st} = 0.29$ [Martin and Feinstein 2014]) were brought to the laboratory and intercrossed in all directions within each lake population for two generations to generate both F2 intercrosses and backcrosses for the second-generation juvenile animals used in the experiment. Hybrid populations were outcrossed in the second generation to avoid any deleterious effects of inbreeding and generated

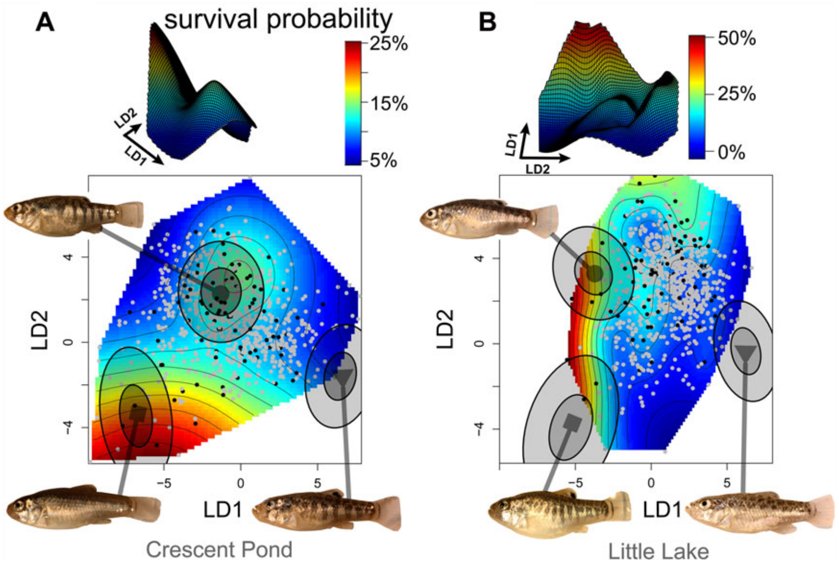

Figure 1. **Fitness landscapes for *Cyprinodon* pupfishes in field enclosures on San Salvador Island, Bahamas (modified from Martin and Wainwright 2013a). Three- and two-dimensional fitness surfaces (thin-plate splines) illustrate the probability of survival (heat color) relative to phenotypic position within discriminant morphospace in high-density field enclosures in (A) Crescent Pond ($n = 796$ hybrids) and (B) Little Lake ($n = 875$ hybrids). Shaded gray 50% (inner) and 95% (outer) confidence ellipses represent the phenotypes for the three parental species in each lake, with representative photographs shown (upper left: ● generalist *Cyprinodon variegatus*; lower left: ■ molluscivore *Cyprinodon brontotheroides*; middle right: ▲ scale-eater *Cyprinodon desquamator*). F2 hybrid survivors (small black circles) and nonsurvivors (small gray circles) after three months in the field enclosures are plotted within the discriminant morphospace.**

independently for each lake. One backcross to the molluscivore species was missing from the Little Lake hybrid population, resulting in a substantial reduction in coverage of morphospace and detection of only a single marginal region of increased fitness (Fig. 1B); thus, I focus on the multipeak fitness landscape measured in the high-density Crescent Pond field enclosure in most analyses and discussion and exclude the low-density treatments in each lake due to their low sample size. Thus, the current study is not replicated within each lake, preventing inference about within-lake differences in fitness.

F2 hybrids were reintroduced to one high-density and one low-density field enclosure in the same original source lake to prevent inadvertent gene flow (Crescent Pond high/low density, $n = 796/96$; Little Lake high/low density, $n = 875/98$ F2 hybrid individuals). The similar numbers of survivors in each high-density enclosure after three months (91/97) suggest that this initial density was about eight times higher than the natural adult carrying capacity of these enclosures, but this juvenile density mimics the very high fecundity of wild populations and low offspring survivorship. Hybridization does occur naturally in the wild at low frequency (Martin and Feinstein 2014) and each lake population reaches tens to hundreds of millions of fish, so reintroducing hybrids poses no risk of hybrid collapse to the wild species. Tagging, photographing, and recapture of surviving hybrids after three months is described in the Supporting Information.

STATISTICAL ANALYSES

A two-dimensional morphospace for each high-density field enclosure was estimated as described previously (Martin and Wainwright 2013a). In brief, all F2 hybrids and parental individuals of all three species in each lake were measured for 16 traits from their prerelease photographs (F2 hybrids) or photographs of juveniles of similar size (for each laboratory-reared parental population). Size-corrected trait values were standardized to an SD of 1 and mean 0 to scale traits equally in all multivariate analyses (Lande and Arnold 1983). Standardized size-corrected trait values were used in a linear discriminant analysis to identify the two multivariate axes best distinguishing among the three parental species using the MASS package (Venables and Ripley 2013) in R statistical software (R Core Team 2015). These two discriminant axes (LD1 and LD2) were used to plot a discriminant morphospace, which functions as a two-dimensional "hybrid index" to visualize overall phenotypic similarity of each F2 hybrid to each of the three parental species (represented by 95% and 50% confidence ellipses in figures).

The shape of the fitness landscape was estimated nonparametrically using a thin-plate spline in the Fields package (Fields Development Team 2011), as originally recommended by Schluter (1988), to avoid forcing quadratic assumptions on highly nonlinear surfaces. The curvature of the surface (defined as the effective degrees of freedom [edf] for a spline) was estimated by generalized cross-validation, a jack-knifing procedure for optimizing the shape of the nonparametric surface to minimize residual prediction error (Fields Development Team 2011). Traditional multiple regression analyses of multivariate selection gradients across the full landscape and specific transects (Lande and Arnold 1983), canonical rotation of the multivariate trait space (Phillips and Arnold 1989), and nonparametric projection pursuit regression analysis (Schluter and Nychka 1994) were described previously (Martin and Wainwright 2013a).

Frequency dependence across the fitness landscape was measured by comparing the residual survival probability of each hybrid with the phenotypic distance to its nearest-neighbors in morphospace. For each hybrid survivor in each high-density enclosure, I calculated the Euclidean phenotypic distance to the 10 nearest-neighbors within the two-dimensional discriminant morphospace using the FNN package in R (Boltz et al. 2007). This distance estimates the density of hybrids within local regions of the discriminant morphospace. Because these local regions are defined by phenotypically similar hybrids, this distance is a measure of the abundance of hybrids with similar phenotypes and will be referred to as "competitor frequency." I also note that this metric is only correlational; it is still necessary to manipulate frequency to assess the frequency dependence of fitness landscapes.

Generalized additive models were used to compare the fit of models with only the thin-plate spline (the fitness landscape within the discriminant morphospace) to models incorporating the additional effect of competitor frequency as a linear fixed effect, smoothing spline, or thin-plate spline for both survival and growth rate using the mgcv package in R (Wood 2011). Nested models were compared using likelihood ratio tests. If the fitness landscape is frequency-dependent, I predict that models incorporating competitor frequency should best explain the fitness data.

To examine the relationship between frequency dependence and survival, I used the residual survival probability of each hybrid relative to the thin-plate spline fitness landscape in Figure 1 (excluding nonsurvivors unlike the previous model comparison tests). Each survivor has a residual survival probability unexplained by the thin-plate spline relating survival to position in the discriminant morphospace. An additional caveat is that these residuals have associated error unaccounted for by this analysis. I tested the relationship between residual survival probability and competitor frequency using nonparametric and parametric models. Generalized additive models were used to estimate a smoothing spline and 95% confidence interval. Generalized linear models were used to test the significance of linear and quadratic terms. Phenotype–diet associations were tested using a similar approach described in the Supporting Information.

Table 1. **Comparison of generalized additive models estimating the effects of hybrid phenotype and competitor frequency (phenotypic distance to the 10 nearest-neighbors) on survival and growth rate in Crescent Pond and Little Lake high-density field enclosures.**

Lake	Model	Deviance	P
Crescent Pond	Survival ~ s(LD1, LD2)	–	
	Survival ~ s(LD1, LD2) + frequency	0.311	0.474
	Survival ~ s(LD1, LD2) + s(frequency)	0.079	0.052·
	Survival ~ s(LD1, LD2, frequency)	5.345	0.090
	Growth ~ s(LD1,LD2)	–	
	Growth ~ s(LD1,LD2) + frequency	0.809	0.318
	Growth ~ s(LD1,LD2) + s(frequency)	−0.00001	**0.001***
Little Lake	Survival ~ s(LD1, LD2)	–	
	Survival ~ s(LD1, LD2) + frequency	1.367	0.243
	Survival ~ s(LD1, LD2) + s(frequency)	0.098	0.058·
	Survival ~ s(LD1, LD2, frequency)	6.973	0.317
	Growth ~ s(LD1,LD2)	–	
	Growth ~ s(LD1,LD2) + distance	2.946	0.219
	Growth ~ s(LD1,LD2) + s(frequency)	1.909	**0.034***

The relationship between hybrid phenotype in the two-dimensional discriminant morphospace and fitness was estimated with a thin-plate spline, s(LD1, LD2), as visualized for survival probability in Figure 1. Competitor frequency was modeled as a fixed effect, smoothing spline, or third dimension of the thin-plate spline, respectively. Relative to the phenotype-only thin-plate spline model, models providing a significantly better fit to the data as indicated by a likelihood ratio test are highlighted with a bold asterisk; trending models are indicated with a dot.

Finally, exploring selection surfaces across the entire 16-dimensional trait space was prohibitive. Existing nonparametric approaches (e.g., projection pursuit regression [Schluter and Nychka 1994], tensor analysis [Calsbeek 2012], and semiparametric models [Wood 2011]) only search for major multivariate axes of selection or make overly restrictive assumptions (such as additivity), rather than systematically exploring the diversity of nonlinear trait interactions with selection. Thus, to make minimal assumptions about the structure of the data and evaluate selection surfaces across subsets of traits, I used thin-plate splines to systematically compare all pairwise interactions for the six traits with the highest loadings on discriminant axes 1 and 2, which best discriminate among the three species in the San Salvador radiation (Martin and Wainwright 2013a). For each pair of traits, I fit a thin-plate spline to the survival data by generalized cross-validation (Fields Development Team 2011) to estimate the fitness landscape and edf of this surface, which provides a quantitative metric of the nonlinearity, curvature, or "complexity" of any spline surface, terms which I use interchangeably here (Martin and Wainwright 2013a). Thin-plate splines were fit separately for each pair of traits and compared to univariate smoothing splines with 95% confidence intervals for each trait and to discriminant axes based on subsets of six traits, rather than the full 16. I assessed statistical confidence in survival surfaces by plotting SE contours estimated from general additive models for each thin-plate spline.

Results

FREQUENCY-DEPENDENT SELECTION

Generalized additive models including the nonlinear effect of competitor frequency on hybrid fitness fit the data only moderately better than models with linear terms or no frequency term for both survival and growth rate in each lake replicate (Table 1). I visualized the relationship between residual hybrid survival probability relative to competitor frequency among only the survivors; however, an important caveat is that these residuals are also sub-

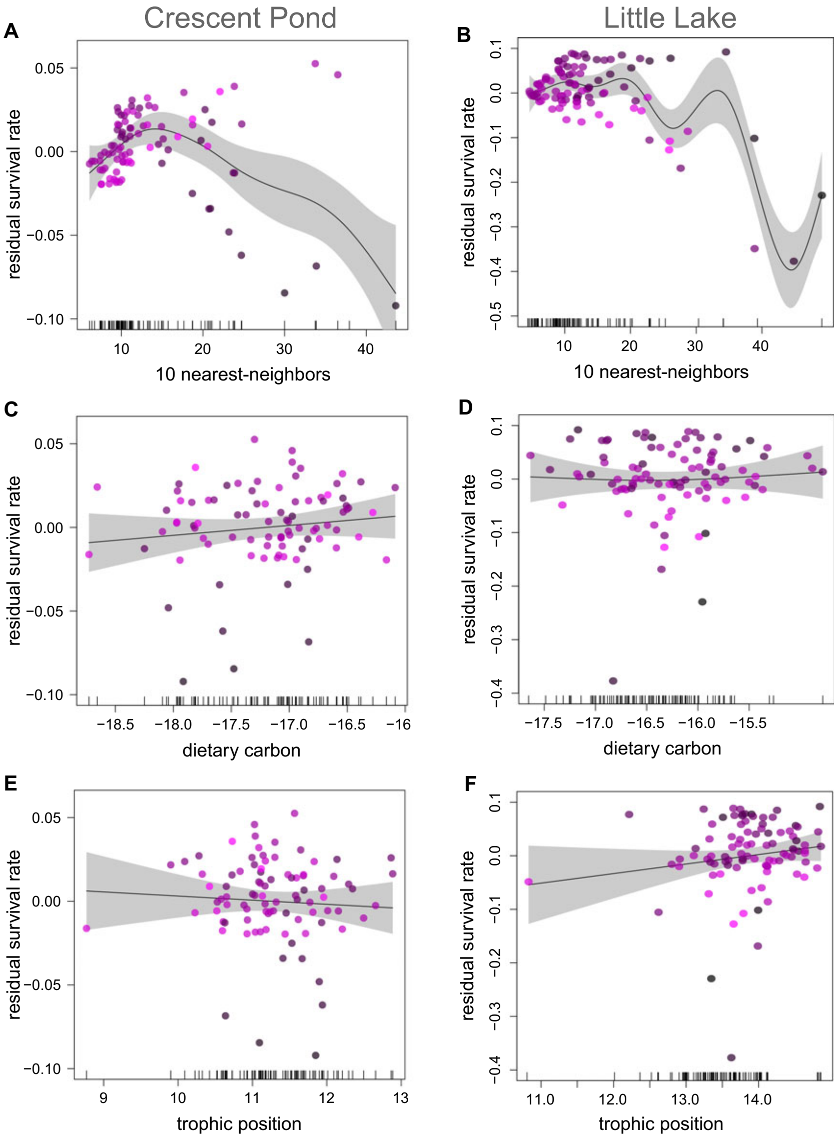

Figure 2. Residual survival probability for each F2 hybrid (relative to the thin-plate splines depicted in Fig. 1) and its association with (A-B) the frequency of competitors estimated from the total morphological distance to the 10 nearest-neighbors, (C-D) dietary carbon source estimated from δ13C stable isotope ratios, and (E-F) relative trophic position estimated from δ15N stable isotope ratios. The first and second columns show results from Crescent Pond ($n = 91$ survivors) and Little Lake ($n = 97$ survivors) high-density field enclosures, respectively. Shaded areas indicate the 95% confidence interval for the smoothing spline (black line) estimated using a general additive model. Magenta coloration indicates hybrid phenotypic position along morphological discriminate axis 2 separating generalist (lighter) from molluscivore (darker) phenotypes.

ject to error. As the frequency of hybrids with similar phenotypes decreased, residual survival probability first increased slightly, consistent with the predicted negative frequency-dependent selection, then leveled off and began to decrease, indicating positive frequency-dependent selection (Fig. 2A, B). This pattern was observed in both high-density enclosures generated from independent F2 hybrid populations. The smoothing spline for competitor frequency was significantly associated with residual survival probability in both lakes (Fig. 2A, B; generalized additive model—Crescent Pond: edf $= 4.081$, $P = 0.00001$; Little Lake: edf $= 8.397$, $P = 2 \times 10^{-16}$). A generalized linear model with linear and quadratic terms for competitor frequency indicated significant stabilizing selection on competitor frequency (quadratic selection gradients for standardized competitor frequency: Crescent Pond, $2\gamma = -0.01 \pm 0.001$, $P = 0.0002$; Little Lake, $2\gamma = -0.038 \pm 0.004$, $P < 0.00001$). Estimating competitor frequency from the five or 20 nearest-neighbors produced qualitatively similar results. Furthermore, this frequency-dependent relationship appeared to be spread throughout the discriminant morphospace; hybrids with low or high residual survival probabilities were not concentrated along discriminant axis 2, such as within the molluscivore fitness peak, as indicated by their relative magenta coloration (Fig. 2A, B).

Thus, among very similar phenotypes within 1 standardized phenotypic SD on the discriminant morphospace (10 nearest-neighbor distance < 10), there was evidence of the predicted negative frequency-dependent selection on survival due to decreasing frequency of competing hybrid phenotypes, providing an approximately 5% increase in survival (Fig. 2A, B; Table 1). In contrast, there was evidence of strong selection against hybrid phenotypes in regions of low competitor frequency (more than 1 SD away from their 10 nearest-neighbors). This was due to decreased survival of the most extreme transgressive hybrid phenotypes at the edges of morphospace outside the parental range. At the edges of the hybrid phenotypic range, survival decreased by 5–10% relative to high-density regions (Fig. 2A, B).

DIET-DEPENDENT SELECTION

Stable isotope analyses of wild-caught individuals indicated substantial separation among the three species within each lake (Fig. 3A, B). The highest trophic position was occupied by adult scale-eating pupfish, molluscivore specialists were intermediate, and generalists were lowest (Martin and Wainwright 2013b). Diets of F2 hybrids in both high- and low-density field enclosures were shifted toward lower trophic positions and macroalgae-dominated carbon sources, suggestive of substantial competition for limited resources (Fig. 3A, B). In Little Lake, F2 hybrid diets overlapped with the isotopic composition of macroalgae, providing some indication of their trophic position within field enclosures, although due to many-to-one mapping of dietary resource to isotopic signature this does not mean that their diets were entirely composed of macroalgae (Post 2002). Despite the shift in absolute isotope ratios, there was substantial variation in F2 hybrid isotopic ratios, comparable to the variation present in wild-caught individuals (Fig. 3A, B).

F2 hybrid morphology was weakly associated with dietary carbon and relative trophic position, indicated by their carbon and nitrogen stable isotope ratios, respectively, at the conclusion of the experiment. In Crescent Pond, dietary carbon was correlated with discriminant axis 1 ($r^2 = 0.045$, $P = 0.045$) but not discriminant axis 2 ($r^2 = 0.0002$, $P = 0.893$), indicating differences between hybrids resembling the scale-eater relative to other hybrids in their sources of dietary carbon (Fig. 3C). Conversely, relative trophic position was correlated with discriminant axis 2 ($r^2 = 0.060$, $P = 0.020$) but not discriminant axis 1 ($r^2 = 0.004$, $P = 0.539$), indicating differences between hybrids resembling the molluscivore relative to other hybrid phenotypes in trophic position (Fig. 3E). However, morphological discriminant axes were not correlated with dietary carbon ($P > 0.20$) or relative trophic position ($P > 0.50$) in Little Lake, perhaps due to the lower coverage of morphospace (Fig. 3D, F). Thin-plate splines fit to isotopic data relative to the discriminant morphospace indicated no nonlinear interactions between morphological discriminant axes and diet, except for stabilizing selection on trophic position in Little Lake (Fig. 3F).

There were no significant associations between residual survival probability and either dietary carbon (Fig. 2C, D: Crescent Pond: edf $= 1$, $P = 0.272$; Little Lake: edf $= 1.342$, $P = 0.76$) or relative trophic position (Fig. 2E, F: Crescent Pond: edf $= 1$, $P = 0.272$; Little Lake: edf $= 1$, $P = 0.134$) in either high-density enclosure. In both lakes, dietary carbon and relative trophic position had significant and interacting effects on growth rate (Table 2). In Little Lake, these dietary isotopes also interacted with competitor frequency (Table 2). As hybrids shifted their dietary carbon source, they also shifted their trophic position with corresponding effects on their growth rates. Interpretation of these patterns is complicated by the strong negative relationship between trophic position and growth rate in Crescent Pond, consistent with enrichment of δ15N due to food limitation (McCue and Pollock 2008). This suggests strong competition for limited resources in Crescent Pond, independent of competitor frequency. In contrast, the greater δ15N enrichment of Little Lake (likely due to longer food chains in this larger lake [Post et al. 2000]) prevents direct comparison between lakes and may have obscured the signal of food limitation.

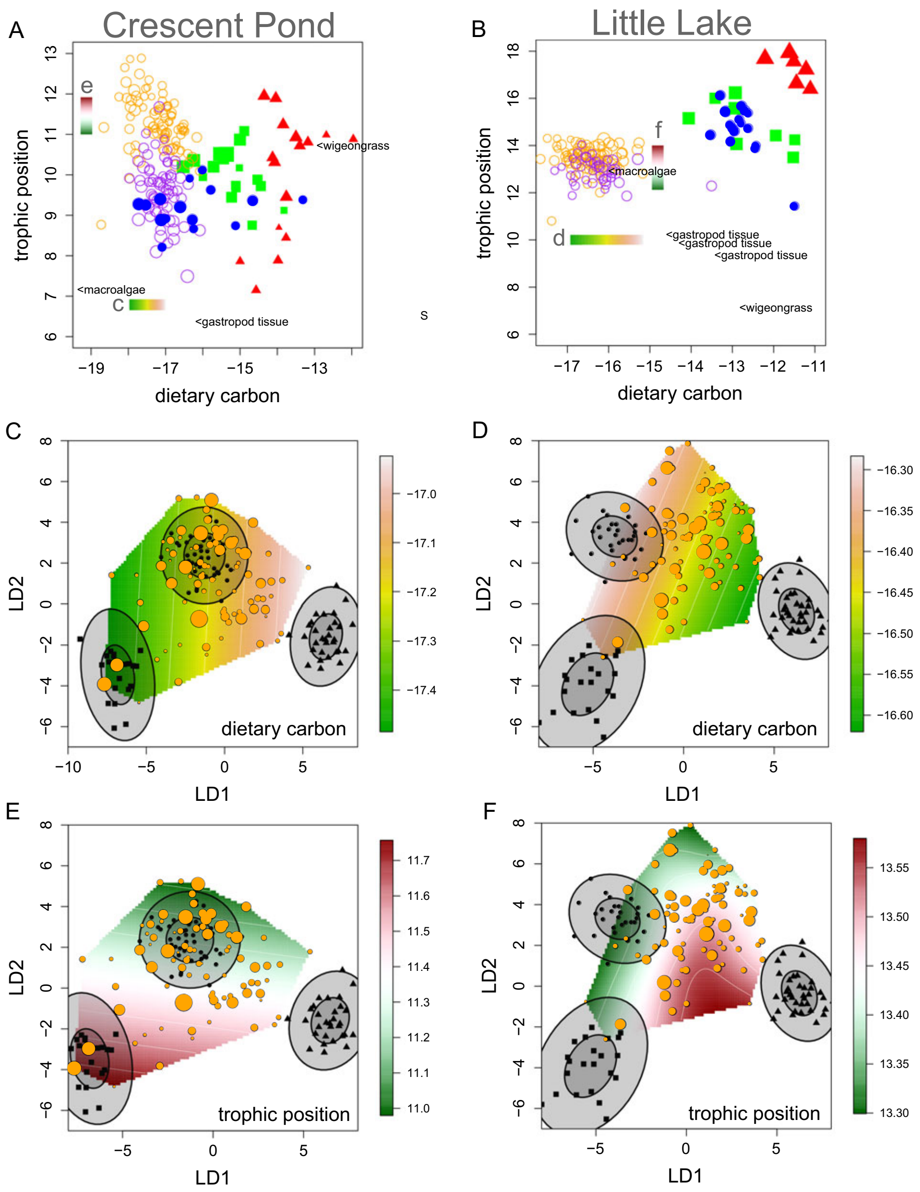

Figure 3. (A-B) Dietary isotope space. Carbon (δ13C) and nitrogen (δ15N) stable isotope ratios in wild-caught pupfishes (●, generalist *Cyprinodon variegatus*; ■, molluscivore *Cyprinodon brontotheroides*; ▲scale-eater *Cyprinodon desquamator*; relative size of each shape is proportional to the standard length of each fish), F2 hybrids in high-density (○; CP *n* = 91; LL *n* = 97) and low-density enclosures (○; CP *n* = 96; LL *n* = 98), and key resources (macroalgae: *Batophora oerstedii*; wigeongrass: *Ruppia maritima*; gastropod tissue: *Cerithium* sp.). Data from Crescent Pond and Little Lake are presented in the first and second columns, respectively. The approximate range of the thin-plate splines from lower panels is represented in the stable isotope dietary space in panels (A-B). (C-D) Dietary carbon source relative to hybrid phenotype. Thin-plate splines estimated for dietary carbon source (δ13C) relative to position in discriminant morphospace for all F2 hybrid survivors in the two high-density enclosures (●, relative size of each circle is proportional to the growth rate of each hybrid survivor) with shaded gray 95% (outer) and 50% (inner) confidence ellipses representing phenotypic position of parental species as in Figure 1 (●, generalist *C. variegatus*; ■, molluscivore *C. brontotheroides*; ▲, scale-eater *C. desquamator*). (E-F) Trophic position relative to hybrid phenotype. Thin-plate splines estimated for trophic position (δ15N) relative to position in discriminant morphospace for all F2 hybrid survivors in the two high-density enclosures. Symbols as described for (C) and (D).

TRAIT-DEPENDENT SELECTION SURFACES

Univariate analyses of selection on each trait generally revealed little variation in selection (Fig. 4). The only exception was body depth, which exhibited fitness peaks for hybrids resembling two of the three parental species (Fig. 4F) and the greatest amount of univariate nonlinearity among the five traits (Table 3). There was

Table 2. **Generalized linear models estimating the effects of dietary isotopes (δ13C: dietary source of carbon; δ15N: relative trophic position or a starvation index) and competitor frequency (phenotypic distance to the 10 nearest-neighbors in the discriminant morphospace) on growth rate in Crescent Pond and Little Lake high-density field enclosures.**

lake	model term	estimate ± SE	*P*
Crescent Pond	frequency	0.01 ± 0.010	0.246
	δ13C	**3.9 ± 1.92**	**0.048**
	δ15N	**−7.0 ± 3.13**	**0.027**
	δ13C: δ15N	**−0.38 ± 0.18**	**0.036**
Little Lake	**Frequency**	**3.2 ± 0.83**	**0.0002**
	δ13C	**−2.5 ± 0.72**	**0.0007**
	δ15N	**2.9 ± 0.87**	**0.001**
	Frequency: δ13C	**0.20 ± 0.05**	**0.0002**
	Frequency: δ15N	**−0.23 ± 0.06**	**0.0003**
	δ13C: δ15N	**0.18 ± 0.05**	**0.0008**
	Frequency: δ13C: δ15N	**−0.01 ± 0.004**	**0.0002**

Significant effects are highlighted in bold. Nonsignificant interaction terms in the full model for Crescent Pond were removed.

substantial variation in the amount of transgressive variation in each trait. For example, lower jaw length was shorter than all three parental species in over half the F2 hybrids (Fig. 4A), whereas nasal protrusion distance did not exceed parental values in any of the F2 hybrids (Fig. 4C).

In contrast to the univariate splines, pairwise interactions between six major functional traits and survival illustrated substantial nonlinearity and dependence of the complexity of fitness landscapes on different trait combinations (Figs. 5 and 6; Table 3). This ranged from flat surfaces with only directional selection (edf = 3 in Table 3), single fitness peaks indicating stabilizing selection, and complex fitness landscapes with two or three fitness peaks. Although it is not typical to assess statistical error of thin-plate splines (e.g., Blows et al. 2003; Keagy et al. 2015; Pfaender et al. 2016), which are sensitive to outliers and largely a visualization technique (Schluter 1988; Boer et al. 2001), I note that there is substantial error associated with these surfaces (Fig. S1), so the observed variation should be interpreted with caution.

Highly nonlinear surfaces were only apparent in Crescent Pond (Fig. S2, Table 3); however, patterns of directional selection in Little Lake were largely consistent with regions of high fitness within Crescent Pond (Fig. S2). Fitness measurements were not replicated within lakes, so it is unclear whether this variation represents differences in selection between lakes or is due to the missing backcross and reduced sampling of hybrid variation in the Little Lake enclosure. Neither F2 hybrid population sampled the extreme large-jawed phenotypes corresponding to the wild-type scale-eater (e.g., note few samples within red scale-eater ellipse in

Table 3. **Nonlinear estimates of the curvature of fitness surfaces (edf = effective degrees of freedom) for six functional traits which discriminate among the three sympatric pupfish species on San Salvador and define four separate functional modules: jaw size, nasal protrusion, eye size, and body elongation.**

	Crescent Pond high-density field enclosure							
	LD1	LD2	Lower jaw	Upper jaw	Nasal protrusion	Nasal angle	Body depth	Orbit diameter
Lower jaw length	−0.08	−0.17	2.1	6.3	6.2	6.2	15.9	3
Upper jaw length	−0.24	−0.71		4.0	6.2	6.8	10.6	6.2
Nasal protrusion	−0.90	−0.78			1.8	3	9.8	3
Nasal angle	−0.21	−1.34				1.1	6.2	3
Body depth	−1.53	−1.04					8.8	3
Orbit diameter	−0.73	0.79						1.8
	Little Lake high-density field enclosure							
Lower jaw length	0.32	−0.65	1.6	3	3	3	3	6.6
Upper jaw length	−0.54	0.35		1.0	3	3	3	3
Nasal protrusion	−0.74	−1.55			1.0	3	3	3
Nasal angle	−0.07	−0.63				1.0	3	3
Body depth	−0.67	0.48					1.5	3
Orbit diameter	−0.50	0.20						1.0

LD1 = loadings on discriminant axis 1 separate the scale-eating pupfish *C. desquamator* from other species (loadings from 10 other traits not shown); LD2 = loadings on discriminant axis 2 separate the molluscivore pupfish *Cyprinodon brontotheroides* from other species. The diagonal shows the estimated nonlinearity of each trait relative to survival (edf estimated from a smoothing spline; 1 indicates a flat line; selection curves with 95% confidence intervals are presented in Fig. 4 for each trait). The off-diagonal triangular matrix shows the estimated nonlinearity between each pairwise trait interaction and survival (edf estimated from a thin-plate spline; 3 indicates a flat surface; selection surfaces for this matrix, including SE contours, are presented in Figs. 5 and 6 and S1 and S2). Moderately and highly nonlinear surfaces are highlighted in gray and dark grey, respectively.

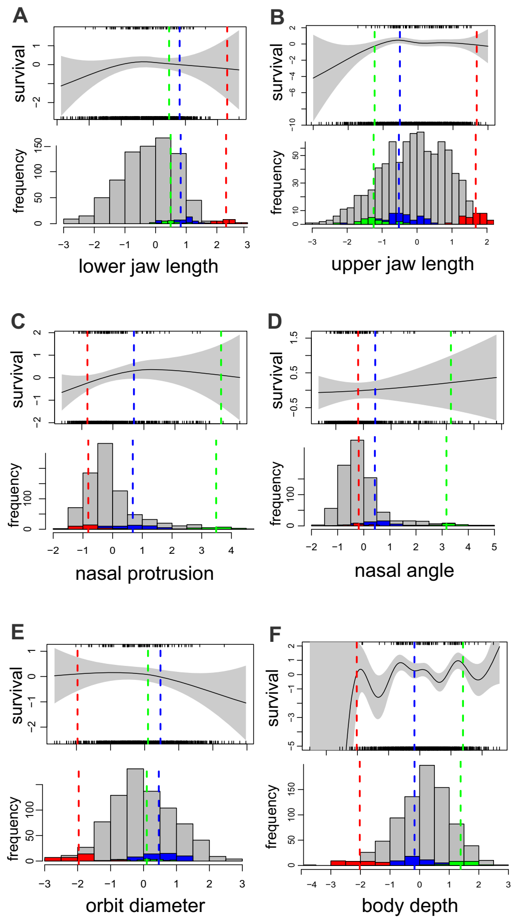

Figure 4. For each of the six major functional traits distinguishing among the three species within the San Salvador pupfish radiation, smoothing splines (black lines) with 95% shaded confidence regions indicate probability of survival (upper panel) and histograms (lower panel) illustrate trait range within the F2 hybrid population relative to parental trait ranges and mean (dashed line) for each parental species (blue, generalist *Cyprinodon variegatus*; green, molluscivore *Cyprinodon brontotheroides*; red, scale-eater *Cyprinodon desquamator*). Rug plots indicate trait values of F2 hybrid survivors (upper axis) and deaths (lower axis). All data presented is from the high-density enclosure in Crescent Pond (n = 796).

Fig. 1), so much remains to be determined about the topography and concordance of fitness landscapes in these lakes.

The complexity of two-way fitness surfaces varied across the different classes of functional traits examined. Body depth exhibited the greatest amount of nonlinearity with four of five trait interactions displaying two or more fitness peaks (Table 1; Figs. 5 and 6); orbit diameter exhibited the least amount, exhibiting flat surfaces ($n = 4$) or stabilizing selection ($n = 1$) in all five trait interactions. Upper and lower jaw lengths exhibited the second highest amount of nonlinearity: out of nine interactions, stabilizing selection was indicated in seven, two-peak landscapes in two, three-peak in one, and flat surfaces in only one interaction. Jaw size is threefold larger in scale-eaters (Martin and Wainwright 2011; Martin 2016), and exhibited the greatest amount of transgressive variation in F2 hybrids (Fig. 4). Finally, the functional module of nasal protrusion and nasal angle loaded strongly on discriminant axis 2, which separates the molluscivore specialist from other species (Fig. 1, Table 3), and defines its unique skeletal nasal protrusion. In contrast to jaw length, four of nine interactions with this pair were flat fitness surfaces (Figs. 5 and 6); however, three of nine interactions were two-peak landscapes.

Despite the complexity and apparent nonadditivity of two-way trait interactions with fitness, combining these six functional traits into two multivariate discriminate axes produced a similar fitness landscape to the full 16-trait dataset, only with less phenotypic separation among the parental species (Fig. S3). A different subset of six traits with less functional relevance produced a landscape with similar patterns of directional selection and the least amount of separation among parental phenotypes (Fig. S3).

Discussion

This study provides one of the first investigations of whether lessons from within-population studies of natural selection in the wild apply to larger fitness landscapes spanning multiple species. I focused on three major predictions from within-population analyses: (1) selection is negative frequency-dependent, (2) selection indirectly shapes trophic morphology through its direct effect on diet, and (3) multivariate selection is additive with a similar form across functionally related traits. Surprisingly, none of these predictions were completely supported, suggesting that the dynamics and context dependence of large-scale fitness landscapes driving adaptive radiation are qualitatively different than the context-dependence of fitness observed within populations. This has important implications for our understanding of the speciation process, adaptive radiation, and the fitness landscape bridge between micro- and macroevolutionary diversification (Arnold et al. 2001; Erwin 2015).

NEGATIVE FREQUENCY-DEPENDENT SELECTION HAS A LOCAL WITHIN-POPULATION SCALE

Within replicated field enclosures in different lakes, increasing frequencies of hybrids with similar phenotypes depressed fitness as predicted only within regions of morphospace with very high densities of hybrids (Fig. 2A, b). This negative-frequency dependence spanned individuals that were within 1 SD within the discriminant morphospace. At the edges of morphospace, which comprised sparse transgressive hybrid phenotypes exceeding the parental range, hybrids survived poorly regardless of the frequency of similar phenotypes (Fig. 2A, B). This observation counters predictions from adaptive dynamics (Dieckmann and

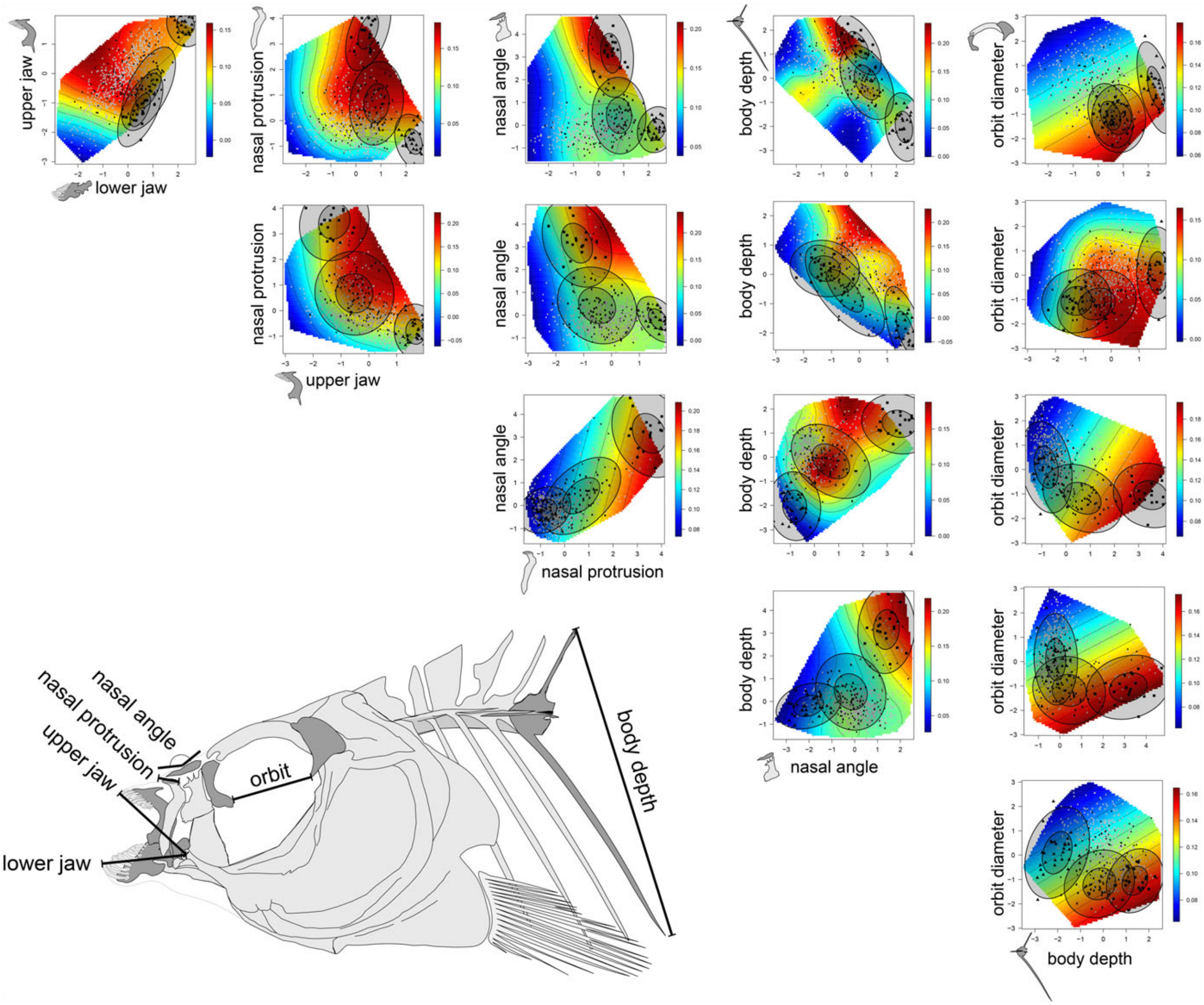

Figure 5. Pairwise interaction plot for all selection surfaces estimated for the six major functional traits best distinguishing among the three San Salvador species (schematic of linear distances measured from prerelease photographs of anesthetized hybrids is presented in lower left). The relationship between each pair of traits and survival in the high-density field enclosure in Crescent Pond was estimated with thin-plate splines (Little Lake presented in Fig. S1); estimated nonlinearity of each selection surface (edf estimated from generalized cross-validation) is presented in Table 3; ±1 SE contours of fitness surfaces are presented in Fig. S1. Shaded gray 95% (outer) and 50% (inner) confidence ellipses and bolded symbols represent phenotypic position of parental individuals (●, generalist *Cyprinodon variegatus*; ■, molluscivore *Cyprinodon brontotheroides*; ▲, scale-eater *Cyprinodon desquamator*). F2 hybrid individuals (n = 796) spanning the range of each two-dimensional morphospace are plotted as small black circles (survivors) or small gray circles (deaths).

Doebeli 1999; Gavrilets 2004; Weissing et al. 2011), widespread expectations of fitness dynamics (Otto et al. 2008; Haller and Hendry 2014), and field experimental results (Hori 1993; Schluter 1994; Bolnick 2004; Olendorf et al. 2006; Koskella and Lively 2009): rare phenotypes, presumably able to exploit underutilized resources, should have a survival advantage. In contrast, in this study the scale of competition appears to be localized to hybrids within about 1 SD away from each other within morphospace (Fig. 2A, B), approximately corresponding to the amount of variation present within each of the purebred populations and the variation spanning a single fitness peak on the fitness landscape (Fig. 1). At larger phenotypic distances, F2 hybrids may no longer directly compete with each other for resources, despite sharing the same 3 m field enclosure.

One important caveat is that phenotypic variation in the F2 hybrid population did not recover the parental scale-eating phenotype for most traits (Fig. 1), most likely due to the genetic architecture of these traits (Albertson and Kocher 2005): none

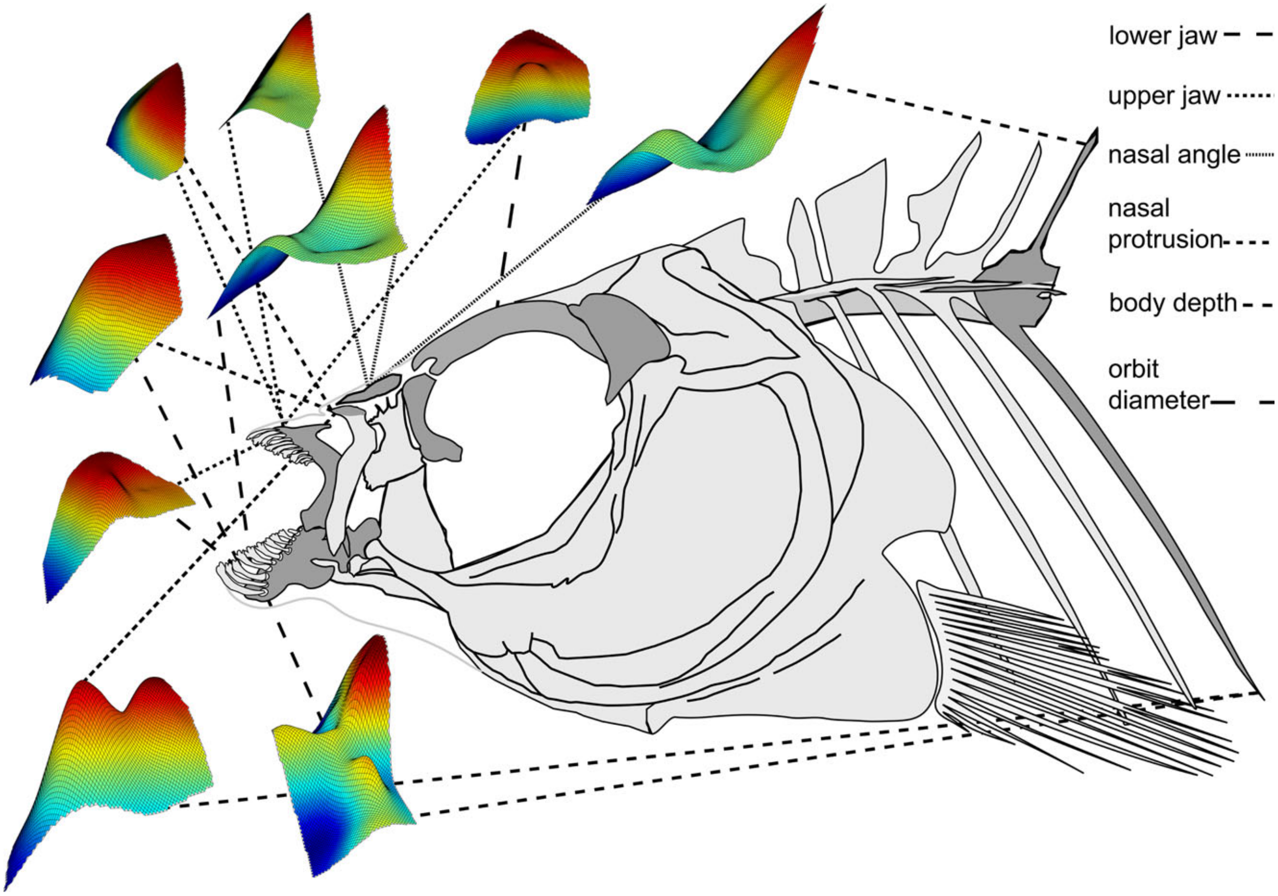

Figure 6. All nonlinear selection surfaces estimated from pairwise interactions among six major functional traits (highlighted in dark gray) separating species within the San Salvador pupfish radiation. The nonlinear relationship between each pair of traits and survival in the Crescent Pond high-density field enclosure was estimated with thin-plate splines; nonlinearity of each selection surface (edf estimated from generalized cross-validation) is presented in Table 3. Pairwise interactions resulting in flat surfaces (presented in Fig. 5) are not illustrated. Dashed lines indicate the two-way trait interactions contributing to each fitness surface.

of the F2 hybrids exhibited jaw morphology as divergent as the wild-type scale-eater phenotype. Thus, there is still no estimate of selection within this region of the morphospace. Nonetheless, negative frequency-dependent selection models predict that hybrids at the edge of this region should still show elevated fitness due to lack of competition for scale resources with the wild-type phenotype.

This analysis suggests a possible estimate of the scale of intrapopulation competition, an important parameter for speciation models (Dieckmann and Doebeli 1999; Arau et al. 2009). Beyond this local scale, overall hybrid performance may be much more important to survival than the abundance of individuals with similar phenotypes. Some theoretical and experimental evidence suggests that F2 phenotypes may have mismatched trophic morphology lacking the integration and coordination necessary for common biomechanical tasks such as suction-feeding. For example, stickleback F2 hybrids have poor growth rates relative to benthic and limnetic ecomorphs (Gow et al. 2007; Arnegard et al. 2014) and increasing phenotypic distance between males was not correlated with increased competitive fitness, suggesting that rarity did not provide a fitness advantage during male competition, but rather specific male trait combinations (Keagy et al. 2015). Alternatively, highly transgressive F2 phenotypes may survive and perform poorly due to weakly deleterious intrinsic incompatibilities resulting from hybridization (Seehausen 2013; Schumer et al. 2014). However, initial F2 hybrid survival in laboratory holding tanks did not indicate strong selection against transgressive phenotypes, but rather a flat fitness surface, suggesting that foraging for diverse resources in the wild caused variable hybrid survival (Martin and Wainwright 2013a).

DIETARY ISOTOPES DID NOT EXPLAIN ADDITIONAL FITNESS VARIATION

I found no evidence to support the prediction that dietary variation, as indicated by stable isotope ratios of carbon and nitrogen, is more directly associated with fitness than trophic morphology

(Arnold 1983; Arnegard et al. 2014). However, the coarse resolution of stable isotope data may obscure fine-scale shifts among resources with similar isotopic compositions (Layman 2007; Martin and Genner 2009b; Layman et al. 2012). Furthermore, dietary isotopes of each hybrid were shifted toward lower trophic positions and macroalgae-dominated carbon sources and were correlated with phenotype in only one of two lakes (Fig. 3). This dietary shift suggests that strong competition within high-density enclosures drove hybrids to switch their foraging behavior to lower trophic levels, perhaps by consuming greater quantities of detritus and macroalgae due to limited abundance of their preferred microinvertebrate prey. Despite this large absolute shift in dietary isotope space, relative dietary isotopes of different hybrid phenotypes still reflected divergence patterns observed in wild-type fish in Crescent Pond (Fig. 3A): hybrids most similar to the molluscivore exhibited higher trophic positions (Fig. 3E) and those most similar to the scale-eater shifted away from macroalgae carbon sources (Fig. 3C). This pattern was not observed in Little Lake, possibly due to reduced hybrid phenotypic variation.

Overall, dietary isotopic data explained surprisingly little variation in survival among hybrids. Competition among hybrids for resources may be much more fine-scaled than can be captured by isotopic variation. Alternatively, efficient foraging performance, such as successful prey capture, may more directly affect hybrid survival than the actual resources consumed and multivariate trophic morphology may best capture this performance ability. Again, these results may point toward the importance of absolute foraging performance, rather than relative competitive ability, in shaping multispecies fitness landscapes.

FUNCTIONAL TRAIT INTERACTIONS REVEAL HIGHLY VARIABLE COMPLEX FITNESS SURFACES

The two-peak fitness landscape estimated for the full 16-trait discriminant morphospace was only apparent in some pairwise interactions between traits and survival. Other interactions exhibited flat, single peak, or even three peak surfaces and these surfaces were nonadditive across different trait pairs (Figs. 5 and 6; Table 3). Caution is also warranted as these nonlinear surfaces require substantial sample sizes to estimate and were associated with statistical error (Fig. S1). This approach contrasts with traditional approaches to selection analysis aimed at extracting multivariate axes that capture the most nonlinear selection surfaces (Phillips and Arnold 1989; Schluter and Nychka 1994). Indeed, combining all six functional traits or a different subset of six traits within a single multivariate analysis produced similar fitness landscapes to the full 16-trait analysis with less phenotypic separation among the three parental phenotypes (Fig. S2). Instead, this systematic survey of fitness surfaces across six major functional traits illustrates a rich landscape of shifting and merging fitness peaks driving trophic diversification in this radiation (Fig. 6). This is particularly surprising given that four of these six traits are components of the oral jaws and should face similar selective pressures, but could also indicate indirect selective constraints due to unmeasured traits.

Nonparametric thin-plate spline analyses of pairwise trait interactions cannot distinguish direct from indirect selection as in traditional selection analyses (Lande and Arnold 1983) because linear multiple regression models cannot be applied to complex fitness surfaces that violate parametric assumptions (Mitchell-Olds and Shaw 1987). Second, these surfaces may be affected by the inclusion or exclusion of additional traits. Third, pairwise trait correlations among these six functional traits were moderate ($r < 0.58$ except for upper and lower jaw lengths: $r = 0.81$). However, previous multivariate analyses of selection indicated multiple independent axes of nonlinear selection across these traits (Martin and Wainwright 2013a) and genetic mapping of some of these traits indicates moderate-effect quantitative trait loci on different chromosomes, suggesting that there are multiple, independent axes of trait diversification within this radiation (Martin CH, Erickson PA, Miller CT in review). Thus, these complex trait interactions with fitness reflect different regions of the high-dimensional fitness landscape shaping diversification in this system. Multivariate summaries of this landscape, such as the discriminant morphospace serving as an index of hybrid similarity to parental species (Fig. 1), are only a reduced-dimensional representation of the true high-dimensional fitness “volume” (originally described by Brodie 1995; also see the empirical example in Keagy et al. 2015). Selection on organismal performance can impose widely varying selective regimes on different component traits within this high-dimensional volume.

It is difficult to interpret the complex survival trade-offs apparent across these trait interactions. No biomechanical model of foraging performance is available for Cyprinodontiform fishes, which is largely composed of biting foragers such as algae-scrapers (Hernandez et al. 2009) and the unique scale-biter (Martin and Wainwright 2013b), to translate trophic morphology into performance predictions as for suction-feeding in Centrarchids (Carroll et al. 2004; Holzman et al. 2012). Furthermore, external measurements of live fish, rather than skeletal anatomy, prevent measurement of in-lever and adductor muscle masses to estimate bite forces. However, the large number of F2 hybrids with lower jaw lengths shorter than any parental species is consistent with quantitative genetic predictions of stabilizing selection: a mix of plus and minus alleles for jaw length in the parental populations due to stabilizing selection produces transgressive segregation in the F2 generation (Rieseberg et al. 1999; Albertson and Kocher 2005). Indeed, jaw length mainly displayed fitness surfaces with a single peak, indicating stabilizing selection across all three species (Figs. 5 and 6). In contrast, nasal protrusion distance and angle, which separates molluscivores from generalists, exhibited minimal transgressive segregation (Fig. 4), suggesting divergent selection on the parental populations. Consistent with this pattern, interactions with these nasal protrusion traits often recapitulated the two fitness peaks observed in the discriminant morphospace for molluscivore and generalist phenotypes. Thus, the amount of transgressive segregation in a trait may provide some clues about the form of selection.

Are fitness landscapes on San Salvador Island unusual?

One might argue that this is an unusual system and that mechanisms of selection uncovered here do not translate to the many studies of population-level fitness in wild populations. However, it is precisely this rare evolution of novelty that enables sampling fitness beyond a single population and across major transitions between highly divergent niches: from generalist algae-scraping to scale-eating and molluscivore specialization. The rarity of novel niches within Caribbean pupfishes makes them an outstanding system for studying the origins of evolutionary novelty, a process that, by definition, should not be common at microevolutionary scales, yet dominates macroevolutionary patterns (Moczek 2008; Erwin 2015) and is a major feature of classic adaptive radiations (Givnish et al. 1997; Grant and Grant 2011; Martin and Wainwright 2011, 2013b).

Thus, it is no coincidence that one of the first investigations of context-dependence in a large-scale fitness landscape defies nearly all expectations from intrapopulation studies. First, most studies of within-population selection are underpowered (less than 500 individuals) and often measure only one fitness component (Kingsolver et al. 2001), which could bias these studies against detecting complex features of fitness landscapes, such as the low fitness regions in this study, which were only detected by tracking hybrid survival rates (Fig. 1, Martin and Wainwright 2013a; but note the much greater skew in reproductive success than survival across many studies: Rundle et al. 2003; Martin and Genner 2009a; Martin 2010; Keagy et al. 2015). Furthermore, many selection studies still do not visualize selection surfaces using nonparametric methods to confirm that quadratic models are appropriate (Schluter and Nychka 1994; Shaw and Geyer 2010).

Second, the adaptive radiation of pupfishes on San Salvador Island presents a rare example of the incipient evolution of novelty, similar to classic examples such as the vampire finch or scale-eating cichlid, but at a more recent microevolutionary scale within a simple environment. Such macroevolutionary transitions cannot ordinarily be studied at the intrapopulation level. For example, scale-eating is not known in any other Cyprinodontiform fishes among the thousands of species distributed globally (Pohl et al. 2015) and is separated from the most closely related scale-eater by 168 million years of evolution (Martin and Wainwright 2013b). Performing constant high-speed strikes to remove the scales of neighboring fish exerts highly divergent performance demands relative to scraping macroalgae and picking small invertebrates from surfaces, the likely ancestral Caribbean pupfish generalist niche. Similarly, the nasal protrusion of the molluscivore pupfish may be a unique trophic innovation. Specializing on crushing hard-shelled prey resulted in a novel skeletal protrusion from the maxillary head as well as a fusion between the maxilla and palatine unknown in other Cyprinodontiform fishes (Hernandez et al., in review), which prevents maxillary rotation during jaw protrusion and may reduce the efficacy of suction-feeding, the predominant mode of feeding in nearly all fishes (Wainwright et al. 2015).

Studying fitness landscape dynamics underlying the rare evolution of such novelties is an excellent approach for investigating the microevolutionary processes underlying macroevolution. This requires examination of rare ecological transitions, rather than frequent shifts among similar niches from standing genetic variation exemplified by many speciation model systems. Although prevalent, these case studies of reproductive isolation as a byproduct of local adaptation may not reflect long-term macroevolutionary trends nor the processes contributing to the long-term stability of biodiversity and diversification (Uyeda et al. 2011; Rosenblum et al. 2012).

Lessons for studies of natural selection in the wild

Intrapopulation studies of fitness, which are often underpowered and sample from a small region of morphospace, emphasize the fragility of selective regimes across space and time with varying environments and competitor frequencies (Grant and Grant 2002; Siepielski et al. 2009; however, note that evidence for temporal variation in selection is weak when taking into account statistical uncertainty: Morrissey and Hadfield 2012). In contrast, examining fitness across much larger regions of morphospace spanning more than three species suggests that the scale of competition is localized and does not extend beyond the phenotypic range of a single species. I speculate that frequency-dependent competition may only affect fitness regimes within populations, such as the very tip of a single fitness peak, whereas the large-scale structure of complex adaptive landscapes, such as the location of fitness peaks and valleys, may be determined mainly by the performance constraints imposed by different ecological niches.

More broadly, the limited reach of frequency-dependent selection and limited explanatory power of dietary variation observed in this study emphasizes the qualitative difference between within-population fitness dynamics and among-population fitness landscapes driving speciation and the evolution of novelty. Similarly, widespread categories for describing the form of selection on a trait—linear/stabilizing/disruptive—cannot begin to capture the complexity of multivariate selective surfaces acting on complex morphological traits across multiple species, even among similar functional trophic traits. This suggests that the form of selection at macroevolutionary scales can best be captured only by the rich visual description of the fitness landscape or other high-dimensional structures, analagous to the ecological niche concept (Hutchinson and Evelyn 1957; Holt 2009). Future studies of selection should attempt to make this distinction between within-population selection dynamics and among-species fitness landscapes. Despite some recent progress (Gimenez et al. 2009; McCandlish 2011; Calsbeek 2012), including aster modeling for combining different fitness components (Geyer et al. 2007; Shaw et al. 2008; Shaw and Geyer 2010), even those measured on different scales (e.g., growth and survival), the field is still in need of quantitative methods for exploring and comparing the diversity of selective regimes within high-dimensional fitness landscapes. More importantly, measurements of the fitness landscape, including my own, require much larger sample sizes than currently employed and incur increasing risk of statistical artifacts with increasing dimensionality and multicollinearity (Mitchell-Olds and Shaw 1987). High-throughput phenomics pipelines are needed (Houle et al. 2010). Finally, although geometric morphometrics facilitates analyses of overall shape variation, relative warp axes are not comparable among studies; instead, fitness measurements of a core set of traits with known biomechanical function within vertebrates, such as jaw length, would facilitate comparison of fitness landscapes across taxa.

Conclusion

Despite sampling a single episode of viability selection in two lake populations, manipulation of phenotypes using F2 hybrids within a young adaptive radiation of three pupfish species provided a surprisingly complex snapshot of the adaptive landscape. In contrast to widespread expectations from intrapopulation studies, (1) negative frequency-dependent selection was moderately supported and only among highly similar phenotypes, (2) there was no evidence of selection on dietary isotopic composition, and (3) fitness landscapes were highly variable even among functionally related traits. In part, these surprising results may reflect the rare evolution of major ecological transitions to novel niches, such as scale-eating, found only on this island, and the large sample sizes and phenotypic manipulation used in this study, which enabled measurement of large-scale fitness landscapes spanning multiple ecologically novel species. Substantial variation in transgressive segregation may also have contributed to variable selective regimes of single or multiple fitness peaks among functional traits. Overall, these analyses suggest an important role for the absolute performance of complex phenotypes, rather than competitor frequency, in dictating the structure of complex fitness landscapes and highlight the hyperdimensionality of the fitness-phenotype map, even within the oral jaws of three small pupfishes in a depauperate lake ecosystem.

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DATA ARCHIVING

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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. SE contour lines (estimated: solid black; +1 SE: green dashed; −1 SE red dashed) for selection surfaces estimated for the six major functional traits best distinguishing among the three San Salvador species for both Crescent Pond and Little Lake.

Figure S2. Pairwise interaction plot for all selection surfaces estimated for the six major functional traits best distinguishing among the three species within the San Salvador pupfish radiation for both Crescent Pond and Little Lake.

Figure S3. Variation in survival fitness landscapes across different trait subsets for *Cyprinodon* pupfishes in high-density field enclosures on San Salvador Island, Bahamas.

Supplemental Methods