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Predictability and Parallelism of Multitrait Adaptation

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Abstract

Environments shape the traits of organisms. Environmental variation may rarely alter selection on only a few traits, but instead precipitate wholesale changes of the multidimensional selective regime—many traits might experience divergent selection across divergent environments. Such changes in selection can elicit multifarious evolution. How predictable (from theory) and how parallel (consistent occurrences) is multitrait divergence across replicated environments? Here, I address this question using the post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*) inhabiting blue holes on Andros Island. These fish independently colonized numerous blue holes, some that harbor a major fish predator (bigmouth sleeper, *Gobiomorus dormitor*) and some that lack any major predators. I used 5 approaches to quantitatively explore the predictability and parallelism of multitrait divergence between predation regimes in Bahamas mosquitofish. Synthesizing data for 90 traits from 13 different types of character suites (e.g., body morphology, life history, genital morphology, coloration, mating preference, habitat use), I found widespread evidence for strong, predictable, and parallel divergence between predation regimes. Yet despite the great majority of traits showing predictable trajectories of change, and the majority of traits showing significant parallelism and strong magnitudes of predictable divergence, I uncovered that over half of the overall phenotypic variation among populations was *not* driven by variation in predation regime. Results suggest that focusing on few traits, or focusing on parallel aspects of divergence, can provide a misleading picture of adaptation, and nonparallel divergence appears widespread and warrants greater attention. Taking a multitrait perspective, and quantifying predictability and parallelism, can yield important insights.

Subject area: Molecular adaptation and selection
Key words: convergent evolution, parallel evolution, Poeciliidae, predictable evolution, unique evolution

Much of biological diversity has arisen through adaptive processes. Environmental variation across time and space drives evolutionary change by altering selection on species traits. Phenotypic diversification typically transpires amidst an array of potentially influential environmental factors (Schluter 2000; Grether et al. 2001; Hendry et al. 2006; MacColl 2011; Heinen et al. 2013; Jourdan et al. 2016). But whether a single environmental factor or many factors influence selection on organismal phenotypes, environmental variation will often lead to changes in multidimensional selection. That is, whole organisms experience selection, not isolated traits, and because organisms typically have many traits, selection can act on many traits simultaneously. This can lead to

multifarious evolution, where numerous traits evolve in response to environmental change.

How predictable and how parallel is multitrait evolution in response to replicated environmental gradients? (Throughout this article, I use "predictable" to refer to the degree to which phenotypic patterns match a priori predictions based on theory, empirical work, and natural history, and I use "parallel" to refer to consistent patterns of phenotypic change, encompassing patterns commonly referred to as convergent, parallel, shared, or replicated.) While many cases of parallel evolution in response to similar environmental shifts are known (Schluter 2000; Langerhans et al. 2007; Palkovacs et al. 2008; Losos 2011; Mahler et al. 2013), few studies take an explicitly *predictive* approach, or attempt to *quantify* the extent of parallelism (Langerhans and DeWitt 2004; Langerhans 2010; Kaeuffer et al. 2012; Franssen et al. 2013; de Visser and Krug 2014; Martin et al. 2015; Stuart et al. 2017). We clearly need such studies to better understand the predictability and parallelism of phenotypic evolution. Natural selection can lead to similar evolutionary outcomes, which might be predictable, but the extent to which organisms respond similarly across shared environmental gradients can be influenced by factors such as selection from unmeasured agents, many-to-one mapping of phenotype to fitness, genetic architecture, gene flow, and genetic drift (Alfaro et al. 2005; Langerhans 2010; Langerhans and Riesch 2013; Oke et al. 2017).

We further need studies that address questions of predictability and parallelism in a multitrait context considering that organisms likely experience selection on multiple, disparate traits in most circumstances. Organisms possess various types of traits—that is, character suites, such as morphology, life history, and behavior—and environmental agents can drive evolution of multiple traits within multiple character suites (Robinson and Wilson 1994; Endler 1995; Forsman and Shine 1995; Riesch et al. 2012; Martin et al. 2015). It is not enough to merely know that a particular trait shows patterns that match a priori predictions of evolutionary change, or that a significant pattern of parallel evolution was uncovered—we need to know the overall degree of predictability and parallelism across numerous traits as populations/species diversify if we wish to gain a fuller appreciation of phenotypic evolution and the process of adaptation.

A powerful way to tackle these questions is to investigate a natural system of recent adaptive divergence with many replicate populations that have experienced divergence in a major environmental factor (Nosil et al. 2002; Boughman et al. 2005; Langerhans 2010; Kaeuffer et al. 2012; Butlin et al. 2014; Martin et al. 2015). In this way, one can compare populations inhabiting similar and different environments (accounting for evolutionary nonindependence among populations) to measure the predictability and parallelism of divergence in *many* traits. Perhaps strong environmental shifts drive predictable, replicated patterns of evolution in one or a few phenotypic dimensions, but how does this scale up to higher numbers of traits? Do few or many traits exhibit predictable trajectories, how large in magnitude is predictable trait divergence, and just how consistently do different traits tend to diverge between environments during adaptive diversification? These are fundamental questions that require empirical studies. Here, I use a post-Pleistocene radiation of fishes to address these questions.

Bahamas mosquitofish (*Gambusia hubbsi*) are small, livebearing fish (family Poeciliidae) that have repeatedly evolved different adaptive traits in blue holes on Andros Island, The Bahamas in either the presence or absence of predatory fish during the past ~15 000 years (e.g., Langerhans et al. 2007; Langerhans 2009,

2010; Heinen-Kay and Langerhans 2013; Riesch et al. 2013; Martin et al. 2014; Anderson and Langerhans 2015). Blue holes are vertical, water-filled caves with an upper freshwater layer resting atop a halocline with mixed water chemistry, and anoxic marine water below (Mylroie et al. 1995; Steadman et al. 2007; Todhunter 2010). A depauperate fish assemblage (typically 1–3 species), including Bahamas mosquitofish, inhabits the upper freshwater regions (Heinen et al. 2013). Previous work has shown that variation in the presence of piscivorous fish represents the primary source of environmental variation in these Bahamas mosquitofish populations, resulting in 2 major categories of populations: 1) populations without predators that experience low mortality rates and consequently have elevated densities and high levels of competition for limited resources, and 2) populations with predators that experience high levels of mortality from predatory fish (primarily bigmouth sleeper, *Gobiomorus dormitor*) and have relatively low densities (Heinen et al. 2013). No other environmental factor measured to date systematically covaries with the presence of predatory fish (e.g., competitors, productivity, salinity, turbidity, water transparency, water color, depth, dissolved oxygen, temperature, pH; Langerhans et al. 2007; Langerhans and Gifford 2009; Heinen et al. 2013; Martin et al. 2014). Moreover, genetic relatedness among populations is not associated with predation regime, gene flow appears low, and no prior study has found any trait to show greater differences between populations with greater genetic distance (Langerhans et al. 2007; Heinen-Kay and Langerhans 2013; Riesch et al. 2013). This permits a more-or-less exclusive focus on the effects of predator presence/absence in driving phenotypic divergence in this system, where numerous hypotheses of divergent selection can be tested. Bahamas mosquitofish inhabit many blue holes, and many traits have been investigated for their divergence in response to predation regime. I have been studying this system for 15 years, and here I use published and unpublished data to synthesize information on trait variation to directly evaluate the predictability and parallelism of multitrait differentiation in Bahamas mosquitofish.

Methods

I collected phenotypic data for adult male and female Bahamas mosquitofish from previous work on 23 blue holes (10 with predators, 13 without predators), and performed 5 types of measurements (Table 1). I first categorized phenotypic data into character suites that describe different types of traits, where traits within suites share functional or conceptual similarities, as well as more likely share genetic/developmental foundations relative to traits in other suites. Data from separate suites also typically derived from separate studies originally designed to directly investigate a particular character suite. For instance, suites include body morphology, life history, genital size, coloration, and in situ behaviors (see Tables 2 and 3 for full list). I only included traits for which data existed for at least 5 populations. Nearly all traits had a priori predictions regarding trajectory of change between predation regimes based on adaptive divergence (86 out of 90 traits)—the original publications should be consulted for detailed descriptions of traits and predictions, as here I am concerned with broader patterns of predictability and not specific details about particular predictions. For all cases where traits were separately measured on males and females, I performed calculations separately for each sex; for the 3 traits in this study for which values were measured without regard for sex (included males and females), I present their results with the females but note that they apply to both sexes. The final dataset included a total of 11 character

Table 1. Description of the 5 approaches employed in this study to assess the predictability and parallelism of multitrait divergence between predation regimes in Bahamas mosquitofish

Question	Measurement
1. Parallelism	Proportion of among-population trait variance explained by environmental factor (R^2)
2. Directional predictability	Frequency with which observations match predicted qualitative trajectory
3. Magnitude of predictability	Signed, standardized effect size of trait divergence associated with environmental factor (Cohen's *d* in this study)
4. Multivariate primacy of environmental factor	Alignment of primary multivariate axis of among-population differentiation (BG-PC1) with primary multivariate axis of among-environment differentiation (**d**)
5. Environmental constraints	Importance of other environmental factors in reducing apparent predictability and parallelism with respect to focal environmental factor (here, interaction of predation and interspecific competition)

suites and 46 traits for males, and 9 character suites and 44 traits for females. Below, I describe the 5 primary measurements/analyses.

First, to quantify the degree of parallelism in phenotypic divergence between predation regimes, for each trait I calculated population means, performed a 1-way analysis of variance (ANOVA), and extracted the proportion of variance explained (R^2) by the predation regime term (presence/absence of predatory fish) (see Oke et al. 2017 for similar method). This value represents the amount of among-population trait variation that is partitioned *between* predation regimes, with the remainder ($1 – R^2$) reflecting variation *within* predation regimes. Rather than focus on statistical significance (majority of traits showed statistically significant differences between predation regimes), I centered on the magnitude of R^2 values, taking evidence for moderate parallel trait divergence between predation regimes as $R^2 \geq 0.33$ (at least one-third of among-population variance), and evidence for strong parallelism as $R^2 \geq 0.50$ (at least half of the variance). Individual trait values were transformed as necessary to improve normality of residuals, as described in the original studies. For instance, size variables were often log-transformed, counts square-root transformed, and proportions arc-sin square-root transformed. For traits that exhibited significant associations with body size (and developmental stage for embryo measurements), I statistically adjusted for body size prior to calculating population means (back-transformed residuals from linear regressions of traits on body size/developmental stage). Thus, all trait differences documented here do not reflect variation in body size—and body size did not differ between predation regimes (see Results). For character suites with numerous component traits, I additionally performed a between-group principal components analysis using all traits within that suite, and examined scores on the first principal component (BG-PC1) as an assessment of the multivariate divergence for that character suite. While an alternative perspective on parallelism could quantify the proportion of among-*individual* trait variation partitioned between predation regimes (instead of proportion of among-*population* trait variation), here I am directly interested in evaluating divergence between populations and not necessarily variation among individuals within populations.

Second, as a qualitative assessment of directional predictability, for each trait I scored whether the trait exhibited significant evidence for the predicted trajectory of difference between predation regimes (Yes/No). This provides a frequency of overall predictability in directional change of traits.

Third, I estimated the magnitude of phenotypic divergence between predation regimes for each trait using Cohen's *d* standardized effect size (difference between means in standard deviation [SD] units; Cohen 1988). I assigned a sign to the effect sizes based on its correspondence to a priori predictions: positive values indicate differences that matched predictions and negative values indicate

differences that were opposite to predictions. These effect sizes employed pooled SDs observed within populations, and were calculated using the individual-level data—thus, effect sizes were not estimated for BG-PC1 scores because those were assigned to populations, not individuals. Cohen's *d* values reported here represent the average magnitude of trait divergence observed between populations living with or without predatory fish using within-population SD units. I treated Cohen's $d \geq 1.0$ as moderate predictable magnitude of divergence, and Cohen's $d \geq 2.0$ as strong predictable magnitude of divergence.

Fourth, for the character suites where a BG-PCA was conducted to evaluate multivariate divergence, I assessed the degree to which multivariate population differentiation aligned with predator-driven divergence. Specifically, I calculated the Pearson correlation coefficient between BG-PC1 scores and scores along the eigenvector of divergence between predation regimes (**d**). The latter divergence vector was derived from a BG-PCA performed on group means (averages for populations with and without predators) and projected onto populations. This directly quantifies the alignment of the primary axis of trait differentiation among populations with the primary axis of trait differentiation between predation regimes, providing an estimate of the degree to which variation in predation regime may drive multivariate trait divergence.

Fifth, I evaluated the role of one particular environmental constraint on predictable and parallel predator-driven divergence: interspecific competition. That is, selection via alternative agents can reduce the parallelism of responses to a focal agent, and here I directly examined one major alternative agent that varies among populations and may drive trait differentiation, especially traits related to resource acquisition (Heinen et al. 2013). I scored the presence/absence of a potential competitor fish species for each blue hole based on visual surveys (fish communities of blue holes are easily and quickly assessed, and appear stable over very long time periods; Langerhans et al. 2007; Langerhans and Gifford 2009; Heinen et al. 2013). Primary competitors of Bahamas mosquitofish in blue holes are the sheepshead minnow (*Cyprinodon variegatus*) and crested goby (*Lophogobius cyprinoides*), which are similarly sized to Bahamas mosquitofish and may represent both exploitative and interference competitors. Potential competitors were found in 13 of the 23 blue holes examined here. To assess the role of interspecific competition in explaining phenotypic differentiation and influencing the effects of predation regime on trait differentiation, I conducted ANOVAs for all traits with data from more than 10 populations, where the independent variables were predation regime (presence/absence), competitor regime (presence/absence), and their interaction. The importance of competitors was assessed by determining whether significantly more variance was explained in this model compared to the original model including only predation regime as the independent

Table 2. Summary of results for the 5 measurements evaluating the predictability and parallelism of multitrait divergence in Bahamas mosquitofish adult males

Character		1	2	3		4	5	N	N
Suite	Trait	(R^2)	Predicted	Cohen's d	SE	(Corr. w/ **d**)	(R^2 w/ C)	Pop.	Fish
Body size	SL	0.01	N	−0.07	0.46		0.06	22	985
	Weight	0.00	N	0.21	0.46		**0.38**	22	985
Body morphology	Body shape	0.82	Y	4.10	0.84	0.998	0.83	21	680
	MB depth	0.66	Y	5.64	1.07		**0.75**	21	680
	CP depth	0.61	Y	5.21	1.00		**0.76**[a]	21	680
	CP area	0.63	Y	5.78	1.09		**0.77**	21	680
	Head depth	0.00	N	0.06	0.46		**0.63**[a]	21	680
	Head length	0.36	Y	3.26	0.72		0.50	21	680
	Head area	0.11	Y	2.14	0.59		**0.55**[a]	21	680
	Eye diameter	0.12	N	0.68	0.73			10	105
Fin morphology	CF length	0.72	Y	2.87	1.07			10	105
	DF length	0.12	N	0.67	0.73			10	105
Life history	Lean weight	0.41	Y	1.56	0.55		0.51	20	200
	Fat content	0.07	N	0.50	0.48		0.08	20	200
	Testes size	0.27	Y	1.15	0.51		0.31	20	200
Ano-urogenital morphology	AU BG-PC1	0.59	Y			0.999	**0.79**	18	272
	Hemal spine #	0.58	Y	2.01	0.63		0.58	18	272
	Gonop. AT	0.27	Y	1.28	0.55		**0.55**	18	272
	15th HS length	0.42	Y	1.64	0.59		**0.73**[a]	18	272
	15th HS UPL	0.46	Y	1.79	0.60		**0.64**	18	272
Genital size	Gonop. area	0.37	Y	2.04	0.55		0.45	23	354
	Gonop. length	0.15	N	−0.73	0.52		0.32	18	270
Genital shape	Gonop. DTS	0.30	Y	1.25	0.49	0.791	0.30	22	123
Coloration	Color BG-PC1	0.59	Y			0.783		10	
	DF redness	0.56	Y	1.47	0.82			10	104
	DF yellowness	0.62	Y	1.87	0.88			10	104
	AF redness	0.21	Y	1.39	0.81			10	93
	AF yellowness	0.24	Y	1.49	0.82			10	93
	Blk patch area	0.44	Y	1.32	0.80			10	105
	Irid. patch area	0.07	Y	0.22	0.71			10	105
	Gonop. black	0.18	N	−1.02	0.76			10	96
	CF black	0.05	N	0.31	0.71			10	105
In situ behavior frequencies	Behav. BG-PC1	0.91	Y			0.899		6	
	Feeding	0.71	Y	2.57	1.50			6	120
	Prey inspection	0.13	N	−0.63	1.04			6	120
	M-F chase	0.14	N	0.67	1.04			6	120
	Copulation	0.10	N	−0.54	1.03			6	120
	Intersex aggr.	0.34	Y	1.18	1.12			6	120
	Intrasex aggr.	0.94	Y	6.57	3.01			6	120
Personality	Pers. BG-PC1	0.68	Y			0.947		6	
	Boldness	0.11	N	0.13	1.00			6	99
	Exploration	0.94	Y	6.39	2.94			6	126
	Activity	0.11	Y (NC)	0.39	1.01			6	120
	Lateralization	0.27	Y	1.29	1.15			6	222
Antipredator behavior	Vis. response	0.54	Y	1.96	1.31			6	97
	Chm. response	0.08	N	0.43	1.02			6	30

Numbers follow Table 1. Corr., correlation; **d**, divergence vector; C, competitor; Body shape: PC 1 of geometric morphometric data of lateral morphology; SL, standard length; MB, mid-body; CP, caudal peduncle; CF, caudal fin; DF, dorsal fin; Gonop., gonopodium; BG-PC1, between-group principal component 1; AU, ano-urogenital morphology; AT, anterior transposition; HS, hemal spine; UPL, uncinate process length; DTS, distal-tip shape (PC1 of geometric morphometric data); AF, anal fin; Blk, black; Irid., iridescent; Behav., behavior; M-F, male-female; Aggr., aggression; Pers., personality; Vis. response: PC1 scores for response to visual cues of predator; Chm. response: PC1 scores for response to chemical cues of predator. Bold values indicate significant increase in explained variance.

[a]Significant interaction between predation regime and competitor regime.

variable. The role of competitors in shaping the effects of predation regime on trait differentiation was assessed by the significance test of the interaction term (revealing that effects of predation regime depended on the competitor regime). I excluded traits with data from 10 or fewer populations to ensure adequate ability to test for the interaction between predation and interspecific competition.

Results

Tables 2 and 3 present the findings for the 5 measured attributes for males and females, respectively. Each attribute is described separately below.

Parallelism

Across a wide range of phenotypes, I observed a wide range of degrees of parallelism in both males (Figures 1a and 3a) and females (Figures 2a and 3d), from 0.00 to 0.95. Thus, while some traits showed no influence of predation regime, despite a priori predictions of divergent selection, many others showed evidence of strong parallelism. Overall, most character suites exhibited evidence of at least moderate parallelism, with 40% of traits showing strong parallelism ($R^2 > 0.5$) (Table 4). Despite the numerous cases of marked parallelism, the majority of traits did not show strong parallelism, the mean R^2 values are moderate for both sexes, and there is considerable variation among traits.

Table 3. Summary of results for the 5 measurements evaluating the predictability and parallelism of multitrait divergence in Bahamas mosquitofish adult females

Character		1	2	3		4	5	N	N
Suite	Trait	(R^2)	Predicted	Cohen's d	SE	(Corr. w/ **d**)	(R^2 w/ C)	Pop.	Fish
Body size	SL	0.00	N	0.23	0.46		0.10	22	852
	Weight	0.02	N	0.29	0.46		**0.24**	22	1210
Body morphology	Body shape	0.76	Y	3.20	0.91	0.985	**0.89**[a]	14	373
	MB depth	0.82	Y	3.88	1.03		**0.93**[a]	14	375
	CP depth	0.67	Y	2.65	0.82		**0.83**[a]	14	375
	CP area	0.80	Y	3.58	0.98		**0.92**[a]	14	375
	Head depth	0.00	N	−0.04	0.58		**0.28**	14	375
	Head length	0.28	Y	1.14	0.63		0.32	14	375
	Head area	0.07	N	0.55	0.59		0.20	14	375
Life history	LH BG-PC1	0.53	Y			0.886	**0.72**[a]	18	225
	Lean weight	0.42	Y	1.76	0.60		**0.84**[a]	18	225
	Fat content	0.01	N	−0.35	0.51		0.03	18	225
	Fecundity	0.40	Y	1.71	0.60		**0.58**[a]	18	225
	Reprod. alloc.	0.04	N	−0.31	0.51		0.08	18	225
	Embryo weight	0.55	Y	2.19	0.65		**0.67**[a]	18	225
	Embryo fat	0.35	Y	1.73	0.60		0.37	18	225
Genital morphology	Gen. BG-PC1	0.55	Y			0.767	0.57	14	109
	UA area	0.44	Y	1.69	0.69		0.51	14	109
	UA open area	0.69	Y	2.67	0.83		0.77	14	109
	UA pap. area	0.35	Y	1.42	0.66		0.42	14	109
	Prp. open area	0.36	Y	1.40	0.66		0.39	14	109
	UA open W	0.51	Y	1.89	0.72		0.51	14	109
	UA open L	0.02	N	0.26	0.59		**0.30**	14	109
	UA open W/L	0.35	NP	1.30	0.65		0.42	14	109
	UA open AR	0.26	NP	1.09	0.63		0.29	14	109
In situ behavior frequencies	Behav. BG-PC1	0.62	Y			0.837		6	120
	Feeding	0.25	N	0.94	1.08			6	120
	Prey inspection	0.05	Y	0.36	1.01			6	120
	M-F chase	0.95	Y	7.11	3.24			6	120
	Copulation	0.84	Y	3.68	1.88			6	120
	Intersex aggr.	0.02	NP	0.22	1.00			6	120
	Intrasex aggr.	0.49	NP	0.71	1.05			6	120
Personality	Pers. BG-PC1	0.85	Y			0.950		6	
	Boldness	0.51	Y	2.14	1.36			6	99
	Exploration	0.93	Y	5.94	2.76			6	123
	Activity	0.19	Y (NC)	0.50	1.02			6	120
	Lateralization	0.90	Y	2.84	1.58			6	155
	Sociability[BS]	0.89	Y	4.53	2.20			6	120[Q]
Antipredator behavior	Vis. response	0.74	Y	2.11	1.36			6	99
	Chm. response	0.03	N	0.55	1.03			6	50
Mating preference	Body shape	0.81	Y	2.02	1.59			5	71
	DF color	0.22	N	−0.58	1.03			6	70
Habitat use	Prp. shallow[BS]	0.63	Y	3.05	0.79		**0.79**[a]	17	1160[Q]
	Prp. offshore[BS]	0.25	Y	1.14	0.56		0.34	17	1160[Q]

Numbers follow Table 1. Abbreviations follow Table 2, with the following additions: LH, life history; Reprod. alloc., reproductive allocation; Gen., genital; UA, urogenital aperture; Pap., papilla; Prp., proportional; W, width; L, length; AR, aspect ratio; BS, both sexes; Q, quadrats. Bold values indicate significant increase in explained variance.

[a]Significant interaction between predation regime and competitor regime.
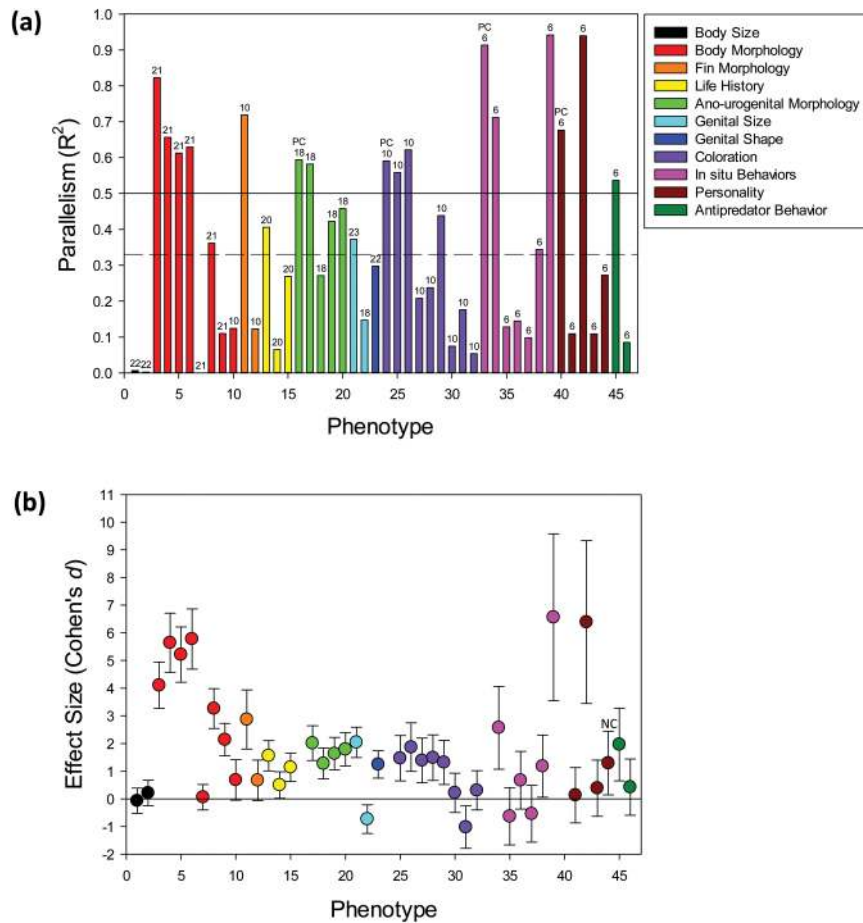
Figure 1. Degree of parallelism and magnitude of predictability for phenotypic differentiation between predation regimes in male Bahamas mosquitofish. (**a**) Proportion of variance among population means explained by the presence/absence of predatory fish for 46 traits across 11 character suites. (**b**) Standardized effect size (difference between predation regimes in SD units) for each trait. Order of traits follows Table 2. Numbers above bars depict the number of populations examined for each trait. "PC" indicates between-group PC 1 scores. The dashed line in (a) represents one-third of the variance, while the solid line represents half. "NC" indicates that no change was predicted for the trait. Error bars are 1 SE.

Directional Predictability

Of the 86 directional predictions of trait divergence between predation regimes, observations for 62 traits matched predictions (72%). Although the traits are not independent, this result greatly exceeds a random expectation, indicating significant directional predictability. However, this also suggests considerable variability, where nearly 3 out of 10 traits do not exhibit predictable trajectories of change between predation regimes. The extent of parallelism (R^2) was much greater, on average, for traits exhibiting directional predictability for males (0.50 vs. 0.09) and females (0.60 vs. 0.07).

Magnitude of Predictability

I found substantial variation in the magnitude of trait differentiation between predation regimes in both males (Figures 1b and 3b) and females (Figures 2b and 3e). While few traits showed trends opposite to predictions, the magnitude of predictability was quite variable across traits (Table 4). That said, the average observed magnitude of divergence was approximately 1.7 SDs, with most character suites showing Cohen's d values of at least 1.0 and 33% of traits exhibiting a difference between predation regimes of more than 2 SDs. Moreover, parallelism (R^2) and magnitude of predictability (Cohen's d) were positively correlated in both males ($r = 0.86$, $P < 0.0001$) and females ($r = 0.88$, $P < 0.0001$) (Figure 3c, f). While not equivalent, and addressing

different questions, the 2 estimates were highly correlated, meaning that traits exhibiting greater parallelism also tended to exhibit greater magnitudes of predictable divergence. Further, it appeared that the magnitude of predictable divergence increased nonlinearly with respect to the degree of parallelism (significance of squared term in quadratic regression, males: $P = 0.0822$ [$P = 0.0457$ ignoring sign of d], females: $P = 0.0058$ [$P = 0.0019$ ignoring sign of d]). Neither of these estimates were heavily influenced by the sample size of populations in either males (R^2: $\rho = -0.11$, $P = 0.4972$; Cohen's d: $\rho = 0.14$, $P = 0.3828$) or females (R^2: $\rho = -0.36$, $P = 0.0165$; Cohen's d: $\rho = -0.21$, $P = 0.1954$).

Multivariate Primacy of Environmental Factor

For all 11 character suites for which I examined the multivariate primacy of predation regime in describing overall population differentiation within the suite, I found high correlation between BG-PC1 and **d** (all $r > 0.75$; Tables 2 and 3). That is, the multivariate axis describing the maximum amount of among-population trait variation typically aligns with the multivariate axis that maximizes differences between predation regimes.

Environmental Constraints

The presence of an interspecific competitor had a significant influence on among-population trait variation in males for 10 of 20 traits
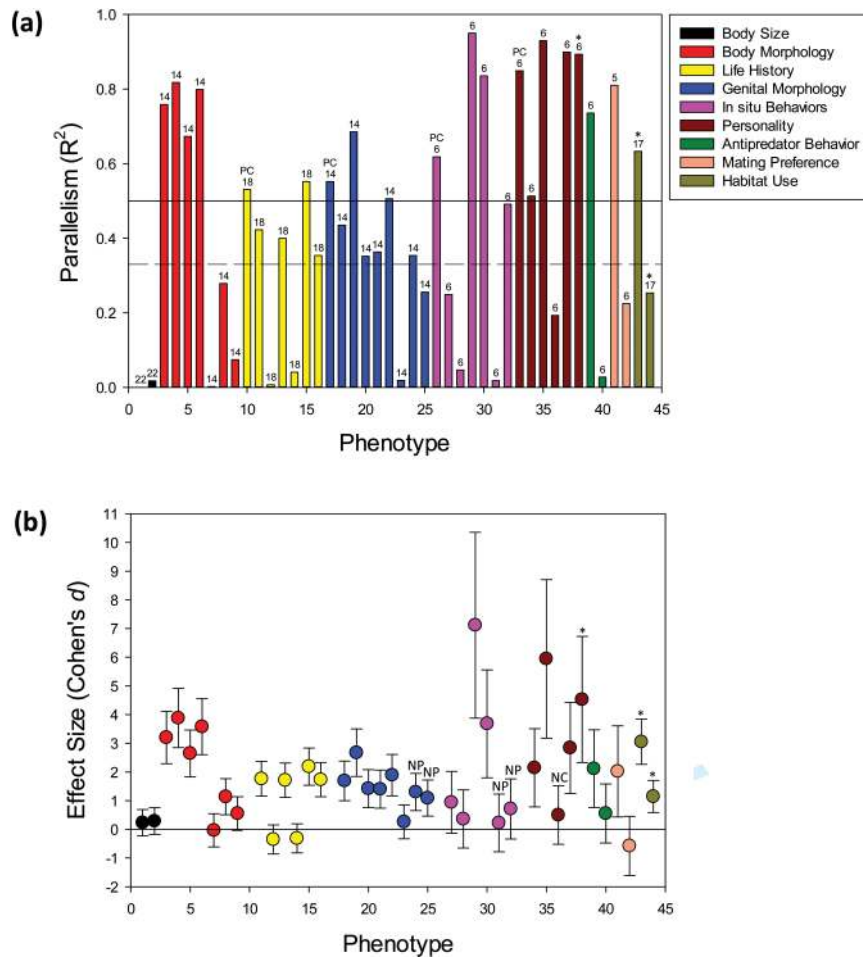
Figure 2. Degree of parallelism and magnitude of predictability for phenotypic differentiation between predation regimes in female Bahamas mosquitofish. (**a**) Proportion of variance among population means explained by the presence/absence of predatory fish for 44 traits across 9 character suites. (**b**) Standardized effect size (difference between predation regimes in SD units) for each trait. Order of traits follows Table 3. Numbers above bars depict the number of populations examined for each trait. "PC" indicates between-group PC 1 scores. Asterisks denote traits measured for both sexes pooled. The dashed line in A represents one-third of the variance, while the solid line represents half. "NC" indicates that no change was predicted for the trait. "NP" indicates that no clear prediction was made for the trait. Error bars are 1 SE.

(3 of 6 suites) and in females for 12 of 27 traits (5 of 5 suites) (Tables 2 and 3). Thus, interspecific competition indeed appeared to represent an important source of divergent selection across blue holes for about half of the phenotypes examined. However, the effects of interspecific competition only altered the degree of parallelism between predation regimes (significant interaction term) for 4 of 20 traits (2 of 6 suites) in males and 9 of 27 traits (3 of 5 suites) in females. In virtually every case, this interaction could be interpreted as the presence of interspecific competitors accentuating trait divergence between predation regimes. Specifically, interspecific competitors had little-to-no effect on trait variation in the presence of predators, but in the absence of predators interspecific competitor presence was associated with greater trait differentiation.

Discussion

Taken together, the results demonstrate widespread evidence for predictable and parallel trait divergence between predation regimes in Bahamas mosquitofish inhabiting blue holes on Andros Island, The Bahamas. Consistent with previous work in this system, adaptive phenotypic differentiation appears commonplace, spanning many,

disparate types of traits. Across phenotypes as varied as body shape, fin morphology, genital shape, coloration, offspring size, sexual behaviors, exploration, behavioral lateralization, and habitat use, I found strong, predictable, and parallel divergence between predation regimes. Importantly, the extent of parallelism and predictability was explicitly quantified here. Particularly, strong parallelism and predictability was evident for some traits: for ~15% of traits, more than 75% of among-population trait variance was explained by predation regime; for ~18% of traits, predictable divergence exceeded 3 SDs. This suggests that fairly simple environmental differences can elicit strong, divergent, multifarious selection and lead to multivariate patterns of highly predictable and consistent adaptive trait changes. However, this synthesis of large numbers of phenotypes has revealed a striking amount of phenotypic variation that is unexplained by this major ecological factor.

Remarkably, in a system well known for the role of predation in driving strong, predictable, and repeatable phenotypic divergence—and demonstrated here for a nontrivial number of traits—I found that the majority of phenotypic divergence among populations is *not* driven by variation in predation regime. That is, much of the trait variation among populations is unrelated to the presence/absence
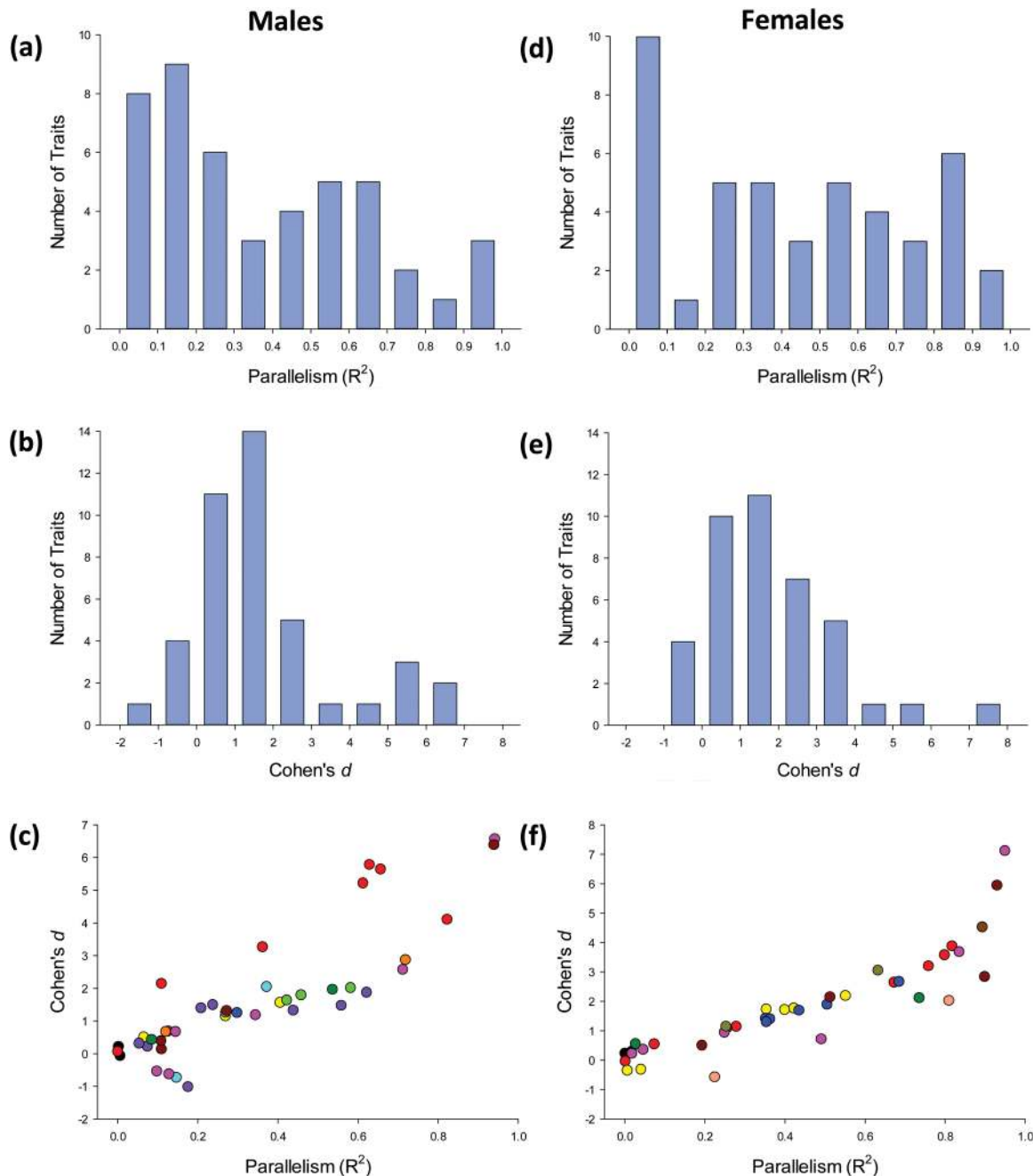
Figure 3. Frequency histograms for parallelism and magnitude of predictable divergence in (**a**, **b**) male Bahamas mosquitofish and female Bahamas mosquitofish (**d**, **e**), and the relationship between parallelism and magnitude of predictable divergence in (**c**) males and (**f**) females. Symbol colors follow Figures 1 and 2.

of predatory fish even though 1) the majority of traits (57%) exhibited at least moderate parallelism ($R^2 > 0.33$), 2) the great majority of traits (72%) matched a priori directional predictions of change, 3) the majority of traits (65%) showed over 1 SD difference between predation regimes, 4) most of the character suites showed both strong parallelism (75% with $R^2 > 0.5$) and divergence (70% with Cohen's $d > 2.0$), and 5) the primary multivariate axis of divergence aligned with the predation-driven divergence vector within each character suite. Why then is there so much unexplained phenotypic differentiation? The key potential culprits for causing nonparallel aspects of divergence are environmental constraints (adaptation to alternative selective agents), complex or rugged fitness landscapes,

genetic constraints, genetic drift, and gene flow. I address each possible source below.

First, the predictability and parallelism of multitrait divergence across a particular environmental gradient can be enhanced or diminished by selection arising from other environmental factors. Researchers typically investigate predictability and parallelism using discrete environmental categories, such as with or without predators, with or without certain competitors, benthic or limnetic habitat, freshwater or saltwater, or toxic or nontoxic environments. However, these categories do not capture all relevant environmental differences between populations, and thus natural (or sexual) selection arising from alternative environmental factors can influence the

Table 4. Summary of key findings for parallelism and magnitude of predictable divergence between predation regimes across all traits for male and female Bahamas mosquitofish

	R^2				Cohen's d			
	Mean	SD	>0.33	>0.50	Mean	SD	>1.0	>2.0
Males	0.37	0.28	23/46 traits 9/11 suites	16/46 traits 7/11 suites	1.68	1.9	27/42 traits 10/11 suites	12/42 traits 6/11 suites
Females	0.44	0.31	28/44 traits 8/9 suites	20/44 traits 8/9 suites	1.78	1.67	26/40 traits 8/9 suites	15/40 traits 8/9 suites

observed predictability and parallelism across the focal environmental gradient. Perhaps much of the nonparallelism observed in many systems reflects such "environmental constraints," meaning that selection might actually be leading to greater determinism than what is apparent because the full "environment" has not been measured (e.g., Stuart et al. 2017). To understand the importance of these environmental constraints in this context, we ideally need to quantify the putatively important selective agents, as well as measure selection on the traits of interest under varying combinations of the putative agents. In the present study, I performed a first step by testing the role of one alternative agent in influencing the observed patterns in Bahamas mosquitofish. Prior work has found that factors such as resource productivity, light environment, salinity, and interspecific competition also influence divergence of some traits (Heinen-Kay and Langerhans 2013; Heinen et al. 2013; Riesch et al. 2013; Martin et al. 2014; Anderson and Langerhans 2015). Examining one factor that is easily quantified across all blue holes, I found that the presence of interspecific competitors affected the observed divergence between predation regimes for 13 of 47 measured traits. These traits mainly included body morphology, female life histories, and habitat use, and explained approximately 18% more phenotypic variance than expected by chance. Considering the average R^2 values observed here (Table 4), this is a nontrivial amount of variance, although it was restricted to about 28% of the traits. These effects can largely be viewed as interspecific competitors strengthening selection from resource competition in the absence of predators where such competition is already relatively high (where Bahamas mosquitofish densities are high), while interspecific competitors had little influence on competition-related selection in the presence of predators (where Bahamas mosquitofish densities are low). Although not widespread across phenotypes, variation in competitors can reduce the overall predictability and parallelism of phenotypic divergence between predation regimes in Bahamas mosquitofish. Similarly, nonfocal ecological variables, or more finely quantified environmental factors, have been shown to influence divergence across focal environments in a number of other systems (Robinson et al. 2000; Landry et al. 2007; Kaeuffer et al. 2012; Fitzpatrick et al. 2014; Heinen-Kay et al. 2014). The potential for similar roles of other factors in this system, as well as the generality of environmental constraints in diminishing parallel responses in other taxa, await further study.

Second, nonparallel responses to common environments can arise through adaptive responses to selection if the fitness surfaces are rugged, where multiple phenotypes have similarly high fitness values within a given population (i.e., multiple fitness peaks). Essentially, there can be multiple phenotypic solutions to the same problem. This can arise from many-to-one mapping of phenotype on performance attributes, such as locomotor ability, predator avoidance/evasion, foraging success, and mate attraction (Alfaro et al. 2005; Wainwright et al. 2005; Mendelson et al. 2014). Thus, even if populations experience the same patterns of selection, they can traverse different peaks owing to their starting points in phenotypic space, genetic (co)variances of traits, or the order of the appearance of mutations. In the present case, multiple fitness peaks within populations could arise via many-to-one mapping, especially when considered in highly dimensional phenotype space, where various phenotypic combinations could result in similar fitness—for example, different morphological, life history, color, and behavioral combinations could represent various equally fit alternative strategies in different predation regimes. Moreover, environments could differ in the ruggedness of fitness surfaces, such as steeper and fewer fitness peaks in the presence of predation than in its absence (e.g., more phenotypic solutions of high fitness in the absence of predators than in their presence). If correct, then we might find lower among-population trait variance in the presence of predators—I tested for heterogeneity of variances in population means between predation regimes using an O'Brien test for all 90 traits and only found 2 traits with significant differences in variances among population means (male standard length: $P = 0.03$, female caudal peduncle depth: $P = 0.04$). One of these traits (male standard length) showed no difference in mean between predation regimes and had greater, not reduced, variance in the presence of predators. Thus, trait variances do not tend to differ between predation regimes in general, nor are any directional trends present. Thus, if divergent responses to similar selection underlies much of the nonparallel responses observed here, it is similar for both predation regimes, and requires further research.

Third, populations could differ in genetic architecture/background, such as variation in heritability of traits and genetic covariances among traits. Even if selection is identical, evolutionary responses will differ if genetic architecture differs (Lande 1979; Arnold 1992). While prior work in this system has demonstrated significant heritability for a large number traits, and shown a genetic basis to differentiation for many traits (e.g., Langerhans 2009; Heinen-Kay and Langerhans 2013; Riesch et al. 2013; Martin et al. 2014; Anderson and Langerhans 2015; Heinen-Kay et al. 2016), whether populations differ in genetic variances and covariances of traits is unknown. Ongoing work is addressing this topic and will help shed light on the possible role of genetic constraints in this system.

Finally, phenotypic variation among populations within similar predation regimes could result from genetic drift or gene flow. Owing to the colonization history of blue holes and the reduced genetic diversity observed within blue holes (Schug et al. 1998; Langerhans et al. 2007), genetic drift could play an important role in determining some phenotypes in at least some populations. For instance, the random loss/fixation of alleles that influence some of the phenotypes examined here is certainly plausible for many or most blue holes. While this could result in genetic drift per se, it can also influence genetic constraints to responses to selection described above. Quantifying the importance of drift across populations (e.g., genomic estimates of effective population sizes) and identifying

genomic bases for traits can help address this notion. While gene flow appears quite low in many cases, genetic exchange is evident among many blue hole populations (Anderson and Langerhans 2015). In other systems, gene flow has been shown to reduce the magnitude of phenotypic divergence between habitat types, but not necessarily the direction of divergence (Hendry and Taylor 2004; Nosil and Crespi 2004; Rasanen and Hendry 2008; Stuart et al. 2017). Further research is required to better understand how gene flow might constrain the magnitude of divergence or otherwise influence trait differentiation in the present system.

An important caveat of this study is that I do not distinguish between genetic divergence and phenotypic plasticity as underlying sources of phenotypic differences observed between populations. This is because previous work in this system has typically found strong genetic bases to trait differences between populations, with magnitudes of divergence being very similar for wild-caught fish and those raised in a common laboratory environment (see references above). Moreover, selection for adaptive plasticity is unexpected in this system because individuals do not experience multiple predation regimes (parent's environment predicts offspring's environment), and a much greater amount of data exists for wild-caught fish. In this study, I used data for wild-caught fish (which could include plasticity) even when common-garden laboratory-raised fish were available because this would ensure greater consistency among variables, because findings were extremely similar for both types of fish (but smaller sample sizes for common-garden fish), and because a recent study showed that parallelism in fishes is similar in magnitude when quantified for wild-caught or lab-raised fish (Oke et al. 2017). That said, phenotypic plasticity could prove important for some traits, and ongoing work is measuring these phenotypes for 8 blue hole populations raised under common laboratory conditions. Plasticity can increase or decrease the predictability and parallelism of trait change, and a recent study in threespine stickleback (*Gasterosteus aculeatus*) found that plasticity typically enhanced the magnitude of predictable trait change, although the strength of plasticity differed among traits and lineages (Oke et al. 2016).

While researchers have been addressing the predictability and parallelism of phenotypic evolution for a long time, surprisingly few studies have explicitly measured the *predictability* of trait changes based on well-articulated a priori expectations (Bull and Molineux 2008; Langerhans 2010; Langerhans and Reznick 2010; Moore et al. 2016), or *quantified* the degree of parallelism in trait differentiation (Langerhans and DeWitt 2004; Kaeuffer et al. 2012; Oke et al. 2017)—and very few have done so in a multitrait context (but see Martin et al. 2015). While more research is needed to gain a better grasp of general patterns of predictability and parallelism of multitrait diversification, I can place this study's findings in the context of existing prior research. First, these results appear to represent a high degree of directional predictability in divergence across many disparate traits, with additionally strong evidence for substantial magnitudes of predictable divergence—for example, 65% of traits differed in the predicted direction between predation regimes by more than 1 SD. Recent work on offspring size and number in livebearing fishes found that trait divergence was not especially predictable, strong, or consistent in response to food limitation, population density, or salinity (mean divergence of –0.63 to 0.98 SDs), while divergence in response to predation regime and hydrogen sulphide toxicity was (1.24–2.81 SDs) (Moore et al. 2016). Further, work on a range of traits (e.g., body morphology, trophic morphology, feeding kinematics, feeding performance, habitat use) in bigmouth sleepers

inhabiting blue holes with different diet regimes found results extremely similar to those reported here: mean divergence of 1.74 SDs compared to 1.73 in this study (Martin et al. 2015). Second, while often not a central focus of studies, it is true that many prior studies have shown that phenotypic responses to common environmental gradients often differ among lineages, even closely related lineages within species, or even among sexes of the same species (Langerhans and DeWitt 2004; Hendry et al. 2006; Langerhans et al. 2006; Langerhans and Makowicz 2009; Franssen et al. 2013; Ravinet et al. 2013; Giery et al. 2015). Langerhans and Riesch (2013) found that 30 separate study systems regarded as examples of parallel evolution and ecological speciation have documented evidence of nonparallel phenotypic divergence between focal environments. In the only other study that I am aware of that quantified parallelism across many traits using the same metric employed here, Oke et al. (2017) synthesized data across 23 fish species and found results very similar to those observed here: average R^2 of 0.46 and SD of 0.32 (0.41 ± 0.29 across all observations in this study), with 54% of traits having R^2 values of less than 0.50 (60% of traits in this study). Thus, nonparallel responses to similar environmental conditions are commonplace, and yet we do not yet possess an understanding of their general magnitudes, causes, or consequences.

A multitrait perspective on the predictability and parallelism of phenotypic evolution, as taken in this study, is important if we want to understand how organisms respond to environmental change, and not simply how particular traits may respond. If we only focus on a small number of traits, or if we focus exclusively on the parallel components of divergence, then we miss much of the story of adaptation. While we do not yet know the extent of parallelism in this system at the genetic or genomic level, if recent work in other systems is any indication, then substantial nonparallelism will be found there as well (Steiner et al. 2009; Tenaillon et al. 2012; Perrier et al. 2013; Soria-Carrasco et al. 2014; Ravinet et al. 2016). Putting this together, accumulating evidence this century is pointing to widespread evidence for nonparallel phenotypic and genomic divergence between what appear to be roughly replicated environmental gradients, despite an apparent focus in the literature on parallel evolutionary change. A greater appreciation and focused study of the causes and consequences of unpredicted and nonparallel divergence appears warranted.

Acknowledgments

References

Alfaro ME, Bolnick DI, Wainwright PC. 2005. Evolutionary consequences of many-to-one mapping of jaw morphology to mechanics in labrid fishes. *Am Nat*. 165:E140–E154.

Anderson CM, Langerhans RB. 2015. Origins of female genital diversity: predation risk and lock-and-key explain rapid divergence during an adaptive radiation. *Evolution*. 69:2452–2467.

Arnold SJ. 1992. Constraints on phenotypic evolution. *Am Nat*. 140(Suppl 1):S85–S107.

Boughman JW, Rundle HD, Schluter D. 2005. Parallel evolution of sexual isolation in sticklebacks. *Evolution*. 59:361–373.

Bull JJ, Molineux IJ. 2008. Predicting evolution from genomics: experimental evolution of bacteriophage T7. *Heredity*. 100:453–463.

Butlin RK, Saura M, Charrier G, Jackson B, André C, Caballero A, Coyne JA, Galindo J, Grahame JW, Hollander J, *et al.* 2014. Parallel evolution of local adaptation and reproductive isolation in the face of gene flow. *Evolution*. 68:935–949.

Cohen JE. 1988. *Statistical power analysis for the behavioral sciences*. Hillsdale (NJ): Lawrence Earlbaum Associates.

de Visser J, Krug J. 2014. Empirical fitness landscapes and the predictability of evolution. *Nat Rev Genet*. 15:480–490.

Endler JA. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol Evol*. 10:22–29.

Fitzpatrick SW, Torres-Dowdall J, Reznick DN, Ghalambor CK, Funk WC. 2014. Parallelism isn't perfect: could disease and flooding drive a life-history anomaly in Trinidadian guppies? *Am Nat*. 183:290–300.

Forsman A, Shine R. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Funct Ecol*. 9:818–828.

Franssen NR, Harris J, Clark SR, Schaefer JF, Stewart LK. 2013. Shared and unique morphological responses of stream fishes to anthropogenic habitat alteration. *Proc R Soc B Biol Sci*. 280:1–8.

Giery ST, Layman CA, Langerhans RB. 2015. Anthropogenic ecosystem fragmentation drives shared and unique patterns of sexual signal divergence among three species of Bahamian mosquitofish. *Evol Appl*. 8:679–691.

Grether GF, Millie DF, Bryant MJ, Reznick DN, Mayea W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*. 82:1546–1559.

Heinen-Kay JL, Langerhans RB. 2013. Predation-associated divergence of male genital morphology in a livebearing fish. *J Evol Biol*. 26:2135–2145.

Heinen-Kay JL, Noel HG, Layman CA, Langerhans RB. 2014. Human-caused habitat fragmentation can drive rapid divergence of male genitalia. *Evol Appl*. 7:1252–1267.

Heinen-Kay JL, Schmidt DA, Stafford AT, Costa MT, Peterson MN, Kern EMA, *et al.* 2016. Predicting multifarious behavioural divergence in the wild. *Anim Behav*. 121:3–10.

Heinen JL, Coco MW, Marcuard MS, White DN, Peterson MN, Martin RA, *et al.* 2013. Environmental drivers of demographics, habitat use, and behavior during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). *Evol Ecol*. 27:971–991.

Hendry AP, Kelly ML, Kinnison MT, Reznick DN. 2006. Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *J Evol Biol*. 19:741–754.

Hendry AP, Taylor EB. 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.

Jourdan J, Krause ST, Lazar VM, Zimmer C, Sommer-Trembo C, Arias-Rodriguez L, Klaus S, Riesch R, Plath M. 2016. Shared and unique patterns of phenotypic diversification along a stream gradient in two congeneric species. *Sci Rep*. 6:38971.

Kaeuffer R, Peichel CL, Bolnick DI, Hendry AP. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. *Evolution*. 66:402–418.

Lande R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution*. 33:402–416.

Landry L, Vincent WF, Bernatchez L. 2007. Parallel evolution of lake whitefish dwarf ecotypes in association with limnological features of their adaptive landscape. *J Evol Biol*. 20:971–984.

Langerhans RB. 2009. Morphology, performance, fitness: functional insight into a post-Pleistocene radiation of mosquitofish. *Biol Lett*. 5:488–491.

Langerhans RB. 2010. Predicting evolution with generalized models of divergent selection: a case study with poeciliid fish. *Integr Comp Biol*. 50:1167–1184.

Langerhans RB, DeWitt TJ. 2004. Shared and unique features of evolutionary diversification. *Am Nat*. 164:335–349.

Langerhans RB, Gifford ME. 2009. Divergent selection, not life-history plasticity via food limitation, drives morphological divergence between predator regimes in *Gambusia hubbsi*. *Evolution*. 63:561–567.

Langerhans RB, Gifford ME, Joseph EO. 2007. Ecological speciation in Gambusia fishes. *Evolution*. 61:2056–2074.

Langerhans RB, Knouft JH, Losos JB. 2006. Shared and unique features of diversification in Greater Antillean Anolis ecomorphs. *Evolution*. 60:362–369.

Langerhans RB, Makowicz AM. 2009. Shared and unique features of morphological differentiation between predator regimes in *Gambusia caymanensis*. *J Evol Biol*. 22:2231–2242.

Langerhans RB, Reznick DN. 2010. Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: P Domenici, BG Kapoor, editors. *Fish locomotion: an etho-ecological perspective*. Enfield: Science Publishers.

Langerhans RB, Riesch R. 2013. Speciation by selection: a framework for understanding ecology's role in speciation. *Curr Zool*. 59:31–52.

Losos JB. 2011. Convergence, adaptation, and constraint. *Evolution*. 65:1827–1840.

MacColl ADC. 2011. The ecological causes of evolution. *Trends Ecol Evol*. 26:514–522.

Mahler DL, Ingram T, Revell LJ, Losos JB. 2013. Exceptional convergence on the macroevolutionary landscape in island lizard radiations. *Science*. 341:292–295.

Martin RA, McGee MD, Langerhans RB. 2015. Predicting ecological and phenotypic differentiation in the wild: a case of piscivorous fish in a fishless environment. *Biol J Linn Soc*. 114:588–607.

Martin RA, Riesch R, Heinen-Kay JL, Langerhans RB. 2014. Evolution of male coloration during a post-Pleistocene radiation of Bahamas mosquitofish (*Gambusia hubbsi*). *Evolution*. 68:397–411.

Mendelson TC, Martin MD, Flaxman SM. 2014. Mutation-order divergence by sexual selection: diversification of sexual signals in similar environments as a first step in speciation. *Ecol Lett*. 17:1053–1066.

Moore MP, Riesch R, Martin RA. 2016. The predictability and magnitude of life-history divergence to ecological agents of selection: a meta-analysis in livebearing fishes. *Ecol Lett*. 19:435–442.

Mylroie JE, Carew JL, Moore AI. 1995. Blue holes: definition and genesis. *Carbonate Evaporite*. 10:225–233.

Nosil P, Crespi BJ. 2004. Does gene flow constrain adaptive divergence or vice versa? A test using ecomorphology and sexual isolation in *Timema cristinae* walking-sticks. *Evolution*. 58:102–112.

Nosil P, Crespi BJ, Sandoval CP. 2002. Host-plant adaptation drives the parallel evolution of reproductive isolation. *Nature*. 417:440–443.

Oke KB, Bukhari M, Kaeuffer R, Rolshausen G, Räsänen K, Bolnick DI, Peichel CL, Hendry AP. 2016. Does plasticity enhance or dampen phenotypic parallelism? A test with three lake-stream stickleback pairs. *J Evol Biol*. 29:126–143.

Oke KB, Rolshausen G, LeBlond C, Hendry AP. 2017. How parallel is parallel evolution? A comparative analysis in fishes. *Am Nat*.

Palkovacs EP, Dion KB, Post DM, Caccone A. 2008. Independent evolutionary origins of landlocked alewife populations and rapid parallel evolution of phenotypic traits. *Mol Ecol*. 17:582–597.

Perrier C, Bourret V, Kent MP, Bernatchez L. 2013. Parallel and nonparallel genome-wide divergence among replicate population pairs of freshwater and anadromous Atlantic salmon. *Mol Ecol*. 22:5577–5593.

Rasanen K, Hendry AP. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecol Lett*. 11:624–636.

Ravinet M, Prodohl PA, Harrod C. 2013. Parallel and nonparallel ecological, morphological and genetic divergence in lake-stream stickleback from a single catchment. *J Evol Biol*. 26:186–204.

Ravinet M, Westram A, Johannesson K, Butlin R, Andre C, Panova M. 2016. Shared and nonshared genomic divergence in parallel ecotypes of *Littorina saxatilis* at a local scale. *Mol Ecol*. 25:287–305.

Riesch R, Barrett-Lennard LG, Ellis GM, Ford JKB, Deecke VB. 2012. Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? *Biol J Linn Soc*. 106:1–17.

Riesch R, Martin RA, Langerhans RB. 2013. Predation's role in life-history evolution of a livebearing fish and a test of the Trexler-DeAngelis model of maternal provisioning. *Am Nat*. 181:78–93.

Robinson BW, Wilson DS. 1994. Character release and displacement in fishes: a neglected literature. *Am Nat*. 144:596–627.

Robinson BW, Wilson DS, Margosian AS. 2000. A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). *Ecology*. 81:2799–2812.

Schluter D. 2000. *The ecology of adaptive radiation*. Oxford: Oxford University Press.

Schug MD, Downhower JF, Brown LP, Sears DB, Fuerst PA. 1998. Isolation and genetic diversity of *Gambusia hubbsi* (mosquitofish) populations in blueholes on Andros island, Bahamas. *Heredity*. 80:336–346.

Soria-Carrasco V, Gompert Z, Comeault AA, Farkas TE, Parchman TL, Johnston JS, Buerkle CA, Feder JL, Bast J, Schwander T, *et al.* 2014. Stick insect genomes reveal natural selection's role in parallel speciation. *Science*. 344:738–742.

Steadman DW, Franz R, Morgan GS, Albury NA, Kakuk B, Broad K, *et al.* 2007. Exceptionally well preserved late Quaternary plant and vertebrate fossils from a blue hole on Abaco, The Bahamas. *Proc Natl Acad Sci USA*. 104:19897–19902.

Steiner CC, Rompler H, Boettger LM, Schoneberg T, Hoekstra HE. 2009. The genetic basis of phenotypic convergence in beach mice: similar pigment patterns but different genes. *Mol Biol Evol*. 26:35–45.

Stuart YE, Veen T, Weber JN, Hanson D, Ravinet M, Lohman BK, *et al.* 2017. Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nat Ecol Evol*.

Tenaillon O, Rodríguez-Verdugo A, Gaut RL, McDonald P, Bennett AF, Long AD, Gaut BS. 2012. The molecular diversity of adaptive convergence. *Science*. 335:457–461.

Todhunter A. 2010. Deep dark secrets. *Natl Geogr*. 8:34–53.

Wainwright PC, Alfaro ME, Bolnick DI, Hulsey CD. 2005. Many-to-one mapping of form to function: a general principle in organismal design? *Integr Comp Biol*. 45:256–262.