



# The cryptic origins of evolutionary novelty: 1000-fold faster trophic diversification rates without increased ecological opportunity or hybrid swarm

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Ecological opportunity is frequently proposed as the sole ingredient for adaptive radiation into novel niches. An additional trigger may be genome-wide hybridization resulting from “hybrid swarm.” However, these hypotheses have been difficult to test due to the rarity of comparable control environments lacking adaptive radiations. Here I exploit such a pattern in microendemic radiations of Caribbean pupfishes. I show that a sympatric three species radiation on San Salvador Island, Bahamas diversified 1445 times faster than neighboring islands in jaw length due to the evolution of a novel scale-eating adaptive zone from a generalist ancestral niche. I then sampled 22 generalist populations on seven neighboring islands and measured morphological diversity, stomach content diversity, dietary isotopic diversity, genetic diversity, lake/island areas, macroalgae richness, and Caribbean-wide patterns of gene flow. None of these standard metrics of ecological opportunity or gene flow were associated with adaptive radiation, except for slight increases in macroalgae richness. Thus, exceptional trophic diversification is highly localized despite myriad generalist populations in comparable environmental and genetic backgrounds. This study provides a strong counterexample to the ecological and hybrid swarm theories of adaptive radiation and suggests that diversification of novel specialists on a sparse fitness landscape is constrained by more than ecological opportunity and gene flow.

**KEY WORDS:** Adaptive radiation, diversification rate, ecological opportunity, ecological speciation, innovation, macroevolution, novelty, trophic divergence.

Adaptive radiation is one of the most fundamental processes producing bursts of species diversification, trait divergence, and niche evolution across the tree of life (Simpson 1944; Schluter 2000). Ecological opportunity, an abundance of available resources in a new environment with few competitors, has long been viewed as the primary force driving diversification through divergent selection on niche use, mediated by competition and predation (Simpson 1944; Schluter 2000; Losos and Mahler 2010; Pfennig and Pfennig 2010). This relationship between new ecological space and divergent selection leading to reproductive isolation forms the foundation of the ecological theory of adaptive radiation (Schluter 2000; Losos and Mahler 2010) and ecological speciation theory (McKinnon et al. 2004; Rundle and Nosil 2005; Nosil 2012).

At microevolutionary scales, substantial observational and experimental evidence demonstrates that resource abundance drives ecological divergence (Schluter 2000; Parent and Crespi 2009; Nosil 2012). For example, parallel ecological diversification and speciation has been observed repeatedly in laboratory microcosms (Rainey and Travisano 1998; Elena and Lenski 2003; Kassen et al. 2004) and natural systems (Gillespie 2004; Langerhans et al. 2007; Losos 2009; Martin 2012) when populations are confronted with similar levels of ecological opportunity, constrained only by altered communities of predators or competitors (Vamosi 2003; Pfennig and Pfennig 2012). Holding lineage-specific factors and biotic communities constant, ecological divergence in response to resource abundance appears much

more predictable than originally imagined (Bolnick and Lau 2008; Nosil et al. 2009; Langerhans 2010).

In contrast, it is still largely unknown what triggers major evolutionary novelties and large-scale adaptive radiation: why do only some lineages rapidly diversify and colonize novel ecological niches in response to resource abundance while others do not (Roderick and Gillespie 1998; Burns et al. 2002; Erwin 2015a)? Many macroevolutionary trends run counter to predictions of the ecological theory of adaptive radiation. First, contrary to Simpson's (1944) paradigm, major ecological transitions to new adaptive zones are only weakly or not at all associated with the availability of ecological opportunity due to colonization of an isolated habitat, following mass extinction, or the evolution of a key innovation (Erwin 2015a; Harmon and Harrison 2015). For example, for nearly every adaptive radiation containing novel ecological specialists, there is a similar lineage that has failed to diversify in the exact same environment (Givnish et al. 1997; Roderick and Gillespie 1998; Arbogast et al. 2006; Losos 2009; Martin and Wainwright 2011, 2013c). Large-scale radiations often originate long before mass extinction events (Schuettelpelz and Pryer 2009; Wilson et al. 2012) or the evolution of key innovations (Alfaro et al. 2009; Erwin 2015a). Moreover, most classic adaptive radiations do not exhibit an early burst of trait diversification, the rapid niche-filling response predicted by the ecological opportunity hypothesis (Harmon et al. 2010; Erwin 2015; Harmon and Harrison 2015).

Second, the origins of adaptive radiation cannot be explained by the same factors that are associated with speciation and morphological diversification rates. Although there is a strong relationship between area and speciation rate (Losos and Schluter 2000; Kisel and Barraclough 2010), island biogeography breaks down when attempting to predict which lineages will radiate and where (Seehausen 2006; Wagner et al. 2012, 2014). Adaptive radiation also appears to defy ecological limits and frequently results in species assemblages exceeding the equilibrium species diversity expected from community assembly processes alone (Gillespie 2004; Gavrillets and Losos 2009; Martin and Genner 2009; Wagner et al. 2014). Thus, while ecological opportunity is sufficient to drive divergent selection among populations, its primary role in triggering macroevolutionary diversification is unclear (Erwin 2015a).

An emerging addition to the ecological opportunity hypothesis is the hybrid swarm hypothesis, which proposes that an influx of genetic diversity and novel allelic combinations after hybridization of distinct lineages (inter- or intraspecific), possibly including segregating postzygotic intrinsic incompatibilities, may trigger rapid diversification in an environment with abundant ecological opportunity (Seehausen 2004; Roy et al. 2015; Schumer et al. 2015). Hybridization is known to play a role in the formation of single ecologically divergent species, even among homoploids

(Rieseberg et al. 2003; but see Schumer et al. 2013). In contrast, although a growing number of studies identify substantial gene flow and adaptive introgression during adaptive radiation (Brawand et al. 2014; Lamichhaney et al. 2015; Malinsky et al. 2015; Martin et al. 2015, 2016; Stankowski and Streisfeld 2015), in general gene flow opposes local adaptation (Lenormand 2002) and there is still no evidence that hybridization specifically triggered adaptive diversification, as opposed to being pervasive throughout the history of any young rapidly diversifying group (Servedio et al. 2013; Berner and Salzburger 2015; Kuhlwilm et al. 2016; but see Kautt et al. 2016). To directly test these hypotheses about the origins of adaptive radiation and novelty, we need to examine systems where speciation has not occurred across many apparently suitable environments. This would allow for comparisons of variation in ecological opportunity and gene flow across similar environments that differ in the presence of nascent adaptive radiations of specialists.

Here I exploit such a pattern of microendemic adaptive radiations and the rare evolution of novel trophic specialists in *Cyprinodon* pupfishes to test the roles of ecological opportunity and hybridization in triggering adaptive radiation and novelty. Pupfishes inhabit coastal areas and salt lakes across the entire Caribbean and Atlantic, from Massachusetts to Venezuela; however, despite the ubiquity of these populations and the abundant ecological opportunity present in most Caribbean salt lakes that lack predators and contain few competing fish species, nearly all pupfishes are generalist omnivores consuming algae and microinvertebrates (Martin and Wainwright 2011). Sympatric radiations of trophic specialists have evolved only twice: once in 10,000-year-old salt lakes on San Salvador Island, Bahamas and independently in the 8000-year-old Laguna Chichancanab basin in the Yucatan (Martin and Wainwright 2011). In addition to a generalist algae-eating species present in all lakes (*Cyprinodon variegatus*), the San Salvador radiation contains two rare specialist species coexisting with generalists in some lakes. These specialists adapted to unique trophic niches using novel skeletal traits: a scale-eating pupfish with enlarged oral jaws (*Cyprinodon desquamator* [Martin and Wainwright 2013a]) and a molluscivore pupfish with a unique nasal/maxillary protrusion that may provide jaw stabilization for crushing (*Cyprinodon brontotheroides* [Martin and Wainwright 2013a]). Similarly, the Chichancanab radiation of five species contains a generalist algae-eating species (*C. beltrani*) and at least two trophic specialists, a zooplanktivore (*C. simus*) and a piscivore (*C. maya*; Humphries and Miller 1981; Humphries 1984; Stevenson 1992; Horstkotte and Strecker 2005). These trophic niches and skeletal phenotypes in each sympatric radiation are unique among all *Cyprinodon* species; furthermore, this is the only specialized scale-eater known among over 1500 species of Cyprinodontiform fishes (Martin and Wainwright 2011, 2013c). These unique traits are hypothesized to be novel, in the sense of a body part

evolving quasi-independence or individuation through modification of gene regulatory networks (following Erwin 2015), and different scaling relationships with body size support this idea (Fig. S4, Fig. S3 Lencer et al. 2016). These traits are also innovations, in the sense of enabling access to new resources (following Liem 1980; Losos 2009; Blount et al. 2012a), but it is too early to determine if they are key innovations causing an increase in species diversification rate (Hunter 1998; Whittall and Hodges 2007).

Trophic specialists in both sympatric pupfish radiations co-occur in all lake habitats, but are largely reproductively isolated with low levels of hybridization (within-lake interspecific  $F_{st} = 0.12\text{--}0.49$ ; Strecker 2006a; Martin and Feinstein 2014). The San Salvador radiation is nested within many outgroup Caribbean populations of *C. variegatus*, strongly indicating that these specialists evolved from a generalist ancestor similar in morphology to *C. variegatus* (Martin and Wainwright 2011; Martin and Feinstein 2014). Similarly, the Chichancanab radiation is nested within outgroup populations of *C. artifrons* along the Yucatan coast, indicating that these specialists evolved from a generalist ancestor resembling *C. artifrons* (Martin and Wainwright 2011). Unfortunately, Chichancanab was colonized by two invasive fish species in the 1990s, which caused the extinction of at least two specialist species (Schmitter-Soto 1999; Strecker 2006b; Martin and Wainwright 2011). Thus, I focus on the San Salvador radiation in this study with occasional comparisons to initial descriptions of the Chichancanab environment before it was impacted by invasives (e.g. Humphries and Miller 1981).

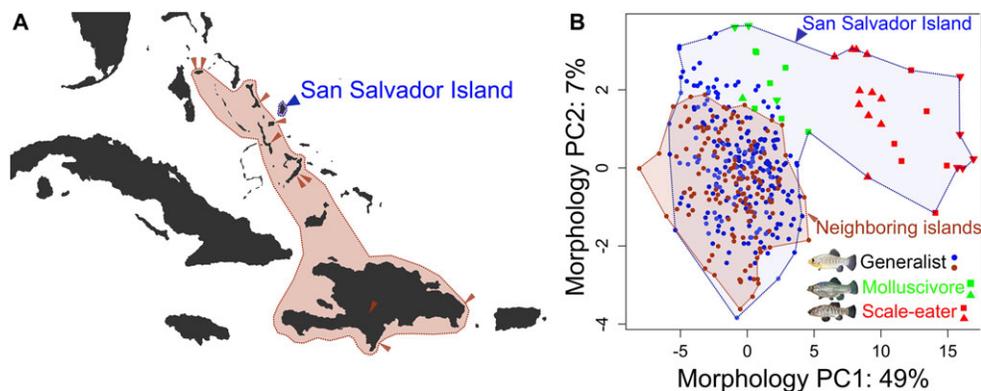
In summary, two unusual features of Caribbean pupfishes provide an outstanding opportunity for investigating the causes of adaptive radiation and the rare evolution of ecological novelty: (1) major evolutionary novelties evolved within 10,000 years and are restricted to isolated locations, (2) each sympatric radiation is surrounded by a large number of comparable environments inhab-

ited by a generalist lineage comparable to the putative ancestor where rapid adaptive diversification has not occurred. To pinpoint where major ecological novelties evolved and identify which ecological and genomic factors are associated with these transitions, I integrated population genomics, phylogenetic comparative methods, and a Caribbean-wide ecological, morphological, and genetic survey of pupfish populations. I first tested for the presence of exceptional trophic diversification and shifts to new adaptive zones by measuring 28 functional traits in 22 pupfish populations on seven islands ( $n = 493$  individuals across all populations). I then tested whether ecological opportunity and hybridization were associated with exceptional trait diversification detected on San Salvador. To test the role of ecological opportunity, I compared the best indicators of ecological opportunity in this system to the number of sympatric pupfish species within each lake: lake area, macroalgae species richness, and the genetic diversity, stomach content diversity, dietary isotopic diversity, and morphological diversity of generalist pupfish populations in each lake. To test the role of hybridization, I used genome-wide sampling of genetic markers to estimate whether the species on San Salvador uniquely showed evidence of hybrid swarm origins relative to neighboring islands using  $f_4$  statistics, principal component analysis, species tree estimation, and Treemix population graphs.

## Methods

### SAMPLING

Twelve hypersaline lakes on San Salvador Island were sampled for both generalist and specialist pupfishes (if present), including four lakes containing only generalists, two containing generalists and molluscivores, two containing generalists and scale-eaters, and four containing generalists and both specialist species in July, 2011 (Fig. 1; Appendix S1). Ten hypersaline lakes



**Figure 1.** (A) Sampling locations of generalist populations across the Caribbean (San Salvador lakes:  $n = 12$ ; lakes on neighboring islands:  $n = 10$ ). (B) First two principal components of morphological diversity for 28 size-corrected skeletal traits measured on 493 alizarin-stained specimens (Fig. S3). Morphological diversity of generalists on San Salvador (●) is equal to the diversity of generalist morphology on neighboring islands (●). Diversity of all three San Salvador species (blue area) greatly exceeds trans-Caribbean diversity (brown area). Shapes indicate different specialist populations on San Salvador.

containing generalist pupfish on five neighboring islands (Rum Cay, Cat, Long, Acklins, New Providence) and the Dominican Republic were sampled between May and July, 2011 (brown arrows: Fig. 1; Appendix S1). Additional outgroup *Cyprinodon* species were sampled across the entire coastal range of *Cyprinodon* from Massachusetts to Venezuela (Appendix S1).

Neighboring lakes were comparable to those on San Salvador: hypersaline due to limited hydrological connectivity with the ocean and dominated by mangroves in predominantly shallow (2–5 m) karst basins. Physiological parameters, including pH, salinity, alkalinity, and temperature, are extremely variable within each lake due to tidal fluctuations and rainfall, but highly comparable across lakes due to their shared carbonate geology and water chemistry (Rothfus 2012). Lakes contained from zero to two insectivorous fish species in addition to *Cyprinodon*: the Bahamian mosquitofish, *Gambusia hubbsi*, and the bighead silverside, *Atherinomorus stipes*, with the exception of both lakes on New Providence Island, which contained invasive fishes such as *Oreochromis mossambicus*, *Xiphophorus maculatus*, and *Poecilia reticulata* and the three large lakes in the Dominican Republic, which contained cichlid, eleotrid, and American crocodile (*Crocodylus acutus*) predators and *Limia* spp. and *Gambusia* spp. competitors. Dominican lakes exhibited similar phenotypic and ecological diversity trends to those in the Bahamas and are included for comparison; removing these lakes did not qualitatively affect the results.

Between nine and 48 specimens (mean = 21.6; SD = 8.8) were sampled from generalist populations in each lake by seine or hand net and euthanized in an overdose of buffered MS-222 (Finquel, Inc.) following animal care protocols approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC protocols #15908 and #17455).

### GENOMIC SEQUENCING AND BIOINFORMATICS

Between one and six wild-caught fish from each of 22 generalist populations, plus specialist *C. desquamator* and *C. bronthoeroides* populations on San Salvador, and Caribbean outgroups ( $n = 112$  individuals; Appendix S1) were sequenced using genotyping by sequencing, aligned to the *C. variegatus* reference assembly (version 1.0), and SNPs (single nucleotide polymorphisms) were called using Stacks (Catchen et al. 2013) as further described in the supplement following previous RADseq studies (Martin and Feinstein 2014; Martin et al. 2015, 2016; Martin et al. in revision). Respectively, 601 million 150-bp and 356 million 100-bp raw reads were sequenced and 651 million reads uniquely aligned to the *C. variegatus* assembly.

### MORPHOLOGICAL DIVERSITY

Multiple individuals from 21 lake populations ( $n = 493$  individuals total; nine to 48 samples per population; mean = 21.6;

SD = 8.8) were cleared and alizarin-stained for measurement of 28 functional skeletal traits (Figs. S2–S4). For each population, adult specimens were cleared and double-stained with alizarin and alcian blue to visualize skeletal morphology (Dingerkus and Uhler 1977). The skull of each specimen was photographed on both lateral sides with jaw adducted for a clear view of the quadrotic region, framing only the head and pectoral girdle for maximum resolution of smaller features (Fig. S3). Specimens were photographed and measured on both lateral sides of the head and the mean was used to reduce measurement error. Thirty-two landmarks (Fig. S3) were digitized using tpsdig2 software (Rohlf 2001) and converted to 29 linear distances (Table S1) describing functional traits and the most divergent traits among the three San Salvador species. Size-corrected residuals were used for analyses (further details in Supporting Information).

### DIETARY DIVERSITY

For a subset of the generalist individuals sampled from each population, dietary diversity was estimated from stomach content analyses ( $n = 359$  individuals total) and stable isotope analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $n = 487$  individuals total; Fig. S5). These complementary approaches reflect short-term fine-grain and long-term coarse-grain estimates of dietary diversity, respectively (Post 2002; Layman 2007). Stomach items were separated into broad taxonomic categories (e.g., macroalgae, seagrass, polychaete, ostracod, gastropod) and total surface areas of each component were estimated under 10–50 $\times$  magnification using a Sedgwick-rafter cell (following Martin and Wainwright 2013c). Dietary diversity within each population was estimated from Simpson's inverse diversity index, the probability that two randomly sampled items do not belong to the same category (DeJong 1975). For stable isotope analyses, dried muscle samples from the caudal peduncle region of each fish were analyzed for natural abundances of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the UC Davis Stable Isotope Facility on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer.

### GENETIC DIVERSITY

Genetic diversity across lake populations of generalist *Cyprinodon* was estimated by exporting 170 loci genotyped completely in 22 populations and using Stacks to calculate  $\pi$ , the average number of pairwise differences between sequences drawn at random within the population (Catchen et al. 2013). Estimates of genetic diversity were similar and qualitatively unchanged when running analyses with more permissive filtering criteria, at the island-level, and without genome alignment. Also note that RADseq is a highly biased sample of the genome (Martin et al. 2016) and will generally underestimate genetic diversity due to restriction site dropout (Arnold et al. 2013), but this should not affect comparative inferences in this case.

## TESTING FOR EXCEPTIONAL DIVERSIFICATION RATES: PHYLOGENETIC COMPARATIVE ANALYSES

I used BEAST (Drummond and Rambaut 2007) to estimate a time-calibrated phylogeny for Caribbean *Cyprinodon* populations from 8352 concatenated RADseq loci (following Martin et al. 2015), detailed in the Supporting Information and presented in Fig. S1. I then used two complementary approaches to assess the distribution of trait diversification rates across this phylogeny. First, I used reversible-jump Markov Chain Monte Carlo (MCMC) sampling of multi-rate Brownian motion models for each trait on the maximum-likelihood phylogeny (Eastman et al. 2011). Nearly all nodes in the time-calibrated tree were resolved with posterior probabilities of 1 (Fig. S1); therefore, phylogenetic uncertainty was not accounted for in these analyses and only a single tree was used. Reversible-jump sampling allows for “jumps” among models with varying numbers of parameters in addition to varying the values of these parameters while sampling likelihood space, enabling the MCMC sampler to explore shifts among different diversification rate regimes on different parts of the phylogeny without a priori specification of these shifts as required in earlier methods (O’Meara et al. 2006). This approach also naturally results in model-averaged parameter estimates (Burnham and Anderson 2002). For each residual trait value measured in 29 taxa (see above Section “Morphological Diversity”), I used the *auteur* suite of methods (Eastman et al. 2011), part of the *geiger2* package (Pennell et al. 2014) in R (R Core Team 2015) to run rjMCMC chains and estimate the placement and magnitude of shifts in diversification rate across the tree. Each chain was run for 10,000 generations, sampling every 100 steps from a relaxed Brownian motion model allowing for the possibility of instantaneous jumps between rate regimes (*type* = “jump-rbm”). The first 50% of each chain was discarded as burn-in and shifts in rate regimes were visualized on the phylogeny for each trait. Chains were run multiple times for each trait to assess convergence. Median diversification rates and their placement on the phylogeny for rapidly diversifying traits were generally robust across runs; however, this is primarily an exploratory technique for visualizing the highest densities of major trait diversification shifts and no explicit model comparisons were performed (e.g., Santini et al. 2013).

Second, I used reversible-jump MCMC sampling of the more generalized Ornstein–Uhlenbeck (OU) model of trait diversification. In addition to a Brownian motion parameter ( $\sigma^2$ ) modeling diffusion rate, the OU model includes the elastic pull of a stabilizing selection regime acting on a trait with two additional parameters:  $\alpha$ , the strength of selection on the trait, and  $\theta$ , the location of the fitness optimum (Hansen 1997; Martins and Hansen 1997; Butler and King 2004). Stronger selection on a trait as it nears its fitness optimum constrains the stochastic diffusion rate of  $\sigma^2$  over time, erasing the phylogenetic signal of a trait (Hansen

et al. 2008). Although a single fitness optimum can be fit to an entire clade, the ecological theory of adaptive radiation predicts that multiple fitness peaks will drive ecological divergence and speciation (Schluter 2000), indicating that multi-optima OU models are needed to model adaptive radiation. Indeed, multiple fitness peaks on a complex fitness landscape were directly measured in this system (Martin and Wainwright 2013c), demonstrating that fitness peaks were non-Gaussian and connected by varying depths of fitness valleys and ridges on a single adaptive landscape for the entire three-species radiation, in contrast to instantaneous transitions among single-optimum Gaussian fitness regimes along branches as specified by the multi-rate OU. Nonetheless, OU models may provide a rough approximation of stabilizing selection acting on different phenotypic optima over evolutionary time and enable estimation of the temporal dynamics of adaptive regime shifts across the *Cyprinodon* phylogeny.

To specifically explore jaw length, the trait with the largest shift in diversification rate on San Salvador, I used *bayou* to conduct reversible-jump MCMC sampling of multi-rate OU models (Uyeda and Harmon 2014) in R. I first specified an uninformative prior on the OU models, using half-Cauchy priors for  $\alpha$  and  $\sigma^2$ , a normal prior for  $\theta$ , a conditional Poisson distribution for the number of shifts between selective regimes ( $\lambda = 15$ ,  $\max = 200$ ), and a maximum of one shift per branch. I also explored more restrictive priors for the number of shifts, but found convergence across runs to be less stable. I ran two chains of 1,000,000 generations each, sampling every 100 steps, and combined chains after discarding the first 50% as burn-in. Convergence was assessed using Gelman and Rubin’s R statistic. Repeated runs indicated that convergence was quickly reached and all runs indicated a robust shift to a new adaptive regime for scale-eaters. Results were plotted as a “traitgram” (Ackerly 2009) using *bayou*’s built-in plotting function (Uyeda and Harmon 2014).

## TESTING THE ECOLOGICAL OPPORTUNITY HYPOTHESIS: MORPHOLOGICAL, DIETARY, AND GENETIC DIVERSITY

To test the hypothesis that ecological opportunity is associated with exceptional diversification rates on San Salvador Island, I used linear models to measure the effects of morphological diversity, dietary diversity, genetic diversity, lake/island areas (from the Google Maps Area Calculator Tool: [daflogic.com](http://daflogic.com)), and macroalgae species richness on the number of pupfish species coexisting within a lake (ranging from one to three species). Multiple regression models were compared using stepwise addition and removal of predictors with the stepAIC function in the MASS package in R (Venables and Ripley 2013). The fit of significant one-way models was compared to full models including all interaction terms.

## TESTING THE HYBRID SWARM HYPOTHESIS: MAJOR AXES OF GENETIC VARIATION AND INTROGRESSION

### *Principal components analysis*

The main prediction of the hybrid swarm hypothesis is that hybridization will facilitate the evolution of ecological specialists. To test for this pattern, I first estimated principal components of genetic variation across the Caribbean, excluding San Salvador populations. Eleven thousand seven hundred six SNPs genotyped in at least 50% of individuals with a minimum depth of 10 aligned reads were exported for principal component analyses (PCA) of genetic variance using probabilistic PCA in the Bioconductor *pcaMethods* package (Stacklies et al. 2007) in R. Only 1 SNP per locus was exported to reduce linkage disequilibrium within this dataset. I then projected all three San Salvador species ( $n = 75$  individuals) onto the first two principal components of Caribbean-wide genetic variation to assess whether specialists shared a greater proportion of their ancestry with any of the outgroups sampled. This approach has previously been used to visualize patterns of shared ancestry within a focal population while avoiding biases introduced by uneven sampling among groups inherent to principal components analysis (e.g. see Discussion in McVean 2009; Lazaridis et al. 2014; Martin et al. 2015). One major caveat is that these analyses only examine genome-wide patterns of relatedness among populations and will miss fine-scale patterns of introgression into specialists and cases where all individuals in the radiation are equally admixed following hybrid swarm (potentially resulting in genomic stabilization: see Schumer et al. 2016).

### *Species tree inference*

To assess uncertainty among gene tree topologies for Caribbean pupfishes, I used SNAPP, which integrates over all sampled gene trees (Bryant et al. 2012). Details are further described in the Supporting Information.

### *Tests of asymmetric gene flow using $f_4$ statistics*

Species trees may still be an inadequate model to understand population history if secondary contact or continuous gene flow is present (Pickrell and Pritchard 2012). I used  $f_4$  statistics to test for additional gene flow between branches within four-population maximum-likelihood trees containing the two specialist species relative to Caribbean outgroups of the form: (*C. brontotheroides*, *C. desquamator*; Caribbean outgroup 1, Caribbean outgroup 2). This statistic measures asymmetry in allele frequency correlations between the (A,B); (C,D) clades on the four-population tree ((A,B); (C,D)) and is expected to be zero in the absence of gene flow with only incomplete lineage sorting (but is sensitive to ancestral population structure: Reich et al. 2009; Durand et al. 2011). Similar to the ABBA-BABA/D-statistic, the  $f_4$  statistic tests for evidence of secondary gene flow in focal taxa, but does not

require a rooted four-taxon tree (Martin et al. 2015). Also note that these statistics will not detect symmetric introgression into multiple taxa, introgression between sister species, or equal amounts of shared hybrid ancestry following hybrid swarm (Reich et al. 2009; Durand et al. 2011).

Treemix software (version 1.12; Pickrell and Pritchard 2012) was used to calculate  $f_4$  statistics from 4213 SNPs genotyped in all focal Caribbean populations (pooled as described for SNAPP analyses) and sampled once per RAD locus. SE was calculated by jackknife estimation in windows of size 1 (i.e., each SNP was treated as an independent sample due to sparse coverage and sampling only one SNP per RAD locus).

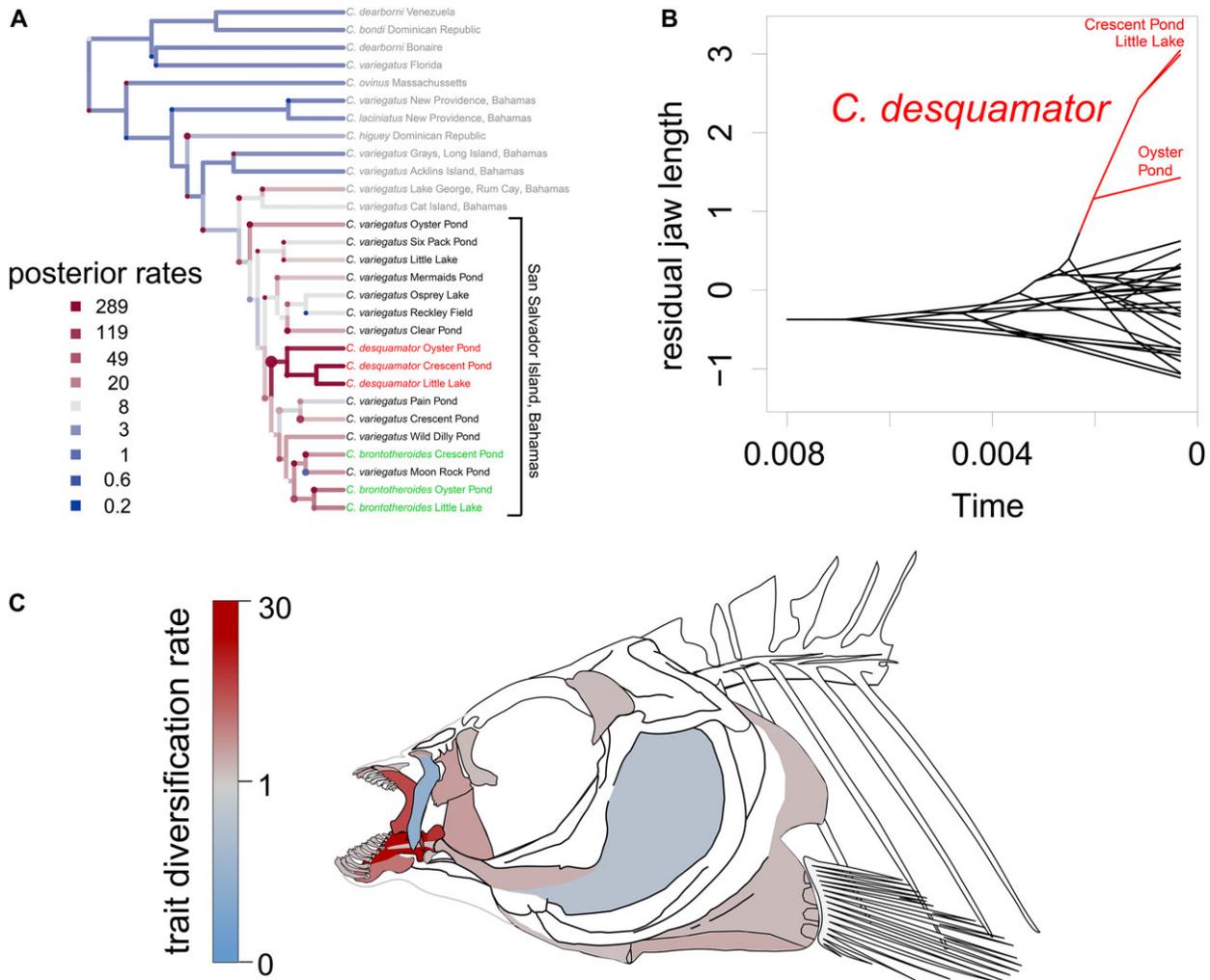
### *Treemix population graphs*

To visualize secondary gene flow among Caribbean populations, I used Treemix (version 1.12; Pickrell and Pritchard 2012) to estimate maximum-likelihood trees with varying numbers of admixture events connecting populations after their divergence, forming graphs of interconnected populations. The number of admixture events was estimated by comparing the log likelihood of different population graphs following global realignment and jackknife estimation in windows of size 1 using the 4213 SNP dataset (following Martin and Feinstein 2014). To estimate the number of admixture events connecting populations, I used an approach similar to Evanno et al. (2005) and compared the rate of change of the log likelihood with the addition of each admixture event; however, note that the ability of Treemix to reliably detect the number of admixture events has never been evaluated and this approach should be viewed only as an exploratory tool.

## Results

### EXCEPTIONAL TROPHIC DIVERSIFICATION RATES WITHIN SAN SALVADOR ISLAND PUPFISHES

I found strong evidence for exceptional rates of trophic diversification and shifts to new adaptive zones localized to San Salvador Island, Bahamas (Fig. 2) despite extensive sampling of hypersaline lakes with identically depauperate fish communities on five neighboring Bahamian islands and the three largest Caribbean hypersaline lakes found in the Dominican Republic (Fig. 1). Sampling from the posterior distribution of multi-rate Brownian motion models indicated that the fastest trait diversification rates occurred for jaw length on the internal branch leading to all three populations of the scale-eating pupfish, *C. desquamator*, endemic to San Salvador (Fig. 2A, Table S1). These analyses must be interpreted with caution due to the small size of our phylogeny and violation of *auteur's* assumption of a bifurcating phylogeny (see Fig. 5); however, the relative rate difference observed was substantially larger than the largest effect size used in *auteur*



**Figure 2.** (A) Shifts in jaw diversification rate painted along each branch of the phylogeny estimated from mean residual jaw size in each population. Heat colors along each branch indicate median rates estimated from the posterior distribution of models and the size of circles at each node indicate the probability of a shift to a different rate regime. There was a 49% posterior probability of a shift to the highest jaw diversification rate at the root node of all three scale-eating pupfish populations, which was 30.8 times faster than median background diversification rates (Table S1) and 1445 times faster than the slowest background rate (legend in panel a: 289/0.2) on most neighboring islands. (B) Traitgram illustrating distribution of mean residual jaw lengths across *Cyprinodon*. Red branches indicate the estimated phylogenetic position of a new adaptive regime corresponding to all three scale-eating populations (*C. desquamator*) with greatly enlarged jaws relative to the ancestral adaptive regime for generalist pupfish indicated by the black branches. (C) *Cyprinodon* “morphogram” illustrating median trait diversification rates on San Salvador relative to background rates on neighboring islands (also see heat map diagram in Martin and Wainwright 2011 for Cyprinodontidae). Warm/cool colors indicate faster/slower rates, respectively. Representative skeletal regions are highlighted for a selection of linear traits presented in Table S1.

simulations and larger effect sizes increase precision in the inferred placement of true rate shifts in this model (see Fig. 2 in Eastman et al. 2011). Nonetheless, comparative methods for population graphs are needed.

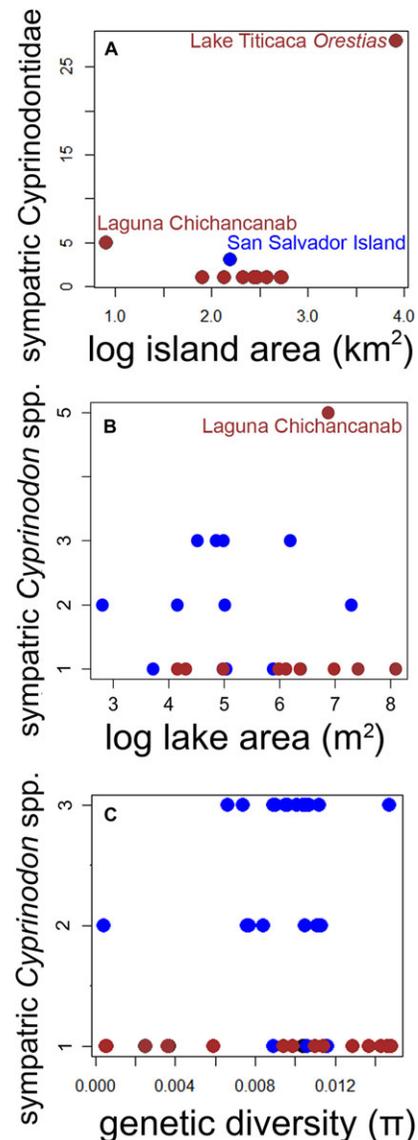
Accelerated trait diversification rates were most pronounced for jaw length and the width of the articular coronoid process (Fig. 2C, Table S1), which forms the base of the jaw closing lever and reflects the biomechanical tradeoff between fast scale-eating strikes and powerful shell-crushing force in the San Salvador

specialists. Only two of the 28 traits measured showed decelerated trait diversification rates on San Salvador relative to other islands (Fig. 2C, Table S1). Sampling from the generalized OU model of trait evolution strongly supported a shift to a new adaptive regime for scale-eating (Fig. 2B). The highest posterior probability of a shift to a new fitness optimum was observed for jaw length along the internal branch leading to scale-eating pupfish (posterior probability = 0.25; Fig. 2B). This new scale-eating optimum jaw size was nearly three SDs away (in mean standardized units)

from the jaw length optimum estimated for generalist pupfish, indicating a major phenotypic transition (Fig. 2B).

### INCREASED ECOLOGICAL OPPORTUNITY IS NOT ASSOCIATED WITH ADAPTIVE RADIATION AND TROPHIC NOVELTY

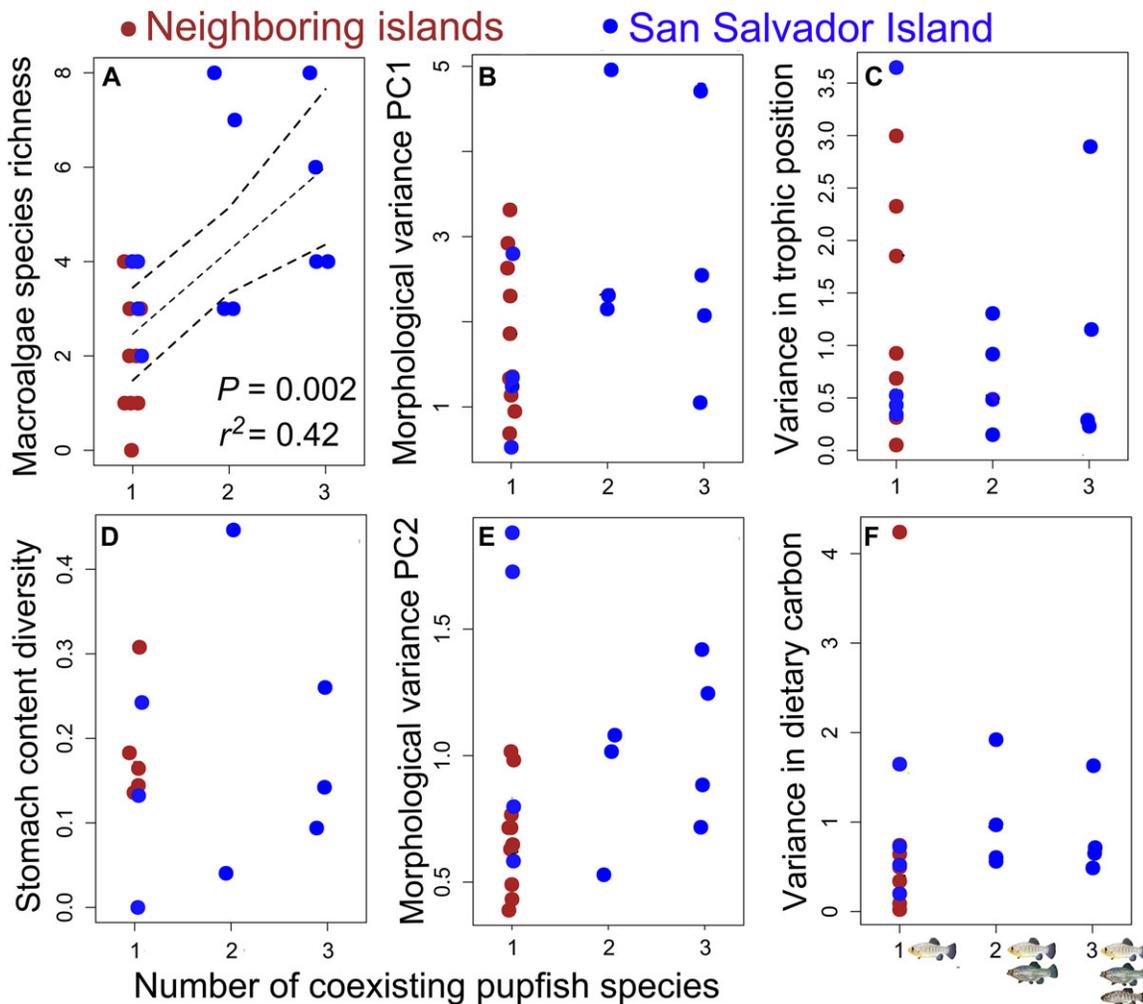
The ecological theory of adaptive radiation predicts that such exceptional rates of morphological, ecological, and sympatric species diversification on San Salvador should be associated with increased ecological opportunity. In contrast, I found no association between the presence of ecological specialist pupfishes and variation in nearly every standard index of ecological opportunity. First, the predicted island biogeographic relationship between island size and speciation rate (Losos and Schluter 2000; Kisel and Barraclough 2010; Wagner et al. 2014) does not hold up in this system. There was no correlation between lake area and the number of sympatric pupfish species and a negative correlation between island area and sympatric pupfish species (Table 1, Fig. 3A and B). Second, despite theoretical predictions (Weinreich and Chao 2005), genetic diversity within lake populations, an indicator of effective population size, was not associated with the number of sympatric pupfish species (Fig. 3C). Comparable levels of genetic diversity were also observed in the Chichancanab radiation (C. H. Martin unpubl. data). Instead, low genetic differentiation among Caribbean islands is consistent with previous demonstrations of pervasive gene flow among islands and may continually renew genetic diversity within hypersaline lakes (Martin and Feinstein 2014; see also Martin et al. 2016). Third, in contrast to many similar studies demonstrating a positive correlation between ecological opportunity and intraspecific or interspecific morphological and dietary diversity (Losos and Schluter 2000; Burns et al. 2002; Parent and Crespi 2009), an extensive survey of generalist populations across San Salvador and neighboring islands provided no evidence of increased ecological or phenotypic diversity in San Salvador lakes supporting specialists (Tables 1 and 2, Figs. 4, S2, S5). The diets of generalist pupfish should most directly reflect the availability of accessible resources within an environment, the best possible measurement of ecological opportunity. Furthermore, in contrast to expectations of character displacement (Pfennig and Pfennig 2012), there was no significant difference in dietary or morphological diversity between generalist populations on San Salvador with and without specialist pupfish species present (Table S2), suggesting that San Salvador generalist populations provide a good ecological baseline for comparison to other islands. There was no association between lakes supporting specialists and stomach content diversity, nitrogen isotopic diversity (indicating relative trophic position [Post 2002]), carbon isotopic diversity (indicating dietary carbon sources), or morphological diversity of generalist populations (estimated from principal component and discriminant axes separating the three San Sal-



**Figure 3.** In contrast to the expected speciation–area relationship, there was no correlation between (A) island area, (B) lake area, or (C) genetic diversity and the number of endemic *Cyprinodon* species (Tables 1 and 2). San Salvador populations are highlighted in blue, all other populations on neighboring islands in brown. The predicted speciation–area relationship was only supported at a much larger scale by including the distantly related radiation of *Orestias* pupfishes in Lake Titicaca (log lake area:  $r^2 = 0.24$ ,  $P = 0.003$ ; log island area:  $r^2 = 0.364$ ,  $P = 0.050$ ; Vila et al. 2013).

vador species based on 28 skeletal traits: Figs. 4, S2, S5; Tables 1 and 2).

The only significant effect among all univariate and multivariate models was the greater species richness of macroalgae communities within some lakes in San Salvador supporting multiple pupfish species (Fig. 4A, Tables 1 and 2). However, these



**Figure 4.** (A) Macroalgae species richness was significantly correlated with the number of coexisting pupfish species within a lake (dashed lines indicate best-fit regression line and 95% confidence interval; Table 1). All other ecological and morphological variables were not correlated with the number of coexisting pupfish species: (B, E) morphological diversity on the first two principal component axes ( $n = 493$  individuals, 21 populations), (D) stomach content diversity (Simpson's index:  $n = 359$  individuals, 13 populations), and (C, F) dietary isotopic diversity ( $\delta^{15}\text{N}$ : trophic position and  $\delta^{13}\text{C}$ : dietary carbon) of generalist populations in Caribbean salt lakes ( $n = 487$  individuals, 20 populations). Only generalist populations were compared among all sites.

additional macroalgae species (e.g., *Ulva fluxuosa*, *Valonia ventricosa*) made up of less than 0.1% of total plant biomass and these lakes were still dominated by three macroalgae species (*Batophora oerstedii*, *Cladophora crispata*, and *Acetabularia crenulata*) and one marine angiosperm (*Ruppia maritima*) found throughout the Bahamas (Godfrey 1994). Furthermore, only half the lakes containing specialists had higher macroalgae richness (Fig. 4A). It is also notable that only a single macroalgae species (*Chara* sp.) occurs in Laguna Chichancanab (Humphries and Miller 1981), demonstrating that a diverse macroalgae community is not necessary for adaptive radiation. Macroalgae richness also exhibited no significant interactions with dietary diversity or lake size (Table 2) and the only other variable with a marginal effect on sympatric species number was morphological diversity

along discriminate axis 1 (LD1), suggestive of slightly increased variation in jaw length (which loads heavily on this axis) in populations with specialists, most likely due to elevated within-lake introgression (demonstrated in Martin and Feinstein 2014).

#### INCREASED GENE FLOW IS NOT ASSOCIATED WITH ADAPTIVE RADIATION AND TROPHIC NOVELTY

The hybrid swarm theory of adaptive radiation predicts that adaptive radiation should be uniquely tied to admixed populations receiving a large influx of gene flow from highly divergent surrounding lineages (Seehausen 2004). This hypothesis must be evaluated with caution because gene flow is pervasive during adaptive radiation, but is often not unique to the radiating lineage (Abbott et al. 2013; Seehausen et al. 2014; Berner and Salzburger 2015). Indeed,

**Table 1.** Association between island/lake area, ecological diversity, genetic diversity, and morphological diversity with the number of sympatric *Cyprinodon* species coexisting within a lake ( $n = 1-3$ ) across 22 lake populations on seven islands (Fig. 4). Except for island area, all linear regressions treated each lake population as independent replicates; however, note that degrees of freedom in these tests may be inflated by the additional covariance among populations due to their varying degrees of shared history (Felsenstein 1985; Revell 2009). The only significant positive correlation is highlighted in bold.

Variable	Correlation with <i>Cyprinodon</i> spp.	$r^2$	$P$
Log <sub>10</sub> island area	<i>negative</i>	0.695	0.003
Log <sub>10</sub> lake area	–	0.006	0.654
Genetic diversity	–	0.030	0.284
<b>Macroalgae richness</b>	<b>positive</b>	<b>0.416</b>	<b>0.002</b>
Stomach content diversity	–	0.003	0.856
δ13C	–	0.001	0.903
δ15N	–	0.012	0.646
PC1 variance	–	0.131	0.107
PC2 variance	–	0.044	0.360
LD1 variance	–	0.017	0.571
LD2 variance	–	0.025	0.496

there was substantial evidence for secondary gene flow into the specialist populations on San Salvador based on asymmetric allele frequency correlations with outgroups (significant  $f_4$  statistics in 20 out of 55 four-population tests: Table S3), but also with gen-

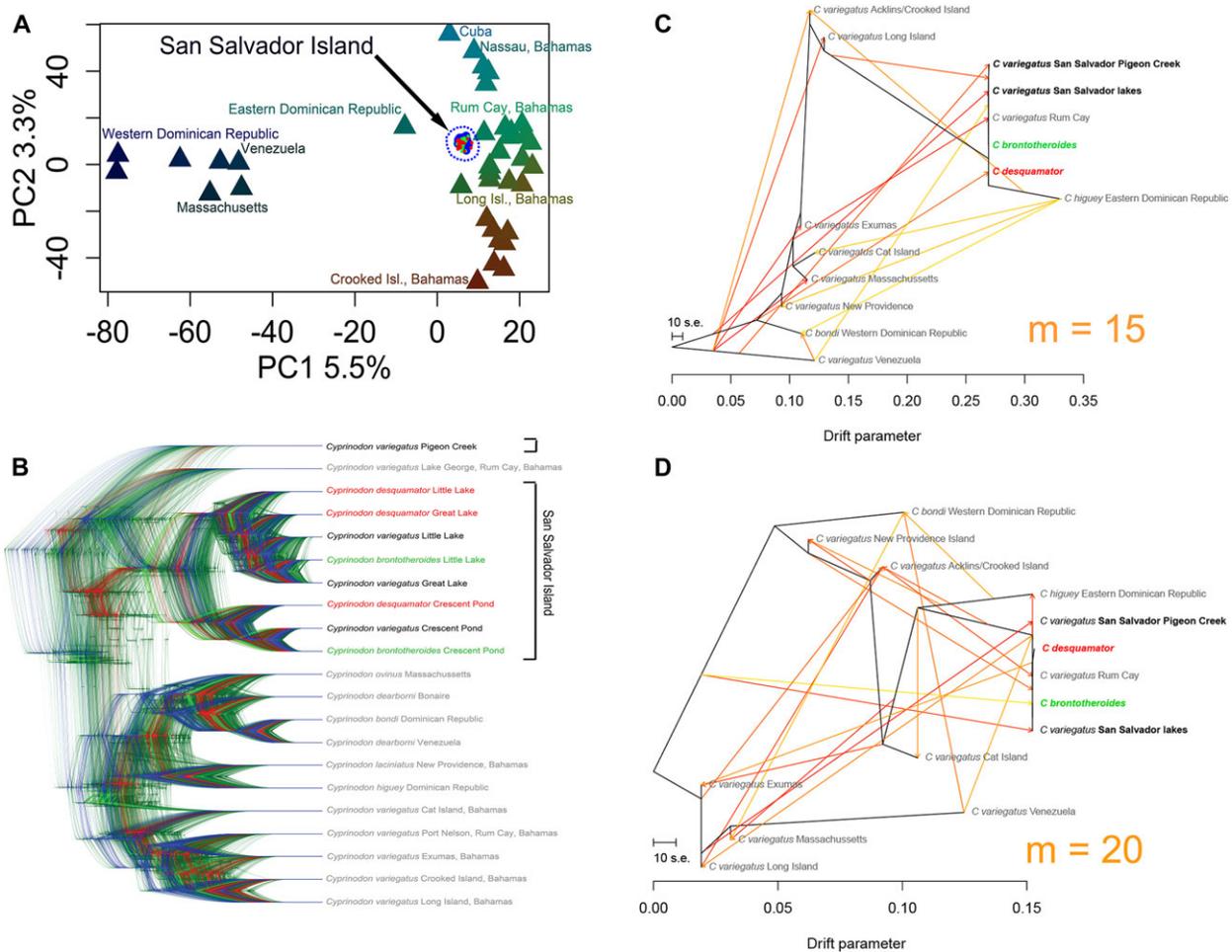
eralist populations on neighboring islands that failed to radiate (Fig. 5). The likelihood of secondary gene flow connecting island populations can be visualized in Treemix population graphs (Fig. 5C and D). Support for highly interconnected population graphs did not subside until reaching 20 admixture connections (Fig. 5C and D), supporting widespread gene flow among Caribbean islands in violation of a bifurcating branching structure assumed by phylogenetic models (in contrast to Figs. 2A, 5B, S1). Importantly, the inferred directions of gene flow events among Caribbean islands crisscrossed the entire archipelago, rather than forming an epicenter at San Salvador (Fig. 5C and D). This suggests that hybridization is prevalent across the Caribbean and not localized to San Salvador Island, in contrast to the pattern expected if a unique hybrid swarm event triggered the microendemic radiation on this island.

Projections of San Salvador populations onto the first two principal components of genetic variation across the Caribbean indicated a pattern of continuous variation across islands, often diverging from geographic proximity (Fig. 5A). For example, most introgression into San Salvador specialists came from *C. higuey* in the Eastern Dominican Republic (Table S3; Fig. 5), which was only distantly related to western Dominican Republic populations.

Species tree analysis illustrated the diversity of majority-consensus phylogenies across the sampled RAD loci, including many different topologies for the sister group relationships among San Salvador specialist populations and outgroups on neighboring islands (Fig. 5B; however, note that a bifurcating tree is an inadequate model of gene flow in this system). For example, there was consistent support for intra-lake clustering of all three species in Crescent Pond, consistent with genome-wide introgression and islands of genetic differentiation (Martin and Feinstein 2014), but

**Table 2.** Models incorporating the effects of lake area, ecological diversity, genetic diversity ( $\pi$ ), and morphological diversity on the number of sympatric *Cyprinodon* species coexisting within a lake (spp. = 1–3). Linear regression models treated the ecology of each lake as independent replicates; however, note that degrees of freedom for comparisons of generalist traits may be inflated by the additional covariance among populations due to their varying degrees of shared history (Felsenstein 1985; Revell 2009). Significant models are highlighted in bold and include presentation of significance, effect sizes, and SE for each term.

Model	df	$r^2$	$P$
Spp. ~ log(lake area) + $\pi$ + macroalgae richness + stomach content diversity + δ13C + δ15N + PC1 variance + PC2 variance + LD1 variance + LD2 variance	10	0.10	0.546
Spp. ~ log(lake area) + $\pi$ + macroalgae richness + PC1 variance + PC2 variance + LD1 variance + LD2 variance	16	0.32	0.216
<b>Spp. ~ macroalgae richness + PC1 variance + LD1 variance</b>	<b>16</b>	<b>0.53</b>	<b>0.005</b>
<b>Macroalgae richness: 0.223 ± 0.07</b>			<b>0.009</b>
PC1 variance: 0.176 ± 0.12			0.162
LD1 variance: 1.054 ± 0.50			0.057
Spp. ~ macroalgae richness × PC1 variance × LD1 variance	16	0.49	0.506



**Figure 5.** (A) Genetic variation within San Salvador populations relative to genetic variation across the Caribbean for 11,706 SNPs. Individuals of all three species on San Salvador (● generalist,  $n = 33$ ; ● molluscivore,  $n = 24$ ; ● scale-eater,  $n = 19$ ) were projected onto the first two principal component axes of Caribbean-wide genetic variation (excluding San Salvador Island). Outgroup populations (▲) are colored according to their values along each principal component axis. (B) Distribution of gene tree topologies estimated from 1534 RAD loci (1 SNP per locus) using SNAPP in BEAST2 (Bryant et al. 2012). (C and D) Treemix population graphs (Pickrell and Pritchard 2012) illustrating gene flow among Caribbean populations based on stepwise fitting of admixture events ( $m$ , shown as colored arrows, heat color indicates amount of gene flow) to a maximum-likelihood phylogeny for 4213 SNPs genotyped in all populations. Graphs are illustrated with 15 and 20 admixture events (c and d, respectively). Following the approach of Evanno et al. (2005) for choosing the number of genetic clusters in a STRUCTURE analysis, the rate of change of the likelihood began to decline as the number of admixture events approached 20 ( $m = 10$ :  $\ln L = 97$ ;  $m = 15$ :  $\ln L = 350$ ;  $m = 20$ :  $\ln L = 552$ ;  $m = 25$ :  $\ln L = 550$ ).

different topologies supported sister relationships between the San Salvador radiation and a coastal population of *C. variegatus* (Pigeon Creek, San Salvador Island), the closest island Rum Cay, or more distant islands (Fig. 5B).

## Discussion

Overall, these results strongly indicate that an exceptional increase in trait diversification rates and a major ecological transition from an ancestral generalist diet of algae and microinvertebrates to a new adaptive zone for scale-eating occurred only on San Salvador Island, Bahamas. This might reflect the extreme functional

demands of scale-eating (Sazima 1983; Janovetz 2005) and the ecological novelty of this niche within pupfishes, which is separated by 168 million years from other extant scale-eating fishes (Martin and Wainwright 2013c). These estimates of relative trait diversification rates are similar to a previous species-level study across *Cyprinodon* using a mitochondrial phylogeny (Martin and Wainwright 2011) and substantially exceed relative diversification rates for the same jaw traits measured in classic examples of adaptive radiation in fishes, including Malawi cichlids (up to nine-fold faster than New World cichlids [Hulsey et al. 2010]) and coral reef fishes (two-fold faster than non-reef [Price et al. 2011]). Indeed, the observed relative rates of trait diversification

are among the fastest ever reported (Barkman et al. 2008; Ackerly 2009; Harmon et al. 2010).

In contrast to the large literature documenting ecological drivers of speciation (Schluter 2000; McKinnon et al. 2004; Parent and Crespi 2009; Rundle and Nosil 2005; Nosil et al. 2009; Nosil 2012), variation in ecological opportunity among depauperate hypersaline lake environments does not appear sufficient to explain the rare occurrence of adaptive radiation in pupfishes. The young age and homogeneity of carbonate hypersaline lakes across the Bahamas suggest that similar levels of ecological opportunity also existed 10 kya at the origins of adaptive radiation on San Salvador; furthermore, field fitness experiments demonstrate that the current environment continues to drive diversification in these lakes in the presence of ongoing gene flow (Martin and Wainwright 2013b; Martin and Feinstein 2014; Martin 2016). Additional unmeasured ecological variables also appear comparable between San Salvador and neighboring hypersaline lakes: all lakes are physiochemically similar with shared carbonate geology, the same dominant macroalgae species, and the same one to two insectivorous fish species (Godfrey 1994; Rothfus 2012). Primary productivity and pupfish population densities also appeared similar across islands based on observations of terrestrial inputs and catch per unit effort. The exceptional diversification rates observed on San Salvador relative to neighboring generalist populations would suggest the presence of exceptional ecological differences unlikely to be overlooked. Surprisingly, this survey indicates that any ecological differences unique to San Salvador are subtle, if present. Perhaps rare macroalgae taxa are necessary to facilitate additional fitness peaks by providing nutritional or structural substrate for critical species of gastropods or ostracods, the predominant food source of the molluscivore pupfish, or shelter for the scale-eating pupfish to successfully ambush its prey. Although alternative stable ecosystem states often exhibit sensitive dependence on initial ecological conditions (Beisner et al. 2003), such subtle thresholds and sensitivity would be unprecedented within the ecological theory of adaptive radiation (Simpson 1944; Schluter 2000; Gavrillets and Losos 2009; Erwin 2015b; but see Chen et al. 2015).

Conversely, the abundance of scales in all pupfish communities and pervasiveness of gene flow would suggest that scale-eating pupfish should colonize the entire Caribbean. There was no unique signature of secondary gene flow into San Salvador as predicted by the hybrid swarm hypothesis (Seehausen 2004); instead, all current evidence suggests that gene flow is ubiquitous and ongoing among islands and lakes (Fig. 5; Martin and Feinstein 2014). If sufficient ecological opportunity and genetic diversity exists in most Caribbean hypersaline lakes, what are the additional constraints on adaptive diversification and dispersal of specialists across the Caribbean? One possibility is that colonization of neighboring populations may be constrained by

the extremely low fitness of scale-eater hybrids (demonstrated in Martin and Wainwright 2013b; Martin 2016) and low probability of dispersal of a scale-eater pair to a neighboring lake due to their low frequency in all lake populations (<5%; Martin and Wainwright 2013c). Indeed, fitness experiments with F2 hybrid pupfish on San Salvador indicated that a large fitness valley separates scale-eating from generalist phenotypes, suggesting that survival in the wild is dependent on an extreme scale-eating phenotype with the full complement of adaptive alleles underlying this complex phenotype, whereas intermediate phenotypes reside in a deep fitness valley (Martin and Wainwright 2013a; Martin 2016). Thus, the complex genetic architecture of the scale-eater phenotype and its interaction with fitness may further constrain its evolution, even in the presence of sufficient ecological opportunity.

Ultimately, this comprehensive examination of the origins of novel ecological niches during adaptive radiation rejects the ecological and hybrid swarm theories of adaptive radiation as necessary but not sufficient. The paradox is why 1000-fold differences in the diversification of trophic traits are not associated with 1000-fold differences in ecological opportunity or genetic diversity? Admittedly, the relationship between these two variables is unlikely to be one-to-one; however, it is worth emphasizing the dramatic nonlinearity between trait diversification rates and ecological or genetic explanatory variables within this system.

An increasing number of case studies suggest that the nonlinear emergence of novel traits leading to evolutionary innovations may be limited by the availability of genetic variation for complex phenotypes in addition to the presence of ecological opportunity (Schuettpeitz and Pryer 2009; Wilson et al. 2012; Erwin 2015a; Harmon and Harrison 2015). For example, in the Lenski long-term evolution experiment, the evolution of citrate feeding was limited by the specific mutations necessary for this major metabolic transition despite the continual presence of citrate (Blount et al. 2008, 2012; Quandt et al. 2015), providing a model for the rare origins of trophic novelty in pupfishes as opposed to the widespread examples of local adaptation in pupfishes (Tobler and Carson 2010). In contrast to parallel ecological speciation across similar environments, major ecological novelties, such as scale-eating, may emerge from nonecological, contingent processes within a long-term background of ecological abundance. The isolation of novel ecological niches on the fitness landscape, as demonstrated for the scale-eating pupfish, may constrain their evolution even in the presence of resource abundance (e.g., scales in all pupfish communities), which could explain the singular evolution of scale-eating among thousands of Cyprinodontiform fishes: perhaps Caribbean pupfishes occupy a sparse fitness landscape dominated by a single wide peak (generalist algae-eating) with very few and difficult-to-access specialist peaks (e.g., scale-eating, molluscivory, piscivory,

planktivory). Abundant ecological opportunity alone should not be expected to trigger such rare transitions.

This counterexample suggests a framework for examining the origins of novelty. First, the repeatability and convergence of adaptive evolution may only have meaning within the context of a particular niche and the local topography of the adaptive landscape surrounding that niche. Thus, the rare emergence of novel niche specialists or repeated parallel speciation of commonly used niches may fundamentally be a question of whether the local adaptive landscape contains sparse peaks or is abundantly rugged. Similarly, strong convergence or weak many-to-one mapping of phenotype to niche may fundamentally be a question of the width of the fitness peak, plateau, or ridge corresponding to a niche. In the same vein, the trigger of an adaptive radiation may depend on the local topography of the adaptive landscape within an environment, the niches accessible to a given radiation, and the niche of the ancestral founders. Developing a predictive theory of adaptive radiation can thus be viewed as an investigation of the diverse mechanisms used to navigate both sparse and rugged adaptive landscapes.

In conclusion, Caribbean pupfishes provide a rare opportunity to examine the origins of evolutionary novelty. Such novelties are commonplace in many classic adaptive radiations (Martin and Wainwright 2013c), but can rarely be observed to vary across very similar populations in comparable neighboring environments. Despite Caribbean-wide gene flow and an abundance of competitor-free environments, trophic innovation is confined to a single island, providing a strong counterexample to the prevailing view of ecologically driven diversification and innovation.

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## DATA ARCHIVING

The data is archived at: doi: <http://dx.doi.org/10.5061/dryad.g26d0>.

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## Supporting Information

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

**Table S1.** Analysis of shifts in trait diversification rates for 28 skeletal traits measured in 24 Caribbean pupfishes (Fig 2).

**Table S2.** Comparison of dietary/morphological diversity of generalist populations between allopatric and sympatric (including one or more pupfish specialist species) lake populations on San Salvador Island (one-way analysis of variance [ANOVA]).

**Table S3.** Tests for differential gene flow into San Salvador specialist species using the  $f_4$  statistic calculated in Treemix (Pickrell and Pritchard 2012).

**Appendix S1.** Species, lake or coastal population, Caribbean island or coastal population location, source/collector, collection date, and sample size for population genetic analyses.

**Figure S1.** Time-calibrated *Cyprinodon* phylogeny across the Caribbean estimated from 8,354 concatenated loci using BEAST.

**Figure S2.** Morphological diversity for 28 size-corrected skeletal traits in *C. variegatus* generalist populations.

**Figure S3.** Lateral photograph of a cleared and stained generalist pupfish specimen illustrating the location of all landmarks used for measurement of skeletal traits.

**Figure S4.** Representative size-correction plot for dentary length.

**Figure S5.** Individual variation in trophic position ( $\delta^{15}\text{N}$  stable isotope ratio) and dietary carbon source ( $\delta^{13}\text{C}$  stable isotope ratio) across *a*) all *C. variegatus* populations sampled across the Caribbean, *b*) all fish species within Crescent Pond, and *c*) all fish species within Little Lake.