The first genotypic fitness network in a vertebrate reveals that hybridization increases access to novel fitness peaks

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Abstract

Estimating the complex relationship between fitness and genotype or phenotype (i.e. the adaptive landscape) is one of the central goals of evolutionary biology. Empirical fitness landscapes have now been estimated for numerous systems, from phage to proteins to finches. However, adaptive walks connecting genotypes to organismal fitness, speciation, and novel ecological niches are still poorly understood and the process by which fitness valleys are crossed remains controversial. One outstanding system for addressing these connections is a recent adaptive radiation of ecologically and morphologically novel pupfishes (a generalist, molluscivore, and scale-eater) endemic to San Salvador Island, Bahamas. Here, we leveraged whole-genome sequencing of 139 hybrids from two independent field fitness experiments to identify the genomic basis of fitness, visualize the first genotypic fitness networks in a vertebrate system, and measure the accessibility of adaptive walks on the fitness landscape. We identified 132 SNPs that were significantly associated with fitness in field enclosures. Six fitness-associated regions contained differentially expressed genes and fixed SNPs between trophic specialists; one gene (*mettle21e*) was also misexpressed in hybrids, suggesting a potential intrinsic genetic incompatibility. We then constructed genotypic fitness networks from adaptive alleles and showed that scale-eating specialists are most isolated on these networks. Furthermore, only introgressed and *de novo* variants, not standing genetic variation, increased the accessibility of genotypic fitness paths from generalist to specialists. Our results suggest that adaptive introgression and *de novo* mutations provided key connections in adaptive walks necessary for crossing fitness valleys and triggering the evolution of novelty during adaptive radiation.

50 **Significance**

51 *The fitness landscape describes the complex relationship between genotype or phenotype and*
 52 *fitness and is the conceptual bridge between micro- and macroevolution. There are many studies*
 53 *of phenotypic fitness landscapes across a range of animal and plant taxa, but studies of the*
 54 *genotypic fitness landscape remain limited to microbial systems and the function of individual*
 55 *proteins. Here, we conducted the first study investigating the genotypic fitness landscape for*
 56 *organismal fitness in an exceptionally young vertebrate adaptive radiation including a generalist*
 57 *algivore, molluscivore, and scale-eating pupfish. We show that hybridization and de novo*
 58 *mutations increase the availability of accessible pathways to new fitness peaks on the fitness*
 59 *landscape, enabling the evolution of novelty.*

Introduction

First conceptualized by Sewall Wright in 1932, the adaptive landscape describes the complex relationship between genotype or phenotype and fitness (1). The landscape is a concept, metaphor, and an empirical measurement that exerts substantial influence over all evolutionary dynamics (2–6). Fitness landscapes were originally depicted as high-dimensional networks spanning genotypic space in which each genotype is associated with fitness (1). Simpson (7) later described phenotypic evolution of populations through time on a rugged landscape, in which isolated clusters of fitness peaks represent ‘adaptive zones’ relative to adjacent regions of low fitness (8). Lande and Arnold formalized the analysis of selection and estimation of phenotypic fitness landscapes (9–11), leading to empirical studies of fitness landscapes in numerous empirical systems (12–18). Fitness surfaces are also central components of speciation models and theory (19–21). Speciation can result from divergent selection (22), either on static landscapes or through negative frequency-dependent disruptive selection (23–28).

A fundamental concept in fitness landscape theory is that not all genotypic trajectories are accessible (5, 29, 30). Rather, only paths that monotonically increase in fitness at each mutational step are evolutionarily accessible, an expectation stemming from Fisher’s fundamental theorem (31). These accessible genotypic trajectories can thus be considered potential adaptive walks under Fisher’s geometric model (31, 32). There are a growing number of experimental studies of adaptive walks in nature, including the evolution of toxin resistance in monarch butterflies (33), alcohol tolerance in *Drosophila* (34, 35), and host-shift in aphids (36). Likewise, the accessibility of genotypic fitness networks has now been explored in numerous microbial systems, including the evolution of antibiotic resistance (29) and metabolism (37), citrate exploitation (38), and glucose limitation in *E. coli* (39), as well as adaptation to salinity in

yeast via evolution of heat shock protein *Hsp90* (40). However, these studies are still limited to the investigation of specific coding substitutions and their effects on fitness in laboratory environments. Nosil et al. (41) estimated genotypic fitness networks for *Timema* stick insects based on a field experiment. However, this study still focused on a single large-effect locus underlying dimorphic coloration between ecotypes. To our knowledge, no study has estimated genotypic fitness landscapes in a vertebrate system among multiple species as originally envisioned by Wright (1) despite the clear utility of this framework in bridging micro- and macroevolutionary dynamics.

One promising system for estimating fitness landscapes is a recent adaptive radiation of *Cyprinodon* pupfishes (17, 18, 42, 43). This radiation is comprised of two endemic specialists, a molluscivore (durophage: *Cyprinodon brontotheroides*) and a scale-eater (lepidophage: *C. desquamator*), derived from a Caribbean-wide generalist ancestor (*C. variegatus*). These three species all occur in sympatry in the hypersaline lakes of San Salvador Island, Bahamas (Fig 1A). Originating less than 10,000 years ago (based on geological age estimates for the lakes: (44)), the functional and trophic novelty harbored within this radiation is the product of exceptional rates of craniofacial morphological evolution (45–49). Furthermore, species boundaries appear to persist across multiple lake populations, despite persistent admixture among species (50, 51). We previously estimated fitness landscapes in these hypersaline lakes from two independent field experiments measuring the growth and survival of hybrids placed in field enclosures. Selection analyses revealed a stable, multi-peaked fitness landscape across lake populations, year of study, and manipulation of the frequency of rare hybrid phenotypes (Fig. 1B: (17, 18, 43)). One of the strongest and most persistent trends across studies and treatments was the apparent isolation of hybrid phenotypes resembling the scale-eater by a large fitness valley from the other two species

(17, 18). Hybrids resembling the generalist occupied a fitness peak and were isolated by a smaller fitness valley from hybrids resembling the molluscivore in a second region of high fitness. Understanding how an ancestral generalist population crossed these fitness valleys is key to understanding the origins of novelty in this system, particularly given the rare evolution of lepidophagy (scale-eating), a niche occupied by less than 0.3% of all fishes (52, 53).

Figure 1. San Salvador Island pupfishes and their hybrids. a. From top to bottom: the generalist, *Cyprinodon variegatus*, the molluscivore *C. brontotheroides*, and the scale-eater *C. desquamator*. b. Representative images of experimental field enclosures. c. Principal component analysis of 1,129,771 SNPs genotyped in hybrids and the three parental species. d. ADMIXTURE analyses for Crescent Pond (top) and Little Lake (bottom). G, M and S indicate individual samples of Generalists (G), Molluscivores (M), and Scale-eaters (S), respectively, followed by all resequenced hybrid individuals from field experiments. Colors correspond to probability of assignment to one of three assumed populations/species (K = 3 in this analysis).

The relative contributions of standing genetic variation, *de novo* mutation, and introgression to the tempo and mode of adaptive radiation are now of central interest to the field of speciation genomics (54–59). Yet to date, no study has investigated the relative influence of each on the accessibility of the fitness landscape, a critical determinant of the dynamics of speciation. The adaptive radiation of San Salvador Island pupfishes, like many others (60–64), appears to have resulted from a complex interplay of abundant standing genetic variation, adaptive introgression from neighboring islands, and several *de novo* substitutions and deletions found only in the scale-eater (51, 65). We have now identified many adaptive alleles showing evidence of hard selective sweeps in each of the trophic specialists (51). Notably, both specialists harbor numerous introgressed adaptive alleles in the regulatory regions of known craniofacial genes (51, 65). In contrast, hard selective sweeps of *de novo* mutations only appear in the scale-eating species, *C. desquamator*; many of these novel mutations also fall in or near craniofacial genes, such as a single non-synonymous fixed substitution in the transcription factor and oncogene *twist1* (51). Here, we leverage whole genome sequencing of 139 hybrids used in these field experiments to identify the genomic basis of fitness differences, infer genotypic fitness networks, and quantify the accessibility of novel fitness peaks and the influence of each source of genetic variation on these adaptive walks.

Results and Discussion

Population structure and ancestry association with fitness

We resequenced 139 hybrids (86 survivors, 56 deaths (Table S1)) from two independent field experiments across a total of six field enclosures and two lake populations (2011: two high-density 3 m diameter enclosures for three months: Crescent Pond $n = 796$; Little Lake = 875 F2 hybrids (17); 2014/2015: four high-density 4 m diameter enclosures: three months in Crescent

Pond n = 923 F4/F5 hybrids; eleven months in Little Lake = 842 F4/F5 hybrids (66)). We then characterized patterns of genetic variation among parental species in each lake and their lab-reared hybrids used in field experiments. We genotyped 1,129,771 SNPs with an average coverage of 9.79x per individual. Principal components analysis (PCA) of genetic variation strongly differentiated pupfishes sampled from Little Lake/Osprey Lake and Crescent Pond (PC1: 22.7% variance explained) and among species within each lake (PC2: 15.9% variance explained: Fig. 1D; Fig. S1-S2). These results were supported by ADMIXTURE analyses (67, 68) (Fig. 1E). However, some hybrids were genotypically transgressive, falling outside the genotypic distributions of the three parental species (Fig. S2), leading ADMIXTURE to attribute the third population to these hybrids, rather than generalists which often contain segregating variation found in the trophic specialists (67).

Neither scale-eater nor molluscivore ancestry estimated by ADMIXTURE analysis was significantly associated with hybrid composite fitness (Fig. S3A, Table S2: Scale-eater $P = 0.164$; molluscivore $P = 0.595$), survival probability (Fig. S3B: Scale-eater $p = 0.804$; molluscivore $P = 0.313$), or growth (Fig. S3C: Scale-eater $P = 0.283$; molluscivore $P = 0.328$), while controlling for effects of lake and experiment. Similarly, neither PC1 nor PC2 was associated with composite fitness (Table S2). Furthermore, pairwise genetic distance did not predict pairwise morphological distance (Fig. S4). Therefore, in contrast to previous studies (70–72), in this system genome-wide ancestry is not associated with fitness, highlighting the complex nonlinear relationship between genotype, phenotype, and fitness within this nascent adaptive radiation. We must look to local ancestry to understand fitness relationships (e.g. (73)).

Figure 2. The genetic basis of fitness variation and improved inference of adaptive landscapes. **a)** Per-SNP $-\log_{10} p$ -values from a genome-wide association test with GEMMA for composite fitness (survival x growth). Lake and experiment were included as covariates in the linear mixed model. SNPs that were significant at $FDR < 0.05$ are indicated in blue; red SNPs above dashed red line cross the threshold for Bonferroni significance at $\alpha = 0.05$. First twenty-four scaffolds are sorted from largest to smallest and remaining scaffolds were pooled. The six genes associated with composite fitness which were both strongly differentiated ($F_{ST} > 0.95$) and differentially expressed between specialists (69) are annotated. **b-c)** Best-fit adaptive landscape for composite fitness using either morphology alone (**b**: flat surface with only directional selection) or morphology in combination with fitness associated SNPs (**c**: highly nonlinear surface). Best-fit model in C was a GAM including a thin plate spline for both LD axes, fixed effects of experiment and lake, and fixed effects of seven (see supplementary methods) SNPs most strongly associated with fitness shown in red. **c)** Three-dimensional view of C with relative positions of the three parental species indicated.

Genome-wide association mapping of fitness

From our LD-pruned dataset we used a linear mixed model in GEMMA to identify 132 SNPs in regions that were strongly associated with composite fitness, including 13 which remained significant at the conservative Bonferroni threshold (Fig. 2A, Table S3: See supplement for results for survival and growth alone [Table S4-S5; Figure S5-6]). Gene ontologies for these 132 fitness-associated regions were significantly enriched for synaptic signaling and chemical synaptic transmission (FDR rate < 0.01 ; Fig. S7; Table S5). Ontologies enriched at an FDR rate

< 0.05 were related to signaling and regulation of cell communication. We did not identify any enrichment for ontologies related to craniofacial development which have previously been identified to play a significant role in the adaptive divergence of these fishes (51, 65, 69, 74, 75). This suggests that fitness-associated regions in our field experiments capture other components of fitness in addition to the external phenotypes measured in previous studies. Surprisingly, our fitness associated regions did not overlap with regions showing significant evidence of a hard selective sweep (51). However, we did find six fitness-associated SNPs near genes differentially expressed between specialists in addition to one that was also misexpressed in F1 hybrids (69). This is particularly intriguing because gene misexpression in hybrids, i.e. genes differentially expressed relative to both parents, could indicate the breakdown of genetic regulatory networks in hybrids and intrinsic genetic incompatibilities that may impact their fitness in field enclosures (69, 76).

We characterized whether genes in or near fitness-associated regions are implicated in adaptive divergence of the specialists. Five fitness-associated genes have previously been shown to include fixed divergent SNPs between specialists (*csad*, *glcci1*, *ino80c*, *mag*, *pim2*), and one contained a fixed deletion between specialists (*med25*) (69). *med25* (Mediator Complex Subunit 25) is a craniofacial transcription factor associated with cleft palate in humans and zebrafish (77, 78). Four of the remaining genes shared across our study and McGirr et al. (69) are associated with growth and/or body size measurements in other fishes. For example, *csad* plays an important role in synthesizing taurine which is a rate-limiting enzyme affecting growth rate in parrotfishes (79), rainbow trout (80), and Japanese flounder (81). Second, *glcci1* is associated with the body depth/length ratio in the large yellow croaker (82). Third, *ino80c* is associated with measures of body size in the Nile tilapia (83). The only gene associated with growth but not

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composite fitness, *mettl21e*, was also misexpressed in F1 hybrids between scale-eaters and molluscivores at eight days post-fertilization, and thus is a putative genetic incompatibility in this system (69). Although it has not been associated with growth-related or body-size traits in fishes, it is associated with intramuscular fat deposition in cattle (84). Taken together, these findings support the interpretation that our fitness-associated regions are associated with unmeasured traits, particularly physiological growth rates, or craniofacial shape in the case of the deletion in *med25*, that affect fitness in our hybrid field experiments.

Fitness-associated SNPs improve inference of the adaptive landscape

We used linear discriminant axes and generalized additive modelling (GAM) to estimate phenotypic fitness landscapes for the sequenced hybrids on a two-dimensional morphospace indicating similarity to each of the three parental populations following previous studies (17, 18). We then tested whether the inclusion of the 13 genomic regions most strongly associated with fitness (red: Fig. 2A) in GAM models improved our inference of the underlying adaptive landscape. Models with fitness-associated SNPs were invariably favored over models with external morphology alone ($\Delta AICc > 8.6$: Tables S8-S9). Morphology-only models predicted a flat linear landscape (Fig. 2B, Fig. S10); whereas including fitness-associated SNPs resulted in inferences of increased curvature and complexity of the fitness surface, despite our limited dataset of 139 hybrids relative to sample sizes in previous studies around 800 hybrids per enclosure.

To reduce complexity of the full model estimated from 31 morphological traits including all 13 fitness-associated SNPs, we fit an additional model including only the seven significant fitness-associated SNPs in the full model. This reduced model was the best-fit; the inferred

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adaptive landscape was complex and characterized by a fitness peak near hybrids resembling generalist phenotypes separated by a small fitness valley from a second region of high fitness for molluscivore hybrid phenotypes. Scale-eater hybrid phenotypes again corresponded to a large fitness valley (Fig. 2B-C: For results pertaining to growth or survival see Supplemental Materials – Fig. S10, Table S10-12). Compared to previous studies, the highest fitness optimum was shifted from molluscivore phenotypes to generalists. This suggests that fitness-associated SNPs increased the fitness of hybrids resembling generalists beyond expectations based on their morphology alone, consistent with the hypothesis that fitness-associated SNPs are associated with unmeasured traits affecting fitness. Indeed, visualization of haplotype frequency on the fitness landscape supported this interpretation: one of the most common haplotypes associated with high fitness was most frequent in hybrids resembling generalists and rare in hybrids resembling specialists (Fig. S11).

Trophic novelty is associated with isolation on the genotypic fitness landscape

We reconstructed genotypic fitness networks from 1,498 candidate adaptive alleles previously identified in this system (e.g. Fig. 3). These regions displayed significant evidence of a hard selective sweep using both site frequency spectrum and LD-based methods, SweeD (85) and OmegaPlus (86), and contained fixed or nearly fixed SNPs ($F_{st} > 0.95$) differentiating trophic specialists across lakes (51). These fitness networks depict both hybrids and parental species in genotypic space, with nodes representing SNP haplotypes and edges connecting mutational neighbors (Fig. 3B). Genotype space is immense; using SNPs coded as homozygous reference, heterozygote, or homozygous alternate, the number of potential haplotypes is equal to $3^{\# \text{ SNPs in network}}$. For instance, to construct a reduced network of even 100 SNPs, there would

be a total of $3^{100} = 5.17 \times 10^{57}$ possible nodes. Thus, unlike experimental studies of individual proteins in haploid *E. coli* (29, 39) or yeast (40), it is not possible for us to investigate the full breadth of genotypic space.

Figure 3. Genotypic fitness networks connecting San Salvador Island pupfishes. **a)** The majority of nearly fixed or fixed variants ($F_{ST} \geq 0.95$) experiencing hard selective sweeps (hereafter ‘adaptive alleles’) originated as standing genetic variation (SGV: molluscivores = 96%, scale-eaters = 92%), followed by introgression (molluscivores = 4%, scale-eaters = 6%), and lastly *de novo* mutation (scale-eaters = 2%)(51). Pie charts shows adaptive alleles retained in our study for each species. **b)** Diagram illustrating genotypic fitness networks and adaptive walks in a pair of species for two SNPs concatenated into haplotypes. Species A and B are separated by four mutational steps. Dashed lines are inaccessible as they result in a decrease in fitness; this leaves a single possible accessible evolutionary trajectory between species A and B (indicated by bold arrows). Each node is associated with an empirical measure of hybrid fitness. Edges are always drawn as directed, from low to high fitness. **c)** The same network as in B, with fitness plotted on the Y-axis and number of mutational steps from species A to B on the X-axis. Dashed lines are inaccessible (decreasing in fitness), solid lines are accessible (increasing in fitness), and the only accessible path between species A and B is indicated by solid arrows. **d)** Genotypic network constructed using a random sample of ten SNPs, sampled from all SNPs shown in **a**. Each edge between nodes is up to five mutational steps away; edge width is proportional to mutational distance: wider edges connect closer haplotypes. Node size (for hybrids) is scaled proportional to mean composite fitness; larger circles correspond to fitter haplotypes. **e)** Median number of mutational steps within or between species (e.g. Fig. 3C). All pairwise comparisons using Tukey’s HSD test (after FDR correction) were significant.

Instead, to understand the distribution of parental species and their hybrids in genotypic space, we began by using a random sample of ten SNPs drawn from our set of candidate adaptive alleles in this system. We chose ten SNPs for this first analysis for a larger sample of SNPs to characterize the overall structure of the genotypic fitness network, while not sampling too vast a space; already, the number of possible genotypes exceeds 59,000. Here, we plotted edges between nodes up to five mutational steps away and found that generalists and molluscivores are closer in the genotypic fitness network than either is to scale-eaters (Fig 4A), as expected based on their genetic distance. Most scale-eaters appear quite isolated in genotypic space, separated from the generalist cluster of nodes by 12.6 ± 0.091 (mean $\pm$ SE: $P < 0.001$) mutational steps and from molluscivores by 16.3 ± 0.060 steps ($P < 0.001$). In contrast, molluscivores were separated from generalists by 5.37 ± 0.103 steps ($P < 0.001$). Generalists show the greatest intrapopulation distances, separated from each other by 6.08 ± 0.088 steps ($P < 0.001$). In contrast, molluscivores exhibited the smallest intrapopulation distances, separated by 1.75 ± 0.021 steps ($P < 0.001$). Scale-eater intrapopulation distances were intermediate (4.71 ± 0.088 steps: $P < 0.001$).

Adaptive introgression and de novo mutations increase accessibility of novel fitness peaks

Evolutionary accessibility reflects the probability that one genotypic trajectory will be favored by natural selection over another, and thus can be related to the predictability of evolution (5, 29, 30, 87, 88). Genotypic trajectories with the minimum number of steps between two states that are characterized by monotonically increasing fitness at each step are considered to be the most accessible (5, 29, 30). That is, each mutational step is favored by natural selection and longer

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paths are less probable (30). This provides two useful metrics of fitness network accessibility: 1) The number of accessible paths and 2) the shortest length of accessible paths.

The adaptive radiation of pupfishes on San Salvador island originated within the last 10,000 years through a combination of selection on standing genetic variation, adaptive introgression, and *de novo* mutations (51). However, it is unclear how each source of genetic variation aided in the traversal of fitness paths or contributed to the colonization of novel fitness peaks. To address this question, we constructed genotypic fitness networks composed of each of the three main sources of adaptive genetic variation: standing genetic variation, introgression from one of four focal Caribbean generalist populations, or *de novo* mutations unique to San Salvador Island. We also examined combinations of these categories to better reflect the stages of adaptive divergence. We compared sets of 1,000 random 5-SNP genotypic networks drawn from different sources of adaptive variation and identified and scored the lengths of accessible paths (i.e. those monotonically increasing in fitness) from generalist to each specialist. We asked whether source of genetic variation affected 1) the number of accessible paths or 2) the shortest length of accessible paths.

We discovered that both scale-eater and molluscivore specialist fitness peaks in genotypic networks constructed from introgressed or *de novo* adaptive mutations were significantly more accessible than networks constructed from standing genetic variation (Fig. 4C, Table S13, S16). Random networks drawn from these sources of adaptive variation contained more accessible fitness paths from generalist to specialists than networks constructed from standing genetic variation, while controlling for differences in overall network size (Figs. S12-S13, Table S13). Specifically, networks constructed from adaptive introgression had 126% more accessible paths than those constructed from standing variation for molluscivores (median: $P <$

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0.0001: Fig. 4C), and 39% more for scale-eaters ($P < 0.0001$: Fig. 4C). Likewise, networks constructed from *de novo* mutations had 42% more accessible paths ($P < 0.0001$: Fig. 4C).

Consistent with past experimental studies in *E. coli* (29, 39) and yeast (40), accessible paths were quite rare; the vast majority of genotypic trajectories were inaccessible, i.e. they included one or more mutational steps that decreased in fitness from generalist to specialist. Compared to standing variation, accessible paths were also significantly shorter in introgressed (mean: 9% shorter in molluscivores: $P < 0.0001$, 23% shorter in scale-eaters: $P < 0.0001$) and *de novo* mutation networks (14% shorter in scale-eaters $P < 0.0001$: Fig. 4D. Fig S12, Tables S13, S17). Thus, introgressed and *de novo* adaptive mutations present more possible trajectories and reduce the number of steps needed to traverse between species and access novel trophic specialist fitness peaks (See Supplemental Materials Fig. S14-S15). These results are consistent with a growing body of evidence that *de novo* and introgressed variation may contribute to rapid speciation and evolution towards novel fitness peaks (38, 56, 62, 89–92).

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Figure 4. Adaptive introgression and *de novo* mutations increase access to specialist fitness peaks. **a)** Example genotypic fitness networks constructed from five SNPs randomly drawn from introgressed adaptive loci for molluscivores (left) or scale-eaters (right). Edges connect nodes (haplotypes) that are separated by a single mutational step, arrows indicate the direction of mutational steps (directed towards higher fitness or parental haplotypes if not observed in hybrids), and red arrows indicate an example accessible trajectory. **b)** Accessibility of genotypic trajectories separating generalists from specialists as measured using networks constructed from all adaptive loci. Left: Proportion of accessible (i.e. monotonically increasing in fitness) paths per network, scaled by the total size of the network. Right: Number of mutational steps in the shortest accessible path. Because of the discrete nature of these data, means are plotted as large circles, with two standard errors shown. Due to the highly skewed nature of these distributions, pairwise significance among all network sets was determined using a nonparametric Kruskal-Wallis one-way analysis of variance following correction for multiple tests using an estimated False Discovery Rate. **c-d)** The same plots as in **b**, but for fitness landscapes constructed from adaptive loci originating from standing genetic variation (SGV), introgression, *de novo* mutation, or a combination.

Conclusion

Here, we have analyzed the first multi-species genotypic fitness landscape based on hybrid fitness across multiple field experiments. We have shown that adaptive introgression and *de novo* mutation increase the accessibility of novel fitness peaks, thus promoting the evolution of novelty. Adaptation from standing genetic variation may initially be more rapid due to its higher frequency, initial availability, and potentially reduced genetic load within a population (93–95). Novel fitness peaks are not inaccessible from standing genetic variation alone – rather, they are simply *less* accessible and thus we predict that standing genetic variation alone is less likely to have triggered adaptive radiation in this system (93).

Once genetic variation arrives from introgression or arises *de novo* in a population, colonization of new adaptive zones may be expedited due to the reduced number of mutational steps in accessible paths. Furthermore, *de novo* mutations that undergo selective sweeps are also predicted to have larger effect sizes (96). These findings are consistent with an interpretation of adaptive radiation unfolding in stages (51, 97), first via standing genetic variation, next introgression from across the Caribbean, and finally *de novo* mutations.

Our findings also provide additional support for the isolation of scale-eaters on the fitness landscape and the rare evolution of scale-eating specialists despite comparable environments across the Caribbean with nearly identical levels of adaptive standing genetic variation. That is, adaptive *de novo* mutations may enable the crossing of a large fitness valley by increasing the number of accessible fitness pathways through genotype space. This in turn may then trap scale-eater populations on this peak by reducing the number of accessible fitness pathways back to the generalist peak. In short, by increasing accessibility of adaptive walks to novel trophic specialist

niches, *de novo* and introgressed adaptive alleles simultaneously reduce the accessibility of reverting to the generalist niche after the population reaches the new adaptive optimum.

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Methods:

Sampling

Our final dataset is comprised of 139 hybrid samples used in two separate field experiments (17, 18) on San Salvador island. Experiments were conducted in two lakes: Little Lake (N = 71) and Crescent Pond (N = 68). Hybrids used in the first field experiment (17) were spawned in the lab between December 2010 and January 2011; juveniles were raised for 2 months, individually

tagged by injecting a stainless steel sequential coded wire tag (Northwest Marine Technologies, Inc.) into their left dorsal musculature, and photographed pre-release for morphometric analyses. Experimental field enclosures consisted of high- and low-density treatments; density was varied by the number of tagged juveniles released into each enclosure. Hybrids in the second field experiment (18) were comprised of F4-F5 outbred juveniles resulting from crosses between all three species. Individuals were spawned, raised, and tagged in an equivalent manner and photographed prior to release. Experimental enclosures included high- and low-frequency treatments in which the frequency of rare transgressive hybrid phenotypes was altered between treatments in each lake.

All hybrids were measured for 32 external morphological traits (see below). Additionally, we included sequenced parental species of the generalist (N = 17), molluscivores (N = 27) and scale-eaters (N = 25) sampled from these two lakes previously included in Richards et al. (51). Note that we treat samples from Little Lake and Osprey Lake as the same population because these two lakes are connected within one hypersaline lake system and fish from these populations are genetically undifferentiated (50, 51). For morphological analyses, we additionally measured samples of 60 generalists, 38 molluscivores, and 60 scale-eaters raised in the same laboratory common garden environment. A full list of samples is included in the supplement (Table S1).

Sequencing, genotyping and filtering

Raw reads from a combined set of 396 samples (see supplement) were first mapped to the *C. brontotheroides* reference genome (genome size = 1.16 Gb; scaffold N50 = 32 Mb) (51) using bwa-mem (v. 0.7.2). Duplicate reads were identified and reads using MarkDuplicates, and BAM

indices were subsequently generated using the Picard software package (98). Samples were genotyped following Richards et al., (51) according to GATK best practices (99). Specifically, Single Nucleotide Polymorphisms (SNPs) were called and filtered using hard-filtering criteria in HaplotypeCaller. We used the following criteria in our filtering approach: QD < 2.0; QUAL < 20; FS < 60; MQRankSum < -12.5; ReadPosRankSum < -8 (99–101).

Following initial genotyping with GATK, we subsequently filtered our data further using VCFtools (102). Specifically, we filtered using the following flags: --maf 0.05; --min-alleles 2; --max-alleles 2; --min-meanDP 7; --max-meanDP 100; --max-missing 0.85. Indels were removed. To reduce non-independence among sites in our final dataset, we conservatively removed sites in strong linkage disequilibrium using plink v1.9 (--indep-pairwise 10['kb'] 50 0.5: (103)). Doing so resulted in the retention of 1,129,771 SNPs across 139 hybrid samples and the 69 samples from Richards et al (51). Unless otherwise specified, these SNPs were used for all downstream analyses.

Hybrid fitness measures

We used three measures of fitness: survival, growth, or a composite measure of the two. Survival was a binary trait indicating whether a fish survived (i.e. a tagged fish was recovered) or not during its exposure period in field enclosures. Growth was a continuous measure, defined as the proportional increase in Standard Length $\left(\frac{Final\ SL - Starting\ SL}{Starting\ SL}\right)$. Lastly, we defined composite fitness as survival * growth, similar to the metric used in (104), and analogous to composite fitness in Hereford (105) who used fecundity as their second fitness measure, rather than growth. Because composite fitness represents the most information rich metric of fitness, we report

composite fitness results in the main text; results for growth and survival are included in the supplement.

Population genetic variation

To visualize genetic variation present in hybrids and across lakes (Crescent Pond and Little Lake), we first used a Principal Components Analysis (PCA) of genetic variation using plink v1.90 ((103), Fig. 1), plotting the first two principal component axes using R (version 3.6.3 (R Core team 2020)). We then estimated admixture proportions in hybrids using ADMIXTURE v1.3.0 (67). Parental species found on San Salvador Island were substantially differentiated among Crescent Pond and Little Lake (50, 51); thus, independent ADMIXTURE analyses were conducted for each lake. Because we were primarily interested in admixture proportions of hybrids, we set $K = 3$ in these analyses, corresponding to the three parental species used in hybrid crosses. Using admixture proportions of hybrid individuals, we tested the hypothesis that ancestry predicts hybrid composite fitness in experimental field enclosures by fitting a generalized additive model including either 1) scale-eater ancestry or 2) molluscivore ancestry with fixed effects for experiment and lake. This was repeated for survival and growth separately.

Genome-wide association tests

To identify SNPs that were most strongly associated with fitness (survival, growth, or composite), we implemented the linear mixed model (LMM) approach in GEMMA (v. 0.98.1: (106)). This analysis was repeated using each fitness measure as the response variable. To account for relatedness among samples we estimated the kinship matrix among all 139 hybrid samples, which in turn were used in downstream LMMs. To account for the potentially

confounding effect of year/experiment and lake on estimated fitness measures, we included each as covariates in the LMMs. To ensure rare variants were not included in these analyses, we only included sites that had a minor allele frequency greater than 5% across all hybrids. A total of 933,520 SNPs were analyzed; 196,251 SNPs were excluded due to allele frequency change following removal of parental species. SNPs strongly associated with fitness were identified as 1) a False Discovery Rate (FDR: Benjamini and Hochberg 1995) less than 0.05, or a 2) P -value < 0.05 following a Bonferroni correction. We focus primarily on the sites identified by the conservative Bonferroni correction, however.

Gene ontology enrichment

We annotated sites that were significantly associated with fitness using snpEff (107) and the annotated *C. brontotheroides* reference genome (51). That is, we constructed a custom database within snpEff using the functional annotations originally produced by Richards et al. (51), and subsequently extracted information on the annotations and putative functional consequences of each variant.

Using genes identified for each SNP that is significantly associated with one of the fitness measures, we performed Gene Ontology (GO) enrichment analyses using ShinyGO v0.61 (108). For genes identified as being intergenic, we included both flanking genes. As in Richards et al. (2021), the gene symbol (abbreviation) database that had the greatest overlap with ours was that of the human database; thus, we tested for enrichment of biological process ontologies curated for human gene functions, based on annotations from Ensembl. Results are reported for biological processes that were significantly enriched with $FDR < 0.05$. We then compared this

list of candidate loci to those identified in past studies of San Salvador Island pupfishes (51, 69, 109).

Morphometrics

We measured 31 external morphological traits for all 139 hybrids and 69 parental individuals from Crescent Pond (30 generalists, 19 molluscivores, and 30 scale-eaters) and 85 from Little Lake (30 generalists, 25 molluscivores, and 30 scale-eaters). We digitally landmarked dorsal and lateral photographs (both sides) of each lab-reared hybrid (pre-release) or parent using DLTdv8 (110). Measurements include 27 linear characters and three angles. For nearly all individuals, lateral measurements were collected from both lateral photographs and averaged. Morphological variables were size-corrected using the residuals of a $\log_{10}(\text{trait}) - \log_{10}(\text{standard length})$ regression standardized for selection analyses as outlined in the supplement. We used these 31 morphological traits to estimate two linear discriminant (LD) axes that best distinguished the generalist, molluscivore, and scale-eater using the LDA function in the mass package in R. We then used the resultant LD model to predict LD-scores for the 139 sequenced hybrids for later use in generalized additive models.

Estimation of adaptive landscapes

We fit generalized additive models (GAMs) using the mgcv package v. 1.8.28 (111) in R to estimate fitness landscapes relating the two discriminant axes (LD1-2) to fitness. All models included a thin-plate spline fitted for the two linear discriminant axes, and we included both lake and experiment in all models as fixed effects. Lake x experiment interaction terms were also included in some models. Models were ranked using the corrected Akaike Information Criterion

(AICc) and were considered to be a significantly worse fit if $\Delta AICc > 4$ from the best-fit model (112). The best-fit model from the above approach was in turn used to visualize fitness landscapes, plotting predicted values of fitness measures across LD space in R.

Using these results, we tested whether inclusion of SNPs that were strongly associated with fitness (i.e., those that surpassed the 0.05 Bonferroni threshold) improved estimation of fitness landscapes. We first extracted genotypes for the highly significant SNPs identified by GEMMA (13 for composite fitness, four for growth: see section *Fitness-genotype association test*), and coded these as either reference, single or double mutants using VCFtools (102). We then used the best-fit models identified above and fit a range of models that included one or all SNPs. Individual fitness-associated SNPs were treated as ordered factors (i.e. transition from homozygous reference to heterozygote to homozygous alternate) and modeled using a factor-smooth in the generalized additive models. Note that factor “smooths” are effectively modeled as step-functions.

Estimation of genotypic fitness networks

We first estimated genotypic networks using sites previously shown to be highly divergent ($F_{ST} > 0.95$) and having undergone hard selective sweeps in specialists. To do so, we identified the SNPs in our unpruned full dataset overlapping with sites inferred to have undergone selective sweeps (51), resulting in 380 SNPs for molluscivores and 1,118 SNPs for scale-eaters. We subsequently constructed genotypic fitness networks in igraph v. 1.2.4.1 (113) following the procedure outlined in the supplement.

To visualize the high-dimensional genotypic fitness network, we randomly sampled ten adaptive loci 100 times and plotted haplotypes connected by edges if they were within five

mutational steps of one another (Figure 3C). Then we calculated the mean distance between all species pairs (in number of mutational steps). We used pairwise Tukey's HSD tests to test whether inter-species distances differed.

Estimation of evolutionary accessibility

We tested whether the evolutionary accessibility of genotypic fitness trajectories through observed hybrid genotypes from generalist to each specialist species differed based on the source of genetic variation. We restricted our investigation to networks composed of adaptive loci as previously described (Figure 3A: Richards et al. 2021). This included a total of 380 SNPs in the molluscivores, and 1,118 in the scale-eaters. The reduced number of adaptive SNPs sites in our dataset as compared to that of (51) is due in large part to the increased stringency of our filtering. We further partitioned these SNPs by their respective sources: standing genetic variation (molluscivore N = 364; scale-eater N = 1,029), *de novo* mutation (scale-eater N = 24), or introgression (molluscivore N = 16; scale-eater N = 65), again using the assignments from Richards et al. (51). For analyses of trajectories between generalists and molluscivores, we included only SNPs found to be sweeping in molluscivores; likewise, we included only SNPs sweeping in scale-eaters for analysis of trajectories between generalists and scale-eaters.

The full procedure for constructing genotypic fitness networks, identifying accessible paths, and quantifying accessibility is outlined in the supplement. Briefly, we randomly generated 1,000 datasets of five SNPs comprised of either 1) standing genetic variation, 2) adaptive introgression, 3) *de novo* mutation, 4) standing genetic variation + adaptive introgression, or 5) standing genetic variation + *de novo* mutation. We additionally repeated this procedure using all three classes of SNPs for scale-eaters to determine whether genotypic

trajectories separating generalists to molluscivores are more accessible than those between generalists and scale-eaters. Because different sets of sites are sweeping in each specialist, we conducted these analyses separately for each species. We then constructed genotypic networks, in which nodes are haplotypes of SNPs encoded in 012 format (0 = homozygous reference, 1 = heterozygote, 2 = homozygous alternate), and edges link mutational neighbors. When determining whether a path was accessible or not, we only included paths for which each mutational step (i.e. each intervening haplotype) between generalist to specialist was observed in at least one hybrid sample.

For each random network sample and for each measure of fitness we calculated 1) the minimum length of accessible paths between a random generalist and specialist sampled from our sequenced individuals, 2) the number of accessible paths between the same generalist and specialist pair, 3) the number of nodes and 4) the number of edges in the network. Larger networks will necessarily have a greater number of potential paths, including both accessible and inaccessible paths (Fig. S16), and we were interested in the relative proportion of accessible adaptive pathways, not just the raw number. For this reason and because network size differs among permutations, we divided the number of accessible paths in each random network sampled by the number of nodes, thus normalizing the counts and making them comparable across replicates and sources of variation. We assessed whether differences in these measures among the three sources of variation or between the two alternate generalist to specialist trajectories were significant using an ANOVA in R; due to the highly skewed nature of these distributions, post-hoc pairwise significance was assessed using a nonparametric Kruskal-Wallis one way analysis of variance in the agricolae package (114) in R.

Literature Cited

1. S. Wright, The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Sixth Int. Congr. Genet.*, 356–366 (1932).
2. S. Gavrillets, Evolution and speciation on holey adaptive landscapes. *Trends Ecol. Evol.* **12**, 307–312 (1997).
3. S. Gavrillets, “High-dimensional fitness landscapes and speciation” in *Evolution—the Extended Synthesis*, M. Pigliucci, G. B. Müller, Eds. (MIT Press, 2010), pp. 51–64.
4. E. Svensson, R. Calsbeek, *The adaptive landscape in evolutionary biology*, E. Svensson, R. Calsbeek, Eds. (Oxford University Press, 2012).
5. I. Fragata, A. Blanckaert, M. A. Dias Louro, D. A. Liberles, C. Bank, Evolution in the light of fitness landscape theory. *Trends Ecol. Evol.* **34**, 69–82 (2019).
6. K. K. Fear, T. Price, The adaptive surface in ecology. *Oikos* **82**, 440–448 (1998).
7. G. G. Simpson, *Tempo and mode in evolution* (Columbia University Press, 1944).
8. S. Kauffman, S. Levin, Towards a general theory of adaptive walks on rugged landscapes. *J. Theor. Biol.* **128**, 11–45 (1987).
9. R. Lande, S. J. Arnold, The measurement of selection on correlated characters. *Evolution* **37**, 1226 (1983).
10. S. J. Arnold, M. E. Pfrender, A. G. Jones, “The adaptive landscape as a conceptual bridge between micro- and macroevolution” in *Microevolution Rate, Pattern, Process*, (Springer, Dordrecht, 2001), pp. 9–32.
11. S. J. Arnold, Performance surfaces and adaptive landscapes. *Integr. Comp. Biol.* **43**, 367–375 (2003).
12. D. Schluter, P. R. Grant, Determinants of morphological patterns in communities of Darwin’s finches. *Am. Nat.* **123**, 175–196 (1984).
13. D. Schluter, Estimating the form of natural selection on a quantitative trait. *Evolution* **42**, 849–861 (1988).
14. A. P. Hendry, S. K. Huber, L. F. De León, A. Herrel, J. Podos, Disruptive selection in a bimodal population of Darwin’s finches. *Proc. R. Soc. B Biol. Sci.* **276**, 753–759 (2009).
15. M. O. Beausoleil, *et al.*, Temporally varying disruptive selection in the medium ground finch (*Geospiza fortis*). *Proc. R. Soc. B Biol. Sci.* **286** (2019).
16. C. W. Benkman, Divergent selection drives the adaptive radiation of crossbills. *Evolution* **57**, 1176–1181 (2003).
17. C. H. Martin, P. C. Wainwright, Multiple fitness peaks on the adaptive landscape drive adaptive radiation in the wild. *Science* **339**, 208–211 (2013).
18. C. H. Martin, K. J. Gould, Surprising spatiotemporal stability of a multi-peak fitness landscape revealed by independent field experiments measuring hybrid fitness. *Evol. Lett.*, **evl3.195** (2020).
19. S. Gavrillets, *Fitness landscapes and the origin of species* (Princeton University Press, 2004).
20. M. Turelli, N. H. Barton, J. A. Coyne, Theory and speciation. *Trends Ecol. Evol.* **16**, 330–343 (2001).
21. M. R. Servedio, J. W. Boughman, The role of sexual selection in local adaptation and speciation. *Annu. Rev. Ecol. Evol. Syst.* **48**, 85–109 (2017).
22. N. H. Barton, “Founder effect speciation” in *Speciation and Its Consequences*, D. Otte, J. A. Endler, Eds. (Sinauer, 1989), pp. 229–256.
23. U. Dieckmann, M. Doebeli, On the origin of species by sympatric speciation. *Nature* **400**,

- 354–357 (1999).
24. D. I. Bolnick, Can intraspecific competition drive disruptive selection? An experimental test in natural populations of sticklebacks. *Evolution* **58**, 608–618 (2004).
25. F. J. Weissing, P. Edelaar, G. S. van Doorn, Adaptive speciation theory: A conceptual review. *Behav. Ecol. Sociobiol.* **65**, 461–480 (2011).
26. J. E. Cresswell, C. Galen, Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. *Am. Nat.* **138**, 1342–1353 (1991).
27. S. P. Otto, M. R. Servedio, S. L. Nuismer, Frequency-dependent selection and the evolution of assortative mating. *Genetics* **179**, 2091–2112 (2008).
28. B. C. Haller, A. P. Hendry, Solving the paradox of stasis: Squashed stabilizing selection and the limits of detection. *Evolution* **68**, 483–500 (2014).
29. D. M. Weinreich, N. F. Delaney, M. A. DePristo, D. L. Hartl, Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* **312**, 111–114 (2006).
30. J. Franke, A. Klözer, J. A. G. M. de Visser, J. Krug, Evolutionary accessibility of mutational pathways. *PLoS Comput. Biol.* **7**, 1002134 (2011).
31. R. A. Fisher, *The genetical theory of natural selection* (Oxford University Press, 1930).
32. H. A. Orr, The genetic theory of adaptation: A brief history. *Nat. Rev. Genet.* **6**, 119–127 (2005).
33. M. Karageorgi, *et al.*, Genome editing retraces the evolution of toxin resistance in the monarch butterfly. *Nature* **574**, 409–412 (2019).
34. M. A. Siddiq, D. W. Loehlin, K. L. Montooth, J. W. Thornton, Experimental test and refutation of a classic case of molecular adaptation in *Drosophila melanogaster*. *Nat. Ecol. Evol.* **1**, 25 (2017).
35. M. A. Siddiq, J. W. Thornton, Fitness effects but no temperature-mediated balancing selection at the polymorphic *Adh* gene of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 21634–21640 (2019).
36. K. S. Singh, *et al.*, The genetic architecture of a host shift: An adaptive walk protected an aphid and its endosymbiont from plant chemical defenses. *Sci. Adv.* **6**, eaba1070 (2020).
37. F. Peng, *et al.*, Effects of beneficial mutations in *pykF* gene vary over time and across replicate populations in a long-term experiment with bacteria. *Mol. Biol. Evol.* **35**, 202–210 (2018).
38. Z. D. Blount, C. Z. Borland, R. E. Lenski, Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* **105**, 7899–7906 (2008).
39. A. I. Khan, D. M. Dinh, D. Schneider, R. E. Lenski, T. F. Cooper, Negative epistasis between beneficial mutations in an evolving bacterial population. *Science* **332**, 1193–1196 (2011).
40. C. Bank, S. Matuszewski, R. T. Hietpas, J. D. Jensen, On the (un)predictability of a large intragenic fitness landscape. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 14085–14090 (2016).
41. P. Nosil, *et al.*, Ecology shapes epistasis in a genotype–phenotype–fitness map for stick insect colour. *Nat. Ecol. Evol.* **4**, 1673–1684 (2020).
42. C. H. Martin, P. C. Wainwright, A remarkable species flock of *Cyprinodon* pupfishes endemic to San Salvador Island, Bahamas. *Bull. Peabody Museum Nat. Hist.* **54**, 231–241 (2013).
43. C. H. Martin, Context dependence in complex adaptive landscapes: frequency and trait-

- dependent selection surfaces within an adaptive radiation of Caribbean pupfishes. *Evolution* **70**, 1265–1282 (2016).
44. B. J. Turner, D. D. Duvernell, T. M. Bunt, M. G. Barton, Reproductive isolation among endemic pupfishes (Cyprinodon) on San Salvador Island, Bahamas: Microsatellite evidence. *Biol. J. Linn. Soc.* **95**, 566–582 (2008).
45. C. H. Martin, P. C. Wainwright, Trophic novelty is linked to exceptional rates of morphological diversification in two adaptive radiations of cyprinodon pupfish. *Evolution* **65**, 2197–2212 (2011).
46. C. H. Martin, The cryptic origins of evolutionary novelty: 1000-fold faster trophic diversification rates without increased ecological opportunity or hybrid swarm. *Evolution* **70**, 2504–2519 (2016).
47. M. E. St. John, K. E. Dixon, C. H. Martin, Oral shelling within an adaptive radiation of pupfishes: Testing the adaptive function of a novel nasal protrusion and behavioural preference. *J. Fish Biol.* **97**, 163–171 (2020).
48. C. H. Martin, J. A. McGirr, E. J. Richards, M. E. St. John, How to investigate the origins of novelty: Insights gained from genetic, behavioral, and fitness perspectives. *Integr. Org. Biol.* **1** (2019).
49. M. E. St. John, R. Holzman, C. H. Martin, Rapid adaptive evolution of scale-eating kinematics to a novel ecological niche. *J. Exp. Biol.* **223** (2020).
50. C. H. Martin, L. C. Feinstein, Novel trophic niches drive variable progress towards ecological speciation within an adaptive radiation of pupfishes. *Mol. Ecol.* **23**, 1846–1862 (2014).
51. E. J. Richards, *et al.*, A vertebrate adaptive radiation is assembled from an ancient and disjunct spatiotemporal landscape. *Proc. Natl. Acad. Sci.* **118**, e2011811118 (2021).
52. C. H. Martin, P. C. Wainwright, On the measurement of ecological novelty: Scale-eating pupfish are separated by 168 my from other scale-eating fishes. *PLoS One* **8**, e71164 (2013).
53. R. Froese, D. Pauly, FishBase. *FishBase* (2021).
54. O. Seehausen, others, Genomics and the origin of species. *Nat. Rev. Genet.* **15**, 176–192 (2014).
55. S. H. Martin, C. D. Jiggins, Interpreting the genomic landscape of introgression. *Curr. Opin. Genet. Dev.* **47**, 69–74 (2017).
56. D. A. Marques, J. I. Meier, O. Seehausen, A Combinatorial View on Speciation and Adaptive Radiation. *Trends Ecol. Evol.* **34**, 531–544 (2019).
57. J. W. Poelstra, E. J. Richards, C. H. Martin, Speciation in sympatry with ongoing secondary gene flow and a potential olfactory trigger in a radiation of Cameroon cichlids. *Mol. Ecol.* **27**, 4270–4288 (2018).
58. T. C. Nelson, W. A. Cresko, Ancient genomic variation underlies repeated ecological adaptation in young stickleback populations. *Evol. Lett.* **2**, 9–21 (2018).
59. K. A. Thompson, M. M. Osmond, D. Schluter, Parallel genetic evolution and speciation from standing variation. *Evol. Lett.* **3**, 129–141 (2019).
60. J. B. Pease, D. C. Haak, M. W. Hahn, L. C. Moyle, Phylogenomics reveals three sources of adaptive variation during a rapid radiation. *PLoS Biol.* **14**, e1002379 (2016).
61. E. J. Richards, J. W. Poelstra, C. H. Martin, Don’t throw out the sympatric speciation with the crater lake water: fine-scale investigation of introgression provides equivocal support for causal role of secondary gene flow in one of the clearest examples of sympatric

- speciation. *Evol. Lett.* **2**, 524–540 (2018).
62. J. I. Meier, *et al.*, Ancient hybridization fuels rapid cichlid fish adaptive radiations. *Nat. Commun.* **8**, 14363 (2017).
63. M. D. McGee, *et al.*, The ecological and genomic basis of explosive adaptive radiation. *Nature* **586**, 75–79 (2020).
64. I. Irisarri, *et al.*, Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. *Nat. Commun.* **9**, 1–12 (2018).
65. E. J. Richards, C. H. Martin, Adaptive introgression from distant Caribbean islands contributed to the diversification of a microendemic adaptive radiation of trophic specialist pupfishes. *PLoS Genet.* **13**, e1006919 (2017).
66. C. H. Martin, K. J. Gould, Surprising spatiotemporal stability of a multi-peak fitness landscape revealed by independent field experiments measuring hybrid fitness. *Evol. Lett.* **4**, 530–544 (2020).
67. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).
68. D. H. Alexander, K. Lange, Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics* **12**, 1–6 (2011).
69. J. A. McGirr, C. H. Martin, Ecological divergence in sympatry causes gene misexpression in hybrids. *Mol. Ecol.* **29**, 2707–2721 (2020).
70. M. E. Arnegard, *et al.*, Genetics of ecological divergence during speciation. *Nature* **511**, 307–311 (2014).
71. S. Wang, J. J. Hard, F. Utter, Genetic variation and fitness in salmonids. *Conserv. Genet.* **3**, 321–333 (2002).
72. R. Leimu, P. Mutikainen, J. Koricheva, M. Fischer, How general are positive relationships between plant population size, fitness and genetic variation? *J. Ecol.* **94**, 942–952 (2006).
73. D. Schluter, *et al.*, Fitness maps to a large-effect locus in introduced stickleback populations. *Proc. Natl. Acad. Sci. U. S. A.* **118**, 1914889118 (2021).
74. C. H. Martin, P. A. Erickson, C. T. Miller, The genetic architecture of novel trophic specialists: larger effect sizes are associated with exceptional oral jaw diversification in a pupfish adaptive radiation. *Mol. Ecol.* **26**, 624–638 (2017).
75. J. A. McGirr, C. H. Martin, Parallel evolution of gene expression between trophic specialists despite divergent genotypes and morphologies. *Evol. Lett.* **2**, 62–75 (2018).
76. J. Kulmuni, A. M. Westram, Intrinsic incompatibilities evolving as a by-product of divergent ecological selection: Considering them in empirical studies on divergence with gene flow. *Mol. Ecol.* **26**, 3093–3103 (2017).
77. Y. Nakamura, *et al.*, Wwp2 is essential for palatogenesis mediated by the interaction between Sox9 and mediator subunit 25. *Nat. Commun.* **2**, 1–10 (2011).
78. L. Mork, G. Crump, “Zebrafish craniofacial development: A Window into early patterning” in *Current Topics in Developmental Biology*, Y. Chai, Ed. (Academic Press Inc., 2015), pp. 235–269.
79. S. J. Lim, *et al.*, Taurine is an essential nutrient for juvenile parrot fish *Oplegnathus fasciatus*. *Aquaculture* **414–415**, 274–279 (2013).
80. T. G. Gaylord, A. M. Teague, F. T. Barrows, Taurine supplementation of all-plant protein diets for rainbow trout (*Oncorhynchus mykiss*). *J. World Aquac. Soc.* **37**, 509–517 (2006).
81. M. Yokoyama, T. Takeuchi, G. S. Park, J. Nakazoe, Hepatic cysteinesulphinate decarboxylase activity in fish. *Aquac. Res.* **32**, 216–220 (2001).

- 766 82. Z. Zhou, *et al.*, Genome-wide association study of growth and body-shape related traits in
767 large yellow croaker (*Larimichthys crocea*) using ddRAD sequencing. *Mar. Biotechnol.*
768 **21**, 655–670 (2019).
- 769 83. G. M. Yoshida, J. M. Yáñez, Multi-trait GWAS using imputed high-density genotypes
770 from whole-genome sequencing identifies genes associated with body traits in Nile tilapia.
771 *BMC Genomics* **22**, 1–13 (2021).
- 772 84. L. F. S. Fonseca, *et al.*, Gene expression profiling and identification of hub genes in
773 Nellore cattle with different marbling score levels. *Genomics* **112**, 873–879 (2020).
- 774 85. P. Pavlidis, D. Živković, A. Stamatakis, N. Alachiotis, SweeD: Likelihood-based
775 detection of selective sweeps in thousands of genomes. *Mol. Biol. Evol.* **30**, 2224–2234
776 (2013).
- 777 86. N. Alachiotis, A. Stamatakis, P. Pavlidis, OmegaPlus: A scalable tool for rapid detection
778 of selective sweeps in whole-genome datasets. *Bioinformatics* **28**, 2274–2275 (2012).
- 779 87. F. J. Poelwijk, D. J. Kiviet, D. M. Weinreich, S. J. Tans, Empirical fitness landscapes
780 reveal accessible evolutionary paths. *Nature* **445**, 383–386 (2007).
- 781 88. L. Ferretti, D. Weinreich, F. Tajima, G. Achaz, Evolutionary constraints in fitness
782 landscapes. *Heredity (Edinb)*. **121**, 466–481 (2018).
- 783 89. T. C. Nelson, *et al.*, Ancient and recent introgression shape the evolutionary history of
784 pollinator adaptation and speciation in a model monkeyflower radiation (*Mimulus* section
785 *Erythranthe*). *PLoS Genet.* **17**, e1009095 (2021).
- 786 90. H. Svardal, *et al.*, Ancestral hybridization facilitated species diversification in the lake
787 malawi cichlid fish adaptive radiation. *Mol. Biol. Evol.* **37**, 1100–1113 (2020).
- 788 91. S. Lamichhaney, *et al.*, Rapid hybrid speciation in Darwin’s Finches. *Science* **359**, 224–
789 228 (2018).
- 790 92. P. R. Grant, B. R. Grant, Hybridization increases population variation during adaptive
791 radiation. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 23216–23224 (2019).
- 792 93. R. D. H. Barrett, D. Schluter, Adaptation from standing genetic variation. *Trends Ecol.*
793 *Evol.* **23**, 38–44 (2008).
- 794 94. J. Hermisson, P. S. Pennings, Soft sweeps and beyond: understanding the patterns and
795 probabilities of selection footprints under rapid adaptation. *Methods Ecol. Evol.* **8**, 700–
796 716 (2017).
- 797 95. P. W. Hedrick, Adaptive introgression in animals: examples and comparison to new
798 mutation and standing variation as sources of adaptive variation. *Mol. Ecol.* **22**, 4606–
799 4618 (2013).
- 800 96. S. Matuszewski, J. Hermisson, M. Kopp, Catch me if you can: Adaptation from standing
801 genetic variation to a moving phenotypic optimum. *Genetics* **200**, 1255–1274 (2015).
- 802 97. J. T. Streebman, P. D. Danley, The stages of vertebrate evolutionary radiation. *Trends*
803 *Ecol. Evol.* **18**, 126–131 (2003).
- 804 98. Broad Institute, Picard Toolkit (2018).
- 805 99. M. A. Depristo, *et al.*, A framework for variation discovery and genotyping using next-
806 generation DNA sequencing data. *Nat. Genet.* **43**, 491–501 (2011).
- 807 100. R. Poplin, *et al.*, Scaling accurate genetic variant discovery to tens of thousands of
808 samples. *bioRxiv*, 201178 (2017).
- 809 101. C. D. Marsden, *et al.*, Diversity, differentiation, and linkage disequilibrium: Prospects for
810 association mapping in the malaria vector anopheles arabiensis. *G3 Genes, Genomes,*
811 *Genet.* **4**, 121–131 (2014).

102. P. Danecek, *et al.*, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).
103. S. Purcell, *et al.*, PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
104. C. T. DiVittorio, *et al.*, Natural selection maintains species despite frequent hybridization in the desert shrub *Encelia*. *Proc. Natl. Acad. Sci. U. S. A.* **117**, 33373–33383 (2021).
105. J. Hereford, A quantitative survey of local adaptation and fitness trade-offs. *Am. Nat.* **173**, 579–588 (2009).
106. X. Zhou, M. Stephens, Genome-wide efficient mixed-model analysis for association studies. *Nat. Genet.* **44**, 821–824 (2012).
107. P. Cingolani, *et al.*, Using *Drosophila melanogaster* as a model for genotoxic chemical mutational studies with a new program, SnpSift. *Front. Genet.* **3** (2012).
108. S. X. Ge, D. Jung, D. Jung, R. Yao, ShinyGO: A graphical gene-set enrichment tool for animals and plants. *Bioinformatics* **36**, 2628–2629 (2020).
109. J. A. McGirr, C. H. Martin, Few fixed variants between trophic specialist pupfish species reveal candidate cis-regulatory alleles underlying rapid craniofacial divergence. *Mol. Biol. Evol.* **38**, 405–423 (2021).
110. T. L. Hedrick, Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspiration and Biomimetics* **3**, 034001 (2008).
111. S. N. Wood, Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *J. R. Stat. Soc. Ser. B (Statistical Methodol.* **73**, 3–36 (2011).
112. K. P. Burnham, D. R. Anderson, *Model Selection and Multimodel Inference* (Springer New York, 2002).
113. G. Csardi, T. Nepusz, The igraph software package for complex network research. *InterJournal Complex Syst.* **1695**, 1–9 (2006).
114. F. de Mendiburu, *agricolae*: Statistical procedures for agricultural research (2020).
115. T. Guillerme, dispRity: A modular R package for measuring disparity. *Methods Ecol. Evol.* **9**, 1755–1763 (2018).
116. B. J. Knaus, N. J. Grünwald, VCFR: a package to manipulate and visualize variant call format data in R. *Mol. Ecol. Resour.* **17**, 44–53 (2017).
117. S. Der Yang, T. S. Lin, F. G. Liu, C. H. Liou, Influence of dietary phosphorus levels on growth, metabolic response and body composition of juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* **253**, 592–601 (2006).
118. J. M. Costa, *et al.*, Inadequate dietary phosphorus levels cause skeletal anomalies and alter osteocalcin gene expression in Zebrafish. *Int. J. Mol. Sci.* **19**, 364 (2018).
119. H. Yu, *et al.*, Effects of stocking density and dietary phosphorus levels on the growth performance, antioxidant capacity, and nitrogen and phosphorus emissions of juvenile blunt snout bream (*Megalobrama amblycephala*). *Aquac. Nutr.* **27**, 581–591 (2021).

Supplementary Methods

Sampling of hybrid individuals

Samples of hybrid *Cyprinodon* pupfish included herein were first collected following two separate fitness experiments, conducted on San Salvador Island in 2011 (17) and 2016 (18) respectively. Experiments were carried out in two lakes: Little Lake (LL), and Crescent Pond (CP). Following their initial collection at the conclusion of their respective experiments (see (17) and (18) for protocols), samples were stored in ethanol. In late 2018, 149 hybrid samples were selected for use in this experiment. Of these, 27 are from the experiment conducted in 2011 (14 from LL, 13 from CP), and the remaining 122 are from the 2016 experiment (58 from LL, 64 from CP). Due to reduced sample size for some species within Little Lake, we include fish obtained from Osprey Lake for downstream analyses comparing hybrids to Little Lake, as the two comprise a single, interconnected body of water.

Genomic Library Prep

DNA was extracted from the muscle tissue of hybrids using DNeasy Blood and Tissue kits (Qiagen, Inc.); these extractions were then quantified using a Qubit 3.0 fluorometer (Thermo Scientific, Inc). Genomic libraries were prepared by the Vincent J. Coates Genomic Sequencing Center (QB3) on the automated Apollo 324 system (WaterGen Biosystems, Inc.). Samples were fragmented using a Covaris sonicator, and barcoded with Illumina indices. Samples were quality checked using a Fragment Analyzer (Advanced Analytical Technologies, Inc.). All samples were sequenced to approximately 10x raw coverage on an Illumina NovaSeq.

Genotyping and filtering

Because we are interested in genomic variants found not only within our hybrid samples but across San Salvador island and the Caribbean, we conducted genotyping including all 247 samples from Richards et al. (51). These samples include members of the three species (*C. variegatus*, *C. brontotheroides*, *C. desquamator*) found on San Salvador Island, as well as individuals of *C. variegatus* found throughout the Caribbean, and numerous outgroups (*C. laciniatus*, *C. higuey*, *C. dearborni*, *Megupsilon aporus*, and *Cualac tessellatus*). We then excluded *M. aporus* and *C. tessellatus* along with 18 additional samples for which necessary data for downstream analyses were missing (e.g. quality photographs for the collection of morphological data). This approach led to the retention of a total of 4,206,786 total SNPs and 139 hybrid individuals.

Morphometrics

Because the morphological measurements used in the 2013 and 2016 experiments differ slightly, we remeasured all sequenced hybrid individuals and up to 30 individuals of each parent species for the 30 morphological characters described in Martin & Gould (18), as well as for standard length (SL).

For each photograph, unit-scale was obtained by additionally landmarking points on a regular grid included in each photograph using DLTdv8a (110). Landmark data (x-y coordinates in units of pixels) were subsequently uploaded into R and converted to millimeters (in the case of linear measurements) or degrees (for angular measurements) using a custom script.

We then assessed, for each trait, the need for size-correction. That is, we sought to avoid an outsized role of body size in downstream interpretation. Thus, if a trait was colinear with SL, we regressed the two (treating SL as the predictor) and took the residuals. In each case, both SL

and the response were Log-10 transformed. Subsequently, residuals were scaled such that their distribution had a mean of 0, and a standard deviation of 1. Traits that did not need size correction were also Log-transformed and unit-scaled.

We used these morphological measurements to estimate two linear discriminant (LD) axes that distinguish the generalist, molluscivore, and scale-eater using the LDA function in R. That is, we used morphological data from the 165 parental fish to estimate LD scores for each individual. Doing so, we were able to correctly assign individual fish to their corresponding species with 99.4% accuracy (Fig. S8-S9: Table S7). Attempting to predict species assignment by lake did not improve this prediction accuracy (instead reducing prediction accuracy to 98.7%); consequently, we proceeded with the LD axes estimated without accounting for lake.

We additionally asked whether 1) specialists were more morphologically constrained than generalists and 2) if hybrids were less constrained than the three parental species. To do so, we calculated morphological disparity per group using the `dispRity.per.group` function implemented in the R package `dispRity` v1.3.3 (115). Specifically, this function calculates the median distance between each row (sample) and the centroid of the matrix per group. By bootstrapping the data 100 times, we tested the hypothesis that each of the three parental species differed significantly in their morphological disparity, first using an ANOVA, followed by a post-hoc pairwise t-test to assess pairwise significance in R. We corrected for multiple tests using an FDR correction.

To test whether genetic distance predicts morphological distance, we calculated two distance matrices. First, we calculated pairwise Euclidean distances between all hybrids using all morphological variables. Then, we calculated pairwise genetic distances between all hybrids using the final set of SNPs described above with the `genet.dist` function implemented in `vcfR` (116). We then fit a simple linear model, regressing genetic distance on morphological distance.

Estimation of Adaptive Landscapes

We sought to characterize the extent to which the three measures of fitness are predicted by morphology alone and to, in turn, visualize fitness landscapes for our sequenced hybrids. To do so, we fitted six generalized additive models (GAMs) using the `mgcv` package v. 1.8.28 (111) in R. All models included a thin-plate spline fitted for the two linear discriminant axes. Because we have strong *a priori* knowledge that fitness outcomes will be contingent to some extent on experiment year and on the lake in which hybrids were placed, we include experiment and lake in all fitted models, modeled either as fixed effects, or as an interaction term between the two. Additionally, we fitted models that included individual splines for each linear discriminant axis, either with or without experiment or lake as a factor smooth. The full list of models and their respective fits are included in the supplement (Supplementary Tables S8-S10).

For composite fitness, we excluded three SNPs that were within close proximity (i.e. < 1000bp) to a SNP that was more significantly associated. Because of the reduced number of significantly associated SNPs identified for growth (four) as compared to composite fitness (ten), we were able to fit and compare all combinations of significantly associated SNPs for the former. The best-fit model for composite fitness was also the most complex, including all fitness associated SNPs. Thus, to reduce model complexity, we fit one additional model, excluding any of the three SNP (fixed effect) that was not significant in the full model. The full range of models and their associated fits are reported in Tables S11-S12. As before, predicted fitness values across LD space were extracted from the best-fit model and plotted using R.

Genotypic Fitness Networks

Recent work has shown that the adaptive radiation of the pupfish of San Salvador island involved selection on standing genetic variation, adaptive introgression, and *de novo* mutation (51). Furthermore, the specialists on San Salvador Island received approximately twice as much adaptive introgression as did generalists on neighboring islands. These findings imply that each source of genetic variation may exert a unique influence on the fitness landscape, in turn facilitating the radiation. We sought to explore this possibility and so estimated genotypic fitness networks using sites previously shown to have undergone hard selective sweeps in specialists. To do so, we identified the SNPs in our un-thinned dataset overlapping with sites inferred to have undergone selective sweeps (51) to produce two datasets (one for each specialist). We then constructed networks using the following procedure:

- 1) Target SNPs were extracted from the vcf file of all sequenced hybrids and parental species from Crescent Pond and Little Lake in 0/1/2 format using VCFtools. That is, individuals genotyped as homozygote reference were coded as 0, heterozygotes as 1, and homozygote alternatives as 2. Note again that the reference genome used was that of the molluscivore, *C. brontotheroides*.
- 2) These SNPs were subsequently loaded into R, and concatenated into haplotypes, such that each sequenced individual has a haplotype. These per-sample haplotypes were subsequently associated with metadata, namely the observed binary survival, growth, assignment as hybrid or one of the three parental species, and lake of origin.
- 3) Each haplotype was subsequently collapsed and summarized, such that mean survival, growth, and composite fitness are retained for each unique haplotype. Additionally, the number of hybrids, generalists, molluscivores and scale-eaters that have the haplotype were recorded.
- 4) The distance, in number of mutational steps was then calculated for each pairwise combination of haplotypes. For example, the distance between haplotype 000 and 001 is a single mutational step, whereas haplotypes 000 and 002 are two steps away.
- 5) Lastly, we constructed networks using the R package igraph v. 1.2.4.1 (113). Specifically, nodes represent haplotypes, and edges are drawn between haplotypes that are mutational neighbors (i.e. are a single mutational step away). Nodes present in hybrids were colored and sized proportional to their respective mean fitness. Nodes present only in parental species were colored according to the species in which that haplotype is unique to.

Estimation of evolutionary accessibility

The large number of SNPs in our dataset above raises numerous challenges in the visualization and summarization of fitness networks. Perhaps most significant, is that as the number of sites assessed increases, the sequence space increases vastly; the number of potential haplotypes is defined by 3 to the power of the number of SNPs, and the number of potential edges is defined by the number of haplotypes choose 2. Consequently, networks constructed from a larger number of focal SNPs are comprised of haplotypes that are separated on average by more mutational steps than those constructed from fewer SNPs. Because we can only interpret the fitness consequences of evolutionary trajectories for which we have data along each mutational step, we restricted analysis to haplotypes that are mutational neighbors (i.e., separated by a single mutational step).

To do so, we developed a permutation approach to construct fitness networks from SNPs that were sourced from the three sources of genetic variation defined above as well as their combinations (i.e. standing genetic variation + adaptive introgression or de novo mutation). Specifically, from each set we sampled five SNPs up to 1000 times. Then, from each permutation, we constructed fitness networks using the five steps defined above; these networks served as the subsequent assessment of evolutionary accessibility of genotypic trajectories separating the generalists from either specialist. Edges were only drawn such that the network is directed; that is, edges were drawn from low to high fitness nodes.

We constructed networks using both parental species and hybrids. For each trajectory under consideration (generalist $\rightarrow$ molluscivore & generalist $\rightarrow$ scale-eater), we first identified all generalist to specialist trajectories that are connected by accessible (monotonically increasing) paths. From these connected generalist-specialist node pairs, we randomly sampled a single pair. We then identified the number and length of accessible paths separating the generalist node from the specialist node and recorded these values using the “all_simple_paths” function in igraph and excluded any paths that traversed through haplotypes not found in any hybrid (i.e., exclusive to parental species) and thus had no information on fitness.

Supplementary Results

Genomic associations recovered for composite fitness and growth, not survival

Whereas we identified 132 SNPs that were associated with composite fitness, only 58 were associated with growth and none were associated with survival. Of the SNPs associated with growth, only four remained significant using the conservative Bonferroni threshold. Across all significant sites (either via FDR or Bonferroni correction) a total of 11 were shared across analyses. The only gene proximate to a growth associated SNP was *csad*. Lastly, we found a single gene shared between our study and the 125 ecological DMIs (putative genetic incompatibilities that are differentially expressed among specialists and misexpressed in F1 hybrids) presented in McGirr & Martin (69). This gene, associated with growth (but not composite fitness) in our study, is *mettl21e*.

When considering SNPs found to be associated with growth, we did not identify any gene ontologies that were significantly enriched at a False Discovery Rate < 0.01 . However, looking at those enriched at an FDR < 0.05 , we do observe a number of ontologies related to biosynthetic processes, and regulation of metabolic processes (Fig. S6). Specifically, the greatest (and most significant) enrichment was for that of phosphorus and phosphate containing compound metabolic processes and their regulation. Phosphorous deficiencies have previously been associated with poor growth in silver perch (*Bidyanus bidyanus*: (117)) and skeletal deformities (including vertebral compression and craniofacial deformities) in zebrafish (*Danio rerio*: (118)). Similarly, blunt snout bream (*Megalobrama amblycephala*) exhibited greater growth rates with increasing phosphorous levels in their diets (119). In short, enrichment of growth-associated SNPs for ontologies pertaining to phosphorous metabolism is consistent with the substantial literature documenting that phosphorous availability and metabolism is a determinant of growth in fishes.

Morphological variation within sampled hybrids

As in the previous two experiments, there is a relative paucity of hybrids exhibiting the morphologies that characterize either specialist. Rather, most hybrids fall near the generalists, with a number exhibiting transgressive morphologies (Fig. S9A). As expected, both specialists

exhibit reduced morphological disparity as compared to generalists, and hybrids show the greatest (Fig. S9B). That is, the specialists appear more morphologically constrained than generalists, falling on average closer to the group centroid. Interestingly molluscivores exhibit the least disparity, even less so than scale-eaters.

Fitness-associated SNPs improve estimation of the adaptive landscape

Using morphology alone, the best fit generalized additive model for survival, growth, and composite fitness were simpler than the model for composite fitness using both morphology and fitness-associated SNPs (Tables S8-10). For survival and composite fitness (Figure S10A & S10B), this model included a thin-plate spline for LD1 & LD2, with experiment and lake included as fixed effects. The resultant landscape was also similar for these two analyses, supporting an interpretation of directional selection, favoring molluscivores. For growth, the best-fit model had the thin-plate spline for LD1 & LD2, but included an interaction term between experiment and lake (Table S9). In contrast to the previous two models, the landscape predicted using growth as our proxy of fitness supported an interpretation of directional selection in favor of hybrids most similar to generalists, and to a lesser extent, scale-eaters.

Notably, model selection using AICc invariably supported the inclusion of fitness associated SNPs for growth and composite fitness (Tables S11-S12). For growth, the best-fit model including genotypes was an improvement of 22.99 AICc over the model including morphology alone, whereas for composite fitness, the improvement was 94.527 AICc. Interestingly, the best-fit models and growth including associated SNPs was similar to that of the landscape without fitness-associated SNPs, but largely supported an interpretation of directional selection in favor of scale-eaters, and to a lesser extent generalists.

Evolutionary accessibility is influenced by the source of genetic variation

As with composite fitness, we find that fitness landscapes including either adaptive introgression or *de novo* mutation are always smaller (i.e. fewer nodes and edges in the network) than networks constructed from standing genetic variation (SGV) alone (Fig. S13; Table S13-S15). In fact, for networks constructed using growth as our fitness proxy, the influence appears even stronger. These results hold even when rarefying our standing genetic variation SNPs to 65, the number of introgressed variants in scale-eaters and the second largest set of variants (data not shown).

Similar to composite fitness, we find that fitness landscapes estimated using both survival (but see generalist to scale-eater) and growth including adaptive introgression have both proportionally more accessible paths between generalists and specialists that are shorter (Fig. S14-S15, Table S13, S16-S17). However, there are several exceptions both for fitness landscapes constructed using survival as our proxy of fitness and looking at genotypic trajectories separating generalists from scale-eaters. First, we found that fitness landscapes constructed using adaptive introgression alone actually had relatively fewer accessible paths than those constructed using standing variation (Fig. S14A, Tables S16), however as with other analyses, these accessible paths were significantly shorter (Fig. S14B, Table S16). Second, we did not observe significant differences between any other group in the relative number of accessible paths (Fig. S14A, Table S16). Lastly, we did not find that accessible paths in networks constructed from a combination of standing genetic variation and adaptive introgression were significantly shorter than those from standing genetic variation alone (Fig. S14, Table S17). One potential explanation for these contrasting findings for accessible paths between generalists and scale-eaters is that because the

Accessibility on the fitness landscape

1082 measure of fitness used to obtain these results, survival, is a binary, the fitness landscape
 1083 essentially converges on that of a holey adaptive landscape (2, 3, 19). Thus, adaptation between
 1084 generalists and scale eaters, which are particularly isolated on these fitness landscapes, may
 1085 occur through predominantly neutral evolution, erasing some of the signal detected for other
 1086 measures of fitness.

Figure S1. Proportion (%) genetic variance explained by the first 20 Principal Components obtained using all SNPs and individuals from Crescent Pond, Little Lake, and Osprey Lake, as well as experimental hybrids. The first two principal component axes are plotted in Figure 1.

Figure S2. Principal components two, three, and four.

Accessibility on the fitness landscape

Figure S3. The proportion of specialist ancestry in hybrids did not predict in experimental hybrids using either A) composite fitness, B) survival or C) growth. Results of a generalized in which survival (modeled as a binomial) is predicted by ancestry proportion, including lake and experiment as fixed effects. Points represent individual hybrids, with each individual represented by two points, one indicating their respective scale-eater (salmon, and molluscivore (blue) ancestry proportions. P -values correspond to the effect of each type of ancestry (scale-eater – red, molluscivore – blue) on survival probability. Lines are predicted values and are colored according to species.

Figure S4. Genetic distance predicts morphological distance among sampled hybrids.

Figure S5. Manhattan plots illustrating the strength of association between individual SNPs and either Survival (A) or Growth (B) as inferred by GEMMA. Significant associations are highlighted in blue (FDR < 0.05) or red (Bonferroni correction $P < 0.05$). The dashed red line indicates the threshold for significance following Bonferroni p-value adjustment. METTL21E and CSAD are both highly differentiated ($F_{ST} > 0.95$) among specialists, differentially expressed. METTL21E is also misexpressed in F1 hybrids, meaning it exhibits gene expression that is higher or lower than observed in both parental species (69), indicating it is involved in intrinsic incompatibilities.

1110

1111 **Figure S6. Gene ontology enrichment for SNPs found to be associated with growth.** Darker colors indicate ontologies that are more significantly
1112 enriched following FDR correction. Length of bar is proportional to the number of genes assigned to an ontology.

1113

1114 **Figure S7. Gene ontology enrichment for SNPs found to be associated with composite fitness.** Darker colors indicate ontologies that are more
 1115 significantly enriched following FDR correction. Length of bar is proportional to the number of genes assigned to an ontology,

Figure S8. The 29 landmarks used to digitally measure thirty traits plus standard length using DLTDV8a (110). The corresponding traits are shown in Table S7.

Figure S9. Morphological variation in the three San Salvador Island pupfish species and their experimentally produced hybrids. A. Linear discriminant (LD) morphospace. Parent species and hybrids are plotted using the two LD axes that together serve to distinguish species with 99.4% accuracy. **B.** Within-group disparity calculated as the median distance between each individual and the groups centroid. Small, semitransparent points are the result of 100 bootstrap replicates, and are summarized by box plots, which in turn show the median and interquartile ranges of these bootstrap replicates. Large, opaque points are the observed disparities using the full dataset per group. All pairwise comparisons using t-tests following correction for the false discovery rate were significant ($P < 0.001$).

Accessibility on the fitness landscape

Figure S10. Best-fit fitness landscapes for composite fitness without fitness associated SNPs (A) survival (B), growth without associated SNPs (C) and growth including associated SNPs (D). Colored points indicate locations of parent species in LD morphospace, and ellipses indicate their 50% (solid) and 95% (dotted) confidence intervals. Grey points indicate location of hybrid individuals, with size proportional to their fitness measure. Cooler colors on the adaptive landscape indicate lower predicted fitness.

Figure S11. The topography of the composite fitness adaptive landscape is influenced by the distribution of a common SNP haplotype. Shown is the same landscape as in Figure 2, but the plotted points are unique SNP haplotypes for the thirteen most strongly fitness-associated SNPs. One haplotype is particularly frequent among hybrids; individuals with this haplotype are closer in morphospace to generalists and drive the emergence of a local fitness optimum for generalists. All other haplotypes (points) are plotted with a distinct color per haplotype.

Figure S12. Genotypic fitness networks (using composite fitness or survival) are smaller both with respect to number of nodes (A) and number of edges (B) when SNPs sourced from introgression or *de novo* mutation are included than networks constructed from standing genetic variation (SGV) alone. Compact letter display reports pairwise significance according to a nonparametric Kruskal-Wallis one-way analysis of variance following correction for multiple tests using an estimated False Discovery Rate. Letters are ordered (i.e. **a** to **c**) in decreasing values of the median. These results are constant whether survival or composite fitness are used because they comprise the same set of samples (both survivors and non-survivors)

Figure S13. Genotypic fitness networks (using growth) are smaller both with respect to number of nodes (A) and number of edges (B) when SNPs sourced from introgression or *de novo* mutation are included than networks constructed from standing genetic variation (SGV) alone. Compact letter display reports pairwise significance according to a nonparametric Kruskal-Wallis one-way analysis of variance following correction for multiple tests using an estimated False Discovery Rate. Letters are ordered (i.e. a to c) in decreasing values of the median. These results include only survivors and thus differ from those shown in Figure S12.

1157

1158 **Figure S14. Accessibility of between-species genotypic trajectories in survival fitness landscapes constructed from SNPs exhibiting**
 1159 **signatures of divergent selection (Richards et al. 2021) that are sourced from either standing genetic variation (SGV), introgression, *de novo***
 1160 **mutation, or a combination. A) The number of accessible (i.e. monotonically increasing in fitness) paths per network, scaled by the size of the**
 1161 **network. Compact letter display reports pairwise significance according to a nonparametric Kruskal-Wallis one-way analysis of variance following**
 1162 **correction for multiple tests using an estimated False Discovery Rate. B) Number of mutational steps in the shortest accessible path.**
 1163

Figure S15. Accessibility of between-species genotypic trajectories in growth fitness landscapes constructed from SNPs exhibiting signatures of divergent selection (Richards et al. 2021) that are sourced from either standing genetic variation (SGV), introgression, *de novo* mutation, or a combination. A) The number of accessible (i.e. monotonically increasing in fitness) paths per network, scaled by the size of the network. Compact letter display reports pairwise significance according to a nonparametric Kruskal-Wallis one-way analysis of variance following correction for multiple tests using an estimated False Discovery Rate. B) Number of mutational steps in the shortest accessible path.

Figure S16. The number of edges and thus potential number of paths (A) and accessible paths (B) increases as network size (number of nodes) increases.

Table S1. Samples of hybrids and parental studies used either in genomic or morphological analyses, along with associated metadata.

ID	Sequenced	Morphology	Survivorship	Experiment	Lake	Species
CP04E02	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP07F05	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP08H06	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP09C02	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP09D01	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP09F10	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11C10	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP13E03	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP13F01	This study	Yes	Non-Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
LL01F03	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL01F05	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL01G05	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02A08	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02D09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02D11	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02E04	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02E07	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02E09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL04E03	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL04E09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL04F02	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL04F07	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05A01	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05C06	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05C09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05C10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05D10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05E06	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05E08	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL05H10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06A10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06B03	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06C10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06D12	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06E10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06F08	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08B09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08B11	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08G07	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08G10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08H01	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09A09	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09A10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09B05	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09C03	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09D01	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09D10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09E01	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09F03	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09H07	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL09H10	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL10A08	This study	Yes	Non-Survivor	Martin & Gould 2020	Little Lake	Hybrid

CP02D01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP02E09	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP03D02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP03G03	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP03G07	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP05B08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP05E02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP05G12	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP05H10	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP06E09	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
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CP06H09	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP07E08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP07H01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP07H11	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP08F07	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP08G08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP09D02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10A03	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10C04	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10C07	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10C12	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10F12	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP10H01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11B03	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11C08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11D04	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11G06	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP11G12	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP12E05	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP12E07	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP12H04	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP13B02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP13B08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP14C04	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP15A10	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP15B08	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP15E01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP15E03	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP17A01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP18B11	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP19B02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP19C02	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP19C07	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP19F01	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CP19G03	This study	Yes	Survivor	Martin & Gould 2020	Crescent Pond	Hybrid
CPH01	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH02	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH03	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH04	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH05	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH07	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH08	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH09	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH10	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH100	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
CPH11	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid

CPH123	This study	Yes	Survivor	Martin & Wainwright 2013	Crescent Pond	Hybrid
LL01H04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL02G09	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06B06	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL06E04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL07E12	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL07G04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08A09	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08B04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL08D05	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
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LL12B04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL13B06	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL13D04	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL17H03	This study	Yes	Survivor	Martin & Gould 2020	Little Lake	Hybrid
LL106	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL114	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL124	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL129	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL175	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL23	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL247	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL251	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LL271	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LLH12	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LLH34	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LLH41	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LLH51	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
LLH94	This study	Yes	Survivor	Martin & Wainwright 2013	Little Lake	Hybrid
CRPA1	Richards et al. 2021	No	NA	NA	Crescent Pond	Generalist
CRPA1000	Richards et al. 2021	No	NA	NA	Crescent Pond	Generalist
CRPA1001	Richards et al. 2021	No	NA	NA	Crescent Pond	Generalist
CRPA1003	Richards et al. 2021	No	NA	NA	Crescent Pond	Generalist
CRPA3	Richards et al. 2021	No	NA	NA	Crescent Pond	Generalist
LILA1	Richards et al. 2021	No	NA	NA	Little Lake	Generalist
OSPA1	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA1000	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA1001	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA11	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA12	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA13	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA4	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA5	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA6	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA8	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
OSPA9	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Generalist
CRPM1	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM10	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM1000	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM1001	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM11	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM2	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM3	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM5	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM6	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM7	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
CRPM8	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore

CRPM9	Richards et al. 2021	No	NA	NA	Crescent Pond	Molluscivore
LILM-QTL	Richards et al. 2021	No	NA	NA	Little Lake	Molluscivore
LILM3	Richards et al. 2021	No	NA	NA	Little Lake	Molluscivore
LILM4	Richards et al. 2021	No	NA	NA	Little Lake	Molluscivore
LILM5	Richards et al. 2021	No	NA	NA	Little Lake	Molluscivore
OSPM1	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM1000	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM1001	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM11	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM2	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
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OSPM4	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM5	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM7	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM8	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
OSPM9	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Molluscivore
CRPP-QTL	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP1000	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP1001	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP2	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP3	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP4	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP5	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP7	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP8	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
CRPP9	Richards et al. 2021	No	NA	NA	Crescent Pond	Scale-eater
LILP-QTL	Richards et al. 2021	No	NA	NA	Little Lake	Scale-eater
LILP3	Richards et al. 2021	No	NA	NA	Little Lake	Scale-eater
LILP4	Richards et al. 2021	No	NA	NA	Little Lake	Scale-eater
LILP5	Richards et al. 2021	No	NA	NA	Little Lake	Scale-eater
OSPP1	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP10	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP1000	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP1001	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP11	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP2	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP3	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP4	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP5	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP7	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
OSPP9	Richards et al. 2021	No	NA	NA	Osprey Pond (Little Lake)	Scale-eater
CPA01	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA03	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA05	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA07	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA09	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA11	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA13	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA15	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA17	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA19	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA21	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA23	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA25	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA27	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA29	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA31	NA	Yes	Parental	NA	Crescent Pond	Generalist

CPA33	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA35	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA37	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA39	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA41	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA43	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA45	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA47	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA49	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA51	NA	Yes	Parental	NA	Crescent Pond	Generalist
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CPA55	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA57	NA	Yes	Parental	NA	Crescent Pond	Generalist
CPA59	NA	Yes	Parental	NA	Crescent Pond	Generalist
LLA20	NA	Yes	Parental	NA	Little Lake	Generalist
LLA21	NA	Yes	Parental	NA	Little Lake	Generalist
LLA22	NA	Yes	Parental	NA	Little Lake	Generalist
LLA23	NA	Yes	Parental	NA	Little Lake	Generalist
LLA24	NA	Yes	Parental	NA	Little Lake	Generalist
LLA25	NA	Yes	Parental	NA	Little Lake	Generalist
LLA26	NA	Yes	Parental	NA	Little Lake	Generalist
LLA27	NA	Yes	Parental	NA	Little Lake	Generalist
LLA28	NA	Yes	Parental	NA	Little Lake	Generalist
LLA29	NA	Yes	Parental	NA	Little Lake	Generalist
LLA30	NA	Yes	Parental	NA	Little Lake	Generalist
LLA31	NA	Yes	Parental	NA	Little Lake	Generalist
LLA32	NA	Yes	Parental	NA	Little Lake	Generalist
LLA33	NA	Yes	Parental	NA	Little Lake	Generalist
LLA34	NA	Yes	Parental	NA	Little Lake	Generalist
LLA35	NA	Yes	Parental	NA	Little Lake	Generalist
LLA36	NA	Yes	Parental	NA	Little Lake	Generalist
LLA37	NA	Yes	Parental	NA	Little Lake	Generalist
LLA38	NA	Yes	Parental	NA	Little Lake	Generalist
LLA39	NA	Yes	Parental	NA	Little Lake	Generalist
LLA40	NA	Yes	Parental	NA	Little Lake	Generalist
LLA41	NA	Yes	Parental	NA	Little Lake	Generalist
LLA42	NA	Yes	Parental	NA	Little Lake	Generalist
LLA43	NA	Yes	Parental	NA	Little Lake	Generalist
LLA44	NA	Yes	Parental	NA	Little Lake	Generalist
LLA45	NA	Yes	Parental	NA	Little Lake	Generalist
LLA46	NA	Yes	Parental	NA	Little Lake	Generalist
LLA47	NA	Yes	Parental	NA	Little Lake	Generalist
LLA48	NA	Yes	Parental	NA	Little Lake	Generalist
LLA49	NA	Yes	Parental	NA	Little Lake	Generalist
CPM01	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM02	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM03	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM04	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM05	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM06	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM07	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM08	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM09	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM10	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM11	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM12	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM13	NA	Yes	Parental	NA	Crescent Pond	Molluscivore

CPM14	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM15	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM16	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM17	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM18	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM19	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
CPM20	NA	Yes	Parental	NA	Crescent Pond	Molluscivore
LLM01	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM02	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM03	NA	Yes	Parental	NA	Little Lake	Molluscivore
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LLM05	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM06	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM07	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM08	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM09	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM10	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM11	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM12	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM13	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM14	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM15	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM16	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM17	NA	Yes	Parental	NA	Little Lake	Molluscivore
LLM18	NA	Yes	Parental	NA	Little Lake	Molluscivore
CPP01	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP02	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP03	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP04	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP05	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP06	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP07	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP08	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP09	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP10	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP11	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP12	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP13	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP14	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP15	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP16	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP17	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP18	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP19	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP20	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP21	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP22	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP23	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP24	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP25	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP26	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP27	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP28	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP29	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
CPP30	NA	Yes	Parental	NA	Crescent Pond	Scale-eater
LLP01	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP02	NA	Yes	Parental	NA	Little Lake	Scale-eater

LLP03	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP04	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP05	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP06	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP07	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP08	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP09	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP10	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP11	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP12	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP13	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP14	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP15	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP16	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP17	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP18	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP19	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP20	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP21	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP22	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP23	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP24	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP25	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP26	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP27	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP28	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP29	NA	Yes	Parental	NA	Little Lake	Scale-eater
LLP30	NA	Yes	Parental	NA	Little Lake	Scale-eater

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Table S2. Models tested to assess the extent to which specialist ancestry are genome-wide variation (PC1/PC2) predicts measures of fitness and their respective fits. Best-fit models are bolded

Fitness Measure	Species	Model	AIC	Ancestry/PC P-value
Composite Fitness	Molluscivore	Composite ~ AncestryProp	77.69	0.5951
		Composite ~ AncestryProp + Lake	78.19	0.4048
		Composite ~ AncestryProp + Experiment	78.28	0.4435
		Composite ~ AncestryProp + Lake + Experiment	78.67	0.2845
		Composite ~ AncestryProp + Lake * Experiment	80.62	0.2797
	Scale-eater	Composite ~ AncestryProp	76.01	0.1641
		Composite ~ AncestryProp + Lake	77.41	0.2283
		Composite ~ AncestryProp + Experiment	77.78	0.3000
		Composite ~ AncestryProp + Lake + Experiment	78.09	0.1715
		Composite ~ AncestryProp + Lake * Experiment	81.12	0.4079
	PC1	Composite ~ PC1	76.32	0.2017
		Composite ~ PC1 + Lake	77.04	0.1778
		Composite ~ PC1 + Experiment	77.43	0.2341
		Composite ~ PC1 + Lake + Experiment	78.64	0.2786
		Composite ~ PC1 + Lake * Experiment	80.39	0.2389
	PC2	Composite ~ PC2	76.32	0.2740
		Composite ~ PC2 + Lake	76.32	0.1124
		Composite ~ PC2 + Experiment	78.15	0.3985
		Composite ~ PC2 + Lake + Experiment	77.99	0.1793
		Composite ~ PC2 + Lake * Experiment	79.96	0.1797
Growth	Molluscivore	Growth ~ AncestryProp	42.52	0.4231
		Growth ~ AncestryProp + Lake	29.24	0.3383
		Growth ~ AncestryProp + Experiment	13.91	0.0041
		Growth ~ AncestryProp + Lake + Experiment	-23.57	0.3932
		Growth ~ AncestryProp + Lake * Experiment	-88.32	0.3280
	Scale-eater	Growth ~ AncestryProp	36.71	0.0122
		Growth ~ AncestryProp + Lake	12.25	0.0000
		Growth ~ AncestryProp + Experiment	21.98	0.4870
		Growth ~ AncestryProp + Lake + Experiment	-22.80	0.9950
		Growth ~ AncestryProp + Lake * Experiment	-88.53	0.2825
	PC1	Growth ~ PC1	30.97	0.0006
		Growth ~ PC1 + Lake	29.37	0.3723
		Growth ~ PC1 + Experiment	-32.37	0.0000
		Growth ~ PC1 + Lake + Experiment	-30.92	0.0055
		Growth ~ PC1 + Lake * Experiment	-87.40	0.7638
	PC2	Growth ~ PC2	23.61	0.0002
		Growth ~ PC2 + Lake	23.61	0.0119
		Growth ~ PC2 + Experiment	14.04	0.0044
		Growth ~ PC2 + Lake + Experiment	-23.65	0.3677
		Growth ~ PC2 + Lake * Experiment	-87.49	0.6724
Survival	Molluscivore	Survival ~ AncestryProp	188.78	0.9643
		Survival ~ AncestryProp + Lake	155.11	0.0936
		Survival ~ AncestryProp + Experiment	161.41	0.3709
		Survival ~ AncestryProp + Lake + Experiment	123.06	0.3127
		Survival ~ AncestryProp + Lake * Experiment	125.06	0.3127
	Scale-eater	Survival ~ AncestryProp	188.61	0.6756
		Survival ~ AncestryProp + Lake	154.93	0.0852
		Survival ~ AncestryProp + Experiment	158.99	0.0828
		Survival ~ AncestryProp + Lake + Experiment	124.04	0.8041
		Survival ~ AncestryProp + Lake * Experiment	126.04	0.8041
	PC1	Survival ~ PC1	156.24	0.0000
		Survival ~ PC1 + Lake	157.87	0.6943
		Survival ~ PC1 + Experiment	121.52	0.0000
		Survival ~ PC1 + Lake + Experiment	123.51	0.4386
		Survival ~ PC1 + Lake * Experiment	125.51	0.4386
	PC2	Survival ~ PC2	157.96	0.0268
		Survival ~ PC2 + Lake	157.96	0.7988
		Survival ~ PC2 + Experiment	160.72	0.2248
		Survival ~ PC2 + Lake + Experiment	123.39	0.4047
		Survival ~ PC2 + Lake * Experiment	125.39	0.4047

1181 **Table S3. SNPs found to be strongly associated with composite fitness using SnpEff (107).** SNPs that were identified as being strongly associated with both growth and composite fitness are
1182 italicized, and those that remain significant after a Bonferroni correction are bolded.

Scaffold	Position	Significance	REF	ALT	Variant Type	Gene Identifier	Gene Card
HiC_scaffold_1	32071263	FDR	A	C	intergenic	CBRO_00000660-CBRO_00000661	Znf250-Ptgr2
HiC_scaffold_1	43866598	Bonferroni	G	A	intergenic	CBRO_00000910-CBRO_00000911	PPM1K-OVCH2
HiC_scaffold_1	43867614	FDR	C	T	intergenic	CBRO_00000910-CBRO_00000911	PPM1K-OVCH2
HiC_scaffold_3	2658774	FDR	T	A	intergenic	CBRO_00017856-CBRO_00017857	KMT2E-Magi2
HiC_scaffold_3	2658775	FDR	T	C	intergenic	CBRO_00017856-CBRO_00017857	KMT2E-Magi2
HiC_scaffold_3	2658793	FDR	C	A	intergenic	CBRO_00017856-CBRO_00017857	KMT2E-Magi2
HiC_scaffold_4	18899496	FDR	T	C	intergenic	CBRO_00012427-CBRO_00012428	UNKNOWN-edc4
HiC_scaffold_5	18306419	FDR	C	G	upstream; intergenic	CBRO_00001232; CBRO_00001231-CBRO_00001232	xlrs1; PPEF2-xlrs1
HiC_scaffold_5	18306428	FDR	A	T	upstream; intergenic	CBRO_00001232	xlrs1; PPEF2-xlrs1
HiC_scaffold_5	18307019	FDR	G	T	intronic	CBRO_00001232	xlrs1
HiC_scaffold_5	18307030	FDR	G	A	intronic	CBRO_00001232	xlrs1
HiC_scaffold_5	18311696	FDR	C	T	intronic	CBRO_00001232	xlrs1
HiC_scaffold_5	40475116	FDR	T	A	intergenic	CBRO_00001627-CBRO_00001628	SLC25A44-UBE2Q2
HiC_scaffold_7	10290141	FDR	T	C	downstream; intergenic	CBRO_00009717; CBRO_00009716-CBRO_00009717	Nfkbie; SLC35B2-Nfkbie
HiC_scaffold_7	10290142	FDR	G	C	downstream; intergenic	CBRO_00009717; CBRO_00009716-CBRO_00009717	Nfkbie; SLC35B2-Nfkbie
HiC_scaffold_7	10290165	FDR	A	G	downstream; intergenic	CBRO_00009717; CBRO_00009716-CBRO_00009717	Nfkbie; SLC35B2-Nfkbie
HiC_scaffold_7	10290166	FDR	T	C	downstream; intergenic	CBRO_00009717; CBRO_00009716-CBRO_00009717	Nfkbie; SLC35B2-Nfkbie
HiC_scaffold_7	10290168	FDR	T	C	downstream; intergenic	CBRO_00009717; CBRO_00009716-CBRO_00009717	Nfkbie; SLC35B2-Nfkbie
HiC_scaffold_7	13815058	FDR	A	G	synonymous; downstream	CBRO_00009805; CBRO_00009804	Aloxe3; UNKNOWN
HiC_scaffold_7	13815326	FDR	C	T	intronic	CBRO_00009805	Aloxe3
HiC_scaffold_7	15349830	FDR	C	A	intergenic	CBRO_00009834-CBRO_00009835	Adcy8-efr3b
HiC_scaffold_7	18378061	FDR	T	A	intergenic	CBRO_00009902-CBRO_00009903	Fam84a-DDX1

HiC_scaffold_8	20263964	Bonferroni	G	A	upstream; intergenic	CBRO_00010647; CBRO_00010647- CBRO_00010648 CBRO_00010835; CBRO_00010834- CBRO_00010835 CBRO_00013361 CBRO_00013361-CBRO_00013362 CBRO_00013404 CBRO_00014134-CBRO_00014135 CBRO_00014134-CBRO_00014135 CBRO_00003226 CBRO_00003289-CBRO_00003290 CBRO_00003383-CBRO_00003384 CBRO_00003384-CBRO_00003385 CBRO_00013239 CBRO_00013239 CBRO_00013239 CBRO_00013240 CBRO_00016084 CBRO_00016084 CBRO_00016304 CBRO_00016304 CBRO_00014375-CBRO_00014376 CBRO_00014421-CBRO_00014422 CBRO_00014601 CBRO_00014635-CBRO_00014636 CBRO_00005887; CBRO_00005886- CBRO_00005887 CBRO_00005998 CBRO_00006026-CBRO_00006027 CBRO_00006131-CBRO_00006132 CBRO_00006685-CBRO_00006686	Srcin1; Srcin1-Srcin1 Gjd3; Gjd3-Gjd3 prkdc prkdc-arhgap29 KAZN UNKNOWN-Abr UNKNOWN-Abr KIF1B Pip5k1c-Polr2e chst10-UNKNOWN UNKNOWN-Carmil3 CSAD CSAD CSAD Znf740 GTF3C4 GTF3C4 MALT1 MALT1 UNKNOWN-UNKNOWN Gal3st3-RIN2 ABCA4 Lrfn2-SNX15 UNKNOWN; hoxb13a-UNKNOWN SMARCA4 ANKFN1-ccdc134 SHISA9-Desi1 med25-Lrrc4b
HiC_scaffold_8	31539262	FDR	G	A	upstream; intergenic		
HiC_scaffold_11	3106766	FDR	T	A	intronic		
HiC_scaffold_11	3138733	FDR	G	A	intergenic		
HiC_scaffold_11	5658921	FDR	A	G	intronic		
HiC_scaffold_14	17556007	FDR	C	T	intergenic		
HiC_scaffold_14	17556026	FDR	C	A	intergenic		
HiC_scaffold_16	32837191	FDR	T	C	intronic		
HiC_scaffold_16	35727503	FDR	G	C	intergenic		
HiC_scaffold_16	40215889	FDR	G	A	intergenic		
HiC_scaffold_16	40300592	FDR	C	A	intergenic		
HiC_scaffold_18	26969972	FDR	T	G	intronic		
HiC_scaffold_18	26970123	FDR	C	T	synonymous		
HiC_scaffold_18	26970601	FDR	T	A	intronic		
HiC_scaffold_18	26978410	FDR	G	A	intronic		
HiC_scaffold_20	332642	FDR	G	A	missense		
HiC_scaffold_20	332689	FDR	A	G	missense		
HiC_scaffold_20	14820437	FDR	A	T	intronic		
HiC_scaffold_20	14820448	FDR	T	C	intronic		
HiC_scaffold_24	1469530	FDR	G	T	intergenic		
HiC_scaffold_24	3223583	FDR	T	A	intergenic		
HiC_scaffold_24	11618442	FDR	T	G	intronic		
HiC_scaffold_24	15964553	Bonferroni	C	T	intergenic		
HiC_scaffold_27	1898180	FDR	G	A	downstream; intergenic		
HiC_scaffold_27	8137335	FDR	G	T	intronic		
HiC_scaffold_27	9065056	FDR	C	A	intergenic		
HiC_scaffold_27	12370585	FDR	C	T	intergenic		
HiC_scaffold_27	32078665	FDR	C	G	intergenic		

HiC_scaffold_27	34904388	FDR	A	C	intergenic	CBRO_00006756-CBRO_00006757	Grin2c-nog3
HiC_scaffold_27	35431570	FDR	G	C	intergenic	CBRO_00006756-CBRO_00006757	Grin2c-nog3
HiC_scaffold_27	35431578	FDR	C	T	intergenic	CBRO_00006756-CBRO_00006757	Grin2c-nog3
HiC_scaffold_27	35431585	FDR	A	G	intergenic	CBRO_00006756-CBRO_00006757	Grin2c-nog3
HiC_scaffold_34	16654675	FDR	G	C	intergenic	CBRO_00001997-CBRO_00001998 CBRO_00002027; CBRO_00002027- CBRO_00002028	MDFIC2-foxp1b
HiC_scaffold_34	19393244	FDR	A	T	upstream; intergenic	CBRO_00002117	SUOX; SUOX-SUOX
HiC_scaffold_34	22010499	FDR	C	T	intronic	CBRO_00002389; CBRO_00002388- CBRO_00002389	GNAI2
HiC_scaffold_34	31220916	FDR	T	A	upstream; intergenic	CBRO_00002519-CBRO_00002520	CTTNBP2NL; Kcnd3-CTTNBP2NL
HiC_scaffold_34	37769304	FDR	A	C	intergenic	CBRO_00011020-CBRO_00011021	ASIC2-asic1
HiC_scaffold_37	5963405	FDR	T	C	intergenic	CBRO_00011155-CBRO_00011156 CBRO_00011221; CBRO_00011220- CBRO_00011221	C14orf93-pim2
HiC_scaffold_37	11017168	FDR	G	A	intergenic	CBRO_00011221; CBRO_00011220- CBRO_00011221	GALNT12-elp2
HiC_scaffold_37	13822135	FDR	A	G	upstream; intergenic	CBRO_00011259-CBRO_00011260	SATB1; KCNH8-SATB1
HiC_scaffold_37	13823678	FDR	G	A	upstream; intergenic	CBRO_00011301-CBRO_00011302	SATB1; KCNH8-SATB1
HiC_scaffold_37	13832007	FDR	T	A	missense	CBRO_00011301-CBRO_00011302	SATB1
HiC_scaffold_37	16920863	FDR	T	C	intergenic	CBRO_00016614; CBRO_00016613- CBRO_00016614	CSMD1-UNKNOWN
HiC_scaffold_37	18591438	Bonferroni	G	A	intergenic	CBRO_00018766; CBRO_00018766- CBRO_00018767	cck-trim71
HiC_scaffold_37	18591463	FDR	C	T	intergenic	CBRO_00018814	cck-trim71
HiC_scaffold_37	18596716	FDR	A	T	intergenic	CBRO_00007439-CBRO_00007440	cck-trim71
HiC_scaffold_40	5885291	FDR	G	T	downstream; intergenic	CBRO_00007448	C14orf93 homolog; HTR2A- C14orf93 homolog
HiC_scaffold_43	2568535	FDR	A	G	intergenic	CBRO_00008059-CBRO_00008060	UNKNOWN-Gpr68
HiC_scaffold_44	25886134	FDR	A	C	intergenic		RAPGEF2-QDPR
HiC_scaffold_45	885834	FDR	A	C	upstream; intergenic		Mog; Mog-EPHB4
HiC_scaffold_45	2213441	FDR	G	T	intronic		Nlrp12
HiC_scaffold_46	856248	FDR	A	G	intergenic		UNKNOWN-NLRP12
HiC_scaffold_46	1232350	FDR	G	A	intronic		NEB
HiC_scaffold_46	30183758	FDR	C	G	intergenic		TFRC-Rgs11

HiC_scaffold_46	32048848	FDR	A	C	intronic	CBRO_00008105	Hsd17b7
HiC_scaffold_46	32050109	FDR	G	T	synonymous	CBRO_00008105	Hsd17b7
HiC_scaffold_46	35151009	Bonferroni	T	C	downstream; intergenic	CBRO_00008165; CBRO_00008165- CBRO_00008166	Klf9; Klf9-Tsen15
HiC_scaffold_46	35163681	FDR	T	C	downstream; intergenic	CBRO_00008166; CBRO_00008167; CBRO_00008166-CBRO_00008167	Tsen15; UNKNOWN; Tsen15- UNKNOWN
HiC_scaffold_46	35164267	FDR	A	G	downstream; intergenic	CBRO_00008166; CBRO_00008167; CBRO_00008166-CBRO_00008167	Tsen15; UNKNOWN; Tsen15- UNKNOWN
HiC_scaffold_47	787141	FDR	T	G	upstream; intergenic	CBRO_00008838; CBRO_00008837- CBRO_00008838	Nlrc3; NLRC3-Nlrc3
HiC_scaffold_47	4300295	FDR	G	A	downstream; intergenic	CBRO_00008933; CBRO_00008933- CBRO_00008934	NEK6; NEK6-Psmb7
HiC_scaffold_47	6767766	FDR	T	C	intergenic	CBRO_00008991-CBRO_00008992	TACR1-Grk5
HiC_scaffold_52	20791197	FDR	T	A	upstream; intronic	CBRO_00011892; CBRO_00011891	rabl3; GTF2E1
HiC_scaffold_52	22551701	FDR	G	C	intergenic	CBRO_00011922-CBRO_00011923	ALS2-Serp2
HiC_scaffold_52	31021517	FDR	C	T	intergenic	CBRO_00012051-CBRO_00012052	Tmeff2-slc39a10
HiC_scaffold_53	11317840	Bonferroni	A	G	intergenic	CBRO_00005178-CBRO_00005179	Fucoatlectin-1-Fucoatlectin-5
HiC_scaffold_53	11326035	FDR	C	T	intergenic	CBRO_00005178-CBRO_00005179	Fucoatlectin-1-Fucoatlectin-5
HiC_scaffold_53	11326410	FDR	G	A	intergenic	CBRO_00005178-CBRO_00005179	Fucoatlectin-1-Fucoatlectin-5
HiC_scaffold_53	11331079	FDR	A	G	intergenic	CBRO_00005178-CBRO_00005179	Fucoatlectin-1-Fucoatlectin-5
HiC_scaffold_53	15966447	FDR	G	A	upstream; downstream; intergenic	CBRO_00005235; CBRO_00005234; CBRO_00005234-CBRO_00005235	Tmem222; WDTC1; WDTC1- Tmem222
HiC_scaffold_53	17413090	FDR	A	G	missense	CBRO_00005274	Mag
HiC_scaffold_53	20715576	FDR	C	A	intergenic	CBRO_00005380-CBRO_00005381	Sert2-Ino80c
HiC_scaffold_53	20715623	FDR	G	A	intergenic	CBRO_00005380-CBRO_00005381	Sert2-Ino80c
HiC_scaffold_53	20715851	FDR	A	G	intergenic	CBRO_00005380-CBRO_00005381	Sert2-Ino80c
HiC_scaffold_53	27386094	FDR	G	T	intronic	CBRO_00005625	Arhgef1
HiC_scaffold_53	27396961	FDR	T	A	intronic	CBRO_00005625	Arhgef1
HiC_scaffold_53	27398605	FDR	T	G	downstream; intergenic	CBRO_00005625; CBRO_00005625- CBRO_00005626	Arhgef1; Arhgef1-CD79A
HiC_scaffold_53	33282501	FDR	A	G	intergenic	CBRO_00005732-CBRO_00005733	mios-GLCCI1
HiC_scaffold_53	39228193	FDR	T	C	intergenic	CBRO_00005830-CBRO_00005831	UNKNOWN-GnRHR2

HiC_scaffold_53	39228264	Bonferroni	C	T	intergenic	CBRO_00005830-CBRO_00005831	UNKNOWN-GnRHR2
HiC_scaffold_53	39228942	FDR	A	T	intergenic	CBRO_00005830-CBRO_00005831	UNKNOWN-GnRHR2
HiC_scaffold_53	39279263	FDR	G	T	intronic	CBRO_00005832	IGDCC3
HiC_scaffold_53	39770914	FDR	A	T	intronic	CBRO_00005849	Cpne4
HiC_scaffold_611	5621	FDR	A	T	intergenic	CHR_START-CBRO_00020243	CHR_START-fzdz-a
HiC_scaffold_611	5625	FDR	G	T	intergenic	CHR_START-CBRO_00020243	CHR_START-fzdz-a
HiC_scaffold_611	5634	FDR	T	A	intergenic	CHR_START-CBRO_00020243	CHR_START-fzdz-a
HiC_scaffold_1053	3494	FDR	A	G	downstream; intergenic	CBRO_00020503; CHR_START-CBRO_00020503	UBE2G2; CHR_START-UBE2G2
HiC_scaffold_1133	9946	FDR	A	G	intergenic	.	.
HiC_scaffold_1371	7318	FDR	C	T	upstream; intergenic	CBRO_00021026; CHR_START-CBRO_00021026	UNKNOWN; CHR_START-UNKNOWN
HiC_scaffold_1848	40119	Bonferroni	C	A	intergenic	.	.
HiC_scaffold_1848	40465	Bonferroni	T	A	intergenic	.	.
HiC_scaffold_1848	40590	Bonferroni	T	C	intergenic	.	.
HiC_scaffold_1848	40877	FDR	T	C	intergenic	.	.
HiC_scaffold_1848	41351	FDR	T	C	intergenic	.	.
HiC_scaffold_2220	10128	FDR	G	T	intergenic	.	.
HiC_scaffold_4461	12939	Bonferroni	T	A	intergenic	.	.
HiC_scaffold_4665	13941	FDR	C	T	intergenic	.	.
HiC_scaffold_6275	6000	FDR	C	A	intergenic	.	.
HiC_scaffold_6337	5745	FDR	T	G	intronic	CBRO_00021217	PKP3
HiC_scaffold_6769	2796	FDR	G	A	intergenic	.	.
HiC_scaffold_6963	5970	FDR	C	A	intergenic	.	.
HiC_scaffold_6963	6101	FDR	A	G	intergenic	.	.
HiC_scaffold_9280	3448	FDR	G	A	intergenic	.	.
HiC_scaffold_9949	52	FDR	C	T	intergenic	.	.
HiC_scaffold_10928	3575	FDR	G	A	downstream; intergenic	CBRO_00021896; CHR_START-CBRO_00021896	CYP2A10; CHR_START-CYP2A10
HiC_scaffold_11921	5560	FDR	A	T	intergenic	.	.
HiC_scaffold_12068	2929	FDR	G	T	intergenic	.	.

HiC_scaffold_1277							
8	1456	Bonferroni	T	A	missense	CBRO_00022026	COL8A1
HiC_scaffold_17578	180	FDR	T	G	intergenic	.	.
HiC_scaffold_18999	1084	Bonferroni	A	G	intergenic	.	.

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1184 **Table S4. SNPs found to be strongly associated with growth SnpEff (107).** SNPs that were identified as being strongly associated with both growth and composite fitness are italicized, and those
1185 that remain significant after a Bonferroni correction are bolded.

Scaffold	Position	Significance	REF	ALT	Variant Type	Gene Identifier	Gene Card
HiC_scaffold_4	8897671	FDR	A	T	intergenic	CBRO_00012254-CBRO_00012255	Megf10-UNKNOWN
HiC_scaffold_4	16057698	FDR	C	A	intergenic	CBRO_00012386-CBRO_00012387	CCND2-Mlycd
HiC_scaffold_4	29273446	FDR	T	C	synonymous	CBRO_00012620	CKAP5
HiC_scaffold_4	29273458	FDR	C	T	synonymous	CBRO_00012620	CKAP5
HiC_scaffold_5	18259775	FDR	C	A	upstream; intergenic	CBRO_00001229; CBRO_00001229- CBRO_00001230	AP1S2; AP1S2-phka2
<i>HiC_scaffold_5</i>	<i>18306419</i>	<i>FDR</i>	<i>C</i>	<i>G</i>	<i>upstream; intergenic</i>	<i>CBRO_00001232; CBRO_00001231- CBRO_00001232</i>	<i>xhrs1; PPEF2-xhrs1</i>
<i>HiC_scaffold_5</i>	<i>18306428</i>	<i>FDR</i>	<i>A</i>	<i>T</i>	<i>upstream; intergenic</i>	<i>CBRO_00001232; CBRO_00001231- CBRO_00001232</i>	<i>xhrs1; PPEF2-xhrs1</i>
<i>HiC_scaffold_5</i>	<i>18307019</i>	<i>FDR</i>	<i>G</i>	<i>T</i>	<i>intronic</i>	<i>CBRO_00001232</i>	<i>xhrs1</i>
<i>HiC_scaffold_5</i>	<i>18307030</i>	<i>FDR</i>	<i>G</i>	<i>A</i>	<i>intronic</i>	<i>CBRO_00001232</i>	<i>xhrs1</i>
HiC_scaffold_5	36619253	FDR	T	C	intergenic	CBRO_00001583-CBRO_00001584	Chst12-ZDHH13
<i>HiC_scaffold_7</i>	<i>13815058</i>	<i>FDR</i>	<i>A</i>	<i>G</i>	<i>synonymous; downstream</i>	<i>CBRO_00009805; CBRO_00009804</i>	<i>Aloxe3; UNKNOWN</i>
HiC_scaffold_7	13823565	FDR	T	C	intronic	CBRO_00009806	Fbxo30
HiC_scaffold_7	13824467	FDR	A	G	missense	CBRO_00009806	Fbxo30
HiC_scaffold_7	19371997	FDR	C	T	upstream; intergenic	CBRO_00009922; CBRO_00009921- CBRO_00009922	ELOVL4; TENT5A-ELOVL4
HiC_scaffold_7	19372002	FDR	T	G	upstream; intergenic	CBRO_00009922; CBRO_00009921- CBRO_00009922	ELOVL4; TENT5A-ELOVL4
HiC_scaffold_8	20265076	FDR	T	C	upstream; intergenic	CBRO_00010647; CBRO_00010647- CBRO_00010648	Srcin1; Srcin1-Srcin1
HiC_scaffold_8	20265098	FDR	G	C	upstream; intergenic	CBRO_00010647; CBRO_00010647- CBRO_00010648	Srcin1; Srcin1-Srcin1
HiC_scaffold_8	20278571	FDR	G	A	intergenic	CBRO_00010647-CBRO_00010648	Srcin1-Srcin1
HiC_scaffold_9	15585466	FDR	C	G	missense	CBRO_00004552	Tmem260
HiC_scaffold_9	18453213	FDR	A	G	intergenic	CBRO_00004639-CBRO_00004640	Bub1b-PAK6
HiC_scaffold_9	28127377	FDR	C	T	intergenic	CBRO_00004857-CBRO_00004858	METTL21E-RASA3
HiC_scaffold_10	193812	FDR	C	G	intergenic	CBRO_00018931-CBRO_00018932	Spsb4-UNKNOWN

HiC_scaffold_11	25632195	FDR	C	T	intergenic	CBRO_00013717-CBRO_00013718	CDH10-Cdh6
HiC_scaffold_11	25632258	FDR	C	T	intergenic	CBRO_00013717-CBRO_00013718	CDH10-Cdh6
HiC_scaffold_11	25632641	FDR	A	G	intergenic	CBRO_00013717-CBRO_00013718	CDH10-Cdh6
HiC_scaffold_14	14624430	FDR	T	C	intergenic	CBRO_00014084-CBRO_00014085	KDM6B-FGF11
HiC_scaffold_18	26970449	FDR	C	T	intronic	CBRO_00013239	CSAD
HiC_scaffold_27	8137335	FDR	G	T	intronic	CBRO_00005998	SMARCA4
HiC_scaffold_27	21919164	FDR	G	T	synonymous	CBRO_00006396	SSTR2
HiC_scaffold_29	2136546	FDR	A	T	upstream; intergenic	CBRO_00016347; CBRO_00016347- CBRO_00016348	RAPGEF6; RAPGEF6-ACSL6
HiC_scaffold_29	2147361	FDR	C	T	intergenic	CBRO_00016347-CBRO_00016348	RAPGEF6-ACSL6
HiC_scaffold_31	6226140	FDR	A	C	intronic	CBRO_00017224	ranbp9
HiC_scaffold_34	7439419	FDR	G	T	intergenic	CBRO_00001828-CBRO_00001829	rnf152-CAMTA1
HiC_scaffold_34	30571660	FDR	C	A	intronic	CBRO_00002374	SHMT2
HiC_scaffold_40	4693917	FDR	G	A	intronic	CBRO_00016589	SLC37A1
HiC_scaffold_40	4694015	FDR	T	C	synonymous	CBRO_00016589	SLC37A1
HiC_scaffold_40	4723358	FDR	A	G	intronic	CBRO_00016591	UBXN4
HiC_scaffold_40	4732538	FDR	T	C	intronic	CBRO_00016591	UBXN4
HiC_scaffold_40	4734825	FDR	G	T	intronic	CBRO_00016591	UBXN4
HiC_scaffold_40	4780518	FDR	G	C	intronic	CBRO_00016592	ITGB2
HiC_scaffold_40	5016538	FDR	C	A	upstream; intergenic	CBRO_00016599; CBRO_00016599- CBRO_00016600	RRP1B; RRP1B-ITGB2
HiC_scaffold_43	26869231	FDR	C	G	synonymous	CBRO_00008677	lrpprc
HiC_scaffold_43	27800569	FDR	C	T	downstream; intergenic	CBRO_00008685; CBRO_00008685- CBRO_00008686	timp3; timp3-ETV6
HiC_scaffold_44	19344526	FDR	C	T	intergenic	CBRO_00007178-CBRO_00007179	ATP11C-sox3
HiC_scaffold_46	16495639	FDR	G	A	intronic	CBRO_00007743	PHLPP1
HiC_scaffold_46	16510323	FDR	G	A	intronic	CBRO_00007743	PHLPP1
HiC_scaffold_46	16512668	FDR	T	A	intronic	CBRO_00007743	PHLPP1
HiC_scaffold_46	16512886	Bonferroni	T	A	intronic	CBRO_00007743	PHLPP1
HiC_scaffold_46	16513809	FDR	T	C	synonymous	CBRO_00007743	PHLPP1
HiC_scaffold_52	19031083	FDR	T	A	intergenic	CBRO_00011872-CBRO_00011873	NRP2-MREG

HiC_scaffold_52	19031216	FDR	A	T	intergenic	CBRO_00011872-CBRO_00011873	NRP2-MREG
HiC_scaffold_1848	40119	Bonferroni	C	A	intergenic	.	.
HiC_scaffold_1848	40465	Bonferroni	T	A	intergenic	.	.
HiC_scaffold_1848	40590	FDR	T	C	intergenic	.	.
HiC_scaffold_1848	40877	FDR	T	C	intergenic	.	.
HiC_scaffold_1848	41351	FDR	T	C	intergenic	.	.
HiC_scaffold_7644	5971	Bonferroni	T	G	intergenic	.	.
HiC_scaffold_12681	4019	FDR	T	C	upstream; intergenic	CBRO_00022068; CBRO_00022068-CHR_END	UNKNOWN; UNKNOWN-CHR_END

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1187 **Table S5. Gene ontology term enrichment for genes associated with growth.**

Functional Category	Enrichment FDR	Genes in list	Total genes	Genes
Negative regulation of intracellular signal transduction	0.0259	6	537	PPEF2, RNF152, RASA3, RANBP9, PHLPP1, TIMP3 PHLPP1, CCND2, PAK6, PPEF2, SHMT2, MLYCD, RRP1B, ITGB2, SRCIN1, PHKA2, ELOVL4,
Phosphorus metabolic process	0.0259	17	3597	BUB1B, RANBP9, TIMP3, CAMTA1, ACSL6, RASA3 PHLPP1, CCND2, PAK6, PPEF2, SHMT2, MLYCD, RRP1B, ITGB2, SRCIN1, PHKA2, ELOVL4,
Phosphate-containing compound metabolic process	0.0259	17	3570	BUB1B, RANBP9, TIMP3, CAMTA1, ACSL6, RASA3
Serine family amino acid catabolic process	0.0259	2	17	SHMT2, CSAD
Organic acid biosynthetic process	0.0259	6	466	ELOVL4, SHMT2, MLYCD, CHST12, ALOXE3, CSAD
Regulation of phosphate metabolic process	0.0259	11	1870	PHLPP1, CCND2, PAK6, PPEF2, SHMT2, RRP1B, ITGB2, SRCIN1, RANBP9, TIMP3, CAMTA1
Nucleoside bisphosphate biosynthetic process	0.0259	3	82	MLYCD, ELOVL4, ACSL6
Ribonucleoside bisphosphate biosynthetic process	0.0259	3	82	MLYCD, ELOVL4, ACSL6
Purine nucleoside bisphosphate biosynthetic process	0.0259	3	82	MLYCD, ELOVL4, ACSL6
Long-chain fatty-acyl-CoA biosynthetic process	0.0259	2	19	ELOVL4, ACSL6
Thioester biosynthetic process	0.0259	3	66	MLYCD, ELOVL4, ACSL6
Positive regulation by host of viral transcription	0.0259	2	18	SMARCA4, RRP1B
Sulfur compound biosynthetic process	0.0259	5	207	MLYCD, CHST12, ELOVL4, CSAD, ACSL6
Regulation of phosphorus metabolic process	0.0259	11	1872	PHLPP1, CCND2, PAK6, PPEF2, SHMT2, RRP1B, ITGB2, SRCIN1, RANBP9, TIMP3, CAMTA1
Acyl-CoA biosynthetic process	0.0259	3	66	MLYCD, ELOVL4, ACSL6
Hypothalamus development	0.0291	2	22	NRP2, SOX3
Sulfur compound metabolic process	0.0312	5	393	MLYCD, CHST12, ELOVL4, CSAD, ACSL6
Long-chain fatty-acyl-CoA metabolic process	0.0343	2	26	ELOVL4, ACSL6
Regulation of mitochondrial translation	0.0343	2	26	LRPPRC, SHMT2
Limbic system development	0.0405	3	115	NRP2, KDM6B, SOX3
Acyl-CoA metabolic process	0.0406	3	119	MLYCD, ELOVL4, ACSL6
Thioester metabolic process	0.0406	3	119	MLYCD, ELOVL4, ACSL6
Protein phosphorylation	0.0412	11	2093	PHLPP1, CCND2, PAK6, PPEF2, ITGB2, SRCIN1, PHKA2, BUB1B, RANBP9, TIMP3, RASA3
Modulation by host of viral transcription	0.0412	2	33	SMARCA4, RRP1B
Carboxylic acid biosynthetic process	0.0412	5	465	ELOVL4, SHMT2, MLYCD, CHST12, ALOXE3
Modulation of transcription in other organism involved in symbiotic interaction	0.0412	2	34	SMARCA4, RRP1B
Modulation by host of symbiont transcription	0.0412	2	33	SMARCA4, RRP1B PHLPP1, CCND2, PAK6, PPEF2, RNF152, ITGB2, SPSB4, SRCIN1, PHKA2, FBXO30, KDM6B,
Cellular protein modification process	0.0495	17	4434	BUB1B, RANBP9, TIMP3, CAMTA1, SHMT2, RASA3 PHLPP1, CCND2, PAK6, PPEF2, RNF152, ITGB2, SPSB4, SRCIN1, PHKA2, FBXO30, KDM6B,
Protein modification process	0.0495	17	4434	BUB1B, RANBP9, TIMP3, CAMTA1, SHMT2, RASA3

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1189 **Table S6. Gene ontology term enrichment for genes associated with composite fitness.**

Functional Category	Enrichment FDR	Genes in list	Total genes	Genes
Anterograde trans-synaptic signaling	0.0060	13	746	GRIN2C, SHISA9, ALS2, KIF1B, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2, ASIC2, PIP5K1C
Synaptic signaling	0.0060	13	762	GRIN2C, SHISA9, ALS2, KIF1B, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2, ASIC2, PIP5K1C
Trans-synaptic signaling	0.0060	13	756	GRIN2C, SHISA9, ALS2, KIF1B, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2, ASIC2, PIP5K1C
Chemical synaptic transmission	0.0060	13	746	GRIN2C, SHISA9, ALS2, KIF1B, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2, ASIC2, PIP5K1C
Regulation of I-kappaB kinase/NF-kappaB signaling	0.0179	7	241	NEK6, SLC35B2, MALT1, NLRP12, NLRC3, DDX1, PIM2
Regulation of trans-synaptic signaling	0.0185	9	468	GRIN2C, SHISA9, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2
Regulation of cell communication	0.0185	32	3903	NEK6, SLC35B2, MALT1, NLRP12, PPEF2, GRIN2C, NLRC3, SLC39A10, SHISA9, ALS2, RAPGEF2, SMARCA4, MAGI2, RGS11, ARHGEF1, DDX1, PIM2, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, PTGDR2, CCK, GPR68, MIOS, SCRT2, PSMB7, ARHGAP29, PIP5K1C, GRK5
Regulation of signaling	0.0185	32	3952	NEK6, SLC35B2, MALT1, NLRP12, PPEF2, GRIN2C, NLRC3, SLC39A10, SHISA9, ALS2, RAPGEF2, SMARCA4, MAGI2, RGS11, ARHGEF1, DDX1, PIM2, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, PTGDR2, CCK, GPR68, MIOS, SCRT2, PSMB7, ARHGAP29, PIP5K1C, GRK5
Modulation of chemical synaptic transmission	0.0185	9	467	GRIN2C, SHISA9, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, RAPGEF2
Sensory perception of sour taste	0.0185	2	5	ASIC2, ASIC1
Regulation of biological quality	0.0185	34	4319	GRIN2C, KCNH8, SLC39A10, SHISA9, PRKDC, TACR1, SMARCA4, KCND3, ABCA4, TRIM71, KMT2E, TFRC, PIM2, MAG, ASIC2, ASIC1, GNAI2, LRRC4B, HSD17B7, WDTC1, ADCY8, LRFN2, ABR, ALOXE3, CSMD1, CCK, SRCIN1, ALS2, GPR68, RAPGEF2, NEB, PSMB7, NFKBIE, PIP5K1C
I-kappaB kinase/NF-kappaB signaling	0.0185	7	280	NEK6, SLC35B2, MALT1, NLRP12, NLRC3, DDX1, PIM2
Positive regulation of catalytic activity	0.0185	17	1514	PPM1K, ALS2, RAPGEF2, MAGI2, SRCIN1, RGS11, ARHGEF1, GNAI2, GTF3C4, RIN2, ARHGAP29, ABR, CCK, SLC39A10, MALT1, ADCY8, NLRP12
Regulation of protein tyrosine phosphatase activity	0.0216	2	6	GNAI2, SLC39A10
Multicellular organismal response to stress	0.0216	4	74	ALS2, ASIC1, TACR1, CCK
Positive regulation of molecular function	0.0270	19	1901	PPM1K, MALT1, ALS2, RAPGEF2, SMARCA4, MAGI2, SRCIN1, RGS11, ARHGEF1, GNAI2, GTF3C4, RIN2, ARHGAP29, ADCY8, ABR, CCK, SLC39A10, MED25, NLRP12
Cell-cell signaling	0.0311	18	1774	GRIN2C, SHISA9, ALS2, KIF1B, ASIC1, GNAI2, TACR1, ADCY8, LRFN2, ABR, GRK5, GPR68, SMARCA4, RAPGEF2, MAGI2, ASIC2, PSMB7, PIP5K1C
Tachykinin receptor signaling pathway	0.0314	2	8	TACR1, GRK5
Cell proliferation	0.0355	20	2165	TFRC, PIM2, RAPGEF2, CD79A, GNAI2, TACR1, COL8A1, MALT1, SATB1, PTGDR2, CCK, SLC39A10, TRIM71, PRKDC, MED25, KLF9, GRK5, NLRC3, MAGI2, ARHGEF1
Behavioral fear response	0.0355	3	39	ALS2, ASIC1, CCK
Response to pH	0.0355	3	41	ASIC1, ASIC2, GPR68
Behavioral defense response	0.0355	3	40	ALS2, ASIC1, CCK
Fear response	0.0355	3	41	ALS2, ASIC1, CCK
Positive regulation of hydrolase activity	0.0401	11	833	ALS2, RAPGEF2, MAGI2, RGS11, ARHGEF1, RIN2, ARHGAP29, ABR, CCK, SLC39A10, NLRP12
NcRNA transcription	0.0457	4	110	GTF3C4, SMARCA4, POLR2E, GTF2E1
Hexose mediated signaling	0.0457	2	13	SMARCA4, ADCY8
Sugar mediated signaling pathway	0.0457	2	13	SMARCA4, ADCY8
Glucose mediated signaling pathway	0.0457	2	13	SMARCA4, ADCY8
Positive regulation of cell communication	0.0457	18	1937	NEK6, SLC35B2, MALT1, GRIN2C, SLC39A10, ALS2, RAPGEF2, SMARCA4, NLRP12, DDX1, PIM2, GNAI2, TACR1, ADCY8, PTGDR2, GPR68, MIOS, PSMB7
Positive regulation of signaling	0.0457	18	1945	NEK6, SLC35B2, MALT1, GRIN2C, SLC39A10, ALS2, RAPGEF2, SMARCA4, NLRP12, DDX1, PIM2, GNAI2, TACR1, ADCY8, PTGDR2, GPR68, MIOS, PSMB7

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Table S7. List of the 31 morphological traits measured for this study, and standard length; corresponding landmark ID's match those shown in Figure S8.

Trait Index	Trait Description	Trait Shorthand	Points	
1	Nasal protrusion	nose	3-4	1193
2	Nasal length	foresnout	2-5	1194
3	Orbit to anal fin insertion	bellylen	6-15	1195
4	Lateral facial length	snoutlen	2-6	1196
5	Upper jaw to pectoral girdle	jaw2pect	2-14	1197
6	Lateral skull length	pmx2add	2-11	1198
7	Premaxilla length	pmxlen	2-9	1199
8	Lower mandible length	jawlen	1-9	1200
9	Jaw joint to orbit	foreeyewidth	6-9	1201
10	Horizontal orbit diameter	eyewidth	6-8	1202
11	Vertical orbit diameter	eyeht	7-10	1203
12	Head height	headht	7-9	1204
13	Suspensorium length	suspensorium	9-11	1205
14	Adductor height	adductorht	11-12	1206
15	Subopercle to pectoral girdle	ad2pect	11-14	1207
16	Pectoral fin insertion width	pectinsertion	13-14	1208
17	Anal to caudal distance	analtocaudal	15-16	1209
18	Caudal peduncle height	caudalpedht	16-18	1210
19	Dorsal to caudal distance	dorsaltocaudal	18-19	1211
20	Body depth	bodydepth	15-19	1212
21	Nasal protrusion angle	nasalangle	7-5-3	1213
22	Premaxilla to orbit angle	topeyeangle	7-2-10	1214
23	Premaxilla to adductor angle	lowereyeangle	7-2-11	1215
24	Dorsal facial length	dorsalsnoutlen	23-24, 25-26	1216
25	Adductor to premaxilla	eyetosnout	21-24, 25-28	1217
26	Neurocranium to premaxilla	headlen	24-20, 25-29	1218
27	Orbit to premaxilla	innereyetosnout	22-24, 25-27	1219
28	Interorbital width	cranialwidth	22-27	1220
29	Orbital neurocranium width	hindeyewidth	21-28	1221
30	Max. neurocranium width	headwidth	20-29	1222
31	Standard length (SL)	SL	2-17	1223
				1230
				1231

Table S8. Generalized additive models fitted to composite fitness. Model fit was assessed using AICc, and Akaike Weights represent proportional model support. A thin plate spline for the two linear discriminant axes s(LD1, LD2) is always included, as is a fixed effect of either experiment (i.e. Martin & Wainwright 2013, Martin and Gould 2020) or Lake (Crescent Pond/Little Lake) or an interaction between the two. In the last two models, Experiment and Lake are included as splines, modeled using a factor smooth (bs = “fs”). The best fit model had 5 estimated degrees of freedom.

Model	AICc	ΔAICc	Akaike Weights
<i>Composite ~ s(LD1, LD2) + Experiment + Lake</i>	99.114	0.000	0.825
<i>Composite ~ s(LD1, LD2) + Experiment * Lake</i>	102.210	3.096	0.175
<i>Composite ~ s(LD1, LD2) + s(LD1) + s(LD2) + Experiment + Lake</i>	131.456	32.342	< 0.001
<i>Composite ~ s(LD1, LD2) + s(LD1) + s(LD2) + Experiment * Lake</i>	135.894	36.781	< 0.001
<i>Composite ~ s(LD1, LD2) + s(LD1, Experiment, bs = "fs") + s(LD2, Experiment, bs = "fs") + Lake</i>	230.428	131.314	< 0.001
<i>Composite ~ s(LD1, LD2) + s(LD1, Lake, bs = "fs") + s(LD2, Lake, bs = "fs") + Experiment</i>	230.868	131.754	< 0.001

Table S9. Generalized additive models fitted to growth. Model fit was assessed using AICc, and Akaike Weights represent proportional model support. A thin plate spline for the two linear discriminant axes s(LD1, LD2) is always included, as is a fixed effect of either experiment (i.e. Martin & Wainwright 2013, Martin and Gould 2020) or Lake (Crescent Pond/Little Lake) or an interaction between the two. In the last two models, Experiment and Lake are included as splines, modeled using a factor smooth (bs = “fs”). The best fit model had 8.93 estimated degrees of freedom.

Model	AICc	ΔAICc	Akaike Weights
<i>Growth ~ s(LD1, LD2) + Experiment * Lake</i>	-44.658	0.000	1
<i>Growth ~ s(LD1, LD2) + Experiment + Lake</i>	3.904	48.562	< 0.001
<i>Growth ~ s(LD1, LD2) + s(LD1) + s(LD2) + Experiment * Lake</i>	46.249	90.907	< 0.001
<i>Growth ~ s(LD1, LD2) + s(LD1) + s(LD2) + Experiment + Lake</i>	89.121	133.779	< 0.001
<i>Growth ~ s(LD1, LD2) + s(LD1, Lake, bs = "fs") + s(LD2, Lake, bs = "fs") + Experiment</i>	690.379	735.038	< 0.001
<i>Growth ~ s(LD1, LD2) + s(LD1, Experiment, bs = "fs") + s(LD2, Experiment, bs = "fs") + Lake</i>	693.748	738.406	< 0.001

Table S10. Generalized additive models fitted to survival. Model fit was assessed using AICc, and Akaike Weights represent proportional model support. A thin plate spline for the two linear discriminant axes $s(\text{LD1}, \text{LD2})$ is always included, as is a fixed effect of either experiment (i.e. Martin & Wainwright 2013, Martin and Gould 2020) or Lake (Crescent Pond/Little Lake) or an interaction between the two. In the last two models, Experiment and Lake are included as splines, modeled using a factor smooth ($\text{bs} = \text{"fs"}$). The best fit model had 5 estimated degrees of freedom.

Model	AICc	ΔAICc	Akaike Weights
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + \text{Experiment} + \text{Lake}$	141.057	0.000	0.849
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + \text{Experiment} * \text{Lake}$	144.504	3.447	0.151
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + s(\text{LD1}) + s(\text{LD2}) + \text{Experiment} + \text{Lake}$	173.399	32.342	< 0.001
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + s(\text{LD1}) + s(\text{LD2}) + \text{Experiment} * \text{Lake}$	178.189	37.132	< 0.001
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + s(\text{LD1}, \text{Experiment}, \text{bs} = \text{"fs"}) + s(\text{LD2}, \text{Experiment}, \text{bs} = \text{"fs"}) + \text{Lake}$	273.547	132.490	< 0.001
$\text{Survival} \sim s(\text{LD1}, \text{LD2}) + s(\text{LD1}, \text{Lake}, \text{bs} = \text{"fs"}) + s(\text{LD2}, \text{Lake}, \text{bs} = \text{"fs"}) + \text{Experiment}$	273.694	132.637	< 0.001

Table S11. Generalized additive models fitted to growth including SNPs most strongly associated with composite fitness. Model fit was assessed using AICc, and Akaike Weights represent proportional model support. The best fit model for composite fitness using morphology alone (see Table S8) was used as the base model. The SNPs that were most strongly associated with composite fitness (following a Bonferroni correction) were included as fixed effects, modeled as splines using a factor smooth, treating genotype as an ordered factor. Note that three SNPs were excluded due to their close proximity to other SNPs that were more strongly associated. All SNPs were considered individually, as well as all SNPs together. We were unable to assess all possible combinations of SNPs due to the vast number of potential models given the number of SNPs under consideration; rather, we fit one final model that only included SNPs found to be significant in the full model. In turn this model led to a substantial improvement in AICc. The best fit model had 20.29 estimated degrees of freedom.

Model	AICc	Δ AICc	Akaike Weights
<i>Composite Fitness</i> ~ <i>s</i> (LD1, LD2) + Experiment + Lake + <i>s</i> (Site1) + <i>s</i> (Site2) + <i>s</i> (Site6) + <i>s</i> (Site7) + <i>s</i> (Site8) + <i>s</i> (Site9) + <i>s</i> (Site10)	4.586	0.000	0.999
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site1) + s(Site2) + s(Site3) + s(Site4) + s(Site5) + s(Site6) + s(Site7) + s(Site8) + s(Site9) + s(Site10)	40.876	36.290	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site3)	55.588	51.001	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site7)	58.386	53.800	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site4)	65.453	60.867	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site2)	71.245	66.658	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site5)	72.329	67.743	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site1)	73.671	69.085	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site8)	74.413	69.827	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site9)	74.680	70.094	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site10)	88.977	84.391	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake + s(Site6)	90.427	85.841	< 0.001
Composite Fitness ~ s(LD1, LD2) + Experiment + Lake	99.114	94.527	< 0.001

Note: Site1 = HiC_Scaffold_1:43866598, Site2 = HiC_Scaffold_53:11317840, Site3 = HiC_Scaffold_46:35151009, Site4 = HiC_Scaffold_8:20263964, Site5 = 37:18591438, Site6 = HiC_Scaffold_37:18591438, Site7 = HiC_Scaffold_24:15964553, Site8 = HiC_Scaffold_1848:40590, Site9 = HiC_Scaffold_12778:1456, Site10 = HiC_Scaffold_18999:1084

Table S12. Generalized additive models fitted to growth including SNPs most strongly associated with growth. Model fit was assessed using AICc, and Akaike Weights represent proportional model support. The best fit model for growth using morphology alone (see Table S9) was used as the base model. Each of the four SNPs that were most strongly associated with growth (following a Bonferroni correction) were included as fixed effects, modeled as splines using a factor smooth, treating genotype as an ordered factor. All SNPs were considered individually, as well as all possible combinations. This was only feasible due to the small number of SNPs assessed (four). The best fit model had 7.97 estimated degrees of freedom.

Model	AICc	Δ AICc	Akaike Weights
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site3) + s(Site4)	-67.649	0.000	0.490
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site3)	-65.634	2.015	0.179
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1)	-64.161	3.488	0.086
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site3)	-63.926	3.723	0.076
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site2)	-63.861	3.788	0.074
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site2) + s(Site4)	-63.503	4.146	0.062
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site2)	-61.849	5.800	0.027
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site4)	-58.044	9.604	0.004
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site4)	-56.068	11.581	0.001
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site3) + s(Site4)	-54.878	12.770	< 0.001
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site2) + s(Site4)	-54.509	13.140	< 0.001
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site2) + s(Site3)	-47.602	20.047	< 0.001
Growth ~ s(LD1, LD2) + Experiment * Lake	-44.658	22.990	< 0.001
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site2) + s(Site3)	-41.689	25.960	< 0.001
Growth ~ s(LD1, LD2) + Experiment * Lake + s(Site1) + s(Site2) + s(Site3) + s(Site4)	-29.801	37.847	< 0.001

Note: Site1 = HiC_Scaffold_46:16512886, Site2 = HiC_Scaffold_1848:40119, Site3 = HiC_Scaffold_1848:40465, Site4 = HiC_Scaffold_7644:5971

Table S13. Summary statistics for fitness networks constructed using adaptive loci. Results for networks using all three measures of fitness (composite fitness, survival, and growth) are reported. Networks were constructed from random draws of five SNPs from either Standing genetic variation (SGV), introgression, or *de novo* mutations. The scaled number of accessible paths was calculated by dividing the number of accessible paths between generalists and specialists by the number of nodes present in a given fitness network.

Fitness Metric	Trajectory	Source	Number of Nodes in Network			Number of Edges in Network			Number of Accessible Paths			Scaled Number of Accessible Paths			Distance of Shortest Accessible Path		
			Median	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median	Mean	SE
Composite	AtoM	SGV	59.000	59.233	10.955	110.000	110.254	28.461	1.000	2.585	8.488	0.019	0.043	0.128	3.000	3.185	1.048
		Introgression	28.000	28.026	7.456	43.000	44.459	16.704	1.000	1.890	6.686	0.043	0.069	0.220	3.000	2.914	0.892
		SGV + Introgression	51.000	50.180	12.763	92.000	92.442	30.504	1.000	5.404	75.895	0.026	0.087	0.930	3.000	3.107	0.945
	AtoP	SGV	61.000	61.324	9.101	116.000	117.164	24.262	1.000	4.015	21.691	0.019	0.063	0.293	4.000	3.978	1.316
		Introgression	46.000	46.785	11.442	77.000	80.066	28.504	1.000	1.664	2.741	0.026	0.036	0.047	3.000	3.248	1.025
		<i>De novo</i>	48.000	47.373	9.243	83.000	83.082	24.048	1.000	2.039	5.624	0.026	0.042	0.098	3.000	3.495	1.052
		SGV + Introgression	57.000	56.858	12.003	107.000	105.897	31.286	1.000	3.589	19.955	0.020	0.059	0.295	4.000	3.701	1.088
		SGV + <i>De novo</i>	58.000	57.200	9.633	108.500	107.732	25.493	1.000	4.396	40.886	0.020	0.076	0.728	4.000	3.768	1.159
Survival	AtoM	SGV	62.000	61.752	9.036	136.000	136.282	29.143	1.000	309.508	4922.357	0.022	4.189	65.732	3.000	3.502	1.469
		Introgression	27.500	27.920	7.145	46.000	48.345	17.452	1.000	2.491	7.478	0.043	0.089	0.240	3.000	2.922	0.952
		SGV + Introgression	51.000	50.655	12.569	103.000	104.784	35.887	1.000	31.986	284.777	0.029	0.477	3.852	3.000	3.264	1.204
	AtoP	SGV	61.000	61.313	9.191	131.500	134.025	28.533	3.000	358.756	3156.552	0.054	5.721	51.543	5.000	5.163	2.077
		Introgression	47.000	47.954	11.559	92.000	94.270	33.744	1.000	15.053	113.473	0.031	0.244	1.630	4.000	4.081	1.626
		<i>De novo</i>	48.000	47.875	9.159	96.000	95.836	28.763	1.000	99.206	1933.358	0.031	1.550	29.317	4.000	4.058	1.690
		SGV + Introgression	58.000	57.379	11.275	124.000	123.244	35.495	2.000	153.381	1199.043	0.038	2.255	17.255	5.000	4.861	1.948
		SGV + <i>De novo</i>	58.000	57.572	9.406	127.000	124.970	29.966	2.000	143.683	1432.083	0.045	2.121	20.358	4.000	4.696	1.848
Growth	AtoM	SGV	38.000	38.166	6.678	48.000	47.959	12.495	1.000	1.142	0.443	0.027	0.031	0.012	2.000	2.347	0.655
		Introgression	17.000	17.670	4.154	16.000	16.227	5.546	1.000	1.011	0.120	0.059	0.061	0.021	2.000	2.151	0.440
		SGV + Introgression	31.000	31.104	7.998	35.000	36.299	13.914	1.000	1.111	0.376	0.033	0.038	0.016	2.000	2.265	0.553
	AtoP	SGV	38.000	37.975	6.154	52.000	51.120	12.469	1.000	1.316	0.749	0.028	0.035	0.020	2.000	2.861	1.143
		Introgression	27.500	28.513	7.232	31.000	33.080	12.378	1.000	1.115	0.359	0.037	0.042	0.016	2.000	2.248	0.453
		<i>De novo</i>	32.000	31.402	5.488	38.000	38.112	9.874	1.000	1.104	0.339	0.032	0.036	0.011	2.000	2.329	0.624
		SGV + Introgression	33.000	34.295	7.648	44.500	43.807	14.444	1.000	1.205	0.590	0.031	0.037	0.018	2.000	2.500	0.816
		SGV + <i>De novo</i>	36.000	35.389	5.713	45.000	45.326	11.109	1.000	1.154	0.519	0.029	0.033	0.015	2.000	2.571	0.812

Table S14. Results of pairwise comparisons using a non-parametric Kruskal-Wallis test for measures of network size (number of nodes and number of edges), using the samples for composite fitness and survival. *P*-values corrected for multiple tests using the false discovery rate. Medians, means, and SE for each group reported in Table S13.

Pairwise Comparison	Number of Nodes in Network		Number of Edges in Network	
	Generalist To Molluscivore	Generalist to Scale-eater	Generalist To Molluscivore	Generalist to Scale-eater
SGV - Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SGV - SGV + Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Introgression - SGV + Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SGV - <i>De novo</i>	-	< 0.0001	-	< 0.0001
SGV - SGV + <i>De novo</i>	-	< 0.0001	-	< 0.0001
<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001	-	< 0.0001
<i>De novo</i> - SGV + Introgression	-	< 0.0001	-	< 0.0001
<i>De novo</i> - Introgression	-	0.6921	-	0.1128
Introgression - SGV + <i>De novo</i>	-	< 0.0001	-	< 0.0001
SGV + <i>De novo</i> - SGV + Introgression	-	0.3068	-	0.0831

Table S15. Results of pairwise comparisons using a non-parametric Kruskal-Wallis test for measures of network size (number of nodes and number of edges), using the samples for growth. *P*-values corrected for multiple tests using the false discovery rate. Medians, means, and SE for each group reported in Table S13.

Pairwise Comparison	Number of Nodes in Network		Number of Edges in Network	
	Generalist To Molluscivore	Generalist to Scale-eater	Generalist To Molluscivore	Generalist to Scale-eater
SGV - Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SGV - SGV + Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Introgression - SGV + Introgression	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SGV - <i>De novo</i>	-	< 0.0001	-	< 0.0001
SGV - SGV + <i>De novo</i>	-	0.0014	-	0.0002
<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001	-	< 0.0001
<i>De novo</i> - SGV + Introgression	-	0.0002	-	0.0001
<i>De novo</i> - Introgression	-	< 0.0001	-	< 0.0001
Introgression - SGV + <i>De novo</i>	-	< 0.0001	-	< 0.0001
SGV + <i>De novo</i> - SGV + Introgression	-	0.0590	-	0.1498

Table S16. Results of pairwise comparisons using a non-parametric Kruskal-Wallis test for differences in the relative number of accessible paths between generalists and specialists in networks constructed using different sources of genetic variation using either composite fitness, growth, or survival as our measure of fitness. *P*-values corrected for multiple tests using the false discovery rate. Medians, means, and SE for each group reported in Table S13.

	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
Composite Fitness	SGV - Introgression	< 0.0001	< 0.0001
	SGV - SGV + Introgression	< 0.0001	0.7060
	Introgression - SGV + Introgression	< 0.0001	< 0.0001
	SGV - <i>De novo</i>	-	< 0.0001
	SGV - SGV + <i>De novo</i>	-	0.0679
	<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001
	<i>De novo</i> - SGV + Introgression	-	< 0.0001
	<i>De novo</i> - Introgression	-	0.0911
	Introgression - SGV + <i>De novo</i>	-	< 0.0001
	<i>SGV</i> + <i>De novo</i> - SGV + Introgression	-	0.1232
Growth	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
	SGV - Introgression	< 0.0001	< 0.0001
	SGV - SGV + Introgression	< 0.0001	0.0145
	Introgression - SGV + Introgression	< 0.0001	< 0.0001
	SGV - <i>De novo</i>	-	< 0.0001
	SGV - SGV + <i>De novo</i>	-	0.5854
	<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001
	<i>De novo</i> - SGV + Introgression	-	0.0996
	<i>De novo</i> - Introgression	-	0.0001
	Introgression - SGV + <i>De novo</i>	-	< 0.0001
	<i>SGV</i> + <i>De novo</i> - SGV + Introgression	-	0.0385
Survival	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
	SGV - Introgression	< 0.0001	0.0425
	SGV - SGV + Introgression	0.0004	0.3872
	Introgression - SGV + Introgression	< 0.0001	0.0468
	SGV - <i>De novo</i>	-	0.0757
	SGV - SGV + <i>De novo</i>	-	0.7967
	<i>De novo</i> - SGV + <i>De novo</i>	-	0.0148
	<i>De novo</i> - SGV + Introgression	-	0.1450
	<i>De novo</i> - Introgression	-	0.5277
	Introgression - SGV + <i>De novo</i>	-	0.0036
	<i>SGV</i> + <i>De novo</i> - SGV + Introgression	-	0.3122

Table S17. Results of pairwise comparisons using a non-parametric Kruskal-Wallis test for differences in the length of the shortest accessible paths between generalists and specialists in networks constructed using different sources of genetic variation using either composite fitness, growth, or survival as our measure of fitness. *P*-values corrected for multiple tests using the false discovery rate. Medians, means, and SE for each group reported in Table S13.

	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
Composite Fitness	SGV - Introgression	< 0.0001	< 0.0001
	SGV - SGV + Introgression	0.0002	0.0020
	Introgression - SGV + Introgression	0.3294	< 0.0001
	SGV - <i>De novo</i>	-	< 0.0001
	SGV - SGV + <i>De novo</i>	-	0.0232
	<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001
	<i>De novo</i> - SGV + Introgression	-	0.0024
	<i>De novo</i> - Introgression	-	0.0002
	Introgression - SGV + <i>De novo</i>	-	< 0.0001
	SGV + <i>De novo</i> - SGV + Introgression	-	0.3535
	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
Growth	SGV - Introgression	< 0.0001	< 0.0001
	SGV - SGV + Introgression	0.0182	0.0073
	Introgression - SGV + Introgression	< 0.0001	0.0215
	SGV - <i>De novo</i>	-	< 0.0001
	SGV - SGV + <i>De novo</i>	-	0.0309
	<i>De novo</i> - SGV + <i>De novo</i>	-	0.0003
	<i>De novo</i> - SGV + Introgression	-	0.0775
	<i>De novo</i> - Introgression	-	0.3384
	Introgression - SGV + <i>De novo</i>	-	0.0001
	SGV + <i>De novo</i> - SGV + Introgression	-	0.3562
	Pairwise Comparison	Generalist To Molluscivore	Generalist to Scale-eater
Survival	SGV - Introgression	< 0.0001	< 0.0001
	SGV - SGV + Introgression	0.0028	0.1279
	Introgression - SGV + Introgression	< 0.0001	< 0.0001
	SGV - <i>De novo</i>	-	< 0.0001
	SGV - SGV + <i>De novo</i>	-	0.0152
	<i>De novo</i> - SGV + <i>De novo</i>	-	< 0.0001
	<i>De novo</i> - SGV + Introgression	-	< 0.0001
	<i>De novo</i> - Introgression	-	0.4422
	Introgression - SGV + <i>De novo</i>	-	< 0.0001
	SGV + <i>De novo</i> - SGV + Introgression	-	0.1454