LETTER

Resource diversity promotes among-individual diet variation, but not genomic diversity, in lake stickleback

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Abstract

Many generalist species consist of specialised individuals that use different resources. This within-population niche variation can stabilise population and community dynamics. Consequently, ecologists wish to identify environmental settings that promote such variation. Theory predicts that environments with greater resource diversity favour ecological diversity among consumers (via disruptive selection or plasticity). Alternatively, niche variation might be a side-effect of neutral genomic diversity in larger populations. We tested these alternatives in a metapopulation of threespine stickleback. Stickleback consume benthic and limnetic invertebrates, focusing on the former in small lakes, the latter in large lakes. Intermediate-sized lakes support generalist stickleback populations using an even mixture of the two prey types, and exhibit greater among-individual variation in diet and morphology. In contrast, genomic diversity increases with lake size. Thus, phenotypic diversity and neutral genetic polymorphism are decoupled: trophic diversity being greatest in intermediate-sized lakes with high resource diversity, whereas neutral genetic diversity is greatest in the largest lakes.

Keywords

adaptive radiation, diversification, effective population size, *Gasterosteus aculeatus*, individual specialisation, intraspecific variation, niche variation.

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INTRODUCTION

Many animal populations that appear to be ecologically generalist are in fact heterogeneous assemblages of relatively specialised individuals (Bolnick *et al*. 2003; Araújo *et al*. 2011). Ecologists have therefore become increasingly interested in evaluating the community and ecosystem consequences of diet variation among co-occurring individuals (Bolnick *et al*. 2011; Des Roches *et al*. 2018). Theory and experiments demonstrate that within-population diet variation (also called 'individual specialization') can increase population stability and reduce extinction risk (Agashe 2009), promote co-existence (Doebeli 1997; Schreiber *et al*. 2011), change community composition (Ingram *et al*. 2011; Start & Gilbert 2017; Start 2018), and even alter ecosystem properties (Vrede *et al*. 2011). As these ecological effects depend on the magnitude of among-individual variation, we need to understand the causes of individual diet specialisation, and in what settings it will be more or less pronounced (Araújo *et al*. 2011).

The most widely accepted explanation of individual specialisation invokes frequency-dependent selection arising from resource competition (e.g. Levene 1953; Wilson & Turelli 1986; Doebeli 1996b; Bolnick 2001). Consider a consumer population inhabiting an environment with two functionally distinct resources. The consumer might specialise entirely on whichever resource is most profitable (taking into account nutritional value and abundance, Stephens & Krebs 1986).

But, as this preferred resource becomes scarcer due to exploitative competition, the alternative resource becomes relatively abundant, favouring niche shift (via evolution, plasticity, or behavioural change) to increasingly use a mixture of the two resources. In population genetic models of a diploid organism, this frequency-dependent competition leads to a balanced polymorphism dominated by heterozygotes, even if they are inefficient at using either resource alone (Levene 1953; Hedrick 1986; Wilson & Turelli 1986). Resource diversity should thus promote genetic polymorphism at certain ecologically functional genes and functional traits. Alternatively, it is possible that ecological opportunity promotes among-individual variation via phenotypic plasticity (Pfennig *et al*. 2006), including behavioural niche shifts (Svanbäck & Bolnick 2007b).

Because this theory was couched in terms of frequency-dependent competition, most studies of individual specialisation have focused on the diversifying effects of intraspecific competition (reviewed in Araújo *et al*. 2011). But, a growing number of comparative studies have found that resource diversity (often termed 'ecological opportunity') also leads to greater population niche breadth via increased individual specialisation (Parent & Crespi 2009; Martin & Pfennig 2010; Araújo & Costa-Pereira 2013; Evangelista *et al*. 2014; Cloyed & Eason 2016; Yurkowski *et al*. 2016; Costa-Pereira *et al*. 2017). This result is in line with theoretical models: at equilibrium, the frequencies of alternatively adapted alleles will depend on

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relative availability of the alternate resources. When one resource dominates, the corresponding allele will be more common or even fix. Genetic diversity at such a locus is therefore expected to be maximised when the resources are equally profitable (taking into account their abundance and value), i.e. when ecological opportunity is the greatest.

Similar logic applies to quantitative genetic traits. Adaptive dynamics theory suggests that a consumer using diverse resources should (given certain conditions) evolve an intermediate trait mean, representing a generalist compromise between the traits required to most efficiently use the alternative prey (Doebeli 1996a; Schreiber et al. 2011). This generalist may be subject to persistent disruptive selection that increases trait variation and individual specialisation (Nuismer et al. 2005), especially when alternative resources are equally available. However, if one resource dominates, the consumer's trait mean should shift correspondingly (by evolution or plasticity) to specialise on that resource, reducing the strength of disruptive selection, which in turn reduces trait and ecological diversity, and the diversity at loci affecting those traits.

An alternative hypothesis is that niche variation among individuals is effectively neutral rather than adaptive, an incidental byproduct of neutral genetic diversity within a population. In this case, larger or more productive habitats that support larger consumer populations should be less subject to genetic drift and thus exhibit greater genome-wide genetic diversity (Kimura 1983). High neutral genetic diversity could impart greater phenotypic and ecological variation as well, with little effect on fitness. This neutral population genomic view should be a null model against which we test adaptive explanations of individual specialisation and diet variation, but this is rarely done.

There is good reason to believe that ecological opportunity and genome-wide genetic diversity may be decoupled. The habitats with greater ecological opportunity may not be those that permit large effective population sizes. To see why, consider the classic but still-controversial 'Niche Variation Hypothesis' (NVH) (Van Valen 1965), which posits that a population colonising a new environment, freed of competitors, should expand its niche width to take advantage of expanded ecological opportunity. Due to trade-offs limiting individual diet breadth, this niche expansion is posited to arise via greater among-individual variation. Experimental and observational data have yielded mixed support for the NVH (Rothstein 1973; Patterson 1983; Swanson et al. 2003; Bolnick 2004; Meiri et al. 2005; Svanbäck & Bolnick 2005, 2007a; Bolnick et al. 2007; Svanbäck et al. 2008; Parent et al. 2014; Jones & Post 2016). However, the classic NVH scenario invokes recent colonisation of a depauperate habitat like islands or post-glacial lakes; this colonisation may entail bottlenecks that reduce genetic diversity, and if the island or lake is small it may only sustain a small population subject to increased genetic drift.

Such decoupling of ecological opportunity and genetic diversity means that comparative studies should be able to tease apart their respective effects on within-population diet and ecomorphological diversity. Specifically, we can test the adaptive versus neutral explanations for niche variation, by evaluating the effect of resource diversity and habitat size on ecological and neutral genetic diversity. Here, we present such a test using lake populations of threespine stickleback (*Gasterosteus aculeatus*). This test takes advantage of a very specific prediction from the models mentioned above. If you consider habitat patches with varying ratios of two alternative resources, there will be an intermediate habitat type where the two resources are equally profitable (considering abundance and nutritional value), maximising ecological opportunity. For example, fish in many lakes around the world have evolved to use a mixture of large benthic invertebrate prey on the lake substrate, and limnetic mid-water zooplankton (Moodie & Reimchen 1976; Lavin & McPhail 1985; Robinson & Wilson 1994; Kusche et al. 2014). Benthic prey tend to be relatively abundant in small lakes dominated by shallow littoral habitat. Limnetic prey dominate in large lakes, where volumes become large relative to the shallow perimeters that support benthic prey. Therefore, ecological opportunity (the evenness of benthic and limnetic prey) should be low in small and large lakes, but maximised in intermediate-sized lakes (Fig. 1). In contrast, fish effective population size typically increases monotonically with lake size (e.g. Caldera & Bolnick 2008). These contrasting effects of lake size on ecological opportunity versus neutral genetic diversity allow us to parse their respective roles in generating among-individual diet diversity.

METHODS

Our overall approach with this study was to sample wild-caught stickleback from numerous lakes. Within each lake, we measured among-individual variation in diet, morphology, and neutral genetic diversity using sparse genomic sequencing. We then compared these diversity metrics to each other, and to lake size which we use as a (tightly correlated) proxy for the balance of benthic/limnetic prey (as first described by Lavin & McPhail 1985, and confirmed again here). Note that because we use wild-caught fish for this study, the trends reported below likely include a mixture of genetic and environmental (e.g. plastic) sources of variation.

Collection

In June 2009, we collected between 60 and 100 threespine stickleback from 33 lakes on Vancouver Island, British Columbia, whose surface areas ranged from 1.5 to 2800 hectares (Table S1). This variation in lake size is uncorrelated with other biogeographic features such as elevation, distance up-river from the ocean, or proximity to other lakes, that also contribute to variation in population genetic diversity (Bolnick et al. preprint). Lake sizes were obtained from the British Columbia Ministry of the Environment HabitatWizard (http://maps.gov.bc.ca/ess/hm/habwiz/), supplemented by surface area analysis of GoogleEarth satellite images for the smallest lakes that were not listed on HabitatWizard. With the exception of a few of the smallest lakes (<10 ha), most lakes are deep enough that benthic habitat used by stickleback is restricted to near the lakes' shorelines. Therefore, area is an effective proxy for the relative availability of benthic and limnetic habitat, to a first approximation. The utility of this proxy has been repeatedly confirmed by diet analysis in previous studies (Lavin & McPhail 1985; Snowberg et al. 2015).
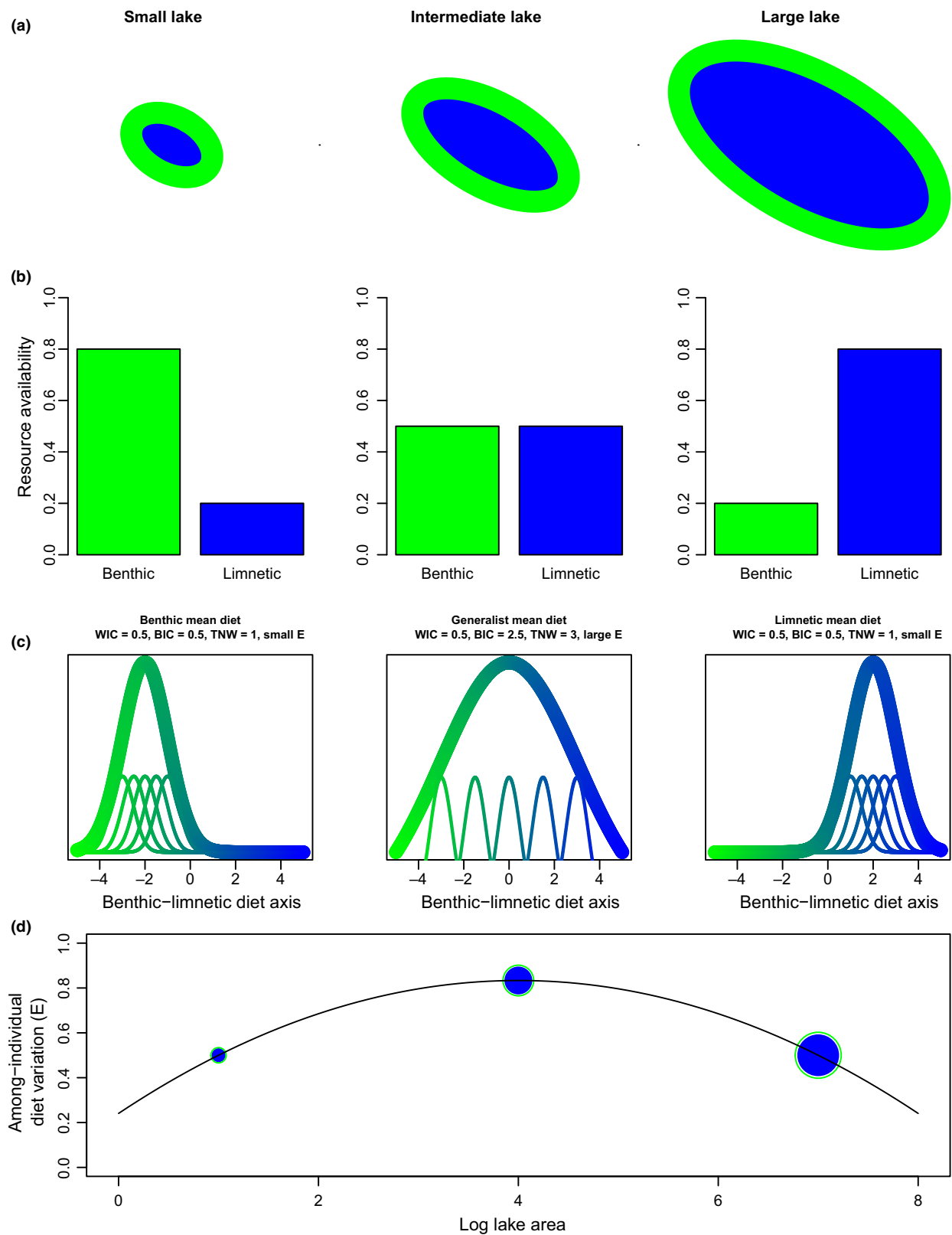
Figure 1 A schematic diagram of the hypothesis tested in this study. Row (a): three lakes of increasing size, with shoreline benthic habitat (green) and mid-water limnetic habitat (blue). Row (b): the corresponding resource availabilities of the three lakes, with decreasing relative abundance of benthic prey in larger lakes. Row (c): Because of the resource availability shifts in Row (b), the populations' diets shift from benthic (in a small lake) to limnetic (in the large lake). The diet distribution of the population as a whole is represented by the thick taller line, and is made up of diets of individuals (smaller lines, colour coded to represent relative use of benthic and limnetic prey). Row (d): As a result of the shifting diet distributions in Row (c), among-individual diet variation is expected to be greatest in the intermediate-sized lake

Previous population genetic analyses of lakes in this region have established the genetic relationships among the sampled populations (Caldera & Bolnick 2008; Stuart *et al.* 2017). There is significant genetic differentiation among all sampled lakes, which tend to cluster by watershed (Stuart *et al.* 2017) and by proximity within watersheds (Caldera & Bolnick 2008). STRUCTURE analyses provide no evidence of multiple colonisation events, as each lake contains a single genetically panmictic population, and the lake fish are genetic subsets of the more diverse marine populations nearby (Caldera & Bolnick 2008). In most of the sampled watersheds, isostatic rebound created low-elevation waterfalls shortly after Pleistocene glacial retreat, curtailing subsequent immigration by marine fish.

Fish were captured in unbaited minnow traps set for less than three hours, and immediately euthanised and preserved in formalin. Collection and animal handling were approved by the University of Texas IACUC (Protocol #07-032201), and a Scientific Fish Collection Permit from the Ministry of the Environment of British Columbia (NA07-32612). Collections occurrrerd in the historical lands of the Kwakwaka'wakw First Nations.

Morphology and diet measurements

All fish were weighed and measured for standard length. We determined sex via dissection. A random subset of 30 fish per lake were also measured for trophic morphology traits: gape width, gill raker number, and gill raker length. Gape width and gill raker length were size-adjusted by calculating residuals of log transformed values regressed on log length. We calculated trait means and variances for each lake.

For a random subset of 23 lakes, we also enumerated stomach contents for 30 fish per lake, using the same individuals subsampled for morphological measurements. We recorded presence or absence of prey taxa in the stomach of each fish. Stomach contents are an admittedly coarse and cross-sectional sample of individuals' diet, in stickleback reflecting approximately the previous 6 h of foraging (Svanbäck & Bolnick 2007b). However, previous work has demonstrated that stomach content variation among trapped individuals is a robust measure of diet variation in the population, being correlated with individual morphology, and with variance in stable isotope signatures that reflect long-term diet over months (Matthews *et al.* 2010; Snowberg *et al.* 2015). Direct observation of foraging individuals also confirms there is variation in choice of foraging microhabitat (benthic versus limnetic zone), which is correlated with those individuals' stomach contents, stable isotopes, and morphology (Snowberg *et al.* 2015).

We categorised prey as benthic or limnetic. For each fish we calculated the proportion of prey taxa that were classified as benthic. This metric was tightly correlated with the first major axis of a non-metric multidimensional scaling analysis of prey composition, so we focus our analysis on the proportion of benthic prey, which is more intuitive. Equivalent results are obtained with NMDS1. The total number of prey taxa observed per fish provides a metric of diet richness, with the recognition that this is a brief cross-sectional sample (for discussion of such caveats, see Bolnick *et al.* 2002; Araújo *et al.*, 2007, 2011). We calculated individual specialisation using the metric E, which measures among-individual diet disparity:

$$E = 1 - \frac{2 \sum_i^{N-1} \sum_{j=i+1}^{N} \left(\sum_k \min(p_{ik}, p_{jk}) \right)}{N(N-1)}$$

For each unique pair of individuals i and j in a population, we calculate the pairwise diet overlap across all k prey taxa (the term in parentheses in the numerator), then average this across all $N(N-1)$ pairwise comparisons between individuals within the population. The resulting metric E ranges from 0 when there is complete diet overlap between individuals, to a hypothetical extreme of 1.0 when every individual uses unique resources with no overlap with other individuals (Araújo *et al.* 2008). We calculated E for each lake, using RInSp (Zaccarelli *et al.* 2013).

Genomic diversity

We sampled fin clips from each fish, and extracted DNA from a random subsample of 12 fish per population. We used ddRAD-seq (Peterson *et al.* 2012) to genotype 175 350 single nucleotide polymorphisms (SNPs) from 336 fish (107 698 SNPs scored per fish on average). Laboratory and bioinformatic protocols are detailed in Stuart *et al.* (2017), who also used these data for a population-level phylogenetic tree for 76 stickleback populations on Vancouver Island. For this paper, we calculated genome-wide heterozygosity for each fish, then averaged among fish to obtain mean heterozygosity for each lake.

Statistical analyses

The focal hypothesis of this study is that individual specialisation (measured by the metric E) will be maximised in intermediate-sized lakes, mid-way along the benthic-limnetic diet continuum where ecological opportunity (resource diversity) should be the highest. We first tested the widely invoked relationship between lake size and population mean diet (Lavin & McPhail 1985), by linear regression of the mean proportion benthic prey as a function of log lake area. Having confirmed this linear trend (and hence the utility of lake size), we next ran a quadratic regression of diet variation, E, as a function of log lake area, anticipating a negative quadratic gradient. To confirm this, we also used quadratic regression relating E to mean proportion benthic prey, and a larger model with linear and quadratic effects of both lake area and mean proportion benthic prey. We then used quadratic regression to test the relationship between individual diet breadth (prey richness) and either log lake area, or mean proportion benthic prey. The NVH predicts this relationship to be flat. Note that the decision to use quadratic or linear regressions, in the above analyses, was determined *a priori*, rather than post hoc.

To test the role of morphological variation in diet diversification, we calculated the standard deviation of each morphological trait (standard length, gill raker number, size-adjusted gill raker length and size-adjusted gill raker number). We used multiple linear regression to test whether E increases monotonically with these traits' standard deviations. We then used quadratic regression to test whether each trait standard deviation is the highest in intermediate-sized lakes.

To test the alternative hypothesis that among-individual variation is a reflection of neutral genome-wide diversity, we

used a linear regression of diet variation E on the mean heterozygosity, with lake as the level of replication. We did the same for each morphological variance, testing for positive linear relationships with heterozygosity. Then, we evaluated the environmental settings that promote neutral genomic diversity, by regressing mean heterozygosity on log lake area. We expected a positive linear relationship, because larger lakes should support larger effective population sizes that result in greater equilibrium neutral genetic diversity. For consistency with the diet variation analyses, we also tested whether genetic heterozygosity exhibited a quadratic relationship with lake size (e.g. greatest in intermediate-sized lakes).

Unlike genome-wide genetic patterns, which on average should primarily reflect neutral population genetic processes, specific loci may be involved in adaptation to benthic or limnetic environments (Arnegard *et al.* 2014). Such non-neutral loci should exhibit allele frequency correlations with lake size, assuming one allele is consistently selectively favoured in benthic lakes, polymorphism favoured in intermediate lakes, and the other allele favoured in limnetic lakes. For each SNP in our dataset, we tested for such correlations using a binomial general linear model in which allele frequency depends on log lake area, with a logit link function. To exclude uninformative SNPs, we only used loci for which we genotyped at least 50 individuals to a depth of a minimum 12 reads, and the minor allele frequency exceeds 10% on average across all samples (e.g. excluding private alleles for one or a couple lakes). Genetic variance is maximised with intermediate allele frequencies ($P \sim 0.5$). If polymorphism at a particular SNP contributes to diet variation among individuals, we should find that allele frequency variance ($p[1 - p]$) is positively correlated with diet variation (E) and has a negative quadratic relationship with log lake size. We tested for these relationships for each SNP, using linear and quadratic regression. To reduce multiple testing, these analyses of single-locus genetic variance were conducted after first excluding SNPs whose allele frequency was clearly unrelated to lake size (uncorrected $P > 0.1$).

RESULTS

Diet differences among lakes

As commonly assumed, stickleback in larger lakes tended to consume relatively more limnetic than benthic prey (Fig. 2a; linear regression log lake effect $P < 0.0001$; all regression results are summarised in Table 1). This trend confirms past studies (Lavin & McPhail, 1986). Because the populations range from using 10 to 90% benthic prey, the intermediate populations are indeed ecological generalists that use roughly equal mixtures of benthic and limnetic resources. This confirms the utility of lake size as a measure of stickleback trophic ecology and ecological opportunity.

Diet diversity

Individual specialisation was most pronounced (highest E values) in intermediate-sized lakes (Fig. 2b; quadratic effect $P = 0.014$,

Table 1). A somewhat redundant test with a different metric (the among-individual standard deviation for the proportion benthic prey) confirms that diet variation is the greatest in intermediate-sized lakes (quadratic $P = 0.015$; Table 1; Fig. 2c). We also confirmed the expectation that individual specialisation (E) is strongest in generalist populations where the average proportion benthic prey is near 0.5 (Fig. 2d). Using multiple regression to partition the effects of lake size and mean diet on E, we find that individual specialisation is strictly a quadratic function of mean diet (diet $P = 0.0002$; diet^2 $P = 0.0001$; area $P = 0.945$; area^2 $P = 0.188$; model $r^2 = 0.727$), which itself depends on lake size (Fig. 2a). In contrast, we found no detectable correlation (linear or quadratic) between within-individual died breadth and log lake size, or with E (both $P > 0.5$; Table 1, Figs S1 and S2). Thus, between-population differences in total diet diversity arise primarily via changes in between-individual variation rather than within-individual diet breadth.

Genomic diversity

Genome-wide mean heterozygosity was greater in larger lakes (Fig. 2e; $P = 0.030$; Table 1). This positive trend is consistent with our expectation that larger lakes support larger effective population sizes that maintain greater genetic diversity (previously supported by smaller studies with a few microsatellites, Caldera & Bolnick 2008). As described in Bolnick *et al.* (preprint), this effect of lake size is independent of variation in lake elevation or distance from the ocean. However, this among-lake variation in heterozygosity had no detectable effect on among-individual diet variation, E ($r = -0.174$; $P = 0.425$; Fig. S3), as would be expected for mostly neutral genetic markers. Likewise, the variance of most ecomorphological traits were uncorrelated with population mean heterozygosity (standard length $r = -0.335$, $P = 0.066$; gape width $r = 0.106$, $P = 0.576$; gill raker number $r = -0.082$, $P = 0.666$; gill raker length $r = -0.402$, $P = 0.0278$; P-values are all non-significant after multiple test correction). From these results, we infer that individual specialisation and trait variance are not a reflection of neutral evolutionary processes. Recall that because the fish were wild-caught, phenotypic variation can include both genetic and environmental (plastic) effects. The latter could, in principle, include counter-gradient adaptive plasticity that can complicate observational correlations.

Single-marker genetic analyses

After filtering out rare or low-coverage loci, we examined a remaining 41 284 SNPs for locus-specific relationships with log lake size that might indicate adaptive function. We found 2149 SNPs with significant ($\alpha < 0.05$) associations with lake size, of which 5 survived arguably over-conservative Bonferroni correction (Fig. S4). None of these alleles exhibited confounding effects of watershed (a covariate in the model). The strongest association was found in two neighbouring SNPs on linkage group 4 (bases 19 204 072 and 19 204 307; $P = 0.0000096$ and 0.000068 respectively), which are within 100 kb of four genes, *foxp2*, *gpr85*, *tmem168a* and *ifrd1*. A second site, on linkage group 20 (site 15 807 116; $P = 0.000074$) lies between the genes *bola1* and *nr2f5*, and
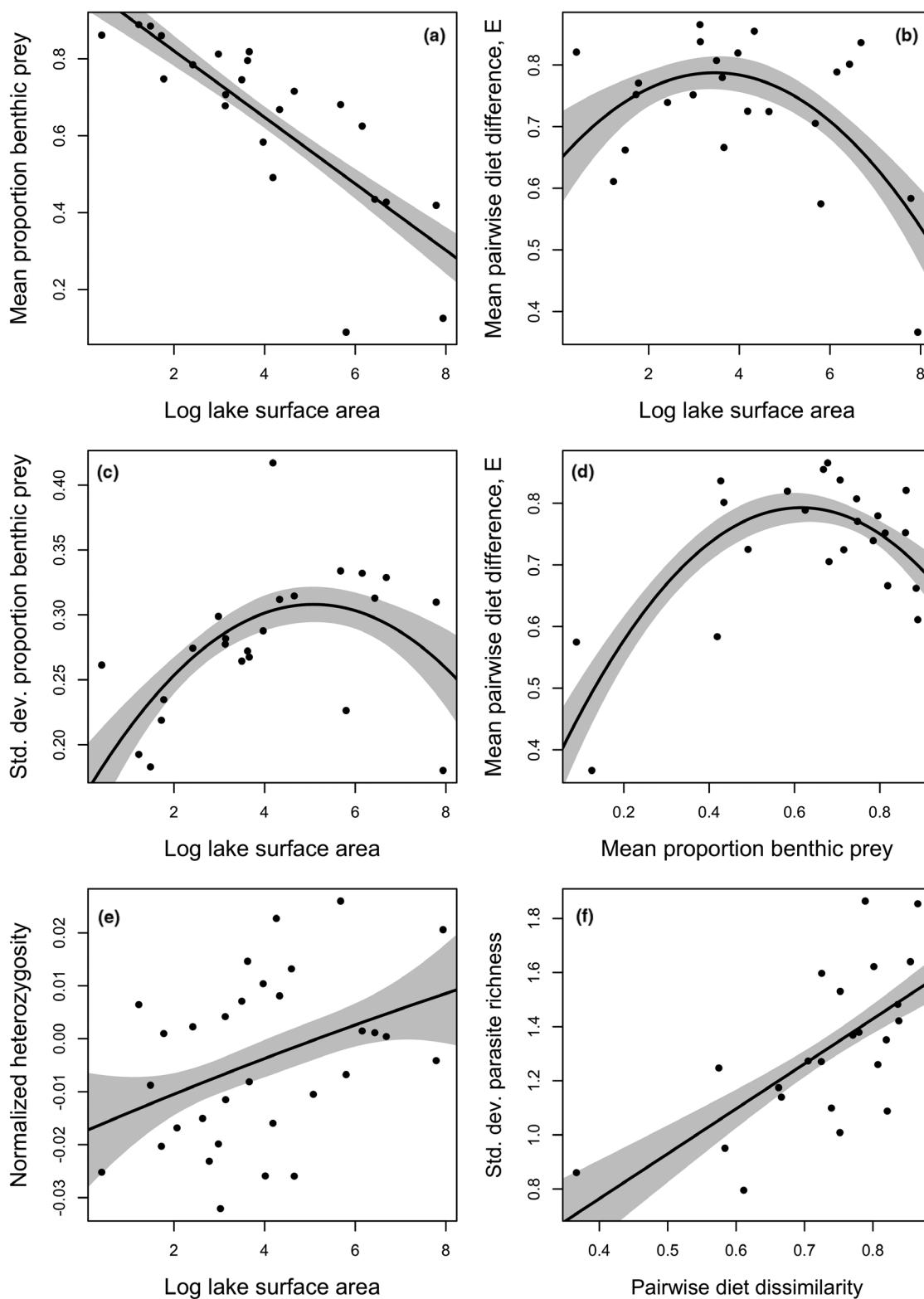
Figure 2 Linear and quadratic regressions examining predicted relationships between population mean diet (proportion benthic prey), pairwise diet dissimilarity (E), genetic diversity (heterozygosity), among-individual variation in per-fish parasite species richness, and lake area (log hectares). Points represent lakes as the level of replication. Lines are linear or quadratic regression estimates, shaded regions are one standard error confidence intervals. Statistical support for the trends reported here are provided in Table 1

Table 1 Results of linear and quadratic regressions between focal variables. Correlation tests (where the direction of causation is ambiguous) are reported only in the main text. Some models test *a priori* linear predictions, so quadratic effects are omitted. For each model, the table lists the figure where the relationship is plotted, the linear (and where relevant quadratic) slope estimate, its standard error, and a *P*-value testing the null hypothesis of zero slope. For each model we provide the r^2. NA denotes relationships not plotted as a figure

Figure	Dependent	Independent	Linear effect	Linear SE	Linear P	Quadratic effect	Quadratic SE	Quadratic P	r^2
2a	Mean proportion benthic prey	Log lake surface area	**−0.086**	**0.013**	**<0.0001**	–	–	–	0.66
2b	E	Log lake surface area	**0.084**	**0.040**	**0.0481**	**−0.012**	**0.004**	**0.0140**	0.36
2c	SD proportion benthic prey	Log lake surface area	**0.058**	**0.019**	**0.0066**	**−0.006**	**0.002**	**0.0159**	0.35
2d	E	Mean proportion benthic prey	**1.540**	**0.325**	**0.0001**	**−1.25**	**0.31**	**0.0005**	0.58
2e	Heterozygosity	Log lake surface area	**0.0003**	**0.001**	**0.0297**	$4.9e^{-5}$	$6.5e^{-4}$	0.939	0.15
2f	SD per fish parasite richness	E	**1.662**	**0.406**	**0.0005**	–	–	–	0.44
S1	Individual diet breadth	Log lake surface area	0.056	0.132	0.677	−0.006	0.015	0.651	0.01
S2	Individual diet breadth	E	0.221	0.581	0.707	–	–	–	0.01
NA	Individual diet breadth	Mean proportion benthic prey	−0.245	1.336	0.857	0.184	1.258	0.885	0.00
S3	E	Heterozygosity	−1.456	1.79	0.425	–	–	–	0.03
S5	SD standard length	Log lake surface area	1.42	0.716	0.0567	**−0.197**	**0.081**	**0.0217**	0.21
S6	SD size-adjusted gape width	Log lake surface area	0.015	0.008	0.0675	**−0.002**	**0.001**	**0.0495**	0.13
NA	SD size-adjusted gill raker length	Log lake surface area	0.051	0.067	0.451	−0.009	0.007	0.241	0.11
NA	SD gill raker number	Log lake surface area	0.005	0.100	0.963	0.003	0.011	0.777	0.05

Bold values are significant at *P* < 0.05.

within 100 kb of *sv2a*. A site on linkage group 1 (site 7 503 783; *P* = 0.000092) sits between *cluha* and *tlcd2*. For each of these top associations, the allele frequency ranged from 0 in some lakes (fixed for the reference genome nucleotide) to 1.0 in other lakes, thus spanning the full range of possible allele frequencies.

For the subset of SNPs with possible correlations between allele frequency and lake size (*P* < 0.1), we then tested whether each SNP's polymorphism (allele frequency variance, $p[1 − p]$) is (1) quadratically related to lake size, or (2) linearly increasing with diet variation (*E*). Focusing first on relationships with lake size, LG1 site 17 861 594 is significantly more polymorphic in intermediate-sized lakes (quadratic *P* < 0.00001) and survives multiple test correction (*q* = 0.0373). This SNP is more than 100 kb from the nearest gene. LG8 site 1 538 664 is also most diverse in intermediate-sized lakes (quadratic *P* = 0.00003; *q* = 0.056). This SNP is close to two coding genes of unknown function, and aldo-keto reductase family 1 member A1b. Other SNPs were most polymorphic in lakes with the greatest diet variation. Both LG4 SNPs mentioned above exhibit positive linear correlations with *E* (*P* = 0.036 and 0.010 respectively), as does the LG20 site listed above (*P* = 0.014), and LG3 site 3 270 353 (*P* = 0.000004; *q* = 0.018). None of these genes has obvious adaptive function in the context of diet variation.

Morphological variation

There was no linear correlation between individual specialisation and the standard deviation of any single phenotypic trait (standard length, *r* = 0.178, *P* = 0.414; size-adjusted gape width *r* = 0.327, *P* = 0.128; size-adjusted gill raker length *r* = 0.137, *P* = 0.532; gill raker number *r* = −0.004, *P* = 0.985). However, some traits were more variable in

intermediate-sized lakes: both standard deviation body length (Fig. S5) and size-adjusted gape width (Fig. S6) were greater in midsized lakes. The standard deviations of gill raker number and size-adjusted gill raker length were unrelated to lake area (Table 1). The most noteworthy correlation between individual specialisation and population phenotypes actually involved parasitism. As described elsewhere (Bolnick *et al.*), we also enumerated parasite infection loads for all sampled fish. Populations with greater individual specialisation exhibited greater among-individual variation in per-fish parasite richness (Fig. 2f, *r* = 0.666, *P* = 0.0005).

DISCUSSION

Many theoretical models suggest that increased resource diversity (a.k.a. ecological opportunity) leads to the evolution of a polymorphic generalist consumer (Levene 1953; Wilson & Turelli 1986; Rueffler *et al.* 2006). These models invoke niche expansion arising via greater among-individual variation rather than greater individual niche width (Van Valen 1965). Our comparative study confirms this expectation: we first confirmed that stickleback in intermediate-sized lakes had the most even mixture of benthic and limnetic prey (e.g. the greatest diet breadth). These generalist populations exhibited the greatest among-individual variation in diet and morphology. Individual diet breadth, in contrast, did not covary with lake size or population diet breadth. While we cannot yet tell to what extent this within-population variation is genetic versus plastic (see below for more discussion of this), these results support the adaptive hypothesis that ecological opportunity promotes variation. In contrast, we found no support for a neutral hypothesis of individual specialisation. Larger lakes tended to support greater population genetic diversity (heterozygosity), consistent with neutral theory's expectation

that large population size reduces drift and thus supports more variation. However, both dietary and phenotypic variation was unrelated to population heterozygosity. Importantly, this result implies that ecological and genomic variation are promoted in different kinds of habitats (intermediate and larger lakes, respectively).

Evidence for adaptive within-population diversification

Our results are consistent with, and build upon, previous work on diet in stickleback. Prior studies have shown that, like many lake fish, stickleback consume a mixture of benthic and limnetic invertebrates (Lavin & McPhail 1985; Lavin & McPhail 1986; Schluter & McPhail 1992; Matthews *et al.* 2010; Snowberg *et al.* 2015). These same studies point out that sticklebacks' relative intake of limnetic : benthic prey increases with lake size, because of the increasing ratio of open water (supporting plankton) to shallow lake perimeter (benthic prey). In this study, we confirmed this relationship ($r^2 = 0.66$ for a positive negative relationship between the proportion benthic prey, and log lake size; equivalently the proportion of limnetic prey increases with lake size). In the intermediate-sized lakes in our sample, we confirmed that stickleback populations consume equal proportions of benthic and limnetic prey.

In principle this broader population niche breadth could arise via increased individual diet breadth. But this is not what we observed: individual diet breadth was unrelated to lake size and population mean diet. The apparent invariance of individual diet breath agrees with experiments showing that individual niche breadth was insensitive to inter or intraspecific competition (Svanbäck & Bolnick 2007b; Araújo *et al.* 2008; Bolnick *et al.* 2010). Individual niche widths may be constrained due to biomechanical or cognitive trade-offs that limit individuals' capacity to efficiently use all potentially suitable available prey (Robinson 2000). Given such trade-offs, theory suggests that ecological opportunity can lead to disruptive selection, favouring increased diet diversity. This disruptive selection indeed occurs in lake stickleback (Bolnick 2004) and is most common in intermediate-sized lakes (Bolnick & Lau 2008). But, until now it remained unclear whether that disruptive selection actually led to greater trait diversity. Our present results therefore add a key piece of evidence, showing that these intermediate-sized lakes support the most functionally diverse populations of stickleback, as measured by diet variation (E), body size variation, and gape width variation. Together with previous evidence for disruptive selection in these lakes (Bolnick & Lau 2008), we conclude that our data support an adaptive explanation for lake-to-lake differences in among-individual diet and trait variation.

One previous study tested for a relationship between morphological variation and lake size. Nosil & Reimchen (2005) found a positive, not quadratic, relationship between body shape variance and lake size in stickleback populations. Their sample was drawn from mostly smaller islands elsewhere in coastal British Columbia. They used lake volume rather than area, so we cannot directly compare our results. However, the islands they surveyed contain only relatively small to intermediate-sized lakes, excluding the very large (> 1000 ha) lakes included in this study. Therefore, our contrasting conclusions are actually mutually consistent, because their positive correlation between lake size and trait variation matches the left half of our quadratic trend.

A neutral model of diet diversity is not supported

In principle, diet and phenotypic variation among individuals might be a non-adaptive consequence of neutral genetic variation. Across the genome, the vast majority of variable loci should have no direct effect on fitness, and should evolve approximately neutrally. Population genetic neutral theory leads us to expect greater heterozygosity where (effective) population size is larger. Larger lakes should support larger census population sizes, and we see a corresponding positive correlation between lake size and heterozygosity, matching previous results based on a more limited survey with microsatellite markers (Caldera & Bolnick 2008). Thus, genomic diversity is maximised in different lakes than diet variation.

Given this difference, it is perhaps unsurprising that we find no association between genome-wide diversity and any measure of diet or trait variation. This negative result illustrates an important point: genome-wide diversity, which is generally expected to be nearly but not completely neutral (Kern & Hahn 2018), is a poor guide for functional diversity. This conclusion serves as a cautionary example for conservation biologists who might seek to use neutral genetic marker diversity as a proxy for functional diversity, which likely matters far more for population persistence.

Heritable or plastic variation?

The phenotypes of wild organisms, such as we sampled for this study, can vary due to both their disparate genotypes and environments (e.g. plasticity). We do not presently know to what extent the trait and diet variation within each lake is heritable or plastic. Past studies provide circumstantial evidence. Diet variation among individuals changes quickly in response to experimental manipulations of competitors, demonstrating a role of behavioural plasticity (Svanbäck & Bolnick 2007b; Bolnick *et al.* 2010). But, we also know that these foraging decisions are conditional on individuals' trophic morphology (Araújo *et al.* 2008), which we know to be partly heritable based on common-garden rearing (Robinson 2000), quantitative trait locus mapping (Arnegard *et al.* 2014), and genome-wide association studies (GWAS; Rennison *et al.* 2019). In our own GWAS analysis, we identified a few genetic markers that are likely to be linked to genes contributing to heritable trait and diet variation. We found loci whose allele frequencies were correlated with lake size, and whose genetic variance was either positively related to diet variation, and/or maximised in intermediate-sized lakes (for more discussion of the nature of these candidate loci, see Supplementary Text A). Some of these SNPs are in close proximity to potentially relevant genes involved in cognition and memory (*foxp2*, *gpr85*), immunity (*ifrdl*, *bola1*) and jaw development (*nr2F5*). Any role of these genes in adaptation to benthic versus limnetic diets, and resource polymorphism, would

have to be confirmed with further genetic mapping and transgenic experiments. Nevertheless, our GWAS results are enough to make us confident there is a heritable component to the variation we document here, though the mechanism remains uncertain. We strongly suspect that the trait and diet variation is also likely to involve behavioural and morphological plasticity, and genotype-by-environment interactions. These GxE interactions might amplify the effects of genetic variance, or might undercut it (e.g. counter-gradient variation).

Implications

Among-individual diet variation can have appreciable effects on ecological dynamics and community composition (Bolnick *et al.* 2011). Experiments show that prey community structure changes depending on the amount of diet variation among stickleback within populations (Ingram *et al.* 2011), between interbreeding populations, or between incipient species (Harmon *et al.* 2009; Matthews *et al.* 2016). Co-occurring individuals with different diets experience different levels of intraspecific competition (Bolnick 2004). Individuals also have different overlap with other species of fish such as trout and sculpin, common intraguild predators on stickleback (Bolnick *et al.* 2010). Thus, we propose that the populations examined here are likely to differ in their sensitivity to interspecific competitors, because greater diet variation means that a smaller subset of individuals overlaps (albeit more strongly) with a given competitor.

Our data indicate that populations with greater diet variation also exhibited greater among-individual variation in parasite richness. Many stickleback parasites are trophically transmitted, so individual differences in diet should yield different infections (Stutz *et al.* 2014). Consequently, the level of individual specialisation will have wide-reaching effects on the composition and diversity of the parasite metacommunity within each lake.

Some theoretical models of sympatric speciation suggest that disruptive selection and niche diversification are initial steps towards the evolution of reproductive isolation. A reasonable question, therefore, is what our results imply about speciation. In particular, are the six lakes in British Columbia that contain pairs of mostly reproductively isolated species (Schluter & McPhail 1992), found in the intermediate-sized lakes where the opportunity for diversification is greatest? The species pair lakes range from 6.7 ha to as large as 44.3 ha (average log surface area of 2.63; Vamosi 2003). These are on the smaller (more benthic) end of the spectrum of the lakes surveyed here. The species pairs are thus not in the lakes with the greatest ecological opportunity (by our measure), at least in the present-day. This finding is consistent with previous studies suggesting that some additional force is responsible for speciation in this handful of lakes (e.g. predation, double-colonisation events, etc; Vamosi 2003; Rundle *et al.* 2003; Bolnick 2011).

Conclusions

Population genetics (Levene 1953; Wilson & Turelli 1986), adaptive dynamics (Doebeli 1996b; Ackermann & Doebeli 2004), and quantitative genetic eco-evolutionary models (Schreiber *et al.* 2011) all suggest that resource diversity can promote within-population variation. A simple corollary is that when there exists a gradient in the ratio of two resources (e.g. benthic : limnetic availability), individual specialisation should be greatest in the middle of the gradient, where the resources are most evenly balanced. The results presented here represent the first test of this theory, confirming that individual specialisation (and some facets of morphological variation) are greatest in intermediate-sized lakes where stickleback populations are generalists using both benthic and limnetic prey. The implication of this finding is that certain geographic settings are more favourable to resource polymorphism. This fits into a broader emerging literature supporting the notion that ecological opportunity promotes variability within populations (Parent & Crespi 2009; Martin & Pfennig 2010; Araújo & Costa-Pereira 2013; Evangelista *et al.* 2014; Cloyed & Eason 2016; Yurkowski *et al.* 2016; Costa-Pereira *et al.* 2017). These shifts in individual specialisation should have cascading effects on prey, competitor, and parasite community structure (Des Roches *et al.* 2018). Our results imply that these ecological impacts of individual specialisation will be strongest in particular kinds of habitats, and these habitats are predictable. Moreover, in the case of lake stickleback, these diet-diversity promoting habitats are not the same places that promote neutral genetic diversity.

AUTHORSHIP

KB collected the data. DIB analysed the data and wrote the manuscript, with editorial feedback from KB.

DATA AVAILABILITY STATEMENT

The data supporting the results of this paper have been archived in a public repository (Dryad): https://doi.org/10.5061/dryad.gmsbcc2j1. Raw sequence reads were previously archived by Stuart *et al.* (2017) Nature Ecology and Evolution https://doi.org/10.1038/s41559-017-0158.

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