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Predictive covariation among trophic, isotopic, and genomic traits is consistent with intrapopulation diversifying selection

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ABSTRACT

Questions: To what extent does intra-sex variation in size-corrected feeding structures covary with stable isotopic values and genotype within threespine stickleback populations?

Hypothesis: Phenotype, isotopic niche space, and genome-wide markers of fish will covary.

Field site: Boulton Lake, a small shallow bog lake on Graham Island, Haida Gwaii, western Canada.

Organism: An endemic population of sexually dimorphic threespine stickleback (*Gasterosteus aculeatus*).

Methods: We sampled 225 male stickleback from 11 microsites on a lake-wide grid that differed in water depth and substrate (mud, sand, hardpan). The fish were scored for: (1) trophic morphology (jaw length, eye size, gill raker length, inter-raker distance); (2) stable isotope signatures (δ^{15}N, δ^{13}C) of muscle tissue; and (3) genome-wide, single nucleotide polymorphisms (SNP) (3072 marker microarray). We reduced all data using principal component analysis (PCA). We compared distance matrices among data sets using Mantel *r*-tests. To visualize the genetic distances among individuals, we also computed unrooted, neighbour-joining trees from the SNPs.

Results: The first three principal components of trophic morphology (PCtroph) accounted for 85% of the variance, with highest loadings for +eye and +gape on PC1, +inter-raker distance and +raker length on PC2, and +raker length and −inter-raker distance on PC3. PC1troph was significantly positively correlated with δ^{15}N and this occurred at each of the three habitat types. Euclidean distance matrices of individuals of PC1troph against PC2troph were correlated with Euclidean distances for δ^{15}N against δ^{13}C arrays. We extracted 25 principal components from the informative SNPs, which accounted for 30% of the total genetic variance. Four principal components, with most SNPs on Chromosomes IV, VIII, and XII, were significantly associated with trophic phenotype variation. Neighbour-joining trees on genomic data yielded branches. These differed in their position in dual isotopic niche space including the extent of dietary specialization.

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Conclusions: The cumulative data indicate that size-corrected phenotypic variation in trophic morphospace represents a combination of niche specialists and generalists with phenotypic outliers concordant with isotopic outliers. These data are largely consistent with diversifying selection for maintenance of intrapopulation, adaptive variation.

Keywords: diversifying selection, ecogenomics, *Gasterosteus aculeatus*, Haida Gwaii, individual niche, intrapopulation variation, stable isotopes, stickleback.

INTRODUCTION

The conceptual framework for analysing the maintenance of genetic variability within populations is largely anchored in mid-twentieth-century population and ecological genetic theory (Ford, 1964). Heterozygote advantage and gene flow continue to provide the major theoretical and empirical mechanisms for the maintenance of this variability, although spatially and temporally heterogeneous (diversifying) selection can broaden the fitness curve among phenotypes and ease the constraints on maintenance (reviewed by Futuyma, 2013). Diversifying selection, initially modelled by Levene (1953) as a 'multi-niche hypothesis', was subsequently framed in an ecological context by Van Valen (1965). Simply stated, each phenotype/genotype in an interbreeding population has elevated fitness in a unique niche space (e.g. dietary or predatory niche axes) and the relative frequencies of the genotypes/phenotypes will reflect the relative proportion of available niche space. While this has received empirical support in intrapopulation variation in diverse habitats including those of Galapagos finches (Grant and Price, 1981; Grant and Grant, 1989), Caribbean anoles (Losos, 1990), and intertidal littorines (Reimchen, 1979, 1989), broad application of the niche-width models has been limited partly because of the difficulty in identifying individual niche space. This remains a greatly understudied topic relative to its importance in the interface between phenotypes and ecology (Bolnick *et al.*, 2003; Layman *et al.*, 2015), and has become particularly relevant given the demographic and genetic bottlenecks that characterize an increasing number of natural populations in fragmented habitats (Violle *et al.*, 2012).

Stable isotope signatures of animal tissues provide a valuable recent proxy for identifying individual trophic niches, as these integrate long-term rather than short-term diet (Chisholm *et al.*, 1982; Hobson *et al.*, 1994; Post, 2002; Bearhop *et al.*, 2004; Fry, 2006). $\delta^{15}N$ values increase approximately 3‰ for each trophic level (Minigawa and Wada, 1984; Vander Zanden and Rasmussen, 1999). $\delta^{13}C$ values, while relatively conserved across trophic levels, provide insight into different carbon sources in the diet. For example, in freshwater lakes, fish and zooplankton become enriched across a gradient from limnetic to littoral zones (France, 1995). Such dual-isotope proxies are particularly useful in studies of intrapopulation variation, as these allow quantification of phenotype-specific niche space (Newsome *et al.*, 2012).

In 1970, a long-term study to test the adaptive variation model was initiated on threespine stickleback (*Gasterosteus aculeatus*) from Boulton Lake, Haida Gwaii, western Canada. This endemic population exhibited a polymorphism for the presence or absence of the pelvic girdle and variability in the dorsal spines (Moodie and Reimchen, 1976). Subsequent study showed that these morphs differed between the sexes and in their spatial distribution in the lake (Reimchen, 1980). In addition, the sexes differed in their diet and in their parasite assemblages (Reimchen, 1982, 1997; Reimchen and Nosil, 2001a, 2001b). Yearly shifts in the morph frequencies occurred with shifts in the habitat and predation regime that altered relative fitness of

the morphs (Reimchen and Nosil, 2004). Competition between the sexes allowed differentiation in niche space and facilitated diversifying selection for dimorphism in this population.

Here, we extend the assessment of this population and ask whether individual morphometric variability in trophic structures within a sex is associated with isotopic variability and whether either of these attributes has any association with genomic markers. Because of the large habitat differences between the sexes in this and in other populations of stickleback (Reimchen *et al.*, 2016), we restrict our analyses to males. If males are composed entirely of dietary generalists, then we predict that size-corrected intrapopulation variability for foraging traits such as gape, eye size, and gill rakers will not be systematically correlated with isotopic niche space, as most fish will converge towards the average isotopic values. In contrast, if some or many of the male stickleback are dietary specialists, then we predict that individual variation in these trophic structures will be statistically associated with distinctive isotopic values and that increased phenotypic distances from the population average will be directly correlated with increased Euclidean isotopic distances from the average isotopic values (geometric centre). This is the first assessment of individual trophic variability within a sex in this population and integrates phenotypes, isotopes, and genotypes.

METHODS

Field data for Boulton Lake were obtained in June 2012 and June 2013. General site description is available in Reimchen (1980). To ensure that we were sampling representatives from the entire population, we set traps at eight littoral and three limnetic sites across the lake (Fig. 1). At each site, we recorded water depth and substrate (mud, sand/gravel, hardpan). In total, 225 male stickleback (average of eight per site per year) were collected (under ACC permit: 2014-003-2, University of Victoria, British Columbia). Fish were anaesthetized (Clove oil) and preserved in 95% ethanol.

Each fish was sexed and processed for morphometrics: standard length (SL), body depth, gape length, eye diameter, gill rakers (bone length, cartilage length for rakers at positions 2, 3, and 4 on the lower arch, basal distances between gill rakers at positions 2/3, 3/4, and 4/5 as numbered from the ventral edge of the lower arch). For each fish, we used the mean raker bone length and the mean raker distance of the three separate measurements for each trait. We counted number of gill rakers on the lower and upper arches but because of the poor repeatability in total counts, we excluded number of rakers from the analyses. A one-centimetre section of trunk musculature beneath the dorsal fin was excised from each fish, dried, powdered, packaged, and processed for carbon and nitrogen stable isotopes at the Mass Spectrometry lab (UC Davis). We did not perform any lipid extraction of the muscle tissue. Standard notations of delta values ($\delta^{13}C$ and $\delta^{15}N$) relative to Pee Dee Belemnite for carbon and atmospheric nitrogen are used. Caudal fins were clipped for DNA extraction and analysed as described in Jones *et al.* (2012) on 3072 genome-wide SNP arrays. Approximately 525 single nucleotide polymorphisms (SNPs) distributed across the genome were polymorphic and used for analysis (10 to 45 per chromosome). Chromosome number, SNP position, and designations are given in the Appendix (evolutionary-ecology.com/data/3173Appendix.pdf). Deficiency in tissue mass for isotopic signature in several fish as well ambiguity in SNP calls reduced the total sample size of stickleback to 217.

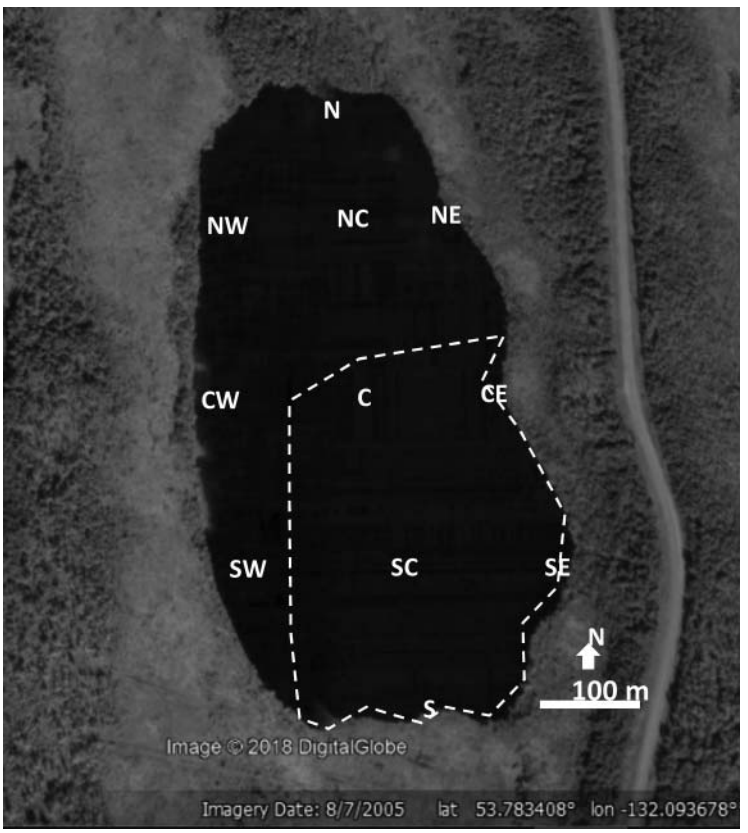

Fig. 1. Boulton Lake, Haida Gwaii, western Canada and locations of the sampling sites. Dashed line approximates area of mud substratum.

Statistical analyses

All statistical analyses were carried out using SPSS v.24 and R (package *Ecodist*). Data for trophic metric traits were body-size-corrected using standardized residuals extracted from a linear regression of each trait (jaw, eye, raker length, inter-raker distance) against SL. We also body-size-corrected carbon and nitrogen isotope values, as these varied with SL. We reduced the variables with principal components (PC). For phenotypic traits, we used the standardized residual values and extracted three PCs (PCtroph). For genotypic traits, we extracted 25 principal components from the polymorphic SNPs (PCsnp). We excluded from the PC analyses any fish with fewer than 400 SNP calls. The extent of genetic clustering was evaluated from the SNP data using R (package *ape*) and STRUCTURE 2.3.4 (Pritchard *et al.*, 2000). Unrooted neighbour-joining trees with cluster identity was used to visualize associations with isotopic niche space. We removed confounding effects of microsite differences on individual isotopic signatures using standardized residuals. For STRUCTURE analyses, we initially used all SNPs in the analyses but subsequently restricted these to the 48 top loading SNPs identified in principal component analysis. We assume no admixture and no *a priori* assignments of individuals to any cluster. In both sets

of analyses with an input of 2–15 clusters, the minimum ln likelihood usually identified four or five clusters over a diverse range of runs (minimum burn in 5000, 5000 to 20,000 Markov chain Monte Carlo runs, three replicates) and we used the minimum number ($K = 4$) for our results. STRUCTURE outputs including cluster membership and F_{st} values are shown in Appendix 2. Comparisons among sites for PCtroph and individual trait values were assessed with analysis of variance (ANOVA) and *post-hoc* tests. Correlations between PCtroph and isotopic values were tested with Pearson's *r* when the data were normally distributed or Spearman's rho for data that departed from normality. We computed Euclidean distance matrices among individuals (partitioned for year) for multiple traits and compared matrices with Mantel tests.

RESULTS

The first three principal components on the four metric trophic traits accounted for 85% of the variance, with highest loadings for +eye and +gape on PC1troph, +inter-raker distance and +raker length on PC2troph, and +raker length and –inter-raker distance on PC3troph (Table 1). Multiple associations occurred among trophic phenotypes and stable isotope signatures but the extent of association varied between years (Table 2). PC1troph was significantly positively correlated with $\delta^{15}N$ in both years and inversely correlated with $\delta^{13}C$ in 2012. PC2troph was inversely correlated with $\delta^{15}N$ in both years and positively correlated with $\delta^{13}C$ in 2012, while PC3troph was inversely correlated with $\delta^{15}N$ in 2012. These pooled samples from all sites show concordance within each of the three sub-areas: south littoral, north littoral, and limnetic (Fig. 2). That is, in different localities in the lake, fish with

Table 1. Principal component scores on residuals of metric traits for 229 male stickleback from Boulton Lake

	PC1troph (35%)	PCtroph (27%)	PC3troph (22%)
Gape	0.731	0.320	0.350
Eye size	0.817	–0.077	0.118
Gill raker length	–0.448	0.587	0.631
Inter-raker distance	0.117	0.787	–0.601

Note: Percentage of variance explained by each principal component is shown in parentheses.

Table 2. Association between first three principal components of trophic traits and residual values for stable isotopes for Boulton Lake stickleback

	2012			2013		
	PC1troph	PC2troph	PC3troph	PC1troph	PC2troph	PC3troph
$\delta^{15}N$	0.27**	–0.30**	–0.27**	0.47**	–0.23*	–0.18
$\delta^{13}C$	–0.28**	0.21*	0.17	0.07	0.05	0.08

Note: PC1troph: +z-eye, +z-gape; PC2: +z-inter-raker distance; PC3: +gill raker length, –inter-raker distance. Shaded cells indicate statistically informative correlations (Spearman's rho).

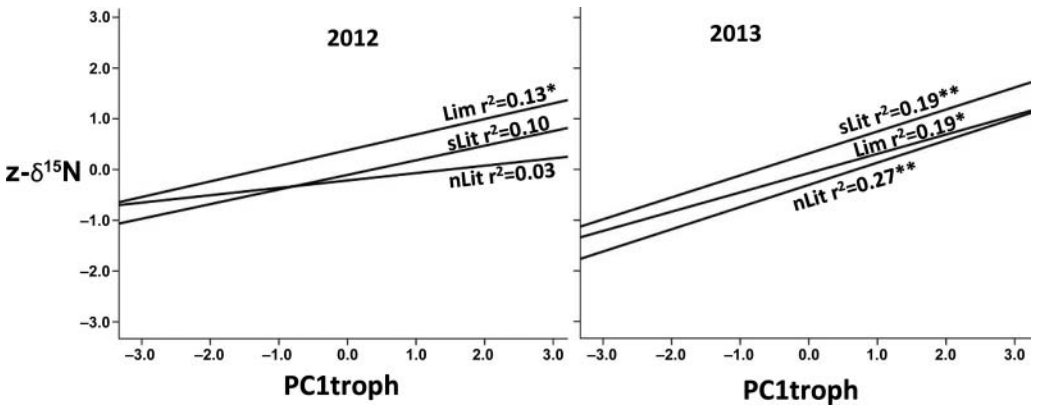

Fig. 2. Nitrogen isotope residual values (z-δ^{15}N) against PC1troph (+eye, +gape) for male stickleback by year and general lake habitat. Regression lines show explained variance and significance for south littoral sites (sLit), limnetic sites (Lim), and north littoral sites (nLit).

elevated PC1troph values (+eye, +gape) had elevated δ^{15}N values, the trends being more highly expressed in 2013 relative to 2012. We also compared (Mantel *r*) individual distance matrices and found the Euclidean distances between PC1troph and PC2troph to be positively correlated to increased Euclidean distances between δ^{15}N and δ^{13}C (2012: $r = 0.11$, $P < 0.02$; 2013: $r = 0.22$, $P < 0.001$).

Three measures of genomic data were examined: (1) genetic distance matrices against geographical distances among sites; (2) principal components of SNPs against trophic phenotypes; and (3) SNP-derived neighbour-joining trees and cluster identity against isotopic niche space. No significant correlations (Mantel *r*) were observed between genetic distances and geographical distances ($P > 0.24$ in both years). The 25 principal components derived from the SNPs accounted for about 30% of the total variance (maximum of 2.4% for PC1). Multiple significant correlations ($P < 0.05$) were identified among PCsnps, trophic traits, and isotopic values (Table 3). PC2snp, PC3snp, and PC7snp were each associated with trophic phenotype variation and had SNPs mainly on Chromosomes IV, VIII, and XII.

Variable numbers of genetic clusters were extracted from the SNP data. Using all SNPs ($N = 567$), unrooted neighbour-joining trees derived from R (package *ape*) yielded 14 clusters that varied in membership and genetic distance (Fig. 3a). To visually determine whether individual branches were associated with isotopic niche space, we overlaid cluster identity for each individual on the dual isotope plot that was partitioned into four categories along a trophic level gradient on the y-axis (δ^{15}N) and limnetic to littoral gradient on the x-axis (δ^{13}C) (Fig. 3b).

The number of individuals within any single cluster was small in most cases and was distributed throughout isotopic space. However, the two largest clusters (10 and 14) were not randomly distributed with respect to each other. Cluster 10 seems to be more prevalent at lower trophic rather than at higher trophic levels (19 vs. 9), while Cluster 14 shows the opposite (8 vs. 17; $\chi^1_2 = 6.8$, $P < 0.01$). Of the four clusters identified in STRUCTURE, there was also an association in cluster identify and ranked isotopic niche space for both the

Table 3. Pearson's correlation among the first 25 PCsnps, trophic phenotypes, and isotopes

PCsnp	Trait	*R*	High loading SNPs (Chromosome)
PC2snp	PC3troph	0.22**	119 (VIII), 918 (VIII), 1028 (VIII), 1711 (VIII), 2011 (VIII), 2068 (VIII)
PC3snp	PC3troph	–0.261**	211 (IV), 297 (IV), 879 (IV), 1157 (IV), 2257 (IV), 2698 (XVII)
PC7snp	PC1troph	–0.14*	2 (VIII), 2330 (XII), 2843 (VIII)
PC7snp	PC2troph	–0.19*	2 (VIII), 2330 (XII), 2843 (VIII)
PC7snp	PC3troph	0.15*	2 (VIII), 2330 (XII), 2843 (VIII)
PC19snp	PC2troph	0.18**	1077 (XII)
PC2snp	z-grbl	0.22**	119 (VIII), 918 (VIII), 1028 (VIII), 1711 (VIII), 2011 (VIII), 2068 (VIII)
PC3snp	z-grbl	–0.18**	211 (IV), 297 (IV), 879 (IV), 1157 (IV), 2257 (IV), 2698 (XVII)
PC4snp	z-grbl	0.14*	120 (un), 124 (XIV), 200 (XVIII), 211 (IV), 296 (IV), 306 (VI), 683 (un), 970 (XVIII), 1157 (IV)
PC7snp	z-gape	–0.14*	2 (VIII), 2330 (XII), 2843 (VIII)
PC7snp	z-grdist	–0.25**	2 (VIII), 2330 (XII), 2843 (VIII)
PC19snp	z-grdist	0.18**	1077 (XII)
PC2snp	z-δ^{15}N	0.19**	119 (VIII), 918 (VIII), 1028 (VIII), 1711 (VIII), 2011 (VIII), 2068 (VIII)
PC5snp	z-δ^{13}C	0.18**	1173 (XX)

Note: PCsnps combined for years. Only significant ($P < 0.05$) values are shown. SNP position on each LG is shown in the Appendix. 'un' represents unassigned LG.
Traits: z-grbl, body-size-adjusted residuals of gill raker bone length; z-gape, body-size-adjusted residuals of gape length; z-grdist, body-size-adjusted distance between rakers; z-δ^{15}N, body-size-adjusted residuals for δ^{15}N; z-δ^{13}C, body-size-adjusted residuals for δ^{13}C.

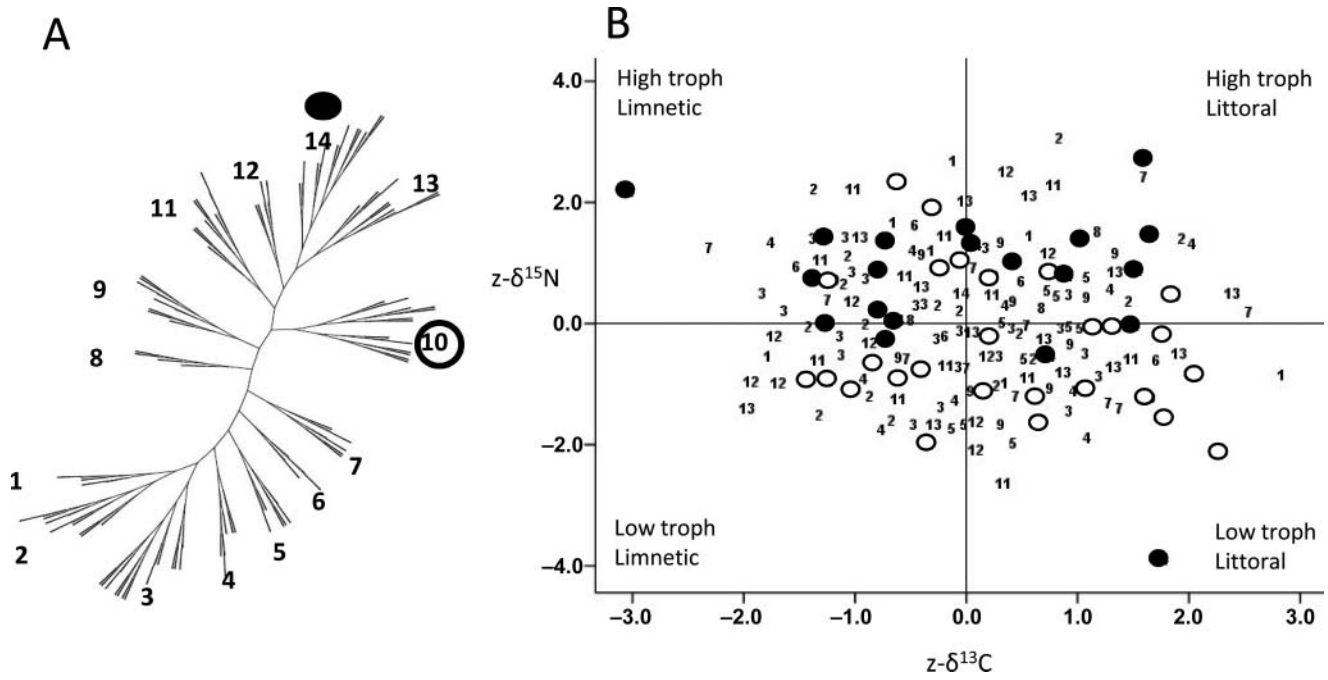

Fig. 3. Genetic data from 566 SNPs for Boulton Lake male stickleback ($N = 225$). (A) Unrooted neighbour-joining tree with assigned clusters. (B) Distribution of individuals in residual isotopic space with assigned genetic cluster. Clusters 10 and 14 are highlighted for comparison. Position in the scatterplot exemplifies on the y-axis relative trophic level and on the x-axis a gradient from limnetic to littoral habitat use. The geometric centre of the 0-intercepts represents the average isotopic niche space, while increased departures represent increased specialization of niche space.

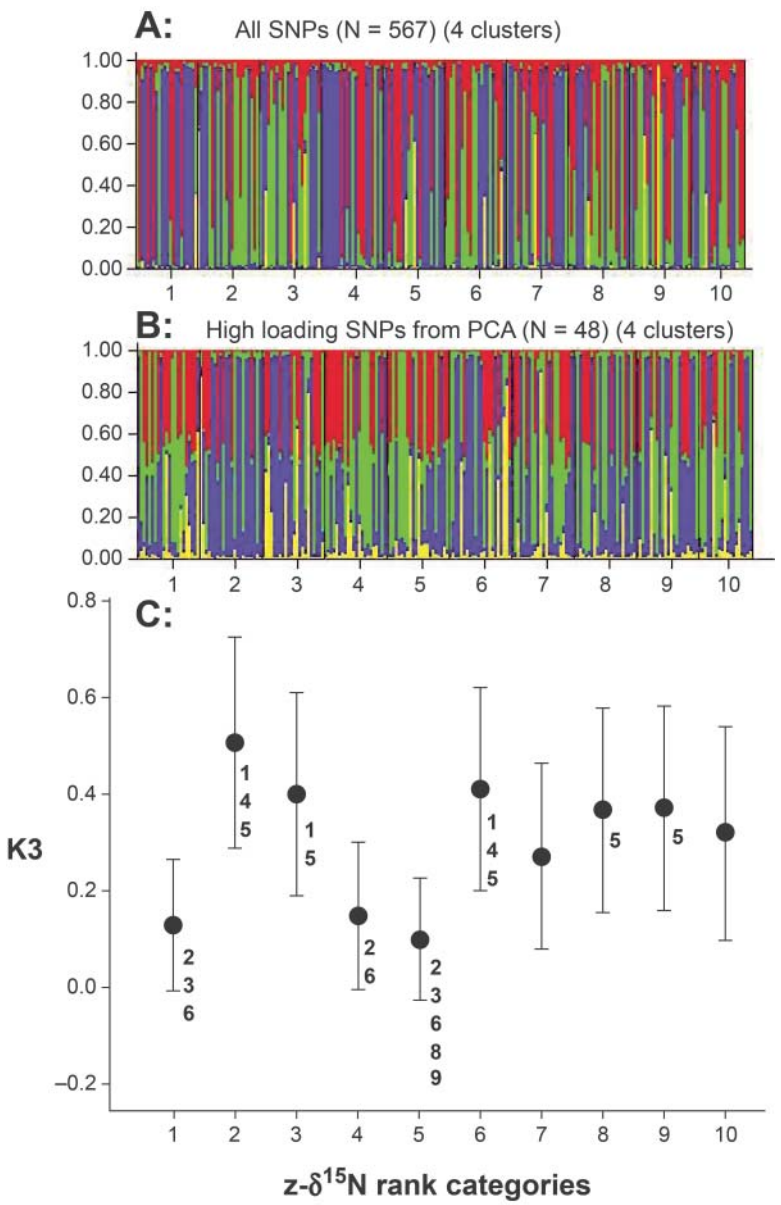

Fig. 4. Genetic cluster (K) membership for each of the 217 stickleback (from STRUCTURE) derived from SNP data in relation to rank of nitrogen isotope signatures as a proxy for trophic niche space. (A) Clusters derived from all SNPs ($N = 587$). (B) Clusters derived from the 48 SNPs that had the highest loading on the first 10 principal components. Each vertical line is an individual fish. Cluster colours are assigned at random among different bar charts. Single colours for an individual indicates 100% membership in that cluster. Mixed bars indicate mixed cluster membership. (C) Mean values (±2 S.E.) of Cluster 3 for nitrogen isotope signatures ranked from low to high as a proxy for trophic level position ($F_{9,207} = 1.95$, $P < 0.05$). Numbers adjacent to isotropic rank categories represent statistically significant differences ($P < 0.05$) of K3 membership (from *post-hoc* LSD tests) among other isotopic category means.

full SNP analyses ($N = 587$, Fig. 4A) and the subset of SNPs ($N = 48$, Fig. 4B) that had the highest loading on the principal components. Significantly depressed frequencies of the third cluster occurred in three of the five lowest ranks of $\delta^{15}N$ (Fig. 4C).

We examined whether Euclidean distances from the isotopic geometric centre (zero intercepts) of each individual varied with the mean distances among the genetic clusters as a proxy for whether individuals were dietary generalists or dietary specialists. These data, ranked for increasing Euclidean distance (Fig. 5), indicate weak but similar trends between years with major variance within each cluster. Overall comparisons of the clusters approach statistical significance ($F_{13,189} = 1.7$, $P < 0.06$; Year: $P > 0.8$), although LSD *post-hoc* tests yielded multiple significant differences among clusters. For example, Cluster 8 is close to the geometric centre (average niche space) and differs significantly from seven other clusters that exhibit increased departure from the population average isotopic niche space. We also examined the four clusters identified in STRUCTURE but found no significant associations with Euclidean distance (all $P > 0.3$).

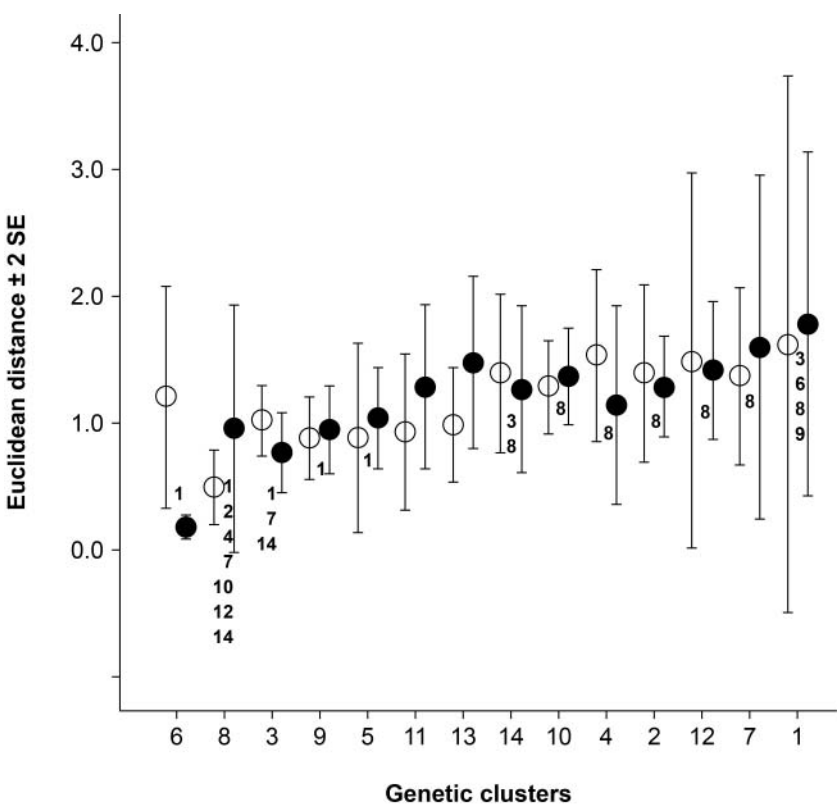

Fig. 5. Euclidean distances of Boulton Lake male stickleback in isotopic niche space from the geometric centre for genetic clusters. Increased distances indicate increased specialization of niche space. Numbers adjacent to mean Euclidean distance for different genetic clusters represent statistically significant differences ($P < 0.05$, *post-hoc* LSD tests) from other clusters. ○, 2012; ●, 2013.

DISCUSSION

Stabilizing and diversifying selection are antagonistic processes influencing natural populations (Hansen, 1997; for reviews, see Kingsolver and Pfennig, 2007; Futuyma, 2013). Stabilizing selection preserves an optimal or modal group of phenotypes with declining fitness functions at increasing distances from optimality. Alternatively, diversifying selection facilitates variation when peripheral phenotypes have the same or greater fitness than more common phenotypes. This is the basis for adaptive variation models (Van Valen, 1965; Grant and Price, 1981).

One of the common vectors of adaptive variation is the taxonomically widespread niche differentiation between the sexes (Butler *et al.*, 2007; Wearmouth and Sims, 2008; Nebel and Thompson, 2011; Dalu *et al.*, 2017). Previous studies of stickleback at Boulton Lake, Haida Gwaii showed substantial niche differentiation between the sexes, including ontogenetic and seasonal shifts in habitat use (Reimchen, 1980), and greater consumption of benthic prey by males and differences in the incidence of parasitic infection (Reimchen and Nosil, 2001a). Although this remains the major support for adaptive variation within this population, in the current study we extended the analyses to assess whether phenotypic and genotypic variation within a sex are also reflective of ecological functionality.

Recent reviews of adaptive variation models conclude that identification of phenotype-specific niches, while anchoring the discipline of ecological genetics, remains understudied relative to its importance in the interface between phenotypes and ecology (Bolnick *et al.*, 2003; Violle *et al.*, 2012; Layman *et al.*, 2015). Isotopic signatures of tissues, which are of particular value in assessing trophic variation, are a time-integrated proxy of diet, and therefore have the potential to identify unique isotopic niche space among phenotypes (Fry, 2006). In dual isotope space, individuals near the geometric centre of isotopic space could be highly specialized if they consumed prey which themselves tended to have average isotopic values; alternatively, individuals near the geometric centre could be generalist foragers when they consumed prey from throughout isotopic space such that multiple trophic levels and multiple carbon sources were averaged. However, isotopic values at increased radial distance from the geometric centre can only occur with increased dietary specialization (see also Bearhop *et al.*, 2004). These methods have become broadly used in eco-evolutionary studies of individuals in terrestrial and aquatic habitats (Bolnick *et al.*, 2002; Darimont *et al.*, 2007; Reimchen *et al.*, 2008; Bolnick and Paull, 2009; Reimchen and Klinka, 2017). Our data on trophic, isotopic, and genomic traits for Boulton Lake stickleback are broadly consistent with phenotype-specific niche specialization, as we observed that trophic morphospace is directly correlated with nitrogen and carbon isotope space. This supports diversifying selection as a proximal cause for variability in natural populations.

However, isotope–phenotype covariation within a population does not exclude the possibility that outliers may be competitively inferior and displaced to low-quality foraging habitats rather than representing a fine-tuned ecological coupling between phenotype and foraging diet. If phenotypic or isotopic outliers are nutritionally compromised, one would predict reduced body condition of such individuals. We did not quantify mass-to-length ratios, a common measure of condition in fishes (Lizama and Ambrósio, 2002) but we were able to test the prediction by evaluating carbon/nitrogen ratios (C/N) of muscle tissue, which can be proxies for condition factor in fishes (Wilder *et al.*, 2016). Higher C/N values in tissues are normally found with greater lipid content and indicate improved condition. We compared C/N values (raw data and residuals of C/N by SL) with isotopic Euclidean distances from

the geometric centre (average isotopic values), predicting a negative relationship if isotopic outliers are nutritionally compromised. This was not the case in either year (2012: $r = 0.15$, $P = 0.10$; 2013: $r = 0.09$, $P = 0.35$) and we infer that isotopic outlines are likely dietary specialists and comprise functional responses to peripheral niche space rather than representing sub-optimal and competitively inferior phenotypes at the edge of the fitness landscape.

The individual trophic differences we observed among male stickleback are probably reflective of both phenotypic plasticity and genetic effects. In fishes such as cichlids, sunfish, and stickleback, trophic traits such as jaw size and length of gill rakers can be substantially altered with differences in diet (Lindsey, 1962; Meyer, 1987; Hegrenes, 2001; Wund *et al.*, 2008). Transplant experiments of Haida Gwaii stickleback from a large lake where zooplankton dominated the diet to a small pond with benthic prey resulted in only minor changes in jaw length but a 40% reduction in gill raker length within one generation, consistent with an important contribution of functional plasticity for gill raker length (Leaver and Reimchen, 2012). Multiple genetic crosses in stickleback show evidence for heritable effects, including jaw size and gill raker number (Gross and Anderson, 1984; Berner *et al.*, 2014; Lucek *et al.*, 2014; Miller *et al.*, 2014). In addition, quantitative trait loci (QTL) studies show that variation in short and long gill rakers map to multiple chromosomes, including Chromosomes I, III, IV, VI, and XX (Miller *et al.*, 2014; Conte *et al.*, 2015) while variation in head foraging traits map to Chromosomes IV, V, VII, VIII, X, and XXI (Miller *et al.*, 2014; Peichel and Marques, 2017). Our data for Boulton Lake male stickleback indicate that SNPs on Chromosomes IV, VII, and XXII exhibit significant associations with individual trophic traits and with principal components of these traits, including isotopic values suggestive of a genomic basis to the covariation between phenotype and habitat in this population.

Neighbour-joining trees based on distance matrices for SNPs from Boulton Lake male stickleback identified up to 14 branches differing in their extent of genetic distance from each other. We assume that the genetic distances between the branches could also indicate phenotypic distances in multiple traits, including trophic morphology, body shape, and behaviour; variation in each of these traits maps to multiple loci throughout the genome (Peichel and Marques, 2017). Our primary interest in using neighbour-joining trees was to assess whether these branches or clusters differed in any ecological attributes such as niche space. We observed that the frequency of each genetic cluster varied across the isotopic landscape and although numbers in each cluster were small, our comparisons demonstrated significant differences in their trophic levels, indicative of long-term niche differentiation among clusters. STRUCTURE analyses suggested that four clusters captured most of the genetic distances in the population, and among these, there were also significant cluster–isotope associations. Independent of their actual positions in dual isotope space, some of the clusters differed in their average Euclidean distances from the geometric centre of isotopic space, although not with the reduced cluster set from STRUCTURE. While this typically will reflect increased dietary specialization (Bolnick *et al.*, 2002), the decreased density of values towards the edge of isotopic space could also suggest reduced availability of trophic niche space. These outliers with the highest Euclidean distances appear to occupy uncommon niche space. As this isotopic niche space also covaries with positions in morphospace, we infer that these genetic clusters could represent functional coupling to niche space and niche abundance.

These cumulative data are consistent with genotype-specific individual niche space as predicted in the adaptive variation models (de León *et al.*, 2012; Layman *et al.*, 2015). If

our data are representative, they imply micro-spatial structuring in the distribution of phenotypes/genotypes in natural populations with frequencies that should generally track the availability of different niches. Consequently, the relative fitness of peripheral phenotypes/genotypes will scale from negative to positive according to niche opportunities. Genetic clusters could represent adaptive units in a locality, each cluster with higher fitness for a particular niche space, where the relative frequencies of each cluster would track the frequencies of available niches. This is conceptually comparable to the original population genetic model of two niches and two alleles (Levene, 1953) expanded to a larger set of continuously distributed phenotypes and genotypes. Under these conditions, diversifying rather than stabilizing selection should dominate the adaptive landscape. Such a theme has been given new momentum (see Bolnick *et al.*, 2003; Violle *et al.*, 2017) and may facilitate improved field methodologies for evaluating form and function of intrapopulation variability.

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