

Ecological predictions lead to the discovery of a benthic–limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia

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Abstract: Sympatric species pairs of benthic and limnetic threespine stickleback (*Gasterosteus aculeatus* L., 1758 complex) are an important example of the role of ecology in speciation in nature. Four endemic pairs are known and each appears to have diverged independently as a consequence of adaptation to alternative environments. Using specific ecological and physical attributes hypothesized to be important to their evolution, we focused a search for further species pairs. Now, two decades after the last discovery, we describe another benthic–limnetic species pair from Little Quarry Lake on Nelson Island, British Columbia, Canada. Bimodality of genetic admixture values provides evidence of strong reproductive isolation between two morphological and genetic clusters, supporting the existence of a sympatric species pair within this lake. Close correspondence in shape to extant benthic and limnetic species pairs confirm their status as such. The remarkable similarity between them and other benthic and limnetic species pairs in levels of morphological differentiation, as well as extent of admixture and hybridization, points to similar processes underlying their origin. This discovery serves as an important reminder of the specificity of ecological factors that promote and maintain biodiversity, as well as the value of habitat conservation.

Résumé : Les paires d'espèces sympatriques benthique et limnétique de l'épinoche à trois épines (complexe de *Gasterosteus aculeatus* L., 1758) constituent un exemple important du rôle de l'écologie dans la spéciation en nature. On connaît quatre de ces paires endémiques et chacune semble avoir divergé de façon indépendante à la suite d'une adaptation à des environnements de recharge. En utilisant des caractéristiques écologiques et physiques considérées par hypothèse comme importantes dans cette évolution, nous avons ciblé notre étude sur la recherche de nouvelles paires d'espèces. Maintenant, deux décennies après la dernière découverte, nous décrivons une nouvelle paire d'espèces benthique–limnétique du lac Little Quarry sur l'île Nelson, Colombie Britannique, Canada. La bimodalité des valeurs de mélange génétique constitue une indication d'un fort isolement génétique entre les deux groupes morphologiques et génétiques. ce qui appuie l'existence d'une paire d'espèces sympatriques dans ce lac. Une forte correspondance de forme avec les paires d'espèces benthiques et limnétiques connues à ce jour vient confirmer leur statut similaire. La ressemblance remarquable entre cette paire d'espèces benthique et limnétique et les autres au niveau de la différenciation morphologique, ainsi que l'étendue du mélange et de l'hybridation, font croire que des processus similaires peuvent expliquer leur origine. Notre découverte constitue un rappel important de la spécificité des facteurs écologiques qui favorisent et maintiennent la biodiversité et de la valeur de la conservation des habitats.

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Introduction

The ecological theory of adaptive radiation postulates that population divergence and speciation are the outcome of divergent natural selection stemming from resource heterogeneity and competitive interactions (Dobzhansky 1951; Simpson 1953; Schluter 2000). Repeatable patterns of population divergence along similar environmental gradients has offered some of the strongest evidence that population divergence has, indeed, been driven by natural selection.

Recently derived postglacial fish populations are among

the most extensively studied systems of ecological speciation in nature (reviewed in McKinnon and Rundle 2002; Nosil et al. 2005; Rundle and Nosil 2005; Rogers and Bernatchez 2007). One such example is the threespine stickleback (*Gasterosteus aculeatus* L., 1758 complex), whereby ecological divergence has resulted in the recent evolution of limnetic and benthic species pairs in several postglacial lakes (McPhail 1993). In each pair, the limnetic stickleback is a small, slender fish that specializes in feeding on zooplankton in the open-water zone of lakes. The benthic stickleback is larger and deeper bodied, foraging mainly on

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large invertebrates from sediment or plants in littoral habitats (Schluter and McPhail 1992).

Their ecological divergence has proceeded independently (Taylor and McPhail 1999) and repeatedly, ultimately as a consequence of adaptation to alternative environments (Rundle et al. 2000) and, since the first description of benthic–limnetic threespine stickleback (McPhail 1984), these sympatric species pairs have become an important example of parallel evolution in nature. However, they have been documented from only four separate water drainages in the central Strait of Georgia region of coastal British Columbia, Canada: Enos Lake on Vancouver Island; the Vanda Creek drainage, including Priest Lake, on Texada Island; Paxton Lake on Texada Island; and Hadley Lake on Lasqueti Island (McPhail 1993). Their limited distribution raises the question: if divergent natural selection for different trophic niches has driven their divergence, why have more species pairs not been found?

Details of the pairs' origins are still not well understood. An earlier description of the geological history of the Strait of Georgia suggested that there had been two marine submergences separated by about 2000 years since the last glaciation (Mathews et al. 1970). This led to the idea that the same marine stickleback species (*Gasterosteus aculeatus*) had simply colonized each lake twice at intervals (the “double invasion hypothesis”; Schluter and McPhail 1992; McPhail 1993; Taylor and McPhail 2000). This scenario explained not only the highly restricted geographic distribution of the species pairs but also the narrow elevation range (50–100 m above sea level) of coastal lakes containing them (McPhail 1993; Vamosi 2003).

However, more exhaustive geological samples have ruled out a second postglacial sea-level rise in this region (Hutchinson et al. 2004) and, therefore, the scenario for the origin of the pairs based upon it. The species pairs nevertheless appear to have resulted from multiple invasions to freshwater by the marine species. For example, nuclear DNA indicates that the limnetic species in each lake is closer genetically to the present-day marine species than is the benthic species (Taylor and McPhail 2000), and it retains a higher tolerance of salt water (Kassen et al. 1995). Atop this uncertainty about the precise historical context of their evolution, specific ecological and physical attributes of lakes are hypothesized to promote the divergence and persistence of the species pair, determining their local distribution. These factors include lake size and depth, habitat diversity (littoral and deeper open-water zones), and depauperate fish communities (McPhail 1993; Vamosi 2003).

There is strong evidence that the disruption of the environmental factors underlying their origin can lead to the reversal of the benthic–limnetic divergence. In Enos Lake, elevated levels of gene flow associated with the appearance of an exotic crayfish (*Pacifastacus leniusculus* (Dana, 1852)) during the 1990s have resulted in their collapse into a hybrid swarm (Gow et al. 2006; Taylor et al. 2006). The increased pressure on these species pairs and their habitat in these coastal areas, which are experiencing rapid increases in human population density, is further exemplified by the extinction of another of the known species pairs. The Hadley Lake species pair on Lasqueti Island was exterminated sometime between 1988 and 1992 following the unauthor-

ized introduction of an exotic catfish, the brown bullhead (*Ameiurus nebulosus* (Lesueur, 1819); Hatfield 2001). With only two extant species pairs remaining on one island, Texada Island, these unique fish are now considered endangered (COSEWIC 2000).

Using the physical and ecological conditions hypothesized to be important to the evolution and persistence of benthic–limnetic species pairs, we identified and explored a lake potentially harbouring such sticklebacks on Nelson Island, British Columbia; the first survey of its kind in these waters. Two decades after the last discovery of benthic–limnetic sticklebacks (Hatfield 2001), we describe another species pair from Little Quarry Lake. Morphological and genetic analyses of threespine stickleback collected from this lake confirm their status as a benthic–limnetic species pair and yield important insights into patterns of evolution in these remarkable fish. Their adaptation to different niches driven by divergent natural selection only under certain environmental conditions serves as an important reminder of the specificity of ecological factors that promote and maintain biodiversity, and the importance of habitat conservation.

Materials and methods

Little Quarry Lake

Bathymetric data for this lake is currently limited. We do, however, know that it is at about the same elevation (53 m) as Enos Lake, which is at the lower end of the elevation range for known species-pair lakes (McPhail 1993; Vamosi 2003). Approximately 1 km at its longest and 350 m at its broadest, Little Quarry Lake is intermediate in surface area compared with other known species-pair lakes, which range up to 43.5 ha in size (McPhail 1993). In similarity to other species-pair lakes, Little Quarry Lake is connected to the sea by a high gradient stream that has been dammed in recent years, resulting in a raised lake level of several feet. As with Enos and Priest lakes, Little Quarry Lake serves as a domestic water supply for local residents. Investigations so far reveal threespine stickleback to be the only fish in this lake. The absence of other fish species (with the exception of cutthroat trout, *Oncorhynchus clarkii* (Richardson, 1836)) is considered crucial to their persistence (Vamosi 2003). As with other known species-pair lakes, Little Quarry Lake contains a range of littoral habitat, ranging from rocky cliffs to shallow beds of vegetation.

Sample collection

We collected adult sticklebacks found in Little Quarry Lake on Nelson Island, British Columbia, in June 2007. Thirty minnow traps distributed approximately evenly along the whole shoreline were used in conjunction with dip-netting to obtain lake-wide samples. We sacrificed fish with an overdose of MS-222 (Tricaine Methane Sulphonate). Tissue samples from fin clips were preserved in 95% ethanol prior to DNA extraction, while fish were soaked in 10% formalin for 2 weeks and stained with alizarin red as previously described (Peichel et al. 2001). While effort was made to balance the proportions of benthic- and limnetic-looking fish in these collections, we did not selectively exclude indeterminate forms, i.e., fish

that appeared to have ambiguous morphology were not discarded.

Shape analysis

We analyzed the shape of 96 fish specimens to determine whether two morphological clusters were present in the samples from Little Quarry Lake. Three specimens with severe bending of shape after preservation were excluded from further analysis. We digitized 25 landmarks on photographs taken of the right side of each specimen (Fig. 1). The landmarks we used are those of Albert et al. (2007), excluding two associated with the pelvis that we did not use, and are similar to those utilized in previous studies of shape (Walker 1997; Schluter 2003; Schluter et al. 2004; Taylor et al. 2006). They include positions at the extremities of bony tissues visible in stained fish, and insertion points of fins and spines. The 50 x and y coordinates for the 25 landmarks were obtained using tpsDig version 2 (Rohlf 2005), and were aligned using generalized least squares and corrected for geometric size in tpsRelw version 1.45 (Rohlf 2006). The aligned landmarks were then imported into R version 2.6.0 for further analysis (R Development Core Team 2007).

We used the model-based clustering method implemented in MCLUST version 3 for R (Fraley and Raftery 2006) to determine the number of distinct morphological clusters present in the landmark data. This method fits the observed frequency distribution of morphological measurements to a series of alternative models incorporating only one or mixtures of two to six Gaussian distributions. The estimated number of clusters is that number maximizing the Bayesian information criterion (BIC, analogous to Akaike's information criterion; Fraley and Raftery 2002). We applied the method to the first two principal components of the aligned landmark data, which captured 28% and 19% (sum: 47%) of the total variance present in all 50 x and y coordinates. The simplest Gaussian mixture model in MCLUST, which assumes that every cluster has the same variance and covariance, fitted the data better than more complex models. We repeated this analysis using the first five principal components rather than the first two, to capture more of the total variance present (71%), but the results were unchanged and we do not present them. A visual summary of the contribution of each landmark to the first two principal components is provided in Fig. S1.² With the most probable number of clusters being two (see Results), we calculated for every specimen the posterior probability of its being of the purported benthic type (P_b). These measures were compared with analogous probabilities from the genetic analysis, described next.

Microsatellite genotyping

We isolated DNA from tissue of the same 96 fish analysed for shape using standard phenol–chloroform protocol (Sambrook et al. 1989) and genotyped it at 11 *G. aculeatus* dinucleotide microsatellites. Eight of these loci constitute a species diagnostic molecular profile for the other benthic–limnetic species pairs (*Stn388*, *Stn295*, *Stn142*, *Stn383*,

Stn254, *Stn216*, *Stn386*, *Stn43*; Gow et al. 2006) and were used alongside three other microsatellites that are highly polymorphic in other benthic–limnetic species pairs (*Gac7*, *Cir51*, and *Gac4*; Gow et al. 2006). Together, they have provided a highly discriminatory tool that has distinguished between benthics, limnetics, and their hybrids (Gow et al. 2006, 2007). We genotyped these loci by using fluorescently labelled primers on a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, California) according to Gow et al. (2006).

Genetic cluster and admixture analysis

We used the program STRUCTURE (Pritchard et al. 2000) to explore the number of discrete genetic clusters (K) present in the genetic data. This Bayesian algorithm, Markov-chain Monte-Carlo-based approach uses a genetic inheritance model to minimize Hardy–Weinberg and linkage disequilibrium within cluster groups. We calculated the probability of there being one to four clusters by running five simulations for each K value, using the admixture and correlated allele frequencies models. Simulations began with a “burn-in” period of 25 000 iterations to minimize the dependence of subsequent parameter estimates on starting values, and parameters were estimated after a further 200 000 iterations. We followed the procedure and guidance of Pritchard and Wen (2003) and Evanno et al. (2005) to estimate the number of clusters given the data; the earlier qualitative method, which estimates the real number of clusters as the K value where the “log probability of data” ($L(K)$) begins to plateau (Pritchard and Wen 2003), has been formalized by the ad hoc statistic ΔK , which is based on the rate of change in $L(K)$ between successive K values (Evanno et al. 2005).

With the most probable number of clusters being two (see Results), we estimated each individual's admixture proportions between benthic and limnetic gene pools for each of the five simulations where $K = 2$. Following this, we calculated each individual's average proportion of ancestry in the purported benthic population ($q_b^{(i)}$).

Comparison with extant benthic–limnetic species pairs

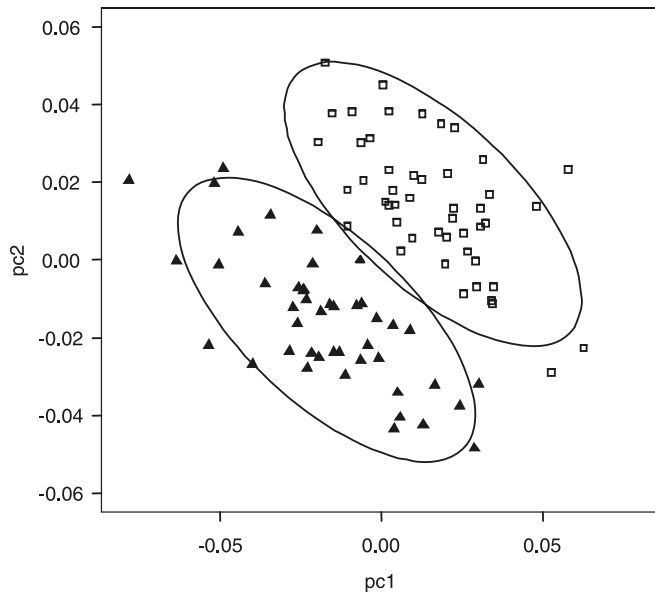
To visualize the relative similarity of samples from Little Quarry Lake to those belonging to the other extant benthic–limnetic species pairs from Priest and Paxton lakes, we compared their patterns of morphological and genetic variation. We repeated the shape analysis using fish collected from Priest Lake (a mixture of 65 benthics and limnetics) and Paxton Lake (a mixture of 70 benthics and limnetics) during 2005. The same preservation and staining procedures ensured samples from different lakes were directly comparable. It was clear by eye that the mean shape of fish in one of the two clusters in Little Quarry Lake resembled that of the limnetic species in the two other lakes, and the second cluster resembled the benthic species from the other lakes. We confirmed our designations quantitatively with a K -means clustering analysis (Hartigan and Wong 1979) on pairwise Mahalanobis distances between mean shapes of all

²Supplementary Fig. S1 for this article is available on the journal Web site (<http://cjz.nrc.ca>) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3752. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.html.

Fig. 1. (A) Little Quarry Lake benthic threespine stickleback (*Gasterosteus aculeatus*) illustrating the 25 landmarks (shaded circles in print; red circles on the Web) used in the shape analysis. (B) Little Quarry Lake limnetic stickleback with the same landmarks.



Fig. 2. The two clusters identified by the MCLUST analyses of shape carried out on the threespine stickleback (*Gasterosteus aculeatus*) samples from Little Quarry Lake. Each symbol indicates the position of an individual fish along the first two principal components of variation among landmark coordinates. Solid triangles indicate fish classified as limnetic and open squares are fish classified as benthic. Ellipses encircle about 90% of the measurements present in each cluster, assuming a Gaussian frequency distribution of measurements.



six populations in the three lakes (results not provided). We then calculated shape differences of sympatric species by the magnitude and direction of difference (“shift”) in the mean position of each landmark between the limnetic and the benthic clusters within each lake. Similarity between lakes in the direction and magnitude of these shifts in different lakes was quantified using correlation.

Table 1. Pelvic girdle presence or absence in three-spine stickleback (*Gasterosteus aculeatus*) specimens analysed from (A) Little Quarry, (B) Priest, and (C) Paxton lakes.

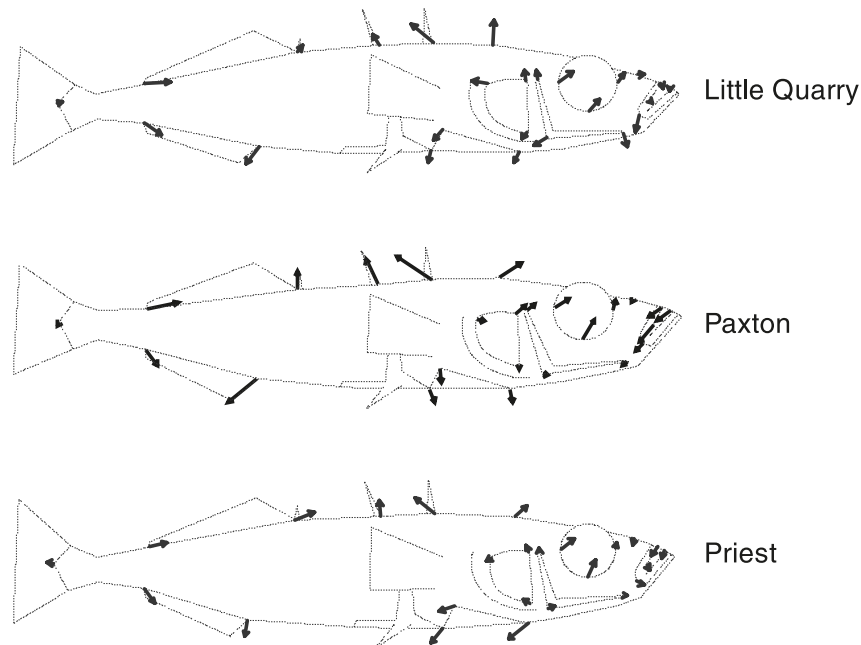
Species	Pelvic girdle	
	Presence	Absence
(A) Little Quarry Lake		
Limnetic	41	0
Benthic	5	43
(B) Priest Lake		
Limnetic	25	0
Benthic	38	0
(C) Paxton Lake		
Limnetic	37	1
Benthic	3	27

Note: Individuals were classified as benthic or limnetic according to their posterior probability of being benthic (P_b). Individuals with ambiguous assignments ($P_b > 0.1$ but < 0.9) were excluded from the analysis.

Staining revealed that the pelvic girdle was absent in a high fraction of benthic individuals from Little Quarry Lake (Fig. 1), an unusual characteristic among threespine sticklebacks in general and within the benthic–limnetic species pairs, occurring only in the Paxton Lake benthic species (McPhail 1992). Therefore, we compared the frequency of limnetics and benthics in all three lakes with and without a pelvic girdle. A pelvic girdle was scored as present if any bony tissue, however diminutive, was evident in the pelvic region. Otherwise the girdle was scored as absent.

Genetic admixture within each species pair was also compared using two alternative approaches. Firstly, we transformed $q_b^{(i)}$ into hybridity (h_i) values using the formula $h_i = 0.5 - |0.5 - q_b^{(i)}|$ (sensu Carney et al. 2000; Duvernell et al. 2007), and calculated mean h_i for each species pair. Ranging from 0 for pure parentals to 0.5 for F_1 hybrids,

Fig. 3. Shape change between benthic and limnetic threespine sticklebacks (*Gasterosteus aculeatus*) within the three species pairs. The base of each arrow indicates the mean position of the corresponding landmark in the limnetic species. Arrows indicate the direction and magnitude of change in landmark position from this limnetic shape to the mean shape of the benthic species. The length of each arrow was multiplied by two to increase visibility.



hybridity provides a measure of how intermediate an individual's multilocus genotype is on an admixture scale. Secondly, we assigned individuals as benthic, limnetic, or hybrid based on their 90% posterior probability interval (90% PI) of $q_b^{(i)}$: a benthic had a 90% PI overlapping 1, a limnetic had a 90% PI overlapping 0, and a hybrid had a 90% PI overlapping with neither 0 nor 1. We calculated the frequency of individuals having a 90% posterior probability interval of $q_b^{(i)}$ overlapping neither 0 nor 1, hereinafter termed hybrid frequency. The admixture calculations for Priest and Paxton lakes species pairs were drawn from data for adult samples from 2004 to 2006 ($n = 439$ and 575 for Priest and Paxton lakes, respectively) published in Gow et al. (2007).

Results

Two distinct morphological clusters within Little Quarry Lake correspond to benthics and limnetics

The MCLUST analysis of the first two principal components indicated that the Little Quarry Lake specimens fell into two distinct morphological clusters (Fig. 2). Two clusters fit the sample of measurements better than any other model. The next best fitting model, containing three clusters, had substantially lower support (difference in BIC from the best model: $\Delta = 10.7$). The single-cluster model had even lower support ($\Delta = 16.6$). Models with Δ scores >10 are considered to be without support (Raftery 1996).

The difference in shape between the two groups of fish from Little Quarry Lake, as classified by the MCLUST analysis, is visualized in the top panel of Fig. 3. Arrows represent the direction and magnitude of shift in mean landmark position from fish in the more limnetic-like cluster to fish in

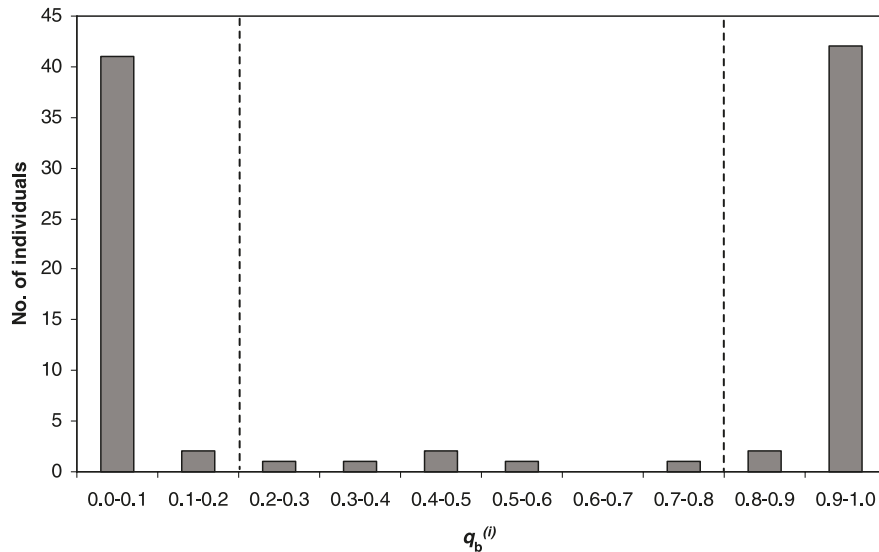
the more benthic-like cluster. Arrow lengths in the figure are doubled to facilitate visibility. Similar diagrams for Paxton and Priest lakes (middle and bottom panels of Fig. 3) reveal that the transitions between limnetic and benthic mean shapes are similar, though not identical, in the three lakes. Shifts in common include a greater overall body depth in the benthic relative to the limnetic species, shorter dorsal and anal fins, a smaller eye, and a shorter jaw that is more downward-oriented. We quantified the resemblance of shifts in different lakes by correlating the magnitudes of the differences in each landmark coordinate (50 in all). The correlation between the shifts in Little Quarry Lake and Paxton Lake was 0.73, and that between Little Quarry Lake and Priest Lake was 0.73. This is virtually identical to the "reference" correlation between the shifts in Paxton and Priest lakes (0.71).

The frequency of benthic individuals lacking a pelvic girdle was high and similar in both Little Quarry and Paxton lakes. Benthics from Priest Lake and limnetics from all three lakes tend to possess a pelvic girdle (Fig. 1, Table 1).

Morphological clusters within Little Quarry Lake correspond to two genetic clusters

The presence of two distinct types within Little Quarry Lake is corroborated by the genetic analysis. Again, the most probable number of clusters was two: the log probability of the data ($L(K)$) (Pritchard and Wen 2003) plateaus at $K = 2$ and ΔK (Evanno et al. 2005) is modal at $K = 2$. A highly significant correlation between an individual's MCLUST posterior probability of being benthic (P_b) and its average proportion of ancestry in the purported benthic population ($q_b^{(i)}$) ($r = 0.96$, $df = 87$, $P = 3.98 \times 10^{-49}$) illustrates concordance between morphological and genetic clusters.

Fig. 4. The average proportion of ancestry of individual threespine sticklebacks (*Gasterosteus aculeatus*) in the benthic population ($q_b^{(i)}$) estimated by STRUCTURE ($K = 2$) for all samples collected from Little Quarry Lake ($n = 93$) species pairs in 2007. The proportion of parental and admixed individuals is illustrated by plotting the frequency of $q_b^{(i)}$ values. A threshold $q_b^{(i)}$ value of 0.2 divides parental and admixed individuals, which are separated by broken vertical lines.



Bimodal admixture values indicate strong reproductive isolation between benthics and limnetics

The distribution of individual genetic admixture values (ranging from 0 to 1 between two parental types) indicates the degree of reproductive isolation between two clusters. If premating reproductive isolation is strong, hybridization will be rare and the distribution of admixture values is expected to be bimodal, with most individuals having values near 0 or 1. If hybridization takes place but postzygotic reproductive isolation is strong, then some adults with admixture values close to 0.5 may be present, but admixture values between 0.5 and the parental extremes will be scarce. Finally, if both premating and postzygotic reproductive barriers are weak, then gene flow will be common and we should see a continuous distribution of admixture values across the range between 0 and 1. Assigned by their average proportion of ancestry in the benthic population ($q_b^{(i)}$), individual admixture values for Little Quarry Lake sticklebacks exhibit a strongly bimodal frequency distribution, with the few intermediate admixture values fairly evenly distributed between 0.2 and 0.8 (Fig. 4).

Little Quarry Lake species pair shares a remarkably similar level of genetic admixture with those from Priest and Paxton lakes; neither estimate of admixture, hybridity (0.04, 0.02, and 0.02 for Little Quarry, Priest, and Paxton lakes, respectively), nor hybrid frequency (4.3%, 3.7%, and 4.7% for Little Quarry, Priest, and Paxton lakes, respectively) varied significantly among species pairs ($F_{[2,6]} = 3.13$, $P = 0.15$ for hybridity; $F_{[2,6]} = 0.43$, $P = 0.68$ for hybrid frequency).

Discussion

Evidence for the parallel evolution of sympatric stickleback species pairs, each consisting of a benthic and limnetic species, has contributed significantly to our understanding of the origin of species and of how divergent natural selection can drive adaptation to alternative environments (McKinnon

and Rundle 2002; Nosil et al. 2005; Rundle and Nosil 2005). The addition of a fifth sympatric pair of benthic and limnetic sticklebacks sheds more light on the environmental context of the evolution of these highly endemic species. We have been able to focus a search and identify a further species pair in Little Quarry Lake on Nelson Island in British Columbia, Canada, based on the knowledge of the ecological and physical factors in lakes thought to drive and maintain their divergence that we have gleaned from the four lakes where these species pairs have been previously known to occur (McPhail 1993; Vamosi 2003).

Little Quarry Lake shares several important features with the other lakes harbouring benthic–limnetic species pairs (McPhail 1993; Vamosi 2003): it falls within their known elevation range of 50–100 m; it is relatively small but includes both littoral habitat and open water; it is close to, but presently inaccessible from, the sea; and it appears to harbour few other fish species. Unlike the other species-pair lakes (Vamosi 2003), Little Quarry Lake appears to have no cutthroat trout. We cannot rule out the possibility of a recent extinction of this species following the creation of a small dam that might prevent cutthroat trout from moving between the lake and the potential breeding habitat in the outlet creek.

By identifying two distinct clusters within the Little Quarry sticklebacks, the morphological and genetic evidence clearly support the existence of a sympatric threespine stickleback species pair within Little Quarry Lake. Their resemblance to the limnetic and benthic species in other lakes allows us to designate the clusters as another benthic and limnetic species pair. The benthic species in this lake exhibits its pelvic reduction, similar to that seen in Paxton Lake.

The bimodality of genetic admixture values provides evidence of strong reproductive isolation between the two clusters. However, the presence of some individuals with intermediate admixture values shows that premating reproductive isolation, although strong, is not complete. The per-

sistence of the species pair in the face of some gene flow implies that there is selection against hybrids. Indeed, selection against wild hybrids has been detected across the stickleback life cycle in Priest and Paxton lakes, and this selection might be pivotal in maintaining their distinct gene pools in sympatry (Gow et al. 2007). Levels of genetic admixture between the limnetics and benthics in Little Quarry Lake are, in fact, very similar to those found in the other species pairs. While this implies that different pairs of benthics and limnetics share a similar degree of reproductive isolation, the contribution made to this reproductive isolation by different pre- and post-mating mechanisms may vary among species pairs.

In addition to its scientific value, this description of another benthic–limnetic species pair comes as welcome news to their conservation, given that two of the four previously described ones are now extinct, with the two extant pairs remaining on one island, Texada Island. Their discovery reinforces the concept that specific environmental conditions are necessary for the existence of benthic–limnetic species pairs, highlighting the importance of habitat conservation and drawing our attention to the fragility of these endangered species. Indeed, the speciation reversal of the Enos Lake species pair caused by diminished reproductive barriers between benthics and limnetics is associated with human-induced environmental change (Gow et al. 2006; Taylor et al. 2006), a phenomenon that is of growing concern to biodiversity loss (Seehausen 2006; Seehausen et al. 2008). A thorough investigation of the Little Quarry Lake species pair's habitat and the threats it may face should now be carried out to assess the conservation status of this species pair. In addition, future surveys of other lakes on Nelson Island may prove fruitful in uncovering yet more remarkable examples of threespine stickleback adaptation to freshwater environments.

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