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Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair

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Abstract

Historically, six small lakes in southwestern British Columbia each contained a sympatric species pair of three-spined sticklebacks (*Gasterosteus aculeatus*). These pairs consisted of a 'benthic' and 'limnetic' species that had arisen postglacially and, in four of the lakes, independently. Sympatric sticklebacks are considered biological species because they are morphologically, ecologically and genetically distinct and because they are strongly reproductively isolated from one another. The restricted range of the species pairs places them at risk of extinction, and one of the pairs has gone extinct after the introduction of an exotic catfish. In another lake, Enos Lake, southeastern Vancouver Island, an earlier report suggested that its species pair is at risk from elevated levels of hybridization. We conducted a detailed morphological analysis, as well as genetic analysis of variation at five microsatellite loci for samples spanning a time frame of 1977 to 2002 to test the hypothesis that the pair in Enos Lake is collapsing into a hybrid swarm. Our morphological analysis showed a clear breakdown between benthics and limnetics. Bayesian model-based clustering indicated that two morphological clusters were evident in 1977 and 1988, which were replaced by 1997 by a single highly variable cluster. The most recent 2000 and 2002 samples confirm the breakdown. Microsatellite analysis corroborated the morphological results. Bayesian analyses of population structure in a sample collected in 1994 indicated two genetically distinct populations in Enos Lake, but only a single genetic population was evident in 1997, 2000, and 2002. In addition, genetic analyses of samples collected in 1997, 2000, and 2002 showed strong signals of 'hybrids'; they were genetically intermediate to parental genotypes. Our results support the idea that the Enos Lake species pair is collapsing into a hybrid swarm. Although the precise mechanism(s) responsible for elevated hybridization in the lake is unknown, the demise of the Enos Lake species pair follows the appearance of an exotic crayfish, *Pascifasticus leniscului*, in the early 1990s.

Keywords: exotic species, extinction, gene flow, introgression, sticklebacks

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Introduction

The persistence of sympatric species that still occasionally hybridize implies that a dynamic balance exists between the occasional production of hybrids and their removal by natural selection. Presumably, there have been cases where completion of speciation in sympatry fails because gene flow overwhelms factors promoting divergence. Perhaps more infrequently, speciation proceeds to completion only to be undone later when environmental conditions change. Such reversals can occur, for instance, if the fitness of hybrids is suddenly improved under the new environmental conditions (e.g. Grant & Grant 2002).

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Alternatively, environmental cues upon which premating isolation is based may suddenly be altered, leading to a burst of hybridization that selection can no longer overcome (discussed in Coyne & Orr 2004). In either instance, the frail integrity of species that lack complete postzygotic isolation demonstrates the contribution of the environment to their maintenance and, perhaps, provides insight to the identity of factors that initiate speciation.

Here we present genetic and morphological evidence of such a breakdown in the three-spined stickleback (*Gasterosteus aculeatus* complex). The complex includes a wide diversity of populations showing morphological and genetic differentiation across the many marine, stream, river and lake environments occupied in coastal areas around the Northern Hemisphere (McPhail 1994; McKinnon & Rundle 2002). Diversity within the complex reaches its peak in several small lakes of southwestern British Columbia (BC), where fully sympatric pairs of species coexist (Schluter & McPhail 1992; McPhail 1994). Each pair consists of a small, slender 'limnetic' species that preys upon zooplankton in the open water zone of lakes, and a larger, deeper-bodied 'benthic' species that feeds on invertebrates associated with the sediment or attached to vegetation in the littoral zone. Six such pairs have been discovered, at least four of which have had independent origins despite parallel differences in morphology, ecology, behaviour, and mating preferences (Taylor & McPhail 2000). Partly because of this replication, the stickleback species pairs have become an important model for the study of speciation in nature and of the ecological factors that cause divergence in general (McKinnon & Rundle 2002).

Recently, the species pairs have faced increased risks of extinction. The lakes they inhabit occur in coastal areas of BC that are experiencing rapid increases in human population density. One of the six stickleback pairs, in Hadley Lake on Lasqueti Island, was eradicated accidentally some time between 1988 and 1992 after the illegal introduction of an exotic catfish, the brown bullhead (*Ameiurus nebulosus*, Hatfield 2001). A second species pair, from Enos Lake on Vancouver Island, now also appears to be at risk of extinction, but by an entirely different cause: elevated levels of gene flow.

The initial indication that Enos Lake might contain two species of sticklebacks was based on morphological observations of 'odd' populations of *Gasterosteus* (Larson 1976; McPhail 1984, 1992). Similarly, the first indication that the distinction within the lake was becoming less clear was based on gross morphological examinations of field-collected specimens and, in particular, the apparently increasing scarcity of pure limnetics (personal observations). The first quantitative assessment of the possible collapse of the Enos Lake species pair was provided by Kraak *et al*. (2001) who reported evidence for an increased number of hybrid individuals inferred from morphological analysis. These authors reported that 12% of a sample of sticklebacks from Enos Lake ($N = 49$) were intermediate to benthics and limnetics in body morphology and were tentatively classified as hybrids. This represented a large increase from previous estimates, also inferred from morphology, of < 1% hybrids (McPhail 1984). Kraak *et al*. (2001) showed that the frequency distribution of gill raker number, a morphological trait strongly connected to feeding (Bentzen & McPhail 1984), showed two clearly distinct clusters in samples collected between 1973 and 1983. By 1997, however, the distribution of gill raker numbers was still bimodal, but the peaks had grown closer and there were no longer two discrete types (Fig. 1, Kraak *et al*. 2001). The analysis of Kraak *et al*. (2001), however, involved only a single point sample and did not include any genetic data that would provide more direct evidence of hybridization. This case of species collapse, if verified, would be interesting because it appears to be caused not by the direct extinction of one or both forms, but by elevated gene flow arising from a change of environment (i.e. speciation 'in reverse' cf. Coyne & Orr 2004 p. 37). Furthermore, genomic extinction via hybridization is one of the major kinds of biotic homogenization negatively impacting native freshwater fish faunas (Rhymer & Simberloff 1996; Taylor 2004).

In this study, we use morphometric and molecular genetic methods to assess the apparent collapse of the species pair and to test the 'collapse by hybridization' hypothesis. Given the importance of distinct trophic resources in the lake to the evolution and maintenance of benthic and limnetic sticklebacks (reviewed in Schluter 2000), however, it is also possible that trophic resources in the lake have changed to the extent that the environment can no longer support the existence of benthics and limnetics. In other words, natural

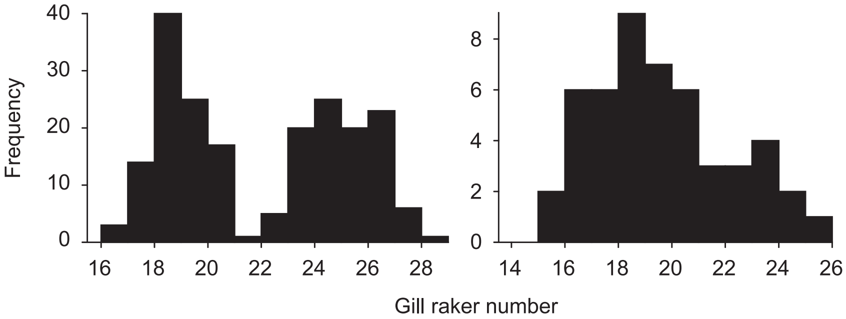

Fig. 1 Frequency distribution of gill raker number in three-spined sticklebacks from Enos Lake. Left panel: fish collected from 1973 to 1983 (redrawn from McPhail 1984). Right panel: fish collected in 1997 (drawn from data in Kraak *et al*. 2001).

selection may have favoured the evolution of a morphologically intermediate phenotype without hybridization. Although these two ideas are not mutually exclusive, only the hybridization hypothesis makes the prediction that selectively neutral molecular traits in Enos Lake sticklebacks should reveal signatures of genetic admixture. We evaluated these hypotheses by testing the following predictions of the collapse via gene flow hypotheses: (i) distinct morphological clusters identified in earlier samples disappear in later samples; (ii) genetically distinct populations collapse into a single hybrid population; (iii) multilocus genetic associations between loci that differ in frequency between the two species deteriorate as hybrid genotypes, previously rare, increase in frequency; and (iv) previous associations between morphology and genotype frequencies break down.

Materials and methods

Field collections

Our study was carried out on sticklebacks from Enos Lake (49°16′05″N, 124°09′02″W) on southeastern Vancouver Island, Canada. Enos Lake is a small (17.6 ha) lake, finger-like in shape, located 48 m above sea level and is connected to the sea by 1.5-km long Enos Creek. The maximum depth is 11 m, but the majority of the lake is less than 10 m in depth (see McPhail 1989 for a bathymetric map). The lake is an oligotrophic slightly acidic lake ‘tea-stained’ in colour from in-wash of organic material. Historically, the lake had extensive beds of *Potamogeton* and *Utricularia*, particularly in the eastern portion of the lake (McPhail 1989). The only native vertebrates in the lake are sticklebacks and the rough-skinned newt, *Taricha granulosa*. Rainbow trout (*Oncorhynchus mykiss*) and coastal cutthroat trout (*Oncorhynchus clarkii clarkii*) have been sporadically introduced, but apparently they have not persisted in appreciable numbers (McPhail 1989). No trout were collected or observed during our studies.

We collected individuals from May to July of 1994, 1997, 2000, and 2002 (Table 1). With the exception of 2000, when minnow traps alone were used to obtain samples, a combination of unbaited minnow traps, dip-netting and seining ensured that limnetics feeding in the open water, as well as breeding limnetics and benthics in the littoral zone, were represented. Morphological measurements from 1997, 2000, and 2002 were compared with collections made in 1977 and 1988 (McPhail 1984; Schluter & McPhail 1992). Our 1994 collection was selective in that we retained only those individuals that could be classified as ‘benthic-’ ($N = 45$) or ‘limnetic-like’ ($N = 29$) based on gross morphological appearance as described by McPhail (1984); 22 individuals were not retained based on visual classification in the field. The 1994 sample was later examined in the laboratory for gill raker counts; individuals with 19 or fewer gill rakers constituted our ‘benthic’ sample and those with more than 21 gill rakers our ‘limnetic’ sample (McPhail 1984). All subsequent collections (1997, 2000, and 2002) were unselective for morphology and morphological measurements of these collections were compared with existing collections from 1977 and 1988 (McPhail 1984; Schluter & McPhail 1992). All collections consisted of adult fish only except for fish collected in 2000 which comprised a mixture of adult and juvenile (young-of-the-year) samples. In this year, only the adult fish were examined morphologically, but adult and juvenile fish both were used for genetic analysis.

All retained individuals were killed with an overdose of buffered MS-222 and placed in 95% ethanol. Pectoral fin clips (post 1997) or liver and muscle tissues (1994) were collected for DNA analysis. Specimens were then fixed in 10% formalin for 2 weeks and stained with alizarin red. Specimens from 1977 and 1988 had been placed in formalin rather than ethanol after collection and before staining and, despite repeated attempts, it was not possible to extract usable DNA from these early samples. After staining, all specimens were stored in 40% isopropyl alcohol.

Shape analysis

We analysed shape changes of fish over time using relative warp analysis (Rohlf 1999). Photographs were taken of the left side of each fish using a digital camera placed overhead. Images were imported into the program TPSDIG (Rohlf 1997) where coordinates of 19 landmarks were obtained (Fig. 2A).

Landmark coordinates for each fish were imported into the program TPSRELW (Rohlf 1997; http://life.bio.sunysb.edu/morph/). The program uses the Procrustes method to centre, scale and align the coordinates and then calculate a mean or ‘consensus’ configuration. The program then compares each set of coordinates to the consensus using thin plate spline analysis (Bookstein 1991). The method deforms each set of coordinates towards the mean configuration, requiring a set of unique energy values called the ‘partial warps’. The principal components of these partial warps, called ‘relative warps’, summarize the major trends of shape variation in the set of specimens (Rohlf 1999). We used the first two relative warps to document shape changes in fish collected in different years.

We employed a model-based clustering method using the program MCLUST (Fraley & Raftery 2003; implemented in SPLUS 6.1; www.stat.washington.edu/fraley/mclust/) to determine how many morphological clusters could be distinguished in each year. The method fits the observed frequency distribution of relative warp scores from a given year to two alternative models. In the first model, only a single cluster is assumed. In the second model, two clusters are assumed to be present. The model with the

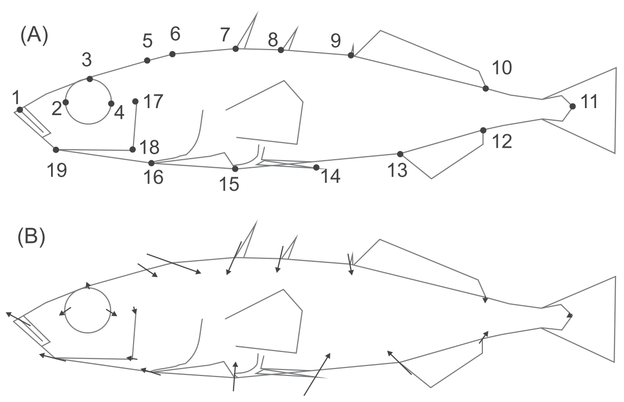

Fig. 2 (A) The 19 morphological landmarks used in morphological analysis of three-spined sticklebacks, shown on the consensus configuration: (1) anterior tip of upper lip; (2) most anterior point of left eye; (3) most dorsal point of left eye; (4) most posterior point of left eye; (5) supraoccipital notch immediately lateral to the dorsal midline (DML); (6) point of intersection between DML and line starting in point 5, tangent to the DML; (7) anterior junction of the first dorsal spine with the DML; (8) anterior junction of the second dorsal spine with the DML; (9) anterior junction of the third dorsal spine with the DML; (10) insertion of the dorsal fin membrane on the DML; (11) caudal border of hypural plate at the lateral midline; (12) insertion of anal fin membrane on the ventral midline (VML); (13) anterior junction of the anal spine with the VML; (14) caudal tip of posterior process of pelvic girdle on VML; (15) posterior tip of ectocoracoid; (16) anterior border of ectocoracoid on VML; (17) dorsal point of angular; (18) anterior edge of angular; (19) posterior edge of angular. (B) Transitions in landmark position along the first relative warp (PC1). Each arrow indicates the average vector of displacement in the X and Y coordinates of each landmark accompanying a position from three standard deviations to the left of the mean to three standard deviations to the right of the mean (PC1).

highest Bayesian information criterion is selected as the most likely.

Finally, we used discriminant analysis to find a single shape axis that best discriminated between limnetics and benthics collected in 1977, prior to any indications of extensive hybridization. To begin, model-based clustering was used to classify individuals as either limnetic or benthic according to shape. We then carried out a discriminant analysis separating limnetics and benthics, using only half the 1977 specimens, randomly chosen. The resulting discriminant function was then used to score the remaining half of the 1977 specimens and all specimens collected in later years. The remaining half of the 1977 specimens control for the possibility that the high level of discrimination between benthics and limnetics in 1977 is due to over fitting of the data.

Microsatellite analysis

We screened each collection from 1994 onwards for allelic variation at five microsatellite loci that have been useful in previous work (Taylor & McPhail 2000; Hendry *et al*. 2002) and provided excellent discrimination between benthics and limnetics (see below). The loci had been isolated from stickleback genomic libraries and were assayed using the polymerase chain reaction (PCR) and radiolabelled primers as described by Rico *et al*. (1993: *Cir*51) and Taylor (1998: *Gac*4, *Gac*7, *Gac*9, and *Gac*14).

We first tested loci for deviations from Hardy–Weinberg equilibrium and linkage equilibrium within each collection using GENEPOP (Raymond & Rousset 2001; version 3.3). For these tests, significance levels were adjusted to a table-wide alpha level of 0.05 using the sequential Bonferroni procedure (Rice 1989; i.e. adjusted for five simultaneous tests within samples).

Assignment tests were performed using the Rannala & Mountain (1997) Bayesian method as implemented in GENECLASS (Piry *et al*. 2004; version 2.0). Assignment of the 1994 samples resulted in a 97% success rate (i.e. in 72 out of 74 cases previous morphological classification as benthics or limnetics matched subsequent genetic classification). This 1994 sample was then used as the 'reference' database to classify individuals collected in 1997, 2000, and 2002 as either 'benthic' or 'limnetic'.

In order to visualize the relative similarity among all samples, we used factorial correspondence analysis (FCA) to project individuals, pooled across all years, in microsatellite allele frequency space using GENETIX (Belkhir *et al*. 2001). FCA is a type of factor analysis that seeks to find the best linear combination of variables (in this case allele frequencies at different loci) that best describe variation between individual observations (fish). In general terms, FCA is best suited for categorical data, such as allele frequency counts, and determines the first K axes of an orthogonal number of axes that describe the most variance from a 'cloud' of observations. To this multiyear 'supersample' of Enos Lake sticklebacks, we added a simulated 'hybrid' sample based on the 1994 samples. To construct the sample of 'hybrids' between benthics and limnetics, we used the program HYBRIDLAB (see Nielsen *et al*. 2001) to generate multilocus F_1 hybrid genotypes between the two species. Alleles are drawn randomly as a function of their estimated frequency distributions, assuming linkage equilibrium among loci, selective neutrality, and random mating.

Finally, we used the program STRUCTURE (Pritchard *et al*. 2000) to estimate the most likely number of populations represented by the samples collected in each year. STRUCTURE is a Bayesian, Markov chain Monte Carlo-based approach that clusters individuals to minimize Hardy–Weinberg and gametic phase disequilibria within groups. The number of populations represented in our samples was estimated by pooling all samples within years and calculating the probability of the data, assuming that they originated from one to three populations in Enos Lake (see Pritchard *et al*. 2000). For each year, we ran STRUCTURE a

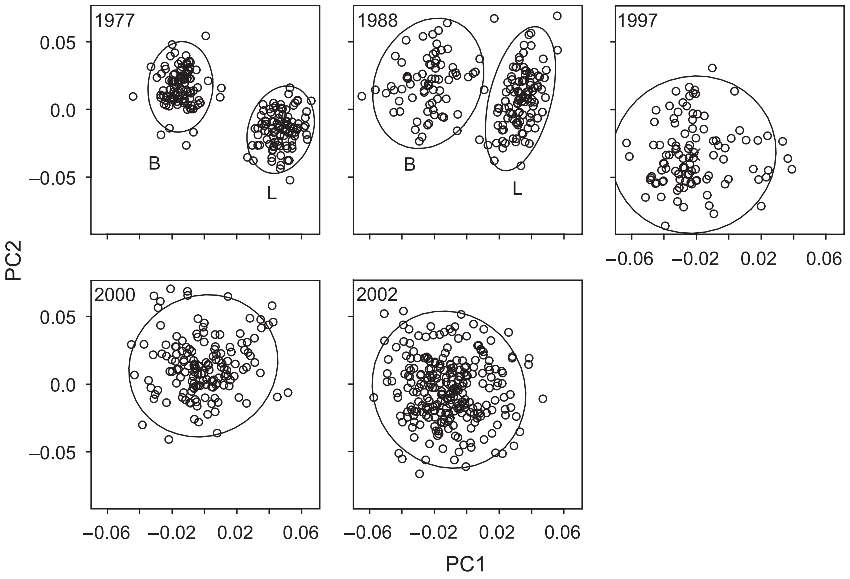

Fig. 3 Annual samples of three-spined sticklebacks plotted along the first and second relative warps (PC1 and PC2). Ellipses encircle about 95% of the measurements present in each cluster of individuals as classified in the MCLUST analysis. B and L refer to the benthic and limnetic species recognized in the earliest samples. Ellipses are based on the best-fit bivariate normal distribution to each cluster.

total of 10 times, each with a 'burn-in' period of 50 000 simulations to minimize the dependence of subsequent parameter estimates on starting values, followed by parameter estimation after a further 450 000 simulations. We used the log-likelihood ratio test to test for differences in the likelihoods of different models of population structure in Enos Lake. The test statistic is 2(ln L1 – ln L2), where ln L1 is the natural logarithm of the likelihood of the simplest model (i.e. one population) and ln L2 (or ln L3) is the log-likelihood of the more general model invoking two (or three) populations (Huelsenbeck & Rannala 1997). The test statistic has an approximately chi-squared distribution with the degrees of freedom equal to the number of additional parameters (populations) in the more complex model (1 in the case of two populations, 2 in the case of three populations). In all cases except for the 1994 baseline sample, genetic analyses were conducted without prior knowledge of the level of morphological distinction within a particular sample.

Results

Morphological variation

The first principal component of shape (relative warp) incorporated all the main shape differences known to distinguish benthics and limnetics (Fig. 2B). The first and second relative warps explained 48% of the variation among specimens in shape. The third and higher relative warps were not associated with differences between species.

The specimens from 1977 and 1988 fell into two distinct morphological clusters (Fig. 3), clearly corresponding to the benthic shape (left) and the limnetic shape (right). Morphological variability was marginally higher in 1988 than 1977, and the difference between species means was slightly less (Table 2). In contrast, the 1997, 2000, and 2002 collections showed only a single cluster of measurements. Morphological variability in the single cluster in these years was substantially higher than in earlier clusters (Table 2).

Table 1 Sample sizes in each year in which three-spined stickleback were sampled from Enos Lake. Dashes (—) indicate that no samples were available

Year	Sample method	*N* morphology	*N* microsatellites
1977	Trap	101	—
	Dip net	97	—
1988	Trap	79	—
	Seine	103	—
1994	Trap	—	47
	Seine	—	27
1997	Trap	103	96
	Seine	—	—
2000	Trap	151	201
	Seine	—	49
2002	Trap	152	60
	Seine	101	51

A discriminant function analysis using half of the 1977 measurements clearly separated remaining measurements from the same year (1977) and measurements from 1988 (Fig. 4). The samples from 1997 and onwards, however, showed little indication of two distinct modes. Unlike samples in 1977 and 1988, those from 2002 showed no separation based on capture location (traps for littoral zone, seines for pelagic zone; Fig. 4).

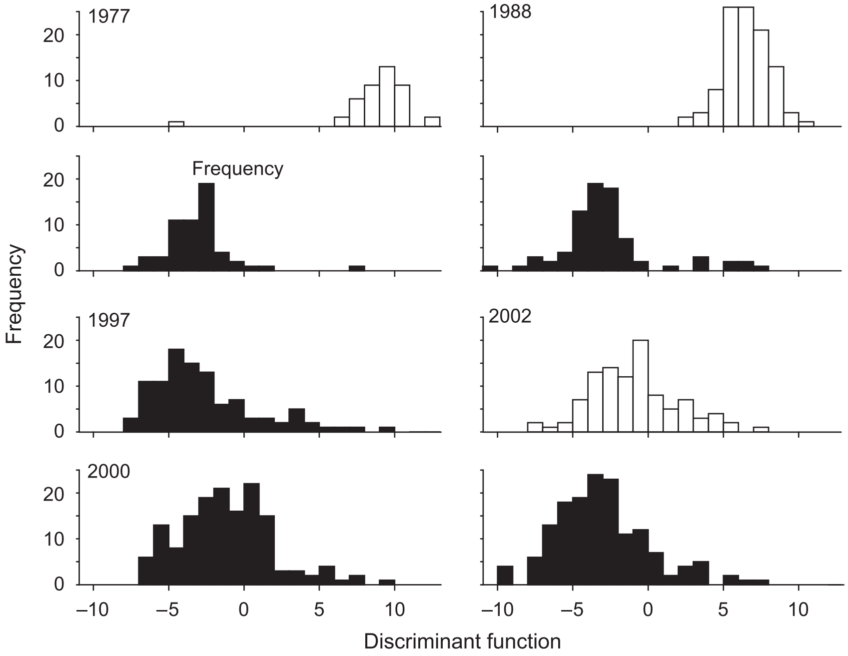

Fig. 4 Frequency distributions of discriminant function scores displayed according to sampling method. The discriminant function was calculated between limnetic and benthic three-spined sticklebacks in 1977 using the partial warps of half the fish, randomly chosen. Scores are displayed for the remaining half of the 1977 measurements and for samples from remaining years. Fish caught in the open water using dip net or seine are shown without shading; the trap samples are indicated by shading. Samples in 1997 and 2000 were obtained only with traps.

Table 2 Mean and variance (× 10^4) of the first relative warp (PC1) of morphological clusters for three-spined sticklebacks in each year

Year	Cluster	Mean coordinates	Variance (× 10^4)	Distance between cluster means
1977	1	–0.015	0.67	0.062
	2	0.047	0.72	
1988	1	–0.024	1.96	0.057
	2	0.033	0.79	
1997	1	–0.022	4.30	—
2000	1	–0.001	3.53	—
2002	1	–0.011	3.76	—

Microsatellite variation

Across the five loci, gene diversity was high and ranged from 0.48 (1997 sample) to 0.61 (Enos limnetic 1994). The number of alleles adjusted to a common sample size of 25 individuals ranged from an average of 4.1 (1997 sample) to a high of 5.8 (Enos limnetic 1994). Tests for deviations from Hardy–Weinberg conditions indicated one significant deviation in the 1994 Enos limnetic sample (at *Cir*51). More deviations, however, were observed in the 1997 (four loci), 2000 (four loci), and 2002 (three loci) samples (Taylor *et al*., unpublished). Within any sample, only one test for linkage disequilibrium was significant (after adjusting for 10 tests within samples); *Gac*7 and *Gac*9 were found to be in significant disequilibrium within the 1997 sample ($P < 0.005$).

Assignment tests

Using the Bayesian assignment criterion within the 1994 samples, 97% of all Enos Lake sticklebacks were correctly assigned to species as previously defined using morphology. Two fish were mis-classified; one fish morphologically classified as a benthic was genetically classified as a limnetic and one fish morphologically classified as a limnetic was genetically classified as a benthic. Another five fish had differences in their log-likelihood benthic vs. limnetic scores of less than 1.0, a value that corresponded to less than an 80% chance of being classified as either a benthic *or* as a limnetic. The average difference in 'benthic' and 'limnetic' log-likelihood scores among the mis-classified fish ($N = 2$) and those that were ambiguous ($N = 5$) was 0.29. We removed these seven fish from the database and used the remaining fish as a baseline with which to classify all further samples. For the subsequent classifications employing the 1994 microsatellite baseline samples of benthics and limnetics, we used a 'cut-off' difference in log-likelihood scores of 0.29 to identify fish of putative hybrid ancestry, i.e. any fish for which the difference in log-likelihood benthic or limnetic was 0.29 or lower was classified as of hybrid ancestry.

In all years the majority of fish sampled were classified as benthics (average = 74.7%), many fewer as limnetics (average = 17.9%), and the fewest as hybrids (average = 8.2%, Table 3). The bias towards benthics was even apparent both when the limnetic (with seines) and littoral (with traps) habitats of the lake were sampled (i.e. years 2000, 2002). In addition, the confidence that individual fish could be classified as either benthic or limnetic dropped

Table 3 Assignment of three-spined sticklebacks from Enos Lake to benthic, limnetic or hybrid categories shown as raw counts with percentages in parentheses

Year	Benthic	Limnetic	Hybrid	Mean difference
1994	36 (48.6)	31 (41.9)	7 (11.1)	3.76 (1.9)
1997	77 (80.2)	12 (12.5)	7 (7.3)	2.23 (1.3)
2000-J	63 (65.0)	27 (28.0)	6 (7.0)	2.23 (1.2)
2000-A	129 (83.7)	15 (9.7)	10 (6.5)	2.44 (1.3)
2002	78 (70.2)	23 (20.7)	10 (9.1)	1.93 (1.3)

Also shown is the mean difference (SD) in individual log-likelihood scores fish assigned as a benthic or limnetic. Assignment was based on variation at five microsatellite loci. All samples are for adult fish except for 2000 where J, juvenile stickleback; A, adult stickleback.

from 1997 to 2002 (Table 3). For instance, the average log-likelihood difference across individuals for the 1997 sample was 2.23 (SD = 1.3) and was significantly smaller (t-test, $P < 0.001$) compared to 3.76 (SD = 1.9) for the 1994 samples. Similarly, the average difference in log-likelihood was 2.23 (1.25) and 2.44 (1.28) for the juvenile and adult samples collected in 2000, respectively (t-test, $P > 0.05$), both of which were significantly lower than for the samples collected in 1994 (both $P < 0.001$). Finally, for the 2002 samples, the difference in log-likelihood scores was the lowest among all years, 1.93 (SD = 1.26) which was significantly lower than for the 1994 baseline samples (t-test, $P < 0.001$). The assignment tests suggested that the level of hybridization was relatively stable despite variation in sampling regime across years, ranging from 6.5% in 2000 to 10% in 1997. In addition, assignment-based identification of hybrids showed similar levels of hybridization between juvenile and adult samples collected in the same year (2000, Table 3).

The genetic variation among all samples was summarized by factorial correspondence analysis (FCA, Fig. 5). The data indicated two clearly distinctive groups of sticklebacks in the 1994 and that all subsequent years' samples were intermediate to the 1994 samples along FCA axis 1, but to varying degrees (Fig. 5). The 'simulated F_1 hybrid' 1994 sample was, as expected, almost exactly intermediate along both FCA axes (Fig. 5). Adult fish collected in 1997 and 2000 were intermediate to benthic and limnetic scores along FCA axis 2, but those for juveniles collected in 2000 were more similar to benthics (Fig. 5). Samples collected in 2002 were highly divergent both from benthics and limnetics along FCA axis 1, and relatively closer to benthics along FCA axis 2 (Fig. 5).

For the 1997 and 2000 samples we had both FCA and warp morphological scores for individual fish (Fig. 6). For 1997, there was no association between morphology and genetic score along FCA axis 1 or axis 2 (i.e. high PCA

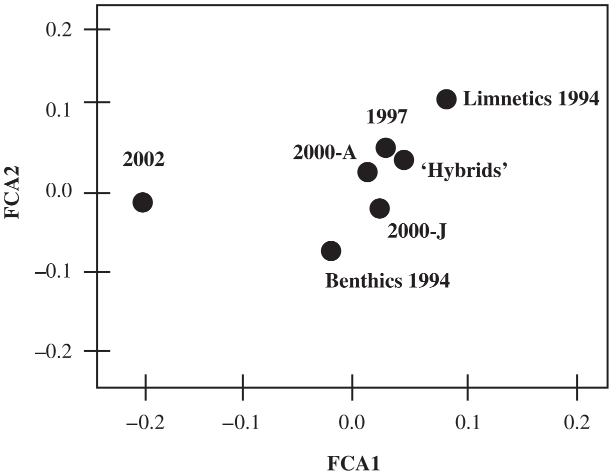

Fig. 5 Plots of the mean scores from a factorial correspondence analysis (FCA) of variation at five microsatellite loci for Enos Lake three-spined sticklebacks collected across four years. Collection year is indicated next to each sample point, 'Hybrids' for simulated hybrids between 1994 Benthic and Limnetic samples. All samples are adult fish except for 2000 where adults ('2000-A') and juvenile ('2000-J') samples are differentiated.

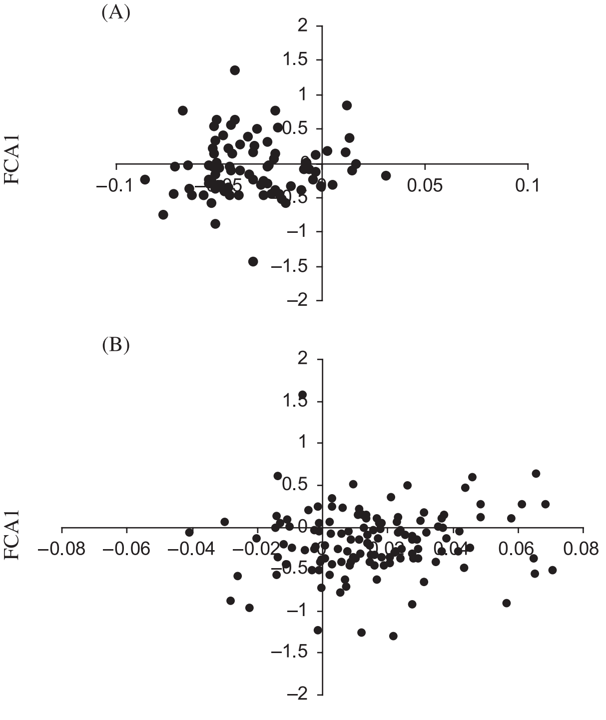

Fig. 6 Bivariate plots of factorial correspondence scores (FCA) along FCA axis 1 for individual Enos Lake three-spined sticklebacks derived from variation at five microsatellite loci and morphological warp score for the same fish for (A) 1997 and (B) 2000.

Table 4 Mean likelihood scores and their standard deviations from 10 runs of the STRUCTURE program for each hypothesized number of populations (*K*) of three-spined sticklebacks inferred from variation at five microsatellite loci. Bold values are the most likely population structure

Sample year	*K*	log-likelihood	SD
1994	1	−840.2	1.85
1995	**2***	**−798.6**	**3.76**
1994	3	−911.8	22.8
1997	**1***	**−854.8**	**5.1**
1998	2	−870.4	92.4
1999	3	−883.6	28.7
2000	**1***	**−2309.2**	**0.98**
2001	2	−2341.7	146.9
2002	3	−2364.5	28.6
2002	**1***	**−1037.34**	**1.9**
2002	2	−1104.2	28.9
2002	3	−1171.5	110.5

*Indicates significantly most likely among models within a year, as determined by likelihood ratio tests.

scores were not consistently associated with particular FCA1 scores, $r = -0.04$ and 0.1, respectively, both $P > 0.01$, Fig. 6A). Similarly, no significant morphological–genetic associations were found for the 2000 adult samples along FCA 1 or 2 ($r = 0.10$ and -0.07, $P > 0.1$, Fig. 6B).

Population structure

The program STRUCTURE performed on each of the temporal samples indicated that, as expected, the most likely number of populations was 2 in the 1994 sample (Table 4). By contrast, for all subsequent samples (1997, 2000, 2002) the most likely number of populations in Enos Lake was 1 and in each year the inference of $K = 1$ was significantly more likely than models involving $K = 2$ or 3 populations (Table 4).

Discussion

Evidence for the collapse of the species pair

Our study provides strong evidence of the apparent fragility of recently derived species and their susceptibility to collapse upon environmental change. Our quantitative morphological and genetic assessment of the collapse of a *Gasterosteus* species pair supported the predictions based on the hypothesis that hybridization between benthics and limnetics is the primary cause of the collapse of the Enos Lake species pair, rather than by biased extinction of limnetics. First, our analyses revealed a pronounced shift in the morphological characterization of sticklebacks in Enos Lake using the relative warp-PC analysis; two distinct morphological clusters (years 1977, 1988) have been replaced by one (1997–2002). The general tendency has been a shift from distinct benthic and limnetic phenotypes to intermediate phenotypes, with a bias towards the benthic phenotype in the later years. The fact that the later year samples (2000, 2002) contain only a single morphological cluster and that the variability within this cluster is larger than in the years with two distinct clusters is consistent with hybridization driving the morphological changes. The presence of parental phenotypes, plus an array of hybrid phenotypes, presumably representing F_1 and post F_1 hybrids, should result in increased morphological variability (cf. McPhail 1984). If, however, there has been biased extinction of limnetic sticklebacks, it is possible that character release in the remaining benthic form could have contributed to increased morphological variability in Enos Lake (Schluter & McPhail 1992).

The results of our microsatellite analyses were strikingly congruent with the morphological data and further strengthen the collapse by hybridization hypothesis; samples examined after 1994 were best characterized by a single genetic population in Enos Lake, as defined by the Bayesian analysis, and supported our second prediction of a single genetic population. In addition, when the data were subject to assignment tests using the 1994 samples as the baseline, the average difference in log-likelihood scores showed a decline indicating reduced genetic differentiation between individuals that were classified as 'benthic' or 'limnetic' among the samples collected between 1997 and 2002. Consequently, both the morphological and genetic data indicate an apparently progressive decline in the distinction between benthic and limnetic sticklebacks in Enos Lake. More importantly, both sets of data are consistent with this change being the result of hybridization and introgression rather than biased extinction; both indicate a decay of distinct morphological and genetic clusters previously associated with benthics and limnetics and the emergence of a single cluster that is intermediate in trait values.

The results of the STRUCTURE analysis are also consistent with our second prediction (a single genetic population) and with our third prediction that linkage disequilibrium evident in precollapse samples should decline with the production of a hybrid swarm by introgression (gene flow). The method resolves the most likely number of populations subject to the constraint that Hardy–Weinberg and linkage disequilibria are minimized within each resolved population (Pritchard *et al.* 2000). Consequently, the only way in which more than one population can be inferred as most likely in a data set, as was the case for only the 1994 sample, is for significant genetic disequilibria to be present in that sample. Because only one population was inferred for the samples from 1997 to 2002, this clearly indicates that Hardy–Weinberg and/or linkage disequilibrium had declined between the 1994 sample and all subsequent samples. This result, however, does not necessarily imply that hybridization became significant only after 1994 because the 1994 samples may not have been representative of the true genotypic distribution in the lake. In fact, although we discarded morphologically intermediate fish or those which were not classified correctly by microsatellites as benthics or limnetics to generate a learning sample for analysis of subsequent years' samples, we still observed 11% hybrids in our 1994 sample. Compared to subsequent years, however, the 1994 sample had about an equal percentage of fish assigned to the benthic and limnetic categories (48% vs. 41%, respectively) when we sampled both the littoral and limnetic habitats of the lake. No subsequent year, however, had more than 28% limnetics. In addition, even when the 11% hybrids identified in the 1994 sample were included, two distinct populations were identified as the most likely population structure in the lake by the STRUCTURE analysis. These observations suggest that hybridization, although clearly evident in 1994, was probably not as extensive as in subsequent years. Extensive hybridization in samples from 1994 and later stands in marked contrast to morphological and genetic indications of two distinct gene pools, and little to no hybridization between them, before this time (McPhail 1984).

Our fourth prediction was that the association between morphological and genetic distinctions between benthics and limnetics should decay with hybridization. Body shape and features of trophic morphology are the hallmark traits that distinguish benthic and limnetic sticklebacks in all the species pair lakes (e.g. Larson 1976; McPhail 1984, 1992; Schluter & McPhail 1992). In addition, multilocus genetic differentiation is apparent between benthics and limnetics (McPhail 1984, 1992; Taylor & McPhail 2000). As a consequence of hybridization and gene flow, morphological–genotype associations that distinguish parental species should decay as recombinant genotypes are produced. Both morphological and genetic data document a decrease in differentiation across time in our samples. We were unable, however, to test this prediction fully because we lack samples for which genetic and morphological data have been collected before hybridization. We were unable to examine the 1994 samples in detail morphologically and repeated attempts at extracting and amplifying DNA from earlier, formalin-preserved samples were too inconsistent for a robust analysis. We found no significant correlations between morphological (individual warp scores) and microsatellite (FCA scores) measures of differentiation in sticklebacks in the two years for which we had comparable data (1997 and 2000). This indicates that there is little to no remaining association between our assays of morphological and genomic differentiation in Enos Lake and suggests that the benthic and limnetic stickleback species pairs may have collapsed into an admixed hybrid swarm.

Mechanisms driving the collapse

Our data confirm Kraak *et al.*'s (2001) suggestion of increased levels of hybridization in Enos Lake sticklebacks. This likely has come about by a breakdown of premating isolation, although increased fitness of hybrids could also have contributed. What ecological factors might be driving such change and what features of Enos Lake have changed over recent times that might promote hybridization? We do not yet have the necessary data to answer this question, but the following observations suggest some potential causal mechanisms. The most obvious change to the lake has been that the American signal crayfish (*Pascifasticus lenisculus*) has become established in Enos Lake since the last intensive field work was undertaken in the late 1980s (Bentzen *et al.* 1984; COSEWIC 2002; personal observations). Although the signal crayfish is native to southwestern BC, it is unclear if it is native to coastal islands such as Vancouver Island (Crandall *et al.* 2000; personal communication). The crayfish may have promoted hybridization by altering: (i) the mating behaviour or habitat choice of benthics, limnetics, or both; (ii) the vegetation abundance or distribution; or (iii) the water clarity and/or distribution of trophic resources. It is known that introduced crayfish, including the signal crayfish, may have extremely detrimental effects on native aquatic faunas through a variety of mechanisms (Rahel 1989; Guan & Wiles 1996; Dorn & Mittlebach 1999; Lodge *et al.* 2000; Carpenter 2005), and this strongly implicates them as a factor in the demise of the Enos Lake species pair. The most obvious possible effect of crayfish in Enos Lake is the destruction of aquatic vegetation and associated increases in turbidity from crayfish movements and omnivorous foraging and the effects of such changes on premating isolation in sticklebacks (e.g. Nystrom *et al.* 1996).

Benthic and limnetic sticklebacks show different microhabitat preferences for construction of nests by males. Benthics nest in heavily vegetated areas in the littoral zone, whereas limnetic males nest in open water portions of the littoral zone (Ridgway & McPhail 1984). In addition, male and female sticklebacks engage in elaborate courtship behaviour during mate choice where visual cues are clearly important (Ridgway & McPhail 1984). Boughman (2001) demonstrated that *Gasterosteus* populations inhabiting different lakes experience distinct light environments and that female perceptual sensitivity to red light varies with the degree of red shift in the light environment. Such perceptual differences probably contribute to divergent preferences and, hence, reproductive isolation. Visual cues and discrimination during mating could be compromised by changes to water turbidity and clarity as has been demonstrated for cichlid fishes in Lake Victoria, eastern Africa (Seehausen *et al.* 1997). If crayfish have caused changes in macrophyte abundance and/or distribution and/or changes in water clarity, then the potential for microhabitat segregation or visual discrimination may have been compromised and isolation reduced. In summary, both changes to vegetation and/or water clarity could provide plausible mechanisms to explain increases in hybridization in the Enos Lake species pair. In addition, it is possible that the distribution of trophic resources has changed such that intermediate resources are now in greater abundance increasing the fitness of morphologically intermediate hybrids. There are, however, presently no data from Enos Lake to assess whether or not these environmental factors have changed from the 1980 to the present day and such a monitoring programme is required to assess future changes.

In addition, crayfish may prey directly on sticklebacks (personal observations) which could influence the relative abundance of either or both species and perhaps promote hybridization. Reduced abundance of one parental species limits homotypic mating opportunities and is often associated with interspecific hybridization (Wirtz 1999). Finally, crayfish feeding may have changed the forage base of macro-invertebrates that may also compromise ecological segregation between benthics and limnetics or alter the relative fitness of hybrids and parental genotypes. While these hypotheses remain to be tested, it is clear that the appearance of crayfish in the lake is associated in time with the increased abundance of hybrids in Enos Lake. The well-known detrimental effects of crayfish on native aquatic faunas strongly implicate them in the demise of the Enos Lake species pair. The extirpation of what used to be an abundant population of the rough-skinned newt, *Taricha granulosa*, in Enos Lake also follows the appearance of the signal crayfish (personal observations, Gamradt & Kats 1996). We cannot, however, rule out the possibility that the appearance of the crayfish and the increased hybridization between stickleback species are two responses to a third change in the environment, e.g. increased nutrient influx to the lake due to the increased urbanization in the area.

Evolutionary and conservation implications

The stickleback species pairs of southwestern BC are key examples of postglacial diversification of north temperate freshwater fishes. Similar combinations of morphological, ecological, and genetic differentiation between sympatric pairs of undescribed taxa that behave as biological species are widespread in at least two other fish families: Salmonidae (*Salvelinus*, *Coregonus*, *Prosopium*, *Oncorhynchus*, *Salmo*), and Osmeridae (smelts, *Osmerus*) (reviewed by Schluter 1996; Taylor 1999). These divergences are usually considered to have been rapid (i.e. occurring since the Wisconsinan glaciation ended some 10 000 years ago) and largely driven by a combination of ecological speciation (*sensu* Schluter 1996) and geomorphological conditions unique to formerly glaciated habitats (e.g. formation of novel habitats, opportunities for multiple invasions, Taylor & McPhail 2000). Consequently, these cases of sympatric pairs are excellent examples of the importance of the environmental context as a driving force in evolutionary divergence rather than by random processes. Our evidence for the collapse of a sympatric pair emphasizes the need to investigate the potential importance of environmental factors both to further our understanding of basic processes of speciation/diversification and to develop responses/management plans that can preserve species.

If our proposed mechanisms for the collapse are substantiated, the Enos Lake situation suggests how ephemeral and reversible such divergences can be. The idea of reversibility is a relatively old one in the literature of sympatric species pairs (e.g. Frost 1965; Savvaitova 1980) and has received renewed attention in the more general literature (Porter & Crandall 2003; Coyne & Orr 2004). In discussing the confused taxonomy of the many sympatric pairs within the 'Arctic charr (*Salvelinus alpinus*) complex', Savvaitova (1980) suggested that the environmental instability of aquatic freshwater habitats in the temperate to high Arctic areas is associated with reproductive barriers that are short lived. Such variable environments may have promoted rapid divergence on the one hand, but also hold the potential for rapid reversal of divergence when these environments change. Such potential collapse of divergent forms is probably related to the relative youth of such systems that are continually 'reborn' following frequent environmental changes throughout the Pleistocene. Bell (2001) made similar arguments when discussing the rapid evolution of lateral plate morphs of *Gasterosteus aculeatus* in ephemeral freshwater habitats and their subsequent extinction when environmental conditions change. Typically, prezygotic isolation and extrinsic postzygotic isolation are thought to evolve before intrinsic postzygotic isolation and characterize relatively recent divergences (e.g. Coyne & Orr 2004). Consequently, the apparent fragility of the Enos Lake species pair following a change in environmental conditions in the lake is consistent with the probable evolutionary youth of divergences in north temperate freshwater fishes, and with observations of other instances of hybridization following environmental changes in fishes (Hubbs 1955; Todd & Stedman 1989; Arnold 1997; Seehausen *et al*. 1997).

Strictly speaking, however, we do not know that the collapse of the Enos Lake species pair of *Gasterosteus* is a case of true speciation in reverse, i.e. a reversal of the processes that lead to the diversification of species pairs. We cannot, for instance, be certain that ecological selection is contributing to the collapse by favouring a return to the ancestral form of solitary stickleback, or that sexual selection is actually favouring nonassortative mating. Further, the collapse may be being driven by a combination of both processes, or by enhanced gene flow owing to a breakdown of reproductive barriers and only weakly opposed by selection against hybrid phenotypes. Much remains to be studied to understand the true mechanisms promoting hybridization and how the relative fitness of parental species and hybrids may be contributing to the collapse and our study of the Enos Lake species pair may promote such investigations.

Our results have implications for the conservation of *Gasterosteus* species pairs. First, we found no correlations between genetic and morphological traits in the Enos Lake sticklebacks. This suggests that even if the environment was restored to such a condition that promoted former levels of reproductive isolation between benthics and limnetics, natural and/or sexual selection may not act quickly to return the species to their previous levels of distinction. A more comprehensive morphology–genetic analysis using many more loci will be required to assess if the apparent lack of an association between neutral molecular and morphological divergence is characteristic of the genome as a whole. Further, divergence of benthics and limnetics following environmental restoration implies that sympatric divergence played a major role in the evolution of stickleback sympatric pairs, yet a period of allopatry appears to be necessary to initiate divergence and promote the evolution of these species pairs (Taylor & McPhail 2000). Second, theoretical and empirical work on disturbed lake ecosystems often indicates that restoring environmental conditions to pre-impact states is no guarantee that biodiversity will also return to pre-impact conditions (Scheffer *et al*. 2001). Third, further sampling of Enos Lake might be directed towards identifying remaining limnetics in the system. Any identified association between genetic and phenotypic differentiation could be exploited in a captive breeding programme to select fish at extreme ends of either axis of differentiation to artificially produce offspring with the most 'benthic-like' and 'limnetic-like' characteristics to use in possible reintroduction programmes. Finally, and more generally, the original sympatric pairs of *Gasterosteus* were endemic to six lakes on three islands in southwestern BC. These pairs diverged postglacially (Taylor & McPhail 1999, 2000) and their recent origin might suggest that they do not represent high conservation priority when measured against the yardstick of evolutionary depth of divergence (Bowen 1999). On the other hand, owing to the fact that the stickleback species pairs can still exchange genes, they represent speciation in progress and have become excellent models for the study of the ecological and genetic basis of adaptive divergence and speciation (e.g. Coyne & Orr 2004; Colosimo *et al*. 2005). Following the extinction of the Hadley Lake (Lasqueti Island) species pair after introduction of a non-native catfish, *Ameiurus nebulosus* (Hatfield 2001), the situation in Enos Lake represents an additional threat to the continued persistence of these young species. Such extinctions are a further example of the increasing threat to freshwater ecosystems from anthropogenically induced habitat changes (Ricciardi & Rasmussen 1999).

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References

Arnold ML (1997) *Natural Hybridization and Evolution*. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Belkhir K, Borsa P, Chikhi N, Raufaste N, Bonhomme F (2001) *GENETIX 4.02, Logiciel sous Windows™ pour la genetique des populations*. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Universite de Montpellier II, Montpellier, France.

Bell MA (2001) Lateral plate evolution in the threespine stickleback: getting nowhere fast. *Genetica*, **112–113**, 445–461.

Bentzen P, McPhail JD (1984) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): specialization for alternative trophic niches in Enos Lake species pair. *Canadian Journal of Zoology*, **62**, 2280–2286.

Bentzen P, Ridgway MS, McPhail JD (1984) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): spatial segregation and seasonal habitat shifts in the Enos lake species pair. *Canadian Journal of Zoology*, **62**, 2436–2439.

Bookstein FL (1991) *Morphometric Tools for Landmark Data*. Cambridge University Press, Cambridge, UK.

Boughman JW (2001) Divergent sexual selection enhances reproductive isolation in sticklebacks. *Nature*, **411**, 944–948.

Bowen BW (1999) Preserving genes, species, or ecosystems? Healing the fractured foundations of conservation policy. *Molecular Ecology*, **8** (s1), S5–S10.

Carpenter J (2005) Competition for food between an introduced crayfish and two fishes endemic to the Colorado River basin. *Environmental Biology of Fishes*, **72**, 335–342.

Colosimo PF, Hosemann KE, Balabhadra S *et al*. (2005) Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. *Science*, **307**, 1928–1933.

COSEWIC (2002) *COSEWIC Assessment and Update Status Report on the Enos Lake Stickleback Species Pair,* Gasterosteus *spp. in Canada*. Committee on the Status of Endangered Wildlife in Canada, Ottawa

Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Sunderland, Massachusetts.

Crandall KA, Harris DJ, Fetzner JW Jr (2000) The monophyletic origin of freshwater crayfish estimated from nuclear and mitochondrial DNA sequences. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267**, 1679–1686.

Dorn NJ, Mittlebach GG (1999) More than predators and prey: a review of interactions between fish and crayfish. *Vie et Milieu*, **49**, 229–237.

Fraley D, Raftery AE (2003) Enhanced software for model-based clustering, discriminant analysis and density estimation: MCLUST. *Journal of Classification*, **20**, 263–286. http://www.stat.washington.edu/fraley/mclust/.

Frost WE (1965) Breeding habits of Windermere charr, *Salvelinus willughbii* (Günther), and their bearing on speciation of these fishes. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **163**, 232–284.

Gamradt SC, Kats LB (1996) Effects of introduced crayfish and mosquitofish on California newts. *Conservation Biology*, **10**, 1055–1061.

Grant PR, Grant BR (2002) Unpredictable evolution in a 30 year study of Darwin's finches. *Science*, **296**, 707–711.

Guan R-Z, Wiles PR (1996) Ecological impacts of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology*, **11**, 641–647.

Hatfield T (2001) Status of the stickleback species pair, *Gasterosteus* spp., in Hadley Lake, Lasqueti Island, British Columbia. *Canadian Field Naturalist*, **115**, 579–583.

Hendry AP, McPhail JD, Taylor EB (2002) Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. *Evolution*, **56**, 1199–1216.

Hubbs CL (1955) Hybridization between fish species in nature. *Systematic Zoology*, **4**, 1–20.

Huelsenbeck JP, Rannala B (1997) Phylogenetic methods come of age: testing hypotheses in an evolutionary context. *Science*, **276**, 227–239.

Kraak SBM, Mundwiler B, Hart PJB (2001) Increased number of hybrids between benthic and limnetic threespined sticklebacks in Enos Lake, Canada: the collapse of a species pair? *Journal of Fish Biology*, **58**, 1458–1464.

Larson GL (1976) Social behavior and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. *Canadian Journal of Zoology*, **54**, 107–121.

Lodge DM, Taylor CA, Holdich DM, Skurdal J (2000) Non-indigenous crayfishes threaten North American freshwater biodiversity: lessons from Europe. *Fisheries*, **25**, 7–20.

McKinnon JS, Rundle HR (2002) Speciation in nature: the three-spine stickleback model systems. *Trends in Ecology & Evolution*, **17**, 480–488.

McPhail JD (1984) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. *Canadian Journal of Zoology*, **62**, 1402–1408.

McPhail JD (1989) Status of the Enos Lake stickleback species pair, *Gasterosteus* spp. *Canadian Field Naturalist*, **103**, 216–219.

McPhail JD (1992) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Paxton Lake, British Columbia. *Canadian Journal of Zoology*, **70**, 361–369.

McPhail JD (1994) Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. In: *The Evolutionary Biology of the Threespine Stickleback* (eds Bell AM, Foster SA). Oxford University Press, Oxford.

Nielsen EE, Hansen MM, Bach LA (2001) Looking for a needle in a haystack: discovery of indigenous Atlantic salmon (*Salmo salar* L.) in stocked populations. *Conservation Genetics*, **2**, 219–232.

Nystrom P, Bronmark C, Graneli W (1996) Patterns in benthic food webs: a role for omnivorous crayfish? *Freshwater Biology*, **36**, 631–646.

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, **95**, 536–539.

Porter ML, Crandall KA (2003) Lost along the way: the significance of evolution in reverse. *Trends in Ecology & Evolution*, **18**, 541–547.

Pritchard J, Stephens K, Donnelly P (2000) Inference of population structure from multilocus genotype data. *Genetics*, **155**, 945–959.

Rahel FJ (1989) Nest defense and aggressive interactions between a small benthic fish (the Johnny darter *Etheostoma nigrum*) and crayfish. *Environmental Biology of Fishes*, **24**, 301–306.

Raymond M, Rousset RR (2001) GENEPOP (version 3.3): Population genetics software for exact tests and ecumenism. Available from http://www.cefe.cnrs-mop.fr/. (updated from Raymond & Rousset 1995, *Journal of Heredity* **86**, 248–249).

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics*, **27**, 83–109.

Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. *Conservation Biology*, **13**, 1220–1222.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Rico C, Zadworny D, Kuhnlein U, Fitzgerald GJ (1993) Characterization of hypervariable microsatellite loci in the threespine stickleback *Gasterosteus aculeatus*. *Molecular Ecology*, **2**, 271–272.

Ridgway MS, McPhail JD (1984) Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): mate choice and reproductive isolation in the Enos Lake species pair. *Canadian Journal of Zoology*, **62**, 1813–1818.

Rohlf FJ (1997) *TPSDIG and TPSRELW programs*. Available from http://life.bio.sunysb.edu/morph.

Rohlf FJ (1999) Shape statistics: procrustes superimpositions and tangent spaces. *Journal of Classification*, **16**, 197–223.

Savvaitova KA (1980) Taxonomy and biogeography of charrs in the Palearctic. In: *Charrs: Salmonid Fishes of the Genus* Salvelinus (ed. Balon EK), pp. 281–294. Dr W. Junk Publishers, The Hague.

Scheffer M, Carpenter S, Foley JA, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. *Nature*, **413**, 591–596.

Schluter D (1996) Ecological speciation in postglacial fishes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **351**, 807–814.

Schluter D (2000) *The Ecology of Adaptive Radiation*. Oxford Series in Ecology and Evolution, Oxford University Press, Oxford.

Schluter D, McPhail JD (1992) Ecological character displacement and speciation in sticklebacks. *American Naturalist*, **140**, 85–108.

Seehausen O, van Alphen JJM, Witte F (1997) Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, **277**, 1808–1811.

Taylor EB (1998) Microsatellites isolated from the threespine stickleback *Gasterosteus aculeatus*. *Molecular Ecology*, **7**, 930–931.

Taylor EB (1999) Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Reviews in Fish Biology and Fisheries*, **9**, 299–324.

Taylor EB (2004) An analysis of homogenization and differentiation of Canadian freshwater fish faunas with an emphasis on British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences*, **61**, 68–79.

Taylor EB, McPhail JD (1999) Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus aculeatus*). *Biological Journal of the Linnean Society*, **66**, 271–299.

Taylor EB, McPhail JD (2000) Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **267** (1460), 2375–2384.

Todd TN, Stedman RM (1989) Hybridization of ciscoes (*Coregonus* spp.) in Lake Huron. *Canadian Journal of Zoology*, **67**, 1679–1685.

Wirtz P (1999) Mother species-father species: unidirectional hybridization in animals with female choice. *Animal Behaviour*, **58**, 1–12.

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