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SHORT COMMUNICATION

Conservation genetics of prickly sculpin (*Cottus asper*) at the periphery of its distribution range in Peace River, Canada

Stefan Dennenmoser · Arne W. Nolte · Steven M. Vamosi · Sean M. Rogers

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Abstract Populations at the edge of their range often invoke taxonomic confusion and are increasingly considered to harbour cryptic genetic diversity of significant adaptive potential. In the Peace River region of northwestern Canada, three sculpin species have been reported: spoonhead (*Cottus ricei*), slimy (*Cottus cognatus*) and prickly (*Cottus asper*) sculpin. Prickly sculpin occurrence in this region represents the most eastern edge of its distribution, but its status has remained uncertain following its initial discovery in 1989. These populations may represent an independently evolving lineage of special conservation concern, or be the consequence of an ongoing range expansion, possibly accompanied by interspecific hybridization with local species. Using a combination of mtDNA sequencing and microsatellite analyses, we did not find peripheral population differentiation or interspecific hybridization, suggesting that the Albertan Peace River population belongs to the same genetic group as its western counterparts. Future studies will benefit from a greater understanding of whether demographically independent prickly sculpin populations established in Alberta without the typical genetic signatures of expansion at the periphery of their range.

Keywords Conservation genetics · Freshwater fishes · Peripheral populations · Hybridization · *Cottus*

S. Dennenmoser (✉) · S. M. Vamosi · S. M. Rogers
Department of Biological Sciences, University of Calgary, 2500 University Dr. NW, Calgary, AB T2N 1N4, Canada
e-mail: sdennenm@ucalgary.ca

A. W. Nolte
Max-Planck Institute for Evolutionary Biology, August-Thienemannstrasse 2, 24306 Plön, Germany

Introduction

The maintenance of genetic variation and evolutionary processes that generate diversity are of major interest in conservation biology, with the aim of enhancing the adaptive potential of threatened species and, ultimately, their chances of long-term survival (Ficetola and Bonin 2011). Populations at the periphery of their distribution range can be small and experience increased genetic drift compared to core populations (Lesica and Allendorf 1995). This may support the emergence of population differentiation and cryptic genetic diversity critical in the face of rapid environmental change (Moritz et al. 2012). However, peripheral populations may not only be sources of adaptive diversity, but can also represent immigrant-dominated sinks where adaptation is hampered by small population sizes and reduced or maladaptive gene flow from central populations (Sexton et al. 2009). Moreover, postglacial colonization may contribute to low genetic diversity and weak divergence of peripheral populations (e.g., Cassel and Tammaru 2003), but surprisingly few studies have examined genetic structure at the leading edge of range expansions (Short and Petren 2011; Nullmeier and Hallatschek 2013). Range expansions may also increase chances of interspecific hybridization with neighboring species (Allendorf et al. 2001), which under some circumstances can facilitate expansion of the invading species (Ellstrand and Schierenbeck 2000). Hence, it is becoming increasingly important to understand the stability and demographic independence of peripheral populations.

Freshwater sculpins are cold-tolerant fishes of northern-temperate regions, and are of conservation interest because they often occur in highly structured populations over small geographic areas (e.g., Nolte et al. 2005a; Junker et al. 2012). Prickly sculpin (*Cottus asper*) occurrence in

the Peace River region of Alberta is thought to represent the most eastern edge of its distribution range, but its status since its initial discovery in 1989 has remained uncertain (Roberts 1990). Albertan prickly sculpin populations are presumably of low abundance, isolated and may have diverged from distant source populations thereby representing independently evolving lineages of special conservation interest. Alternatively, their presence could be the consequence of an ongoing range expansion and be reflected by high immigration rates and weak population differentiation. Hybridization with local *Cottus* species appears possible given numerous reports of hybridization among even distantly related clades of *Cottus* species (Nolte et al. 2005a; McPhail 2007). Herein, we used microsatellites and mitochondrial DNA to test for peripheral population differentiation and interspecific hybridization of *C. asper*.

Methods

Sampling and DNA analysis

We sampled sculpins non-invasively from the Peace River in British Columbia in 2009 near Hudson's Hope, and in 2010 in Alberta near Dunvegan and Many Islands Park with minnow traps or backpack-electrofishing (Fig. 1; Table 1). Samples from British Columbia were exclusively *C. asper*, whereas Albertan samples also included *C. cognatus* and *C. ricei*. Additionally, we included *C. asper* samples from the southwestern periphery of the Peace River watershed (McLeod Lake), and provide genetic diversity indices from two coastal (Little Campbell River, Mosquito Lake) and two inland (Okanagan Lake, Lakelse Lake) populations across the distribution range of *C. asper* in British Columbia (Fig. 1; Table 1). Tissues from the second dorsal fin were used for genetic analyses and stored in 95 % ethanol. DNA was extracted using a standard phenol–chloroform technique. Using the general fish primers FishF1 (5′-TCAACCAACCACAAAGACATTG GCAC-3′) and FishR1 (5′-TAGACTTCTGGGTGGCCAA AGAATCA-3′) (Ward et al. 2005), 583 base pairs of mitochondrial DNA (Cox1) was PCR-amplified and sequenced for species verification and a comparison of *C. asper* populations from the Peace River in British Columbia ($n = 10$) and Alberta ($n = 12$). PCR reactions were performed in 25-µl volumes containing 1× PCR-Buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer, 0.625 U of TAQ-polymerase, and ~200 ng genomic DNA. Cycling conditions consisted of 95 °C for 2 min, followed by 35 cycles of 94 °C (30 s), 52 °C (40 s), 72 °C (60 s), and a final elongation of 72 °C for 10 min. A total of 19 microsatellite loci from Nolte et al. (2005b) were used to estimate population differentiation between *C. asper* populations (*Cott170*, *Cott207*, *Cott255*, *Cott54*, *CottE30*, *LCE54*, *Cott214*, *Cott224*, *Cott78*, *Cott348*, *CottE11*, *CottE20*, *Cott153*, *Cott213*, *CottE13*, *LCE81*, *Cott50*, *Cott686*, *CottE23*). Interspecific cross amplification of the following subsets of loci was experimentally validated and permitted assessing hybridization among species: *C. asper–Cottus cognatus*: 16 loci (*Cott170*, *Cott207*, *Cott255*, *Cott54*, *LCE54*, *Cott214*, *Cott224*, *Cott78*, *CottE11*, *CottE20*, *Cott153*, *Cott213*, *CottE13*, *LCE81*, *Cott686*, *CottE23*), *C. asper–Cottus ricei*: 8 loci (*Cott255*, *CottE30*, *Cott224*, *CottE20*, *CottE13*, *LCE81*, *Cott50*, *Cott686*), *C. ricei–C. cognatus*: 6 loci (*Cott207*, *Cott255*, *Cott224*, *CottE20*, *CottE13*, *LCE81*). Microsatellites were multiplexed with up to six fluorescently-labeled primers using QIAGEN Multiplex Kits, and amplified in 10 µl-reactions (5 µl Qiagen-Multiplex-Kit, 0.3–0.6 µM primer, 40 ng DNA-template). PCR protocols consisted of an initial denaturation step of 95 °C for 15 min, followed by 38 cycles of 94 °C (30 s), 60 °C (90 s), 72 °C (60 s), and a final elongation of 72 °C for 30 min. PCR products were run on an ABI 3730xl sequencer, and allelic sizes (in base pairs) were determined by reference to the internal sizing standard in the software GENEMAPPER version 4.1 (Applied Biosystems). Microsatellite data files are deposited under Dryad (doi:10.5061/dryad.9061f).

Statistical analyses

Mitochondrial DNA sequences were aligned and visually inspected using ClustalX 2.1 (Larkin et al. 2007). We used an extended version of Fisher's exact test for testing microsatellite loci for Hardy–Weinberg equilibrium (HWE, 10^5 Markov chain steps, 10^4 dememorization steps) and linkage disequilibrium (LDE, 10^5 Markov chain steps, 10^4 dememorization steps) implemented in the software ARLEQUIN v. 3.5.1.2 (Excoffier et al. 2005). Sequential Bonferroni correction was applied to P values to correct for multiple comparisons in the same dataset. Pairwise F_{st} values were calculated in ARLEQUIN to test for differentiation between *C. asper* populations. Allelic richness (mean number of alleles, corrected for smallest sample number of ten) was calculated in FSTAT 2.9.3.2 (Goudet 2001). We used a permutation test (15,000 permutations) in FSTAT to compare allelic richness and genetic diversity between (1) coastal and inland populations, and (2) Peace River and inland populations. The possibility of null alleles, large allele drop-outs and scoring errors was evaluated using MICROCHECKER v. 2.2.3 (Van Oosterhout et al. 2004). No evidence for scoring errors or large allele drop-outs was found, and only three loci with potential null alleles were identified: *Cott54* and *CottE13* in *C. cognatus*, and *Cott686* in *C. ricei*. Significant (Bonferroni-corrected)

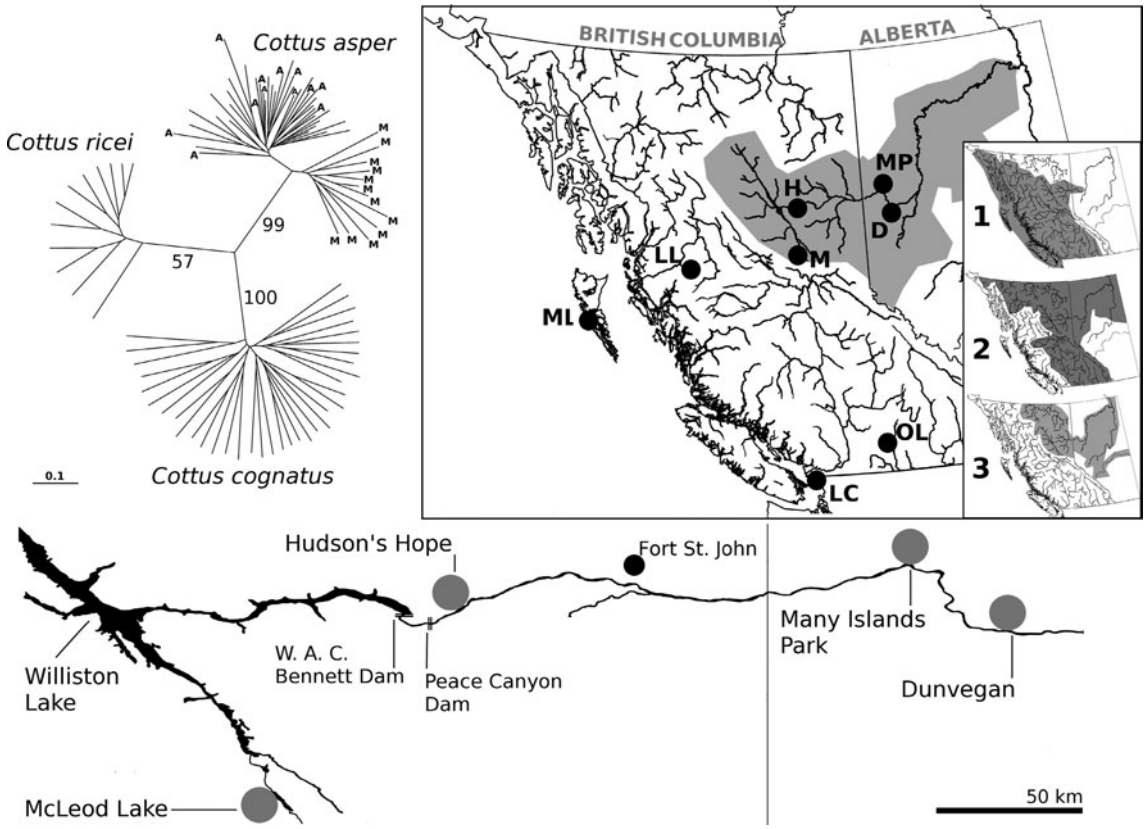

Fig. 1 Map showing sampling sites and study area (Peace River watershed shaded in *grey*). *Abbreviations* represent Mosquito Lake (*ML*), Lakelse Lake (*LL*), Little Campbell River (*LC*), Okanagan Lake (*OL*), McLeod Lake (*M*), Hudson's Hope (*H*), Many Islands Park (*MP*) and Dunvegan (*D*). *Inlet picture* shows distribution ranges of *C. asper* (*1*), *C. cognatus* (*2*) and *C. ricei* (*3*) in Alberta and British Columbia. A bootstrapped Neighbor-Joining tree based on D_C distances of microsatellites is presented on the upper left (for *C. asper*, Albertan samples are marked as "A", and McLeod Lake samples as "M" at branch tips)

departures from HWE were found in four comparisons (three in *C. cognatus*, one in *C. ricei*). Two pairs of loci showed evidence for linkage disequilibrium (*Cott255–CottE30* in *C. asper*, and *CottE20–CottE13* in *C. cognatus*). We calculated individual-based chord distance measures (D_C, Cavalli-Sforza and Edwards 1967) for all three species in MSA 4.05 (Dieringer and Schlötterer 2003). Distance measures were used for construction of a neighbor-joining tree in POPULATIONS 1.2.32 (Langella 1999), which was bootstrapped 1,000 times and visualized in TREEVIEW v. 1.6.6 (Page 1996). An individual-based assignment approach implemented in STRUCTURE 2.2.3 (Pritchard et al. 2000) was used to test for hybridization among species-pairs. Assuming an admixture model and independent allele frequencies, we set the number of clusters to $K = 2$ (two parental species), applied 2×10^4 burn-in steps followed by 10^5 Markov-Chain Monte-Carlo steps, and replicated each analysis five times. Individuals were assigned to parental species for $q > 0.9$, while hybrids were expected to fall within a threshold of $0.1 < q < 0.9$ (Vähä and Primmer 2006).

Results and discussion

Freshwater sculpins have typically been found to exhibit strong population genetic structure on small geographic scales (<50 km), reaching extremes between headwaters of small streams separated by only a few kilometers (Nolte et al. 2005a; Junker et al. 2012). In contrast, we found only weak within-watershed genetic structure over a larger geographic scale (>200 km) between Peace River and McLeod Lake ($F_{st} = 0.013$, $P = 0.015$; Fig. 1). This is particularly surprising given the presence of two dams separating Peace River populations from Williston Lake and adjacent populations such as McLeod Lake. No genetic differentiation was found between Peace River populations of *C. asper* in British Columbia and Alberta, corresponding to the finding of just one mitochondrial DNA haplotype, and a pairwise F_{st} value for microsatellites of zero. Consistently, genetic diversity indices were highly similar (Table 1). Albertan populations of *C. asper* are apparently not demographically independent from distant (>250 km) putative source populations in British Columbia, which

Table 1 Sampling sites for *Cottus asper*, *C. cognatus and C. ricei* with respective geographic location (lat./long.), number of samples (*N*), allelic richness (A_R) and average expected heterozygosity (H_E)

Location	Lat., Long.	*N*	A_R	H_E
Cottus asper				
Dunvegan (D)	55°55.1′ N, 118°36.4′ W	12	3.66	0.37
Hudson's Hope (H)	56°3.9′ N, 121°50.5′ W	30	3.68	0.36
McLeod Lake (M)	54°59.4′ N, 123°2.1′ W	12	3.89	0.37
Lakelse Lake (LL)	54°22.6′ N, 128°33' W	30	4.01	0.43
Okanagan Lake (OL)	50°12.5′ N, 119°27.9′ W	14	4.37	0.47
Mosquito Lake (ML)	53°4.2′ N, 132°4.2′ W	30	5.15	0.59
Little Campbell River (LC)	49°0.9′ N, 122°46.7′ W	30	7.11	0.76
Cottus cognatus				
Dunvegan (D)	55°55.1′ N, 118°36.4′ W	10	4.5	0.49
Many Islands Park (MP)	56°18.9′ N, 119°8.9′ W	26	4.9	0.52
Cottus ricei				
Dunvegan (D)	55°55.1′ N, 118°36.4′ W	12	3.1	0.38

could reflect an ongoing range expansion into Alberta with high immigration rates. An extended planktonic larval stage of 30–35 days and subsequent juvenile upstream migration has been described for coastal populations (Krejsa 1967) and may contribute to the absence of genetic structure, but this remains speculative because we are unaware of any studies that have examined life history characteristics of inland populations. Given the possibility of high migration rates or high numbers of founders overriding effects of genetic drift (e.g., Nullmeier and Hallatschek 2013), more data are needed to make inferences about the timing of range expansion (postglacial or very recent) into Alberta. In contrast to conservation concerns attributed to recent and ongoing expansions into neighboring species ranges (Allendorf et al. 2001), we found no indications of hybridization among species in the STRUCTURE analyses (Fig. 2). This may suggest that local sculpin species in Alberta are not currently threatened by hybridization with *C. asper*, and that hybridization has not facilitated the expansion of *C. asper* into Alberta.

Genetic diversity indices of *C. asper* were lower in inland populations with an average allelic richness of 3.9 compared to 6.1 in coastal populations (Fstat permutation test, two-sided, $P = 0.044$), and a genetic diversity of 0.39 compared to 0.65, respectively ($P = 0.049$). Peace River did not show a significantly lower allelic richness ($P = 0.81$) or genetic diversity ($P = 0.83$) than other inland populations. Hence, low genetic diversity is more likely a consequence of postglacial (<15,000 y BP) colonization from coastal populations via temporal connections

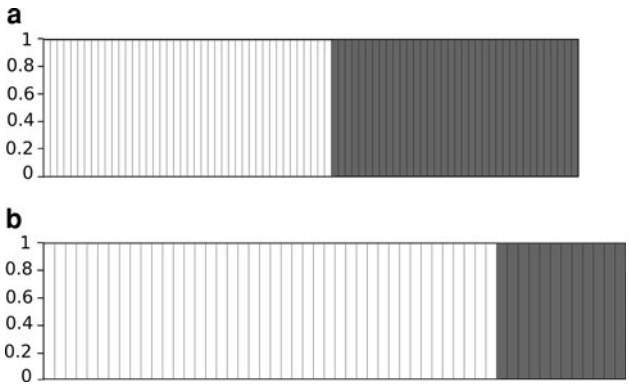

Fig. 2 STRUCTURE analyses. **a** Comparison of *C. asper* (*white*) and *C. cognatus* (*grey*). **b** Comparison of *C. asper* (*white*) and *C. ricei* (*grey*)

between the Peace and Fraser/Skeena River systems (McPhail and Lindsey 1970). Similarly, other studies have suggested that postglacial colonization, often in concert with founder effects, may contribute more to low genetic diversity than the peripheral location of a population (e.g., Cassel and Tammaru 2003). While we found no evidence for a marginal population of *C. asper* in the Peace River that might qualify as a conservation designation unit (Dalton 1991; COSEWIC 2005), the lack of genetic differentiation at neutral genetic markers does not necessarily inhibit the adaptive potential of peripheral populations (Hendry and Taylor 2004). Source-sink dynamics have repeatedly been suggested to favor local maladaptation in peripheral populations (Anderson and Geber 2009), but the ecological and demographic conditions that allow peripheral sink populations to establish and to expand without diverging from source populations need further research.

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