The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **103**, 514–529. With 3 figures

Phylogeography and phenotypic diversification in the Patagonian fish *Percichthys trucha*: the roles of Quaternary glacial cycles and natural selection

DANIEL E. RUZZANTE[1]*, SANDRA J. WALDE[1], PATRICIO J. MACCHI[2], MARCELO ALONSO[2] and JUAN P. BARRIGA[3]

[1]*Department of Biology, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada*
[2]*Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, Bariloche, Rio Negro, Argentina*
[3]*Instituto de Investigaciones en Biodiversidad y Medioambiente (CONICET – Universidad Nacional del Comahue), Bariloche, Río Negro, Argentina*

Received 4 March 2011; accepted for publication 4 March 2011

Current patterns of genetic and morphological diversity are the product of historical climatic and geomorphological events, and of contemporary selection processes acting upon this diversity. Here we examine the phylogeographic and phenotypic patterns of diversity within *Percichthys trucha*, a widely distributed Patagonian fish species complex that inhabits Andean and steppe freshwater environments. Molecular analysis (mtDNA control region) of 21 populations distributed throughout its latitudinal range revealed little evidence of phylogeographic structure and no evidence of species-level genetic divergence east of the Andes. The complex, however, exhibits high levels of intra- and interpopulation phenotypic variation. Patterns of among-population divergence in morphology were most easily explained by differences in predation pressure among populations; dorsal fin spines (commonly a defensive characteristic) were longer in environments with greater densities of potentially piscivorous fish. Trophic characters were highly variable within populations, suggesting an important role for resources in generating within-population morphological variation. The very shallow levels of divergence shown by the molecular data most likely reflect the historical mixing of populations as a result of the climatic and landscape changes that affected Patagonia throughout the Quaternary. The phenotypic divergences, in contrast, are probably the result of differing contemporary selection regimes acting on currently disjoint populations. © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2011, **103**, 514–529.

ADDITIONAL KEYWORDS: adaptive radiation – Patagonia – predation – resource competition – Salmonidae.

Los patrones de diversidad genética y morfológica que pueden observarse en poblaciones existentes son el producto de la influencia conjunta de procesos históricos (climáticos, geomorfológicos) y de la selección natural. En este trabajo examinamos los patrones de diversidad filogeográfica y fenotípica en *Percichthys trucha,* una especie o complejo de especies de amplia distribución en Patagonia andina y esteparia. Análisis molecular (Region de Control ADN mitocondrial) de 21 poblaciones a lo largo y ancho del rango distribucional del grupo reveló poca evidencia de estructura filogeográfica (estructura poco profunda) y ninguna evidencia de divergencia genética a nivel de especie al este de los Andes. El complejo exhibe sin embargo, altos niveles de variación fenotípica tanto intra-, como interpoblacional. Los patrones de divergencia morfológica entre poblaciones se correlacionan con diferencias interpoblacionales en la intensidad de predación; las espinas dorsales (comúnmente una característica defensiva) son más largas en ambientes con mayor densidad de peces potencialmente piscívoros. Los caracteres tróficos exhiben alta variación intrapoblacional sugiriendo que los recursos tróficos cumplen un rol importante en

---

*Corresponding author. E-mail: daniel.ruzzante@dal.ca

la generación de variación morfológica dentro de poblaciones. Los bajos niveles de divergencia molecular y de estructura filogeográfica son probablemente el resultado de la mezcla histórica de individuos y poblaciones como consecuencia de los cambios climáticos y geográficos (paisaje) que afectaron la región Patagónica durante el Cuaternario. Las divergencias fenotípicas por el contrario, son el resultado de diferencias en los regímenes de selección natural operantes en poblaciones de *Percichthys trucha* actualmente disjuntas.

PALABRAS CLAVE: competencia por recursos – Cuaternario – filogeografia – predacion – radiación adaptativa – Patagonia – Salmónidos – trucha criolla (Percichthys) – variación fenotípica.

INTRODUCTION

Current patterns of genetic and morphological diversity are the product of historical processes that have constrained, moulded and dispersed that diversity, and of the selection processes (biotic and abiotic) that are currently acting upon it. The Quaternary glacial cycles are among the most important of the major natural historical processes known to have influenced diversity patterns worldwide, and their effects on phylogeographic patterns have been well documented for the biota of the northern hemisphere (Avise, 2000; Hewitt, 2000). Comparable information for the southern hemisphere is sparser (Beheregaray, 2008), but has recently been accumulating, particularly for the fauna and flora of Patagonia (Ruzzante *et al*., 2008; Zemlak *et al*., 2010; Pardiñas *et al*., 2011, Sérsic *et al*., 2011).

It is critical to recognize that, concomitant with shifts in climate during the Quaternary, the biota of Patagonia experienced large-scale and important changes in geography and landscape. First, and most obviously, as the glaciers advanced, some existing habitat was eliminated or rendered uninhabitable, leading to the elimination of local populations and shifts in the distribution of most species. Icefields were mostly restricted to the Andes through much of their latitudinal range, but glaciers calved into the Pacific Ocean south of 39°S, and reached the Atlantic Ocean in southernmost Patagonia and Tierra del Fuego during the most extensive glaciations (Clapperton, 1993; Sugden *et al*., 2005; Rabassa, Coronato & Martínez, 2011). However, the total area of exposed and potentially habitable land actually increased dramatically during glacial periods, as the sea level fell. In fact, Patagonia doubled its current surface area during the most recent glacial cycle (Cavallotto, Violante & Hernández-Molina, 2011; Ponce *et al*., 2011). Thus, there was considerable potential for terrestrial refugia, and perhaps even the potential for the expansion of some populations during glacial periods. Glaciers largely eliminated the current headwater lakes and upper reaches of the major river systems of Patagonia (Rabassa *et al*., 2011). However, the exposure of much of the continental shelf to the east would have also allowed for the extension and perhaps expansion of aquatic habitats. The changing size of these aquatic environments and the routes of the now-submerged river systems would have had major effects on the distribution of diversity within the aquatic biota of Patagonia.

Current ecological forces, i.e. the physical environment and biotic factors, such as competition and predation, act upon and may modify the patterns of morphological and/or genetic diversity produced by historic processes. For example, previously divergent populations may converge phenotypically when exposed to a similar environment, or marked phenotypic differences might emerge among currently isolated populations for which there is no evidence of phylogeographic structure. Differences among habitats in resources, competitive regimes, and number and type of predators can rapidly lead to divergent morphology through phenotypic plasticity and/or selection, and differences in competitive and predation regimes among lakes have repeatedly been associated with morphologically and behaviourally distinct fish populations (Milano *et al*., 2002, 2006; Vamosi, 2002; Langerhans *et al*., 2004; Andersson, Johansson & Söderlund, 2006; Eklöv & Svanbäck, 2006; Svanbäck & Persson, 2009).

In the present study we examine phylogeographic patterns within *Percichthys*. Although the genus is present on both sides of the Andes in Patagonia, here we focus primarily on populations in Argentina, where they are found in both Andean and steppe freshwater environments. Our goal is to interpret the molecular genetic and phenotypic diversity within the group in the light of known historic and current forces. We thus look at the effects of historic changes in aquatic landscapes as well as the potential consequences of current environments on the observed patterns of genetic and morphological diversity. *Percichthys* exhibits such high levels of intra- and interpopulation phenotypic variation in characters such as mouth structure, body depth, and dorsal fin spine, that it has historically been divided into various species (Ringuelet, Aramburu & Alonso de Aramburu, 1967; López-Albarello, 2004). The number and identities of recognized morphological species has varied through time. López-Albarello (2004) collapsed three of the species originally identified by Ringuelet *et al*. (1967) (*Percichthys trucha*, *Percichthys vinciguerrai*, and *Percichthys altispinnis*) into a single species (*P. trucha*), and created a new one (*Percichthys laevis*), to conclude that three species of *Percichthys* are present in Patagonia east of the Andes: *P. trucha*, *Percichthys colhuapensis*, and *P. laevis*. Here we describe the available molecular genetic information for the group along with information on morphological diversity and its ecological correlates. Our objectives were to: (1) interpret current phylogeographic patterns in *Percichthys* in the light of the historic changes in the aquatic environment in Patagonia; and (2) determine if there is molecular support for multiple species as described by Ringuelet *et al*. (1967) or López-Albarello (2004). A finding of significant genetic divergence and concordance between patterns of genetic and morphological diversity would lend support to the proposal that several species of *Percichthys* are present east of the Andes. Alternatively, the absence of geographic patterning in genetic diversity coupled with shallow phylogeographic structure would indicate that only a single species of *Percichthys* is found in the region.

MATERIAL AND METHODS

COLLECTION OF FISH

A total of 25 locations spanning the latitudinal and altitudinal range of *Percichthys* east of the Andes, and including four locations west of the Andes in Chile, were sampled between 1996 and 2007 (Fig. 1). Molecular genetics and morphological analyses were conducted on samples from 21 and 12 of these locations, respectively, with eight of them analysed for both molecular and morphological diversity. The four locations for which only morphological data are available all belong to the Limay river basin (Fig. 1). The eight locations for which both genetic and morphological data were available include populations from the six major Patagonian river drainages with headwaters east of the Andes: (1) Limay or Negro drainage (Lakes Quillén and Espejo); (2) Puelo (Lake Puelo); (3) Futaleufú (Lakes Rivadavia and Futalufquen-Kruger); (4) Chubut-Chico-Senguerr (Lake Musters); (5) Baker (Lake Pueyrredón); and (6) Santa Cruz (Lake Argentino). The Puelo, Futaleufú, and Baker rivers drain into the Pacific Ocean, whereas the rest drain into the Atlantic. Details on sampling procedures were presented in Ruzzante *et al*. (1998, 2003, 2006, 2008). Briefly, fish were collected with sets of gillnets placed at between three and five sites per lake (2–5 nights per lake, depending on the catch). Nets were set before dusk and hauled in after dawn the following morning. Fish were weighed, sexed, and assessed for reproductive status immediately upon retrieval. Blood or muscle tissue samples were taken and stored in ethanol for DNA analysis. Stomachs were removed, and stomach contents and fish were preserved in 4% formaldehyde. Estimates of catch per unit effort (CPUE) were based on gillnet surface area and the number of hours of deployment.

MOLECULAR ANALYSIS

DNA extraction and mitochondrial DNA sequencing

Detailed procedural information on DNA extraction and amplification is available in Ruzzante *et al*. (2006, 2008). Briefly, a fragment of the mtDNA control region (~380 bp) was amplified from all samples using the polymerase chain reaction (PCR) and standard methods on an MJ PTC-225 Thermocycler in 25-μL volumes using 2 μL of DNA extract as template. The primers L19 and MT16498H (Jerry & Baverstock, 1998) were used under the following conditions: an initial denaturing cycle of 94 °C for 5 min, 35 cycles with denaturation at 94 °C for 1 min, annealing at 51 °C for 1 min, and extension at 72 °C for 1 min, followed by a final extension step of 5 min at 72 °C. PCR products were purified using QIAGEN MinElute 96 PCR purification plates. For all samples, sequencing was conducted in both directions.

VISUALIZATION OF MOLECULAR DATA: GENGIS

The pattern of phylogeographic structure was visualized with GenGIS v1.08, a free downloadable bioinformatics application that provides a 3D graphical interface for the merging of information on molecular diversity (DNA sequences) with the geographic location from which the sequences were collected (http://kiwi.cs.dal.ca/GenGIS/Main_Page; Parks *et al*., 2009). A permutation test implemented in the application is designed for the testing of phylogeographic structure. In the present study we tested whether the latitudinal position of different haplotypes is correlated with their phylogenetic relatedness according to the maximum likelihood phylogram constructed with MEGA v5 (Tamura *et al*., 2011), and assuming default settings of uniform rates among sites and a nearest-neighbour interchange (NNI) as the tree inference option. Disagreement between the geographic gra-

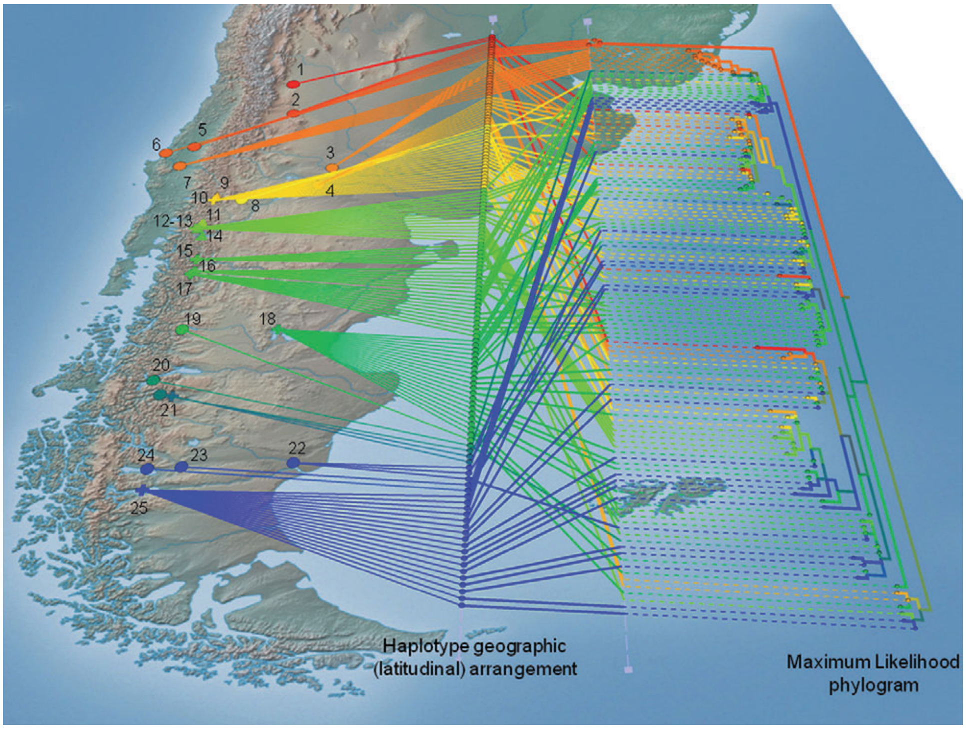

Figure 1. Sampling locations (25) for *Percichthys trucha*. The species is found throughout Patagonia in Andean as well as steppe freshwater environments. Collections span the altitudinal and latitudinal distributional range for this species east of the Andes, and took place between 1996 and 2007. Molecular genetic variation (Control Region (CR) haplotypes) was examined in individuals collected from 21 sites, phenotypic variation was examined in individuals collected from 12 sites, with overlapping molecular and phenotypic information available for eight sites. Key: ●, sites with molecular information (13); +, sites with molecular and morphological information (8); ▲, sites with morphological but no molecular information (4). The figure associates the phylogenetic relationship among *Percichtys* mtDNA CR haplotypes with the geographic distribution of those haplotypes. Colours reflect latitude, going from red in the north to dark blue in the south. There is no relationship between the latitude (or population) at which haplotypes were found and the phylogenetic relationships among haplotypes (permutation test between latitude and genetics: $P > 0.333$). The results suggest thorough mixing among ancestral *Percichthys* populations throughout Patagonia east of the Andes. Mixing among populations from different drainages is likely to have taken place at times of high meltwater discharge during glacial stabilization and/or termination periods (Martínez & Kutschker*,* 2011) on the current Patagonian steppe, and/or the exposed continental shelf (Ponce *et al*., 2011). Locations: 1, River Tunuyán (El Carrizal Reservoir); 2, River Atuel (El Nihuil Reservoir); 3, River Colorado; 4, River Negro; 5, River Itata (Chile); 6, River Andalién (Chile); 7, River Bio-Bio (Chile); 8, Lake Blanca; 9, Lake Ruca Choroi; 10, Lake Quillén; 11, Lake Falkner–Villarino; 12, Lake Espejo; 13, Lake Correntoso; 14, Lake Morenito; 15, Lake Puelo; 16, Lake Rivadavia; 17, Lake Futalaufquen-Kruger; 18, Lake Musters; 19, Lake La Plata; 20, Lake Silvia (Chile); 21, Lake Pueyrredón (Argentine)/Cochrane (Chile); 22, Rio Chico (near highway 3); 23, River Chalia (in Tres Lagos); 24, Lake Viedma (Lake de los Toros); 25, Lake Argentino.

dient and the ordering of haplotypes in the tree is assessed by counting the number of crossings (a reflection of rank-order differences) that occur between the two orderings, and comparing this count with the counts obtained from 1000 random permutations of the leaf labels of the tree. The resulting P value reflects the number of replicates that have equal or fewer crossings than the true tree, and $P \leq 0.05$ was taken as an indication of phylogeographic structure.

MORPHOLOGICAL AND DIET ANALYSIS

Morphological measurements were carried out on a total of $N = 1067$ individuals, from 12 populations, all inhabiting lakes. All measurements were performed on formalin-preserved material by the same person. We measured standard length (SL), head length (HL), length of the upper jaw (UJ), depth of the caudal peduncle (CP), and length of the longest (usually the second) spine of the first dorsal fin (DF) (Fig. 2). Characters were selected based on their likely

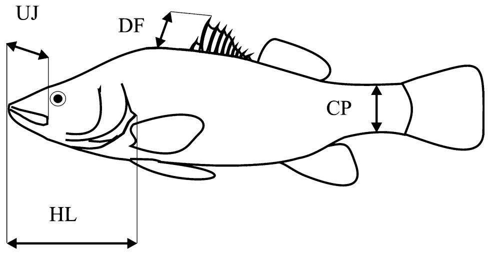

Figure 2. External morphological measurements of *Percichthys trucha*; CP, caudal peduncle depth; DF, length of dorsal spine; HL, head length; SL, standard length; UJ, length of upper jaw.

relationship with feeding and/or swimming ability (HL, UJ, CP), as well as predator avoidance (DF), and represent the most repeatable of the phenotypic traits used in previous studies (Ruzzante *et al*., 1998, 2003). These traits have also been used as diagnostic variables for species identification within the Percichtyidae (Ringuelet *et al*., 1967). In addition, we measured the length of the four longest gill rakers on the first left branchial arch. In early collections (1996, 1998), gill rakers were drawn with a camera lucida attached to a stereomicroscope and then measured. Later collections (2000, 2001, and 2005) were digitally photographed under a stereomicroscope and measured using image analysis software. Prior to analysis, all variables were standardized to a common fish size using the relationship:

$$y_i = \log(x_i) - b\{\log(SL_i) - \text{mean}[\log(SL)]\}, \qquad (1)$$

where y_i and x_i are the adjusted and original values for the character in individuals i ($i = 1, \ldots, N$), SL_i is the individual standard length, and b is the regression coefficient of the logarithm of x on the logarithm of SL. Allometric relationships did not differ between sexes for any lake, but they differed slightly among lakes for some of the variables. Variables were therefore standardized with sexes pooled, using individual lake allometric relationships. For the comparisons among populations we conducted a principal component analysis on standardized variables and ANOVA.

We obtained information on diet for the 12 populations where morphological variation was measured. For each prey category present in the fish stomachs i (i.e. family, genus, or species), we calculated an index of relative importance, RI, based on prey number and size (Pinkas, Oliphant & Iverson, 1971): $RI_i = (N_i + V_i)F_i$, where N_i is the percentage of total prey items that were prey of type i, V_i is the percentage of total prey volume occupied by prey of type i, and F_i is the percentage of non-empty guts that contained at least one prey of type i. For each population, RI indices were then expressed as a proportion of the sum of all RI indices (proportional RI) for that population (Appendix). Diet analyses for the lakes that were visited twice were conducted separately for each year of collection, and then the proportional RI values were averaged across the years. Diet data were analysed using principal component analysis.

RESULTS

PHYLOGEOGRAPHIC PATTERN OF *PERCICHTHYS TRUCHA* IN PATAGONIA

Analysis of $N = 107$ haplotypes from the mtDNA control region for the individuals collected from 21 lakes and rivers shows no evidence of geographic patterning in the distribution of genetic diversity within *Percichthys* east of the Andes (Fig. 1). In Figure 1, the haplotypes are linked to the population (lake or river) the individuals were collected from. Haplotypes found in more than one location are indicated by the presence of multifurcating nodes as terminals. Haplotype colour in this figure reflects latitude, with the northernmost locations shown in red and the southernmost locations shown in dark blue. Related haplotypes are widely distributed across latitude, as can be seen in the crossing of the lines connecting sampling location, and the terminal nodes in the maximum-likelihood haplotype tree. No latitudinal or geographical pattern emerges among haplotypes collected from locations east of the Andes (Fig. 1). Three closely related haplotypes collected from a single Chilean location (location 5, River Itata; Fig. 1) are slightly more divergent, as reflected by their relatively long branches in the maximum likelihood haplotype tree (see also Ruzzante *et al*., 2006), although the permutation test designed to examine the relationship between latitudinal position and genetic relationship overall indicated no significant evidence of structure ($P > 0.333$). The relationship between mitochondrial control region haplotypes and the location from which they were sampled therefore provides no evidence of phylogeographic structure or of species-level differentiation among populations of *Percichthys* east of the Andes in Patagonia.

MORPHOLOGICAL DIFFERENCES AMONG POPULATIONS AMONG AND WITHIN DRAINAGES

As stated above, previous authors identified several morphological distinct groups within *Percichthys* east of the Andes, with characters different enough to be designated as distinct species (Ringuelet *et al*., 1967; López-Albarello, 2004). Here we address the question of morphological variability in the light of the little genetic variation described above.

A principal components analysis, using the five standardized traits, suggests that morphologically, the 12 populations fall into three groups (Fig. 3). The separation is primarily along the first component, which accounted for 61% of the total variance, and was most strongly influenced by differences in dorsal fin spine length ($DF_{loading} = 0.907$), and to a lesser extent by gill raker and upper jaw lengths (Table 1). The first group (shortest dorsal spines, and relatively long gill rakers and upper jaw) consisted of the northern populations (Limay and Futalaufquen basins), the second group (intermediate dorsal fin spine, gill rakers, and upper jaw) contained the two southern and one central lake populations (Argentino, Pueyrredón, and Puelo), and the third group (longest dorsal fin spines) comprised the only lake located on the Patagonian steppe (Musters).

The second principal component (23.4% of the variance) was influenced most strongly by gill raker length ($MGR_{loading} = -0.875$) and, to a lesser extent, by dorsal spine length and upper jaw length (Table 1). Most of the variation along this axis was among the northern lakes (Fig. 3). The most northerly population, from Lake Ruca Choroi, had the shortest gill rakers, but otherwise there seemed to be no geographic pattern. If the dorsal fin spine is excluded from the principal component analysis, there are no distinct population groupings along either PC1 or PC2 (result not shown).

FISH COMMUNITY COMPOSITION, DENSITY, AND MORPHOLOGICAL DIFFERENCES AMONG *PERCICHTHYS* SP. POPULATIONS FROM 12 LAKES

The composition of the fish community differed significantly among the 12 lakes. *Galaxias platei* (puyen) were present in eight lakes, silverside (pejerrey) *Odontesthes hatcheri* were present in five lakes, and catfish (bagre) *Diplomystes viedmensis* were present in one lake (Table 2). All twelve lakes contained introduced salmonids, but differed in abundance and species composition (Table 2). Rainbow trout (*Oncorhynchus mykiss*) were present in 11 of the 12 lakes, and were usually the most abundant salmonid (Table 2). Brook trout (*Salvelinus fontinalis*) were present in eight of the 12 lakes, and were often abundant, and brown trout (*Salmo trutta*), although collected from nine of the 12 lakes, were usually found in small numbers. Lake trout, *Salvelinus namaycush*, were found only in Lake Argentino.

Our CPUE estimates are only rough approximations of population density; much more intensive sampling, including temporal replication, would be required to get accurate abundance estimates. However, they do provide an index of the variation among lakes in densities of *Percichthys* and other species. There was almost a 20-fold difference among lakes in CPUE for *Percichthys*, from a low of 1.0 fish per unit effort in Lake Espejo to a high of 19 fish per unit effort in Lake Musters (Table 3). Salmonid densities also varied among lakes, with CPUEs ranging from 0.6 to 4.3. Single estimates were averaged for lakes with temporal replication (Espejo, Quillén, and Rivadavia). Lake Morenito is a shallow lake, used as the breeding area for fish from Lake Moreno, to which it is connected through a short passage (Buria *et al.*, 2007). Individuals spend most of their time in the larger Lake Moreno, and thus we used a CPUE estimate for Lake Moreno (CPUE = 1.92), obtained for collections not used in the present study.

Variation among populations in morphology (PC1 scores) was highly correlated with the variation in density of *Percichthys* ($CPUE_{Percichthys}$) ($r = -0.90$, d.f. = 9, $P < 0.0001$), and of salmonids ($CPUE_{salmonide}$) ($r = -0.90$, d.f. = 9, $P \leq 0.0002$; Fig. 3C). Thus, individuals from populations of *Percichthys* in lakes with high densities of conspecifics or of introduced salmonids tended to have longer dorsal spines than individuals from lakes with low densities of conspecifics and/or salmonids.

DIET AND MORPHOLOGICAL DIFFERENCES AMONG POPULATIONS

A principal component analysis of diet (based on the proportional *RI* of prey types) indicated that variation in the importance of three prey types (Odonata, Amphipoda, and Chironomidae) was

Table 1. Principal component loading for five phenotypic traits measured across all 12 *Percichthys trucha* populations and percentage of the total variance explained by each of the principal components

Phenotypic trait	PC 1	PC 2	PC 3	PC 4	PC 5
Head length (HL)	0.113	–0.110	–0.422	0.051	0.891
Upper jaw length (UJ)	0.231	–0.231	–0.797	0.240	–0.449
Caudal peduncle length (CP)	0.004	–0.116	–0.222	–0.967	–0.064
Dorsal fin Spine length (DF)	–0.907	–0.395	–0.128	0.071	0.002
Mean gill raker length (MGR)	0.334	–0.875	0.349	0.020	0.014
% of total variance explained	61.4	23.4	8.3	5.2	1.8

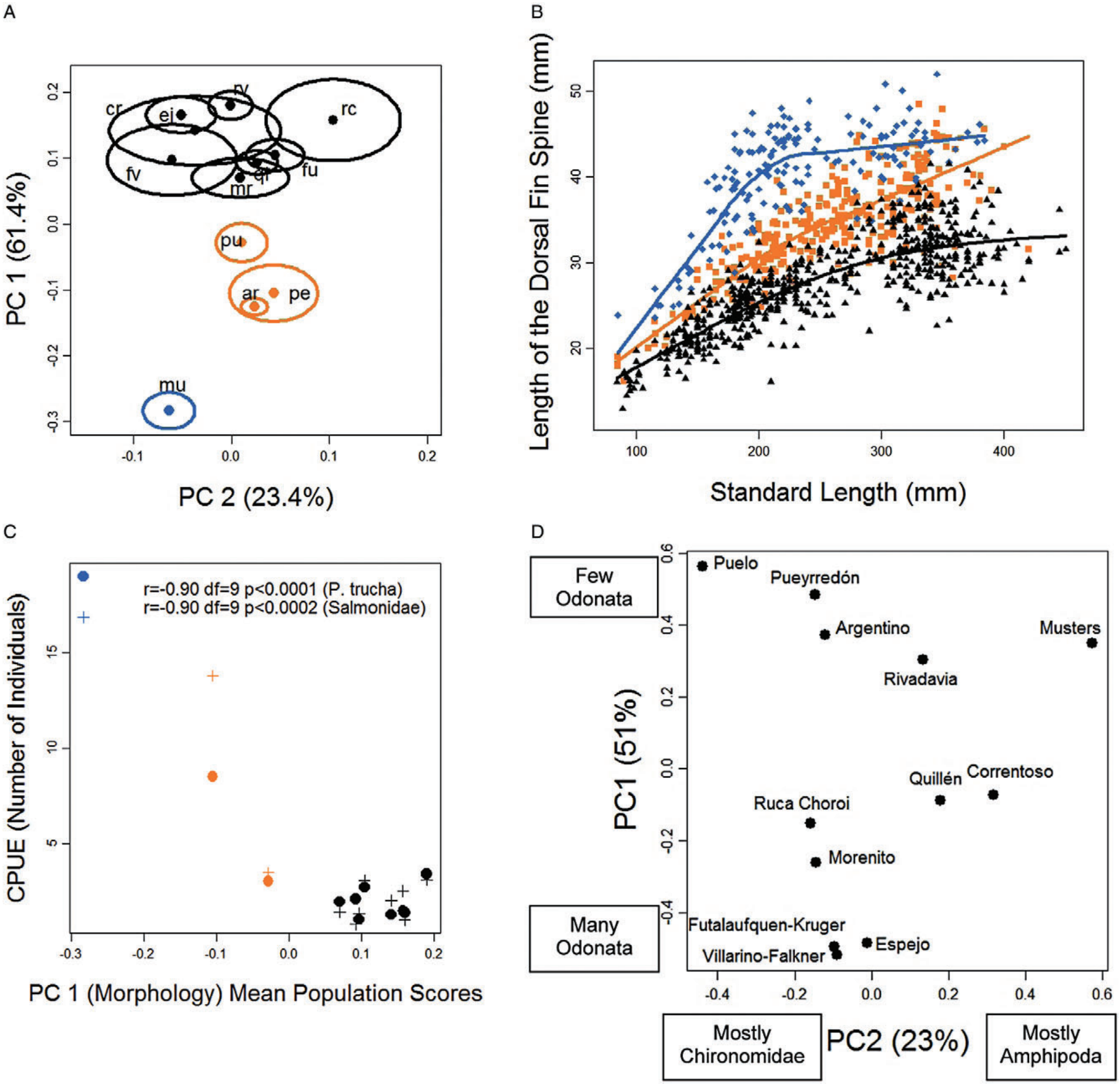

Figure 3. A, among-lake variation in morphology. Principal component analysis based on five phenotypic traits: length of dorsal fin spine (DF); length of upper jaw (UJ); length of the head (HL); depth of caudal peduncle (CP); and length of gill rakers (average of four longest rakers; MGR). Scatterplot of PC1 versus PC2 (together these two PCs explain ~85% of the total phenotypic variation). Length of the dorsal fin spine (DF) and mean gill raker length (MGR) are the traits with the highest loading on PC1 (–0.907) and PC2 (–0.875), respectively. Lake abbreviations are as listed in Table 2. B, length of the dorsal fin spine (DF) plotted against standard length of the fish. C, scatterplot of catch per unit effort (CPUE) in numbers of individuals [●, *P. trucha*; +, Salmonids) versus morphology (principal component 1)]. Fish density correlates with PC1 scores (morphology), for which the length of the dorsal fin spine (DF) has the highest loading. D, among-lake variation in the diet of *P. trucha.* The principal component analysis is based on the relative index (%) of prey items listed in Table 4. Scatterplot of PC1 versus PC2. Together these two principal components explain 74% of the total variance in the diet of *P. trucha* among lakes. The two diet items with the highest loading on PC1 are Odonata (–0.888) and Chironomid larvae and pupae (0.376), and on PC2 are Amphipoda (0.746) and Chironomid larvae and pupae (–0.555). See Table 4. Key for panels A, B and C: black, *Percichthys trucha* from Limay and Futalaufquen systems pooled (Limay – lakes Ruca Choroi, Quillén, Falkner–Villarino, Espejo, Correntoso, and Morenito; Futalaufquen – lakes Rivadavia and Futalaufquen); brown, *P. trucha* from Lakes Puelo, Pueyrredón, and Argentino; blue, *P. trucha* from Lake Musters. In panel C, no CPUE was available for Lake Argentino where *P. trucha* were caught by seine netting.

Table 2. Fish assemblage composition: number of fish captured and standard length [mean (SD) and range in mm] of native and non-native fish caught in gill-net sampling in 12 Patagonian lakes spanning the distribution of *Percichthys trucha*

Lake	*Percichthys trucha*	*Oncorhynchus mykiss*	*Salvelinus fontinalis*	*Salmo trutta*	*Salvelinus namaycush*	*Odontesthes hatcheri*	*Galaxias platei*	*Diplomystes viedmensis*
Ruca Choroi (rc)								
Number of fish	15	16	11	–	–	–	2	–
Mean length (SD)	251 (74)	302 (66)	336 (51)	–	–	–	298	–
Range	125–395	185–435	255–400	–	–	–	270–325	–
Quillén 96 & 98 (ql)								
Number of fish	171	44	11	12	–	1	–	–
Mean length (SD)	242 (71)	346 (85)	284 (61)	497 (68)	–	345	–	–
Range	84–375	120–490	180–355	350– 593	–	–	–	–
Falkner–Villarino (fv)								
Number of fish	22	13	17	1	–	–	1	–
Mean length (SD)	321 (43)	260 (138)	254 (82)	420	–	–	200	–
Range	195–390	100–470	160–420	–	–	–	–	–
Correntoso (cr)								
Number of fish	21	12	4	2	–		–	–
Mean length (SD)	326 (32)	371 (135)	281 (43)	433 (110)	–	–	–	–
Range	230–370	105–485	255–345	355–510	–	–	–	–
Espejo 96 & 98 (ej)								
Number of fish	82	44	41	6	–	–	41	**1**
Mean length (SD)	327 (64)	379 (108)	308 (81)	431 (131)	–	–	185 (36)	**161**
Range	149–420	115–525	157–473	265–565	–	–	115–275	–
Morenito (mr)								
Number of fish	50	–	2	–		15	–	–
Mean length (SD)	311 (34)	–	263 (11)	–		267 (38)	–	–
Range	196–390	–	255–270	–		210–330	–	–

Table 2. *Continued*

Lake	*Percichthys trucha*	*Oncorhynchus mykiss*	*Salvelinus fontinalis*	*Salmo trutta*	*Salvelinus namaycush*	*Odontesthes hatcheri*	*Galaxias platei*	*Diplomystes viedmensis*
Puelo (pu)								
Number of fish	175	9	–	18	–	–	34	–
Mean length (SD)	271 (72)	NA	–	NA	–	–	NA	–
Range	95–460	NA	–	NA	–	–	NA	–
Rivadavia 98 & 00 (rv)								
Number of fish	174	145	25	6	–	39	37	
Mean length (SD)	209 (83)	300 (81)	258 (57)	550 (95)	–	241 (62)	200 (47)	
Range	89–450	105–447	172–380	395–650	–	136–371	127–290	
Futalaufquen–Kruger (fu)								
Number of fish	71	33	1	29	–	–	5	–
Mean length (SD)	218 (84)	333	370	409 (68)	–	–	144	–
Range	90–386	122–460	–	195–550	–	–	117–195	–
Musters (mu)								
Number of fish	166	38 [20]*	–	–	–	285 [67]*	–	–
Mean length (SD)	230 (64)	321 (85)	–	–	–	207 (38)	–	–
Range	85–384	225–550	–	–	–	138–332	–	–
Pueyrredón (pe)								
Number of fish	54	20	–	–	–	19	19	–
Mean length (SD)	298 (45)	429 (73)	–	–	–	291 (48)	294 (64)	–
Range	170–380	320–630	–	–	–	203–365	175–334	–
Argentino (ar)								
Number of fish	204	37	–	–	17	–	1	–
Mean length (SD)	245 (69)	230 (101)	–	–	382 (90)	–	145	–
Range	80–384	101–403	–	–	260–516	–	–	–

*Actual number of fish measured out of those captured.

primarily responsible for distinguishing diets of the different populations (Fig. 3D). Populations in the Limay river basin were distinguishable from southern or steppe populations along PC1 (51% of total variance). Odonata were the prey item with the highest loading on PC1 (PC1 $\text{Odonata}_{\text{loading}} = -0.888$; Table 4): Limay river basin populations tended to rely more heavily on Odonata. The second principal component (23% of total variation) mostly reflected differences in the importance of Chironomidae versus Amphipoda in the diet (Table 4). For example, *Percichthys* from Lake Musters fed mostly on Amphipoda and to a lesser extent also on Cladocera, with little contribution from Chironomidae, whereas individuals from Lake Puelo relied mostly on Chironomidae (Fig. 3D; Table 4).

We estimated correlations between diet and morphology after the exclusion of the length of the dorsal fin spine from the suite of morphological traits, as this trait is unlikely to be related to resource use. In this analysis PC1 (45% of total variation) for morphology chiefly reflected variation in gill raker length and, to a lesser extent, upper jaw length ($\text{loading}_{\text{MGR}} = 0.902$; $\text{loading}_{\text{UJ}} = 0.382$). There was a negative correlation between morphology ($\text{PC1}_{\text{morph}}$) and diet ($\text{PC1}_{\text{diet}}$) ($r = -0.61$, d.f. = 10, $P < 0.036$), indicating that populations that relied more heavily on Odonata tended to have shorter gill rakers and shorter jaws. Diet varies seasonally, but differences in sampling time among lakes were minor (all in summer – January and February).

Table 3. Catch per unit of effort (CPUE) in numbers of individuals

	CPUE
Ruca Choroi	1.44
Quillén	2.07
Falkner–Villarino	1.00
Espejo	1.35
Correntoso	1.25
Morenito (Moreno)	1.92
Puelo	8.5
Rivadavia	3.38
Futalaufquen–Kruger	2.68
Musters	19.00
Pueyrredón	3.00
Argentino	Not available

MORPHOLOGICAL DIVERSITY WITHIN VERSUS DIVERGENCE AMONG POPULATIONS

We used ANOVA to partition the variance for each morphological trait between the among- and within-population levels. Only the dorsal fin spine (DF) was more variable among populations than within populations, with 72% of the total variance for this trait explained by differences among populations (Table 5). Two traits are much more variable within than among populations: gill raker length and caudal peduncle depth (Table 5).

DISCUSSION

In this study, we show what might be considered a paradox: a high level of morphological variability among and within *Percichthys* populations, sufficiently high that variants from different drainages have been

Table 5. Percentage of total variance explained by differences among and variation within populations in an ANOVA framework for each of the five measured morphological characters

Morphological character	Among populations (% variance)	Within populations (% variance)
Dorsal fin (DF)	72	28
Head length (HL)	41	59
Upper jaw (UJ)	35	65
Mean gill raker length (MGR)	19	81
Caudal peduncle (CP)	12	88

Table 4. Principal component loading for the six most important prey items in the diet of *Percichthys trucha* in 12 lakes distributed throughout the range for the species in Patagonia

Prey type	Comp1	Comp2	Comp3	Comp4	Comp5
Odonata	**–0.888**	–0.145	0.132	–0.052	0.061
Amphipoda	0.117	**0.746**	0.168	–0.214	0.437
Chironomid Larvae & pupae	0.376	**–0.555**	0.343	–0.472	0.116
Chilina	0.116	–0.137	**–0.780**	0.124	0.144
Tricoptera	0.184	–0.070	0.389	**0.824**	0.004
Cladocera	0.078	0.289	–0.009	–0.139	**–0.842**
Percentage of Total Variance explained	50.7	23.2	13.6	9.6	2.2

deemed to be different species, yet we find no evidence of deep divergence in the entire haplotype tree for *Percichthys* east of the Andes, and only a very shallow phylogeographic structure for the region. We argue here that this pattern has been produced by two very different processes, operating on different time scales.

The morphological trait identified as best distinguishing groups of populations, and the only trait that differed more among than within populations, was the length of the dorsal fin spine. Long dorsal spines have been associated with predator avoidance in fish (Januszkiewicz & Robinson, 2007), and we found that variation among populations in spine length correlates with the density of conspecifics and introduced salmonids. Small *Percichthys* are vulnerable to predation by larger conspecifics, and perhaps also by some salmonids. Longer spines could be a defensive trait that has evolved in populations subject to high predation pressure, or could be a developmental response to predators, as has been shown for sunfish (Januszkiewicz & Robinson, 2007). Superimposed on the phenotypic differences among populations, there was strong evidence of marked intrapopulation phenotypic diversity involving mainly gill raker length, a trait often linked to resource acquisition in fish. Thus, it appears that the relative importance of the various processes responsible for the generation of within-population diversity may differ from those involved in allopatric differentiation among populations (see also Calsbeek & Cox, 2010). Regardless of which processes have produced phenotypic diversification within and among *Percichthys* populations east of the Andes, our molecular analysis indicates that the phenotypic differences do not correlate with genetic differentiation (as assessed from variation at the mitochondrial control region) (Fig. 1). Below we discuss the potential reasons for, and implications of, our results.

ABSENCE OF PHYLOGEOGRAPHIC STRUCTURE WITHIN THE *PERCICHTHYS TRUCHA* COMPLEX

As we found previously with a smaller data set (Ruzzante *et al*., 2006), analysis of 21 populations of *Percichthys*, encompassing their full distributional range east of the Andes as well as some western populations, demonstrates little to no phylogeographic structure. The very shallow structure east of the Andes suggests that there must have been relatively recent mixing of populations throughout the region. We suspect that this mixing took place via two non-exclusive mechanisms. Firstly, there may have been exchange among now disjunct lakes that were part of larger proglacial lakes formed in front of the ice during the retreat of the glaciers. Secondly, and probably more importantly, individuals were probably able to move between current drainages via the many braided and deltaic connections that formed on the exposed continental shelf during glacial periods (Martínez & Kutschker, 2011; Ponce *et al*., 2011).

Species respond in different ways to repeated glacial advances and retreats, depending, in part, on characteristics such as cold tolerance and dispersal ability. Recent studies suggest that the various terrestrial Patagonian taxa did show distinctive responses, with some species surviving glacial periods in one to a few southern refugia, whereas others survived in and subsequently recolonized from northern areas (Sersic *et al*., 2011, Pardiñas *et al*., 2011). Likewise, there is evidence that some aquatic species appear to have survived in southern refugia (Zemlak *et al*., 2008, 2010), but it is likely that others were driven extinct locally. *Percichthys* is a relatively warm water-adapted Patagonian fish that reaches very high densities in warmer steppe lakes and reservoirs. If it survived in southern drainages through the glacial cycles, the refugia must have been on the expanded Patagonian surface that included exposed areas of the currently submerged continental shelf (Cavallotto *et al*., 2011; Ponce *et al*., 2011).

Several lines of evidence suggest that the exposed continental shelf may have provided refugial habitat as well as opportunities for the movement of *Percichthys* and other freshwater fauna among river drainages, from the Colorado River in the north to the Santa Cruz and Gallegos Rivers in the south. Bathymetric images provide evidence for the presence of endorheic basins with a circular morphology and a deeper centre than periphery in the present-day San Jorge, San Matías, and San José gulfs (Ponce *et al*., 2011). These areas would have been exposed during glacial periods, and were probably filled with shallow, relatively warm water. Shallow, productive basins are an ideal habitat for *Percichthys*. The exposed shelf is relatively homogeneous and flat, with a west–east gradient of generally $< 1\%$, and is thus conducive to channel shifts and overflow during wetter periods. In addition, there is sedimentary evidence for the formation of braided river systems (which typically have unstable, shifting channels) and deltaic fronts (e.g. the Colorado and Negro systems; Martínez & Kutschker, 2011). Thus, there was considerable potential for the large, currently disjoint, Patagonian river systems on the continental shelf to have merged continually or intermittently during the extended full glacial periods that lasted tens of thousands of years, and probably also during the relatively short glacial termination periods, when river flows were high, up to ten times greater than today (Cavallotto *et al*., 2011; Martínez & Kutschker, 2011). The shallow phylogeographic structure that we see in *Percichthys* is thus likely to have been maintained through periodic mixing, produced by large-scale landscape changes that occurred as a function of changing climate through the Quaternary (Rabassa, Coronato & Salemme, 2005; Rabassa, 2008). The extent of mixing probably varied among freshwater species, and we speculate that species that inhabited the Patagonian steppe and/or the shallow lake and river environments on the now submerged continental shelf were most susceptible.

Phenotypic variation

The remaining question, then, is what processes were likely to have produced such divergent morphology among and within populations. The lakes we surveyed span the latitudinal and elevational range of *Percichthys* in Patagonia east of the Andes, and differ greatly in the abiotic conditions that are associated with productivity. Deep Andean lakes of glacial origin are ultraoligotrophic–oligotrophic, with nitrogen levels sufficiently low so as to limit productivity (Soto *et al*., 1994; Diaz *et al*., 2007). Steppe lakes, on the other hand, tend to be warmer, to have higher levels of dissolved nutrients, and to be much more productive (Quirós & Drago, 1999; Diaz *et al*., 2007). Differences in productivity are likely to lead to differences in both resource/competitive and predation regimes.

By far the most important morphological character differentiating populations was the length of the dorsal fin spine, suggesting that predation (predator defence or predator avoidance) may underpin much of the among-population morphological diversity (see also Reimchen 1983 and Reimchen & Nosil 2002, and references therein). Percids typically erect their dorsal spine in response to piscivorous fish (Ylönen *et al*., 2007): longer spines presumably reduce predation risk, perhaps in part through increases in apparent size to gape-limited predators. Predators have been shown to induce morphological changes in fish through water-born chemicals, including the induction of longer dorsal spines in sunfish (Januszkiewicz & Robinson, 2007), and greater body depth in sunfish and other species (Brönmark & Miner, 1992; Langerhans *et al*., 2004; Januszkiewicz & Robinson, 2007). Body depth can also be related to predation risk through its effects on swimming performance and escape success (Domenici *et al*., 2008). The principal predation threats to young *Percichthys* in the study lakes are conspecifics (large *Percichthys* are partially piscivorous) and, perhaps, introduced salmonids (Macchi *et al*., 1999). The much longer spines in high-density populations of *Percichthys*, such as the steppe Lake Musters, probably result from an induced or evolutionary response to high predation intensity. The lack of genetic differentiation of this population, together with a plausible ecological explanation for its morphological divergence argues that the *Percichthys* species described for this lake, *P. colhuapensis*, is no more than a morphotype of *P. trucha* that develops under particular environmental conditions, i.e. high densities of conspecific and salmonid predators and/or competitors.

The other characters that differed somewhat among populations (head and upper jaw lengths and, to a lesser extent, mean gill raker length) are usually related to feeding, and differences probably resulted from variation in resources among the lakes. Resource availability and type can induce variation in trophic morphology in fish: individuals that feed primarily on zooplankton tend to develop more streamlined bodies, and a head morphology that can efficiently consume small pelagic prey (many long, closely-spaced gill rakers), whereas those that feed on benthic prey tend to develop deeper bodies, and sometimes longer, more robust jaws (Adams, Woltering & Alexander, 2003; Andersson *et al*., 2005; Yonekura, Kohmatsu & Yuma, 2007; Berner *et al*., 2008). In oligotrophic Andean lakes, *Percichthys* feed primarily on benthic macroinvertebrates, although small crustaceans (e.g. cyclopoid copepods and cladocerans) are consumed by juveniles (Ruzzante *et al*., 2003). Lake productivity affects the age at which young percids begin feeding on larger benthic invertebrates (Persson, 1987; Huss, Byström & Persson, 2008), and variation in the timing of diet shifts can lead to differences in trophic morphology. The most productive lake in our study (Lake Musters) was the only lake in which small Crustaceans (Cladocera) were a significant part of the diet of adult *Percichthys*, perhaps because of a greater availability of plankton or perhaps because of more intense competition for benthic resources, and the very distinctive morphology of individuals in this lake might therefore be resource related as well as predation related.

Other marked differences among lakes in the type of benthic prey consumed by *Percichthys* probably also reflect variation among lakes in resource availability/abundance. (As all fish were collected in the summer, in January and February, differences in diet across lakes are not likely to be greatly confounded by seasonal differences in composition of prey community). Some of the variation in diet could be associated with variation in morphology: for instance, populations that relied heavily on Odonata tended to have relatively short gill rakers and jaws compared with those that did not feed on Odonata. We do not know the nature of any links between diet and trophic morphology for *Percichthys*: adult morphology is almost certainly influenced by the diet of early developmental stages, and diet can also be affected by predation regime. Thus, some combination of differences in predation and resource regimes, both related to lake productivity, are likely to be responsible for the morphological diversity within the species. What is very clear is that very different morphologies have emerged in different aquatic environments without concomitant genetic divergence.

Percichthys is also known to have variable morphology within lakes (Ruzzante *et al*., 1998, 2003; López-Albarello, 2004). Our analysis showed that two characters (gill raker length and caudal peduncle depth) were much more variable within than among populations. Both traits are commonly linked to resource use. Competition for resources may promote diversification of resource use and associated divergence in phenotype (Lack, 1947; Schluter & McPhail, 1992; Schluter, 1994), although other processes such as predation can also play a significant role (Jablonski & Sepkoski, 1996; Rundle, Vamosi & Schluter, 2003; Langerhans *et al*., 2004; Nosil & Crespi, 2006; Meyer & Kassen, 2007). The population with the greatest interindividual variation in trophic morphology, the steppe Lake Musters (D. E. Ruzzante, unpubl. data), was also the most productive lake with the highest density of potential predators (*Percichthys* and salmonids). Thus, ecological factors (resource competition and/or predation) are likely to be responsible for within-population morphological variation as well as the differences among populations.

SPECIATION WITHIN PATAGONIAN *PERCICHTHYIDAE*?

Several of the morphological characters examined in this study (upper jaw length, body depth, and dorsal spine length) have been used to define different species within the genus *Percichthys* (Ringuelet *et al*., 1967; López-Albarello, 2004). We found similarly high levels of within- and among-population variability in these traits, and identified three broad morphological groups: one encompassing all *Percichthys* populations from the Limay and Futalaufquen river basins; one encompassing *Perichthys* from lakes Argentino, Pueyrredón, and Puelo; and one type from Lake Musters (Fig. 3A). In a recent and thorough attempt to sort out species designations within Argentine *Percichthys*, López-Albarello (2004) collapsed most of the previous species into a single species, *P. trucha*, but designated the *Percichthys* from Lake Argentino as *P. laevis*, whereas those from Lake Musters were presumed to be *P. colhuapensis.* We also found these populations to be morphologically distinct. However, the molecular differences (mtDNA and nuclear sequences) among individuals and populations that we describe here and in previous studies (Ruzzante *et al*., 2006, 2008) are much smaller than would be expected for species, or even subspecies, designation. All *Percichthys* populations in Patagonia east of the Andes thus appear to belong to the same species: *P. trucha*. We argue that different processes produced the spatial patterns of genetic versus morphological variation. Shifting aquatic landscapes during the Quaternary mixed the populations, producing a very shallow phylogeographic structure east of the Andes, whereas ecological factors (perhaps differences in predation and competition regimes) most likely account for the current morphological differences. We expect that populations of *P. trucha* do diverge over time in response to different ecological pressures in different environments, and that current *P. trucha* populations in Patagonia may be in some intermediate stage of an adaptive radiation. Divergence at the molecular level appears to be considerably short of speciation, however, perhaps because of the relatively young age of individual populations, or perhaps because of inconsistency through time in the direction or strength of selection pressures (e.g. Svanbäck & Persson, 2009). We suspect that these processes may have been repeated multiple times in the past: populations in different environments underwent partial but incomplete divergence caused, at least in part, by natural selection, but the divergence was then lost as climate change altered the landscape, allowing haplotypes from different populations to mix.

ACKNOWLEDGEMENTS

We thank Guillermo Ortí (George Washington University) and Evelyn Habit (Universidad de Concepción) for insightful comments on the article, and Robert Beiko and Donovan Parks (Faculty of Computer Science, Dalhousie University) for assistance with GenGIS and Figure 1. We also thank the Committee for Research and Exploration of the National Geographic Society, Washington, for generous support for fieldwork in 2000–01 (NGS 6799–00) and 2007–08 (8168–07), NSERC Discovery grants, and a Special Research Opportunity award (SROPJ/326493–06) to D.E.R. and S.J.W. as well as Universidad Nacional del Comahue, FONCYT, and CONICET (Argentina) grants for the support of fieldwork. We also acknowledge an NSF-PIRE award (OISE 0530267) for the support of collaborative research on Patagonian Biodiversity granted to the following institutions (listed alphabetically): Brigham Young University, Centro Nacional Patagónico, Dalhousie University, Instituto Botánico Darwinion, Universidad Austral de Chile, Universidad Nacional del Comahue, Universidad de Concepción, and University of Nebraska. We thank Evelyn Habit for collections from Chile, and Juana Aigo, Miguel Battini, Víctor Cussac, Amalia

Denegri, Maria Lattuca, Daniela Milano, and Pablo Vigliano for assistance in the field in Argentina, and Tyler Zemlak for assistance in the laboratory. We thank Mauricio Uguccioni and José 'Pepe' De Giusto for their logistical support for more than a decade of the fieldwork in Patagonia.

REFERENCES

Adams CE, Woltering C, Alexander G. 2003. Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinnus alpinus*. *Biological Journal of the Linnean Society* **78:** 43–49.

Andersson J, Byström P, Persson L, De Roos AM. 2005. Plastic resource polymorphism: effects of resource availability on Arctic charr (*Salvelinus alpinus*) morphology. *Biological Journal of the Linnean Society* **85:** 341–351.

Andersson J, Johansson F, Söderlund T. 2006. Interactions between predator- and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society of London B* **273:** 431–437.

Avise JC. 2000. *Phylogeography: the history and formation of species*. Cambridge, MA: Harvard University Press.

Beheregaray LB. 2008. Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology* **17:** 3754–3664.

Berner D, Adams DC, Grandchamp A-C, Hendry AP. 2008. Natural selection drives patterns of lake–stream divergence in stickleback foraging morphology. *Journal of Evolutionary Biology* **21:** 1653–1665.

Brönmark C, Miner JG. 1992. Predator-induced phenotypical change in body morphology in Crucian Carp. *Science* **258:** 1348–1350.

Buria L, Walde SJ, Battini MA, Macchi PJ, Alonso M, Ruzzante DE, Cussac VE. 2007. Movement of a native perch (*Percichthys trucha*) in a montane Patagonian lake during spawning and pre-spawning periods. *Journal Fish Biology* **70:** 215–230.

Calsbeek R, Cox RM. 2010. Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* **465:** 613–616.

Cavallotto JL, Violante RA, Hernández-Molina FJ. 2011. Geological aspects and evolution of the Patagonian Continental Margin. *Biological Journal of the Linnean Society* **103:** 346–362.

Clapperton CM. 1993. *Quaternary Geology and Geomorphology of South America*. Amsterdam: Elsevier.

Diaz M, Pedrozo F, Reynolds C, Temporetti P. 2007. Chemical composition and the nitrogen-regulated trophic state of Patagonian lakes. *Limnologica* **37:** 17–27.

Domenici P, Turesson H, Brodersen J, Brönmark C. 2008. Predator-induced morphology enhances escape locomotion in crucian carp. *Proceedings of the Royal Society of London B* **275:** 195–200.

Eklöv P, Svanbäck R. 2006. Predation risk influences adaptive morphological variation in fish populations. *American Naturalist* **167:** 440–452.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907–913.

Huss M, Byström P, Persson L. 2008. Resrouce hetergeneity, diet shifts andintra-cohort competition: effects on size divergence in YOY fish. *Oecologia* **158:** 249–257.

Jablonski D, Sepkoski JJ Jr. 1996. Paleobiology, community ecology, and scales of ecological pattern. *Ecology* **77:** 1367–1378.

Januszkiewicz AJ, Robinson BW. 2007. Divergent walleye (*Sander vitreus)*-mediated inducible defenses in the centrarchid pumpkinseed sunfish (*Lepomis gibbosus*). *Biological Journal of the Linnean Society* **90:** 25–36.

Jerry DR, Baverstock PR. 1998. Consequences of a catadromous life-strategy for levels of mitochondrial DNA differentiation among populations of the Australian bass, *Macquaria novemaculeata*. *Molecular Ecology* **7:** 1003–1013.

Lack D. 1947. *Darwin's finches*. Cambridge: Cambridge University Press.

Langerhans RB, Layman CA, Langerhans AK, DeWitt TJ. 2004. Predator-driven phenotypic diversification in *Gambusia affinis*. *Evolution* **58:** 2305–2318.

López-Albarello A. 2004. Taxonomy of the genus *Percichthys* (Perciformes: Percichthyidae). *Ichthyological Exploration of Freshwaters* **15:** 331–350.

Macchi PJ, Cussac VE, Alonso MF, Denegri MA. 1999. Predation relationships between introduced salmonids and the native fish fauna in lakes and reservoirs in northern Patagonia. *Ecology of Freshwater Fish* **8:** 227–236.

Martínez O, Kutschker A. 2011. The 'Rodados Patagónicos' (Patagonian Shingle Formation) of Eastern Patagonia: environmental Conditions of gravel sedimentation. *Biological Journal of the Linnean Society* **103:** 336–345.

Meyer JR, Kassen R. 2007. The effects of competition and predation on diversification in a model adaptive radiation. *Nature* **446:** 432–435.

Milano D, Cussac VE, Macchi PJ, Ruzzante DE, Alonso MF, Vigliano PH, Denegri MA. 2002. Predator associated morphology in *Galaxias platei in* Patagonia lakes. *Journal of Fish Biology* **61:** 138–156.

Milano D, Ruzzante DE, Cussac VE, Macchi PJ, Ferriz RA, Barriga JP, Aigo JA, Lattuca ME, Walde SJ. 2006. Latitudinal and ecological correlates of morphological variation in *Galaxias platei* (Pisces: Galaxiidae) in Patagonia. *Biological Journal of the Linnean Society* **87:** 69–82.

Nosil P, Crespi BJ. 2006. Experimental evidence that predation promotes divergence in adaptive radiation. *Proceedings of the National Academy of Sciences, Washington* **103:** 9090–9095.

Pardiñas UFJ, Teta P, D'Elía G, Lessa EP. 2011. The evolutionary history of sigmodontine rodents in Patagonia and tierra del Fuego. *Biological Journal of the Linnean Society* **103:** 495–513.

Parks DH, Porter M, Churcher S, Wang S, Blouin C, Whalley J, Brooks S, Beiko RG. 2009. GenGIS: a geospatial information system for genomic data. *Genome Research* **19:** 1896–1904.

Persson L. 1987. Competition-induced switch in young of the year perch *Perca fluviatilis*: an experimental test of resource limitation. *Environmental Biology of Fishes* **19:** 235–239.

Pinkas L, Oliphant MS, Iverson ZL. 1971. Food habits of albacore bluefin tuna and bonito in California water. *Department of Fish and Game Fisheries Bulletin* **152:** 1–105.

Ponce JF, Rabassa J, Coronato A, Borromei AM. 2011. Paleographic evolution of the Atlantic coast of Pampa and Patagonia from threLast Glacial Maximum to the Middle Holocene. *Biological Journal of the Linnean Society* **103:** 363–379.

Quirós R, Drago E. 1999. The environmental state of Argentinean lakes: An overview. *Lakes & Reservoirs: Research and Management* **4:** 55–64.

Rabassa J. 2008. Late Cenozoic Glaciations in Patagonia and Tierra del Fuego. In: Rabassa J, ed. *The Late Cenozoic of Patagonia and Tierra del Fuego*. Developments in quaternary sciences. Vol. 11. Amsterdam: Elsevier, 151–204.

Rabassa J, Coronato A, Salemme M. 2005. Chronology of the Late Cenozoic Patagonian Glaciations and their correlation with biostratigraphic units in the Pampean Region (Argentina). *Journal of South American Earth Sciences* **20:** 81–103.

Rabassa J, Coronato A, Martínez O. 2011. Late Cenozoic Glaciations in Patagonia and Tierra del Fuego: an updated review. *Biological Journal of the Linnean Society* **103:** 316–335.

Reimchen TE. 1983. Structural relationships between spines and lateral plates in threespine stickleback (*Gasterosteus aculeatus*). *Evolution* **37:** 931–946.

Reimchen TE, Nosil P. 2002. Temporal variation in divergent selection on spine number in threespine stickleback. *Evolution* **56:** 2472–2483.

Ringuelet RA, Aramburu RH, Alonso de Aramburu A. 1967. *Los Peces Argentinos de Agua Dulce*. Gobernación: Provincia de Buenos Aires. Comisión de Investigación Científica.

Rundle HD, Vamosi SM, Schluter D. 2003. Experimental test of predation's effect on divergent selection during character displacement in sticklebacks. *Proceedings of the National Academy of Sciences USA* **100:** 14943–14948.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF. 1998. Trophic polymorphism, habitat and diet segregation in *Percichthys trucha* (Pisces: Percichthyidae) in the Andes. *Biological Journal of the Linnean Society* **65:** 191–214.

Ruzzante DE, Walde SJ, Cussac VE, Macchi PJ, Alonso MF, Battini M. 2003. Resource polymorphism in a Patagonia fish *Percichthys trucha* (Percichthyidae): phenotypic evidence for interlake pattern variation. *Biological Journal of the Linnean Society* **78:** 497–515.

Ruzzante DE, Walde SJ, Cussac VE, Dalebout ML, Seibert J, Ortubay S, Habit E. 2006. Phylogeography of the Percichthyidae in Patagonia: roles of orogeny, glaciation, and volcanism. *Molecular Ecology* **15:** 2949–2968.

Ruzzante DE, Walde SJ, Gosse JC, Cussac VE, Habit E, Zemlak TS, Adams EDM. 2008. Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography. *Molecular Ecology* **17:** 2234–2244.

Schluter D. 1994. Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **266:** 798–801.

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

Sérsic AN, Cosacov A, Cocucci AA, Johnson LA, Pozner R, Avila LJ, Sites JW Jr, Morando M. 2011. Emerging phylogeographic patterns of plants and terrestrial vertebrates from Patagonia. *Biological Journal of the Linnean Society* **103:** 475–494.

Soto D, Campos H, Steffen W, Parra O, Zuniga L. 1994. The Torres del Paine lake district (Chilean Patagonia): a case of potentially N-limited lakes andponds. *Archiv fur Hydrobiologie Supplement* **99:** 181–197.

Sugden DE, Bentley MJ, Fogwill CJ, Hulton NRJ, McCulloch RD, Purves RS. 2005. Late-glacial glacier events in southernmost South America: a blend of 'northern' and 'southern' hemispheric climatic signals. *Geografiska Annaler* **87A:** 273–288.

Svanbäck R, Persson L. 2009. Population density fluctuations change the selection gradient in Eurasian perch. *American Naturalist* **173:** 507–516.

Vamosi SM. 2002. Predation sharpens the adaptive peaks: survival trade-offs in sympatric sticklebacks. *Annales Zoologici Fennici* **39:** 237–248.

Ylönen H, Kortet R, Myntti J, Vainikka A. 2007. Predator odor recognition and antipredatory response in fish: does the prey know the predator diel rhythm? *Acta Oecologia* **31:** 1–7.

Yonekura R, Kohmatsu Y, Yuma M. 2007. Difference in the predation impact enhanced by morphological divergence between introduced fish populations. *Biological Journal of the Linnean Society* **91:** 601–610.

Zemlak TS, Habit EM, Walde SJ, Battini MA, Adams EDM, Ruzzante DE. 2008. Across the southern Andes on fin: glacial refugia, drainage reversals & a secondary contact zone revealed by the phylogeographic signal of *Galaxias platei* in Patagonia. *Molecular Ecology* **17:** 5049–5061.

Zemlak TS, Habit E, Walde SJ, Carrea C, Ruzzante DE. 2010. Surviving historical Patagonian landscapes and climate: molecular insights from *Galaxias maculatus*. *BMC, Evolutionary Biology* **10:** 67. Available at http://www.biomedcentral.com/1471-2148/10/67.

APPENDIX

Table A1. Proportional relative indices (proportional *RI*s) for each prey category by lake population

Lake	Odonata (nymphs)	Amphipoda	Chironomid larvae & pupae	Trichoptera	Aegla sp	Ephemeroptera	Samastacus	Chilina sp.	Diplodon sp.	Cladocera	Plancton	Oligochaetes	Organic matter	Fish
Ruca Choroi	0.542	0.024	0.222	0	0.020	0.192	0	0	0	0	0	0	0	0
Quillén	0.476	0.356	0.085	0.077	0.003	0.002	0	0	0	0	0	0	0	0.001
Villarino–Falkner	0.862	0	0	0.008	0	0	0.019	0	0	0	0	0	0	0.017
Espejo	0.842	0.102	0.001	0.025	0.003	0	0.024	0	0	0	0	0	0	0
Correntoso	0.429	0.462	0	0.045	0	0	0.063	0.001	0	0	0	0	0	0
Morenito	0.656	0.022	0.147	0	0	0.015	0.001	0.145	0	0	0	0	0	0.015
Puelo	0.005	0.001	0.840	0.014	0	0.028	0	0.030	0.074	0	0	0	0	0.003
Rivadavia	0.117	0.347	0.246	0.143	0	0	0	0.001	0	0.005	0	0.015	0.114	0.001
Futalaufquen–Kruger	0.845	0.001	0.016	0.028	0	0.001	0	0	0	0	0.097	0.012	0	0
Musters	0.001	0.582	0.016	0.004	0	0	0	0.002	0	0.389	0.001	0	0	0
Pueyrredón	0	0.042	0.307	0.643	0	0	0	0.001	0	0	0	0	0	0.007
Argentino	0	0.003	0.127	0	0	0	0	0.602	0	0	0	0.214	0	0.048