

Climate control on ancestral population dynamics: insight from Patagonian fish phylogeography

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Abstract

Changes in lake and stream habitats during the growth and retreat of Pleistocene glaciers repeatedly altered the spatial distributions and population sizes of the aquatic fauna of the southern Andes. Here, we use variation in mtDNA control region sequences to infer the temporal dynamics of two species of southern Andean fish during the past few million years. At least five important climate events were associated with major demographic changes: (i) the widespread glaciations of the mid-Pliocene (c. 3.5 Ma); (ii) the largest Patagonian glaciation (1.1 Ma); (iii) the coldest Pleistocene glaciation as indicated by stacked marine $\delta^{18}\text{O}$ (c. 0.7 Ma); (iv) the last southern Patagonian glaciation to reach the Atlantic coast (180 ka); and (v) the last glacial maximum (LGM, 23–25 000 years ago). The colder-water inhabitant, *Galaxias platei*, underwent a strong bottleneck during the LGM and its haplotype diversity coalesces c. 0.7 Ma. In contrast, the more warm-adapted and widely distributed *Percichthys trucha* showed continuous growth through the last two glacial cycles but went through an important bottleneck c. 180 000 years ago, at which time populations east of the Andes may have been eliminated. Haplotype diversity of the most divergent *P. trucha* populations, found west of the Andes, coalesces c. 3.2 Ma. The demographic timelines obtained for the two species thus illustrate the continent-wide response of aquatic life in Patagonia to climate change during the Pleistocene, but also show how differing ecological traits and distributions led to distinctive responses.

Keywords: Bayesian skyline plots, *Galaxias platei*, Patagonia, *Percichthys trucha*, phylogeography, Quaternary glacial cycles

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Introduction

Environmental changes associated with Quaternary glacial cycles are known to have altered the distribution and genetic structure of fauna and flora in North America and Europe (Avice 2000; Hewitt 2000), but little information exists on their effects in the Southern Hemisphere. In South America, the extent of glaciation varied significantly during the Quaternary and glaciers are known to have altered drainage patterns, lake distributions and even the position of the continental divide (Clapperton 1993; Turner *et al.* 2005). Patagonian glaciers were mostly restricted to

the Cordillera Principal along much of their latitudinal range, and parts of the Argentine steppe to the east and coastal Chile to the west remained ice free. During the largest glaciations, glaciers calved into the Pacific Ocean south of 39°S and reached the Atlantic Ocean in southernmost Patagonia and Tierra del Fuego (Clapperton 1993; Sugden *et al.* 2005). The effects of the repeated glacial advances and retreats on the fauna and flora of Patagonia are largely unknown. Different taxa likely had distinctive responses, for example, while some terrestrial plants appear to have survived the last glacial cycle in small southern refugia west of the Andes (Allnutt *et al.* 1999; Muellner *et al.* 2005); current Patagonian rodent populations are the result of re-colonization from the nonglaciated north (Palma *et al.* 2002). Even less is known about the effects of Quaternary

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glacial cycles on the aquatic fauna of Patagonia; the degree to which populations were reduced in size, the extent to which they survived within southern drainages, or the routes through which re-colonization occurred are unknown. In this study we compare the long-term temporal dynamics of two widespread but ecologically different Patagonian fishes, and show how their demographic histories through the Quaternary covary with the spatial and temporal variations in glacier extent that characterized this period.

The glacial history of Patagonia

Precise long-term records of the palaeoclimate of southern South America are not available, and terrestrial palaeoclimate proxies from palynology, geomorphology and limnology rarely extend past the last glacial maximum (LGM), dated at between 23 and 25 000 years ago for Patagonia, depending on the latitude (Sugden *et al.* 2005). The timing of glacial advances and retreats is known well for only the last few glaciations, and their spatial extents, while generally known, is still being mapped in detail (Caldenius 1932; Rabassa & Clapperton 1990; Clapperton 1993; Singer *et al.* 2004; Sugden *et al.* 2005). However, the correlation of glacial and fluvial sediment fluxes to the Chilean margin (ODP-1233) with Sea Surface Temperature (SST) from the same core (Lamy *et al.* 2004) suggests that southern-ocean marine sediments may provide good proxies for glacial fluctuations on land. A stacked benthic $\delta^{18}\text{O}$ record from 57 globally distributed marine sediment cores thus provides the best currently available representation of the timing and extent of glaciations in southern South America over the past 4 Ma (Fig. 2a). Five conspicuous anomalies evident in the $\delta^{18}\text{O}$ record are supported by the geological record in multiple localities in the southern Andes; their timing has been directly dated or bracketed between dated lavas: (i) the high-amplitude events around 3.5 Ma provide the first evidence of widespread glaciation; (ii) the most extensive Andean glaciation ('Largest Patagonian Glaciation', LPG), commonly dated between 1 and 1.1 Ma; (iii) the high-amplitude benthic $\delta^{18}\text{O}$ peaks in the mid-Pleistocene, correlated with extensive glaciations between 0.9 and 0.6 Ma; (iv) the most extensive glaciations of the past half million years, known as the Oxygen Isotope Stage 6 (OIS 6) glaciation; and (v) the last glaciation (OIS 2) (Rabassa & Clapperton 1990; Singer *et al.* 2004). However, imprecisions in the $\delta^{18}\text{O}$ chronology, and possible slight antiphasing of high-latitude southern hemisphere glaciations from the global ice volume and temperature record, result in chronological uncertainties of at least several thousand years. The Antarctic Vostok ice core provides a more precise indication of recent climate change (past 400 000 years ago) in the southern Andes; the Vostok δD regional record is correlative with southern ocean core data

(ODP-1089) (Mortyn *et al.* 2003). We thus used the temperature history from the Vostok δD record (represented as temperature departure from present day temperature, Petit *et al.* 1999), with the timescale modified according to Mortyn *et al.* (2003), to provide a record of climate change in the southern Andes over the last 250 ka (Fig. 3a).

Genetic imprints from demography

Cycles of population reduction and expansion leave phylogenetic signatures that can be used to reconstruct demographic histories (Abbott *et al.* 2000; Avise 2000; De Chaine & Martin 2006). Differences among species in life history (fecundity, dispersal ability) or ecological traits (e.g. temperature tolerance) can alter responses to historical events such as climate or drainage change, resulting in sympatric taxa that display quite different phylogeographical structures (Turner *et al.* 1996; Bermingham & Martin 1998; Hodges *et al.* 2007). A comparative approach can thus greatly enhance the credibility of links between demographic change and climatic or geomorphological events (Avise 2000; Hodges *et al.* 2007). Here, we examine the long-term demographic histories of two of the most abundant and wide-spread fish endemic to Patagonia. *Percichthys trucha* is found throughout most of the region but is absent from Tierra del Fuego and southern Chile, where glaciers were particularly extensive during the last glaciations (Ruzzante *et al.* 2006; Fig. 1). *Galaxias platei*, the more cold-adapted species, is found only in postglacial lakes and streams (Fig. 1). Both feed predominantly on benthic macroinvertebrates (Ruzzante *et al.* 2003; Milano *et al.* 2006) and spawn in shallow waters (Barriga *et al.* 2002; Buria *et al.* 2007). *G. platei* has few obvious defences (lacks scales or protective spines) but its low metabolic rate and tolerance of low-oxygen environments aid its survival in silty, glacier-fed lakes (reviewed by Cussac *et al.* 2004). Where the species are sympatric, *G. platei* occupies deeper habitats. Large *P. trucha* have no native predators and feed readily on other fish, including small galaxiids (Ruzzante *et al.* 2003).

We carried out extensive collections of *P. trucha* and *G. platei* from throughout the altitudinal and latitudinal ranges of each species (Fig. 1 and Table 1). We then constructed demographic timelines for each species, using the Bayesian Skyline approach (Drummond *et al.* 2005) based on mitochondrial DNA (mtDNA) control-region sequences of *P. trucha* and *G. platei*, and associated the timelines with the glacial history of the region.

Materials and methods

Sample collection

Percichthys trucha ($N = 552$) and *Galaxias platei* ($N = 166$) were collected from a total of 36 and 25 locations throughout

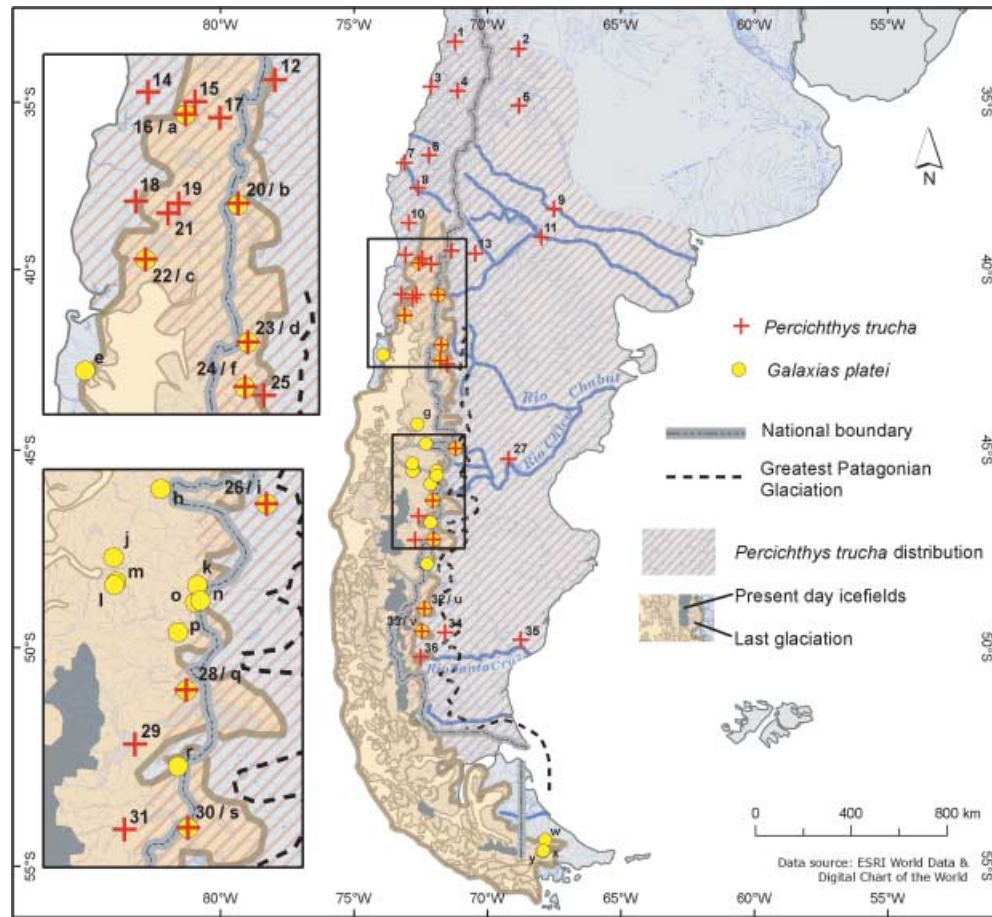


Fig. 1 Sampling locations and distributions of *Percichthys trucha* and *Galaxias platei*. Both species are endemic to the southern cone of South America. *P. trucha* is found in lakes and rivers throughout Patagonia; within, as well as outside, the area covered by the last glacial maximum (LGM), with two exceptions: (i) the island of Tierra del Fuego, extensively glaciated c. 180 ka, 0.7 Ma and 1.1 Ma; and (ii) west of the Andes south of 42°S, where the LGM reached the edge of the continental shelf. In contrast, *G. platei* is found only within the area covered by the LGM. Collections span the altitudinal and latitudinal distributional ranges of both species and took place between 1998 and 2007 in Argentina, and between 2004 and 2007 in Chile (see Table 1 for location details).

Argentina and Chile, respectively, which collectively represent the full altitudinal and latitudinal range of each species in South America (Fig. 1 and Table 1). Samples were collected using gillnets or electrofishing equipment between 1998 and 2007 in Argentina, and between 2004 and 2007 in Chile. Each individual was subsampled (fin clip, gill tissue or blood) into 95% ethanol and stored at -20 °C in the GeneProbe laboratory at Dalhousie University, Halifax, Nova Scotia.

DNA extraction

Prior to DNA extraction, all tissue samples were dried of ethanol either by incubation for 20–40 min at 55 °C or by exposure to ambient temperature for 60–120 min. Several DNA-extraction techniques were employed in this study. Total genomic DNA was isolated from subsamples (5 µL of

blood or 2 × 2 mm² gill tissue) of *P. trucha* individuals using the Hot-SHOT procedure (Truett *et al.* 2000). The glassmilk procedure (Elphinstone *et al.* 2003) was used to extract genomic DNA from fin or gill tissue (approximately 10 mg) derived from *G. platei* samples. Blood samples from *G. platei* were extracted using the DNeasy® (Qiagen) procedure, following manufacturer's instructions for isolating genomic DNA from whole nucleated blood.

Mitochondrial control region amplification and sequencing

Mitochondrial (mt) control-region sequences were amplified for each species using an PTC-225 DNA Engine Tetrad™ Cycler from Bio-Rad (formerly MJ Research), using 25 µL reaction volumes: 2.5 µL 10X Reaction Buffer [100 mM KCl, 100 mM (NH₄)₂SO₄, 200 mM Tris HCl (pH 8.75, 22 °C), 1%

	Number in Fig. 1	Latitude (S)	Longitude (W)
In Argentina			
R Tunuyán (El Carrizal Reservoir)	2	-33.30812	-68.71697
R Atuel (El Nihuil Reservoir)	5	-35.06592	-68.70408
R Colorado	9	-38.20000	-67.40000
R Negro	11	-39.02000	-67.87000
L Quillén	12	-39.41670	-71.25000
Lag Blanca	13	-39.50000	-70.35000
L Espejo	20	-40.69330	-71.75000
L Puelo	23	-42.09340	-71.61515
L Rivadavia	24	-42.60000	-71.65000
L Futalaufquen	25	-42.50000	-71.40000
L La Plata	26	-44.91667	-71.08333
L Musters	27	-45.20000	-69.10000
L Pueyrredón (AR)/Cochrane (CH)	30	-47.30000	-71.91670
L San Martín (AR)/O'Higgins (CH)	32	-49.03183	-72.24448
R Chalia (in 3 Lagos)	33	-49.60408	-71.48329
Rio Chico (near highway 3)	34	-49.78018	-68.63998
L Viedma (Lag de los Toros)	35	-49.57381	-72.34769
L Argentino	36	-50.20000	-72.40000
In Chile			
R Maipo (E Belloto)	1	-33.09718	-71.10858
E Nilahue	3	-34.48354	-72.01623
R Rapel (R Tinguiririca)	4	-34.61147	-71.00131
R Itata	6	-36.58333	-72.07500
R Andalién	7	-36.81720	-73.01570
R Bio Bio	8	-37.56590	-72.48980
R Imperial (R Chol Chol)	10	-38.61078	-72.84322
R Valdivia (R Cruces)	14	-39.54281	-72.95889
L Panguipulli	15	-39.64463	-72.32338
L Riñihue	16	-39.77479	-72.45289
L Neltume	17	-39.80991	-71.99450
R Rahue	18	-40.67147	-73.12497
L Puyehue	19	-40.69093	-72.54541
L Rupanco	21	-40.78966	-72.68628
L Llanquihue	22	-41.26208	-72.99454
L Gral. Carrera (CH)/Buenos Aires (AR)	28	-46.29790	-71.93713
Lag Silvia	29	-46.69337	-72.47825
Lag Esmeralda	31	-47.31151	-72.59156

Table 1 (a) *Percichthys trucha* sampling locations in Argentina and Chile. Latitude and Longitude are given in decimal degrees. The second column indicates the location in Fig. 1 of the paper. L, lake; Lag, lagoon; R, river; E, 'Estero'. Cross-border lakes are listed with their names in both countries

Triton X-100, 1 mg/mL BSA], 2.5 µL dNTPs (2 µM), 3.75 µL MgSO₄ (20 mM), 13 µL ddH₂O, 0.25 µL of each 10 µM primer, 1 U of Tsg Polymerase (Bio Basic Inc.) and 2.0–2.5 µL of DNA template. The primer pair *MT16498H* (5'-CCT GAA GTA GGA ACC AGA TG-3', Meyer *et al.* 1990) and *L19* (5'-ACC ACT AGC ACC CAA AGC TA-3', Bernatchez & Danzmann 1993) was used to amplify a 368 bp fragment from *P. trucha* samples under the following thermal regime: 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. A larger fragment (*c.* 800 bp) was amplified from *G. platei* extracts using the primers *S-phe* (5'-GCT TTA GTT AAG CTA CG-3', Nielsen *et al.* 1994)

and *P3* (5'-AAC TTC CAT CCT CAA CTC CCA AAG-3'; Sang *et al.* 1994), using the following thermal profile: an initial denaturing temperature of 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min 30 s, 72 °C for 1 min 30 s and a final extension at 72 °C for 5 min. Polymerase chain reaction (PCR) products were visualized using 1% agarose gels and subsequently purified using Qiagen MinElute 96 PCR purification plates (Qiagen). Sequencing reactions for the majority of the *P. trucha* samples used Beckman dye terminator cycle fluorescent chemistry (DTCS) and were run on a Beckman CEQTM 8000 automated capillary sequencer. A fraction of the *P. trucha* samples and all the *G. platei* samples were sequenced at the McGill University and Génome Québec Innovation Centre,

	Letter in Fig. 1	Latitude (S)	Longitude (W)
In Argentina			
L Espejo	b	-40.68333	-71.66667
L Puelo	d	-42.09340	-71.61515
L Rivadavia	f	-42.50000	-71.75000
L La Plata	i	-44.91667	-71.08333
L Pueyrredón (AR)/Cochrane (CH)	s	-47.30000	-71.91670
L Belgrano	t	-47.91667	-72.15000
L San Martín (AR)/O'Higgins (CH)	u	-49.03183	-72.24448
L Viedma (Lag de los Toros)	v	-49.57381	-72.34769
L Yehuin (Tierra del Fuego)	w	-54.40000	-67.73333
L Escondido (Tierra del Fuego)	x	-54.63333	-67.80000
Lag Margarita (Tierra del Fuego)	y	-54.66670	-67.83330
In Chile			
L Riñihue	a	-39.77479	-72.45289
L Llanquihue	c	-41.26208	-72.99454
R Cudil	e	-42.37461	-73.80614
L Risopatrón	g	-44.26404	-72.52232
L Las Torres	h	-44.80125	-72.20633
L Riesco	j	-45.49910	-72.67790
Laguna Alta	k	-45.52098	-72.69588
Laguna Escondida	l	-45.52395	-71.81790
L Los Palos	m	-45.31827	-72.70749
L Thompson	n	-45.63841	-71.78750
L Pollux	o	-45.65056	-71.84026
Laguna Azul	p	-45.86988	-72.02527
L Gral. Carrera (CH)/Buenos Aires (AR)	q	-46.29790	-71.93713
L Jeinimeni	r	-46.85390	-72.02632

Table 1 (b) *Galaxias platei* sampling locations in Argentina and Chile. Latitude and Longitude are given in decimal degrees. The second column indicates the location in Fig. 1 of the paper. L, lake; Lag, lagoon; R, River; E, 'estero'. Cross-border lakes are listed with their names in both countries

Montreal, Quebec, using a 3730XL DNA Analyser System (Applied Biosystems). All samples were bidirectionally sequenced.

Sequence analysis

Alignment, confirmation of polymorphic sites, contig assembly and haplotype designation for all *P. trucha* sequences was conducted using the program MEGA version 3.0 (Kumar *et al.* 2004). For *G. platei*, sequences were aligned and edited for errors using SEQUENCHER™ (Gene codes, 2000), and contigs were formed using BIOEDIT version 7.0.5.3 (Hall 1999) and aligned with CLUSTALW (Thompson *et al.* 1994). All sequences have been deposited in GenBank. GenBank Accession nos DQ324213–DQ324115 represent existing entries of *P. trucha* haplotypes 1–99 published in (Ruzzante *et al.* 2006). GenBank Accession nos EU069871–EU069916 and EU069832–EU069870 represent new entries of *P. trucha* ($N = 46$) and of *G. platei* ($N = 39$) haplotypes generated for the current study.

Regional analyses

Of the 39 haplotypes described for *G. platei*, 23 and 19 were present in systems west and east of the Andes, respectively,

with three haplotypes, Haplotypes 1, 4 and 16 present on both sides of the Andean divide. Western *G. platei* populations exhibited Haplotypes 1–4, 6, 8, 14, 16, 18–23, 31–34, 36–37, 42 and 49–50, while eastern *G. platei* populations exhibited Haplotypes 1, 4–5, 7, 9–13, 15–17, 43–48 and 51.

Of the 145 *P. trucha* haplotypes, 95 were present in populations east of the Andes in Argentina, while the remaining 50 haplotypes were present in populations west of the Andes in Chile. The 95 haplotypes described for Argentina include Haplotypes 1–86, 98, 100–105, 107 and 112. The 50 haplotypes described for Chile include Haplotypes 87–97, 99 and 113–150. The 95 *P. trucha* haplotypes from Argentina were examined altogether (see Results, Figs 2 and 3c) as well as separately for haplotypes present in populations north and south of Lat 42°S (See Results, Fig. 3d). This latitude was chosen because it separates the lakes draining into the major northern Patagonian rivers, the Negro and Colorado, from those lakes to the south that drain through other systems into the Pacific or Atlantic Oceans. There were 48 haplotypes present in the northern systems and 54 haplotypes present in the southern systems with Haplotypes 2, 11–14, 46 and 71, present in both regions. Northern populations had Haplotypes 1–14, 44–57, 70–86, 101, 102 and 112; southern populations had Haplotypes: 2, 11–43, 46, 58–71, 83, 100, 103–105 and 107. Of the 50 *P. trucha*

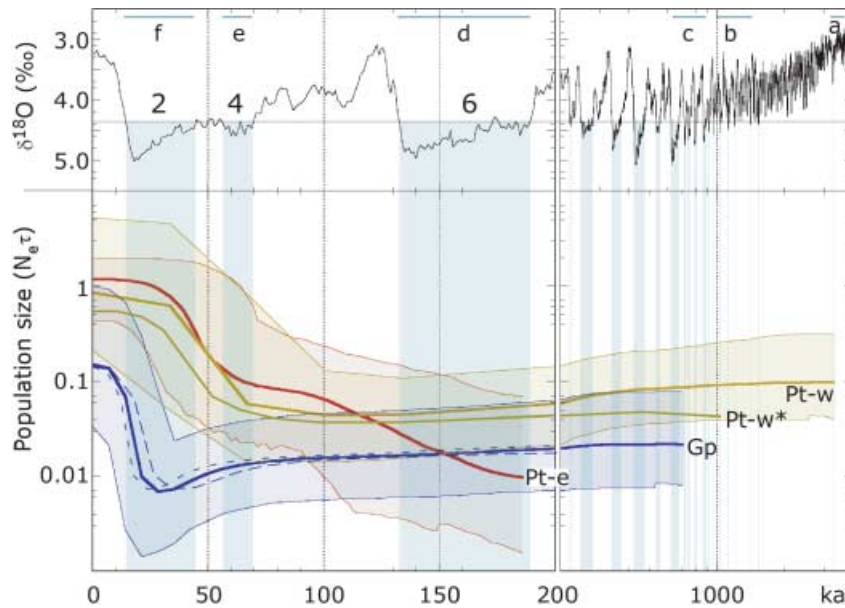


Fig. 2 Climate change and the demographic histories of two Patagonian fish over the past 3 Ma. Top panel: Inverted $\delta^{18}\text{O}$ time series of 57 stacked globally distributed benthic $\delta^{18}\text{O}$ records (Lisiecki & Raymo 2005). Bottom panel: Bayesian skyline plots for *Galaxias platei* (blue) and for *Percichthys trucha* populations west of the Andes (Chile) (brown) and east of the Andes (Argentina) (red). The most significant glaciations in the southern Andes are shaded in blue. Glaciations with prominent geological records throughout the region (Rabassa & Clapperton 1990; Singer *et al.* 2004) are labelled with letters (a) earliest evidence of widespread glaciations; (b) Largest Patagonian Glaciation; (c) extensive mid-Pleistocene glaciations; and (d–f) OIS-6, 4 and 2. The time series on the X axis are presented on a linear-scale for the time period 0–200 000 years ago but on a log-scale for 200 000 to 4 Ma. The Bayesian skyline plots (Drummond *et al.* 2005) are derived from mtDNA control region sequences, and the y axis represents the product of effective population size and generation length. The thick solid lines are median estimates, and shaded areas indicate 95% highest posterior density (HPD) regions. Gp, *Galaxias platei*: $n = 39$ haplotypes obtained from $N = 166$ individuals collected from 25 locations in Chile and Argentina. The dashed lines in the *G. platei* skyline plot show the pattern (median) obtained under the assumption of per site mutation rates of 6%/Ma (short dashes) and 4%/Ma (long dashes) Pt, *Percichthys trucha*: $n = 145$ haplotypes obtained from $N = 552$ individuals, collected from 18 locations in Argentina ($n = 95$ haplotypes, Pt-e) and 18 locations in Chile ($n = 50$ haplotypes, Pt-w). Pt-w* are Chilean samples with the haplotypes found in three most northern locations excluded, as indicated in text.

haplotypes described for Chile, the highly divergent Haplotypes 140–150 were present exclusively in the three northernmost rivers Maipo, Rapel (Tinguiririca) and Estero Nilahue and were excluded from some of our analyses.

Population dynamics through time

We examined past population dynamics for both, *P. trucha* and *G. platei*, using the Bayesian skyline plot approach of Drummond *et al.* (2005) implemented in the program BEAST (Drummond & Rambaut 2006). This method uses standard Markov Chain Monte Carlo (MCMC) sampling procedures to estimate a posterior distribution of effective population size through time from a sample of gene sequences, given a previously specified nucleotide substitution model (Drummond *et al.* 2002). We used the HKY + G, and the HKY + I + G as the most appropriate models of sequence evolution for *P. trucha* and for *G. platei*, respectively, as derived by MODELTEST 3.06 (*P. trucha*) and 3.07 (*G. platei*) (Posada & Crandall 1998). For *P. trucha* ($N = 145$ haplotypes),

nucleotide frequencies were, A = 0.305, C = 0.224, G = 0.194 and T = 0.284, and total average sequence length was 363.6. For *G. platei* ($N = 39$ haplotypes), nucleotide frequencies were, A = 0.2980, C = 0.224, G = 0.194 and T = 0.284, and total average sequence length was 819.2. Throughout our analysis, we assumed a within-lineage per site mutation rate of 5%/Ma for both, *P. trucha* and *G. platei*. We address the uncertainty around this estimate by (i) also presenting results assuming within-lineage per site mutation rates of 4%/Ma and 6%/Ma (values within square brackets below); and (ii) by discussing the implications for coalescence of halving or doubling the per site mutation rate to 2.5% and 10%/Ma, respectively.

Results

The significant events (bottlenecks, coalescence of haplotype diversity) identified in the demographic histories of *Galaxias platei* and *Percichthys trucha* coincide with five major climatic events over the past 4 Myrs. The coldest Pleistocene glaciation

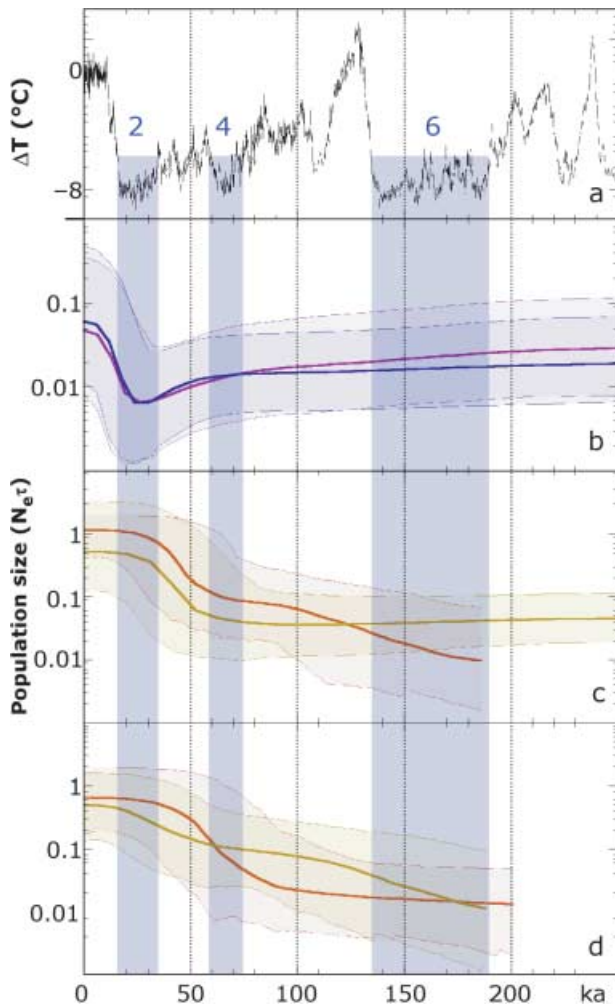


Fig. 3 Climate change and the demographic histories of two Patagonian fish over the past 250 000 years ago. (a) Vostok ice core δ D record of temperature departure from today (Petit *et al.* 1999) shown with recalibrated timescale according to Mortyn *et al.* (2003) and (b–d) Regional Bayesian skyline plots over the past 250 000 years ago. The most significant glaciations are shaded blue and labelled according to OIS. Bayesian skyline plots ($m = 10$) are derived from samples of mtDNA control region sequences: (b) *Galaxias platei* west (blue) (Chile, $n = 23$ haplotypes from 14 locations) and east (purple) (Argentina, $n = 19$ haplotypes from 11 locations) of the Andes, (c) *Percichthys trucha* from west (brown, $n = 50$ haplotypes from 18 locations) and east (red, $n = 95$ haplotypes from 18 locations) of the Andes and, (d) *Percichthys trucha* from Argentina north and south of Lat. 42°S (north: red, 48 haplotypes from seven locations; south: brown, 54 haplotypes from 11 locations). See Table 1 for location names and coordinates. Skyline plot axes, curves, and confidence bands as in Fig. 2.

(0.7 Ma) and the LGM (23–25 000 years ago, Rabassa *et al.* 2005) were implicated in the dynamics of *G. platei*, while the large Pliocene glaciations (3.5 Ma), the LPG (1 Ma) and the most recent glaciation to reach the Atlantic coast (140–180 ka) left genetic imprints on *P. trucha*.

Haplotypes of the cold-adapted species, *G. platei*, coalesced at approximately 0.7 Ma [0.58–0.88 Ma] (Fig. 2). In addition, *G. platei* underwent a major demographic bottleneck during the LGM, with populations of less than 10% of the current size (Fig. 2). The rapid demographic expansion that began at this time slowed during the Holocene. Populations east and west of the Andes showed very similar timelines, coinciding in time of coalescence and the recent bottleneck (Fig. 3b).

In contrast, demographic patterns of the more warm-adapted species, *P. trucha*, differed regionally (Fig. 3c, d). The most divergent haplotypes were found west of the Andes; in the northernmost part of the species range (Maipo, Rapel and Nilahue systems). With these samples included, coalescence occurred at 3.2 Ma [2.7–4.0 Ma], broadly coinciding with the time of the mid-Pliocene glaciations (Fig. 2). For populations south of 36°S latitude (excluding the northernmost collections), coalescence was more recent (1.1 Ma) [0.86–1.29 Ma] (Fig. 2), coinciding with the time of the LPGs. Western *P. trucha* populations underwent a bottleneck 100–140 000 years ago, with rapid expansion beginning c. 60 000 years ago (OIS 4) (Fig. 3c).

Populations east of the Andes are much younger; eastern haplotypes coalesced c. 180–200 years ago [155–233 000 years ago], during the OIS 6 glacial advance (Fig. 3c). South of 42°S, eastern populations showed nearly continuous exponential growth since this time, although the irregularities in the curve suggest slightly slower growth during times of glacial advance, OIS 4 and 2 (Fig. 3d). North of 42°S, the eastern populations (Tunuyán, Atuel, Colorado and Negro river systems) show a similar coalescence time, but expansion was relatively slow until c. 80 000 years ago (OIS 5 interglacial), accelerated thereafter and slowed again prior to OIS 2 (Fig. 3d). Unlike *G. platei*, none of the *P. trucha* populations showed evidence of a significant decline during the LGM.

Discussion

While many local factors have disrupted aquatic ecosystems in the Andes over the past few million years, including large landslides, volcanic and tephra events, as well as tectonic-induced drainage changes, the wide geographical extent of synchronous demographic changes we see for *Galaxias platei* and *Percichthys trucha*, and the coincidence of the timing of those changes with significant Andean glaciations, strongly suggest a broad-scale causal factor such as climate change. During the glacial cycles of the Quaternary, ice expanded east and west from the Andean divide, with lobes first advancing along existing valleys, covering upland lakes and streams. The cooling climate, accompanied by regional increases in aridity, presumably caused aquatic species to shift away from the divide, perhaps expanding onto the newly exposed continental shelf as sea level

dropped. During glacial retreat, rivers charged with meltwater and newly formed lakes provided new habitat. East of the Andes, the formation of extensive systems of pro-glacial lakes and streams (Clapperton 1993) probably facilitated interdrainage exchange. The large proglacial and postglacial lakes were contained within the large canyons carved in the past 3–4 Ma, in large part by the glaciers themselves. The timelines of the species in this study do not extend beyond 4 Ma, but the dynamics and response to climatic change of the aquatic fauna present in the earlier, purely fluvial landscape was probably quite different.

The close association of coalescence times, the major bottlenecks and periods of expansion for *G. platei* and *P. trucha* with some of the strongest signals in the marine $\delta^{18}\text{O}$ and Vostok temperature record is striking. In particular, coalescence times (3.2 Ma, 1.1 Ma, 0.7 Ma and 180 000 years ago), times at which populations began growing from small remnant populations, coincide with times known to have been particularly cold (Fig. 2). Cold periods tended to be very dry outside the glacial limits in much of Patagonia east of the Andes, while remaining much wetter west of the Andes, during the last two glacial cycles, and perhaps before (Heusser *et al.* 2000).

The most recent glacial advance (OIS 2, *c.* 15–35 000 years ago) produced a marked population bottleneck in the cold-water adapted *G. platei* but had apparently little effect on population size of the more widely distributed *P. trucha*. The current distribution of *G. platei* lies entirely within the area covered by ice during the LGM, indicating that the dynamics of *G. platei* and its response to glacial cycles may be related to its 'fugitive life-style', a tolerance of harsh conditions coupled with the ability to colonize newly opened habitat (e.g. postglacial lakes). *G. platei* has likely experienced repeated episodes of population reduction, as ice cover forced it to shift away from the Andes during each glacial cycle. Its tolerance of glacial water probably enabled it to survive many of the glaciations within the drainages and pro-glacial lakes to the east and west of the ice. *G. platei*, unlike *P. trucha*, is found in southern Chilean drainages, where glaciers reached the Pacific during the LGM, and in Tierra del Fuego, mostly covered by ice during the very large OIS 6 glaciation (*c.* 140–180 000 years ago) (Clapperton 1993). Unidentified local refugia suitable for *G. platei* may have been present. Alternatively, there may have been postglacial dispersal among neighbouring drainages through river capture, or dispersal via the sea: although current *G. platei* populations are mostly lacustrine, a marine larval phase is a primitive character in the Galaxiidae (Waters & Wallis 2001); and many populations of a related species, *G. maculatus*, as well as other Australian/New Zealand Galaxiidae, are diadromous (McDowall 1990).

The very large glacial expansion during OIS 6 (*c.* 140–180 000 years ago) had the greatest impact on the demo-

graphic history of the more warm-adapted *P. trucha*, greatly reducing populations east and west of the Andes (Fig. 3). The coalescence of eastern haplotypes at this time, including those from the northern limit of the species distribution, suggests the (near) elimination of eastern *P. trucha* populations. The close link between some eastern and western haplotypes (Ruzzante *et al.* 2006) indicates that colonization could have occurred from the west. The apparent elimination of eastern populations during OIS 6, even those outside the glacial limits, together with the bottleneck experienced by western populations at the same time, suggests that climate, rather than ice cover *per se*, produced the decline. *P. trucha* probably survived OIS 6 west of the Andes in the coastal Cordillera in Chile. This region may have been a critical refuge for many aquatic species during the larger glacial expansions, as suggested by the presence of several species within the Percichthyidae and Galaxiidae found only west of the Andes, and with very restricted geographical distributions (*Percichthys melanops*, *Galaxias globiceps*, *Brachygalaxias bullocki*, *B. gothei*, Dyer 2000).

Recent glaciations (OIS 4 and 2) had much less effect on *P. trucha*. Population expansion in southern populations east of the Andes appears to slow during the glacial advances, but populations did not decline (Fig. 3d). Loss of habitat for *P. trucha* due to glacial advance (and concomitant changes in moisture) may have been partially compensated by increased exposure of the eastern steppe as the sea level dropped. In addition, the development of extensive pluvial lakes in the steppe probably contributed to rapid population expansion, providing habitat and increasing opportunities for interdrainage dispersal. The delayed recovery of northern and western populations following the OIS 6 glaciation may be attributed to slower increases in habitat recovery or to more limited opportunities for interdrainage dispersal (large pluvial lakes were largely restricted to southern areas east of the Andes). The rapid expansion of western populations, through the OIS 4 glaciation (60–70 000 years ago) and into the following interglacial probably reflects the continuity of wet southern Chile climate, and the consequent maintenance of aquatic environments, since at least OIS 4 (Heusser *et al.* 2000).

Uncertainties in the demographic timelines

We have shown a marked correspondence between major historical climate events and coalescence times, population bottlenecks and periods of rapid expansion for *G. platei* and *P. trucha* in Patagonian lakes and rivers. Our conclusions must however, be tempered by the consideration of potential effects of three different sources of uncertainty: (i) evolutionary variability around the coalescence: different loci evolve at different rates and are thus likely to exhibit different coalescence time; (ii) inadequate sampling may have missed some haplotypes and may thus have led to

underestimates of population age; and (iii) uncertainty of the molecular clock of the mtDNA control region of the two species may have led to uncertainty in estimates of the timing of demographic events. Of these three, we regard evolutionary variability around the coalescence as the most important source of uncertainty in our study; nuclear loci in particular are likely to exhibit different coalescence and perhaps a slightly different temporal dynamics of effective population size (N_e) as inferred from the Bayesian skyline approach. Ongoing work in our laboratory is aimed at addressing this. Incomplete sampling of haplotypes is inevitable in any study of wild populations. However, the extensive geographical coverage we obtained for both species should have ensured that most or all common haplotypes were included. Additional haplotypes almost certainly exist, but they are most likely to be rare and terminal haplotypes in star phylogenies, and thus they are not likely to significantly affect the overall shape(s) of the skyline plots, although they may have had some effect on the time to coalescence. We address the third source of variability, variability due to uncertainty in the calibration of the molecular clock for the control region of mtDNA in the following section.

The molecular clock for the control region of mtDNA is uncertain and seems to vary across taxonomic groups and as a function of the timescale over which mutation rates are estimated (Penny 2005). Across fish taxonomic groups, estimates of mutation rates per site for cichlid fishes include 2.2–4.5%/Ma for cichlids inhabiting two East African crater lakes (Sato *et al.* 2003), 5.6%/Ma for cichlids of Lake Victoria (Nagl *et al.* 2000), 6.5–8.8%/Ma for cichlids of Lake Malawi (Sturmbauer *et al.* 2001) and 7.08%/Ma for New World cichlids (Barluenga & Meyer 2004). Mutation rates for the mtDNA control region in other fish groups range from 3.6%/Ma for snooks (Percoidei: Centropomidae; Donaldson & Wilson 1999) to 5%/Ma for Pygmy Angelfish (Genus Centropyge; Bowen *et al.* 2006). Outlier estimates from the fish literature include estimates of 15–20%/Ma for sardines (Bowen & Grant 1997) and even 33%/Ma for butterflyfishes (Chaetodontidae; McMillan & Palumbi 1997). Molecular rates estimated from population-level analyses and over genealogical timescales (< 1 Ma) have also been observed to be an order of magnitude higher than rates estimated over geological timescales (> 1 Ma; Ho *et al.* 2005; Penny 2005; Ho & Larson 2006).

Following Bowen *et al.* (2006), we assumed a within-lineage per site mutation rate of 5%/Ma for the mt control region of both species *P. trucha* and *G. platei*. We currently have no locus information with which to refine our estimates of mutation rate; thus, we regard our estimates of coalescence as approximate. To indicate the sensitivity of our results to this assumption, we bracket estimates by reporting values based on per site mutation rates of 4%/Ma and 6%/Ma. Our main conclusions regarding the palaeoclimate influence on ancestral population dynamics remain robust.

Estimates of coalescence time are directly related to mutation rates in the Skyline approach. For example, halving the within-lineage per site mutation rate to 2.5%/Ma would translate into a doubling of the time to coalescence, i.e. from 700 000 years ago to 1.4 Ma for *G. platei* and from 3.2 Ma to 6.4 Ma for western *P. trucha* populations. Environment (including temperature) affects mutation rate (Gillooly *et al.* 2005), and any single estimate is probably an approximation, as mutation rates are not constant over time, even for a single species. For example, we suspect that the mutation rate for *G. platei* is slightly lower than that of *P. trucha*, given the affinity of *G. platei* for the benthic environments of cold-water postglacial lakes (Milano *et al.* 2006). However, given the lack of specific information for the species under consideration, a rate of 5%/Ma is a reasonable starting point (Bowen *et al.* 2006). The historic climate events to which we link demographic events lasted thousands or tens of thousands of years, and some error in the mutation rate would not greatly change the associations. For instance, the Bayesian skyline plot for *G. platei* shown in Fig. 2 for per site mutation rates of 4%/Ma (long dashes), 5%/Ma (solid line), and 6%/Ma (short dashes) suggests a link between the LGM and the bottleneck experienced by this species, regardless of variation in mutation rate within this range. However, given the other two sources of uncertainty, we regard our conclusions as our best 'educated guess' as to the identity of the key events during the Quaternary that controlled the population dynamics of the aquatic fauna of Patagonia.

Conclusion

In summary, the timelines of demographic change obtained using a Bayesian Skyline approach suggest that two of the most wide-spread species of fish in Patagonia responded differently to the climatic and geomorphological events that characterized the Quaternary, and that the more warm-adapted species, *Percichthys trucha*, was less affected by recent glacial advances than the cold-adapted *Galaxias platei*. *P. trucha* appears to have had a permanent refuge northwest of the Andes throughout the Quaternary, and the large refuge areas east and north of the glacial limit likely reduced the impact of recent glacial cycles on its population size and genetic diversity. In contrast, use of temporary habitats by *G. platei* (postglacial lakes and rivers) led to dramatic reductions in population size for this species during the LGM and probably during most previous glacial stages. The glaciations most prominently represented in the geological record had disproportionately large effects on *G. platei* and *P. trucha*, and perhaps on the aquatic fauna of the region in general, but some effects varied regionally, pointing to the need for additional information on the palaeoclimates of these periods. Our results thus illustrate the potential strengths of an interplay between the study

of phylogeography and the study of landscape evolution. Ancient demographic histories can only be understood in light of the geomorphologic and palaeoclimatic history of the region. In turn, phylogeography – especially comparative phylogeography – may indicate the times and rates of geomorphologic and palaeoclimatic change of most relevance to the history of life in our planet.

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