Historical biogeography and post-glacial recolonization of South American temperate rain forest by the relictual marsupial *Dromiciops gliroides*

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**ABSTRACT**

**Aim** Long-term climatic variation has generated historical expansions and contractions of species ranges, with accompanying fragmentation and population bottlenecks, which are evidenced by spatial variation in genetic structure of populations. We examine here hypotheses concerning dispersal and vicariance in response to historical geoclimatic change and potential isolation produced by mountains and water barriers.

**Location** The temperate rain forest of southern South America, which is distributed from coastal Chile, including the large continental island of Chiloé, across the Andes into Argentina.

**Methods** We investigated our hypotheses in the phylogenetically and biogeographically relictual marsupial *Dromiciops gliroides*. We examined 56 specimens, which resulted from field samples and museum study skins from 21 localities. We evaluated the influence of two major barriers, the Andean cordillera and the waterway between the mainland and the large island of Chiloé, by performing Bayesian and maximum-likelihood phylogenetic analyses on sequences of 877 base pairs of mitochondrial DNA. We further tested the contribution of the proposed geographical barriers using analysis of molecular variance (amova). We also evaluated the responses of populations to historical north–south shifts of habitat associated with glacial history and sea-level change.

**Results** Our analyses revealed a phylogeny with three clades, two of which are widespread and contain nearly all the haplotypes: a northern clade (36–39° S) and a southern clade (40–43° S). These two clades contain forms from both sides of the Andes. Within the southern clade, island and mainland forms were not significantly differentiated. Tests of recent demographic change revealed that southern populations have experienced recent expansion, whereas northern populations exhibit long-term stability. The direction of recent gene flow and range expansion is predominantly from Chile to Argentina, with a modest reciprocal exchange across the Andes. Recent gene flow from the island of Chiloé to the mainland is also supported.

**Main conclusions** The genetic structure of contemporary *D. gliroides* populations suggests recent gene flow across the Andes and between the mainland and the island of Chiloé. Differences in demographic history that we detected between northern and southern populations have resulted from historical southward shifts of habitat associated with glacial recession in South America. Our results add to a growing literature that demonstrates the value of genetic data to illuminate how environmental history shapes species range and population structure.

**Keywords**

*Dromiciops gliroides*, genetic population structure, historical biogeography, Pleistocene climate change, Pleistocene glaciation, southern South America, species range.
INTRODUCTION

The distribution of populations across the geographical range of a contemporary species is the result of historical processes, the influence of which can be inferred from patterns of genetic population structure and phylogenetic lineages (Avise, 2000; Knowles & Maddison, 2002). Geographical analysis remains fundamental to the study of evolution and speciation (Mayr, 1942). Historical climate change, interacting with geological features and ecosystems, has played a major role in the expansion and contraction of species ranges (Riddle, 1998; Hewitt, 2004). The associated habitat shifts have generated transient range fragmentation, variation in gene flow and genetic drift, and population bottlenecks. Phylogeography assesses the effects of these evolutionary processes on intraspecific population histories, and contributes to the biogeographical concerns of vicariance and dispersal.

South America’s temperate rain forests (35°–55°S) date from the late Cretaceous Gondwanan supercontinent, and were later influenced by the Oligocene uplift of the Andes (Simpson, 1980). The presence of the Andes created altitudinal zonation that restricted the distribution of certain biota to lowlands on the east or west of the cordillera (Vuilleumier & Monasterio, 1986; Webb, 1991). On the other hand, a variety of small mammals with geographical ranges that encompass both sides of the Andes have apparently recently utilized low-elevation southern mountain passes to extend their range across the Andes (Smith et al., 2001; Palma et al., 2002, 2005). The climatic and glacial cycles of the Pleistocene produced periods of expansion, reduction, fragmentation and isolation of this forest habitat; geographical shifts of the forest biota from north to south occurred along with these changes (Villagrán & Hinojosa, 1997; Heusser et al., 1999). During the last glacial maximum (c. 18,000 years ago), ice sheets covered both the lowlands and the Andean cordillera in the south (Heusser et al., 1999), but at latitudes north of c. 41°S, ice sheets were confined to the cordillera, leaving much of the lowland forest intact. This northern lowland forest represents a major refugium for the forest and its inhabitants, but other refugia to the south have been identified in ice-free coastal regions including the north-western portion of the island of Chiloé (Villagrán, 1988).

The endemic arboreal marsupial Dromiciops gliroides (Thomas, 1894), known as monito del monte, is restricted to a narrow strip of temperate rain forest on the western side of South America that includes Chile and extends over the Andes into Argentina from c. 36 to 43°S (Fig. 1a; Muñoz-Pedreros & Palma, 2000). Dromiciops gliroides is the sole extant representative of the order Microbiotheria, which shares a stronger phylogenetic affinity with the Australasian marsupials than with the Neotropical orders (Palma & Spotorno, 1999; Asher et al., 2004; Phillips et al., 2006). Dromiciops gliroides is thus both a biogeographical and a phylogenetic relict. The dietary ecology of this frugivorous and insectivorous mammal and its co-occurrence with southern beech and bamboo represent a unique ecological tie to the southern temperate rain forest (Amico & Aizen, 2000; Muñoz-Pedreros & Palma, 2000). Anthropogenic reduction and fragmentation of this forest habitat (Armento et al., 1998), along with the limited geographical range of D. gliroides, have resulted in the IUCN listing of this species as ‘vulnerable’ (New World Marsupial Specialist Group, 1996). Given the geographical and phylogenetic distinction of D. gliroides, and its ecological uniqueness, it seems especially useful to understand the genetic structuring of populations over the geographical range of the species. Such an analysis provides both an indication of the historical dynamics of this forest system and a basis for conservation assessment.

We use a phylogeographical approach, based on mitochondrial DNA, to examine several hypotheses concerning the influence of proposed geographical barriers and environmental history on the recent evolution of populations of D. gliroides in Chile and Argentina. We explicitly test the potential influence on population structure of the Andean Cordillera and the waterway separating continental Chile and the island of Chiloé. If these two potential geographical barriers have restricted gene flow over an extended period, we expect evidence of reciprocal monophyly and genetic divergence associated with each barrier. On the other hand, lack of monophyly would suggest either retention of ancestral lineages in the populations or recent gene flow across the proposed geographical barriers. Additionally, we attempt to understand the role of historical habitat shifts and sea-level changes associated with the recent geoclimatic cycles of the Quaternary. We hypothesize that the north–south pattern of glacial coverage across the species range of the monito del monte has produced contrasting population histories in the north and south. We predict positive evidence for demographic and geographical expansion over the southern extent of the species range in contrast to long-term stability of the northern populations. Our study documents the interplay of both climatic changes and geographical features in southern South America on the structure and history of an endemic marsupial.

MATERIALS AND METHODS

Sampling

We collected 29 specimens and tissue samples of D. gliroides from eight localities in Chile, which were deposited in the Burke Museum of Natural History and Culture, University of Washington (Table 1). Liver or kidney tissues were excised and stored in 100% ethanol in the field and later held at −80°C in an ultra-cold freezer at the Burke Museum.

We complemented our field samples with an additional 27 tissue and museum-skin samples from Chile and Argentina from four collections: Instituto de Ecología y Evolución, Universidad Austral de Chile, Valdivia, Chile; Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn, Argentina; Field Museum of Natural History, Chicago, IL, USA; Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA (Table 1).
Laboratory techniques

We extracted whole genomic DNA using the prescribed protocol of DNeasy Tissue Kit (Qiagen, Valencia, CA, USA) from 41 tissue samples. Working at a separate time and location, we used a similar protocol to extract DNA from 15 museum study skins, except that we tripled the volumes of the following reagents: ATL buffer, proteinase K, AL buffer and ethanol. For these skin extractions we also purified and concentrated the extracted DNA using the Bio101 GeneClean Spin Kit (Qbiogene Inc., Carlsbad, CA, USA); finally we performed a negative control during all skin extractions to ensure detection of any possible contamination.

We amplified two portions of the mitochondrial genome, the first domain of the control region (CR) and the first section of cytochrome b (cyt-b). We used polymerase chain reaction (PCR) with the primer combination TDKD (5'-CCTGAACTAGAACACCATG-3'; Kocher et al., 1993) and LCNTL (5'-CACYTAYACWCCCAAAGCT-3'; Bidlack & Cook, 2001) for CR; MVZ 5 (5'-CGAAGCTTGATATCGAATGATATTTGTCCTC-3') and MVZ 4 (5'-GCAGCCCTTGAGAATGTATTGTCCCTC-3'; Smith & Patton, 1993) for cyt-b. PCR reactions were run in a total volume of 10 or 25 µL for cyt-b and CR, respectively. Each reaction contained 1× PCR buffer, 2.0 mM MgCl₂, 0.2 mM of each dNTP, 1.0 µM of each primer and 1 U Tag Jump Start DNA polymerase (Sigma, St Louis, MO, USA). For amplification we used 1–2 µL genomic DNA for CR and 1 µL for cyt-b. The PCR reactions were carried out in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). The cycle conditions for CR were: 94°C for 45 s; 35 cycles of 94°C for 10 s, 50°C for 15 s and 72°C for 30 s; 72°C for 3 min. The cycle conditions for cyt-b were: 94°C for 3 min; 30 cycles of 94°C for 40 s, 50°C for 40 s, 72°C for 40 s; 72°C for 5 min. All reactions included a negative control to check for contamination.

We removed unincorporated bases from PCR products with QIAquick PCR Purification Kit (Qiagen) prior to sequencing. Sequencing reactions for each sample were performed with the same forward and reverse primers as used in amplification. Each primer was run separately in a total volume of 10 µL using a Big Dye Terminator Cycle Sequencing Ready Reaction Mix 3.1 (Applied Biosystems). Sequences were visualized on an ABI 3100 sequencer (Applied Biosystems). Sequences were aligned using the software by the University of Washington. Degradation of DNA isolated from museum skins limited the total number of base pairs analysed to 877 (CR = 427 bp; cyt-b = 450 bp). All sequences were deposited in GenBank (accession numbers CR:EU481860–EU481915; Cyt-b:EU481916–EU481971).

Phylogenetic analysis

The model of DNA evolution for our analysis was determined using the hierarchical likelihood ratio test implemented.
Table 1  Specimens of *Dromiciops gliroides* analysed in this study with locality numbers (Fig 1a), country of origin, Chilean region names and Roman numeral designation or Argentine province names and abbreviations, latitude and longitude.

<table>
<thead>
<tr>
<th>Locality number</th>
<th>Country</th>
<th>Region (Chile) or province (Argentina)</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Specimen number</th>
<th>Clade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chile</td>
<td>Bio Bio (VIII)</td>
<td>36°35' S</td>
<td>71°28' W</td>
<td>UWBM 78633</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>Chile</td>
<td>Bio Bio (VIII)</td>
<td>37°26' S</td>
<td>73°21' W</td>
<td>IEEUACH 1053*, IEEUACH 1054*</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>Chile</td>
<td>Araucania (IX)</td>
<td>37°50' S</td>
<td>72°52' W</td>
<td>UWBM 78634, UWBM 78635</td>
<td>A</td>
</tr>
<tr>
<td>4</td>
<td>Chile</td>
<td>Araucania (IX)</td>
<td>38°14' S</td>
<td>71°44' W</td>
<td>UWBM 78636, UWBM 78637, UWBM 78638, UWBM 78639, UWBM 78640</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>Chile</td>
<td>Araucania (IX)</td>
<td>38°32' S</td>
<td>71°11' W</td>
<td>IEEUACH 6161</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>Chile</td>
<td>Araucania (IX)</td>
<td>39°06' S</td>
<td>71°52' W</td>
<td>UWBM 78641</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>Argentina</td>
<td>Neuquen (NQ)</td>
<td>39°22' S</td>
<td>71°14' W</td>
<td>MVZ-RDS 17584, MVZ-RDS 17593, MVZ-RDS 17602, MVZ-RDS 17603</td>
<td>A</td>
</tr>
<tr>
<td>8</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>39°39' S</td>
<td>73°11' W</td>
<td>UWBM 78623, UWBM 78625, UWBM 78626, UWBM 78627, UWBM 78628, UWBM 78629, UWBM 78630, UWBM 78631</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>39°43' S</td>
<td>73°07' W</td>
<td>IEEUACH 5731</td>
<td>C</td>
</tr>
<tr>
<td>10</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>40°40' S</td>
<td>72°16' W</td>
<td>UWBM 78644, UWBM 78645</td>
<td>C</td>
</tr>
<tr>
<td>11</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>40°46' S</td>
<td>72°30' W</td>
<td>IEEUACH 1056*</td>
<td>C</td>
</tr>
<tr>
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<td>40°53' S</td>
<td>71°17' W</td>
<td>MVZ 184914</td>
<td>C</td>
</tr>
<tr>
<td>13</td>
<td>Argentina</td>
<td>Rio Negro (RN)</td>
<td>41°03' S</td>
<td>71°34' W</td>
<td>MVZ-RDS 17491</td>
<td>C</td>
</tr>
<tr>
<td>14</td>
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<td>Los Lagos (X)</td>
<td>41°11' S</td>
<td>72°33' W</td>
<td>IEEUACH 2144*, IEEUACH 2145*, IEEUACH 2146*, IEEUACH 2147*, IEEUACH 2148*, IEEUACH 2149*, IEEUACH 5732, IEEUACH 7027, IEEUACH 7028</td>
<td>C</td>
</tr>
<tr>
<td>15</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>41°11’ S</td>
<td>72°33’ W</td>
<td>UWBM 78624, UWBM 78643</td>
<td>C</td>
</tr>
<tr>
<td>16</td>
<td>Argentina</td>
<td>Rio Negro (RN)</td>
<td>41°33' S</td>
<td>71°45' W</td>
<td>CNP 890</td>
<td>C</td>
</tr>
<tr>
<td>17</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>41°53' S</td>
<td>73°41' W</td>
<td>UWBM 79629, UWBM 79630, UWBM 79634, UWBM 79635, UWBM 79859, UWBM 79638, UWBM 79639, UWBM 79642</td>
<td>C</td>
</tr>
<tr>
<td>18</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>41°58’ S</td>
<td>72°36’ W</td>
<td>FMNH 129812*, FMNH 134624*</td>
<td>C</td>
</tr>
<tr>
<td>19</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>42°03’ S</td>
<td>73°58’ W</td>
<td>IEEUACH 6998*, IEEUACH 6999*</td>
<td>C</td>
</tr>
<tr>
<td>20</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>42°38’ S</td>
<td>74°07’ W</td>
<td>IEEUACH 6997*</td>
<td>C</td>
</tr>
<tr>
<td>21</td>
<td>Chile</td>
<td>Los Lagos (X)</td>
<td>43°08’ S</td>
<td>73°46’ W</td>
<td>FMNH 127465*</td>
<td>C</td>
</tr>
</tbody>
</table>

Specimen numbers are preceded by museum codes. Specimens with an asterisk indicate DNA extraction from museum skins. Clade (A–C) assignment corresponds to phylogenetic results illustrated in Fig. 1(b).

UWBM, University of Washington, WA, USA; IEEUACH, Instituto de Ecología y Evolución, Universidad Austral de Chile, Valdivia; CNP, Colección de Mamíferos del Centro Nacional Patagónico, Puerto Madryn, Argentina; FMNH, Field Museum of Natural History, Chicago, IL, USA; MVZ, Museum of Vertebrate Zoology, University of California, Berkeley, CA, USA.

The phylogenetic relationships among haplotypes were reconstructed using maximum likelihood (ML; Felsenstein, 1981) and Bayesian methods in *paup* ver. 4.0b10 (Swofford, 2002) and MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck, 2003). We performed the ML analysis with tree bisection and reconnection (TBR) branch swapping and a heuristic search with 10 random replicates. The Bayesian analysis was performed using four replicates with four chains (three heated and one cold); the analyses were run for a million generations sampling every 100 generations with a burn-in of 5000 generations. Nodal support was determined by performing an ML bootstrap analysis (Felsenstein, 1985) with 1000 replicates in *paup* and by using Bayesian posterior probabilities as provided by MrBayes. In order to validate the concatenation of sequence data from both cyt-b and control regions, we ran separate ML analyses for the combined data set and for each locus independently. For all three analyses, we obtained the same major clades with only minor variation in the number of unique haplotypes and in branch lengths. Similarly a Bayesian analysis of the combined data set generated a phylogeny with the same topology as the ML analysis. We present our ML phylogeny with both Bayesian posterior probabilities and bootstrap support.

**Population structure**

We investigated the structure of populations to reveal correlations with genetic variation and the geographical landscape...
of Chile and Argentina by using a hierarchical approach implemented through analysis of molecular variation (AMOVA) (Excoffier et al., 1992) employing ARLEQUIN ver. 2.001 (Schneider et al., 2000). This analysis partitions the total variance observed into covariance components that result from the variance among regions, among populations within regions, and within populations. The covariance components are then used to calculate fixation indices, \( \Phi \) statistics, that are equivalent to Wright’s \( F \) statistics (Wright, 1978). The significance of the fixation indices is tested using a nonparametric permutation approach (Excoffier et al., 1992).

We were interested in two potential geographical barriers to the movement of haplotypes: the waterway separating mainland Chile from the island of Chiloé, and the cordillera of the Andes that separates Chile and Argentina. For the examination across these two proposed barriers, we used sample sites as the criterion for population designation, and grouped the populations by location with respect to the barrier of interest. For the waterway separating Chiloé and the mainland, we grouped all specimens from the island (locality numbers 17 and 19–21, Fig. 1a). We compared these with mainland specimens from southern continental Chile (locality numbers 9–11, 14, 15 and 18). Similarly, for the Andean cordillera as a barrier we grouped populations immediately west (numbers 1, 4–6, 10, 11, 14, 15 and 18) and east (7, 12, 13 and 16) of the Andes. We ran this with ARLEQUIN, calculating a distance matrix using the DNA model of Tamura with a gamma correction (Tamura, 1992). This model was the most similar model of DNA evolution available within ARLEQUIN to that selected by MODELTST. The two separate analyses were run with 16,000 permutations. We also report standard \( \Phi_{ST} \) scores.

We also tested for isolation by distance (IBD) using a Mantel test (Mantel, 1967) implemented through the program ALLELES IN SPACE (Miller, 2005). The Mantel test explores the significance of correlation between two data matrices; here we use genetic distance and geographical distance. The two major clades, determined by phylogenetic analysis, were examined independently with 1000 permutations to test whether populations within either clade conformed to IBD by exhibiting a positive correlation between genetic and geographical distance. The IBD is anticipated when populations have been stable over a long period and when gene flow occurs more often among geographically neighbouring populations.

**Molecular diversity and historical population dynamics**

We used ARLEQUIN to estimate haplotype diversity and nucleotide diversity for the combined data set. We also examined corrected percentage sequence divergence for the cyt-\( b \) data set alone. For the combined data set, we further compared the two major clades using four methods. We examined the frequency distribution of pairwise differences between haplotypes, the mismatch distribution (Rogers & Harpending, 1992). A unimodal distribution is expected when a population has experienced recent expansion. We further tested for patterns of demographic history with Fu’s \( F_S \) test of neutrality (Fu, 1997), which is based on an infinite-sites model without recombination. This test evaluates the probability of observing a random neutral sample with a number of alleles that is equal to or smaller than the observed number of alleles. When the \( F_S \) statistic is large and negative, one can infer demographic expansion. We further examined the hypothesis of demographic expansion using FLUCTUATE (Kuhner et al., 1998). This program uses a coalescent approach to produce ML estimates of a contemporary value of \( \Theta \) (where \( \Theta = 2 \mu N_0 \)) and of exponential growth rate (\( g \)). The mutation rate per nucleotide is represented by \( \mu \), and \( N_0 \) is the contemporary effective population size of females. To explore our hypotheses of geographical expansion further, we used the program MIGRATE ver. 2.3 (Beerli, 1998; Beerli & Felsenstein, 1999, 2001), which produces ML estimates of migration rates among populations where the migration rate (\( M = m/m_d \)) is set to 4.9133 and for the other clade (southern) to 4.6123. We obtained these values using MODELTST.

**RESULTS**

To investigate the relationship of *D. gliroides* populations over the geographical range of the species, we sampled 56 individual specimens from 21 localities (Table 1; Fig. 1a). Maximum-likelihood and Bayesian analyses of the mtDNA data set revealed three distinct and well supported clades (Fig. 1b). Two of these clades (A and C) include 36 of the 41 total haplotypes, represent most of the samples (48 out of 56 total), and cover the majority of the geographical range. Surprisingly, the five haplotypes found in the small portion of the species range that lies east of the Andes, within Argentina, are split between clades A and C.

The southern clade C (south of 39°40’ latitude) contains 25 unique haplotypes from southern Chile, Argentina and the island of Chiloé. The island haplotypes are all nested in a subclade of C that shows low-resolution, ambiguous relationships with mainland haplotypes, and an overall star-like phylogeny (Fig. 1b). This indicates that *D. gliroides* on the island of Chiloé does not form a genetically distinct population from that of the mainland, with which it shares a recent history. The geographically restricted clade B is represented by only five haplotypes (from eight specimens) at a single locality (Fig. 1a, site 8) that is geographically intermediate between the larger northern and southern clades. The corrected percentage sequence divergence between clades A and C is 11.3%; 15.1% between A and B; and 8.2% between B and C. The lack of further resolution to clade B is probably due to the absence of additional intervening samples to the north and to the east.

The AMOVA examining populations across two potential geographical barriers (the Andean cordillera and the waterway
between the Chilean continent and the island of Chiloé) did not reveal significant variation between the regions (Table 2). This result corroborates the outcome of the phylogeny (Fig. 1b) regarding the lack of distinct structure across the trans-Andean and island-continental regions. The greatest source of variation revealed by AMOVA was among the populations within regions, amounting to 68–96% of variance explained. The genetic structuring within populations, as indicated by ΦST, was significant for both analyses (Table 2). It is interesting that whereas ΦST of trans-Andean populations was > 0.85, ΦST of insular vs. mainland populations was only about 0.6, indicating that population substructuring is present, but at a relatively lower level in the south than in the other geographical regions. The Mantel test revealed no correlation between genetic distance and geographical distance for the southern populations (r = 0.0354; P = 0.215), whereas a positive correlation between the two distance parameters was found for the northern populations (r = 0.5767; P = 0.001), indicating that with increasing geographical distance individuals in the north are increasingly genetically isolated.

Our analysis of haplotype distribution, molecular diversity and demographic parameters consists of comparisons among the three major clades represented in Fig. 1 (Table 3). Haplotype diversity was high across the entire geographical range (Table 3). Nucleotide diversity of the northern and southern clades (A and C) was much greater than that of the small population making up clade B. However, the southern clade (containing the greatest number of haplotypes) actually showed lower nucleotide diversity than the northern clade.

We found that for both major clades the distributions of pairwise differences were multimodal, as expected when a population has not experienced recent expansion. The result for the identified southern population may have been influenced by population substructuring, which is apparent in the phylogenetic analysis and suggested by ΦST values. Therefore we continued the evaluation of demographic history with the following three analyses. The large significant negative value of Fu’s Fs indicates recent population expansion in the southern clade (Table 3). Using an exponential model of population expansion (employed with the program FLUCTUATE), we found an indication of population growth (g = 86.842278) in the south in contrast to a modest decline (g = −7.51442) in the north. Using the program MIGRATE, we examined gene flow among three geographical components of the southern clade C to test for geographical patterns and directionality of this southern expansion. This analysis revealed that the strongest pattern of gene flow has occurred from Chile to Argentina (M = 1640) across the Andes, whereas gene flow in the opposite direction is relatively low (M = 399; Table 4). Relatively weak gene flow is also indicated from the island of Chiloé to continental Chile (M = 139), while the estimate of the reciprocal exchange suggests a lack of historical gene flow from the mainland to the island.

**DISCUSSION**

Across the entire species range of *D. gliroides* in Chile and Argentina, populations are divided at c. 40° S into a large northern and a large southern clade. Our results suggest that northern populations of *D. gliroides* persisted in ice-free areas during the most recent glacial maximum, whereas southern populations expanded upon glacial retreat. Percentage sequence divergence was c. 11% for the cyt-b data set between the northern and southern clades. Palma *et al.* (2002) report similar sequence divergence values occurring among species within the South American mouse-opossum genus *Thylamys*. The authors estimated the timing of the species-level

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**Table 2** Analysis of molecular variance (AMOVA) for regions across two prospective geographical barriers.

<table>
<thead>
<tr>
<th>Clade</th>
<th>Variance components</th>
<th>Percentage explained</th>
<th>Variance components</th>
<th>Percentage explained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among regions</td>
<td>−0.31</td>
<td>−7.91</td>
<td>−1.288</td>
<td>−10.36</td>
</tr>
<tr>
<td>Among populations within regions</td>
<td>2.644*</td>
<td>67.72*</td>
<td>11.940*</td>
<td>96.1*</td>
</tr>
<tr>
<td>Within populations</td>
<td>1.576*</td>
<td>40.29*</td>
<td>1.772*</td>
<td>14.26*</td>
</tr>
<tr>
<td>ΦST</td>
<td>0.597*</td>
<td>8.57*</td>
<td></td>
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</tbody>
</table>

*P < 0.0001.

**Table 3** Haplotype distribution, molecular diversity and demographic parameters within the three major clades represented in Fig. 1.

<table>
<thead>
<tr>
<th>Clade</th>
<th>No. individuals</th>
<th>No. haplotypes</th>
<th>Haplotype diversity</th>
<th>Nucleotide diversity</th>
<th>Fu’s Fs</th>
<th>Exponential expansion model</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>16</td>
<td>11</td>
<td>0.958 ± 0.0312</td>
<td>0.018452 ± 0.009792</td>
<td>0.74876 (n.s.)</td>
<td>0.018972 −7.51442</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>5</td>
<td>0.8214 ± 0.1007</td>
<td>0.004838 ± 0.003067</td>
<td>1.71498 (n.s.)</td>
<td>−</td>
</tr>
<tr>
<td>C</td>
<td>32</td>
<td>25</td>
<td>0.9716 ± 0.0152</td>
<td>0.008476 ± 0.004574</td>
<td>−10.38526</td>
<td>0.43381 86.842278</td>
</tr>
</tbody>
</table>
divergence to have occurred during the Quaternary. Assuming a similar evolutionary history, the divergence of the three D. gliroides clades also probably occurred in the Quaternary, however, the divergence would have pre-dated the last glacial maximum. Despite the high level of divergence of clades A and C from clade B, we cannot interpret the evolutionary history of clade B due to the limited geographical sampling. Within both northern and southern clades, haplotypes are shared east and west across the Andes, indicating cross-cordilleran gene flow. Within the southern clade, individuals on the island of Chiloe have not differentiated significantly from those on the nearby continent. The southern clade further shows recent population growth, which probably has occurred as a response to post-glacial habitat expansion. In contrast, the northern clade has experienced a relatively stable long-term history.

The temperate rain forest of southern South America, including taxa such as southern beech trees (Nothofagus spp.), dates from Cretaceous Gondwanan times (Markgraf et al., 1996; Heads, 2006). This ecosystem has been shaped by subsequent geological events, including the Oligocene uplift of the Andean cordillera (Simpson, 1980) and Pleistocene glacial cycles (Villagrán & Hinojosa, 1997; Heusser et al., 1999). The dynamic changes of the Pleistocene have included expansion, reduction, fragmentation and isolation of this forest habitat (Heusser et al., 1999). At the last glacial maximum (c. 18,000 years ago), the southern reaches of the continent experienced extensive glacial coverage, except for limited ice-free coastal refugia. These are known from the north-western portion of the island of Chiloe, near 42° S, and from nearby coastal pockets of mixed forest and grassland (Villagrán, 1988; Heusser et al., 1999). Furthermore, sea levels may have dropped enough during this time to provide connections between Chiloe and the continent. North of 41° S, glaciers covered less land, left more forest intact, and were increasingly associated with higher elevations in the Andes (Heusser et al., 1999).

The demographic history, genetic differentiation and degree of population structure of D. gliroides contrast sharply in the northern vs. southern portions of the species range due to differences in their Quaternary environmental history. The endemic coniferous tree alerce (Fitzroya cupressoides) of the southern temperate forest shows similar genetic distinctions between northern and southern populations, also influenced by the same environmental history (Allnutt et al., 1999). Historical pollen records have also documented survival of southern beech trees (Nothofagus) in the southern refugia (Markgraf et al., 1996; Premoli, 1997). The ranges of the monito del monte and these trees probably expanded more or less in common out of the southern refugia, which included north-western Chiloe and other nearby continental areas (Villagrán, 1988; Heusser et al., 1999). Our genetic characterization of the southern clade of D. gliroides is consistent with the scenario of refugial survival and expansion. The northern clade shows indications of long-term stability shown by a lack of population growth and isolation by distance.

Our results provide evidence for trans-Andean gene flow within both northern and southern clades of D. gliroides. Although gene flow across mountain tops is generally not expected, it has been reported for the lower southern Andean passes in South America. Two widespread sigmodontine rodents (Oligoryzomys longicaudatus and Abrothrix olivaceus) have expanded their range through the continuous forest habitat that crosses the southern passes of the Andes (Gallardo & Palma, 1990; Smith et al., 2001; Palma et al., 2005). Dromiciops gliroides appears to have used these southern passes similarly. The phylogenetic examination of clade C shows a subclade containing both Argentine and Chilean haplotypes that is sister to another clade of exclusively Chilean haplotypes. This suggests a Chilean origin of the populations now present in Argentina. This Chilean origin of D. gliroides is also corroborated by our analysis of historical gene flow, which indicated directionality eastward from Chile to Argentina.

Isolation of the island of Chiloe may have been interrupted during Pleistocene glacial oscillations (Heusser et al., 1999). Lowered sea levels presumably provided opportunities for exchange between insular and continental biota. Such intermittent connections between Chiloe and the mainland explain the existence of two disjunct populations of Darwin’s fox (Dusicyon fulvipes), one on Chiloe and the other 600 km to the north on the continent; mitochondrial DNA evidence demonstrates that these two populations belong to the same species (Yahnke et al., 1996). Intervening continental populations of Darwin’s fox have gone extinct since the time of a more widespread geographical range. The minimal genetic differentiation between island and mainland D. gliroides suggests that

### Post-glacial recolonization of Dromiciops gliroides

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Gene flow, represented by ‘migration rates’ estimated with the program Migrate, for three populations within the southern clade C: continental Chile, Argentina and the island of Chiloe.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>Migration rate (M = m/my)</td>
</tr>
<tr>
<td></td>
<td>Chile to receiving population</td>
</tr>
<tr>
<td>Chile</td>
<td>0.00712 (0.00438, 0.01108)</td>
</tr>
<tr>
<td>Argentina</td>
<td>0.00948 (0.00259, 0.08992)</td>
</tr>
<tr>
<td>Chiloe</td>
<td>0.00695 (0.00454, 0.01218)</td>
</tr>
</tbody>
</table>

Direction of gene flow is from source populations receiving populations as listed in the first column. Θ, effective female population size. For each estimated parameter, 95% confidence intervals are given in parentheses.
both populations share a common history that was facilitated by a recent period of connection between the island and the mainland. The evidence of gene flow from Chiloé to the mainland further supports the hypothesis of historical biotic exchange between the mainland and island. The unidirectional nature of gene flow in this region suggests that the mitochondrial diversity of the southern clade may result in part from a population that persisted in an island refugium during one or more glacial maxima.

Deforestation, plantations of exotic tree species, and agricultural practices have reduced and fragmented the southern temperate forest ecosystem (Armesto et al., 1998). As a result of forest loss, the IUCN (New World Marsupial Specialist Group, 1996) has listed D. gliroides as ‘vulnerable’ (VU A1c). Our documentation of variation in genetic structure across the range of this species, and our interpretation of the historical responses of D. gliroides to changes in distribution of its habitat, should be useful to the evaluation of conservation plans.

Dromiciops gliroides has a strong ecological association with the temperate rain forest of southern South America (Amico & Aizen, 2000; Muñoz-Pedreros & Palma, 2000). Other endemic species of this forest share the same environmentally driven Quaternary history of habitat expansion and contraction. The analysis of genetic structure of multiple co-occurring species that are linked to the same habitat allows us to test hypotheses concerning the response of communities to environmental change. This approach, ‘comparative phylogeography’ (Arbogast & Kenagy, 2001; Solis et al., 2006), can assess the replication of common genetic patterns. For example, the north–south genetic break of D. gliroides could be matched by other forest endemics. An explicit comparative phylogeographical analysis of South America’s temperate rain forest, with its rich and complex geological and climatological history, has not yet been conducted. However, our results for D. gliroides are concordant with patterns documented for other species within this region and thus should contribute to a broader understanding of the evolution of this major southern temperate ecosystem.

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