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Pleistocene glaciation is implicated in the phylogeographical structure of *Potamopyrgus antipodarum*, a New Zealand snail

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Abstract

Pleistocene glaciation has been identified as an important factor shaping present-day patterns of phylogeographical structure in a diverse array of taxa. The purpose of this study was to use mitochondrial sequence data to address whether Pleistocene glaciation is also a major determinant of phylogeographical patterns in *Potamopyrgus antipodarum*, a freshwater snail native to New Zealand. We found that haplotypes were separated by no more than 3.7% sequence divergence, and major genetic divisions tended to occur on a north–south axis. These data fit the predictions of the hypothesis that isolation of *P. antipodarum* in glacial refugia at the northern and southern tip of the South Island of New Zealand during the Pleistocene glaciation underlies the present-day phylogeographical structure. Because sexual *P. antipodarum* occasionally produce asexual offspring, we also used these data to show that the appearance of asexuality is not phylogeographically constrained. This means that the maintenance of sex in *P. antipodarum* cannot be wholly due to limited contact between sexual and asexual lineages and must instead be linked to a selective advantage of sexual reproduction.

Keywords: phylogeography, Pleistocene glaciation, *Potamopyrgus antipodarum*, sex

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Increasingly sophisticated molecular techniques and rising awareness of how phylogeography unites disparate evolutionary fields has fuelled an explosion of interest in the nature of the historical forces underlying present-day phylogeographical patterns (Avise 2000). Recent evaluation of phylogeographical patterns of mitochondrial sequence diversity point to Pleistocene glaciation as a key force shaping current distributions of many species (e.g. Zink 1996; Avise *et al*. 1998; Schneider *et al*. 1998; Taberlet *et al*. 1998; Trewick & Wallis 2001; Church *et al*. 2003). It is now clear that many modern species communities were assembled following range expansion from glacial refugia into formerly uninhabitable areas after the Pleistocene glaciation ended ~14 000 years ago (reviewed in Avise 2000). This connection between present-day species assemblages and the retreat of the Pleistocene glaciers has been detected on a continental scale in a variety of North American (e.g. Gill *et al*. 1993; Zink 1996; Wooding & Ward 1997; Avise & Walker 1998), Australian (Joseph *et al*. 1995; Schneider *et al*. 1998) and European taxa (Hewitt 1996; Taberlet *et al*. 1998; Trewick *et al*. 2003).

During the Pleistocene period of 1.8 million to 14 000 years ago, much of the South Island of present-day New Zealand was rendered uninhabitable by dramatic climactic and vegetational changes, as well as by the physical presence of ice, tundra, and glacial outwash and aggredation (Fig. 1) (Willett 1950; Fleming 1979; McGlone 1985; Pillans *et al*. 1992). Although controversy remains (e.g. Heads 1998; Trewick & Wallis 2001), recent studies of invertebrate phylogeography by Buckley *et al*. (2001) and Trewick & Wallis (2001) have pointed to Pleistocene glaciation as an important factor in determining the present-day distribution of many invertebrate species in New Zealand.

The question of whether Pleistocene glaciation is also a primary determinant of the current distribution of *Potamopyrgus antipodarum*, a freshwater snail native to New Zealand, remains. This gap in knowledge is particularly pressing because populations of this ancestrally sexual species often contain a large number of asexual individuals (e.g. Lively 1987; Dybdahl & Lively 1996). Without detailed

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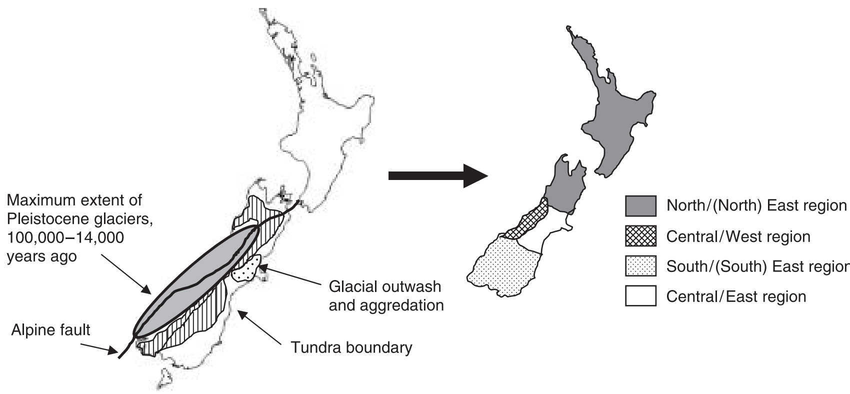

Fig. 1 Schematic representing the extent of Pleistocene glaciation and the location of the Alpine Fault, which marks the Southern Alps. These physical features define the regions outlined on the right-hand side of the figure (modified from Wardle 1963; Pillans *et al*. 1992; Trewick & Wallis 2001). The phylogeographic relevance of these regions is supported by a variety of studies showing that major phylogeographic divisions often occur among these regions (e.g. Heads 1998; Trewick & Wallis 2001; Wallis *et al*. 2001).

Mechanism	Timing of event	Maximum sequence divergence	Major genetic divisions
Pleistocene glaciation	14,000–1.8 million years ago	2–4%	North/South
Late Pliocene climactic and geological changes	2.5 million years ago	4.5–8%	North/South
Alpine Fault and Southern Alps	11–16 million years ago	> 15%	East/West

Fig. 2 Predictions of the amount of genetic divergence and patterns of phylogeographic structure predicted under Pleistocene glaciation, late Pliocene climatic and geological changes, and the formation of the Alpine fault and concurrent upthrust of the Southern Alps.

phylogeographical information, the key assumption that 'all else is equal' (Maynard Smith 1978) between competing sexual and asexual lineages may be subject to question. More specifically, without knowledge of the origins and phylogeographical distribution of asexuality, it is impossible to know whether sexual and asexual reproduction have actually had the opportunity to be engaged in 'fair competition.' This means that although many insights have come from research showing that sexual *P. antipodarum* seem to be favoured by negative frequency-dependent selection exerted by a virulent coevolved parasite (e.g. Lively 1987, 1992; Dybdahl & Lively 1995b, 1998; Lively & Jokela 2002), the possibility remains that the current distribution of sex has more to do with an historically limited distribution of asexual individuals than the assumed elimination of asexuals from sexual populations by parasite pressure.

Accordingly, the goal of this research was to determine the phylogeographical structure of *P. antipodarum* in its native New Zealand with respect to the distribution of sex, as well as to identify factors that seemed likely contributors to the present-day distribution of the species. We approached the latter question by considering discriminatory predictions for genetic structure and divergence under three scenarios: (i) Pleistocene glaciation, (ii) Pliocene mountain building and climatic changes, and (iii) formation of the Alpine Fault and upthrust of the Southern Alps (McGlone 1985; Trewick *et al*. 2000; Buckley *et al*. 2001; Trewick & Wallis 2001) (Fig. 2).

Predictions

Very different degrees of genetic divergence between the most genetically distinct populations are expected under these three hypothesized mechanisms for phylogeographical structure (McGlone 1985; Trewick & Wallis 2001; Wallis & Trewick 2001). Because the Pleistocene glaciation is a

relatively recent event, even the most genetically distinct lineages will be closely related if Pleistocene glaciation is the primary determinant of present-day phylogeographical patterns in *P. antipodarum*. Under the assumption that cytochrome *b* sequence divergence in *P. antipodarum* occurs at a rate of ~1.5–2.5%/Myr, as estimated from studies of the rate of mitochondrial divergence in snails (Murray *et al*. 1991; Collins *et al*. 1996) and in other invertebrates (Brown *et al*. 1979; Brower 1994; Trewick & Wallis 2001), major genetic divisions following Pleistocene glaciation should be marked by at most ~4% sequence divergence. This corresponds to a situation where genetic mixing between northern and southern South Island populations ended no more than ~1.5 Ma.

In contrast, genetic divergence between the most genetically distinct lineages during late Pliocene climactic and geological changes is predicted to date to a much earlier time, around 2.5 Ma (McGlone 1985; Taberlet *et al*. 1998; Avise 2000; Buckley *et al*. 2001; Trewick & Wallis 2001). This means that sequence divergence demarcating major genetic divisions between haplotypes should fall within the range of 4–8% if phylogeographical structure was largely caused by Pliocene events.

Finally, genetic divergence between the most genetically distinct haplotypes should be at least 15–20% if geological activity related to the formation of the Alpine Fault and consequential upthrust of the Southern Alps is the primary determinant of *P. antipodarum* phylogenetic structure (Trewick & Wallis 2001; Wallis & Trewick 2001).This prediction follows from geological evidence showing that these events occurred no later than 11–16 Ma, when the Pacific and Australasian continental plates collided (McGlone 1985; Kamp 1992; Heads 1998; Sutherland 1999).

Characteristic and contrasting patterns of phylogeographical structure are also expected under these three different mechanisms (McGlone 1985; Trewick & Wallis 2001; Wallis & Trewick 2001). If Pleistocene glaciation underlies much of the current distribution of *P. antipodarum*, present-day populations are likely descendents of snail lineages that endured glaciation in refugia in the extreme north and south of the South Island. In addition, the North Island was largely glacier free (McGlone 1985) and was intermittently connected to the north part of the South Island via land bridges as sea levels fell during the Pleistocene (McGlone 1985; Lewis *et al*. 1994; Nodder 1995; J. Diamond, pers. commun.). Thus, under glaciation, major genetic divisions are expected between populations from the historically connected North Island and northern third of the South Island and populations from the southern third of the South Island, which would have been diverging since the Pleistocene glaciation began ~100 000 years ago (Trewick & Wallis 2001; Wallis & Trewick 2001). Similar north–south divisions within the South Island are expected under Pliocene mountain building and climactic cooling, which caused dramatic shifts towards Alpine-adapted vegetation communities in the central third of the south island at this time (Mildenhall 1980, 1999; as cited by Newnham *et al*. 1999; Wallis & Trewick 2001).

The formation of the Alpine Fault and concurrent upthrust of the Southern Alps created a formidable barrier between the northwestern third of the South Island and the remainder of the island. Accordingly, if the Southern Alps are in fact the main factor shaping present-day genetic structure in *P. antipodarum*, major genetic divisions between lineages should occur between snails from lakes located to the northwest of the Southern Alps and lakes to the south and east of the Alps (Wallis & Trewick 2001). This pattern is very different from the north/south divisions predicted under Pleistocene glaciation and Pliocene climate change.

It is more difficult to formulate predictions of genetic structure for snail lineages originating from lakes in the central third of the South Island (hereafter referred to as the 'central region'). This region is 300 km long from north to south, and is characterized by low genetic diversity, endemism and gaps in the distribution of many poorly dispersing species (Wardle 1963; Burrows 1965; McGlone 1985; Heads 1998; Trewick & Wallis 2001; Wallis *et al*. 2001). Although the nature of the mechanism underlying this phenomenon remains controversial (McGlone 1985; Trewick & Wallis 2001), it is clear that many species have only recently re-entered the central region (McGlone 1985; Trewick & Wallis 2001; Wallis *et al*. 2001).

Here, we use mitochondrial sequence data to test the assumption that sexual and asexual *P. antipodarum* have in fact met in fair competition and to attempt to determine the factors that led to the current phylogenetic distribution of *P. antipodarum*. We show that: (i) asexual individuals are widely represented geographically and across the phylogeny, indicating that the maintenance of sex cannot be attributed to limited distribution of competing asexual lineages; and (ii) the degree of genetic divergence, geographical placement of major genetic divisions, and patterns of genetic diversity and endemism suggest that Pleistocene glaciation has played a key role in determining the present-day phylogeographical distribution of *P. antipodarum*.

Materials and methods

Collection

Snails were collected from 20 lakes across the North and South Islands of New Zealand (Fig. 3). All South Island snails were collected from 'shallow' habitats (< 0.5 m) in January or February of 2000–2003 by washing snails from small rocks into kick nets or by pushing the nets through aquatic vegetation. Following collection, snails were snap frozen with liquid nitrogen, transported by air to Indiana University, and stored in a −80 °C freezer until DNA

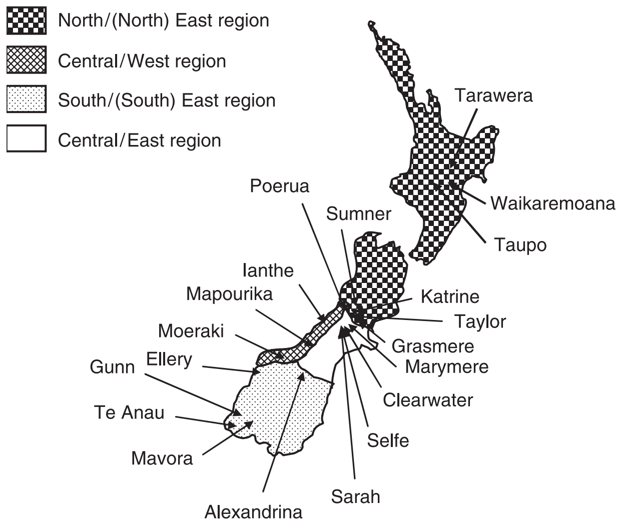

Fig. 3 Locations of the 20 sampled lakes with regard to the biogeographical regions defined in Figs 1 and 2.

extraction. All North Island snails were collected in a similar manner to South Island snails, but were maintained in freshwater aquaria for several years. We used the descendents of these snails as representatives of North Island populations.

DNA extraction

We extracted genomic DNA using the Puregene Cell and Tissue DNA extraction kit (Gentra Co.). Field-collected snails that had been previously frozen with liquid nitrogen and stored in a −80 °C freezer were individually placed into 1.5 mL Eppendorf tubes containing 300 μL chilled cell lysis buffer. The snails were crushed with a mortar and pestle, and the tubes were inverted 25 times and incubated for 1 h in a 65 °C water bath. Following incubation, and after the tubes had been cooled to room temperature, we added 1.5 μL RNase solution (Gentra Corp.) to the tubes, inverted 25 times, and incubated the tubes in a 37 °C water bath for ~45 min. After the tubes had cooled to room temperature, we added 100 μL Protein Purification Solution to the tubes and vortexed each tube vigorously for 20 s. Next, the tubes were centrifuged at 14 196 *g* for 3 min. Following centrifugation, the supernatant was applied to new 1.5 mL Eppendorf tubes that each contained 300 μL chilled isopropanol. The tubes were inverted 50 times and centrifuged at 14 196 *g* for 5 min. The isopropanol was poured off and 300 mL of 70% ethanol was added. Each tube was inverted several times and centrifuged at 14 196 *g* for 1 min, after which the ethanol was drained and the tube was left open and orientated downward for 30 min in order to evaporate any remaining ethanol. Finally, 50 μL DNA hydration solution was added to each tube, and, after 8–12 h of rehydration at room temperature, the tubes were stored at –4 °C. These procedures were identical for snails that had been stored live in tanks following their collection from New Zealand (rather than being stored frozen), except that these snails were frozen with liquid nitrogen immediately before the cell lysis step.

Mitochondrial sequencing

We began by using primers developed for the gastropod genus *Nucella* by Collins *et al*. (1996) that targeted a segment of the mitochondrial cytochrome *b* gene. We then designed internal primers from conserved areas within the 718 bp region amplified using the Collins *et al*. primers. From 5′ to 3′, the forward internal primer was TTCTTTATTAGGAC-TTTGTTTAGG, and the reverse internal primer was TTTCACCGTCTCTGTTTAGCC. These primers amplified a 497 bp region in *Potamopyrgus antipodarum*. Polymerase chain reaction (PCR) amplification was performed in 25 μL volumes consisting of ~50 ng DNA, 2.5 μL 10× tricine *Taq* buffer, 1.5 mM $MgCl_2$, 0.005 μg each primer, 100 μM each dNTP and 0.5 unit *Taq* DNA polymerase. Amplification began with an initial denaturation of 2 min at 94 °C, followed by 40 cycles of 94 °C for 30 s, 55 °C for 1 min and 72 °C for 2 min, and ended with a final extension period of 72 °C for 8 min. The PCR products were purified using the Qiagen Qiaquick PCR purification kit following the manufacturer's instructions. The purified DNA was then cycle-sequenced in both directions in 10 μL reactions with 0.0002 μg of either the forward or reverse primer solution, 1 μL v 3.1 BigDye terminator ready reaction mix (Applied Biosystems, Inc.; Perkin-Elmer), and 1.5 mM $MgCl_2$. The samples were sequenced with either an ABI Biosystems 3700 or 3730 automated sequencer. Sequences were aligned with SEQUENCHER V. 4.1 (Gene Codes Corp.). Ambiguous base calls were corrected manually.

Microsatellite genotyping

Asexual *P. antipodarum* are thought to arise via the normal fertilization of an unreduced (diploid) egg produced by a sexual female, producing a triploid individual. These triploid snails, which are exclusively female, mature to produce offspring via the development of parthenogenetically produced eggs (Wallace 1985, 1992; Phillips & Lambert 1989). We used microsatellite markers to determine the ploidy, and thus the sexuality, of each sequenced snail by genotyping all individuals at three microsatellite loci (*PA112*, *PA143* and *PA254*) developed for *P. antipodarum* by Weetman *et al*. (2001). Snails with three microsatellite alleles at at least one locus, as indicated by three peaks or by two peaks with unequal peak intensity biased towards the longer fragment (short-allele dominance, Armour *et al*. 1996; Wattier *et al*. 1998), were identified as triploid.

We chose loci that we were able to amplify reliably and that were relatively diverse in order to maximize the chances of detecting triploid individuals. *PA112* has at least seven alleles, *PA143* has at least 18 alleles and *PA254* has at least 43 alleles. The sequence characteristics and primers for these microsatellites are specified in Weetman *et al*. (2001). The forward primer from each primer pair was 5′-labelled with one of two fluorophores (HEX or 6-FAM). PCR amplification was performed in 10-μL volumes with 1 ng of template DNA, 1.5 mM $MgCl_2$, 1 μL Promega *Taq* buffer B, 0.5 unit Promega buffer B *Taq*, 100 μM each dNTP, 0.0001 μg reverse primer and 0.0002 μg forward primer. We used the thermocycler profiles for each locus as specified in Weetman *et al*. (2001). PCR products were diluted 20-fold, and 1 μL of the dilution was combined with 0.2 μL GenSize R350 Rox size standard (GenPak) and 9.8 μL ddH_2O. The samples were then denatured by heating at 95 °C for 3 min, snap-cooled on ice and genotyped on an ABI 3700 using GENESCAN v. 3.5 and GENOTYPER v. 3.6 (Applied Biosystems).

Phylogenetic analysis

Phylogenetic analyses and trees were generated using the maximum parsimony (MP), maximum likelihood (ML) and neighbour joining (NJ) functions of PAUP* v. 4.04b (Swofford 1998). For the ML tree, we used likelihood-ratio tests (within PAUP) to determine the model of nucleotide evolution that best fit the data. First, we used the most complex model (general time reversible with gamma distributed rate variation and estimating the proportion of variable sites) and then compared the likelihood of the data from this test with likelihood ratios from increasingly simple models. For our purposes, the best model of evolution was the general-time-reversible + invariable sites (0.78) (GTR + I). ML analyses were performed with a heuristic search with 10 random addition sequences and tree-bisection–reconnection algorithms (TBR). For the NJ analyses, total uncorrected character differences were used to calculate distance. Bootstrap resampling with 1000 replicates was used to determine the degree of support for each node in MP and NJ trees, and 100 replicates were used for the ML tree. Bootstrap support values were calculated from a 50% majority-rule consensus tree. *P. estuarinus*, a close sexual relative of *P. antipodarum*, was used as the outgroup (following Phillips & Lambert 1990). A haplotype network was drawn by hand based on the topology of the best-supported tree. We also used PAUP* v. 4.04b to test the monophyly of the asexual haplotypes by constraining the asexual haplotypes to form a monophyletic group and comparing the likelihood score of the unconstrained tree with the constrained tree with a Kishino–Hasegawa test (Kishino & Hasegawa 1989).

We used AMOVA (Excoffier *et al*. 1992) as implemented by ARLEQUIN v. 2.0 (Schneider *et al*. 2000), to determine whether there was phylogeographical structure among lake populations from different regions within New Zealand according to the predictions detailed above. All AMOVAs were conducted with sexual and asexual snails together as well as separately. We also used ARLEQUIN to calculate pairwise F_{ST} values for each pair of populations and to perform a Mantel's test examining the correlation between pairwise population F_{ST} and geographical separation in kilometres.

The lakes were designated as north or south (to test for north–south division as expected under Pleistocene glaciation and Pliocene climactic and geological changes) and as east or west (to test for the east–west divisions expected under the uplift of the Southern Alps) (Fig. 2). Lakes north of the northern boundary of the previously discussed region of low diversity and endemism (estimated at 42.75°S, Grasmere, Katrine, Poerua, Sumner, Taupo, Tarawera, Taylor, Waikeremoana) were designated as north, and snails south of the southern boundary of this region (estimated at 43.75°S, Alexandrina, Ellery, Gunn, Mavora, Moeraki, Te Anau) were designated as south. Lakes that fell within the central region (Clearwater, Ianthe, Mapourika, Marymere, Sarah and Selfe) were excluded from the north–south analysis because their placement with regard to north/south divisions is unclear (Figs 2 and 3). Lakes to the west of the Southern Alps (Poerua, Ianthe, Moeraki and Mapourika) were classified as west; all other South Island lakes were classified as east. North Island lakes were excluded from the east–west analysis because putative biogeographical divisions related to the formation of the Southern Alps are restricted to the South Island (Figs 2 and 3). We used 100 000 permutations for each AMOVA.

We calculated haplotypic diversity with the formula $h = 1 - \Sigma f_i^2$, where f_i = the frequency of the ith haplotype, and nucleotide diversity with the formula $p = \Sigma f_i f_j p_{ij}$, where p_{ij} = the sequence divergence between the ith and jth haplotypes (Nei & Tajima 1981; Nei 1982). Finally, we used MEGA (Kumar *et al*. 2001) to calculate genetic distances within and between lake populations. We used the Tamura–Nei model of nucleotide substitution (Tamura & Nei 1993), which corrects for multiple hits, accounts for substitutional rate differences between nucleotides and inequality of nucleotide frequencies, distinguishes between transitions and transversions and between substitutions between purines and between pyrimidines, and assumes equality of substitution rates among sites. This model was chosen because it best represented the model selected for ML analysis (GTR + I).

We used the genetic code of the snail *Cepaea nemoralis* (Terrett *et al*. 1996), which differs from the universal code in that ATA = methionine instead of isoleucine, TGA = tryptophan rather than stop, and AGA and AGG = serine instead of stop (Goodacre & Wade 2001).

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Results

We sequenced 497 bp pairs from 638 individuals from 20 different lakes across New Zealand. At least 23 individuals from each lake were sampled. Of the 497 bp amplified by the primer set, 430 were unambiguously readable. We found 44 variable sites, 26 of which were parsimony informative. As expected for protein-coding mitochondrial genes (Brown 1985; Collins *et al*. 1996), the polymorphisms were not distributed randomly with respect to site; there were 32 synonymous and 12 nonsynonymous substitutions. Forty-five different haplotypes were detected. There were 22 fixed nucleotide differences between *Potamopyrgus antipodarum* and the outgroup, *P. estuarinus*, corresponding to 5.1% sequence divergence between the two species. This value suggests that *P. antipodarum* and *P. estuarinus* have been diverging for at least 2 Myr. The greatest amount of pairwise divergence between any two *P. antipodarum* haplotypes was 3.72%, between a haplotype found only in the north biogeographical region and two haplotypes confined to a lake in the west biogeographical region.

MP, ML and NJ trees were similar topologically, especially in clades that received > 50% bootstrap support. ML analysis resulted in a tree with –ln L = 1072.8. Mean (± SE) sequence divergence between haplotypes was 1.61 ± 0.27%. Pairwise F_{ST} comparisons indicated that there was significant genetic differentiation between most populations. The mean (± SE) pairwise F_{ST} between populations was 0.417 ± 0.017. When the asexual haplotypes were constrained to be monophyletic, the resulting tree had a –ln L = 1120.76. The difference between the constrained tree and the most likely tree, which had a –ln L = 1072.8, was highly significant (KH test, $P < 0.0001$). This indicates that the asexual haplotypes do not have a monophyletic origin.

Haplotype and nucleotide diversity varied widely among lakes. Mean (± SE) within-lake haplotype diversity was 0.413 ± 0.05, and mean (± SE) within-lake nucleotide diversity was 0.008 ± 0.0003. On average, 1.6 ± 0.26 endemic haplotypes were sampled from each lake. North and south region lakes tended to have the highest levels of haplotype and nucleotide diversity, and south and east region lakes tended to have the most endemic haplotypes. Central region lakes had the lowest genetic diversity and the fewest endemic haplotypes. This information is summarized in Tables 1–3.

Phylogeographical structure

Haplotypes were not distributed randomly with respect to geography. There was more genetic differentiation among snails from different lakes ($D = 0.014 \pm 0.002$ SE) than within lakes ($D = 0.004 \pm 0.001$). There was a tendency for

Table 1 Lakes, their location according to the described biogeographical regions, and the haplotypes found in each lake

Lakes	Location	Haplotype ID (number of individuals)
Alexandrina	S/E	1(16)A, 4(1)A, 10(1)A, *11(1)S*, *12(2)M*, 18(1)A, *23(8)M*, 25(3)M, 28(1)S, *31(1)A*
Clearwater	C/E	1(17)M, *6(1)S*, *9(2)M*, 28(22)M, *30(1)A*
Ellery	S/E	1(4)M, 18(1)S, *29(1)S*, *33(2)S*, 38(1)S, 39(29)M
Grasmere	N/E	*2(28)M*, *5(2)M*, 8(1)A, 25(2)M, 28(2)M
Gunn	S/E	1(6)A, 10(10)M, 18(9)M, 22(5)M, *41(2)A*
Ianthe	C/W	*32(1)A*, 38(13)M, 39(31)M, *40(1)S*
Katrine	N/E	1(11)M, 15(1)A, 16(1)A, 18(1)A, 25(10)M, 28(1)A, 35(1)A, 36(1)A
Marymere	C/E	1(25)M, 28(1)A
Mapourika	C/W	1(13)M, 4(2)M, *24(1)A*, 25(3)M, 28(3)M, *43(1)A*
Mavora	S/E	1(18)A, *13(1)S*, *14(2)M*, 17(1)S, 18(2)M
Moeraki	S/W	1(18)M, *7(1)A*, 18(6)M
Poerua	N/W	1(37)M, *3(1)S*, *19(1)A*
Sarah	C/E	1(35)M
Selfe	C/E	1(19)M, 25(4)M, *42(1)A*
Sumner	N/E	1(3)M, 25(23)M, *44(2)M*
Tarawera	N	35(27)A
Taupo	N	1(1)S, 8(1)A, 15(2)A, 16(1)A, *26(1)A*, *27(1)A*, 35(15)A, *37(2)A*
Taylor	N/E	1(25)M, 18(1)A, 25(1)S, 35(2)A, 36(1)A
Te Anau	S/E	17(16)M, 22(12)M, *45(4)A*
Waikeremoana	N	*20(33)A*, *21(1)A*, *34(6)A*, 35(5)A

In the location column, N = North, S = South, C = Central, W = West and E = East. North Island lakes (Tarawera, Taupo and Waikeremoana) do not have an east/west designation because the North Island was not affected by the mountain building processes that created the Southern Alps. Haplotypes endemic to a lake are italicized. The number of individuals with each haplotype is given in parentheses following the haplotype ID number. The letter immediately to the right of the parentheses indicates whether the haplotype is represented by sexual (S), asexual (A) or both types (M) of individuals.

Table 2 Mean haplotype diversity (*h*), nucleotide diversity (*p*), endemic haplotype number, genetic distance (*D*) and sequence divergence calculated from snails sampled from lakes within each of the five biogeographical regions (± SE)

Region	Haplotype diversity (*h*)	Nucleotide diversity (*p*)	Endemic haplotypes	Genetic distance (*D*)	Sequence Divergence
North	0.55 ± 0.1	0.008 ± 0.0003	1.38 ± 0.46	0.017 ± 0.002	1.67%
South	0.41 ± 0.07	0.0024 ± 0.0004	2.17 ± 0.88	0.016 ± 0.002	1.43%
Central	0.33 ± 0.09	0.0008 ± 0.0003	1.33 ± 0.49	0.008 ± 0.002	1.49%
West	0.39 ± 0.1	0.001 ± 0.0004	1.75 ± 0.25	0.013 ± 0.002	1.51%
East	0.44 ± 0.07	0.0019 ± 0.0004	2.46 ± 0.35	0.01 ± 0.001	1.62%

Mean haplotype diversity was 0.413 ± 0.05 SE, and mean nucleotide diversity was 0.008 ± 0.0003. Mean genetic differentiation among snails from different lakes was 0.014 ± 0.002 and was 0.004 ± 0.001 among snails within lakes. On average, 1.6 ± 0.26 SE endemic haplotypes were sampled from each lake. Mean sequence divergence between haplotypes was 1.61 ± 0.27% SE.

Table 3 Mean genetic distance and sequence divergence (± SE) among snails from lakes from each of the regional comparisons used in our analyses

Regional comparison	Genetic distance (*D*)	Sequence divergence
North vs. South	0.017 ± 0.001	1.97%
West vs. East	0.011 ± 0.002	1.61%
North vs. Central	0.017 ± 0.002	1.91%
South vs. Central	0.012 ± 0.002	1.56%

more genetic distance and sequence divergence among snails from lakes within the north region and within the south region than among lakes from other regions. There was also more genetic distance and sequence divergence between snails from lakes from the north and south regions than between any other two regions. This result was corroborated by a Mantel's test indicating that there is a positive correlation between pairwise population F_{ST} and geographical distance ($r = 0.41$, $P = 0.004$, 1000 permutations), which is in line with the fact that the north and the south regions are more geographically distant than any other pair of regions. This information is summarized in Tables 1–3.

Despite the relatively high genetic distance and sequence divergence between the north and south regions, the amount of variance contributed by region was not significant when all haplotypes were considered (AMOVA, $P = 0.117$). Nor was the east/west division a significant source of genetic variation (AMOVA, $P = 0.944$). There was significant genetic differentiation among populations ($P < 0.0001$), as well as significant genetic variation within populations ($P < 0.0001$) in both cases. This information is summarized in Tables 4 and 5. AMOVAs conducted with sexual and asexual snails considered separately did not differ from AMOVAs conducted with sexual and asexual snails together, and are not presented here.

We suspected that the large number of endemic haplotypes and the frequency and wide distribution of an unusually common haplotype may have contributed so much to between-lake (43.21% of all variation) and within-lake (53.21% of all variation) genetic variation that these values swamped out much of the contribution from between-region genetic variance. In particular, the 'common' haplotype is notable in the degree to which it did not conform to the general pattern of geographically restricted distribution. This particular haplotype was found in nearly one third of all sampled snails, and in 15 of the 20 lakes. The next most common haplotype was shared by 60 individuals across

Table 4 Results of AMOVA among all snails from lakes designated as north and south

Source of variation	df	SS	Variance components	% variation
Among north and south regions	1	7.606	0.0006 Va	0.41
Among lakes within north and south regions	12	86.7	0.2294 Vb	50.01***
Within lakes	427	95.141	0.22281 Vc	49.85***
Total	366	159.638	0.46116	
Fixation indices				
F_{SC}	0.50081			
F_{ST}	0.5015			
F_{CT}	0.0014			

***Significant at $P < 0.0001$. There is significant differentiation between lakes within the north and south regions and within lakes.

Table 5 Results of AMOVA among all snails from lakes designated as east and west

Source of variation	df	SS	Variance components	% variation
Among east and west regions	1	3.052	−0.0178 Va	−4.86
Among lakes within east and west regions	15	89.625	0.189241 Vb	47.93***
Within lakes	525	113.395	0.21599 Vc	56.75***
Total	541	206.072	0.3806	
Fixation indices				
F_{SC}	0.45786			
F_{ST}	0.43250			
F_{CT}	0.04678			

***Significant at $P < 0.0001$. There is significant genetic differentiation between lakes within the east and west regions and within lakes.

Table 6 Results of AMOVA among all lakes designated as north and south

Source of variation	df	SS	Variance components	% variation
Among north and south regions	1	13.713	0.0821 Va	16.64
Among lakes within north and south regions	11	39.523	0.2418 Vb	49.03***
Within lakes	187	31.659	0.1693 Vc	34.33***
Total	199	84.895	0.4931	
Fixation indices				
F_{SC}	0.588			
F_{ST}	0.657			
F_{CT}	0.166			

Snails with the 'common' haplotype or with endemic haplotypes were excluded. This resulted in Lake Poerua (north region) being excluded from the analysis because all snails sampled from this lake had either the common or an endemic haplotype.
***Significant at $P < 0.0001$, '*' indicates significance at $P < 0.015$. This result remained significant following a Bonferroni correction for the two north/south tests, in that $P < 0.025$. There is significant genetic differentiation between the north and south regions, among lakes within the north and south regions, and within lakes.

two lakes, and the second most widely distributed haplotype was found in only six lakes.

We conducted an AMOVA with endemics and the common haplotype removed to test the hypothesis that these haplotypes were overwhelming any regional contribution to genetic structure. We found that there was significant division between the north and south regions ($F_{CT} = 0.166$, $P = 0.014$, Table 6). This result remained significant following a Bonferroni correction for the two north/south tests, in that $P < 0.025$. This means that haplotypes that are shared between lakes (with the exception of the common haplotype) tend to be located within either the north or the south region. Though this analysis was conducted with modified data, and as such must be viewed with caution, it does indicate that there is genetic differentiation between northern and southern populations. Removing the common haplotypes and endemics from an AMOVA between east and west lakes did not improve the ability of the analysis to detect genetic divisions between these regions ($F_{CT} = -0.098$, $P = 0.928$).

Sexuality

All individuals that had three alleles at least one of the genotyped microsatellite loci were classified as triploid. Given the high allelic diversity of the surveyed loci, the probability is low that individuals that never had more than two alleles at any one locus were in fact triploid. Thus, we classified these snails as putative diploids, although the possibility remains that these individuals are in fact cryptic triploids. Thirty-seven of the 45 haplotypes were represented by at least one triploid snail, indicating that asexuality has arisen multiple times in lakes all over New Zealand (Figs 4 and 5).

Discussion

We determined that major divisions in *Potamopyrgus antipodarum* phylogenetic structure appear to have occurred recently on a north–south axis, which suggests that isolation of snails into northern and southern glacial refugia during

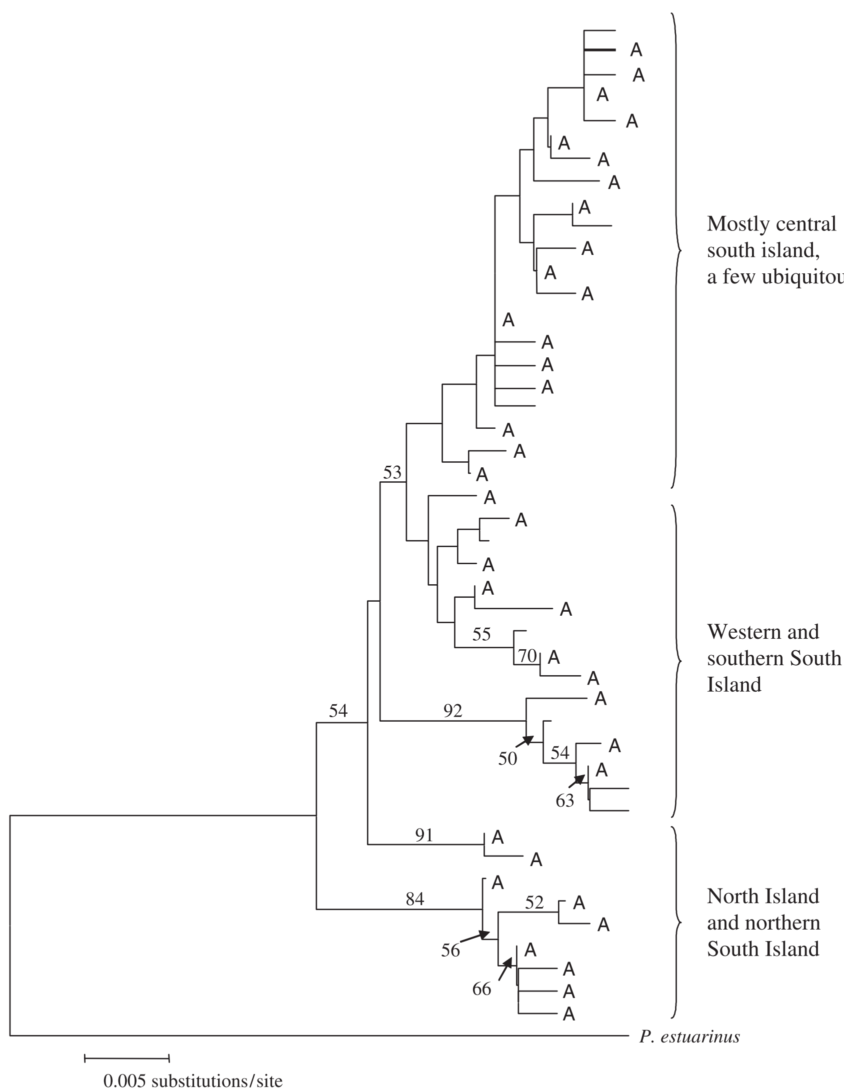

Fig. 4 ML tree showing the phylogenetic relationships between the 45 Potamopyrgus antipodarum haplotypes. The letter 'A' at the end of a branch indicates that the haplotype is represented by at least one triploid (asexual) snail. Numbers above the nodes indicate bootstrap support for each node; bootstrap values below 50% are not shown. The emboldened haplotype is the 'common' haplotype.

the Pleistocene glaciation is likely to underlie present-day patterns of *P. antipodarum* population structure, and that there is strong genetic differentiation among and within lakes. We also showed that genetically distinct *P. antipodarum* cytochrome *b* haplotypes with asexual representatives are widely distributed on a phylogeographical scale and in lakes known to have a high frequency of sexual conspecifics. This indicates that asexual snails have indeed had a 'fair' chance to out-compete their sexual counterparts in many lakes where sex predominates today. Thus, the current distribution of sexual reproduction in *P. antipodarum* cannot be attributed to phylogenetically or geographically limited distribution of asexual snails. In addition, most lakes contained endemic haplotypes characterized by both triploid (asexual) and diploid (putatively sexual) individuals. This result indicates that many asexual lineages have recently arisen from local sexual progenitors, and provides support for the contention that the current distribution of sex is related to selection for sex in these lakes rather than limited distribution of asexual individuals (also see Jensen *et al*. 2002).

Although most asexual lineages appear to have been derived within the last 40 000–70 000 years from sexual lineages, there are two genetically distinct, all-asexual clades that exceed 1.2% sequence divergence from any haplotype with a diploid representative. These clades are confined to the north region and to lakes with significantly lower frequency of sexual conspecifics and coevolving, virulent parasitism than other sampled lakes (Neiman *et al*. manuscript under review).

In general, our results corroborate and expand upon the research of Dybdahl & Lively (1995a, 1996), who used allozymes to genotype *P. antipodarum* from four South Island lakes and found that: (i) *P. antipodarum* populations are genetically structured with regard to lake and geography (Dybdahl & Lively 1996), (ii) most asexual *P. antipodarum* lineages are recently derived from sympatric sexual populations, and (iii) many asexual *P. antipodarum* lineages are

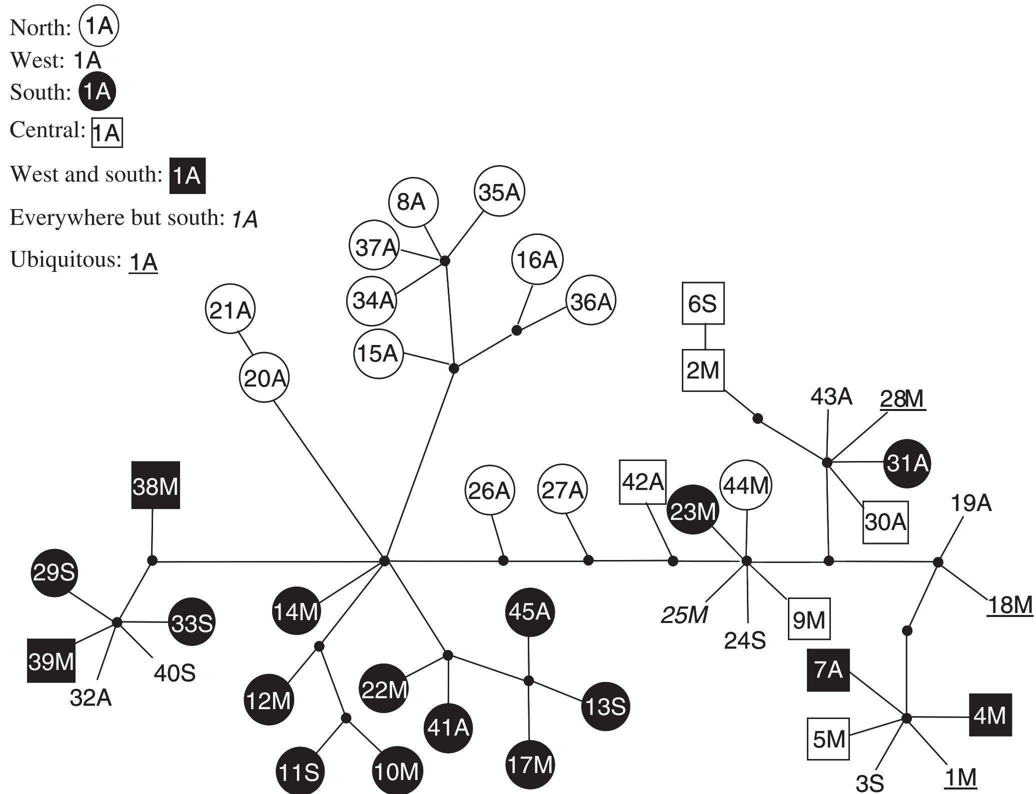

Fig. 5 Haplotype network drawn from the best-supported tree showing how haplotypes are distributed with regard to biogeographic region, as indicated by the colour and shape surrounding each numerical haplotype ID number. The letter immediately to the right of each numerical haplotype ID indicates whether the haplotype is represented by sexual (S), asexual (A) or both types (M) of individuals (see Table 1 for ID numbers).

endemic to particular lakes (Dybdahl & Lively 1995a). Molecular data have shown that asexual lineages from a variety of other taxa are often locally and recently derived from sexual ancestors (Crease *et al*. 1989; Quattro *et al*. 1991; Moritz *et al*. 1992; Vrijenhoek 1998).

Interpreting phylogeographical data

The phylogeographical pattern of *P. antipodarum* cytochrome *b* haplotypes was characterized by a diverse and generally shallow phylogeny. Most haplotypes were closely related and localized geographically, and many haplotypes (31/45) were endemic. This pattern fits 'phylogeographical pattern III', as described by Avise *et al*. (1987) and Avise (2000), and can be interpreted to mean that contemporary gene flow has been low enough to permit lineage sorting and drift to cause small-scale divergence between geographically close populations and larger divergence between biogeographically distant populations.

Much of the observed cytochrome *b* genetic diversity has probably not evolved *in situ*, especially given that present-day lakes in the areas most affected by glaciation are probably less than 23 000 years old (Gage 1975). Rather, haplotypes that existed together in a glacial refugium (which themselves were likely to often be closely related) during the Pleistocene and then dispersed to newly formed lakes may have done so by chance. This would explain the observed pattern of present-day lake populations that are largely comprised of closely related, but not monophyletic, assemblages of haplotypes that often feature endemic lineages. Mutations occurring in the short time since *P. antipodarum* reached its current distribution are likely to be geographically confined (Castelloe & Templeton 1994; Templeton 1998), which may also contribute to the large number of endemic lineages.

We found no more than 3.7% sequence divergence between any two haplotypes, and mean sequence divergence between haplotypes was ~1.6%. This degree of pairwise sequence divergence is in line with genetic divergences estimated from several species in other studies of New Zealand phylogeography (e.g. Trewick & Wallis 2001). Our estimates of maximal divergence time, under the assumption that sequence divergence occurs at a rate of 1.5–2.5%/Myr, indicate that the mechanism(s) underlying major genetic divisions within *P. antipodarum* operated within the last ~1–2 Myr.

The relatively low genetic differentiation between the east and west regions also indicates that there was not a large-scale perturbation of *P. antipodarum* phylogeny on the east/west axis defined by the Southern Alps. We can thus eliminate the formation of the Alpine Fault and the consequential upthrust of the Southern Alps from consideration as the primary force driving present-day patterns of *P. antipodarum* phylogeographical structure because the relative antiquity of this event should result in much deeper genetic divergence, at least 15%, between the most diverged snail lineages. These results support and extend the findings of Dybdahl & Lively (1996), who showed that populations from relatively distant lakes on the same side of the Southern Alps are often more genetically distinct from one another than populations from similar latitudes but on opposite sides of the Alps. It is reasonable, however, that the Southern Alps constitute a formidable barrier to dispersal for a small aquatic snail, and may contribute to more recent divergence between eastern and western populations (also see Dybdahl & Lively 1996).

Unlike the phylogeography of some other New Zealand invertebrates (e.g. Trewick *et al*. 2000; Trewick & Wallis 2001), which is dominated by the effects of events taking place during both the Pleistocene and Pliocene, we found no evidence for intraspecific genetic divergence dating to Pliocene times. If late Pliocene climactic changes and mountain building are actually the source of current *P. antipodarum* phylogenetic distribution, genetic divergence should exceed ~4–5% to correspond with the several million years that have elapsed since this event.

Thus, our data point to Pleistocene glaciation as the most likely factor (along with subsequent lineage sorting and genetic drift) shaping present-day patterns of *P. antipodarum* phylogeographical structure. Pleistocene glaciation is also implicated by the substantial genetic distance and sequence divergence between the north and south regions, which easily exceeds the divergence between any other two regions, and by our finding that haplotypes that are shared between lakes (with the exception of the common haplotype) tend to be confined to either the north or south regions.

An additional line of evidence that lends support to the hypothesis that isolation in northern and southern glacial refugia during the Pleistocene underlies present-day patterns of phylogeographical structure in *P. antipodarum* is that there is higher genetic diversity and more endemic haplotypes in these north and south regions relative to the central part of the South Island of New Zealand. Relatively high levels of genetic diversity are expected to characterize older and/or more stable populations that have not endured recent bottlenecks, in marked contrast to newly colonized areas, which are often settled by a small group of individuals carrying a relatively small subset of the allelic diversity contained in the source population (e.g. Hewitt 1996, 1999; Grant & Bowen 1998; Avise 2000; Guiller *et al*. 2001; Haase *et al*. 2003). High genetic diversity in populations located in areas of former glacial refugia has been reported in a variety of other taxa for which Pleistocene glaciation has been identified as an important force underlying present-day phylogeographical structure (e.g. Cwynar & MacDonald 1987; Hewitt 1996; Wallis *et al*. 2001; Church *et al*. 2003).

These patterns of genetic diversity and endemism are also informative in that they enable discrimination between genetic differentiation due to geographical distance *per se* and genetic differentiation due to an event occurring on a north/south axis (like the Pleistocene glaciation) in which north/south distances are large compared with east/west differences. More specifically, differentiation due to isolation-by-distance alone would not be expected to lead to a situation where the two most geographically distant regions are also characterized by the most genetic diversity and endemic lineages. Rather, the combination of relatively high north/south divergence and high diversity in the north and south point to a scenario in which Pleistocene glaciation, which most severely affected the central third of the south island, led to isolation between northern and southern populations.

As for several other New Zealand invertebrate species (e.g. Heads 1998; Trewick & Wallis 2001; Wallis *et al*. 2001), *P. antipodarum* populations within the central third of the South Island of New Zealand were characterized by relatively low genetic diversity and endemism. Some believe that this phenomenon is linked to the severity of Pleistocene glaciation in the central region of the South Island. They suggest that this glaciation ended so recently (~14 000 years ago) that there has been little time for species, especially poor dispersers, to diverge genetically following dispersal from glacial refugia in the northern and southern extremes of the island (Willett 1950; Wardle 1963; Burrows 1965; Trewick & Wallis 2001). Others have speculated that low diversity and endemism in the centre of the South Island could be related more to Pliocene climactic changes and mountain building (McGlone 1985; Newnham *et al*. 1999; Buckley *et al*. 2001). In our case, the relatively recent genetic divergence between even the most divergent haplotypes points to events taking place in the Pleistocene. Populations from this middle region of the South Island were more closely linked genetically to populations from the region of the southern glacial refugia than from the northern populations, suggesting that expansion from the southern region of the South Island is the likely source for many of the present-day central South Island populations.

In summary, we present evidence supporting the hypothesis that Pleistocene glaciation forced *P. antipodarum* into northern and southern glacial refugia. This event was likely an important contributor to present-day phylogeographical structure in *P. antipodarum*. We also showed that

asexuality is not phylogeographically constrained; rather, genetically distinct asexual *P. antipodarum* lineages occur all over the *P. antipodarum* cytochrome *b* phylogeny and across New Zealand. This finding indicates that the maintenance of sex in *P. antipodarum* must be linked to selective advantages of sex rather than limited phylogeographical distribution of asexual invaders.

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References

Armour JA, Crosier LM, Jeffreys AJ (1996) Distribution of tandem repeat polymorphisms within minisatellite MS621 (D5S110). *Annals of Human Genetics*, **7**, 11–20.

Avise JC (1992) Molecular population structure and the biogeographical history of a regional fauna: a case history with lessons for conservation biology. *Oikos*, **63**, 62–76.

Avise JC (2000) *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge, MA.

Avise JC, Arnold J, Ball RM *et al.* (1987) Intraspecific phylogeny: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics*, **18**, 489–522.

Avise JC, Walker D (1998) Pleistocene phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London B, Series*, **265**, 457–463.

Avise JC, Walker D, Johns GC (1998) Speciation durations and Pleistocene effects on vertebrate phylogeography. *Proceedings of the Royal Society of London Series B*, **265**, 1707–1712.

Brower AVZ (1994) Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proceedings of the National Academy of Sciences of the USA*, **91**, 6491–6495.

Brown WM (1985) The mitochondrial genome of animals. In: *Molecular Evolutionary Genetics* (ed. MacIntyre RJ), pp. 95–130. Plenum Press, New York.

Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the USA*, **76**, 1967–1971.

Buckley TR, Simon C, Chambers GK (2001) Phylogeography of the New Zealand cicada *Maoricicada campbelli* based on mitochondrial DNA sequences: ancient clades associated with Cenozoic environmental change. *Evolution*, **55**, 1395–1407.

Burrows JC (1965) Some discontinuous distributions of plants within New Zealand and their ecological significance. II. Disjunctions between Otago–Southland and Nelson–Marlborough and related distribution patterns. *Tuatara*, **13**, 9–29.

Castelloe J, Templeton AR (1994) Root probabilities for intraspecific gene trees under neutral coalescent theory. *Molecular Phylogenetics and Evolution*, **3**, 102–113.

Church SA, Kraus JM, Church DR, Taylor DR (2003) Multiple Pleistocene refugia in the postglacial expansion of the eastern tiger salamander, *Ambystoma tigrinum tigrinum*. *Evolution*, **57**, 372–383.

Collins TM, Frazer K, Palmer AR, Vermeij GJ, Brown WM (1996) Evolutionary history of northern hemisphere *Nucella* (Muricidae): molecular, morphological, ecological, and paleontological evidence. *Evolution*, **50**, 2287–2304.

Crease TJ, Stanton DJ, Hebert PDN (1989) Polyphyletic origins of asexuality in *Daphnia pulex*. II. Mitochondrial–DNA variation. *Evolution*, **43**, 1016–1026.

Cwynar LC, MacDonald GM (1987) Geographical variation of lodgepole pine in relation to population history. *American Naturalist*, **129**, 463–469.

Dybdahl MF, Lively CM (1995a) Diverse, endemic and polyphyletic clones in mixed populations of a freshwater snail. *Journal of Evolutionary Biology*, **8**, 385–398.

Dybdahl MF, Lively CM (1995b) Host–parasite interactions: infection of common clones in natural populations of a freshwater snail (*Potamopyrgus antipodarum*). *Proceedings of the Royal Society of London B, Series*, **260**, 99–103.

Dybdahl MF, Lively CM (1996) The geography of coevolution: comparative population structures for a snail and its trematode parasite. *Evolution*, **50**, 2264–2275.

Dybdahl MF, Lively CM (1998) Host–parasite coevolution: evidence for rare advantage and time–lagged selection in a natural population. *Evolution*, **52**, 1057–1066.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial restriction data. *Genetics*, **131**, 474–491.

Fleming CA (1979) *The Geological History of New Zealand and its Life*. Aukland University Press/Oxford University Press, Auckland.

Gage M (1975) Glacial lakes. In: *New Zealand Lakes* (eds Jolly VH, Brown JMA), pp. 57–69. Auckland University Press, Auckland.

Gill FB, Mostrom AM, Mack AL (1993) Speciation in North American chickadees. I. Patterns of mtDNA divergence. *Evolution*, **47**, 195–212.

Goodacre SL, Wade CM (2001) Patterns of genetic variation in Pacific island land snails: the distribution of cytochrome *b* lineages among Society Island *Partula*. *Biological Journal of the Linnean Society*, **73**, 131–138.

Grant WAS, Bowen BW (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.

Guiller A, Coutellec-Vreto MA, Madec L, Deunff J (2001) Evolutionary history of the snail *Helix aspersa* in the western Mediterranean: preliminary results inferred from mitochondrial DNA sequences. *Molecular Ecology*, **10**, 81–88.

Haase M, Misof B, Wirth T, Baminger H, Baur B (2003) Mitochondrial differentiation in a polymorphic land snail: evidence for Pleistocene survival within the boundaries of permafrost. *Journal of Evolutionary Biology*, **16**, 415–428.

Heads M (1998) Biogeographic distribution along the Alpine Fault, New Zealand. *Biological Journal of the Linnean Society*, **63**, 161–176.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt GM (1999) Post-glacial recolonization of European biota. *Biological Journal of the Linnean Society*, **68**, 87–112.

Jensen LH, Enghoff H, Frydenberg J, Parker ED (2002) Genetic diversity and the phylogeography of parthenogenesis: comparing bisexual and thelytokous populations of *Nemasoma varicorne* (Diplopoda: Nemasomatidae) in Denmark. *Hereditas*, **136**, 184–194.

Joseph L, Moritz C, Hugall A (1995) Molecular support for vicariance as a source of diversity in rainforest. *Proceedings of the Royal Society of London Series B*, **260**, 177–182.

Kamp PJJ (1992) Tectonic architecture of New Zealand. In: *Landforms of New Zealand* (eds Soons JM, Selby MJ), pp. 1–30. Longman Paul, Auckland.

Kishino H, Hasegawa M (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in *Hominoidea*. *Journal of Molecular Evolution*, **29**, 170–179.

Kumar S, Tamura K, Jakobsen IB, Nei M (2001) *MEGA2: Molecular Evolutionary Genetics Analysis Software*. Arizona State University, Tempe.

Lewis KB, Carter L, Davey FJ (1994) The opening of the Cook Strait: interglacial scour and aligned basins at a subduction to transform plate edge. *Marine Geology*, **116**, 293–312.

Lively CM (1987) Evidence from a New Zealand snail for the maintenance of sex by parasitism. *Nature*, **328**, 519–521.

Lively CM (1992) Parthenogenesis in a freshwater snail: reproductive assurance versus parasitic release. *Evolution*, **46**, 907–913.

Lively CM, Jokela J (2002) Temporal and spatial distributions of parasites and sex in a freshwater snail. *Evolutionary Ecology Research*, **4**, 219–226.

Maynard Smith J (1978) *The Evolution of Sex*. Cambridge University Press, London.

McGlone MS (1985) Plant biogeography and the late Cenozoic history of New Zealand. *New Zealand Journal of Botany*, **23**, 723–749.

Mildenhall DC (1980) New Zealand Late Cretaceous and Cenozoic plant biogeography: a contribution. *Mammology, Palaeoclimatology, Palaeoecology*, **31**, 197–233.

Mildenhall D (1999) Pollen analysis of the Plio-Pleistocene Kowhai formation (Kurow Group), Mackenzie Basin Central Otago. In: *Institute of Geological and Nuclear Science, Report 99/17*. IGNS Ltd, Lower Hutt.

Moritz C, Wright JW, Brown WM (1992) Mitochondrial DNA analyses and the origin and relative age of parthenogenetic *Cnemidophorus:* phylogenetic constraints on hybrid origins. *Evolution*, **46**, 184–192.

Murray J, Stine OC, Johnson MS (1991) The evolution of mitochondrial DNA in *Partula*. *Heredity*, **66**, 93–104.

Nei M (1982) Evolution of human races at the gene level. In: *Human Genetics, Part A: The Unfolding Genome, Proceedings of the Sixth International Congress of Human Genetics* (eds Bonné-Tamir B, Cohen T, Goodman RM), pp. 167–181. Alan Liss, New York.

Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. *Genetics*, **97**, 145–163.

Newnham RM, Lowe DJ, Williams PW (1999) Quaternary environmental changes in New Zealand: a review. *Progress in Physiological Geography*, **23**, 567–610.

Nodder SD (1995) Later Quaternary transgressive/regressive sequences from Taranaki continental shelf, western New Zealand. *Marine Geology*, **123**, 187–214.

Phillips NR, Lambert DM (1989) Genetics of *Potamopyrgus antipodarum* (Gastropoda: Prosobranchia): evidence for reproductive modes. *New Zealand Journal of Zoology*, **16**, 435–445.

Phillips NR, Lambert DM (1990) A cladistic analysis of species of the molluscan genus *Potamopyrgus* based on allozyme data. *New Zealand Journal of Zoology*, **17**, 257–264.

Pillans RB, Pullar WA, Selby MJ, Soons JM (1992) The age and development of the New Zealand landscape. In: *Landforms of New Zealand* (eds Soons JM, Selby MJ), pp. 31–62. Longman Paul, Auckland.

Quattro JM, Avise JC, Vrijenhoek RC (1991) Molecular evidence for multiple origins of hybridogenetic fish clones. *Genetics*, **127**, 391–398.

Schneider CJ, Cunningham N, Moritz C (1998) Comparative phylogeography and history of endemic vertebrates in the wet Tropics rainforests of Australia. *Molecular Ecology*, **7**, 487–498.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN: A Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, Department of Anthropology, Geneva.

Sutherland R (1999) Cenozoic banding of New Zealand basement terranes and Alpine fault displacement: a brief review. *New Zealand Journal of Geology and Geophysics*, **42**, 295–301.

Swofford D (1998) Phylogenetic analysis using parsimony (and other methods) PAUP 4.04 beta version. Sinauer Associates, Sunderland, MA.

Taberlet P, Wust-Saucy A-G, Cosson JF (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453–462.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, **10**, 512–526.

Templeton AR (1998) Nested clade analyses of phylogeographic data: testing hypotheses about gene flow and population history. *Molecular Ecology*, **7**, 381–397.

Terrett JA, Miles S, Thomas RH (1996) Complete DNA sequence of the mitochondrial genome of *Cepaea nemoralis* (Gastropoda: Pulmonata). *Journal of Molecular Evolution*, **42**, 160–168.

Trewick SA, Morgan-Richards M, Russell SJ *et al.* (2003) Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. *Molecular Ecology*, **11**, 2003–2012.

Trewick SA, Wallis GP (2001) Bridging the 'beech-gap': New Zealand invertebrate phylogeography implicates Pleistocene glaciation and Pliocene isolation. *Evolution*, **55**, 2170–2180.

Trewick SA, Wallis GP, Morgan-Richards M (2000) Phylogeographic pattern correlates with Pliocene mountain building in the Alpine scree weta (Orthoptera, Anostostomatidae). *Molecular Ecology*, **9**, 657–666.

Vrijenhoek RC (1998) Animal clones and diversity. *Bioscience*, **48**, 617–628.

Wallace C (1985) On the distribution of the sexes of *Potamopyrgus jenkensi*. *Journal of Molluscan Studies*, **51**, 290–296.

Wallace C (1992) Parthenogenesis, sex, and chromosomes in *Potamopyrgus*. *Journal of Molluscan Studies*, **58**, 93–107.

Wallis GP, Judge KF, Bland J, Waters JM, Berra TM (2001) Genetic diversity in New Zealand *Galaxias vulgaris sensu lato* (Teleostei: Osmeriformes: Galaxiidae): a test of a biogeographic hypothesis. *Journal of Biogeography*, **28**, 59–67.

Wallis GP, Trewick SA (2001) Finding fault with vicariance: a critique of Heads. *Systematic Biology*, **50**, 602–609.

Wardle P (1963) Evolution and distribution of the New Zealand flora, as affected by Quaternary climate. *New Zealand Journal of Botany*, **1**, 3–17.

Wattier R, Engel CR, Saumitou-Laprade P, Valero M (1998) Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus *Gv1CT* in *Gracilaria gracilis* (Rhodophyta). *Molecular Ecology*, **7**, 1569–1573.

Weetman D, Hauser L, Carvalho R (2001) Isolation and characterization of di- and tri-nucleotide microsatellites in the freshwater snail *Potamopyrgus antipodarum*. *Molecular Ecology Notes*, **1**, 185–187.

Willett RW (1950) The New Zealand Pleistocene snow line, climatic conditions, and suggested biological effects. *New Zealand Journal of Science and Technology*, **32**, 18–48.

Wooding S, Ward R (1997) Phylogeography and Pleistocene evolution in the North American black bear. *Molecular Biology and Evolution*, **14**, 1096–1105.

Zink RM (1996) Comparative phylogeography in North American birds. *Evolution*, **50**, 308–317.

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