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Spatial genetics of a high elevation lineage of Rhytididae land snails in New Zealand: the *Powelliphanta* Kawatiri complex

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ABSTRACT

Powelliphanta is a genus of large carnivorous land snails endemic to New Zealand which display phenotypic variation within comparatively small geographic distances. The diversity within these snails has become a matter of high interest to conservation, as many lineages occupy small (or highly fragmented) ranges that render them vulnerable to ongoing habitat loss and predation by exotic pests. Combining *Powelliphanta* mitochondrial sequence data and genotypes of microsatellite loci we document the genetic structure within a species complex dubbed 'Kawatiri'. All populations (with one exception) within the Kawatiri lineage are restricted to subalpine habitat (at elevations over 600 m above sea level). The ranges of some Kawatiri complex populations are adjacent to the congeneric lowland species *Powelliphanta lignaria*. Improved understanding of the distribution of this complex and the level and structure of genetic diversity provided a picture of a naturally fragmented lineage, restricted to a particular ecological zone. We identified six genetic clusters associated with population connectivity orientated north–south along mountain ranges, with east–west divisions between ranges. Future management should aim to retain the evolutionary potential within this young radiation by actively conserving the variation encompassed by each of the six clusters identified here.

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Introduction

Molluscs have the highest number of documented animal extinctions of any major taxonomic group (Lydeard et al. 2004). They suffer the dual disadvantages of extreme sensitivity to habitat modification and pollution, and under-recognition of taxonomic diversity. The grossly disproportionate distribution of taxonomic and conservation effort toward vertebrates and higher plants (Gaston 1992) means that we remain ignorant of the ecology of most mollusc species (Lydeard et al. 2004). Among gastropods, defining taxa has traditionally relied on shell morphology, often in association with spatial distribution. However, geographic distance does not correlate directly with population partitioning as genetic cohesion may reflect environmental heterogeneity (e.g., Mitchell et al. 2015; Weber et al. 2017). For example, landscapes that appear homogeneous by virtue of topographic uniformity might encompass cryptic ecological heterogeneity and thus evolutionary and taxonomic diversity among the inhabitants. Similarly, topographic variation might not be as influential as other factors such as vegetation or historical subdivision. Habitat fragmentation generates physical fragmentation of populations and can pose a serious threat to the genetic diversity of a species (Frankham 2005; Willi et al. 2007), but the effects of habitat fragmentation on patterns of genetic diversity and the genetic structure of resulting populations are difficult to predict (Gibbs 2001; Nelson-Tunley et al. 2016; Ricketts 2001; Willi et al. 2007). Consequently, understanding and ameliorating impacts of anthropogenic activity requires an understanding of the underlying 'natural' patterns and processes.

Here we examine the link between population fragmentation and landscape, in a group of endemic land snails that occupies mountainous habitat on the west coast of South Island, New Zealand. *Powelliphanta* O'Connor, 1945 is an endemic genus of carnivorous snails belonging to the Rhytididae (Mollusca; Gastropoda; Eupulmonata) that occurs in South Africa, Australia, New Zealand and nearby South Pacific islands (Powell 1979; Spencer et al. 2009). The systematics and nomenclature of *Powelliphanta* are undergoing molecular and morphological study (e.g., Walker 2003; Trewick et al. 2008; Walker et al. 2008) that has so far provided support for much of Powell's classification of 41 taxa (9 species, 34 subspecies, 4 forms (Powell 1979)). Similar progress is being made in Rhytididae elsewhere (e.g., Herbert and Moussalli 2010; Moussalli and Herbert 2015).

Powelliphanta is mostly associated with the high precipitation western half of South Island where

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species tend to be restricted to lowland forest, montane forest or the subalpine zone. *Powelliphanta* species typically have small ranges that render them vulnerable to habitat loss and predation by introduced pests, and many members of this genus are ranked as threatened (Hitchmough et al. 2007). The *Powelliphanta* Kawatiri complex refers to a set of subalpine populations spread over less than 11,250 km^2 of the northern West Coast region, South Island. They are united by spatial proximity, broadly similar morphology and mtDNA monophyly. Powell (1979) treated some northern populations of these snails as *Powelliphanta rossiana patrickensis* and one on Kirwins Hill as *P. rossiana gagei*, but most populations were unknown at the time that Powell was working. Walker (2003) suggested that these northern subalpine snails belonged to a new taxon with separate eastern and western groups. Here we use *Powelliphanta* Kawatiri complex to refer to the clade using a regional name linked to the Buller River that flows through the geographic range of this complex. Members of the complex are characterised by an inky to bright blue mantle, with blue, purple or clear mucus, but variation in size (35–45 mm diameter), patterning and colouration of shells led to the recognition of eight informal sub-groups, which are taxonomic hypotheses reflecting this morphological variation (Table 1, Walker 2003).

All populations of the Kawatiri complex exist at high-elevation, just above and below the tree line. Field searches over the last 20 years have increased the known ranges and number of populations but have not revealed snails within this complex in intervening lower elevation forest habitat. The elevational range over which these snail populations occur is in fact quite broad, extending from 550 to 1500 m asl in subalpine forest and tussock grassland. Elevation is an inexact proxy for habitat distribution that is strongly influenced by local rock type and drainage. For instance, snail populations at 550–600 m asl on the Stockton and Denniston plateaux are nevertheless ecologically situated above the local tree line because high rainfall and acidic coal measure soils have depleted soil nutrients and suppressed forest growth (Overmars et al. 1992). By contrast a population referred to as 'Buller River' lives at just 50 m asl in tall forest on recent alluvium beside the Buller River.

The fragmented distribution of snail populations in the *Powelliphanta* Kawatiri complex could result from either of two processes. They might be high-elevation tussock grassland specialists whose evolution and distribution originated during tectonic upheavals in the region about 1–2 mya (Pleistocene), and was therefore subject to latitudinal and elevation shifts during Pleistocene climate cycling (Trewick et al. 2000). Alternatively, isolation of populations might be much more recent; due to anthropogenic habitat modification involving deforestation and introduction of mammalian predators that extirpated snails from lowland areas. The impact of introduced predators such as rats is much lower in high-elevation snail habitat than in the surrounding lowlands (Meads et al. 1984; Walker 2003).

Table 1. *Powelliphanta* Kawatiri complex snail morphology by informal tag-names and location sample.

Powell's name	Tag names	Elevations	Diameter	Height	*d/h*	Populations	Mucus coloration	Soft tissue coloration
	P. "Garabaldi"	1200–1400	38	18	2.11	Garabaldi	4 faint blue	4 blue
	P. "Baton"	1100–1200	39	19	2.05	Baton	3 faint blue	3 blue
	P. "Martiri"	1200–1500 (uncommonly to 600)	42.5	21	2.02	Matiri	1 blue 2 clear 2 nr	1 blue 2 grey 2 nr
	P. "Matakitaki"	1200–1700 (at one site to 1000)	44.5	21.5	2.07	Braeburn Matakitaki Springs Junction	nr nr 4 faint blue	nr nr 4 blue-green
	P. "Kirwans"	1250–1280	40	18	2.22	Nth Brunner Mid Brunner Kirwans (Sth Brunner)	1 blue 1 nr nr 4 clear	1 blue 1 nr nr 4 blue
P. rossiana gagei	*P. gagei*	800–1450	42.5	24	1.77	Gagei * Mounts Three Sisters (no micros) Bucklands Peak	* 1 purple 1 clear 1 nr 2 purple 2 clear 2 purple 1 dark blue 3 nr	* 1 purple 2 nr nr nr
	P. "Buller River"	50–100	40	20	2	Buller	5 blue 2 clear 3 nr	5 grey 2 nr
P. rossiana patrickensis	*P. patrickensis*	550–850	35	21	1.67	Sth Denniston Nth Denniston Stockton	4 faint blue 3 faint blue	4 blue 3 blue

Notes: Elevations and shell morphology from Walker (2003), mucus and soft tissue colouration noted at time of specimen collection. Measurements in millimetres. nr = not recorded. * = not included in this study as they fall outside the Kawatiri mtDNA clade.

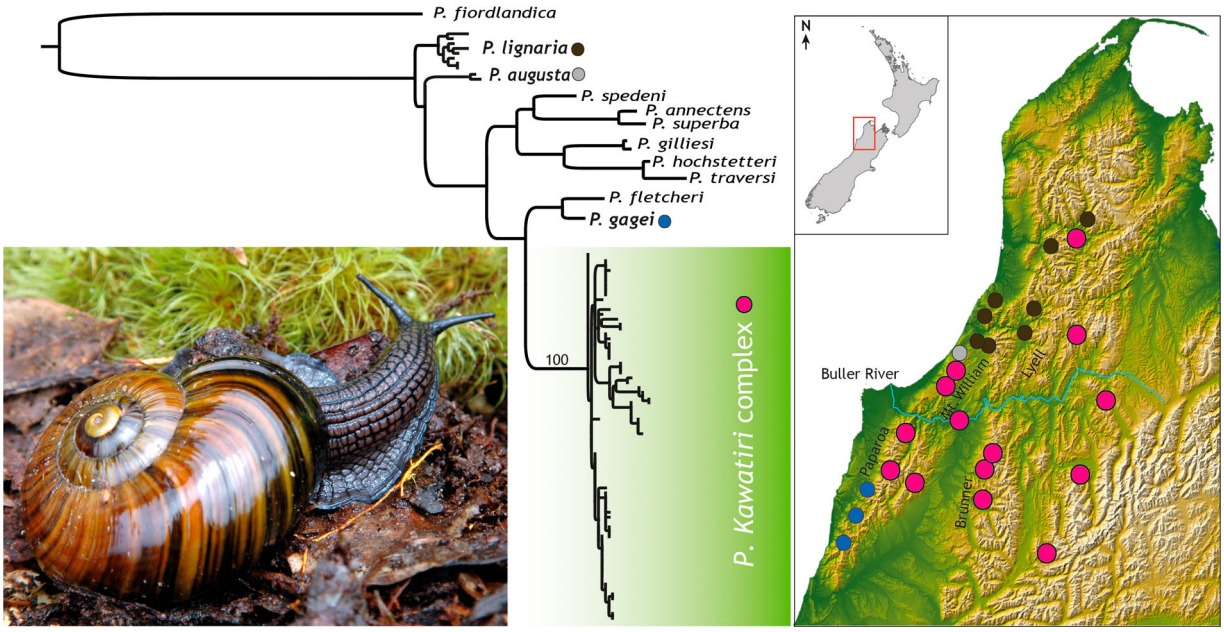

Figure 1. Sampling locations and phylogenetic relationships among representative *Powelliphanta* taxa in northwest South Island, New Zealand. Members of the Kawatiri complex (magenta filled circles) occur in close proximity to three other species. Maximum likelihood phylogeny of mitochondrial COI haplotypes from these species and representative of *Powelliphanta* diversity that are geographically more distant (Trewick et al. 2008). False colour map shows landscape relief with higher elevation areas graded yellow to white, lower in green. The course of the Buller River is indicated in blue, and key mountain ranges are indicated (Paparoa Range, Mt William Range, Brunner Range, Lyell Range). The snail shown is an adult *Powelliphanta patrickensis* (credit Kathleen Walker).

Here we use spatial distance, mtDNA and microsatellite variation over the small geographical area of the *Powelliphanta* Kawatiri complex, to explore its genetic structure (Figure 1). Should the current pattern of population fragmentation result from geologically recent isolation we expect population genetic structure to reflect a pre-existing pattern of isolation by distance, for all markers (Charlesworth 2009). Alternatively, partitioning that results from high-elevation specialisation over a longer time frame would be inferred if some populations were differentiated by mtDNA, but retained the signs of isolation by distance only at slower evolving nuclear loci. Isolated taxa would be expected to have concordant patterns of nuclear and mitochondrial markers and morphology due to relatively long isolation.

Methods

Material

We explored genetic structure in a sample of *Powelliphanta* snails previously revealed by preliminary analysis of mtDNA sequence data to be a distinct monophyletic clade (Trewick et al. 2008). Snails in this clade occur in allopatric populations across a topographically heterogeneous landscape that has contiguous native vegetation (Figure 1).

Snails were located in the field by hand searching. A minority of specimens (~35) were whole snails, killed by freezing and stored at −80°C for genetic analysis, while 73 (47 from a single population on the Stockton Plateau) were sampled as tissue biopsies taken from live snails that were released at their collecting location (Trewick et al. 2008). Tissue samples were stored in 95% ethanol at 4°C. GPS coordinates were recorded for each sample with data on mucus and mantle colour, and shell morphology. The long lifespan and naturally overlapping generations of these snails mean that samples from different years can effectively be treated as the same generation in downstream analysis.

Laboratory protocols

Genomic DNA was extracted using incubation at 55°C with Proteinase K in a CTAB buffer (2% Hexadecyltrimethyl ammonium bromide, 100 mM Tris-HCl pH8.0, 1.4M NaCl, 20 mM EDTA). This was followed by a combined phenol/chloroform/isoamyl alcohol (25:24:1) cleanup based on previously described methods (Stine 1989; Terret, 1992; Thomaz et al. 1996). DNA was precipitated with ethanol, resuspended in a TE buffer (10 mM Tris, 0.1 mM EDTA) and the quantity and quality checked using a Nanodrop® ND-1000 (NanoDrop Technologies) and electrophoresis on 1% agarose gels. DNA was diluted to approximately 10 ng/µl to provide templates for amplification of specific fragments.

PCR targeted a fragment of the mitochondiral Cytochrome Oxidase subunit I gene, using a new

Powelliphanta specific primer (POWCO1r) that replaces H7005 (Hafner et al. 1994) used in previous studies. Together with LCO1490 (Folmer et al. 1994), POWCO1r (5′ GCA ATT ACT ATC GTT GCA GCA G 3′) targets a fragment of about 700 bp at the 5′ end of COI. PCR reactions were performed in 10 µL volumes with Red Hot® Taq polymerase using thermocycling conditions 94°C for 2 min followed by 36 cycles of 94°C for 15 s, 50°C for 30 s, and 72°C for 1 min 30 s with a final extension of 72°C for 8 min. PCR products were purified using SAP/EXO (Shrimp Alkaline Phosphatase/exonuclease) enzymatic digest (USB Corporation) and sequenced using BigDye® chemistry (Perkin Elmer) following the manufacturer's protocols on an ABI3730 DNA analyser (Applied Biosystems Inc. California).

Sequence reads were checked against ABI trace files using Sequencher v4.6 (Applied Biosystems Inc., Foster City, California) and aligned by eye in SeAl v2.0a11 (SeAl 2007) and Geneious v6.05 (Kearse et al. 2012). The nucleotide sequences for COI were translated using the invertebrate mitochondrial amino acid code to check for the presence of stop codons and frame shifts that might be indicative of nuclear copies (Blanchard and Lynch 2000; Bensasson et al. 2001). Data for representative outgroup taxa were obtained from Genbank.

mtDNA analysis

The *Powelliphanta* Kawatiri complex clade was identified using phylogenetic analysis of a 622 bp alignment of COI DNA sequence. A maximum likelihood tree was generated by PhyML in GENEIOUS v6.0.5 using the general time reversible (GTR) model, with outgroup sampling as previously reported (Trewick et al. 2008). As phylogenetic trees are not optimal for describing the relationship among haplotypes within closely related taxa where ancestral sequence variants might persist in extant populations, we used POPART (Leigh and Bryant 2015) to construct a median joining network among the mtDNA COI ingroup haplotypes. Networks inferred using other algorithms available in POPART (Minimum Spanning, Ancestral MP, Integer NJ, Tight Span Walker, TCS) were also examined.

Genetic diversity among the ingroup COI sequences was estimated using the Geneious v6.0.5 tree builder. To assess mtDNA sequence variation present within each population sample, nucleotide (π) and haplotype (h) diversities and Φ_{ST} were calculated in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010) (Supplementary Table S1). Population pairwise Φ_{ST} were estimated and significant deviations from 0 determined. A standard AMOVA was used to test for significant genetic differences based on the estimate of genetic partitioning among groups (Φ_{CT}). For this, population samples were alternatively grouped according to informal names that reflect shell traits, geographic clusters or other characteristics.

Evidence of isolation by distance (IBD) was sought using a Mantel test of the correlation of pairwise linear geographic distances and pairwise Φ_{ST} from the COI haplotype data implemented in IBDWS v3.23 (Jensen et al. 2005) using 1000 permutations. MtDNA haplotypes were imported to IBDWS as a text alignment. Geographic distances were log transformed as recommended for an expanded stepping stone model (Slatkin 1993).

Microsatellite genotyping

Taxon specific microsatellite (SSR) markers were developed using a modified enriched microsatellite library protocol (Supplementary Table S2). Whole genomic DNA was digested with the restriction enzyme BfuCI and fragments ligated to a linker. Probe hybridisation used 5′ biotin-labelled (CA_{12} or GA_{12}) oligonucleotides selectively captured using Streptavidin magnetic beads (Promega). Enriched DNA was subjected to PCR amplification to generate double stranded DNA and cloned using the TOPO® Cloning Kit (Invitrogen). Putative microsatellite loci were tested by PCR amplification across seven individuals, comprising five from the *Powelliphanta* Kawatiri complex from separate geographical locations and two *P. lignaria johnstoni*. Each forward primer was labelled with either of the fluorescent dyes, 6-Fam or HEX (Invitrogen), during PCR using M13 tails on the forward primers. PCR reactions were performed in 10 µL volumes with Red Hot® Taq DNA polymerase using the cycling conditions 94°C for 2 min followed by 36 cycles of 94°C for 15 s, 50°C for 30 s, and 72°C for 1 min 30 s with a final extension of 72°C for 8 min. Loci were pooled using combinations of fluorophore and allele size range, genotyped on an ABI 3730 with an internal LIZ size standard and scored using GENEMAPPER® v4.7 (Applied Biosystems).

Five reliable polymorphic loci were used to genotype population samples. Loci were assessed for large allele dropout and stuttering using 1000 randomisations in the programme MICROCHECKER v2.2.3 (Van Oosterhout et al. 2004). Evidence of linkage disequilibrium among loci was sought using the largest sample of 31 snails from the Stockton population and the software GENEPOP ON THE WEB (Raymond and Rousset 1995; Rousset 2008). We compared observed genotype distributions in each population sample with those expected under Hardy-Weinberg equilibrium using χ^2 tests. Population pairwise F_{ST} estimates were examined to see which were significantly greater than zero as expected if gene flow was limited (ARLEQUIN v3.5.1.2). Evidence for Isolation by Distance (IBD) was sought using a Mantel test of the correlation of pairwise geographic distances and pairwise F_{ST} with 1000 permutations implemented in IBDWS v3.23 (Jensen et al. 2005). Diploid genotypes were imported into IBDWS as raw data. Geographic distances were

calculated and log transformed as described above. Loci were examined separately and as a single group.

We searched for evidence of population structuring among our multi-locus genotype data using Bayesian assignment in STRUCTURE v2.3.2 (Pritchard et al. 2000). This approach infers genotype similarity and assigns individuals to clusters (populations), as well as determining isolation-by-barrier. We used a Bayesian Markov Chain Monte Carlo (MCMC) simulation chain of 100,000 steps following a burnin of 10,000 steps. The number of clusters (K) tested was set at between 1 and 15 in separate runs with 11 iterations of each. This analysis was performed using the no-admixture model, appropriate for studying fully discrete populations and suitable for detecting subtle structure. Analysis was repeated with both correlated and independent allele frequency models and then repeated using the admixture model. We also examined the effect of including sampling location information as recommended for analyses with relatively small sample sizes (Hubisz et al. 2009). Including sampling location categories or phenotype-based groups allows the models implemented by STRUCTURE v2.3.2 to modify the prior distribution for each individual's population assignment. Results were processed in STRUCTURE HARVESTER v0.6.93 (Earl and vonHoldt 2011) to assess and visualise likelihood values across multiple values of K and hundreds of iterations to determine the number of genetic clusters that best fitted the genotypic data. This used the DeltaK statistic that considers the rate of change in log probability between successive values of K. In addition, STRUCTURE HARVESTER v0.6.93 was used to reformat data for optimal K for analysis in CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). This cluster matching and permutation program deals with label switching and multimodality in analysis of population structure. DISTRUCT v1.1 (Rosenberg 2003) was used to illustrate results.

To determine which populations lent significance to the genetic structure of the group we used OBSTRUCT (Gayevskiy et al. 2014). CSV files were manually created from STRUCTURE output files for hypothesised numbers of populations (K) of 2, 5, 6 or 7 and processed with OBSTRUCT. The overall R^2 values for each K were compared with each other and to R^2 values calculated either without predefined populations or without inferred populations. Results were graphically visualised using R (R Core Team 2013).

Hierarchical analyses of haplotype and microsatellite data were performed separately across hypothesised groupings of populations in ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010). Population samples were grouped according to closest proximity to the eight recognised informal names (eight groups), the clusters identified in the mtDNA analysis (seven groups), microsatellite data (six groups), and into groups based on geographic proximity (six groups) and by the axial mountain range on which they occurred (three groups).

Results

Mitochondrial DNA

Phylogenetic analysis of the mtDNA COI sequences resolved a distinct monophyletic lineage comprising *Powelliphanta* Kawatiri complex that was sister to a clade comprising *P. fletcheri* and *P. gagei* (Figure 1). Within the Kawatiri complex clade were 101 snails collected from 16 locations (Supplementary Table S1). All these snails were collected from subalpine vegetation at elevations ranging from 550 m asl to 1500 m asl, with the exception of the lowland snail population sample 'Buller River' ($n = 7$).

No frameshifts, deletions, inversions or insertions were detected in the nucleotide alignment of mtDNA COI sequences. There were 36 haplotypes that differed by up to 3%, (HKY distance) and had nucleotide diversity (π) of 0.009. Nucleotide diversity was low within population samples from the Kawatiri complex with six population samples being monomorphic. The highest sequence divergence within a putative population sample was between two snails from two different mountains in the eastern Paparoa Ranges, aggregated here as the 'Mounts' (0.013; Supplementary Table S1). Haplotype sharing among population samples of the Kawatiri complex snails was limited, with most samples having private haplotypes. Even the most densely sampled location (Stockton), which had eleven haplotypes, shared only one (Hap12) with the adjacent population sample (Denniston). In most cases haplotypes from the same location grouped together on the mtDNA network (Figure 2). Most genetic variation in our sample was therefore partitioned among population samples with many pairwise Φ_{ST} estimates significantly greater than zero (Supplementary Table S2). There was strong evidence of genetic partitioning among the population samples with an overall Φ_{ST} estimate of 0.737. The haplotype network showed some evidence of geographic partitioning, with in particular a western cluster encompassing population samples on the Paparoa, Brunner and Mount William ranges (Figure 1). Overall mtDNA variation was nevertheless consistent with a model of isolation by distance (Mantel test: $r = 0.453$; $P < .001$) such that geographically close population samples are genetically more similar to each other than to more geographically distant population samples.

Microsatellites

All 101 snails were genotyped for five microsatellite loci. No evidence of long allele drop out was detected for any of these loci. However, there was evidence of

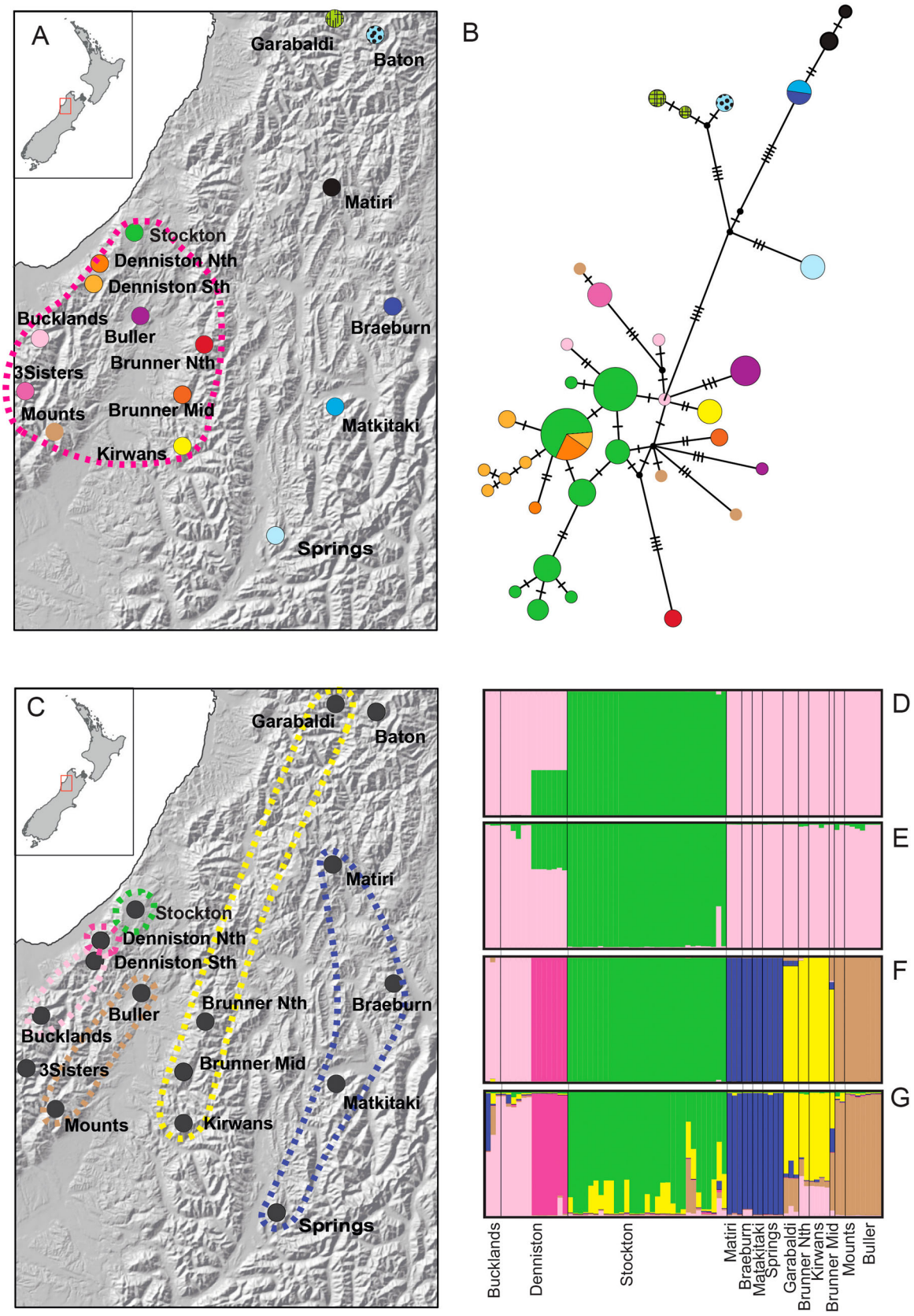

Figure 2. Population genetic structure among land snail populations in the New Zealand *Powelliphanta* Kawatiri complex. (A) Population sampling for mtDNA haplotypes; (B) median joining network of mtDNA COI (622bp) haplotypes with colours indicating geographic source shown on map; (C) population sample clusters indicated by Bayesian genotype assignment of five microsatellite loci shown in graphs; (D) no-admixture model, $K = 2$; (E) admixture model, $K = 2$; (F) non-admixture model, $K = 6$; (G) admixture model, $K = 6$. Map C shows population samples sharing genotypic clusters (dashed lines). Colours represent alternative genotype assignments, at each *K*.

Table 2. Genetic diversity at five microsatellite loci among population samples of the New Zealand *Powelliphanta* Kawatiri landsnail complex.

Location	*n*	Ar	Ho	He	G
Garabaldi	3	2.00	0.11	0.47	0.28
Matiri	3	4.00	0.33	0.87	0.17
Braeburn	2	1.00	*	*	0.00
Matakitaki	2	1.00	*	*	0.00
Springs Junction	4	3.00	0.00	0.71	0.14
Brunner Nth	1	2.00	1.00	1.00	0.40
Brunner Mid	2	2.60	0.60	0.73	0.73
Kirwans	4	3.50	0.31	0.62	0.49
Mounts	2	2.50	0.63	0.67	0.53
Bucklands Peak	3	2.75	0.17	0.68	0.55
Buller	7	2.75	0.61	0.52	0.42
Sth Denniston	7	2.67	0.52	0.54	0.32
Nth Denniston	6	3.40	0.33	0.56	0.56
Stockton	31	6.20	0.25	0.60	0.60

Notes: Monomorphic samples are indicated by *. Number of individuals (*n*), average allelic richness (Ar), observed heterozygosity (Ho), expected heterozygosity (He), and average gene diversity (G).

departure from the expectations of the Hardy-Weinberg equilibrium for four of the five loci within the population sample at Stockton, probably caused by the presence of null alleles. Pairwise linkage disequilibrium tested using the genotypes of the 31 Stockton snails indicated that the alleles at different loci randomly associated. The number of alleles per locus ranged from 4 to 20. Per locus, allelic richness from all genotyped population samples ($n = 15$) reached eight (Supplementary Table S3). Average gene diversity (Table 2) was highest in the sample from Brunner Mid (0.733). Braeburn and Matakitaki samples had no allelic variation, but each consisted of only two snails. Allele sharing among population samples was common but half of the population pairwise F_{ST} estimates were significantly greater than zero (46/91; Table S4). Each population was significantly differentiated from at least three other populations, with the exception of Brunner North ($n = 2$; Supplementary Table S3). Stockton differed significantly from all other population samples (pairwise F_{ST} 0.247–0.504). Even Denniston South and Denniston North differed significantly although the collection sites of these samples were only ~5.4 km apart. Microsatellite differentiation followed a model of isolation by distance (Mantel test: $r = 0.3207$; $P = .0190$).

Population structure

Bayesian assignment of microsatellite genotypes using STRUCTURE v2.3.2 yielded similar results using correlated and independent allele frequency models. The maximum value of Delta *K* determined using the independent allele frequency model, which is reported as less likely to overestimate *K*, was for $K = 2$ with the next optimal value for $K = 6$. Visual examination of output from DISTRUCT (Figure 2) shows that $K = 6$ captured more information about subdivision of population with similar clustering of samples in analyses with or without admixture. There was little difference in the R^2 values for $K = 2$ (0.84), $K = 5$(0.84) or $K = 6$ (0.83), but these were all higher than the R^2 value for $K = 7$ (0.69) returned by OBSTRUCT. We infer from this steep decline in R^2 that $K = 6$ captures the majority of population structuring. For $K = 6$ the Stockton population had the greatest average contribution to structure within the data, followed by Buller and Springs Junction. Other samples had little effect on the structure of the data. Results were similar for $K = 5$ and $K = 2$ where it was again the Stockton and Buller populations that contributed most to the structure of the group. Most individuals had high assignment probabilities to a single cluster but at low *K* values the sample from Denniston revealed mixed genotypes, as expected of gene flow among conspecific populations.

We explored support for alternative clustering hypotheses using hierarchical analysis with seven

Table 3. Hypothetical groupings of *Powelliphanta* Kawatiri population samples used for hierarchical analysis of data from mtDNA haplotypes and five microsatellite loci, and their Φ_{CT} / F_{CT} scores.

Populations	Structure $K = 2$	Other $K = 2$	Mount $K = 3$	Geo $K = 6$	Micro $K = 6$	Haplo $K = 7$	Tagnames $K = 8$
Denniston Nth	1	1	1	1	1	1	1
Dennsiton Sth	1	1	1	1	2	1	1
Stockton	1	1	1	1	3	1	1
Buller River	1	1	1	4	5	5	6
3 Sisters	1	1	1	5	1	6	7
Bucklands Peak	1	1	1	5	1	6	7
Mounts	1	1	1	5	5	6	7
Brunner Nth	1	2	2	6	6	7	8
Brunner Mid	1	2	2	6	6	7	8
Kirwans	1	2	2	6	6	7	8
Braeburn	2	2	3	2	4	2	2
Matakitaki	2	2	3	2	4	2	2
Springs Junction	2	2	3	2	4	3	2
Matiri	2	2	3	3	4	2	3
Garabaldi	2	2	3	3	6	4	4
Baton	2	2	3	3	6	4	5
FCT mt DNA	0.44	0.21	0.36	0.40	*	0.61	0.50
FCT micros	0.22	0.14	0.19	0.15	*	0.13	0.11

Notes: Hypothetical groupings used: Structure, $K = 2$ analysis of microsatellites; Other, east/west geographic split; Mount, main mountain ranges; Geo, geographic proximity of sample sites; Micro, $K = 6$ analysis of microsatellites; Haplo, mitochondrial haplotype lineages; Tag-names, closest proximity to prior informal taxon names.

alternative arrangements of population samples that reflect taxonomic and geographical hypotheses (Table 3). Unsurprisingly the highest Φ_{CT} was obtained for mtDNA data using the structure within the haplotype network, $K = 7$ (0.60605; $P < .001$). The highest F_{CT} for microsatellite data was obtained with two groups ($K = 2$) based on the clusters identified by Bayesian assignment (0.22413; $P < .005$). Mitochondrial DNA and genotype data were thus not concordant although they were consistent. The lowest estimate of F_{CT} / Φ_{CT} (which did not differ significantly from zero) was obtained for mtDNA and microsatellite data using a grouping hypothesis based on the six populations inferred using STRUCTURE (Table 3). The informal name hypothesis ($K = 8$) provided reasonable support for mtDNA (0.49809; $P < .001$), and grouping by mountain ranges ($K = 3$) provided the second highest F_{CT} for microsatellite data (0.18708; $P < .001$).

Discussion

The *Powelliphanta* Kawatiri complex mtDNA lineage comprises snails from high elevations and subalpine habitat in northwest South Island. The lowland species *P. lignaria* is found in the adjacent lowland areas towards the northern end of the distribution of Kawatiri snails, within just 3 km of the Garabaldi and Baton populations and 15–20 km from the Stockton population. The Kawatiri complex also includes snails in the Northern Paparoa Range separated from populations of *Powelliphanta gagei* only by elevation (Figure 2). The only lowland snail population within the Kawatiri mtDNA clade that we encountered was 'Buller River'. This unusual low elevation population sample also grouped with the nearby Kawatiri complex populations (Mounts) in our microsatellite analysis, consistent with it being a recent accidental colonisation of this small area.

Genetic diversity within our sampling of the Kawatiri complex follows a model of moderate isolation by distance suggesting population connectivity in the geologically recent past, and lack of concordance among markers is consistent with a single broad genotypic cluster (Mallet 1995) or single species. Despite the signature of isolation by distance, there was evidence of restricted gene flow allowing differentiation of populations. In particular this has resulted in an east/west division of population samples in the haplotype network and to some extent within the genotype analysis (Figure 2). The uneven sampling regime (with many snails in the sample from Stockton and small sample sizes from several other populations) may have influenced results, as small sample sizes can over-estimate population genetic structure if few markers are included (Sinclair and Hobbs 2009; Willing et al. 2012). Caution is therefore needed when interpreting the patterns observed, but there are real limitations on the scale of sampling that is possible with these scarce, protected and cryptic snails.

On the data found here we can dismiss the theory of deeply diverged lineages within the complex as both nuclear and mitochondrial markers would be expected to be showing the same clusters if this was so. However, extremely recent fragmentation of the species, since human colonisation, also looks unlikely. The existence of other *Powelliphanta* species in low elevation forest habitat suggests that mammalian predation does not explain the current fragmented distribution of Kawatiri complex. This is supported by the level of differentiation described here that is unlikely to have accumulated in just ~800 years since the arrival of rats in New Zealand or in the ~250 years, since the start of European habitat modification. In fact, the genetic structure and differentiation described among Kawatiri complex populations suggests that there has been restricted gene flow for many generations and we therefore infer that the eastern and western populations began separating during rapid uplift and faulting of the Buller region mountain ranges in the Early-Mid Pleistocene (Nathan et al. 2002) and through the Mid-Late Pleistocene climate cycling (Trewick and Bland 2012). Climate-linked habitat adaptation will have been influenced by the repeated changes in conditions through the Pleistocene (Hewitt 2000), and episodes of climate change are likely to have had a major influence on population fragmentation and thus population size. We note that the implied north–south trend in gene flow is consistent with the intersection of altitudinal and latitudinal range shifting (Bulgarella et al. 2014). This contrasts with the deeper genetic divergences and more pronounced spatial pattern seen among other invertebrates in the Southern Alps such as alpine scree weta (Trewick et al. 2000), cicadas (Hill et al. 2009) and stoneflies (McCulloch et al. 2010), which suggest earlier Pliocene separation and persistence of isolated populations through repeated climate cycles of the Pleistocene (Trewick et al. 2011).

We saw a tendency for north–south connectivity (east/west division), sometimes over quite large distances, rather than more regional groups which might have been expected. This is in accord with a landscape where ridges and mountain ranges have a strong north/south orientation. Thus, when the climate was warmest we expect there was a retreat of grassland to the mountain tops as the intervening depressions filled with unsuitable tall forest habitat, while glacial ice, snow and fellfield confined the populations to small parts of each range during the glacial maxima. It is interesting to note that even a large river, such as the Buller, does not appear to have provided as much of a barrier to gene flow in this snail lineage as the extensive basins of the Grey-Inangahua and Maruia-Murchison Depressions (Nathan et al. 2002). As a result there

are two major groupings seen within the genetic diversity of the Kawatiri complex lineage, one comprises the populations found on the western edge of their range (Paparoa, Mount William and Brunner ranges), and the other includes the more inland (eastern) populations with six shallower clusters. We advocate management that retains the evolutionary potential of these clusters and recognises their variation.

Currently two of the most distinctive clusters within the Kawatiri complex (Stockton and Denniston) are threatened by loss of habitat through coal mining (Trewick et al. 2008; Walker et al. 2008). Until opencast coal mining became economically viable the high-elevation and remoteness of their habitat provided some protection for these snail populations and other subalpine fauna and flora. Ideally, decisions about how conservation effort is distributed should be based on genetic, morphological and ecological evidence, in the context of ecological and genetic exchangeability. However, there may also be other non-evolutionary (geological, economic, aesthetic) reasons for ascribing conservation value to particular populations (Moritz 1994). Conservationists should explicitly separate taxonomic diversity from recovery planning, and recognise that different units are appropriate to each purpose (Mace 2004). This study goes some way towards understanding the genetic basis of the ecological, morphological and distributional clustering noted in this complex (Walker 2003). As such it is an essential first step in the classification and protection of one of New Zealand's more interesting recent species radiations.

Disclosure statement

No potential conflict of interest was reported by the authors.

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