Zoologischer Anzeiger 255 (2015) 71–79

Contents lists available at ScienceDirect

Zoologischer Anzeiger

journal homepage: www.elsevier.com/locate/jcz

Zoologischer Anzeiger

Intercontinental island hopping: Colonization and speciation of the grasshopper genus *Phaulacridium* (Orthoptera: Acrididae) in Australasia

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ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 22 February 2015
Accepted 22 February 2015
Available online 24 February 2015

Keywords:
Orthoptera
Acrididae
Australia
New Zealand
Lord Howe Island
Chatham Island
Dispersal
LGM

ABSTRACT

Due to their distance from the pole and extent of surrounding oceans, southern hemisphere lands were not subjected to such severe climatic conditions in the Pleistocene as those in the northern hemisphere. Pleistocene climate cycling did however result in extensive shifts in habitat zones due to fluctuation of rainfall and temperature. Warm and wet conditions during interglacials supported southward extension of forests, whereas cooler and drier environments during glacial maxima increased the extent of dry grassland and scrub conditions. Such fluctuations are likely to have influenced the spatial distribution and evolution of the fauna of Australasia. Using data from four genes (two mitochondrial and two nuclear) the genetic structure of the *trans*-Tasman grasshopper genus *Phaulacridium* was used to infer phylogeographic patterns.

The widespread New Zealand species *Phaulacridium marginale* shows a phylogeographic pattern typical of recent range expansion, with low genetic diversity within the species. In stark contrast, the other New Zealand species *Phaulacridium otagoense* is extremely localized in two small areas in southern New Zealand where it exhibits very high genetic diversity. The phylogeographic patterns in Australian *Phaulacridium*, however, show deeper divergence within the most widespread species, than between different species in the same area. Mismatched correlation between geographic scale and genetic diversity imply that populations' genetics contain the signature of past species ranges.

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1. Introduction

Since Darwin (1809–1882) and Wallace (1823–1913), a fundamental objective for biologists has been to understand the diversity and distribution of biota. This endeavor received a substantial stimulus from phylogeographic approaches (e.g., Avise et al., 1987; Avise, 2000) which continue to develop and improve (e.g., Hickerson et al., 2010). An initial emphasis of phylogeographic research was in North America and Europe, where studies have provided a degree of consensus on patterns and processes (e.g., Taberlet et al., 1998; Hewitt, 1999, 2000, 2004; Schmitt, 2007). Phylogeographic structuring in these landscapes has been predominantly influenced by two extrinsic factors. The first is the impact of extreme climate cycling throughout the Quaternary period with extension of polar ice sheets, vast periglacial regions with permafrost and lower global temperatures during glacials. The second is the largely continuous distribution of land in northern latitudes across thousands of kilometers of longitude. The biology of temperate northern hemisphere regions is to a large extent the story of dispersal and range shifting since the last glacial maximum (LGM). In the southern hemisphere, the situation is rather different. There was no extension of polar ice sheets to continents beyond Antarctica, although some small adjacent islands were affected (McIntosh et al., 2009; Nevill et al., 2010). Furthermore, landmasses are widely spaced and separated around longitude by wide expanses of ocean. This probably restricts dispersal of terrestrial organisms, and also ameliorates the local intensity of climate fluctuations. The Australasian region therefore provides a strong

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http://dx.doi.org/10.1016/j.jcz.2015.02.005
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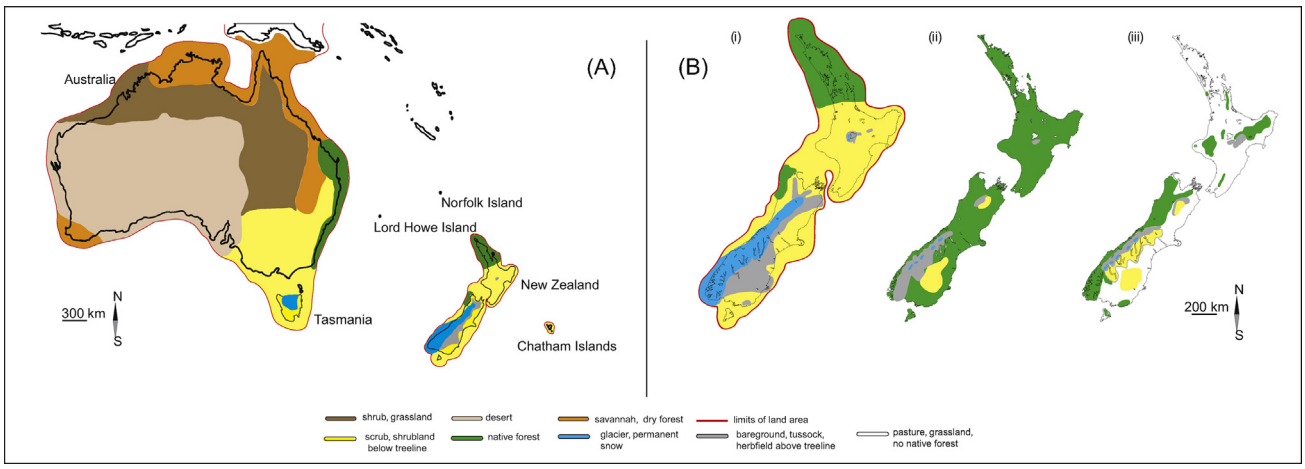

Fig. 1. Vegetation habitat zones in Australia and New Zealand; (A) Last glacial maximum (LGM), modified from Hope et al. (2004) and Alloway et al. (2007). (B) Approximate distribution of vegetation zones in New Zealand at (i) LGM, (ii) pre-human, (iii) modern (post human settlement) New Zealand (modified from Alloway et al., 2007).

and intriguing contrast to most studied northern hemisphere systems, as it includes land areas that differ extensively in size and degree of spatial isolation, ranging from the continent of Australia (7.6 million km^2) in the west to New Zealand (268.000 km^2) and the Chatham Islands (900 km^2) in the east (Fig. 1).

During the LGM, extensive montane glaciers developed on the Southern Alps in South Island, New Zealand (Fig. 1A and B(i)) (Alloway et al., 2007). Australia on the other hand was little affected by ice, but experienced with New Zealand a general aridification and cooling resulting in expansion of shrub and dry grassland and restriction of high forest (Fig. 1A) (Lancashire et al., 2002; McKinnon et al., 2004; Williams et al., 2009; Nevill et al., 2010). Lowered sea level resulted in land connection between North and South Islands of New Zealand (Trewick and Bland, 2012) and between Tasmania and mainland Australia (Chappell and Shackleton, 1986), but no significant increase in the proximity of Australia, New Zealand and Chatham Island. Climate warming in the Holocene resulted in retraction of montane glaciers and expansion of forests which covered about 85% of New Zealand (Fig. 1B(ii)) prior to the arrival of humans (Trewick and Morgan-Richards, 2009). The maritime climate of the low lying Chatham Islands minimized habitat variation through the Pleistocene (Holt et al., 2010).

New Zealand is a continental island that is an emergent part of Zealandia, a sunken continent separated from other parts of Gondwana for approximately 80 Myr (million years) (Trewick et al., 2007). Its biota comprises representatives of lineages that are elsewhere extinct, and organisms that are more typical of an isolated archipelago (Daugherty et al., 1993; Goldberg et al., 2008; Wallis and Trewick, 2009). Extensive marine inundation of Zealandia culminating in the late Oligocene (Cooper and Millener, 1993; Landis et al., 2008) may explain, at least in part, the absence of many higher-level taxa in New Zealand (Daugherty et al., 1993), radiation of others (Cooper and Cooper, 1995), and the close relationships of the New Zealand fauna and flora to that of Australia (Pole, 1994; Knapp et al., 2007; Trewick and Morgan-Richards, 2009).

Miocene New Zealand was a relatively low-lying landmass with a temperate or subtropical climate (Fleming, 1979; Daugherty et al., 1993; Campbell and Hutching, 2007), modified in the Pliocene by climate cooling and formation of the South Island Southern Alps (Batt et al., 2000; Chamberlain and Poage, 2000), and subsequently by Pleistocene climate cycling and glaciation (Carter, 2005). Ecological and molecular evidence indicates that plant and animal inhabitants of the subalpine/alpine zone of the Southern Alps radiated during this time (Buckley et al., 2001; Lockhart et al., 2001; Meudt and Simpson, 2006; Goldberg et al., 2008; Wallis and Trewick, 2009). We infer a shift from low lying habitat dominated by forests to a more diverse and heterogeneous one that provided opportunities for both colonization and radiation in the Plio-Pleistocene. Substantial and rapid fluctuations in the distribution of vegetation communities during the Pleistocene (McGlone, 1985) are thought to explain the modern distribution of many cold-sensitive taxa as a result of their survival in glacial refugia (Wardle, 1963; Burrows, 1965; Dumbleton, 1969). Studies of phylogeographic patterns in alpine and forest species (e.g., Trewick, 2008; Hill et al., 2009; O'Neill et al., 2009; Goldberg et al., 2011, 2014; Marske et al., 2011) show little consensus among taxa (see also Goldberg et al., 2008; Wallis and Trewick, 2009; Trewick et al., 2011). Less is known about the response of terrestrial taxa in lowland non-forest habitat. This in part reflects a natural scarcity of such open habitats in Holocene New Zealand, but also lack of clarity about the evolutionary association between animals and habitat types. For instance, many insect species that can persist on scrub vegetation or are primarily soil dwellers may not require mature forest to persist in a region. Taxa in such a setting provide the most direct means for comparison of responses to Pleistocene climate cycling that had a major impact on the distribution of non-forest habitats.

We focus this research on the grasshopper genus *Phaulacridium* Brunner v. Wattenwyl, 1893 (Orthoptera: Acrididae) that comprises two species in Australia [*Phaulacridium vittatum* (Sjöstedt, 1920) and *P. crassum* Key, 1992], one on Lord Howe Island (*P. howeanum* Key, 1992) and two in New Zealand [*Phaulacridium marginale* (Walker, 1870) and *Phaulacridium otagoense* Westerman and Ritchie, 1984] (Key, 1992). The species are relatively small (<12 mm) and inhabit native and mixed exotic grasslands (Clark, 1967; Key, 1992). *Phaulacridium* are primarily lowland grasshoppers occurring up to ~1200 m above sea-level in mainland New Zealand (Westerman and Ritchie, 1984) but can reach higher altitudes in equitable areas of southern New Zealand, South Australia and Tasmania (Key, 1992; Harris et al., 2013). Most *Phaulacridium* have reduced, non-functional wings, but macropterous (fully-winged) individuals of *P. vittatum*, *P. crassum* and *P. marginale* do sporadically occur (Westerman and Ritchie, 1984; Key, 1992). In general, fewer than 3% of *P. crassum* and 10% of *P. vittatum* are macropterous (Key, 1992), although in some populations of *P. vittatum* the macropterous form can be more abundant (Clark, 1967). Winged *P. marginale* are probably quite rare (Bigelow, 1967). Hutton (1897) recorded only two winged animals (both female) in a sample of 218 specimens, but a 2011 population in Hawkes Bay appears to have had a higher

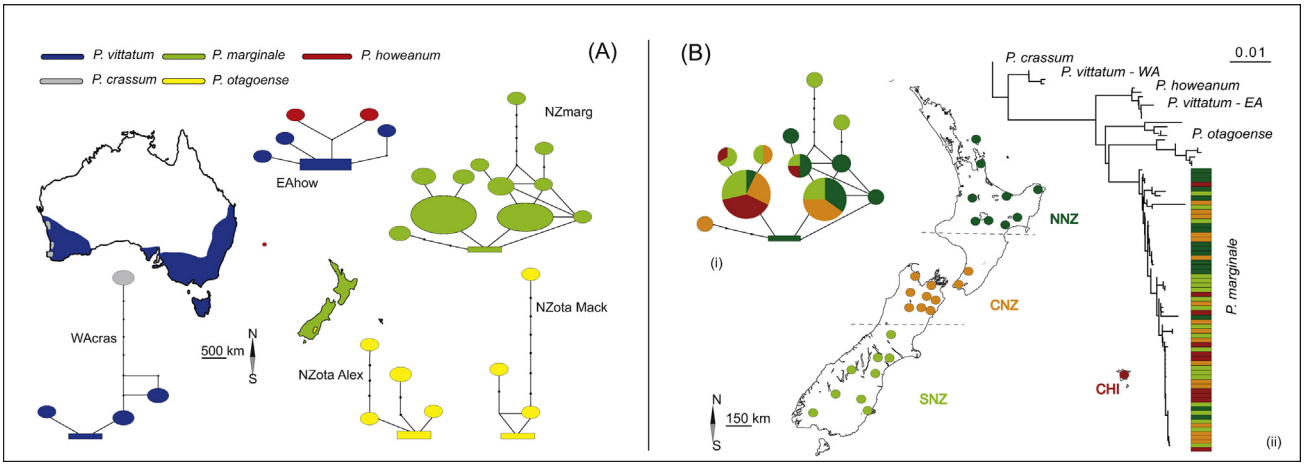

Fig. 2. Distribution of mtDNA COI diversity among *Phaulacridium* grasshoppers in Australia and New Zealand with haplotype networks for each taxon. (A) Australian and New Zealand region with distribution of *Phaulacridium* taxa, with matching colored circles in corresponding haplotype networks (NZ*marg* = New Zealand *P. marginale*, NZ*ota* Alex = New Zealand *P. otagoense* from Alexandra area, NZ*ota* Mack = New Zealand *P. otagoense* from Mackenzie area, EA*how* = *P. vittatum* eastern Australia and *P. howeanum*, WA*cras* = *P. vittatum* western Australia and *P. crassum*). (B) Map of New Zealand showing the four main sampling areas (three in mainland New Zealand, the fourth being the Chatham Islands) of *P. marginale* with sampling locations (colored circles); inset (i) shows haplotype network for *P. marginale*, with colors corresponding to sampling areas and depicting the frequency of the different areas within one haplotype; inset (ii) shows a neighbour-joining tree representing all sampled taxa, with colored labels of *P. marginale* representing haplotypes according to sampling areas and colored circles in Fig. 2B(i).

density of macropterous individuals (M. Lusk pers. comm.). In Australia, *P. vittatum* is the most widespread species and often becomes abundant enough to be considered a significant pest on pasture land (Australian Department of Agriculture and Food, http://agspsrv34.agric.wa.gov.au/ento/pestweb/Query1_1.idc?ID=307365355), whilst *P. crassum* has a very narrow range (Fig. 2A). On Lord Howe Island, approximately 575 km east of Australia in the Tasman Sea the endemic species *P. howeanum* is restricted to arid rock outcrops (Key, 1992). In New Zealand, *P. marginale* is today widespread in open grasslands on the three main islands and the smaller islands in the north. It can also be found on the Chatham Islands, approximately 850 km east of New Zealand in the Pacific Ocean. In contrast, *P. otagoense* is confined to semi-arid parts of central Otago and central Canterbury in South Island, New Zealand (Fig. 2A). Both of the New Zealand species occupy areas that now include exotic herbs and grasses.

New Zealand *Phaulacridium* are thought to have been derived from Australian lineages. Bigelow (1967) suggested that *P. marginale* either arrived in New Zealand during the last 10,000 years (i.e., after the LGM), or that it arrived earlier and persisted through the Pleistocene in northern refugia. Westerman and Ritchie (1984) favored the latter hypothesis. They also proposed evolution of their newly described species, *P. otagoense*, in situ during the Pleistocene. They noted as an alternative, that morphological evolution of *P. otagoense* might have been unusually rapid and extremely recent (~200 ybp) in response to human modification of habitat (Westerman and Ritchie, 1984), but conceded this to be unlikely given the extent of morphological and molecular change indicated by their allozyme data. Key (1992) agreed that *P. otagoense* was probably derived from *P. marginale* during the Pleistocene, and proposed that, of the two, *P. marginale* was morphologically more similar to *P. vittatum*. Westerman and Ritchie (1984) cited comparatively warm Pliocene climate as providing suitable conditions for colonization of New Zealand during that time. Lowland grassland/scrub was rare in Holocene New Zealand but the semi-arid environment occupied today by *P. otagoense* in central South Island was likely to have been more extensive during Pleistocene 'glacial' phases (Fig. 1). These areas were not glaciated but associated cooler, drier climate is thought to have resulted in expansion of scrub/grassland (McGlone et al., 2010). Thus, *P. marginale* is hypothesised to have evolved from an Australian lineage, and *P. otagoense* to have subsequently evolved from the *P. marginale* lineage.

Based on four genes (two mitochondrial and two nuclear) we examined the genetic structure of the *trans*-Tasman grasshopper genus *Phaulacridium* to asses if its genetic diversity correlates with its current ranges and to infer phylogeographic patterns in Australasia in relation to changing environments.

2. Material and methods

2.1. Sampling and DNA extraction

Phaulacridium grasshoppers (*P. vittatum*, *P. marginale* and *P. otagoense*) were collected by hand from lowland grasslands and stored in 95% Ethanol. DNA from fresh ethanol material was extracted from muscle tissue of one hind femur using a salting-out method (Sunnucks and Hales, 1996). Specimens from Western Australia (*P. crassum*) and from Lord Howe Island (*P. howeanum*) were previously pinned and dried; here an entire hind leg was used for DNA extraction using CTAB and phenol/chloroform extractions (Trewick, 2008). Species were identified by morphological character differences, e.g., size, sculpturing of pronotum and position of tegmina (in Australian species) following Bigelow (1967), Westerman and Ritchie (1984) and Key (1992).

2.2. Polymerase chain reaction and sequencing

Molecular analyses used primers that target the mitochondrial DNA gene cytochrome oxidase I (COI). For a subset of samples from all species cytochrome oxidase II (COII) and nuclear ITS and 18S genes were also amplified. In total 95 individuals, and two representative outgroup taxa (Supplementary Table 1) were employed. Additional to 72 available sequences of COI on GenBank (JN409741–JN409816; Goldberg & Trewick, 2011) we sequenced 21 individuals for COI using primers C1-J2195 and L2-N-3014 (Simon et al., 1994). The total sampling comprised 65 specimens of *P. marginale* from New Zealand (including Chatham Islands), 11 *P. otagoense* from southern New Zealand, 15 *P. vittatum* from western

Table 1
DNA variation and haplotype diversity within and between regional samples of *P. marginale* in the New Zealand region and the two populations of *P. otagoense*, with the sample size for each region (n), number of observed haplotypes (N_{haps}), number of unique haplotypes ($N_{unihaps}$) per population, average number of nucleotide differences (k), nucleotide diversity ($\pi \times 10^{-3}$), number of polymorphic sites (S), Tajima's D ($^{*}P < 0.05$) and haplotype diversity (h). Region abbreviations correspond to abbreviations in Supplementary Table 1 and Fig. 2B (NNZ = Northern North Island, CNZ = Central Mainland New Zealand, SNZ = Southern South Island, CHI = Chatham Islands; NZ*ota* Alex = *P. otagoense* population in Alexandra, Otago, NZ*ota* Mack = *P. otagoense* population in Mackenzie, Canterbury).

Area	n	N_{haps}	$N_{unihaps}$	k	π	S	Tajima's D	h
NNZ	16	7	4	0.358	0.81	2	−0.3817	0.342
CNZ	17	4	1	0.353	0.80	3	−1.706	0.118
SNZ	22	7	2	0.273	0.62	3	−2.140*	0.177
CHI	10	3	0	0	0	0	−1.401	0
Total population	65	12	7	0.274	0.62	7	−2.051*	0.180
CHI/NNZ				0.225	0.45			
CHI/CNZ				0.296	0.65			
CHI/SNZ				0.438	0.82			
NNZ/CNZ				0.360	0.80			
CNZ/SNZ				0.308	0.70			
NNZ/SNZ				0.360	0.75			
NZ*ota* Alex	7	5	3	2.095	3.50	6	−0.863	0.905
NZ*ota* Mack	4	4	4	4.667	7.79	9	−0.491	0.833
Total NZ*ota*	11	9	7	9.800	16.36	23	0.680	0.945

and eastern Australia, two *P. crassum* from western Australia, two *P. howeanum* from Lord Howe Island and the outgroup (*Minyacris*) from Australia. Twelve of these grasshoppers were sequenced for a 760 bp fragment of COII using primers TL2-J-3037 and C2-N-3661 (Simon et al., 1994), and four taxa were sequenced for a 730 bp fragment of ITS using primers ITS4 and ITS5 and a 1095 bp fragment of 18S using primers 18S-S22 and 18S-A1984. PCR reactions were performed in 10 μl volumes. The amplified products were checked on 1% agarose gels and purified using high pure purification columns (Roche Applied Science, Mannheim, Germany) or SAP/EXO1 digest (USB Corporation) following the manufacturer's instructions. Purified PCR products were sequenced using standard protocols for the ABI Prism BigDye Terminator Ready Reaction Kit (Applied Biosystems, Mulgrave, Australia) and run on an ABI Prism 377 automated sequencer (Applied Biosystems). Sequence identity was confirmed by comparison with published data, checked for nucleotide ambiguities in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI, www.genecodes.com) and aligned by eye using Se–Al v2.0a11 (Rambaut, 1996). Sequences newly generated for this study have been deposited at GenBank (accession numbers: KP784376–KP784415).

2.3. Population and phylogenetic analyses

The program TCS 1.21 (Clement et al., 2000) was run with a 95% connection limit to construct parsimony haplotype networks of *Phaulacridium* COI sequences to assess the geographic structuring of the genus. Furthermore *P. marginale* haplotype frequencies were calculated for sampled regions in New Zealand. Using a prediction of spatial partitioning, mainland New Zealand was divided into three sampled zones of similar area, using latitudinal breaks that transected the islands east to west (Fig. 2B); northern North Island (NNZ), central New Zealand (CNZ), southern South Island (SNZ). An additional zone was designated for the Chatham Islands (CHI). These were used to test for differences in genetic composition spanning New Zealand. Under panmixis it is expected that no significant partitioning of genetic diversity would exist (thus $\_\Phi_{ST} = 0$), however, any reduction in gene flow is expected to result in genetic diversity (haplotype variation and frequency) being partitioned in space ($\Phi_{ST} > 0$).

DnaSP v5.0 (Rozas et al., 2003) was used to calculate haplotype diversity (h), nucleotide diversity (π, Nei, 1987) and the average number of nucleotide differences (k) within *P. marginale* populations and the two distinct populations of *P. otagoense*. Additionally, Tajima's D statistic (Tajima, 1989) was calculated for the different regions as it provides a useful indicator for neutral markers, such as synonymous changes within mitochondrial DNA, of population range expansion and exchange (Ray et al., 2003; Wegmann et al., 2006). Mismatch distributions for the mainland New Zealand species *P. marginale* and *P. otagoense* were calculated and evidence of isolation-by-distance was sought using a Mantel test of the correlation of pairwise geographic distances and pairwise $\_\Phi_{ST}$ for mtDNA with 1000 permutations for mainland *P. marginale* population samples using IBDWS v.2 (Jensen et al., 2005).

Distance estimation and phylogenetic analyses were performed using PAUP* 4.0b10 (Swofford, 1998). We conducted neighbor-joining (NJ) and maximum likelihood (ML) analyses, as implemented in PAUP* with the entire COI dataset and with a subset of 31 samples, respectively. We used 1000 bootstrap replicates to test the tree topology under ML and NJ criteria.

We employed Mr. Bayes 3.1.2 (Ronquist and Huelsenbeck, 2003) to examine tree topology with a COI–COII dataset under a six parameter model similar to that selected for ML analysis by Modeltest 3.7 (Posada and Crandall, 1998). We used the GTR model with gamma-distributed rate variation across sites and a proportion of invariable sites. The same model was applied to the two partitions (COI and COII) with rates and nucleotide frequencies for each gene unlinked. We used four independent MCMC runs for ten million generations with a burn in of 25%. Resulting posterior probabilities on the nodes were recorded.

Data for two nuclear genes (ITS and 18S) were examined to help resolve the phylogenetic relationships within this genus. Analyses were run in Paup* with a subset of four species from New Zealand and Australia.

3. Results

3.1. Population analyses

Statistical parsimony networks were generated in TCS containing COI sequences of all *Phaulacridium* species (Fig. 2A). In a sample of 65 specimens of *P. marginale* from mainland New Zealand we found 12 different COI haplotypes (Fig. 2A and B inset (i), Supplementary Table 1) with uncorrected genetic distances up to 0.018 (1.8%). Two of these haplotypes were observed at high frequency ($n = 20$ (31%) and 28 (43%) (Supplementary Table 1)), and both were distributed throughout New Zealand (Fig. 2B, inset (i), Supplementary Table 1), one including samples from the Chatham Islands. Additionally, unique haplotypes were encountered in all regional samples except the Chatham Islands (Fig. 2B, Table 1). Strikingly, in contrast to the situation in *P. marginale*, diversity in the more range restricted *P. otagoense* splits into two separate haplotype

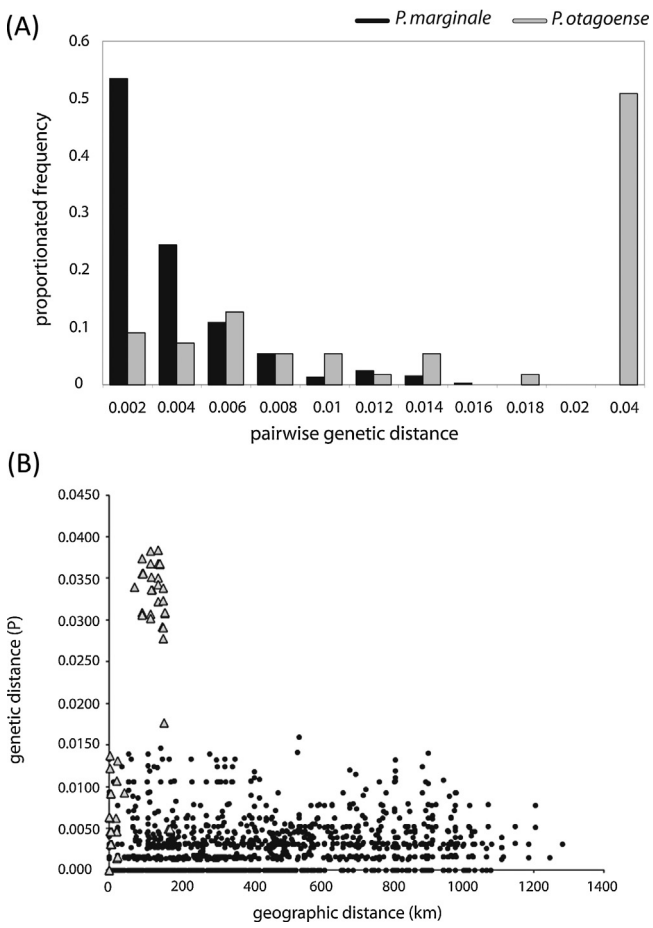

Fig. 3. Mismatch distributions calculated for *P. marginale* (black) and *P. otagoense* (grey) from COI mtDNA illustrating the differences in genetic divergence within the two species. (A) Frequency distribution plot based on proportionated frequencies and pairwise genetic distances highlighting the difference in frequency distribution in the two species, with *P. marginale* mostly exhibiting low genetic distances (up to 1.5%) and *P. otagoense* showing the highest frequency at approximately 4% pairwise genetic distance. (B) Distribution plot based on genetic and geographic distances of individuals of the two species, depicting the high genetic divergence coupled with a narrow geographic range in *P. otagoense* (grey triangles) and the opposite – low genetic distance within a wider geographic distribution – in *P. marginale* (black dots).

networks, each representing grasshoppers from one of the two small areas in southern New Zealand where this species is found (Fig. 2A). This species displays higher genetic diversity than the widespread *P. marginale* with nine haplotypes found in a sample of 11 grasshoppers (Fig. 2A, Table 1). Of these nine haplotypes seven were unique within the respective population (Fig. 2B inset (i), Supplementary Table 1, Table 1). The calculated uncorrected pairwise genetic distances within this species reached 0.015 (1.5%) in the Alexandra population (NZ*ota*Alex) and 0.018 (1.8%) in the more northern Mackenzie population (NZ*ota*Mack) (Fig. 2A). The maximal genetic distance between any two *P. otagoense* haplotypes was 0.037 (3.7%) across a spatial range of about 180 km. This is similar to the maximum genetic distance between *P. otagoense* and *P. marginale* (0.035/3.5%). Mismatch distributions calculated for *P. marginale* and *P. otagoense* highlights the difference in genetic divergence within these two species (Fig. 3A and B).

Populations of *P. marginale* displayed very low levels of DNA nucleotide diversity (π) within mainland New Zealand and the Chatham Islands (Table 1). The sequences of the northern area had the highest level of haplotype and nucleotide diversity. These results of mtDNA differentiation refute the prediction of *P. marginale* partitioning into northern, southern and Chatham Island populations, but rather show panmixis in New Zealand. In the current data set no isolation by distance is apparent. Tajima *D* tests (Tajima, 1989) were only significant for the SNZ dataset and the total population (Table 1). Negative Tajima's *D* statistics for the different populations indicate an excess of sites with low frequency polymorphisms, i.e., the population has yet to reach equilibrium (Tajima, 1989). The two *P. otagoense* population samples on the other hand show higher estimates of π than *P. marginale* populations in their small modern range (Table 1), implying that this species had a larger population size and range until relatively recently.

The Australian species *P. vittatum* cannot be regarded as monophyletic. The western population of *P. vittatum* is sister to the western parapatric species *P. crassum* (WA*cras*), while the eastern *P. vittatum* is sister to *P. howeanum* on Lord Howe Island (EA*how*; Fig. 2A). Genetic distances within the Australian haplotype networks reached a maximum of 0.013 (1.3%) and 0.006 (0.6%), respectively. In comparison, the western Australian *Phaulacridium* show genetic distances of up to 0.044 (4.4%) to eastern Australian samples, and only 0.05 (5%) to *P. marginale* and 0.055 (5.5%) to *P. otagoense* from New Zealand.

3.2. Phylogenetic analyses

Phylogenetic analysis illustrates the closer relationship of the western Australian *P. vittatum* specimens to individuals of *P. crassum* compared to eastern populations of the same species, and the closer affinity of the *P. howeanum* to the eastern Australian samples of *P. vittatum* (Fig. 2B inset (ii)). Bootstrap support (1000 replicates) was not high for all internal nodes, a common problem encountered

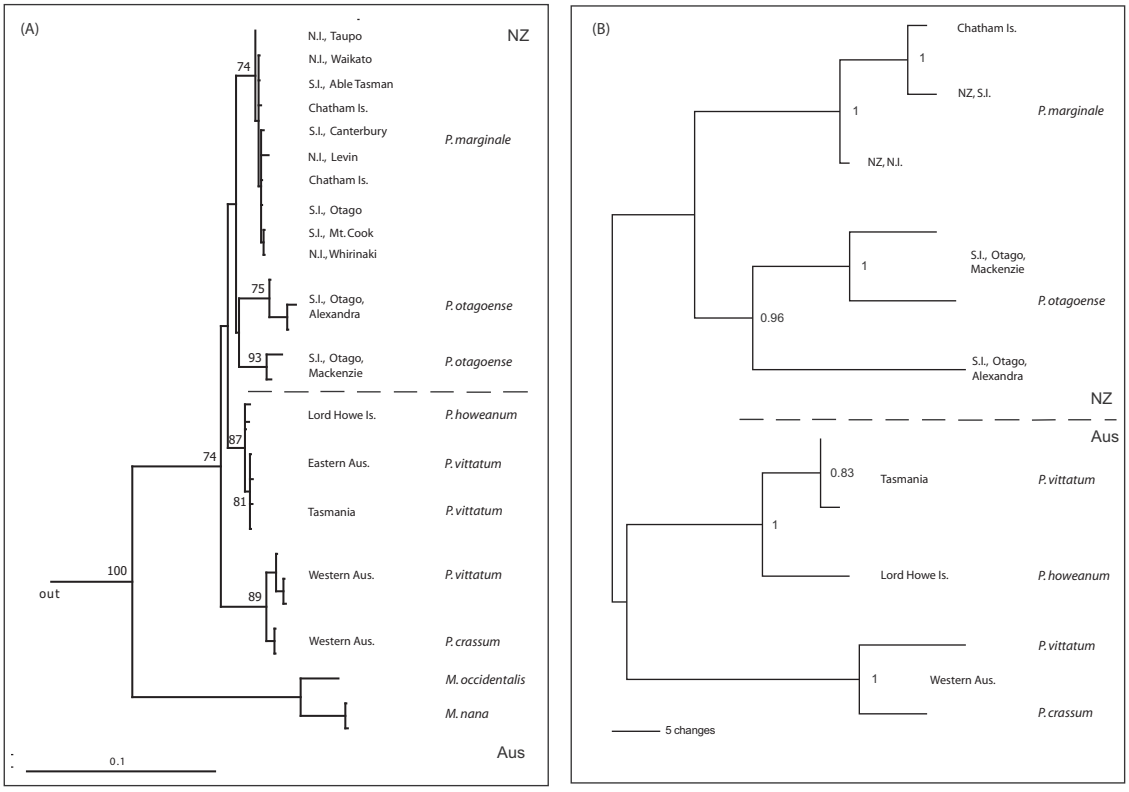

Fig. 4. (A) COI ML tree of 31 individuals representing all species of *Phaulacridium* used in this study. Numbers on branches show bootstrap support for the nodes (1000 replicates); the tree was rooted with outgroup taxa *Minyacris nana* and *Minyacris occidentalis* from Australia; (B) Midpoint rooted phylogeny of 11 individuals representing *Phaulacridium* species used in this study for COI–COII generated in Mr. Bayes with values for Bayesian posterior probabilities mapped to the branches.

when lineages are closely related (ML tree; Fig. 4A). However, *P. crassum* was resolved with good support as sister to western Australian *P. vittatum* and *P. howeanum* as sister to eastern Australian *P. vittatum*.

Bayesian analysis with longer DNA sequences (COI and COII) for a set of 11 specimens representing the taxonomic and geographic range of the genus returned a tree with strong support for the internal nodes, but little support for separation of New Zealand and Australian clades (Fig. 4B).

The two nuclear genes (ITS and 18S) provided little phylogenetic signal as the sequences were identical or differed by at most three substitutions (ITS had 3 variable sites; 18S sequences were identical). The ITS variation was consistent with the inferences of shallow evolutionary history of the genus indicated by mitochondrial DNA data (results not shown).

4. Discussion

Phaulacridium is an interesting example of a trans-Tasman distribution in animals as there are few instances where species of the same genus naturally occur in Australia and New Zealand. Although these grasshoppers are only rarely fully winged this does not appear to have prevented their dispersal across wide expanses of land and ocean. The geographic range of *Phaulacridium* is large by southern hemisphere standards as it extends approximately 4000 km from west to east Australia, a further 1500 km across the Tasman Sea to New Zealand and 850 km to the Chatham Islands. Within this range, five species are recognised, but the scale of the species' ranges differs enormously. Three species have small geographic ranges, *P. crassum* near the coast in Western Australia, *P. howeanum* on a rock outcrop on Lord Howe Island (Tasman Sea) and *P. otagoense* in semi-arid land in central South Island, New Zealand (Fig. 2A). The other two species are more widespread but comprise three disjunct geographic populations, *P. vittatum* west and east of the Nullarbor Plain in southern Australia, and *P. marginale* in New Zealand. Eastern *P. vitattum* also extend to Tasmania (Fig. 2A), and *P. marginale* to the Chatham Islands (Fig. 2A and B). These species distributions include two cases of parapatry (*P. crassum* surrounded by *P. vittatum* in Western Australia and *P. otagoense* by *P. marginale* in southern New Zealand). Apparent reproductive isolation and ecological specialisation is consistent with them having speciated in response to local microenvironments (Westerman and Ritchie, 1984).

Phylogeographic data add to this picture, revealing that in Australia neighboring populations are more closely related to one another than geographically more distant populations, even to the extent that western *P. vittatum* are sister to parapatric *P. crassum*, and eastern *P. vittatum* are sister to *P. howeanum*. Consequently *P. vittatum* mtDNA is paraphyletic with respect to these two species. However, the situation in New Zealand is rather different, as the two local endemics are, by comparison, more deeply diverged from one another. There is higher genetic diversity in the locally restricted *P. otagoense*, compared to the widespread *P. marginale*, and support for monophyly of the New Zealand taxa is rather weak. Thus the mitochondrial data do not support the hypothesis that *P. otagoense* is derived from *P. marginale* (Key, 1992; Westerman and Ritchie, 1984) as it only shows that both genetic lineages of *P. otagoense* are sisters to *P. marginale* and had to shared a common ancestor with *P. marginale* at some stage. While *P. marginale* is widespread today, population genetic analyses suggest this is the result of recent population expansion. Genetic diversity in this species is low, even when New Zealand and Chatham Islands samples are compared; implying *P. marginale* lineages constitute a continuous entity in New Zealand. On the other hand, high genetic diversity in *P. otagoense*, despite a small modern range, suggests that this species had until recently a comparatively large population size and range. Support for this idea comes from the observation that

the habitat/region occupied by *P. otagoense* today probably represents the most extensive natural lowland grass/scrubland available in New Zealand prior to human colonization. Other extensive native grasslands are those that developed above the treeline during the Pleistocene (Heenan and McGlone, 2013), but these are occupied by a separate unrelated group of 'alpine' grasshoppers (Bigelow, 1967; Trewick, 2008; Trewick and Morris, 2008). This pattern emphasizes the need to consider past climatic and geological events when interpreting extant patterns of diversity (Graham et al., 2006).

Vegetation patterns inferred for the Pleistocene LGM in New Zealand indicate that during glacials, cooling and drying in the east led to the development of extensive grass- and shrublands (McGlone et al., 1993, 2010). An increase in grassland habitat in concert with a westerly airflow (Sanmartin et al., 2007) may have improved the chances of long distance dispersal and establishment in New Zealand by *Phaulacridium*. The near complete coverage of non-alpine New Zealand by forest during interglacials would, conversely, have severely limited habitat availability for *Phaulacridium*. Consequently, it is likely that the range of *P. marginale* was most limited during interglacial rather than glacial episodes (in contrast to at least one alpine orthopteran; Trewick et al., 2000). Suitable natural habitat was probably limited to grassland patches associated with disturbed environments around rivers and frost flats. Even by 1840 when Polynesia and European colonizers were well established, it is estimated that lowland swards covered no more than 2% of mainland New Zealand (Mark and McLennan, 2004). The pattern of the widespread New Zealand species *P. marginale* does not reveal specific locations of refugia, as has been observed in northern hemisphere Pleistocene phylogeography, but does exhibit the typical pattern of recent range expansion, with low genetic diversity. The fact that, until the arrival of humans in New Zealand (~1000 years ago), the only "substantial area below tree line without complete forest cover was central Otago" (McGlone et al., 1993) suggests that this semi-arid (e.g., Walker et al., 1995) or rain-shadow grassland region (McGlone, 2001) may have been the refuge for *P. otagoense* during interglacials, as now. Distance from oceans and presence of mountain ranges, means that grassland habitat could have existed here since formation of the Southern Alps in the Pliocene (i.e., before Pleistocene global climate cooling). Forest clearance by humans in New Zealand resulted in the expansion of native and subsequently mixed exotic grassland habitat was available for *P. marginale* to expand its range. During the 1800s grassland expanded in mainland New Zealand (Mark and McLennan, 2004), and the process continues today (Scott, 1979).

The two distinct populations of *P. vittatum* in eastern and western Australia are today separated by the Nullarbor Plain, a large semi-arid area that stretches approximately 1200 km from Western Australia to Southern Australia (Key, 1992). Many other grasshoppers occupy this region including plague forming species of *Austroicetes*. Mitochondrial lineages of *P. vittattum* are distinctive to west and east Australia, lack monophyly and are likely to have been separated at least since the LGM as vast areas of southern and Western Australia, including the area that is now the Nullarbor, supported arid desert habitat (Hope et al., 2004; Byrne et al., 2008). We find two morphologically distinct species (*P. howeanum* and *P. crassum*) differ from the respective *P. vittatum* populations by small mitochondrial DNA distances, so they are within the respective mitochondrial clades of *P. vittatum* regional populations.

The New Zealand lineages might be sister to the eastern Australian lineages as suggested by NJ and ML analyses (Fig. 2B (ii); Fig. 4A and B), which indicates a general west to east pattern of range extension for the genus. Although, the number and direction of *trans*-Tasman dispersal events cannot be confirmed with the current data, the presence of the closest relative to *Phaulacridium* and a diverse non-alpine grasshopper fauna in Australia support expansion from Australia to New Zealand. Additionally, shallow mitochondrial DNA genetic distance between New Zealand and Chatham Island populations and lack of unique Chatham haplotypes suggest that these islands have been recently colonized by numerous *P. marginale* individuals migrating from mainland New Zealand.

The population composition of the New Zealand species is contrary to their current range sizes, with two lineages of *P. otagoense* apparently occupying distinct areas and retaining high genetic diversity ($\pi = 16.36$) in a relatively small space, compared to a very widespread lineage (*P. marginale*) with relatively low genetic diversity ($\pi = 0.62$) (Figs. 2 and 3; Table 1). The genetic diversity within the two *P. otagoense* populations is more or less as high as the diversity in the *P. marginale* population (Table 1). In fact the genetic diversity is so high and the phylogenetic signal deeply structured that the possibility arises that the two *P. otagoense* lineages from Alexandra and Mackenzie are actually two distinct biological entities. Other examples of paired species endemics in this area include chafer beetles (Emerson and Wallis, 1995) and *Sigaus* grasshoppers (Trewick, 2008).

Phaulacridium exemplifies the manner in which phylogeography can be driven by multiple factors on different time scales. The most important factors in geologically recent times appear to have been the uplift of the Southern Alps in the Pliocene (5 Ma) creating new alpine habitat and rain-shadow habitat, and Pleistocene (2.4 Ma) climate cooling events restricting available habitat in New Zealand. Both factors likely favored species that are more adapted to dry and cold environment. In Australia, Pleistocene climate cycling had similar effects on the biota but on a larger geographic scale, with locally distributed high haplotype diversity within populations in many taxa (Byrne, 2008), resulting in divergent lineages. This structure is evident in the present sampling of Australian *Phaulacridium* with deeply divergent lineages within populations.

When compared to northern hemisphere grasshoppers, there are stark contrasts at the population and species level depending on habitat zones occupied. In Europe low genetic diversity in the lowland *Chorthippus* meadow grasshopper (Lunt et al., 1998) indicates widespread extirpation in the LGM, followed by extensive range expansion. Diversity within *Phaulacridium* is more like that of northern lowland species, but intriguingly this pattern of range expansion and speciation has operated across a region with habitat patches widely separated by desert and ocean.

Acknowledgements

We are indebted to many people who provided specimens for this study and helped with collection of specimens (in alphabetical order): Nathan Curtis (Lincoln University, Canterbury, NZ), Rowan Emberson (Lincoln University, Canterbury, NZ), Rebecca Harris (University of Tasmania, Aus), Stewart Learmouth (Department of Agriculture, WA, Aus), Simon Morris, Jacquie Recsei (ANIC, Canberra, Aus), Phil Sirvid (Te Papa, Wellington, NZ), Mike Westerman (La Trobe University, Aus) and Frank Wieland (Pfalzmuseum, Bad Dürkheim, GER).

This work was supported by the Marsden Fund of the Royal Society of New Zealand (03-GNS-302).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcz.2015.02.005.

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