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Identity of an endangered grasshopper (Acrididae: Brachaspis): Taxonomy, molecules and conservation

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Abstract

Brachaspis robustus is an endangered grasshopper endemic to South Island, New Zealand. It is both rare and localised; occupying low altitude floodplain terraces and braided riverbeds of the Mackenzie Basin. This is in stark contrast to the two other species in this genus (*B. nivalis* and *B. collinus*) which occupy montane habitats. Mitochondrial and nuclear sequence data were employed to explore genetic diversity and phylogenetic relationships of populations of *Brachaspis* with a view to establishing the status of *B. robustus*. Molecular evidence indicates that *Brachaspis* probably radiated during the Pliocene and that divisions within the genus relate more to spatial distribution developed during the Pleistocene than to ecology. The mitochondrial (Cytochrome oxidase I) and nuclear (ITS) sequence data indicate that *Brachaspis nivalis* is divided into northern and southern populations. The northern clade is further subdivided geographically. The southern clade comprises alpine populations of *B. nivalis* and includes the lowland *B. robustus*. Additionally, it is observed that some morphological features previously thought to be specific to *B. robustus* also occur in members of the southern *B. nivalis* clade. It is suggested that the taxon *B. robustus* should include all of the southern *Brachaspis* populations. But it is argued that the absence of genetic evidence distinguishing the endangered population does not preclude it from conservation effort. A combination of morphological and habitat peculiarities indicate that the survival of *B. robustus* (*sensu lato*) is important to the maintenance of diversity.

Introduction

Molecular techniques have provided markers that have proven valuable in identification and confirmation of cryptic taxa (e.g. tuatara – Daugherty et al. 1990; turtles – Bowen et al. 1991; peripatus – Trewick 1998). Conversely the same methods have also cast doubt on the identity of taxonomically recognised species (e.g. pocket goffers – Laerm et al. 1982; seaside sparrows – Avise and Nelson 1989; beetles – Emerson and Wallis 1994). Although evolutionary geneticists are well placed to undertake systematic revision, taxonomy has so far been considered outside their field of expertise. However, aspects of evolutionary study including conservation genetics emphasise weaknesses of the Linnean classification in light of phylogenetic evidence. The typological approach has in many cases proven to be an ineffective basis for the identification of diversity, and conservation (Avise 1989). For example, the dusky seaside sparrow (*Ammodramus maritimus*) was the subject of (unsuccessful) conservation effort on the basis of minor plumage differences that endowed it with taxonomic distinction (Avise 1989, 1994). However, subsequent genetic analysis fortunately revealed that the now extinct dusky seaside sparrow was essentially identical to other populations in the region irrespective of colour variation (Avise and Nelson 1989).

The subspecies concept favours analyses that use geographic and genetic variation (Wilson and Brown 1953). Challenges to the concept were followed by the proposal of the evolutionarily significant unit (ESU) as a conservable, definable entity within a species (Ryder 1986). One of the advantages of the ESU approach

is that emphasis is placed upon genetic and thus evolutionary distinctiveness rather than nomenclature (Moritz 1994). In addition to having a broad role in the study of evolution, molecular evidence in combination with geographical data has therefore assisted in the recognition of cryptic diversity and targeting of conservation effort. ESUs and management units (MUs) provide the opportunity for a relatively empirical approach to defining diversity (Moritz 1994). However, these units are not a universal panacea and there are situations where they are not applicable, for example the polar bear (*Ursus maritimus*) which whilst a good species by other standards (habitat, morphology, behaviour) does not satisfy the ESU requirements of mtDNA reciprocal monophyly with respect to the brown bear (Talbot and Shields 1996; Paetkau 1999).

Debate continues about the definition and applicability of such units (see for instance Molecular Ecology 12, Supp. 1 1999), but most attention has been focused upon vertebrate taxa and in particular large, charismatic species (e.g. Coelacanth, Komodo dragon – King and Burke 1999). It could be argued that for such taxa their phylogenetic distinctiveness allows for treatment as special-cases irrespective of whether they satisfy ESU and MU criteria (as with the Polar bear). But many difficulties exist in the diagnosis of conservation units among speciose groups (e.g. among insects) that have less distinctive relationships (Vogler and DeSalle 1994).

The acridid grasshopper genus *Brachaspis* is one of four endemic to New Zealand, that consist of species that are largely restricted to subalpine or alpine habitats of South Island. *Brachaspis* species are relatively large (female body length ~30 mm), rugose and flightless (as are all endemic genera). Three species of *Brachaspis* were recognised by Bigelow (1967). Two, *B. nivalis* (Hutton) and *B. collinus* (Hutton) are common in rocky montane habitats in the northern two-thirds of South Island. The third species, the robust grasshopper *B. robustus*, was described on the basis of just three museum specimens (Bigelow 1967), and has not subsequently been reappraised. These specimens differed from others of the genus by virtue of their relatively broad pronota, short hind femura, extremely short tegmina and it is thought that they had been collected at low altitude, although their provenance is unclear. Intensive field searches in the last two decades recorded low numbers of *B. robustus* in floodplain terrace and braided river bed habitats in an area of central South Island known as the Mackenzie Basin (Figure 1) (Davis 1986; Maloney 1993; White 1994; Fraser 1999). The low frequency, apparent morphological distinctiveness and unusual habitat of *B. robustus* led to it being listed as a threatened species of the highest conservation priority by the New Zealand Department of Conservation (Tisdall 1994). No other New Zealand grasshoppers and relatively few other insects are afforded this status. Since observations began the range of *B. robustus* appears to have decreased, probably as the result of the combined effects of predators (native and introduced), vegetation changes and hydroelectric development (White 1994). Although intensive field observation has been undertaken the avoidance of handling individual grasshoppers means there are very few empirical data on the morphology of living *B. robustus*.

This paper describes discoveries about the nature of *B. robustus* that arose from a wider study of New Zealand grasshopper phylogeography. Because of the extreme rarity of the robust grasshopper this species was originally exempted from study. However, phylogenetic analysis of mitochondrial DNA sequence data revealed three well-supported clades within a sample that consisted of just the two common morphospecies (*B. nivalis* and *B. collinus*). This prompted efforts to include *B. robustus* in order to determine its status and/or characterise a further undescribed taxon. The present analysis includes alpine *Brachaspis* populations not documented by Bigelow (1967) that encircle the low altitude range of *B. robustus*, and raises interesting questions about perceptions of habitat specificity and morphological distinctiveness, species definition and identification of units of conservation.

Methods

Collecting

Specimens of *Brachaspis collinus* and *B. nivalis* were collected by hand from rock and scree habitats above 1500m asl on mountain ranges in South Island, New Zealand (Figure 1). *B. collinus* with pale-yellow longitudinal stripes were easily distinguished from *B. nivalis* which do not have them, but some *B. collinus* also lacked stripes (Green 1967). At Mt Lyford all *B. collinus* were the unstriped, slate-grey form and these were sympatric with *B. nivalis*, which look very similar. Here, identification was reliant upon the shape of the female subgenital plate and male epiproct following Bigelow's (1967) descrip-

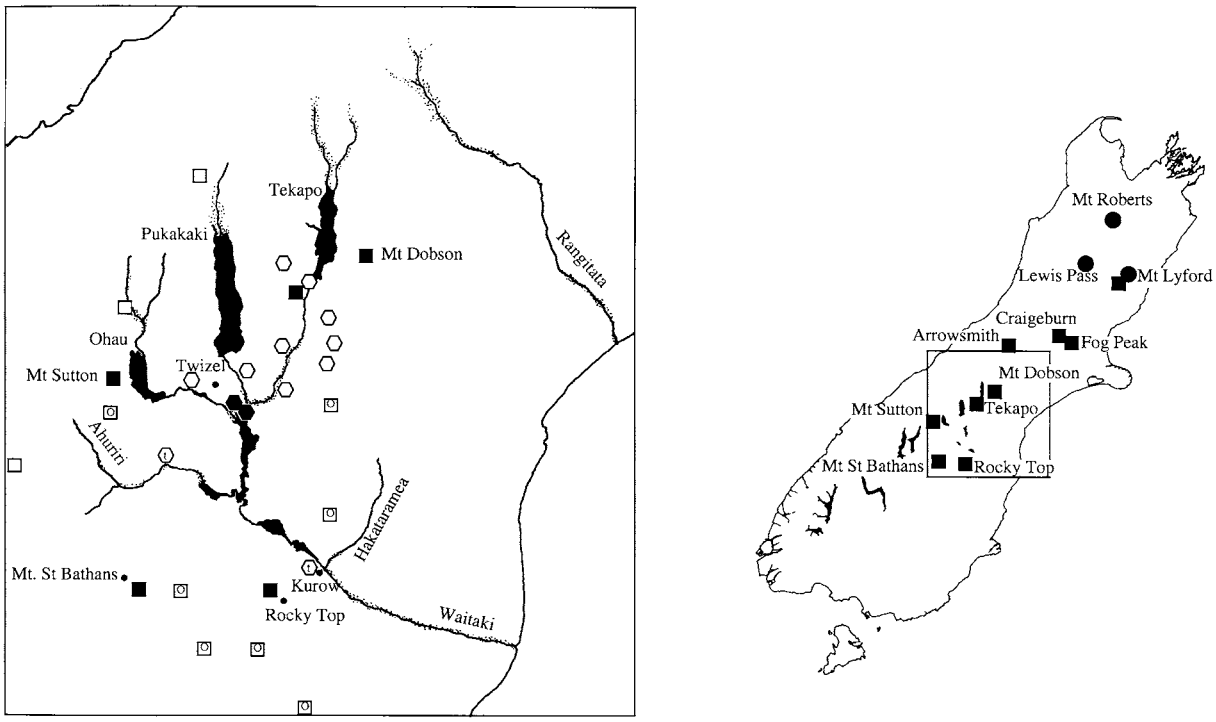

Figure 1. Distribution of sites sampled for *Brachaspis* grasshoppers in South Island, New Zealand. ■ *B. nivalis*, ● *collinus*. The enlarged inset map indicates recorded localities of *B. nivalis* and *B. robustus* in the Mackenzie Basin and the surrounding mountains. *B. robsustus*: ⬢ sampled this study, ⬡ recent localities (White 1994), ⓣ type localities (Bigelow 1967). *B. nivalis*: ■ sampled this study, □ known localities (Bigelow 1967), 🄾 known localities (Otago Museum of New Zealand).

tions, although these characters were also found to be subtle and sometimes unreliable for diagnosis as previously noted by Irving (1967). The flash-display of the hind legs was reddish brown in *B. collinus* but scarlet, purple or indigo-black among the *B. nivalis* individuals collected at various sites. Thus, the full range of flash-display colours exhibited by *B. nivalis* includes those (purple or indigo-black) of the endangered grasshopper *B. robustus* that were previously thought to be distinctive (White 1994). Three *Brachaspis* individuals were obtained from the Mackenzie Basin area (the lowland range of *B. robustus*). One of these could not be morphologically distinguished from *B. nivalis* (Figure 1). Department of Conservation (DoC) staff collected two *Brachaspis* specimens in October 1999 and March 2000 from the Tekapo Delta and Ohau River. These were identified as *B. robustus* by the location (Mackenzie Basin) and habitat in which they were found, plus the combination of short hind femur, pronotum wider than long and very short tegmina (relictual wings).

Molecular methods

Following euthanasia with ether, muscle tissue was removed from hind femora and stored at −80 °C or extracted immediately. DNA was extracted using a salting-out method (Sunnucks and Hales 1996). Tissue was macerated and incubated with 5 μL of 10 mg/mL proteinase-K in 600 μL of TNES buffer (20 mm EDTA, 50 mm Tris, 400 mm NaCl, 0.5% SDS) at 50 °C for 1–4 h. 10% 5 m NaCl was added and the extractions shaken vigorously for 20 s followed by spinning at 14,000 rpm for 5 min. The supernatant was removed and precipitated with an equal volume of cold 100% ethanol. DNA was collected by spinning and washed with 70% ethanol, then dried and dissolved in water.

Molecular analysis used DNA sequences obtained using primers that target part of the mitochondrial cytochrome oxidase I gene (COI). These primers are known to be highly conserved and applicable to a wide range of invertebrate taxa (Lunt et al.

1996), and COI data have been successfully utilised in intra- and interspecific studies of many invertebrates including orthopterans (e.g. Szymura et al. 1996; Zhang and Hewitt 1996; Funk et al. 1995; Trewick et al. 2000). Single stranded conformational polymorphism (SSCP) was used to screen for variant haplotypes (Trewick et al. 2000). For this purpose the mitochondrial primers SR-J-14233 (AAG AGC GAC GGG CGA TGT GT) and SR-N-14588 (AAA CTA GGA TTA GAT ACC CTA TTA T) (Simon et al. 1994) were used to amplify a ~380 bp fragment of the 3′ end of the small ribosomal subunit (12S) as it was found that SSCP using COI gave less consistent and legible banding patterns in these grasshoppers. As the mitochondrion is inherited as a single non-recombining unit, signal from these two genes is expected to be complementary, and because COI evolves more rapidly than 12S, diversity of COI is expected to nest within diversity of 12S. PCR (polymerase chain reaction) products were isotopically labeled by incorporation of αdATP^{33}P. 10 μL reactions (200 μM dNTPs, 2.5 mM $mgCl_2$, 0.25 U Qiagen Taq) were treated to 40 cycles of 94 °C for 15 s, 50 °C for 30 s, 72 °C for 90 s with an initial denaturation of 94 °C for 60s. Amplification products were denatured for 5 min at 95 °C in the presence of an equal volume (10 μL) of 95% formamide loading buffer. These were loaded from ice into vertical, non-denaturing polyacrylamide gels consisting of 6% 37.5:1 bis/acrylamide, 5% glycerol and 0.5x TBE. Gels were electrophoresed at 4 °C for 200 W/h at approximately 13 W and then lifted on blotting paper, dried and exposed with Biomax (Kodak) film for 24 to 48 h. Individuals were scored for haplotype by comparison of re-natured singlestrand DNA migration patterns.

A minimum of two representatives of each SSCP haplotype were sequenced for a longer and more variable fragment towards the 3′ end of COI using the primers, C1-J-2195 (TTG ATT TTT TGG TCA TCC AGA AGT) and L2-N-3014 (TCC AAT GCA CTA ATC TGC CA TAT TA) (Simon et al. 1994). In order to see whether similarity of mtDNA in *B. robustus* and neighbouring *B. nivalis* was the result of recent introgression, representative individuals were also sequenced for a (nuclear) internal transcribed spacer (ITS) using primers ITS4 (TCC TCC GCT TAT TGA TAT GC) and ITS5 (GGA AGT AAA AGT CGT AAC AAG G) (White et al. 1990). In both instances PCR reactions were performed in 25 μL volumes using the same parameters as amplifications for SSCP. Products were either gel-purified using Qiaquick spin columns (Qiagen) or cleaned directly using High Pure purification columns (Roche). Cycle sequencing used Bigdye chemistry (Perkin Elmer) following the manufacturer's protocols. Sequences were aligned manually using SeqEd. v1.0.3 (ABI, PE).

Sequences from species representing the other endemic New Zealand grasshopper genera (*Alpinacris*, *Sigaus* and *Paprides*) were applied as outgroups without altering the topology of *Brachaspis* phylogenies obtained. *Paprides nitidus* was chosen as the outgroup for the figures presented as it is sympatric with many *Brachaspis* populations. Distance estimation and phylogenetic analyses were performed using PAUP 4.0 (Swofford 1998). Population genetic analyses were implemented by Arlequin 1.0 (Schneider et al. 1997). All primers used were sourced from the insect primer sets (John Hobbs, UBC).

Results

Distribution

Although some of the sites from which *Brachaspis* specimens were collected for this study are new locations, the majority are within the known range. Most significant in the context of this study is the extension of the range of the genus southward beyond the Waitaki River (Figure 1). The Rocky Top and Mt St Bathans populations were discovered during the present study and examination of collections at the Otago Museum revealed additional locations for putative *Brachaspis nivalis* on other peaks in that area (Figure 1). Some of these localities have been documented in Department of Conservation internal reports (Patrick 1991, 1994) but have not been further investigated.

Morphology

Gross external morphology of the specimens collected revealed that for at least some of the characters considered to be diagnostic for *Brachaspis* species, *B. robustus* and *B. nivalis* are apparently polymorphic. One individual (of three) that had the form typical of *B. nivalis* (hind femura extending to the distal end of abdomen, and tegmina reaching to the 2nd abdominal tergite) was found within the range and habitat of *B. robustus* (Mackenzie basin, near the outflow of

L. Tekapo). Similar individuals have on rare occasions been observed in this area and classified as *B. robustus* (G. White, pers. comm.). This was presumably by virtue of their presence in that location and implies that the species has previously been accepted as polymorphic. Conversely, several individuals observed and collected at Rocky Top in alpine habitat had unusually short tegmina, similar to typical *B. robustus*. The Rocky Top population was also polymorphic for distinct colour-patterns having mottled dark brown and pale slate-grey forms. These extremes and other subtler colour variants were observed separately in other populations of *B. nivalis*. All Mackenzie basin *B. robustus* were slate-grey. The two individuals of *B. robustus* (one male and one female) collected by DoC staff were of the expected phenotype, although no formal description of the male of this species exists. All other *B. nivalis* individuals collected in the course of this study (with the exception of the Rocky Top variants) conformed to the species description in terms of overall shape and colour, but as noted above, some characters expected to differentiate *B. collinus* and *B. nivalis* were not reliable in all cases.

Sequence data

Twenty-one different COI haplotypes (540bp length) were detected using a combination of SSCP and sequencing from 51 *Brachaspis* individuals. These individuals represented three described species collected from 12 locations (sample sizes given in Figure 2). In cases where SSCP indicated more than one individual with the same haplotype, COI sequences from additional individuals confirmed their identity. ITS sequences (440bp length) were obtained from two *B. robustus*, two *B. collinus* and eight *B. nivalis*. Sequence data have been deposited on Genbank (AY 042370–042390).

Genetic diversity and phylogenetics

Maximum pairwise genetic distance calculated using the Kimura 2-parameter model (K2P) from COI sequence haplotypes was 9.0% among all *Brachaspis* (Table 1). Genetic distances within clades (Figure 2) ranged between 0.2 and 4.8% K2P although the maximum distance among haplotypes of the clade that included *B. robustus* was 2.8%. The two grasshoppers of typical *B. robustus* form had the same COI haplotype but differed from the third Mackenzie specimen by 2.5%, from other clade members by 0.7–2.5% (being most similar to Sutton haplotypes), and from other haplotypes by 4.7–8.3%. A heuristic maximum parsimony (MP) search of the COI haplotypes that comprised 69 informative sites yielded a single shortest tree of 131 steps (Figure 2). Eight well-supported branches were revealed from 500 bootstrap replicates utilising the heuristic search option of PAUP 4.0 with 3:1 tv:ti weighting (bootstrap values = 70, Hillis and Bull 1993). Neighbor-Joining analysis produced a tree of similar topology that differed only in the relative placement of haplotypes within one clade (Fog Peak, Arrowsmith, Craigeburn) (Figure 2). The use of alternative New Zealand endemic grasshoppers (i.e. species of *Sigaus* and *Alpinacris*) as outgroups did not alter tree structure. COI haplotypes obtained from *B. nivalis* fell into two principal, well-supported northern and southern clades, one of which included the *B. robustus* haplotype. In the north, Lyford *B. nivalis* comprised 3 haplotypes (n = 4) that formed a well-supported monophyletic clade that grouped with a clade consisting of haplotpes from the three other northern locations (Fog Peak, Craigeburn and Arrowsmith). All of the southern *Brachaspis* (St Bathans, Rocky Top, Dobson, Tekapo, Ohau River, Tekapo Delta and Sutton) formed a will supported clade of closely related haplotypes. Rocky Top *B. nivalis* comprised 5 haplotypes (n = 8) which were paraphyletic with respect to haplotypes from three other southern locations (St Bathans, Dobson and Tekapo). Haplotypes from the four southern alpine locations of *B. nivalis* (St Bathans, Rocky Top, Dobson, Sutton) were paraphyletic with respect to the lowland Mackenzie Basin *Brachaspis* (Tekapo, Tekapo Delta and Ohau River). Rocky Top had the highest nucleotide diversity of the populations surveyed (π = 0.00725). Constraining the MP tree to be consistent with the existing taxonomy (i.e. all *B. nivalis* haplotypes monophyletic with respect to *B. collinus* and *B. robustus*) resulted in a tree 13 steps longer than the unconstrained tree.

Eight distinct unambiguous ITS sequences were obtained from eleven individuals (Table 2). Ambiguous sequences were obtained from two Lyford *B. nivalis*, and this ambiguity apparently resulted from length polymorphism of the alleles typical of *B. nivalis* and *B. collinus* in this area, and indicated that these individuals were hybrids. The majority of variation among ITS sequences from *Brachaspis* resulted from INDELs. *B. collinus* (Roberts) was distinct from all *B. nivalis* and *B. robustus*. ITS sequences from southern *B. nivalis* populations (Rocky Top and Sutton) and

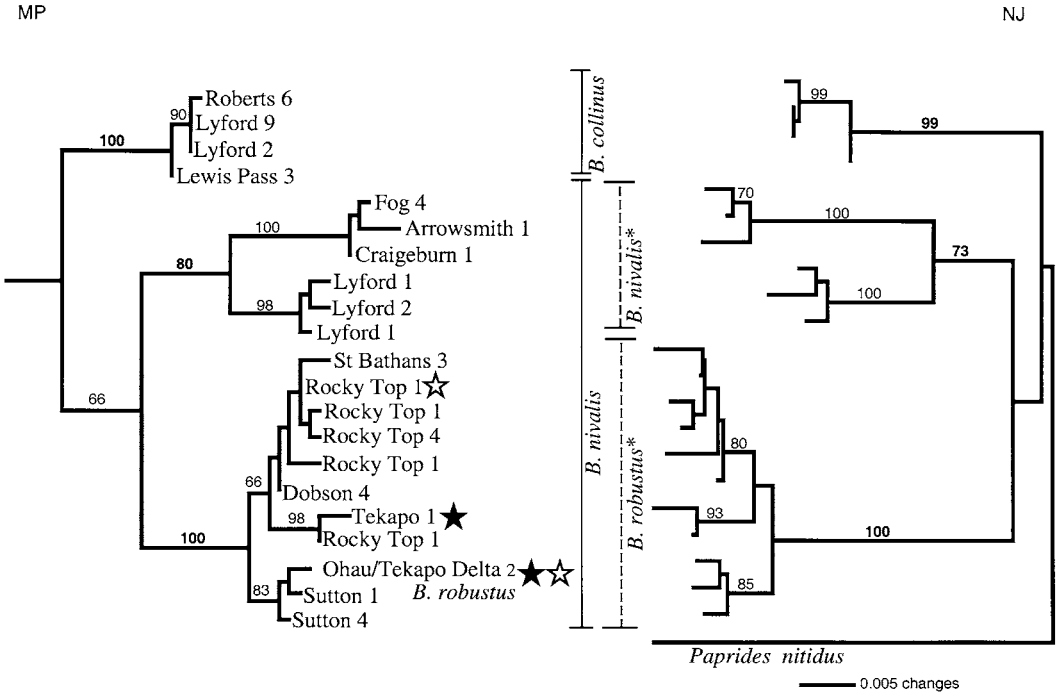

Figure 2. Phylograms resulting from analysis of *Brachaspis* COI sequences using Maximum parsimony and Neighbor-joining of Kimura 2-parameter distances. Numbers on edges indicate level of support from 500 heuristic bootstrap replications using the full heuristic with T3 weighting, and Neighbor-joining options of PAUP 4.0 respectively. Termini are labelled with location names and sample sizes. Species names are shown and taxonomy indicated by phylogenetic analysis is shown *B. robustus**, *B. nivalis**. Lowland habitat/collection site is indicated by a black star, and presence on individual grasshoppers of short tegmina by white star. Rooting used the New Zealand grasshopper *Paprides nitidus*.

Table 1. Pairwise K2P genetic distances among *Brachaspis* COI haplotypes. Fine boxes indicate distances within species based on phylogenetic analysis (Figure 2), and bold box indicates distiances between *B. robustus* and other haplotypes. The existing taxonomy is indicated in the vertical column beside location names (c – *B. collinus*, n – *B. nivalis*, r – *B robustus*)

		Rob	Lyf	Lyf	Lew	Fog	Arr	Lyf	Lyf	Lyf	Lyf	St B	Roc	Roc	Roc	Roc	Dob	Tek	Roc	Sut	Sut	Mac
Roberts	*collinus*																					
Lyford	*collinus*	0.2	*B. collinus*																			
Lyford	*collinus*	0.2	0.0																			
Lewis Pass	*collinus*	0.4	0.4	0.4																		
Fog Peak	*nivalis*	7.7	7.9	7.9	6.4																	
Arrowsmith	*nivalis*	7.7	7.9	7.9	6.4	0.4		*B. nivalis**														
Lyford	*nivalis*	8.0	8.2	8.2	6.6	0.9	0.9															
Lyford	*nivalis*	6.0	6.3	6.3	5.1	4.2	3.8	4.7														
Lyford	*nivalis*	6.4	6.6	6.6	5.5	4.2	4.2	4.6	0.8													
Lyford	*nivalis*	5.8	6.0	6.0	4.9	4.2	3.8	4.3	0.7	0.7												
St Bathans	*nivalis*	7.3	7.1	7.1	6.6	7.9	7.5	7.9	6.3	6.6	6.2											
Rocky Top	*nivalis*	7.5	7.3	7.3	6.4	7.7	7.3	7.7	6.5	6.8	6.4	0.6			*B. robustus**							
Rocky Top	*nivalis*	7.5	7.3	7.3	6.4	8.1	7.7	8.2	6.5	6.8	6.4	0.9	0.4									
Rocky Top	*nivalis*	7.1	6.9	6.9	6.0	7.7	7.3	7.8	6.5	6.9	6.5	1.3	0.7	1.1								
Rocky Top	*nivalis*	7.1	6.8	6.8	5.9	7.7	7.3	7.7	6.0	6.4	6.0	0.9	0.4	0.4	1.1							
Dobson	*nivalis*	7.1	6.9	6.9	6.0	7.3	6.8	7.3	6.1	6.4	6.0	0.9	0.4	0.7	0.7	0.4						
Tekapo	*nivalis*	7.0	6.8	6.8	5.9	7.5	7.5	7.3	6.7	7.4	6.6	2.5	1.9	2.3	2.3	2.1	1.7					
Rocky Top	*nivalis*	7.3	7.0	7.0	6.1	7.7	7.7	7.5	6.9	7.2	6.4	1.9	1.3	1.7	1.7	1.5	1.1	0.6				
Sutton	*nivalis*	7.1	6.8	6.8	6.0	7.7	7.3	7.3	6.0	6.4	6.0	2.1	1.9	2.3	2.3	1.9	1.5	2.6	2.1			
Sutton	*nivalis*	6.8	6.6	6.6	5.7	7.1	6.6	6.7	5.8	6.6	6.2	2.3	1.7	2.1	2.1	1.7	1.3	2.1	1.9	0.6		
Mackenzie Basin	*robustus*	7.7	7.5	7.5	6.6	7.9	7.5	7.5	6.7	7.0	6.6	2.3	1.7	2.1	2.1	1.7	1.3	2.5	1.9	0.6	0.7	

the Mackenzie Basin *Brachaspis* individuals (Tekapo, Tekapo Delta and Ohau River) differed from northern *B. nivalis* (Fog Peak, Arrowsmith, Craigeburn) by two 3bp INDELs. *B. nivalis* from Mount Dobson had a distinct ITS genotype. Bootstrap MP analysis of the unambiguous ITS sequences revealed a pattern consistent with that observed from COI data (not shown).

Thus, the two Mackenzie Basin grasshoppers (Tekapo Delta and Ohau River) that looked like *B. robustus* had a COI haplotype very similar to the Sutton haplotypes, and the Mackenzie Basin individual that looked like *B. nivalis* (Tekapo) had a haplotype most similar to one of the Rocky Top haplotypes. All three Mackenzie Basin *Brachaspis* (Tekapo, Tekapo Delta and Ohau River), consisting of two morphs, had identical ITS sequences, and shared the two 3bp INDELs with individuals from Rocky Top and Sutton.

Discussion

COI and ITS sequences indicate the existence of at least three *Brachaspis* lineages. However, the relationship of populations implied by the genetic and geographic evidence is not consistent with the three species recognised in the current taxonomy. Phylogenetic evidence for distinct lineages is supported by COI genetic distance data that reveal levels of divergence ($\sim$7%) between clades (species) similar to those encountered in studies of other insect close-species using COI data (e.g. Langor and Sperling 1997; Wirth et al. 1998). However, overall genetic distances are smaller than those encountered within many insect genera (see examples in Funk 1999) and even within some species (e.g. Trewick et al. 2000). Divisions within *Brachaspis* appear to relate more to spatial distribution (allopatry) than to the ecological traits that form part of the diagnoses posited in the existing taxonomy.

Hybrids between *B. collinus* and *B. nivalis* were indicated by the presence of two length-polymorphic ITS sequences in some *Brachaspis* individuals from Lyford where these species are sympatric. If the hybrids are not sterile, this would suggest that under the biological species concept, *B. collinus* and *B. nivalis* are not good species. However, even if this were so it does not preclude their retention as phylogenetic species on the basis of mitochondrial and geographical evidence. The fact that hybrids may be readily detected using ITS polymorphism, and that *B. robustus* individuals examined here did not appear to be heterozygous for distinct ITS sequences suggests the observed similarity of mtDNA genomes of *B. robustus* and southern *B. nivalis* is not the result of recent introgression. Both the mitochondrial and nuclear genomes of *B. robustus* and southern *B. nivalis* are very similar or identical.

The comparatively low genetic diversity within *Brachaspis*, paraphyly of *B. nivalis* and *B. robustus*, the evidence of hybridisation between two species and the instability of several supposedly diagnostic morphological characters all suggest that the entire radiation is shallow. The evidence to support the protected Mackenzie Basin lowland grasshopper (*B. robustus* Bigelow) as a distinct species is correspondingly weak. Inconsistencies of morphology, habitat and molecules among and within the existing taxonomic boundaries underscore the difficulty of defining meaningful conservation units, and the two issues of taxonomy and conservation status need to be addressed.

Two alternative approaches may be taken. 1) Retain the existing taxonomy, and thus conservation status of *B. robustus sensu lato*. This would entail the acceptance of *B. nivalis* as a widespread, genetically diverse (in comparison to other *Brachaspis*) and paraphyletic (with respect to *B. robustus*) species. *B. nivalis* would also be polymorphic for colour pattern and length of tegmina, with most variability among the southern populations. 2) Adopt a phylogenetic approach such that *B. robustus* (hereafter *B. robustus**) includes all the southern *Brachaspis* populations, as these appear to be closely related and form a monophyletic clade. *B. nivalis* (hereafter *B. nivalis**) would be stable with respect to colour pattern and length of tegmina. *B. robustus** would remain polymorphic as it currently is (i.e. long tegmina morph at Tekapo, short tegmina and black/indigo femura on grasshoppers at Rocky Top). This latter option (2) appears to be the optimal way to recognise and summarise morphological, geographical and molecular diversity under the current evidence (see Figure 2, Figure 3).

Interestingly, this will mean that *B. nivalis** (the northern *B. nivalis* populations) and some *B. robustus** individuals will be difficult to distinguish on morphological criteria, but poor morphological differentiation among allopatric *Brachaspis* is consistent with the time frame and manner in which they probably evolved. Similar levels of population differentiation (morphological and genetic) exist within another

Table 2. Aligned ITS sequences from *Brachaspis* from locations in South Island. Species under the existing taxonomy are indicated by: c – *B. collinus*, n – *B. nivalis*, r – *B. robustus*

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                        10        20        30        40        50        60        70        80
                         .         .         .         .         .         .         .         .
Arrowsmith      n TAGTCTCGCCTGCTCTGAGGTCGTTCTTACGAGGTCAAGTG-AAGGCAGGCCATCGCCACGCGGCTGTGCACGCTACCGAGACAT
Craigeburn      n ........................................-.......................................
Lyford          n ........................................-.......................................
Rocky Top       n .....................................---.-.-.....................?.............
Rocky Top       n .....................................---.-.-.......................................
Dobson          n ....................................G.G..-.........G.............................
Tekapo          n .....................................---.-.-.......................................
Sutton          n .....................................---.-.-.......................................
Ohau river      r .....................................---.-.-.......................................
Tekapo delta    r .....................................---.-.-.......................................
Roberts         c ........................G..-----...--..CA.....................................

                     90       100       110       120       130       140       150       160       170
                      .         .         .         .         .         .         .         .         .
Arrowsmith      CAGTACCGGTATGCGAACCGCCACGCGACGGCCACGCCACACGTGTTTAAGGAGACGCAGCC---CACAGGCCACGACGCTCCCA
Craigeburn      ?.............................?............T.................---.....................
Lyford          ..........................................?.................---.....................
Rocky Top       ...............................A...........A.................ACAG....................
Rocky Top       ..........................................C.................ACAG....................
Dobson          .......................G..................................---G..-..................
Tekapo          ..........................................C.................ACAG....................
Sutton          ..........................................C.................ACAG....................
Ohau river      ..........................................C.................ACAG....................
Tekapo delta    ..........................................C.................ACAG....................
Roberts         .......................G.........G..........C.................---G..-..................

                            180       190       200       210       220       230       240       250
                             .         .         .         .         .         .         .         .
Arrowsmith      AGTCTCCGAAAACGCCTGAGCGCTTCAGTATACGTAGCCGACCCTCAGCCAGACGTGGCCCGGGAACGGAATCCATGGACCGCAA
Craigeburn      ..........................................................................................
Lyford          .....G....................................................................................
Rocky Top       ..........................................................................................
Rocky Top       ..........................................................................................
Dobson          ................C.........................................................................
Tekapo          ..........................................................................................
Sutton          ..........................................................................................
Ohau river      ..........................................................................................
Tekapo delta    ..........................................................................................
Roberts         ................C.........................................................................

                        260       270       280       290       300       310       320       330       340
                         .         .         .         .         .         .         .         .         .
Arrowsmith      TGTGCGTTCGAAACGTCGATGTTCATGTGTCCTGCAGTTCACATGTCGACGCGCAATTTGCTGCGTTCTTCATCGACCCACGAGC
Craigeburn      ..........................................................................................
Lyford          ..........................................................................................
Rocky Top       ..........................................................................................
Rocky Top       ..........................................................................................
Dobson          ..........................................................................................
Tekapo          ..........................................................................................
Sutton          ..........................................................................................
Ohau river      ..........................................................................................
Tekapo delta    ..........................................................................................
Roberts         ..........................................................................................

                            350       360       370       380       390       400       410       420
                             .         .         .         .         .         .         .         .
Arrowsmith      CGAGTGATCCACCGTCCTGGGTGATCTTTTTCAACAGTTGTTTTGCGTAAAGCAAAGCTGCGGGACTGGGGCGTTTGACGGCCC
Craigeburn      ...............................A.......................?..............................
Lyford          ..........................................................................................
Rocky Top       ..........................................................T.................................
Rocky Top       ..........................................................T.................................
Dobson          ..........................................................................................
Tekapo          ..........................................................T.................C.................
Sutton          ..........................................................T.................................
Ohau river      ..........................................................T.................C.................
Tekapo delta    ..........................................................T.................C.................
Roberts         ..........................................................................T.................
```

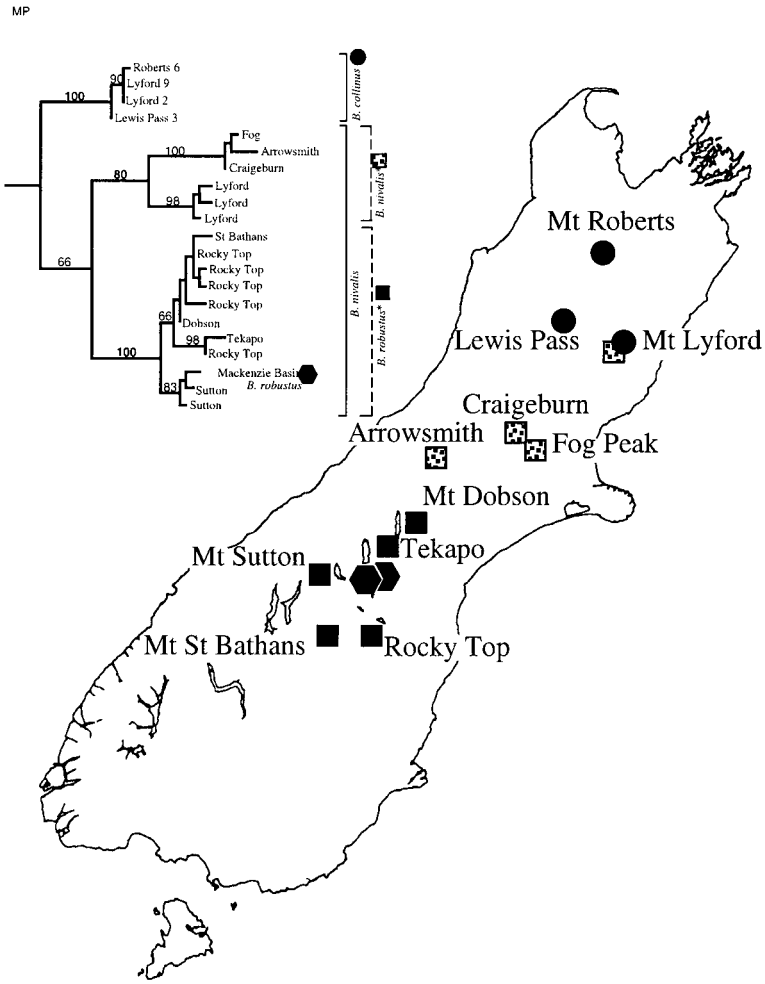

Figure 3. Brachaspis species distribution in South Island as indicated by phylogenetic analyses of COI and ITS sequence data. Inset phylogram shows site names and species symbols (black circle – *B. collinus*, stippled square – *B. nivalis**, black polygons – *B. robustus**).

alpine orthopteran species (*Deinacrida connectens*) over the same landscape (Trewick et al. 2000). Genetic distances among *Brachaspis* (~7%) suggest radiation in the Pliocene (3–5 mya) assuming rates of mtDNA divergence between 1.4 and 2.4% per million years (Brown et al. 1979; Brower 1994; Knowlton and Lee 1998). In contrast, genetic distances among alpine and low altitude individuals of *B. robustus** (with differing phenotypes) are as low as 0.7% and indicate isolation in the late Pleistocene (<500 kya). Natural isolation of the lowland Mackenzie Basin and alpine populations, now apparently separated by ~1000 m of altitude, may not have occurred until the end of the last glacial (~10 kya). It is conceivable that gene flow was not significantly reduced until even more recently; since the arrival of humans (<1000 ya). Changes to vegetation and the introduction of predators are strongly implicated as having localised the Mackenzie Basin grasshoppers.

The Mackenzie Basin *B. robustus** appear to be a localised and genetically weakly differentiated population of a widely distributed and relatively abundant species. But, the purpose of conservation is to retain diversity and while it has been recognised that taxonomic nomenclature often masks diversity, the failure of some populations to meet ESU and MU, let alone species criteria might result in their loss. Moritz (1994) highlighted the benefits of considering pattern rather than extent of sequence divergence, but the approach still necessitates the use of a sufficiently rapidly evolving marker. Suitable genetic markers may be unavailable or prohibitively expensive

in many situations so recognition needs to be given to other types of markers, even if these are difficult to quantify (Vogler and DeSalle 1994). Mackenzie Basin *B. robustus** appear to have uniquely short hind femura, a high frequency of a short-tegmina morph, and perhaps most importantly are distinctive in the type of habitat they occupy. These features imply behavioural and physiological differences and may be just as meaningful indicators of heritable diversity as neutral DNA sequence mutations.

The expansion of molecular based research fuelled by the increasing simplicity and utility of the techniques has allowed the development of theory with respect of units of conservation to proceed toward a simplified (and economically/politically convenient) unitary framework that may often be inapplicable or misleading (Taylor and Dizon 1999). Molecular genetic assessments provide a more consistent and rigorous basis for the description of biological diversity than traditional taxonomy and should continue to have an every increasing role in systematics. However, other less easily quantified characters such as continuous morphological characters and behavioural and ecological characters of uncertain heritability (Vogler and DeSalle 1994), and social, economic and aesthetic aspects (Avise 1989) also ought to have a role in the targeting of conservation effort.

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