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Population Genetic Structure of Antarctic Springtails (Collembola) and New Zealand Damselflies (Odonata)

A thesis

submitted in fulfillment

of the requirements for the degree

of

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at

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By

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The University of Waikato

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Introduction

Since Darwin (Darwin, 1859), the process of speciation and maintenance of biological diversity has caused intensive debate in the scientific and non-scientific communities alike. The ability to analyze differences in the molecular structure of enzymes and DNA sequences has provided an extremely sensitive tool for investigating gene flow within and among populations, a key facet of the genetic divergence required for speciation to occur. Using such techniques, we are now able to gain a snapshot of the genetic structure of a population and its geographical distribution (Hewitt, 2001)

In this way, the phylogeography of species from many environments around the globe have now been studied in fine detail. It has also been possible to generate hypotheses proposing restricted distribution in 'refugia' and subsequent recolonisation after climatic events such as glaciation have occurred. Refugia appear to be particularly important in shaping high latitude biodiversity (Willis & Whittaker, 2000). On such occasions the distribution of different populations may ultimately overlap again at a contact zone, and the species geographical subdivision may provide enough evidence to suggest speciation or the creation of a hybrid zone (Hewitt, 2001).

In addition, the DNA sequences may yield important information on the evolutionary history, dispersal and taxonomy of various species. Morphologically indistinguishable organisms may be discovered as 'cryptic species', and vice versa, taxa considered to be very different based on observable characteristics can be found to be genetically similar and not reproductively isolated (e.g. Trewick, 2000; Witt & Hebert, 2000).

In this way, the genetic diversity within and among closely related species may be determined to a high degree of resolution. In today's climate of human interference and relatively rapid environmental change, it is vital that we appreciate and make full use of the detailed population information available to us. In this way, we may be able to predict and potentially mitigate the consequences of environmental change for organisms by analyzing their evolutionary past.

This thesis contains an analysis of molecular data (mtDNA and allozymes) on two arthropod taxa. The thesis consists of two chapters. Chapter I describes the distribution of mitochondrial (mt) DNA haplotypes for the Antarctic springtail *Gomphiocephalus hodgsoni* (Collembola) in Taylor Valley, southern Victoria Land. The observed distribution was congruent with a hypothesis of multiple refugia during the Pleistocene glaciations and a barrier to gene flow by a glacial lake. Chapter II assesses the genetic variability of the New Zealand damselfly genera (Odonata) from sites throughout the North, South and Chatham islands using both allozyme and mtDNA analyses. All morphologically recognized species were clearly discernible on the basis of both mtDNA and allozymes. However, variability within and among sites was limited for all species, and may have implications from a conservation perspective.

The thesis ends with a brief summary section highlighting the main findings contained in the thesis and outlining potential future research directions

References:

Darwin C (1859) *On the Origin of Species by Means of Natural Selection*. John Murray, London.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology*, **10**, 537-549.

Trewick, S. (2000) Molecular evidence for dispersal rather than vicariance as the origin of flightless insect species on the Chatham Islands, New Zealand. *Journal of Biogeography,* **27,** 1189-1200.

Willis KJ, Whittaker RJ (2000) The refugial debate. *Science*, **287**, 1406-1407.

Witt JDS, Hebert PDN (2000) Cryptic species diversity and evolution in the amphipod genus *Hyallela* within central glaciated North America: a molecular phylogenetic approach. *Canadian Journal of Fisheries and Aquatic Science*, **57**, 687-698.

Role of co-authors:

I have been very fortunate in having the opportunity to work closely with several people who have offered guidance while I conducted the work contained in this thesis. Accordingly, both of the chapters have been progressed to the stage of complete manuscripts ready for submission to refereed international journals. Indeed Chapter 1 has already been accepted for publication in the journal *Polar Biology*. All co-authors for the two chapters are provided in footnotes of the title pages for the chapters.

As with most theses, the discussion of concepts and the review and revision of manuscripts was primarily conducted between myself and my thesis supervisor (I. Hogg). Comments were also sought from the other authors where appropriate. Other input from co-authors was as follows: A small number of samples and mtDNA sequences were provided by M. Stevens and I. Hogg for Chapter I, as recorded in Table 1 of that chapter. Samples and allozyme analysis were provided by I. Hogg and K. Schnabel for Chapter II. Guidance in methodology was provided by M. Stevens (Chapter I and II).
Guidance in analysis was provided by M. Haase (Chapter I) and D. Sutherland (Chapter II).

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Chapter I

Fine scale distribution of mtDNA haplotypes for the springtail *Gomphiocephalus hodgsoni* (Collembola) corresponds to an ancient shoreline in Taylor Valley, continental Antarctica

To be published under the same title as Nolan, L., Hogg, I.D., Stevens, M.I., Haase, M. in Polar Biology

Abstract

We examined the fine scale distribution of the endemic Antarctic collembolan *Gomphiocephalus hodgsoni* in Taylor Valley, southern Victoria Land using the mitochondrial DNA cytochrome *c* oxidase I (COI) gene. We found an area of sympatry in the mid-region of the valley between two common haplotype groups (2.4% sequence divergence). The area of sympatry coincided with the extent of proglacial Lake Washburn (approx. 8,000 ya). This lake existed as a result of the damming of lower Taylor Valley by the grounding of the Ross Ice Sheet and may have acted as an isolating barrier to dispersal/gene flow. We suggest that the phylogenetic break occurring in the vicinity of the ancient shoreline may be the result of previous isolation of refugial allopatric populations, followed by recolonisation into a secondary contact zone during the Holocene.

Introduction

The impacts of palaeoclimatic oscillations on phylogeography have been extensively studied in the past 15 years (see Hewitt 2001 for review). In particular, the role of late Pleistocene glaciations in shaping the genetic structure of populations has been examined for a variety of taxa, especially in high latitudes of the Northern Hemisphere (e.g. Knowles 2001). Fragmentation of populations into local refugia during periods of glaciation, and subsequent recolonisation of previously unavailable habitat can lead to the formation of secondary contact zones. In these zones, genetically isolated populations may overlap in an area of sympatry (e.g. Pfenninger and Posada 2002). The likelihood of observing genetic isolation in such a contact zone increases as dispersal ability and population size decrease (Irwin 2002). If the populations are not reproductively isolated a hybrid zone may form (Hewitt 2001).

Despite the increasing number of phylogeographic studies in the Northern Hemisphere (see Avise 1998; Hewitt 2001), comparatively fewer studies have been undertaken to determine the effect of glaciations on genetic differentiation among terrestrial invertebrate populations in Antarctica (Courtwright et al. 2000; Frati et al. 2001; Stevens and Hogg 2003, see also Stevens and Hogg 2006 for review). This is unfortunate because Antarctica provides an ideal opportunity to study such events owing to its well studied glacial history (e.g. Denton and Hughes 2000). For example, palaeoclimatic oscillations resulted in periodic fluctuations of the sea ice level in McMurdo Sound and in lake levels in the adjacent Dry Valleys. The formation of the proglacial Lake Washburn in Taylor Valley in the late Pleistocene (Denton and Hughes 2000; Denton and Marchant 2000) is now well documented and consequently

provides an historical record of terrestrial habitat availability throughout Taylor Valley at the end of the Pleistocene. The lake formed due to the grounding of the West Antarctic Ice Sheet at the mouth of the Valley during the last glacial maximum. Detailed studies of the terrestrial fauna in this region would therefore provide valuable information on the phylogeographic history of the region, and more importantly, the role of glaciation on processes of recolonisation, genetic differentiation, and ultimately speciation.

One potential target organism is the springtail *Gomphiocephalus hodgsoni* Carpenter, 1908 (Collembola: Hypogastruridae), which is endemic to southern Victoria Land and adjacent islands in the Ross Sea region of Antarctica (Wise 1967). As with other continental Antarctic Collembola, relative humidity is one of the most important factors determining the distribution of *G. hodgsoni* (Sinclair 2002). The Ross sea region experiences a mean annual temperature of about -20°C (Thompson et al. 1971) and minimal precipitation (approx. 0.7 to 8.2 mm yr^{-1} water equivalent, Thompson 1973). Consequently, distribution of *G. hodgsoni* is extremely patchy, being limited to the edges of streams, lakes and snow patches which have liquid water available during part of the summer. However, many species of Collembola can survive for extended periods while floating on water and this may provide a viable means of dispersal (Coulson et al. 2002). By contrast, overland transport by wind appears unlikely due to the desiccation of the organisms (Gressit et al. 1960). Accordingly, *G. hodgsoni* may provide an excellent model to study the effects of past glaciations in southern Victoria Land.

Here, we examine the fine scale distribution of distinct mtDNA haplotypes previously identified for *G. hodgsoni* in Taylor Valley (Stevens and Hogg 2003). As we show, the observed distribution of these haplotypes is consistent with a secondary contact zone along an ancient shoreline since the last glacial maximum.

Materials and methods

Sample collection

Structured sampling for terrestrial arthropods was undertaken at several locations in Taylor Valley, southern Victoria Land (Fig. 1). Individuals of *G. hodgsoni* were collected from 3 sites in January 1999 (Stevens and Hogg 2003) (TV26-28), and at a further 25 sites (TV1-25) in January 2003 (Table 1). To minimize disturbance, approximately 10 individuals were collected at each site from the underside of stones using a miniaturized aspirator (Stevens and Hogg 2002). This sample size generally represents much less than 5% of the population at any given site and hence any long-term impact was likely to be minimal. Samples were placed in 70% ethanol before being transferred to 100% ethanol on return from the field, where they were stored at -20°C until needed for molecular analyses.

Mitochondrial DNA analyses

In addition to eight Taylor Valley individuals from Stevens and Hogg (2003), we extracted total genomic DNA from a further 40 individuals, comprising of one to six individuals from each site (Table 1). This was based on previous sampling in this Valley (Stevens and Hogg 2003), indicating that variability within any given site was limited to one (rarely two) haplotypes. Extractions were modified from Sleigh and Cursons (2000), and consisted of homogenizing an entire individual in 80 μL of lysis buffer (5 ml 1M Tris, 5 ml 10% SDS, 5 ml 0.5M EDTA, 1 ml 5M NaCl, 34 ml H_2O) and 20 μL of proteinase K (100 μg/mL). This was then incubated at 56°C for 12

hours, followed by the addition of 100 µL LiCl (5M), then rotated for 30 min; then 200 µL of chloroform was added, and rotated for a further 30 min; then centrifuged for 10 min at 10,000 rcf after which the aqueous phase was removed to a new tube and DNA precipitated by using an equal volume of isopropanol and left for 2 hrs; DNA was pelleted by centrifugation (15 min at 13,000rcf) and washed with 70% ethanol. The DNA pellet was air dried and re-suspended in 50 µL TE (Sleigh and Cursons 2000).

PCR amplifications (Saiki et al. 1998) were carried out using a 50 µL reaction volume consisting of 3 µL DNA (not quantified), 1xPCR buffer (Roche), 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer Mannheim), 1.0 µM of each primer and 1.0 unit *Taq* polymerase (Roche) on an Eppendorf Mastercycler gradient thermocycler. A 710-bp fragment of the mtDNA (COI) gene was amplified (via PCR) using the universal primers LCO1490 (5'- ggt caa caa atc ata aag ata ttg g -3') and HCO2198 (5'- taa act tca ggg tga cca aaa aat ca -3') (Folmer et al. 1994). The thermal cycling conditions were: 94°C for 1 min, followed by five cycles of (94°C for 1 min, 45°C for 1.5 min and 72°C for 1 min), followed by 35 cycles of (94°C for 1 min, 51°C for 1.5 min and 72°C for 1 min) followed by 5 min at 72°C. All reaction products were purified using a DNA purification kit (QIAGEN: Qiaquick). Sequencing was performed using the same primers as those used for PCR amplification on a MegaBACE DNA sequencer (Amersham Biosciences) at the University of Waikato DNA sequencing facility.

Sequences were aligned using SEQUENCHER (Gene Codes ver. 4.1.2) sequence editor. Sequences were verified as being consistent with collembolan DNA using the

GenBank BLASTn search and these data were analysed using PAUP* 4.0b10 (Swofford 2002). All sequences are accessible from GenBank (accession numbers AY294602-AY294603 (Stevens and Hogg 2003); and DQ309560-DQ309567). We used χ^2-tests, as implemented in PAUP* to determine whether the assumption of equal base frequencies among sequences was violated on all sites, or using third codon positions only. A distance matrix of pairwise nucleotide sequence divergence was calculated using uncorrected distance, in addition to a corrected model (TRN+I, -lnL = 761.7747: base frequencies set to A = 0.2699, C = 0.1921, G = 0.1558, T = 0.3823; I = 0.8044), as indicated by Modeltest ver. 3.7 (Posada and Crandall 1998). The corrected model was used to estimate a maximum likelihood (ML) phylogram (heuristic search), all other settings remained as default in PAUP*. Confidence in the tree was assessed using bootstrap analysis with 500 pseudoreplicates (Felsenstein 1985). We constructed a haplotype network using the programme TCS ver. 1.21 (Clement et al. 2000). Based on this network, we performed a nested clade analysis (NCA) (Templeton et al. 1987, Templeton and Sing 1993, Templeton 2004) using GeoDis ver. 2.0 (Posada et al. 2000). However, this analysis resulted in an 'inconclusive outcome'. Accordingly, here we present only the results of the ML analysis. A detailed NCA of *G. hodgsoni* across a broader geographic scale will be presented elsewhere (A. McGaughran unpubl. data).

Results

A 484-bp fragment (161 codons) of the COI gene was used in these analyses and no insertions, deletions, or stop codons (using MacClade ver. 4; Maddison and Maddison 2000) were detected. The nucleotide composition of all sequences was biased for A and T (A = 27%, T = 38%, C = 19%, G = 16%), and is similar to other Collembola (e.g. Hogg and Hebert 2004; Stevens et al. 2006). No significant differences in base composition were detected for all sites (χ^2_{141}=4.87, *P*=1.00), or among third codon positions (χ^2_{141} = 39.66, *P*=1.00). Across all 484 characters, 18 were variable and 10 were parsimony informative nucleotide substitutions (Table 2). The number of nucleotide substitutions between the ten unique *G. hodgsoni* haplotypes in Taylor Valley ranged from 1 to 8, and sequence divergence ranged from 0.2% (one substitution) to 2.4% (8 substitutions)(Table 3). Table 3 shows the pairwise divergences among the 10 unique *G. hodgsoni* haplotypes.

The ML phylogram (Fig. 2) shows two distinct haplotype groupings in Taylor Valley. With few exceptions, haplotypes with little divergence group together according to their location (Fig. 2). For example, individuals from the lower Taylor Valley fall into group "X" (haplotypes A-F), whereas haplotypes from the upper Taylor Valley fall into group "Y" (haplotypes G-J). Figure 2 also shows the distribution of haplotypes in the Valley, and indicates the approximate shoreline of Lake Washburn at the end of the last glacial maximum. An area of sympatry occurs between the two most divergent haplotype groups along the shoreline of Lake Washburn (Fig. 2). In addition, all haplotypes shared by more than one site can be "linked" by either streams flowing down the valley, and/or lakes (Fig. 2).

Discussion

Sequence divergence among the 10 different haplotypes ranged from 0.2 to 2.4%, and is comparable to rates reported for other arthropods with a Pleistocene coalescence (Knowles 2001). A molecular clock rate for arthropods of 1.5-2.3% sequence divergence per million years (e.g. Quek et al. 2004) suggests that the two divergent *G. hodgsoni* groups ("X" and "Y") in the present study diverged less than one million years ago. The geographic grouping of similar haplotypes indicates minimal gene flow among locations. This is expected due to the potentially limited dispersal opportunities for these springtails. However, while dispersal of springtails is generally low, it may be possible for individuals to be carried relatively large distances by melt-water streams. For example, Borns Glacier (TV28) and Lake Chad (TV25, TV27) share haplotypes I and J and are connected by a stream and lake network. Likewise, Howard Glacier (TV2, TV26) and Delta Stream (TV3) share the unique haplotype D (Fig. 2). Furthermore, only two individuals from Group "X" were found above Howard Glacier (TV16, TV18b), yet representatives of all haplotypes (G, H, I, J) from the upper Taylor Valley Group "Y" were found on the southern side of Lake Fryxell (Fig. 2). Consequently, it appears that dispersal down Taylor Valley is more likely than movement up the valley.

The two common *G. hodgsoni* haplotype groupings in Taylor Valley ("X" and "Y") have distinct geographic ranges, indicative of parapatric populations or metapopulations (Levins 1970). Such a distribution may be interpreted as divergence associated with displacement to multiple glacial refugia followed by recolonisation

converging at a contact zone in an area of sympatry (Fig. 2). Genetic drift appears to be the dominant force structuring genetic variation in *G. hodgsoni* whereby haplotypes have diverged in allopatric refugia (see also Stevens and Hogg 2003). Hence, it is likely that at least two geographically isolated refugia existed within Taylor Valley, which individually harboured individuals of the two haplotype groupings ("X" and "Y").

Recolonisation of an area of sympatry requires the previous existence of a barrier to gene flow. At the last glacial maximum (LGM) the West Antarctic Ice Sheet advanced across the Ross Sea continental shelf and an ice sheet formed in the Ross Sea Embayment (Hall et al. 2000). A lobe of the ice sheet grounded at the mouth of Taylor Valley inland from the present day sea-level (Fig. 2), and dammed Glacial Lake Washburn, which extended to at least 336m elevation. The perennially ice-covered lake had a moat, and meltwater from the surrounding glaciers formed deltas where they met the lake. The grounded ice sheet blocked the mouth of the valley between 8,340 and 23,800 yr BP, reaching its maximum between 12,700 and 14,600 yr BP (Hall and Denton 2000).

The westward extent of Glacial Lake Washburn coincides well with the region of sympatry between the "X" and "Y" haplotype groups of *G. hodgsoni* (Fig. 2). The lake effectively rendered the lower part of Taylor Valley uninhabitable to an elevation of 336m until around 8,000 years ago. During this time, refugia may have existed for haplotype group "Y" in upper Taylor Valley, and for haplotype group "X" at higher altitude (where they are presently found) in lower Taylor Valley, most likely around moist habitats in close proximity to melt-water streams, or glacial foregrounds. As the

ice sheet lifted from the mouth of the valley, facilitating drainage of the lake, streams would have provided a rapid means of transport (see Coulson et al. 2002) enabling recolonisation along melt-water streams resulting in the present distribution. Subsequently, the distribution of the upper Taylor Valley haplotypes from group "Y" has spread further into the Lake Washburn area. This is most likely the result of individual springtails being transported in melt-water streams, which flow from the upper to lower Taylor Valley. Only two individuals (haplotypes A, E) from group "X" were found outside of the lower Taylor Valley on the eastern side of the nearby Goldman Glacier (TV16, 18) (Fig. 2), and may represent local (e.g. wind-mediated) dispersal.

The strength of a phylogenetic break due to a significant barrier tends to decline rapidly after the disappearance of the barrier, except for taxa with very low dispersal rates, although reproductive isolation or selection against hybrids may stabilize such a break (Irwin 2002). Little data exist on the actual seasonal dispersal ability of *G. hodgsoni* in the Dry Valleys, although it is assumed to be low in the absence of direct water flow. The possibility of reproductive isolation (assortative mating) has previously been suggested by Stevens and Hogg (2003), (based on fixed allelic differences at an allozyme locus) for individuals at Howard Glacier (TV26) collected in the contact zone of Taylor Valley.

To further clarify the processes that have shaped the genetic structure of the regional fauna, future work should target: (1) the phylogeography of other species in Taylor Valley that could be transported by melt-water (e.g. mites); and (2) sampling of mtDNA haplotypes north and south of Taylor Valley to clarify the potential existence

of glacial refugia and further examine patterns and process of long-term glaciation on the endemic Antarctic fauna.

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References

Avise JC (1998) The history and purview of phylogeography: a personal reflection. Mol Ecol 7:371-379

Clement MD, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657-1659

Coulson SJ, Hodkinson ID, Webb NR, Harrison JA (2002) Survival of terrestrial soil-dwelling arthropods on and in seawater: implications for trans-oceanic dispersal. Funct Ecol 16:353-356

Courtwright EM, Wall DH, Virginia RA, Frisse LM, Vida JT, Thomas WK (2000) Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode *Scottnema lindsayae*. J Nematol 32:143-153

Denton GH, Hughes TJ (2000) Reconstruction of the Ross ice drainage system, Antarctica, at the last glacial maximum. Geografiska Annaler 82A:143-166

Denton GH, Marchant DR (2000) The geological basis for a reconstruction of a grounded ice sheet in McMurdo Sound, Antarctica, at the last glacial maximum. Geografiska Annaler, 82A:167-211

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for the amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotech 3:294-299

Frati F, Spinsanti G, Dallai R (2001) Genetic variation of mtCOII gene sequences in the collembolan *Isotoma klovstadi* from Victoria Land, Antarctica: evidence for population differentiation. Polar Biol 24:934-940

Gressitt JL, Leech RE, O'Brien CW (1960) Trapping of air-borne insects in the Antarctic area I. *Pacific Insects*, **2**, 245-250.

Hall BL, Denton GH, Hendy CH (2000) Evidence from Taylor Valley for a grounded ice sheet in the Ross Sea, Antarctica. Geografiska Annaler 82A:275-303

Hall BL, Denton GH (2000) Radiocarbon chronology of Ross Sea drift, Eastern Taylor Valley, Antarctica: Evidence for a grounded ice sheet in the Ross Sea at the last glacial maximum. Geografiska Annaler 82A:305-336

Hewitt GM (2001) Speciation, hybrid zones and phylogeography – or seeing genes in space and time. Mol Ecol 10:537-549

Hogg ID, Hebert PDN (2004) Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. Can J Zool 82:749-754

Irwin DE (2002) Phylogeographic breaks without geographic barriers to gene flow. Evolution 56:2383-2394

Knowles LL (2001) Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. Mol Ecol 10:691-701

Levins R (1970) Extinction. Lecture Notes in Mathematics 2:75-107

Maddison DR, Maddison WP (2000) MacCalde version 4. Sinauer Associates Inc., Sunderland MA.

Pfenninger M, Posada D (2002) Phylogeographic history of the land snail *Candidula unifasciata* (Helicelinae, Stylommatophora): Fragmentation, corridor migration, and secondary contact. Evolution 56:1776-1788

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818

Posada D, Crandall KA, Templeton AR (2000) GeoDis: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology 9:487-488

Quek S-P, Davies SJ, Itino T, Pierce NE (2004) Codiversification in an ant-plant mutualism: stem texture and the evolution of host use in *Crematogaster* (Formicidae: Myrmicinae) inhabitants of *Macaranga* (Euphorbiaceae). Evolution 58:554–570

Saiki RK, Gelfand DH, Stoffel S (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491

Sinclair BJ (2002) Effects of increased temperatures simulating climate change on terrestrial invertebrates on Ross Island, Antarctica. Pedobiologia 46:150-160

Sleigh JW, Cursons RT (2000) Generic Polymerase Chain Reaction followed by DNA sequencing as a means of diagnosing bacteraemia. Anaesth. Intensive Care 28:54-57

Stevens MI, Hogg ID (2002) Expanded distributional records of Collembola and Acari in Southern Victoria Land, Antarctica. Pedobiologia 46:485-496

Stevens MI, Hogg ID (2003) Long-term isolation and recent range expansion from glacial refugia revealed for the endemic springtail *Gomphiocephalus hodgsoni* from Victoria Land, Antarctica. Mol Ecol 12:2357-2369

Stevens MI, Hogg, ID (2006) Molecular Ecology of Antarctic terrestrial Invertebrates. In: Huiskes A, Convey P, Bergstrom D (eds) Trends in Antarctic Terrestrial and Limnetic Ecosystems. Kluwers.

Stevens MI, Greenslade P, Hogg ID, Sunnucks P (2006) Southern Hemisphere springtails: could any have survived glaciation of Antarctica? Mol Biol Evol 23: ın press

Swofford DL (2002) PAUP*: Phylogenetic Analysis using Parsimony, Version 4.0b10 for Macintosh. Sinauer Associates Inc., Sunderland MA.

Templeton AR (2004) Statistical phylogeography: methods of evaluating and minimizing inference errors. Mol Ecol 13:789-809

Templeton AR, Sing CF (1993) A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombinations. Genetics 134:659-669

Templeton AR, Boerwinkle E, Sing CF (1987) A cladistic-analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol-dehydrogenase activity in Drosophila. Genetics 117: 343-351

Thompson DC (1973) Climate of the Dry Valleys area of South Victoria Land. New Zealand Geographical Society Conference Series 7:259-265

Thompson DC, Craig RF, Bromley AM (1971) Climate and surface heat balance in an Antarctic Dry Valley. New Zealand Journal of Science 14:245-251

Wise KAJ (1967) Collembola (Springtails). Antarctic Research Series 10:123–148

Table 1. Sampling locations in Taylor Valley for *Gomphiocephalus hodgsoni* and number of individuals analyzed (n). ‡ Samples collected January 1999 (Stevens and Hogg 2003).

Site No.	n	Location	Latitude (S)	Longitude (E)
TV1	1	L. Fryxell	77°37.599'	163°06.901'
TV2	1	Howard Gl.	77°37.599'	163°05.930'
TV3	6	Delta Stream	77°38.577'	163°07.978'
TV4	2	Canada Gl.	77°36.579'	163°00.548'
TV5	1	Mt Falconer	77°34.864'	163°06.575'
TV6	1	Commonwealth Gl.	77°36.743'	163°24.137'
TV7	1	Mt Falconer	77°34.135'	163°09.347'
TV8	1	Mt Falconer	77°34.635'	163°05.526'
TV9	1	L. Fryxell	77°37.932'	163°12.757'
TV10	1	L. Fryxell	77°38.478'	163°17.681'
TV11	1	Mt Coleman	77°38.506'	163°17.837'
TV12	1	Mt Coleman	77°38.281'	163°21.516'
TV13	1	Mt Coleman	77°33.085'	163°20.474'
TV14	1	Mt Coleman	77°33.008'	163°20.859'
TV15	1	Mt Coleman	77°32.758'	163°21.038'
TV16	1	Goldman Gl.	77°41.199'	163°52.334'
TV17	1	Goldman Gl.	77°41.154'	162°55.298'
TV18	6	Goldman Gl.	77°41.224'	162°56.996'
TV19	1	Goldman Gl.	77°41.322'	162°58.241'

TV20	1	Mt Barnes	77°36.429'	163°30.053'
TV21	1	Mt Barnes	77°36.770'	163°31.617'
TV22	1	Mt Barnes	77°37.158'	163°29.579'
TV23	1	Mt Barnes	77°37.267'	163°29.348'
TV24	1	L. Fryxell	77°37.199'	163°11.101'
TV25	1	L. Chad	77°38.740'	162°45.780'
TV26	4	Howard Gl. ‡	77°39.735'	163°05.835'
TV27	1	L. Chad‡	77°38.571'	162°46.492'
TV28	3	Borns Gl.‡	77°45.833'	162°02.240'

Table 2. The 18 variable nucleotide sites among the 10 haplotypes for the 48 *Gomphiocephalus hodgsoni* individuals. Haplotype A is used as a reference sequence (identical character states are indicated by dots). Locations where each haplotype was found are indicated using location codes from Table 1, and the lower-case letter indicates multiple individuals analysed from a location.

			2	4	6	9	1	1	1	2	2	2	2	2	2	2	3	3	4	4
			1	2	6	3	4	4	3	1	7	2	7	3	8	5	3	9	8	0
	HAPLOTYPE	LOCATION																		
Lower TV	A	TV3b, TV4a-b, TV6-TV8, TV10, TV12-TV16, TV20-23	T	C	G	C	C	G	G	G	A	A	C	T	G	T	G	G	A	G
	B	TV11	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
	C	TV3a	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
	D	TV3c, TV26d	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.
	E	TV18b	.	T	.	.	.	A	.	A	.	G	.	.	.	.	.	A	.	.
	F	TV5	.	.	.	.	T	.	.	.	.	G	.	C	A			A	.	.
Upper TV	G	TV3d	C	.	A	T	T	A	.	.	.	.	T	.	.	C	A	A	.	.
	H	TV1	C	.	A	T	T	A	.	.	.	.	T	.	.	.	A	A	G	.
	I	TV2, TV3e-f, TV9, TV17, TV18a, TV18c-f, TV19, TV24-TV25, TV26a, TV28a, TV28c	C	.	A	T	T	A	.	.	.	.	T	.	.	.	A	A	.	.
	J	TV26b, TV26c, TV27, TV28b	C	.	A	T	T	A	.	.	.	.	T	.	.	.	A	A	.	A

Table 3. Genetic distance (lower = TRN+I ML model; upper = uncorrected distances) based on sequence variation in the mtDNA COI sequences (484 aligned sites) among the 10 identified *Gomphiocephalus hodgsoni* haplotypes. Haplotype codes refer to Table 3, and Fig. 2.

Haplotype	A	B	C	D	E	F	G	H	I	J
A		0.002	0.002	0.002	0.01	0.01	0.019	0.019	0.017	0.019
B	0.002		0.004	0.004	0.012	0.012	0.021	0.021	0.019	0.021
C	0.002	0.004		0.004	0.012	0.012	0.021	0.021	0.019	0.021
D	0.002	0.004	0.004		0.008	0.008	0.017	0.017	0.014	0.017
E	0.011	0.013	0.013	0.009		0.012	0.021	0.021	0.019	0.021
F	0.011	0.013	0.013	0.009	0.013		0.021	0.021	0.019	0.021
G	0.021	0.024	0.024	0.019	0.024	0.024		0.004	0.002	0.004
H	0.021	0.024	0.024	0.019	0.024	0.024	0.004		0.002	0.004
I	0.019	0.021	0.021	0.016	0.021	0.021	0.002	0.002		0.002
J	0.021	0.024	0.024	0.019	0.024	0.024	0.004	0.004	0.002	

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Fig. 1. Location of Taylor Valley and sampling area in southern Victoria Land, Ross Dependency.

Fig. 2. Maximum likelihood phylogram (ML; TRN+I) for the 48 individuals of *Gomphiocephalus hodgsoni* collected in Taylor Valley; bootstrap support (500 replicates) shown below branches. Approximate shoreline of Lake Washburn in Taylor Valley is indicated (dotted line). Unique haplotypes (A-J) are indicated and the haplotype groupings ("X" and "Y") indicate sites between the ML phylogram and distribution map of Taylor Valley where haplotypes were found. Black-filled boxes with white text correspond to "Group X" and white-filled boxes with black text correspond to "Group Y". Combination black and white-filled boxes correspond to sites where representatives from both haplotype groupings ("X" and "Y") were found.

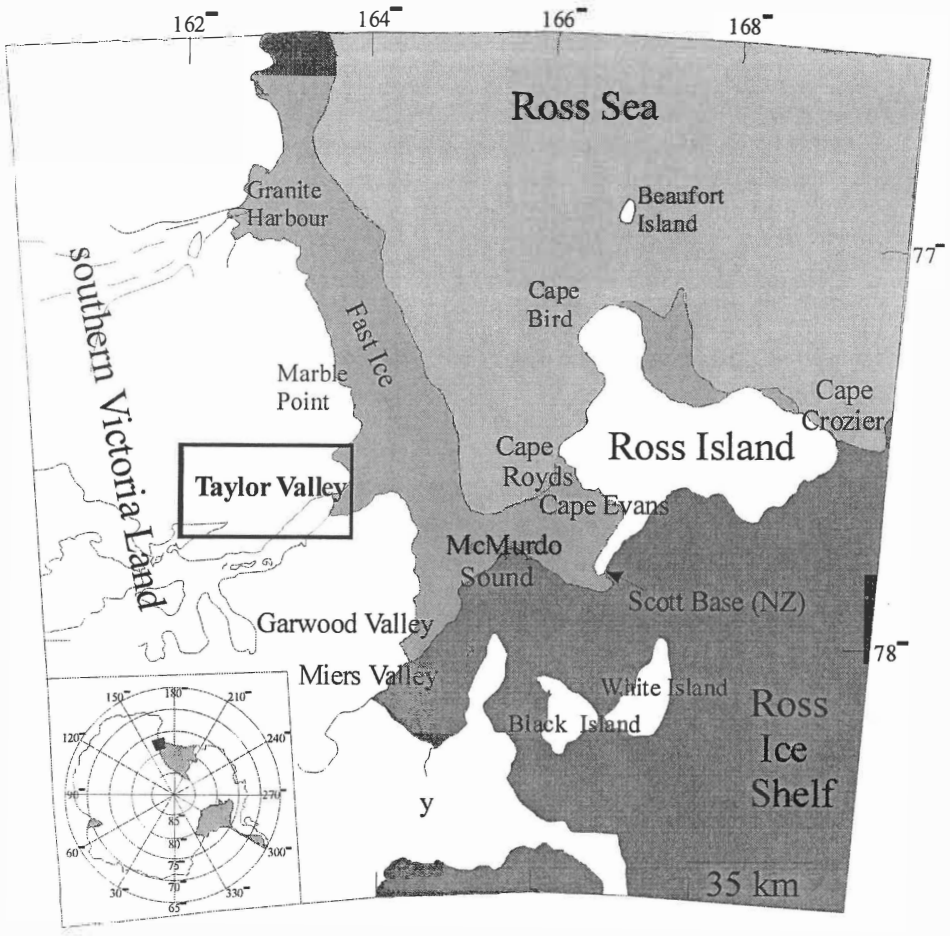

Fig. 1. Nolan et al.

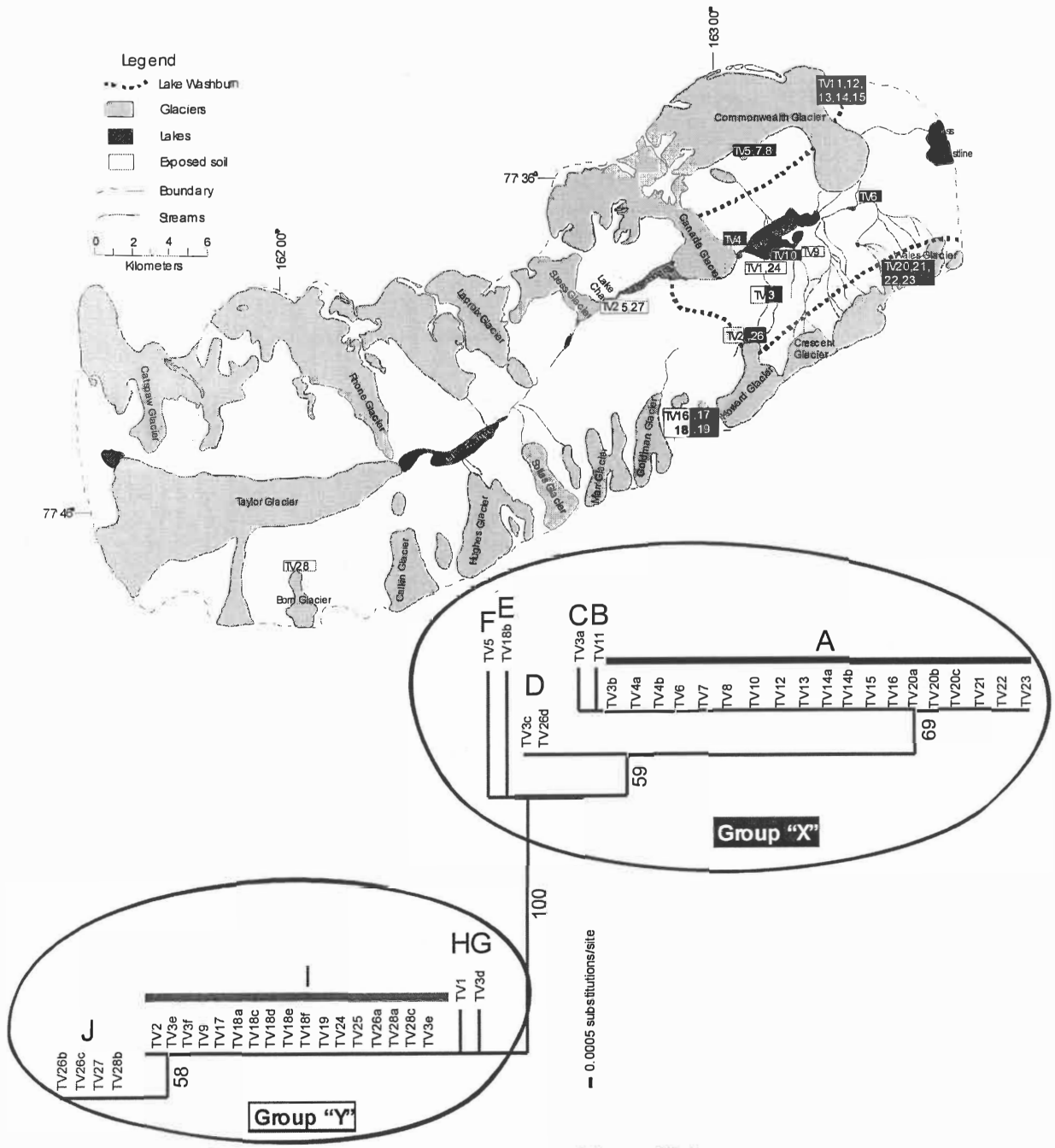

Fig. 2. Nolan et al.

Chapter II

Limited genetic variability within the New Zealand damselfly genera *Xanthocnemis*, *Austrolestes,* and *Ischnura* (Odonata), revealed using allozyme and mitochondrial DNA analyses

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Abstract

We collected larval damselflies from 17 sites in the North, South and Chatham Islands, and using 11 allozyme loci and the mitochondrial cytochrome *c* oxidase subunit I (COI) gene tested the hypothesis that species would have limited variability among locations. Four species from three genera were all clearly discernible on the basis of allozyme and mtDNA data. We found evidence for very limited genetic variability based on allozyme data ($H_{exp} < 0.06$ in all cases) and low to moderate differentiation among locations based on allozyme (mean $F_{ST} = 0.09$) and sequence (COI) divergence (< 0.034). No obvious patterns with respect to geographic location were detected, although slight differences were found between New Zealand's main islands and the Chatham Islands for *A. colensonis* (sequence divergence 0.030-0.034). We suggest that levels of gene flow/dispersal have been adequate to maintain a homogeneous population structure. However, limited levels of genetic variability may be a concern from a conservation perspective particularly if other less common odonate species in New Zealand have similarly limited levels of variability. Given the conservation concern for odonates globally, we suggest that future studies be targeted towards genetically assessing the taxonomic status and variability of other, less common, species in New Zealand.

Introduction

Several studies now exist on the population genetic structures of aquatic insects in North America (e.g., Sweeney et al. 1986; Jackson & Resh 1992), Europe (e.g., Wilcock et al. 2002) and Australasia (e.g. Hughes et al. 1998; Smith & Collier 2001; Hogg et al. 2002). However, until recently comparatively few studies have focused on odonates (e.g., Geenen et al. 2000). This is unfortunate as many species of odonates are now considered to be endangered or undergoing serious range restrictions (Freeland & Conrad 2002; Watts et al. 2004). Furthermore, it would allow testing the generality of hypotheses regarding population structure and variability relative to taxonomic group, gene flow/dispersal capabilities and adult longevity. Odonates are relatively long lived as adults compared to the other "palaeoptera" (e.g., mayflies), but similar to some stonefly taxa (e.g., Nemouridae). Adult dragonflies and damselflies may live for more than 30 days actively hunting for food and mating, and some are known to undergo annual migrations (Freeland et al. 2003). Accordingly, the potential for dispersal/gene flow among discrete habitats would seem great. Furthermore, their presence on distant offshore islands of relatively recent geological origin (e.g., Chatham Islands, Rowe 2000), would seem to suggest the likelihood of minimal genetic structure among populations.

Previous Australasian studies of aquatic insects have found evidence of limited differentiation among systems for some taxa (e.g., Ephemeroptera, Trichoptera), a pattern attributed to adult flight capabilities (Hughes et al. 1998), and/or the evolutionary history of the taxon (Hogg et al. 2002). However, these studies have also found evidence of strong genetic structure among discrete aquatic systems (Smith &

Collier 2001; Hogg et al. 2002), and even among reaches of the same river system (Hughes et al. 1998). For example, Smith & Collier (2001) and Hogg et al. (2002), found a north-south pattern of differentiation in New Zealand for *Orthopsyche fimbriata* (Trichoptera) and *Archicauliodes diversus* (Megaloptera), respectively, with greatest differences found between sites of increasing geographic distance. Similar patterns have been observed in North America with strong differentiation among geographically discrete sites (Sweeney et al. 1987). In some cases, the levels of genetic differentiation have been so strong the authors have suggested the existence of possible cryptic species for some groups (Sweeney & Funk 1991; Jackson & Resh 1998). The possibility that levels of biodiversity are being underestimated has serious consequences from a conservation perspective. However, too few studies have been undertaken on aquatic insects in New Zealand and Australia to determine the prevalence of such cryptic species. Accordingly, we targeted the New Zealand damselfly genera as the focus of our study.

Six species of damselflies in three genera are recognised in New Zealand; *Austrolestes colensonis* (White, 1846) which is widespread throughout New Zealand, *Ischnura aurora aurora* (Brauer, 1865) which is found only on the North Island and four species of *Xanthocnemis*. Of the four *Xanthocnemis* species, *X. zealandica* (McLachlan, 1873) is the most widely distributed, and likely the most common odonate species in New Zealand (Rowe 1987). The other described species are much more restricted. For example, *X. sobrinia* (McLachlan, 1873) is known only from sites in northern North Island, *X. sinclairi* (Rowe, 1987) has been recorded from alpine tarns in the headwaters of a single river (Rowe 2000), and *X. tuanuii* (Rowe, 1981) is known only from Chatham Islands. The restricted distribution of these latter species

would seem to suggest that dispersal capabilities/behaviour or larval habitat requirements for some species are limited.

On the basis of these available biogeographic data, we targeted *X. zealandica* in particular and tested the hypothesis that New Zealand damselflies would show limited levels of genetic variability among habitats. We further tested the utility of the molecular genetic techniques (allozyme electrophoresis, mtDNA sequencing) in discriminating between the New Zealand taxa.

Materials and methods

Sample collection

Following extensive sampling of suitable aquatic habitats throughout New Zealand (including Chatham Islands), larval damselflies were collected from a total of 17 sites (Fig. 1, Table 1). Animals were collected using a sweep net in submergent aquatic macrophytes or overhanging bankside vegetation. For the allozyme analyses, efforts were made to collect up to 40 individuals. However, in most instances this was not possible due to the relatively low densities and patchy distribution of the animals. This was particularly true for individuals of *Austrolestes*, and *Ischnura*, which were recovered in very low numbers (<5 per site in all cases). All generic designations were morphologically confirmed using Rowe (2000). For the mitochondrial DNA (mtDNA) analyses, individuals of *Austrolestes* were taken from Sites C1, N10 and S4, individuals of *Ischnura* from N3 and N9, and individuals of *Xanthocnemis* were taken from Sites C1, N1, N2, N3, N5, N6, N8, N9, N11, S1 and S5 (Table 1).

Allozyme analyses

Heads of individual damselflies were removed and homogenised in 15µl of buffer containing distilled water (100ml), NADP (10mg), B-mercaptoethanol (100µl), and detergent Tween 80 (100µl) (Richardson et al. 1986). We used cellulose acetate gel electrophoresis (Helena Super Z-12 applicator kit; 76x76mm Titan III cellulose acetate plates) and staining recipes outlined in Hebert & Beaton (1993). Running conditions consisted of 15-20 minutes at 200mV. Animals were screened for allozyme

activity for several enzyme systems and following this preliminary screening, we selected the following nine enzyme systems for analyses: isocitrate dehydrogenase (IDH: EC 1.1.1.42), lactate dehydrogenase (LDH: EC 1.1.1.27), 6-phosphogluconate dehydrogenase (6PGDH: EC 1.1.1.44), Glycerol-3-phosphate dehydrogenase (GPDH: EC 1.1.1.8), peptidase (PEP: EC 3.4.11/13), phosphoglucomutase (PGM: EC 2.7.5.1), malate dehydrogenase (MDH: EC 1.1.1.37), phosphoglucose isomerase (PGI: 5.3.1.9). Three enzymes (IDH, PEP, MDH) were scorable at two loci each providing a total of 11 presumptive loci. However, in some cases it was not possible to score all enzymes/loci for a particular individual. Accordingly, sample sizes vary slightly among individuals. Alleles were scored in order of increasing mobility such that those migrating closest to the anode were designated "A", and sequentially slower alleles "B", "C" etc. For enzymes with more than one locus (e.g., IDH), these were assigned in order of increasing electrophoretic mobility such that *IDH-2* was closer to the anode that *IDH-1*. In order to verify the accuracy of allelic designations, gel lineups (*sensu* Richardson et al. 1986), were used, and at least one individual from a previous run was included on subsequent gels.

We used BIOSYS-1 (Swofford & Selander 1981) to calculate: 1) allele frequencies; 2) measures of genetic variability (mean number of alleles per locus, percentage of polymorphic loci where the frequency of the most common allele was <95%, expected and observed heterozygosity); 3) agreement of genotype frequencies with predictions of Hardy-Weinberg equilibrium; 4) Wright's (1978) F_{IS}, and F_{ST}; and 5) Nei's (1978) unbiased genetic distance. *F*-statistics for each locus were tested for significance using the formulae given by Waples (1987).

Mitochondrial DNA analyses

DNA was extracted from entire individuals (1-5 individuals per site) using the DNeasy Tissue kit (Qiagen Inc., Hilden, Germany) as per the manufacturer's instructions, with the exception that we incubated the sample at 56°C for 24 hours and used 60 µl to elute the DNA. PCR amplification was carried out using a 50 µl reaction volume consisting of 2 µl of DNA, 1×PCR buffer plus $MgCl_2$ (Roche, Penzberg, Germany), 2.2 mM $MgCl_2$, 0.2 mM of each dNTP (Boehringer Mannheim, Mannheim, Germany), 1.0 µM of each primer, and 1.0 unit of *Taq* DNA polymerase (Roche) on a Eppendorf Mastercycler gradient thermocycler. A 680 base pair fragment of the mitochondrial gene cytochrome *c* oxidase I (COI) gene was amplified using the primers CO1-2F (5'-TYGAYCCIDYIGGRGGAGGAGATCC-3') and CO1-2R (5'-GGRTARTCWGARTAWCGNCGWGGTAT-3')(Otto & Wilson 2001). The thermal cycling conditions were: initial denaturation at 94°C for 60 s; followed by 40 cycles of denaturation at 94°C for 20 s; annealing at 50°C for 30 s; and extension at 72°C for 90 s; with a final extension at 72°C for 5 min. Sequencing was performed using the same primers as those used for PCR amplification on an ABI 377XL automated sequencer (Applied Biosystems Inc., Foster City, CA) or a MegaBACE DNA Analysis System (Amersham Biosciences, Buckinghamshire, England) at the University of Waikato DNA sequencing facility.

Sequences were aligned using SEQUENCHER (Gene Codes ver. 4.1.2) and verified as being derived from insect DNA using GenBank BLASTn searches. We used χ^2-tests, as implemented in PAUP* 4.0b10 (Swofford 2002), to determine whether the assumption of equal base frequencies among sequences was violated on: (1) all sites;

(2) parsimony-informative sites only; and (3) with the third codon position only. We then constructed phylogenetic trees using PAUP* 4.0b10 (Swofford 2002). A maximum likelihood (ML) phylogram was constructed using the GTR+Γ model (-1nL = 2390: ti/tv = 3.106; Γ = 0.2286; with base frequencies A = 0.3273, C = 0.1606, G = 0.1596, T = 0.3524) (selected using Modeltest 3.7; Posada & Crandel 1998). A maximum parsimony (MP) analysis using the branch and bound search option with unweighted characters was also implemented. One dragonfly species (*Aeshna brevistyla*) was sequenced by us for use as an outgroup taxon (accession number: to be added), in addition to *Orthetrum triangulare melania* obtained from GenBank (accession number: AB126005). We compared the ML and MP trees in order to minimise the potential for error that may arise from assumptions inherent in phylogenetic reconstruction. Confidence in the cladistic analyses was assessed by estimation of the g_1 skewness statistic from 100,000 random tree length distributions (Hillis & Huelsenbeck 1992), and by bootstrap analysis with 100 pseudoreplicates (identical sequences were deleted to decrease analysis time) for the ML tree (Felsenstein 1985). A Templeton (Wilcoxon signed-ranks) test was used to determine if significant differences existed between the trees. All unique sequences have been submitted to GenBank (accession numbers to be added).

Results

Allozymes

Austrolestes colensonis and *Xanthocnemis zealandica* were the most widely distributed taxa and were collected from the North, South and Chatham Islands (Table 1). *Ischnura aurora aurora* was found only on the North Island (Table 1). All three genera were clearly discernible on the basis of allozyme data with fixed differences observed at several loci (Table 2). In the case of *I. a. aurora* and *X. zealandica*, fixed differences occurred at all loci. Two species of *Xanthocnemis* were found on Chatham Islands; one having the same allelic composition to *X. zealandica* found elsewhere in New Zealand, and a single individual (likely *X. tuanui*), showing fixed differences at 10 of 11 loci (Table 2). Of the 11 loci assessed, both *A. colensonis* and *I. a. aurora* showed no within-species allelic variability among sites (and hence appear as single entries in Tables 2 and 3). *Xanthocnemis zealandica* also showed limited allelic variability with only five of 11 loci showing more than one allele among all sites. Indeed, individuals from the South Island and Chatham Island sites showed variability at only two of 11 loci (Table 2). Measures of genetic variability were similarly limited (Table 3). The mean number of alleles per locus was < 1.3 in all cases and observed/expected heterozygosity was low (< 0.06 in all cases, and usually < 0.03)(Table 3). Percentage of polymorphic loci (95% criterion) was also low and ranged from 0-18% for *X. zealandica*. Frequencies of genotypes for all variable loci were in agreement with Hardy-Weinberg expectations (exact probability method).

Differentiation among populations of *X. zealandica* was low to moderate (mean F_{ST} = 0.09). Nei's (1978) distance values were < 0.01 in all cases suggesting minimal allelic differences among sites. No obvious pattern was found with respect to geographic location or habitat type (e.g., lotic versus lentic habitats).

mtDNA

A 598 base pair fragment of the COI mitochondrial gene was used in all analyses for the 34 damselflies from 15 sites. No insertions, deletions or stop codons were detected. There were 164 parsimony-informative sites with an additional 36 variable sites. A total of 27 different haplotypes were found with pairwise sequence divergences ranging from 0% to 18% (Table 5). The nucleotide composition of all sequences was: A = 31%, T = 33%, C = 17% and G = 18%. The assumption of homogeneity of base frequencies was supported using all sites (χ^2_{105} = 26.72, P = 1.00), parsimony informative sites (χ^2_{105} = 94.40, P = 0.76), and for the third codon sites (χ^2_{105} = 70.22, P = 1.00).

Divergence values (uncorrected) ranged from 0 to 1.6% among individuals of *X. zealandica* to greater than 15% between *Xanthocnemis* spp. and *Austrolestes*. Divergences between *X. zealandica* and a second *Xanthocnemis* species found in Chatham Islands (presumably *X. tuamuii*) was 7%. A Templeton (Wilcoxon signed-ranks) test examining tree similarity showed there were no significant differences between the ML and one of the 345 most parsimonious (chosen by closest similarity to the consensus tree) trees (P < 0.05). Here, we present the ML tree (Fig. 3), which

clearly showed the three genera forming separate groups, with *X. tuanuii* forming a sister group with *X. zealandica*.

Discussion

Levels of allozyme variability were low within all four discernible species, particularly in the case of *Austrolestes* and *Ischnura*. One possible explanation is that our sample size was too small for these latter species to detect any allelic variation (n = 11 and 10, respectively). However, the absence of variation even among widely separated sites (e.g., Chatham Islands versus North and South Islands), was surprising given that differences were partially expected based on previous work (e.g., Trewick 2000, Hogg et al. 2002). Accordingly, these available data suggest limited genetic variability within these genera. This was also true for *Xanthocnemis zealandica* (n = 151), which had very limited levels of allelic variation, low percentages of polymorphic loci (≤9% at 12 of 13 sites) and low heterozygosity (average = 2%). Low heterozygosity levels were slightly lower than those reported for other New Zealand aquatic insects (e.g., 5.7% for *Archicauliodes diversus*; Hogg et al. 2002), but comparable to those reported for the North American mayfly *Dolania americana* (average = 3%; Sweeney & Funk 1991). Allelic variability was comparable to levels previously reported for New Zealand freshwater insects (Smith & Collier 2001; Hogg et al. 2002). However, it was lower than the 3 to 4 alleles per locus reported for North American taxa (e.g., Robinson et al. 1992; Plague & McArthur 1998). Accordingly, limited genetic variability among New Zealand freshwater insects may be a prevalent feature and possibly the result of the insular nature of island habitats.

Levels of differentiation among habitats were also low. Wright's (1978) F_{ST} values were similar to those reported for New Zealand mayflies, but lower than the caddisfly *Orthopsyche fimbriata* and megalopteran *Archicauliodes diversus* (Smith & Collier

2001; Hogg et al. 2002). This suggests that levels of dispersal and hence gene flow, are adequate to maintain an almost homogenous population structure throughout New Zealand. The presence of both *Xanthocnemis* and *Austrolestes* on Chatham Islands also suggests that they have moderate dispersal capabilities as these islands have only been available for colonisation in the last 3-5 million years (Nelson et al. 1999). However, the limited distribution of some *Xanthocnemis* species (e.g., *X. sobrinia*)(Rowe 2000) suggests that dispersal or other factors (e.g., behaviour or habitat requirements) may have some influence on distribution.

On the basis of our survey, we were unable to find any genetic evidence for more than two *Xanthocnemis* species, although individuals of *Ischnura* and *Austrolestes* were commonly found. We caution, however, that we did not specifically target other *Xanthocnemis* species in our sampling protocol. Hence, the absence of these additional species, particularly in the case of *X. sinclairi*, could be an artefact of our sampling programme. However, it may also indicate that morphological differences (phenotypic variation) among some species are not related to any discernible genetic variability. Indeed, larvae of the four described species are morphologically indistinguishable (Rowe 2000). Furthermore, our work has highlighted the usefulness of genetic techniques in discerning between closely related species (e.g., *X. zealandica* and *X. tuanuii*). Future work specifically targeting other less common odonate species may be useful to more accurately assess their taxonomic status as well as levels of diversity within New Zealand.

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References

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39*: 783-791.

Freeland, J. R.; Conrad, K. F. 2002: Genetic similarity within and among populations of the Variable and Azure damselflies (*Coenagrion pulchellum* and *C. puella*). *Hydrobiologia 479*: 69-73.

Freeland, J. R.; May, M.; Lodge, R.; Conrad, K. F. 2003: Genetic diversity and widespread haplotypes in a migratory dragonfly, the common green darner *Anax junius*. *Ecological Entomology 28*: 413-421.

Geenen, S.; Jordaens, K.; De Block, M.; Stoks, R.; De Bruyn, L. 2000: Genetic differentiation and dispersal among populations of the damselfly *Lestes viridis* (Odonata). *Journal of the North American Benthological Society 19:* 321-328.

Hebert, P. D. N.; Beaton, M. J. 1993: Methodologies for allozyme analysis using cellulose acetate electrophoresis: a practical handbook. Beaumont, Texas, Helena Laboratories.

Hogg, I. D.; Willmann-Huerner, P.; Stevens, M. I. 2002: Population genetic structures of two New Zealand stream insects: *Archicauliodes diversus* (Megaloptera) and *Coloburiscus humeralis* (Ephemeroptera). *New Zealand Journal of Marine and Freshwater Research 36:* 491-501.

Hughes, J. M.; Bunn, S. E.; Hurwood, D. A.; Cleary, C. 1998: Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. *Freshwater Biology 39:* 117-127.

Jackson, J. K.; Resh, V. H. 1998: Morpologically cryptic species confound ecological studies of the caddisfly genus *Gumaga* (Trichoptera: Sericostomidae) in northern California. *Aquatic Insects 20:* 69-84.

Nei, M. 1972: Genetic distance between populations. *The American Naturalist 106*: 283-292.

Otto, J. C. & Wilson, K. J. 2001: Assessment of the usefulness of ribosomal 18S and mitochondrial COI sequences in Prostigmata phylogeny. In: Halliday, R.B., Walter, D.E., Proctor, H.C., Norton, R.A., Colloff, M.J. (eds) Acarology: Proceedings of the 10th International Congress. pp.100-109, CSIRO publ. Melbourne 2001.

Plague, G. R.; McArthur, J. V. 1998: Genetic diversity versus geographic distribution of five congeneric caddisflies. *Hydrobiologia 362:* 1-8.

Richardson, B. J.; Baverstock, P. R.; Adams, M. 1986: Allozyme electrophoresis. A handbook for animal systematics and population studies. California, Academic Press Inc.

Robinson, C. T.; Read, L. M.; Minshall, G. W. 1992: Influence of flow regime on life history, production, and genetic structure of *Baetis tricaudatus* (Ephemeroptera) and *Hesperoperla pacifica* (Plecoptera). *Journal of the North American Benthological Society 11:* 278-289.

Rowe, R. J. 1987: The dragonflies of New Zealand. Auckland University Press. Auckland.

Rowe, R. J. 2000: Odonata (damselflies and dragonflies) key to larvae. *Bulletin of the Entomological Society of New Zealand 13:* 13-16.

Smith, P. J.; Collier, K. J. 2001: Allozyme diversity and population genetic structure of the caddisfly *Orthopsyche fimbriata* and the mayfly *Acanthophlebia cruentata* in New Zealand streams. *Freshwater Biology 46:* 795-805.

Sweeney, B. W.; Funk, D. H. 1991: Population genetics of the burrowing mayfly *Dolania americana*: geographic variation and the presence of a cryptic species. *Aquatic Insects 13:* 17-27.

Sweeney, B. W.; Funk, D. H.; Vannote, R. L. 1987: Genetic variation in stream mayfly (Insecta: Ephemeroptera) populations of eastern North America. *Annals of the Entomological Society of America 80:* 600-611.

Swofford, D. L.; Selander, R. B. 1981: BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and biochemical systematics. *Journal of Heredity 72:* 281-283.

Trewick, S. 2000: Molecular evidence for dispersal rather than vicariance as the origin of flightless insect species on the Chatham Islands, New Zealand. *Journal of Biogeography 27:* 1189-1200.

Waples, R.S. 1987: A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution, 41:* 385-400.

Watts, P. C.; Rouquette, J. R.; Saccheri, J.; Kemp, J.; Thompson, D. J. 2004: Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion mercuriale*. *Molecular Ecology 13:* 2931-2945.

Wilcock, H.R.; Hildrew, A.G.; Nichols, R.A. 2001: Genetic differentiation of a European caddisfly: past and present gene flow among fragmented larval habitats. *Molecular Ecology 10*: 1821-1834.

Wright, S. 1978: *Evolution and the genetics of populations, vol. 4. Variability within and among natural populations.* University of Chicago Press, Chicago.

Table 1 Sampling sites for damselflies *Xanthocnemis* spp. (X), *Austrolestes colensonis* (A), and *Ischnura aurora aurora* (I). Site codes refer to Figure 1 (C = Chatham Island; N = North Island; S = South Island).

Site	Name	Date	Lat (S)	Long (E)	Habitat	Species	Allozyme	mtDNA
C1	Point Gap	25/2/00	44°04'	176°23'	2nd order stream	X	Xx11 Ax8	Xx3 Ax1
N1	Dargarville	16/2/00	35°55'	173°54'	2nd order farm stream	X,A	Xx13 Ax4	Xx2
N2	Whangerai	18/2/00	35°43	174°19'	4th order stream	X,I	Xx22 Ix1	Xx2
N3	Whirinaki Armco culvert	17/2/00	35°28'	173°28'	2nd order stream	X,I	Xx6 Ix3	Xx2 Ix1
N4	Mangatina Scenic reserve	17/2/00	35°12'	173°30'	Spring-fed 1st order stream	X	Xx12	Xx1
N5	Nr Kaitia	17/2/00	35°07'	173°17'	2nd order stream	X	Xx17	Xx1
N6	Houhora	17/2/00	34°48'	173°07'	2nd order stream	X,I	Xx4 Ix1	Xx1
N7	Waiwhakaiho River	19/9/99	39°02'	174°08'	2nd order stream	X	Xx3	
N8	Lake Waahi	11/8/ 98	37°34'	175°08'	Small Lake	X,I	Xx5 Ix4	Xx3
N9	Waitoa River	12/8/ 98	37°31'	175°38'	2nd order stream	X,I	Xx20 Ix2	Xx5 Ix1
N10	Whanganui River	18/11/01	39°55'	175°01'	3rd order stream	A		Ax1

N11	Piako River	9/5/05	37°39'	175°32'	River	X		Xx3
S1	Heritage Park Lodge Pond	18/1/98	43°52'	169°01'	Pond	X	Xx9	Xx1
S2	Lake Ruatanewha (Twizel)	18/1/98	44°17'	170°04'	Lake	X,A	Xx17 Ax1	
S3	Butchers Dam	16/1/98	45°18'	169°20'	Small lake	X	Xx12	Xx2
S4	Waihopai River	1/04/01	46 °39	168 °38	River	A		Ax2
S5	Lee Creek	12/12/98	45 °55	170 °10	Creek	X		Xx2

Table 2 Allele frequencies at 11 allozyme loci for damselflies (Odonata) collected from 14 sites in New Zealand. Site codes (for *Xanthocnemis zealandica*) refer to Fig. 1, A = *Austrolestes*, I = *Ischnura*, Xt = *X. tuanuii*. Sample sizes (*N*) are shown immediately below header row

	CI	N1	N2	N3	N4	N5	N6	N7	N8	N9	S1	S2	S3	A	I	Xt
N	11	13	22	6	12	17	4	3	5	20	9	17	12	13	11	1
IDH-1																
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
B														1.00	1.00	1.00
IDH-2																
A															1.00	
B												0.03				
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00		
D																1.00
LDH																
A															1.00	
B			0.16	0.17	0.21	0.03		0.17		0.08						
C	1.00	1.00	0.84	0.83	0.79	0.97	1.00	0.83	1.00	0.92	1.00	1.00	1.00			1.00
D														1.00		
6PGDH																
A															1.00	
B														1.00		
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00
GPDH																
A														1.00		
B															1.00	
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00

PEP-1																
A								0.17								1.00
B	0.95	1.00	1.00	1.00	1.00	1.00	1.00	0.83	1.00	1.00	1.00	1.00	1.00	1.00		
C	0.05															
D															1.00	
PEP-2																
A															1.00	
B														1.00		1.00
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
PGM																
A														1.00		
B															1.00	
C	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00			
D																1.00
MDH-1																
A															1.00	
B	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
C																1.00
MDH-2																
A	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.78	1.00	1.00			
B											0.22				1.00	
C										0.03				1.00		1.00
GPI																
A		0.04	0.02												1.00	
B										0.03						
C	0.94	0.96	0.98	1.00	1.00	1.00	0.87	1.00	1.00	0.97	0.94	0.97	1.00			
D														1.00		
E	0.06						0.13				0.06	0.03				
F																1.00

Table 3 Measures of genetic variability based on 11 allozyme loci for *Xanthocnemis zealandica* collected from 13 sites throughout New Zealand. Site designations refer to Table 1.

Population	Mean sample size/ locus	Mean no. of alleles/locus	% polymorphic loci	H_{obs}	H_{exp}
C1	10.4 (0.4)	1.2 (0.1)	9	0.02 (0.01)	0.02 (0.01)
N1	11.9 (1.0)	1.1 (0.1)	0	0.01 (0.01)	0.01 (0.01)
N2	18.5 (1.2)	1.2 (0.1)	9	0.03 (0.02)	0.03 (0.03)
N3	6.0 (0.4)	1.1 (0.1)	9	0.03 (0.03)	0.03 (0.03)
N4	9.6 (0.9)	1.1 (0.1)	9	0.04 (0.04)	0.03 (0.04)
N5	15.4 (1.0)	1.1 (0.1)	0	0.01 (0.01)	0.01 (0.01)
N6	3.7 (0.1)	1.1 (0.1)	9	0.02 (0.02)	0.02 (0.02)
N7	2.9 (0.1)	1.2 (0.2)	18	0.06 (0.04)	0.06 (0.04)
N8	5.0 (0.0)	1.0 (0.0)	0	0.00 (0.00)	0.00 (0.00)
N9	19.3 (0.3)	1.3 (0.1)	9	0.02 (0.01)	0.02 (0.01)
S1	6.8 (0.2)	1.1 (0.1)	9	0.01 (0.01)	0.01 (0.01)
S2	16.1 (0.7	1.2 (0.1)	0	0.01(0.01)	0.01 (0.01)
S3	11.2 (0.4)	1.0 (0.0)	0	0.00 (0.00)	0.00 (0.00)

Table 4 Wright's (1978) F_{IS} and F_{ST} values based on 11 allozyme loci for *Xanthocnemis zealandica* collected from 13 sites in New Zealand. Site designations refer to Table 1. Only significant deviations from zero are shown, all others were not significantly different to zero ($P > 0.05$).

Locus	F_{IS}	F_{ST}
IDH-2	-0.03	0.027
LDH	0.13	0.107 ($P < 0.01$)
PEP-1	-0.16	0.129 ($P < 0.001$)
MDH-2	0.03	0.02
GPI	-0.08	0.05
Mean	-0.12	0.09 ($P < 0.05$)

Table 5 Average pairwise divergence values (uncorrected p values) for *Austrolestes colensonis*, *Ischnura aurora aurora*, *Xanthocnemis tuanuii* and *Xanthocnemis zealandica*.

	A. a. aurora	*I. colensonis*	*X. tuanuii*	*X. zealandica*
A. a. aurora	0.007-0.034			
I. colensonis	0.180-0.185	0.002		
X. tuanuii	0.176-0.184	0.140-0.142	-	
X. zealandica	0.160-0.180	0.151-0.158	0.063-0.074	0.002-0.010

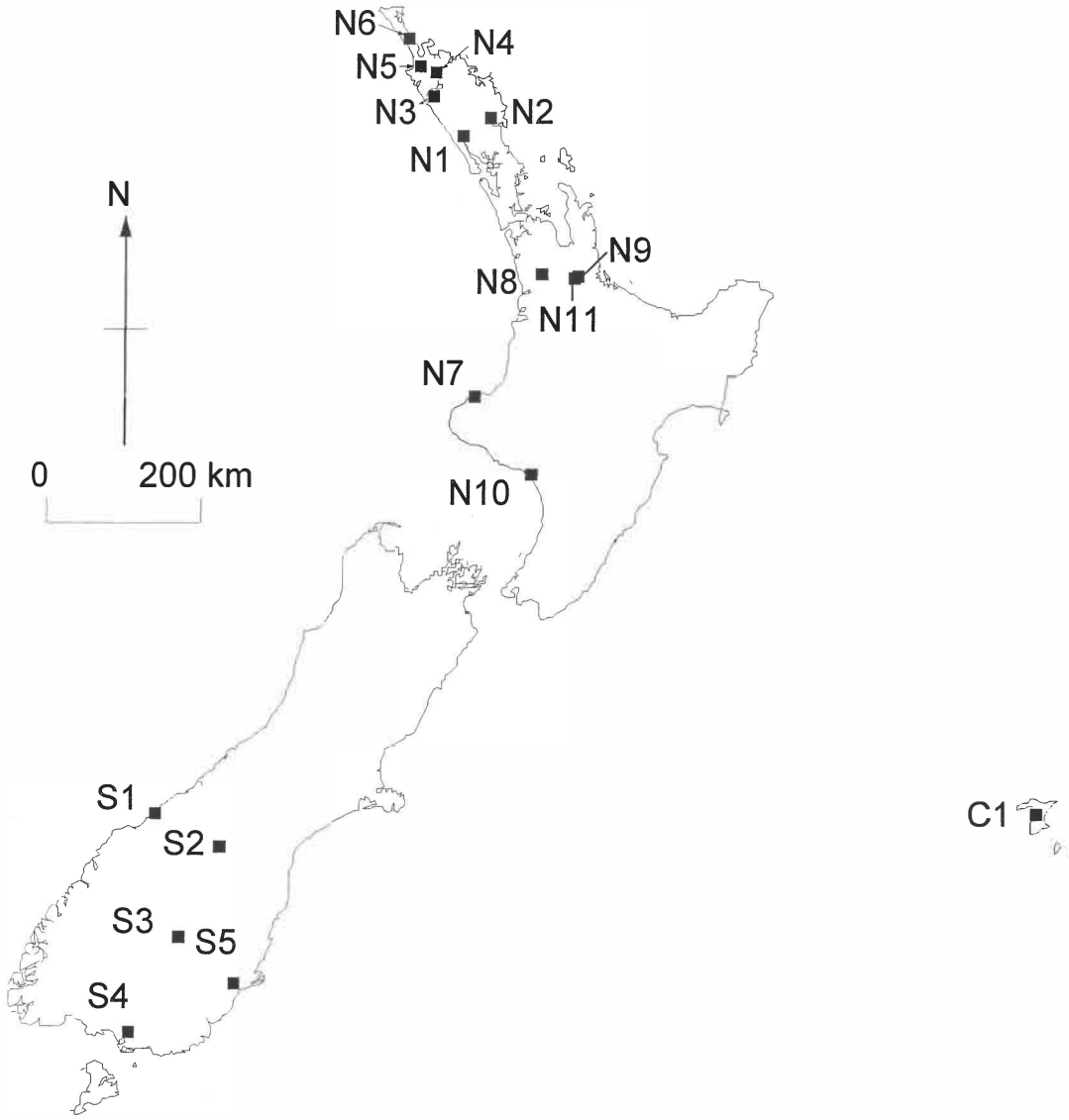
List of Figures:

Fig. 1 Study area, showing collection sites for damselflies on the North Island and South Island of New Zealand. Further details for each site are provided in Table 1.

Fig. 2 UPGMA dendrogram for *Xanthocnemis zealandica* on the North Island and South Island of New Zealand based on an analysis of 11 allozyme loci using Nei's (1978) genetic distance. Site codes refer to Figure 1.

Fig. 3 Maximum likelihood (ML) tree for the three New Zealand damselfly genera: *Xanthocnemis, Ischnura* and *Austrolestes* based on a 598-bp fragment of the mitochondrial DNA (COI) gene with bootstrap values (100 replicates) shown at the nodes. Site codes refer to Figure 1.

Fig. 1 Nolan et al.



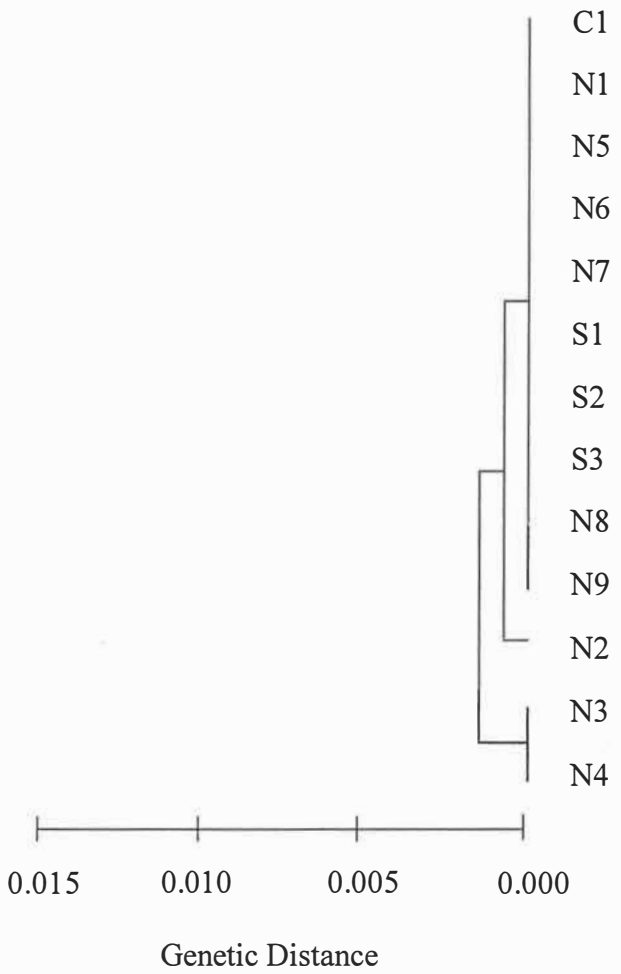


Fig. 2 Nolan et al.

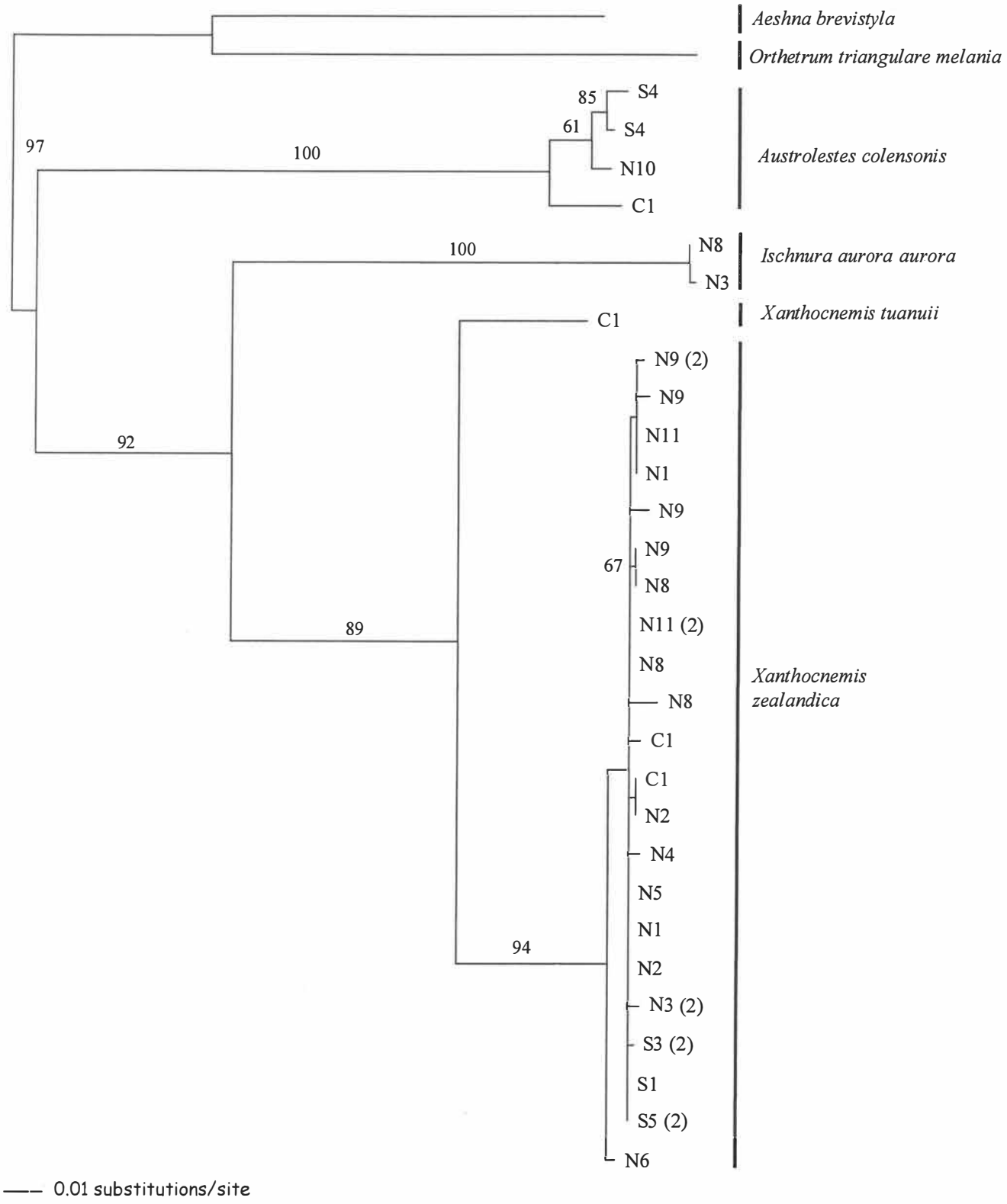


Fig. 3 Nolan et al.

Summary

This thesis demonstrates the application of molecular techniques by examining the genetic variability found within and among invertebrate populations in Antarctica and New Zealand. Such data enable us to suggest how the distribution of organisms has been affected by past environmental change, which in turns allows us to predict future consequences for populations which exist in increasingly insular situations. Fragmentation of habitat has occurred in the past during glacial periods (as discussed in Chapter 1); but the impact of human activity is accelerating the isolation of populations. If we are to be concerned with the fate of other species in the biosphere, it is vital that we understand the role of genetic variability relative to species' responses to environmental change. Such information will be critical in making informed decisions for the conservation of the earth's biological resources.

Chapter I used this information to examine how historic environmental change affected the genetic structure of an Antarctic springtail. It suggested a possible dispersal mechanism that may have maintained gene flow among populations and explained their current distribution relative to glacial refugia during the Pleistocene. By understanding how this species was affected during past environmental change, we will be able to more meaningfully assess the 'health' of the species and to manage effectively for the future to mitigate any undesirable ecological consequences.

Chapter II used the information to demonstrate that the targeted damselfly species can be clearly identified on the basis of molecular techniques (i.e. allozymes, mtDNA). It also suggested relatively low levels of genetic variability within species as well as potentially high levels of gene flow among

locations. In addition it provides a benchmark for assessing the genetic variability of other, less common odonate species in New Zealand.

Future work based on the results of this thesis could include: 1) further sampling of mtDNA haplotypes to the north and south of Taylor Valley, in Victoria Land Antarctica to further clarify the role of glacial refugia and to identify areas as priorities for conservation efforts (i.e. biodiversity "hotspots"); and 2) targeting other less common odonate species to more accurately assess existing levels of genetic and species diversity within New Zealand.