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Kelvin M. Lloyd
Landcare Research, Dunedin, New Zealand

Angela M. Hunter
University of Otago, Dunedin, New Zealand

David A. Orlovich
University of Otago, Dunedin, New Zealand

Suzanne J. Draffin
University of Otago, Dunedin, New Zealand

Alan V. Stewart
PGG Seeds, Christchurch, New Zealand

See next page for additional authors

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PHYLOGENY AND BIOGEOGRAPHY OF ENDEMIC *FESTUCA* (POACEAE) FROM NEW ZEALAND BASED ON NUCLEAR (ITS) AND CHLOROPLAST (*trn*L–*trn*F) NUCLEOTIDE SEQUENCES

KELVIN M. LLOYD,[1,4] ANGELA M. HUNTER,[2] DAVID A. ORLOVICH,[2] SUZANNE J. DRAFFIN,[2] ALAN V. STEWART,[3] AND WILLIAM G. LEE[1]

[1]*Landcare Research, Private Bag 1930, Dunedin, New Zealand*; [2]*Department of Botany, University of Otago, PO Box 56, Dunedin, New Zealand;* [3]*PGG Seeds, PO Box 3100, Christchurch, New Zealand*
[4]*Corresponding author (lloydk@landcareresearch.co.nz)*

ABSTRACT

We investigated the phylogenetic relationships of the endemic New Zealand (NZ) species of *Festuca* (Poaceae, Pooideae) by assessing sequence variation from the nuclear internal transcribed spacers (ITS) and a chloroplast intergenic spacer (*trn*L–*trn*F) and by measuring DNA content using flow cytometry. The ITS and *trn*L–*trn*F data sets were congruent in showing that the NZ species of *Festuca* have two origins. One group, containing *F. coxii, F. luciarum, F. multinodis,* and *F. ultramafica,* is closely related to *Festuca* sect. *Aulaxyper.* The other group includes a clade of five endemic species (*F. actae, F. deflexa, F. madida, F. matthewsii, F. novae-zelandiae*) and one species (*F. contracta*) with a circum-Antarctic distribution. The North American species *F. californica* is sister to the latter group in the *trn*L–*trn*F phylogeny but not so in the ITS phylogeny. The differentiation of endemic NZ species into two groups is supported by differences in chromosome number and genome size, the latter showing an inverse relationship to ploidy level. We discuss the ecology and biogeography of NZ's endemic species of *Festuca.* Origin from Northern Hemisphere ancestors via dispersal to NZ through the American continents is a plausible hypothesis based on current information.

Key words: *Austrofestuca,* biogeography, ecology, *Festuca,* genome size, ITS, phylogeny, ploidy, Poeae, *trn*L–*trn*F.

INTRODUCTION

New Zealand (NZ) has nine endemic species of *Festuca* (Poaceae, Pooideae), a large (ca. 450 spp.) genus of grasses that is prominent in temperate regions of the world. The phylogenetic relationships of the NZ species have been unknown. Morphologically, species of *Festuca* can be separated into two major groups: those with broad leaves and those with fine leaves. New Zealand's indigenous species are all fine leaved (Edgar and Connor 2000), most having an erect, tufted habit but others forming swards. They occur from sea level to the alpine zone, and are found in a range of habitats including coastal rocks, inland cliffs, ultramafic (serpentine) and calcareous areas, and tall-tussock (dominated by *Chionochloa* Zotov) and short-tussock (dominated by *Festuca*/*Poa*) grasslands. One species (*F. coxii*) previously has been placed in *Agropyron* Gaertn. on account of morphological features not shared with other NZ species of *Festuca,* and uncertainty has existed regarding its generic position (Edgar and Connor 2000).

Molecular evidence has shown that the broad-leaved species of *Festuca* are related to *Lolium* and *Schedonorus* and that *Festuca* s.l. does not form a monophyletic group without inclusion of these genera (Darbyshire and Warwick 1992; Stammers et al. 1995; Charmet et al. 1997; Gaut et al. 2000; Torrecilla and Catalán 2002). *Dactylis* is either sister to, or nested within, the broad-leaved *Festuca*/*Lolium*/*Schedonorus* group (Hsiao et al. 1995; Charmet et al. 1997; Torrecilla and Catalán 2002). Most analyses place fine-leaved species of *Festuca* (and related ephemerals) as sister to the broad-leaved *Festuca*/*Lolium*/*Schedonorus* complex (Charmet et al. 1997; Gaut et al. 2000; Torrecilla and Catalán 2002), but relationships within the group of fine-leaved *Festuca* have only recently begun to receive attention (Torrecilla and Catalán 2002; Torrecilla et al. 2003, 2004; Catalán et al. 2004). Three groups of fine-leaved *Festuca* corresponding to sect. *Aulaxyper,* sect. *Eskia,* and sect. *Festuca* were identified on the basis of internal transcribed spacer (ITS) region (nrDNA) sequence data by Torrecilla and Catalán (2002). More recently, Catalán et al. (2004) have shown that the fine-leaved *Festuca* clade includes a large number of additional, mostly annual, genera (*Ctenopsis, Cutandia* Willk., *Helleria, Micropyrum, Narduroides, Psilurus, Vulpia, Wangenheimia*), while Torrecilla et al. (2004) showed that *Vulpia* is polyphyletic within the clade of paraphyletic fine-leaved *Festuca.*

Northern Hemisphere taxa have dominated previous molecular studies of *Festuca.* This geographical bias is unfortunate because *Festuca* and other members of Pooideae are also important genera in temperate areas of the Southern Hemisphere (Hartley 1961, 1973). Inclusion of species from both Northern and Southern hemispheres will help to evaluate hypotheses concerning processes of speciation and biogeography. Indeed, Catalán et al. (2004) have recently found a close and unexpected relationship between three South American species (the fine-leaved *Helleria fragilis* and broad-leaved *F. coromotensis* and *F. elviae*) and Northern Hemisphere species in the fine-leaved *Festuca* sect. *Aulaxyper.*

While the origin of the grass family is thought to be South American (Soreng and Davis 1998; Hsiao et al. 1999), the center of diversity for Pooideae is Eurasia (Hartley 1961, 1973; Soreng 1990), and Loliinae lineages such as *Festuca* appear to have arisen in the Mediterranean region (Catalán et al. 2004). Connor and Edgar (1986) assumed that Australasian *Festuca* and *Poa* originated from Northern Hemi-

sphere ancestors that dispersed southward. Chloroplast restriction site data for one Australasian species of *Poa* supports this assertion (Soreng 1990), but it is not known whether the NZ species of *Festuca* have arisen from northern forms that dispersed southward relatively recently or whether they are the evolutionary products of long isolation in the Southern Hemisphere.

Accordingly, we included all nine endemic NZ species of *Festuca* (*F. actae, F. coxii, F. deflexa, F. luciarum, F. madida, F. matthewsii, F. multinodis, F. novae-zelandiae, F. ultramafica*) in this study, as well as one fine-leaved circum-Antarctic species that is indigenous to the NZ region (*F. contracta*), and one species of *Austrofestuca,* an Australasian genus that was segregated from *Festuca* by Alexeev (1976) but which has recently been shown to be aligned with *Poa* (Hunter et al. 2004). Also included were Northern Hemisphere representatives of the following genera: *Ctenopsis, Dactylis, Festuca, Lolium, Micropyrum, Narduroides, Poa, Vulpia,* and *Wangenheimia,* as well as South American *Helleria fragilis,* weighting our taxonomic sampling toward the fine-leaved lineages.

Our primary aims were to (1) test whether NZ's endemic *Festuca* taxa form a monophyletic group and examine the generic placement of *F. coxii,* (2) discover the closest relatives of these taxa and thus identify their putative origins, and (3) examine relationships between Southern and Northern hemisphere *Festuca* lineages. We present the results of phylogenetic analyses using sequences of the ITS region of nuclear ribosomal DNA and the *trn*L–*trn*F intergenic spacer of chloroplast DNA. We also used flow cytometry to measure the DNA contents of the endemic NZ species of *Festuca* and compared these with chromosome numbers to examine variation in genome size.

MATERIALS AND METHODS

Our study included representatives from six subgenera and nine sections of *Festuca,* three species of *Schedonorus,* two species of *Vulpia,* and a single species each of *Austrofestuca, Brachypodium, Ctenopsis, Dactylis, Helleria, Lolium, Micropyrum, Narduroides, Poa, Psilurus,* and *Wangenheimia.* Three populations of *Austrofestuca littoralis* from NZ and Australia were included, and all four subspecies of the NZ endemic, *F. matthewsii,* resulting in a total of 81 sampled taxa used in the analyses. A list of DNA sequences and their sources is shown in Table 1. The majority of these were obtained from the National Center for Biotechnology Information database (GenBank), but we sequenced all of the indigenous species of *Festuca* (13 taxa), as well as *F. rubra* and *Vulpia bromoides,* two species that are naturalized in NZ. Live specimens were obtained from field collections or grown from seed and propagated outdoors in pots. For *F. contracta,* DNA was extracted from a herbarium specimen.

DNA was extracted from small samples of young leaf-base material following the method of Carlson et al. (1991). RNA was removed by incubation with DNase-free RNase (F. Hoffman La Roche, Ltd., Basel, Switzerland) for 15 min at 37°C. Polymerase chain reactions were done in 20 μL volumes, using AmpliTaq Gold PCR Master Mix (Applied Biosystems, Inc., Foster City, California, USA) following the manufacturer's instructions. Each reaction contained 8 pmol of each primer. The ITS regions were amplified using the primers ITS-1 and ITS-4 from White et al. (1990) and the following amplification protocol: 95°C for 5 min, 35 cycles of 94°C for 45 sec, 48°C for 45 sec, 72°C for 2 min 10 sec, followed by a final extension of 72°C for 10 min. To amplify the spacer between the *trn*L (UAA) 3′ exon and *trn*F (GAA) gene of chloroplast DNA, we followed the protocol of Taberlet et al. (1991) using primers ''e'' and ''f'' and an annealing temperature of 53°C. PCR products were purified using spin column purification (QIAquick PCR Purification Kit, QIAGEN Pty, Ltd., Clifton Hill, Victoria, Australia). The approximate concentration of each amplification product was determined by electrophoresis with a known amount of molecular marker (1 kilobase ladder, Invitrogen New Zealand Limited, Auckland, NZ). The purified PCR products were sequenced, using the original amplification primers, by the Centre for Gene Research, Department of Microbiology, University of Otago, NZ. Reactions were done in 20 μL volumes using Big Dye Terminator vers. 3.0 cycle-sequencing chemistry (Applied Biosystems) following the manufacturer's protocol. Electropherograms were edited and assembled using Autoassembler vers. 1.3.0 (Applied Biosystems).

Seventy-seven taxa were included in the ITS data set and 74 in the *trn*L–*trn*F data set. Seven species (*Festuca dalmatica, F. filiformis, F. heterophylla, F. lemanii, F. pallens, F. pseudodalmatica, F. rupicaprina*) were represented only by ITS sequences, while *F. ampla, F. caerulescens, F. coromotensis, F. elviae,* and the Kaitorete Spit population of *Austrofestuca littoralis* were only represented in the *trn*L–*trn*F data set. The *trn*L–*trn*F data set included many sequences from GenBank that incorporated the *trn*L intron. This region was included in the analysis, although the corresponding sequences were missing for the species sequenced in this study. Sequences were aligned initially using CLUSTAL_X vers. 1.83 (Thompson et al. 1997) followed by manual adjustment using Se-Al vers. 2.0a11 (Rambaut 2002). Gaps were treated as missing data and informative insertions/deletions (indels) were coded as binary (or in two cases, multistate) characters and added to the data matrix. We analyzed the ITS and *trn*L–*trn*F data sets separately by maximum parsimony using PAUP* vers. 4.0b10 (Swofford 2002), with *Brachypodium distachyon* (Brachypodieae) used as the outgroup in each analysis. Within both data sets we conducted heuristic searches using 1000 random-addition replicates and the tree-bisection-reconnection branch-swapping algorithm. Branch support was assessed by parsimony bootstrap analysis with 1000 replicate heuristic analyses using two random-addition replicates in each bootstrap replicate.

Relative nuclear DNA content was determined for the NZ taxa (Table 2) by flow cytometry (PA-II, Partec, Münster, Germany). Plant nuclei were first isolated by chopping leaf tissue in an extraction buffer using a razor blade. DNA was stained with a 4′,6′-diamidino-2-phenylindole dye and the extract was filtered through a 0.30 μm sieve prior to analysis. The nuclear extraction buffer and staining solution were supplied in a kit (Partec CyStain UV Precise T kit) and used according to the manufacturer's instructions. All samples were analyzed immediately after isolation and the instrument was aligned daily with flow check beads (Partec) labeled with a defined fluorescence intensity. An internal standard

was used for each sample to account for machine drift and/or differences in the turbidity of the sample flow. *Trifolium repens* L. var. 'Grasslands Huia' was used as the standard as it was known to have a 2C-value of 2.38 picograms (pg) (Campbell et al. 1999). For each sample, a leaf segment of this reference tissue was co-chopped with the test sample, and the ratio of the test sample to the standard peak channel was used to compute the 2C-values for each test sample. Between 5000 and 10,000 nuclei were analyzed from each sample. The coefficient of variation of the 2C peaks used to estimate C-values in all samples was below 6%.

Festuca madida, listed as tetraploid by de Lange and Murray (2002), had a 2C-value similar to those of hexaploid taxa. Consequently, the chromosome number for *F. madida* was re-determined by R. Pickering (Crop and Food Research, Lincoln, NZ). Mitotic chromosome preparations were made from root tips that had been pretreated in iced water for 24 hr, fixed in 3 : 1 ethanol : acetic acid for 48 hr, hydrolyzed in 1N HCl for 10 min at 60°C, and squashed in 1% aceto-carmine.

RESULTS

ITS Analysis

In most species, amplification produced a fragment 630–700 base pairs (bp) long that included partial sequences of the 18S and 26S genes flanking the ITS region. The ITS-1 region was 216–219 bp long in most taxa, but longer in *Vulpia bromoides* (220 bp) and *Festuca madida* (225 bp). The ITS-2 region was generally 211–213 bp long, but shorter in *F. madida* (209 bp) and longer in *F. multinodis* (225 bp). Sequences were truncated for *F. matthewsii* subsp. *latifundii* and the Wekakura population of *Austrofestuca littoralis* (ITS-1 only), *F. deflex*a and *F. ultramafica* (partial ITS-1 and partial ITS-2), and *F. contracta* (partial ITS-2 only). Alignment of trimmed ITS sequences and insertion of gaps yielded a matrix with 660 characters, of which 157 (including 19 binary indel characters) were parsimony informative. A heuristic search found 480 equally parsimonious trees of length 622 and with a consistency index of 0.466 excluding uninformative characters.

In the ITS strict consensus tree (Fig. 1) the group of broad-leaved taxa is well resolved and supported by bootstrap (bs) values, but resolution and support was generally poor among fine-leaved taxa. A strongly supported (bs 100%) *Austrofestuca/Poa pratensis* clade is sister to the other ingroup taxa, which are also well supported (bs 80%) as a group. *Dactylis glomerata* is sister (bs <50%) to the remaining taxa in this group, which are split into two clades corresponding to broad-leaved *Festuca/Lolium/Schedonorus* (bs <50%), and fine-leaved *Festuca* and allied genera (the FEVRE group of Catalán et al. 2004 and Torrecilla et al. 2004). A feature of the generally well-resolved broad-leaved clades is the mixing of species representing different subgenera of *Festuca* (Table 1).

The clade of fine-leaved FEVRE (*Festuca, Vulpia,* and Related Ephemerals) taxa is well supported (bs 79%) as a group, but its higher order branches lack bootstrap support and only a few clades are resolved within it. Notably, the NZ taxa are split into two different groups. Four endemic species (*F. coxii, F. luciarum, F. multinodis, F. ultramafica*) are included within a weakly supported (bs 68%) clade loosely corresponding to *Festuca* sect. *Aulaxyper.* The remaining indigenous species (the endemic *F. actae, F. deflexa, F. madida, F. matthewsii,* and *F. novae-zelandiae,* and circum-Antarctic *F. contracta*) are resolved together within a well-supported (bs 90%) clade in which *F. madida* has a sister relationship (bs 74%) to the other ''southern endemic'' taxa, which are unresolved. Three additional lineages of fine-leaved taxa are identifiable. The first corresponds to a weakly supported (bs 61%) *Festuca* sect. *Festuca* and includes a strongly supported (bs 93%) group of mainly eastern European species (*F. dalmatica, F. pseudodalmatica, F. lemanii, F. pallens, F. rupicaprina*). The second clade (bs <50%) comprises species from *Festuca* sects. *Eskia* and *Amphigenes* (*F. carpathica, F. dimorpha, F. eskia, F. gautieri, F. quadriflora*). The third clade (bs 100%) contains two ephemeral species (*Psilurus incurvus, Vulpia myuros*).

trn*L*–trn*F Analysis*

Amplification produced a fragment 331–465 bp long, which in most species included a portion of the *trn*L intron, the *trn*L 3′ exon, the *trn*L–*trn*F intergenic spacer, and a portion of the *trn*F gene. Among NZ taxa of *Festuca,* the *trn*L–*trn*F spacer was generally 251–252 bp long, but was slightly longer in *F. ultramafica* (259 bp) and much longer (282 bp) in *F. deflexa, F. matthewsii* subsp. *latifundii,* and *F. matthewsii* subsp. *pisamontis.* These sequences were aligned with the longer sequences, incorporating the whole *trn*L intron, obtained from GenBank (Table 1). Twenty-eight informative indel characters (26 binary, 2 multistate) were added to the aligned matrix to give 1112 characters in total, of which 161 were parsimony informative. A heuristic search found 6520 equally parsimonious trees of length 416 and with a consistency index of 0.534 excluding uninformative characters. Figure 2 shows the strict consensus of these trees.

The *trn*L–*trn*F tree (Fig. 2) is broadly similar to the ITS tree (Fig. 1), with a strongly supported (bs 100%) *Austrofestuca littoralis/Poa pratensis* clade again occupying a sister relationship to the rest of the ingroup, and *Dactylis glomerata* again sister (bp 75%) to the remaining species. The chief difference surrounds the ''southern endemic'' clade, which here, along with *Festuca californica,* are sister (bp <50%) to the broad-leaved *Festuca/Lolium/Schedonorus* clade. Together, these clades are sister to the large FEVRE clade of fine-leaved *Festuca* and allied genera. *Festuca californica* occupies a weakly supported (bp 67%) sister position to the well-supported (bp 80%) ''southern endemic'' clade. The *trn*L–*trn*F data provide some structure within the ''southern endemic'' clade: *Festuca deflexa* and two subspecies of *F. matthewsii* (subspp. *latifundii* and *pisamontis*) form a clade that is sister to the other taxa and share a unique 30 bp repeat near the 3′-end of the intergenic spacer.

The broad-leaved *Festuca/Schedonorus/Lolium* subclades are nested in this analysis, with *Festuca font-queri/F. mairei/Lolium/Schedonorus* occupying a derived position, and *F. altissima, F. caerulescens, F. drymeja, F. lasto, F. scariosa,* and *F. triflora* unresolved at the base. Each division in the broad-leaved clade is well supported by a bootstrap value (71–94%), unlike the ITS tree where relationships among the

Table 1. Sources of DNA sequences used in the analyses. Voucher numbers cited refer to specimens in the University of Otago (OTA) and Landcare Research New Zealand (CHR) herbaria.

Taxon	Source/Voucher	Reference	GenBank accession number	
			ITS	*trn*L–*trn*F
Austrofestuca E. B. Alexeev				
A. littoralis (Labill.) E. B. Alexeev	GenBank	Hunter et al. (2004)		AY528933
	GenBank	Hunter et al. (2004)	AY327791	AY327796
	GenBank	Hunter et al. (2004)	AY524824	AY528934
Festuca L.				
Subgen. *Festuca*				
Sect. *Festuca*				
Subsect. *Festuca*				
F. alpina Suter	GenBank	Torrecilla and Catalán (2002)	AF303415	AF478524
F. clementei Boiss	GenBank	Catalán et al. (2004)	AF478482	AF478524
F. dalmatica (Hack.) Richt.	GenBank	Galli et al. (unpubl. data)	AY254371	
F. filiformis Pourr.	GenBank	Charmet et al. (1997)	AJ240160	
F. frigida (Hack.) K. Richt.	GenBank	Torrecilla et al. (2003)	AF478481	AF478521
F. glacialis Miégev. ex Anon.	GenBank	Torrecilla and Catalán (2002)	AF303428	AF478523
F. idahoensis Elmer	GenBank	Catalń et al. (2004)	AF147177	AF533064
F. indigesta Boiss.	GenBank	Torrecilla and Catalán (2002)	AF303426	
	GenBank	Catalán et al. (2004)		AF478519
F. lemanii Bastard	GenBank	Tredway et al. (unpubl. data)	AF147136	
F. longiauriculata Fuerte, Ortúñez et Ferrero	GenBank	Catalán et al. (2004)	AF478479	AF478518
F. ovina L.	GenBank	Hunter et al. (2004)	AY327792	AY327798
F. plicata Hack.	GenBank	Catalán et al. (2004)	AF478483	AF478525
F. pseudodalmatica Krajina ex Domin	GenBank	Galli et al. (unpubl. data)	AY254374	
F. rupicaprina (Hack.) A. Kern.	GenBank	Gaut et al. (2000)	AF171145	
Subsect. *Exaratae* St.-Yves				
F. borderei (Hack.) K. Richt.	GenBank	Torrecilla and Catalán (2002)	AF303403	AF478510
F. capillifolia Dufour	GenBank	Torrecilla and Catalán (2002)	AF303419	AF478511
F. querana Litard.	GenBank	Catalán et al. (2004)	AF532957	AF533057
Sect. *Aulaxyper* Dumort				
F. ampla Hack.	GenBank	Catalán et al. (2004)		AF543516
F. heterophylla Lam.	GenBank	Charmet et al. (1997)	AJ240519	
F. juncifolia Chaub.	GenBank	Torrecilla et al. (2003)	AF478478	AF478515
F. nevadensis (Hack.) Markgr.-Dann.	GenBank	Catalán et al. (2004)	AF478477	AF478514
F. pyrenaica Reuter	GenBank	Torrecilla and Catalán (2002)	AF303423	AF478517
F. rivularis Boiss.	GenBank	Torrecilla et al. (2003)	AF478475	AF478512
F. rothmaleri (Litard.) Markgr.-Dann.	GenBank	Catalán et al. (2004)	AF478476	AF478513
F. rubra L.	GenBank	Torrecilla and Catalán (2002)	AF303422	
	New Zealand, Margot Forde Forage Germplasm Centre (OTA057896)	This study		AY528950

Table 1. Continued.

Taxon	Source/Voucher	Reference	GenBank accession number	
			ITS	*trn*L–*trn*F
Sect. *Eskia* Willk. p. p.				
F. burnatii St.-Yves	GenBank	Catalán et al. (2004)	AY099007	AY099002
F. eskia Ramond ex DC.	GenBank	Torrecilla and Catalán (2002)	AF303412	AF478508
F. gautieri (Hack.) K. Richt.	GenBank	Torrecilla and Catalán (2002)	AF303414	AF478506
F. quadriflora Honck.	GenBank	Torrecilla and Catalán (2002)	AF303413	AF478506
Sect. *Pseudatropis* Krivot.				
F. elegans Boiss.	GenBank	Torrecilla and Catalán (2002)	AF303406	AF478509
Sect. *Scariosae* Hack.				
F. mairei St.-Yves	GenBank	Torrecilla and Catalán (2002)	AF303424	
	GenBank	Torrecilla et al. (2003)		AY098996
F. scariosa (Lag.) Asch. et Graebn.	GenBank	Torrecilla and Catalán (2002)	AF519977	
	GenBank	Torrecilla et al. (2003)		AY098999
Sect. *Amphigenes* Janka				
F. agustinii (C. M. Sm. ex Link) Linding.	GenBank	Catalán et al. (2004)	AY099005	AY099003
F. carpathica Dietr.	GenBank	Catalán et al. (2004)	AY099006	AY099001
F. dimorpha Guss.	GenBank	Torrecilla et al. (2003)	AF519982	AF519987
F. pulchella Schrad. subsp. *pulchella*	GenBank	Torrecilla and Catalán (2002)	AF519980	
	GenBank	Torrecilla et al. (2003)		AF519985
F. spectabilis Jan	GenBank	Torrecilla et al. (2003)	AF519977	AF519984
Sect. *Subbulbosae* Hack.				
F. caerulescens Desf.	GenBank	Catalán et al. (2004)		AF533054
F. durandoi Clauson	GenBank	Catalán et al. (2004)	AF543514	AF533047
F. paniculata L. subsp. *baetica* (Hack.) Markgr.-Dann.	GenBank	Catalán et al. (2004)	AF303405	AF533049
F. triflora Desf.	GenBank	Catalán et al. (2004)	AF538362	AF533052
Subgen. *Drymanthele* Krechetovich et Bobrov				
F. altissima All.	GenBank	Catalán et al. (2004)	AF303411	AF478505
F. drymeja Mert. et Koch	GenBank	Torrecilla and Catalán (2002)	AF303411	
	GenBank	Catalán et al. (2004)		AY098997
F. lasto Boiss	GenBank	Torrecilla and Catalán (2002)	AF303418	
	GenBank	Torrecilla et al. (2003)		AY098998
Subgen. *Helleria* E. B. Alexeev				
F. contracta Kirk	Australia, Maquarie Island (CHR385372)	This study	AY524830	AY528944
Subgen. *Leucopoa* (Griseb.) Hack.				
Sect. *Leucopoa* (Griseb.) Krivot.				
F. kingii (S. Watson) Cassidy	GenBank	Torrecilla and Catalán (2002)	AF303410	
	GenBank	Torrecilla et al. (2003)		AY099004

Table 1. Continued.

Taxon	Source/Voucher	Reference	GenBank accession number	
			ITS	*trn*L–*trn*F
Sect. *Breviristatae* Krivot.				
F. altaica Trin.	GenBank	Catalán et al. (2004)	AF532952	AF533055
F. californica Vasey	GenBank	Catalán et al. (2004)	AF532956	AF533054
Subgen. *Subulatae* (Tzvelev) E. B. Alexeev				
F. subulata Trin.	GenBank	Catalán et al. (2004)	AF532953	AF533056
Subgen. *Schedonorus* (P. Beauv) Peterm.				
F. font-queri St.-Yves	GenBank	Torrecilla and Catalán (2002)	AF303404	
	GenBank	Catalán et al. (2004)		AF533044
Incertae sedis				
F. actae Connor	New Zealand, Canterbury, Lake Forsyth (OTA057589)	This study	AY524829	AY528949
F. coromotensis Briceño	GenBank	Catalán et al. (2004)		AF543518
F. coxii (Petrie) Hack.	New Zealand, Chatham Island (OTA057956)	This study	AY524825	AY528937
F. deflexa Connor	New Zealand, Nelson, Mt. Owen (OTA057588)	This study	AY524838	AY528942
F. elviae Briceño	GenBank	Catalán et al. (2004)		AF543517
F. luciarum Connor	New Zealand, Hawkes Bay, Maungaharuru Range (OTA057621)	This study	AY524828	AY528939
F. madida Connor	New Zealand, Otago, Rock and Pillar Range (OTA057627)	This study	AY524833	AY528943
F. matthewsii (Hack.) Cheeseman subsp. *matthewsii*	New Zealand, Fiordland, Takahe Valley (OTA057938)	This study	AY524836	AY528948
subsp. *aquilonia* Connor	New Zealand, Marlborough, Mt. Fyffe (OTA057936)	This study	AY524835	AY528946
subsp. *latifundii* Connor	New Zealand, Otago, Remarkables Range (OTA057625)	This study	AY524837	AY528945
subsp. *pisamontis* Connor	New Zealand, Otago, Pisa Range (OTA057945)	This study	AY524831	AY528947
F. multinodis Petrie et Hack.	New Zealand, Wellington, Baring Head (OTA057958)	This study	AY524827	AY528940
F. novae-zelandiae (Hack.) Cockayne	New Zealand, Fiordland, Takahe Valley (OTA057939)	This study	AY524832	AY528941
F. ultramafica Connor	New Zealand, Nelson, Windy Point (OTA057629)	This study	AY524826	AY528938
Brachypodium P. Beauv.				
B. distachyon (L.) P. Beauv.	GenBank	Torrecilla and Catalán (2002)	AF303399	AF478500
Ctenopsis De Not.				
C. delicatula (Lag.) Paunero	GenBank	Catalán et al. (2004)	AF478499	AF478537

Table 1. Continued.

Taxon	Source/Voucher	Reference	GenBank accession number	
			ITS	*trn*L–*trn*F
Dactylis L.				
D. glomerata L.	GenBank	Hsiao et al. (1995)	L36512	
	GenBank	Hunter et al. (2004)		AY327794
Helleria Fourn.				
H. fragilis Luces	GenBank	Catalán et al. (2004)	AF532960	AF533059
Lolium L.				
L. perenne L.	GenBank	Catalán et al. (2004)	AF303401	
	GenBank	Hunter et al. (2004)		AY327799
Micropyrum Link				
M. tenellum (L.) Link	GenBank	Catalán et al. (2004)	AF478494	AF478534
Narduroides Rouy				
N. salzmanii (Boiss.) Rouy	GenBank	Catalán et al. (2004)	AF478497	AF478535
Poa L.				
P. pratensis L.	GenBank	Gaut et al. (2000)	AF171182	
	GenBank	Stoneberg Holt et al. (2004)		AY061957
Psilurus Trin.				
P. incurvus (Gouan) Schinz et Thell.	GenBank	Catalán et al. (2004)	AF478493	AF478533
Schedonorus P. Beauv.				
S. giganteus (L.) Holub	GenBank	Torrecilla and Catalán (2002)	AF303416	
	GenBank	Catalán et al. (2004)		AF533043
S. phoenix (Scop.) Holub	GenBank	Charmet et al. (1997)	AJ240153	
	GenBank	Torrecilla et al. (2003)		AY098995
S. pratensis (Huds.) P. Beauv.	GenBank	Torrecilla and Catalán (2002)	AF303421	
	GenBank	Catalán et al. (2004)		AF478503
Vulpia C. C. Gmel.				
Sect. *Vulpia*				
V. bromoides (L.) Gray	New Zealand, Otago, Cromwell Gorge (OTA057652)	This study	AY524834	AY528936
V. myuros (L.) C. C. Gmel.	GenBank	Charmet et al. (1997)	AJ240162	
	GenBank	Hunter et al. (2004)		AY327797
Wangenheimia Moench				
W. lima (L.) Trin.	GenBank	Catalán et al. (2004)	AF478498	AF478536

Table 2. DNA content, ploidy level, and genome size of endemic New Zealand species of *Festuca.*

Taxon	2C-value (pg)	Ploidy	2*n*	DNA/ genome (pg)
F. actae	9.4	6*x*	42[b]	1.57
F. deflexa	10.45	6*x*	42[b]	1.74
F. madida	9.7	6*x*	42[c]	1.62
F. matthewsii subsp. *aquilonia*	9.98	6*x*	42[b]	1.66
F. matthewsii subsp. *latifundii*	9.89	6*x*	42[a]	1.65
F. matthewsii subsp. *matthewsii*	10.13	6*x*	42[b]	1.69
F. matthewsii subsp. *pisamontis*	10.02	6*x*	42[b]	1.67
F. novae-zelandiae	9.08	6*x*	42[a]	1.51
F. coxii	8.74	8*x*	56[a]	1.09
F. luciarum	8.06	8*x*	56[b]	1.01
F. multinodis	7.65	8*x*	56[a]	0.96
F. ultramafica	8.5	8*x*	56[b]	1.06

[a] Edgar and Connor (2000).

[b] Unpublished data. Brian Murray and Peter de Lange, University of Auckland, Private Bag 92019, Auckland, New Zealand.

[c] This study.

four broad-leaved subclades were not supported by bootstrap analysis.

The large FEVRE clade of fine-leaved *Festuca* and allied genera is supported by a bootstrap value of 75%, but basal nodes within the clade lack support. The only well-resolved lineages are a sect. *Festuca* clade (bs 78%) to which *Wangenheimia* is a weakly supported (bs 58%) sister, and a clade (bs 75%) comprising the two species of *Vulpia* (which form a monophyletic group in this analysis) and *Psilurus incurvus.* A weakly supported (bs 67%) red fescue clade includes six species from sect. *Aulaxyper,* one species each from sect. *Amphigenes* (*F. agustinii* and *F. querana,* respectively), two broad-leaved South American species (*F. coromotensis* and *F. elviae*), *Helleria fragilis,* and four fine-leaved NZ endemics (*F. coxii, F. luciarum, F. multinodis, F. ultramafica*) and subsect. *Exaratae.* This clade lacks further resolution except for a weak (bs 63%) relationship between *F. juncifolia* and *F. rothmaleri.*

DNA Content (C-Value) and Chromosome Number in New Zealand Festuca

Three ploidy levels have been recognized among NZ endemic species (Table 2), but the $2n = 28$ (tetraploid) count obtained from *F. madida* by de Lange and Murray (2002) appears mistaken. Our own chromosome count gave $2n = 42$ (hexaploid), as in *F. actae, F. deflexa, F. matthewsii,* and *F. novae-zealandiae.* The remaining species (*F. coxii, F. luciarum, F. multinodis, F. ultramafica*) are octoploids. The hexaploid/octoploid split corresponds with the separation of NZ *Festuca* into two groups based on ITS and *trn*L–*trn*F sequence variation, with *F. madida* aligning with the hexaploids (Fig. 1, 2). DNA content (2C-value) ranges from 7.65 pg per cell in *F. multinodis* to 10.45 pg per cell in *F. deflexa.* Hexaploid taxa have larger genome sizes (2C-value/ploidy level) than octoploids (Table 2). The genome size of *F. madida* (1.62 pg), which we measured on the same plant used for the chromosome count, matches the average of 1.64 pg for the remaining hexaploids. The octoploid species have an average genome size of 1.03 pg.

DISCUSSION

Phylogeny of Festuca *Sensu Lato*

We included a wide selection of taxa in our analyses, but our intention was to provide a broad context in which to place the NZ taxa rather than to examine relationships among Northern Hemisphere groups, which have been documented by others, particularly in relation to the broad-leaved *Festuca/Lolium* complex (Darbyshire and Warwick 1992; Stammers et al. 1995; Charmet et al. 1997; Gaut et al. 2000; Torrecilla and Catalán 2002; Catalán et al. 2004). The relationships we obtained among the broad-leaved taxa were similar to those obtained by Catalán et al. (2004). In our analyses, the monophyly of *Schedonorus* was precluded by either *F. font-queri* or *L. perenne,* but wider sampling within this group will be necessary to accurately determine the relationships among these three genera and whether any new nomenclatural combinations are justified. Both ITS and *trn*L–*trn*F analyses show a close relationship between *Austrofestuca littoralis* and *Poa,* supporting the findings of Hunter et al. (2004).

Phylogenetic Relationships of New Zealand Festuca

Among fine-leaved *Festuca* and related genera, we obtained very similar relationships to those of Catalán et al. (2004) and Torrecilla et al. (2004), who denoted the large fine-leaved clade containing *Festuca* s.s., *Vulpia,* and related ephemerals as FEVRE (Fig. 1, 2). Relationships within the FEVRE clade are described in detail by the authors. The relationships between the fine-leaved species of NZ *Festuca* and the FEVRE clade differed according to the data set used. In the ITS tree (Fig. 1), all of the NZ taxa are included in the FEVRE clade. The *trn*L–*trn*F tree (Fig. 2) shows one group of NZ taxa within the FEVRE clade and one outside the FEVRE clade and sister to *F. californica.*

The evidence is compelling that NZ's endemic species of *Festuca* do not form a monophyletic group and have at least two origins. One group has affinities to *Festuca* sect. *Aulaxyper* (red fescues). The other species form a group (''southern endemic'' clade) with the circum-Antarctic *F. contracta,* and may have a close relationship to *F. californica* of the western USA (Fig. 2), but are not well resolved with respect to any previously recognized clade of fine-leaved *Festuca.* The evidence for two or more origins is congruent between nucleotide sequences from both the nuclear (ITS) and chloroplast (*trn*L–*trn*F) phylogenies (Fig. 1, 2), and is supported by concordant differences in ploidy level and genome size (Table 2).

Aulaxyper-*related taxa.*—The first association between northern species of *Festuca* sect. *Aulaxyper* and southern species and relatives of *Festuca* was reported by Catalán et al. (2004) for three South American species, *F. coromotensis, F. elviae,* and *Helleria fragilis.* Our results confirm this relationship and extend it across the South Pacific Ocean to four fine-leaved NZ species (Fig. 1, 2). Two of these species (*F. luciarum, F. multinodis*) are strongly rhizomatous/stoloniferous, whereas the other two (*F. coxii, F. ultramafica*)

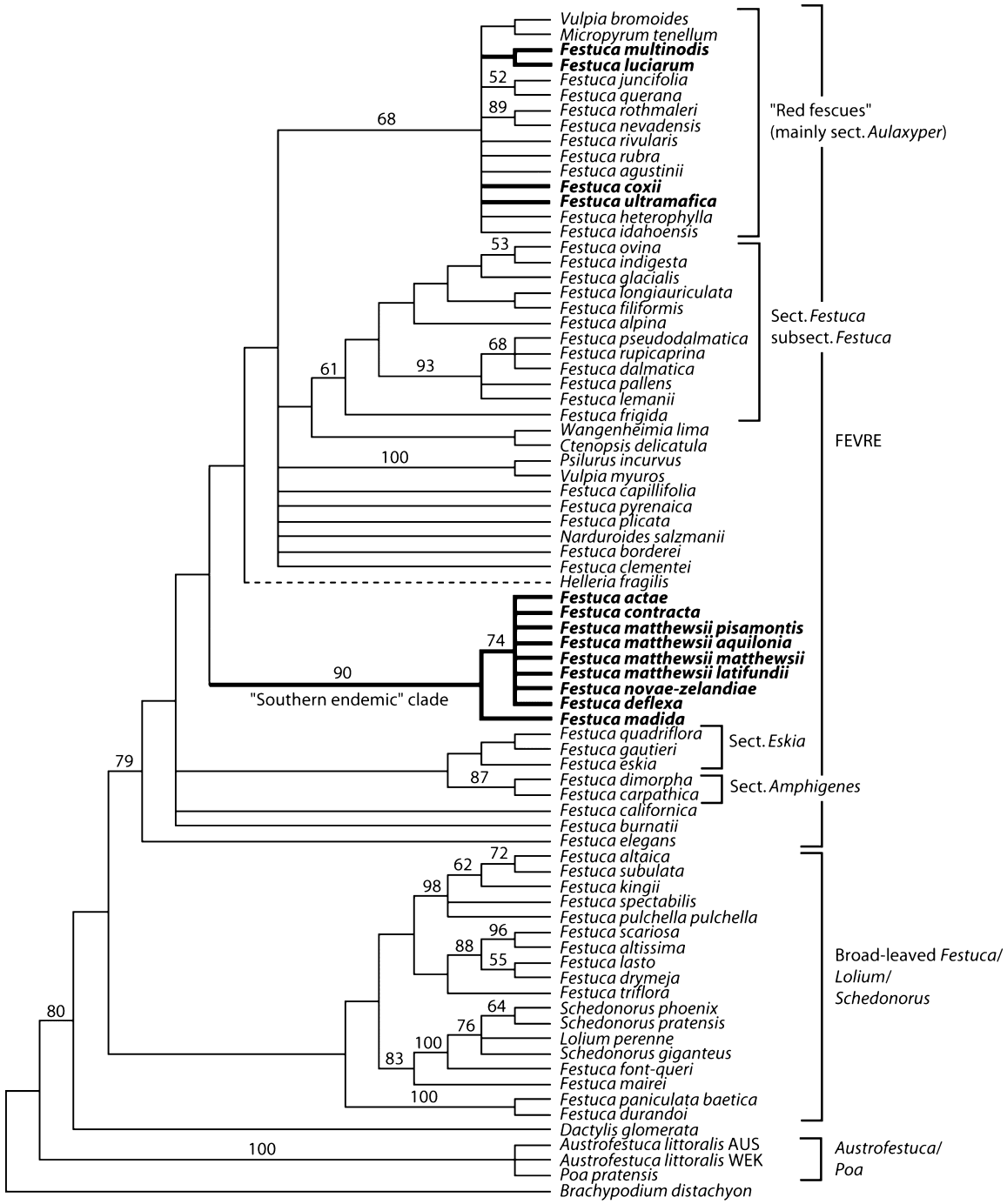

Fig. 1.—Strict consensus of 480 trees derived from parsimony analysis of ITS sequences. Indigenous New Zealand taxa and branches are shown in bold. Branch support is indicated by bootstrap values above 50%. Consistency index = 0.466, retention index = 0.749, rescaled consistency = 0.349.

have a short-tussock (bunchgrass) habit that is shared with the remaining NZ endemics. Extravaginal branching is typical of taxa in sect. *Aulaxyper* (Torrecilla and Catalán 2002), but while three of the endemic *Aulaxyper*-related species have extravaginal branching, *F. coxii* does not (Connor 1998). *Festuca coxii, F. luciarum,* and *F. multinodis* have lax leaves, while those of *F. ultramafica* are conduplicate and pungent. Leaves are smooth in all four species, apart from hairs present on the lower portions.

Most studies are similar to ours in showing that sect. *Aulaxyper* taxa occupy a derived position among fine-leaved taxa of *Festuca* and their allies (Darbyshire and Warwick 1992; Charmet et al. 1997; Torrecilla and Catalán 2002; Torrecilla et al. 2003, 2004; Catalán et al. 2004), although Gaut et al. (2000) found the phylogenetic position of the red fescues to be equivocal, depending on the type of analysis or whether indels were used as characters. The placement of *F. coxii* among sect. *Aulaxyper* taxa confirms its standing with-

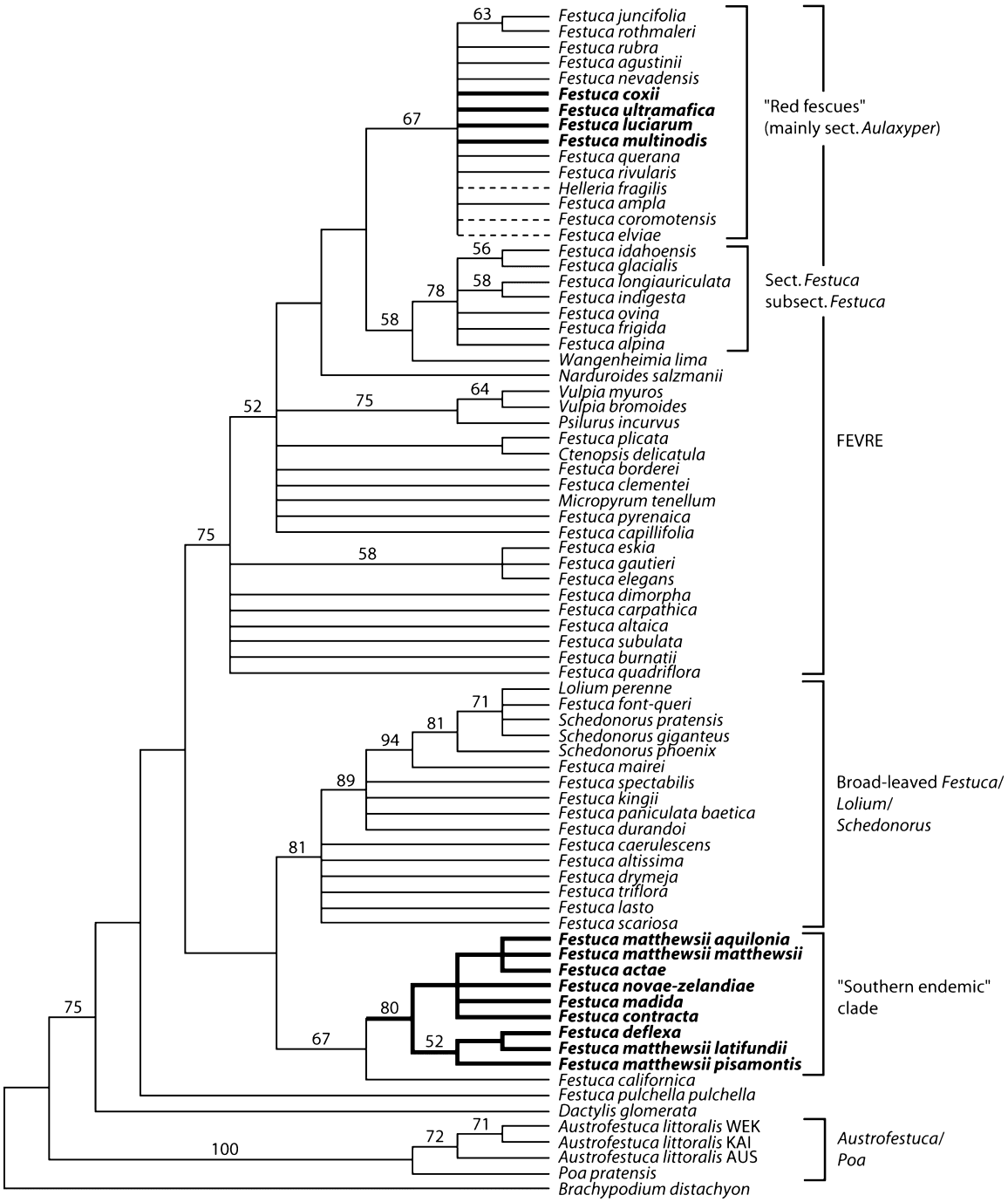

Fig. 2.—Strict consensus of 6520 trees derived from parsimony analysis of *trn*L–*trn*F sequences. Indigenous New Zealand taxa and branches are shown in bold. Branch support is indicated by bootstrap values above 50%. Consistency index = 0.534, retention index = 0.798, rescaled consistency = 0.426.

in *Festuca,* despite its atypical morphological features (Connor 1998). The isolation and exposed nature of the Chatham Island group, which lies 870 km east of the NZ mainland, may have given rise to selective pressures that affect *F. coxii* uniquely among the endemic taxa.

''Southern endemic'' taxa.—The ''southern endemic'' clade is a novel clade that has not been recognized in previous molecular analyses involving fine-leaved *Festuca* (Darbyshire and Warwick 1992; Gaut et al. 2000; Torrecilla and Catalán 2002; Torrecilla et al. 2003, 2004; Catalán et al. 2004). This clade contains the endemic species *F. actae, F. deflexa, F. madida, F. matthewsii,* and *F. novae-zelandiae,* as well as circum-Antarctic *F. contracta* (Fig. 1, 2). In the *trn*L–*trn*F tree (Fig. 2), *F. californica* is sister to this clade, and *F. matthewsii* is paraphyletic, in part due to a 30 bp repeat that is shared by only two of its four subspecies and *F. deflexa.* No morphological features appear to be correlated with this split among the subspecies of *F. matthewsii.* Geographically, the distribution of subsp. *pisamontis* is nest-

ed within that of subsp. *latifundii* and these two taxa do not overlap with subspp. *aquilonia* or *matthewsii* (Fig. 3C). Apart from *F. madida,* all of the ''southern endemic'' species have intravaginally branching tillers. Leaves of all taxa are erect and, apart from *F. madida* and *F. matthewsii* subsp. *matthewsii,* scabrid. *Festuca contracta* and *F. madida* share a cleistogamous breeding system, whereas the other taxa are chasmogamous.

Festuca contracta is restricted to subantarctic islands and occurs on Macquarie Island (Australian territory) in the New Zealand Botanical Region, as well as elsewhere in the Southern Ocean (Tierra del Fuego, Falkland Islands, South Georgia, Kerguelen Islands). It is a hexaploid (Dubcovsky and Martínez 1991), in common with other members of the ''southern endemic'' clade (Table 2). Phenetic analysis of morphological characters among several species of Patagonian *Festuca* placed *F. contracta* in a clade with Andean taxa belonging to sect. *Festuca* (Dubcovsky and Martínez 1988). These authors doubted such a placement, and their view is confirmed by our molecular analyses, which show *F. contracta* to be related to species in the ''southern endemic'' clade rather than to species in sect. *Festuca* (Fig. 1, 2). *Festuca californica* is sister to the ''southern endemic'' clade in the *trn*L–*trn*F tree (Fig. 2), but is unresolved among the fine-leaved fescues in the ITS tree (Fig. 1). The species is found in dry grassland, shrubland, and woodland habitats in the Coast, Cascade, and Sierra Nevada ranges of California and Oregon (Walsh 1994). It shares a bunchgrass (tussock) habit, tall culms, and openly branched panicles with species in the ''southern endemic'' clade, but unlike them, it is either tetraploid (Aiken et al. 1996) or octoploid (Hickman 1993), rather than hexaploid.

DNA Content and Genome Size of Endemic New Zealand Festuca

The 2C DNA contents we report (Table 2) correspond closely with those obtained by flow cytometry for *Festuca madida* and *F. novae-zelandiae* (Murray et al. 2003), while the hexaploid taxa have slightly higher DNA contents than those calculated from Feulgen staining of two fine-leaved hexaploids (Seal 1983). All of our values, however, are lower than the flow cytometry values reported by Huff and Palazzo (1998) for hexaploid and octoploid species of fine-leaved *Festuca.* Nonetheless, all four studies show the same trend of declining genome size with increasing ploidy level. Possibly, the differences in reported values between the flow cytometry studies relate to the different external standards used for calibration of DNA amounts; Huff and Palazzo (1998) used animal cells (chicken red blood cells), whereas this study and Murray et al. (2003) used plant cells (*Trifolium repens* and *Actinidia chinensis* Planchon, respectively).

Ecology of Endemic New Zealand Species of Festuca

For most of the Tertiary, the NZ landmass was largely low lying and covered with dense forest (Lee et al. 2001). Habitats for nonforest species would have been limited until tectonic mountain building in the late Pliocene created extensive mountainous regions, providing widespread areas where species of open habitats could persist (Raven 1973). Glacial activity in the Pleistocene would have resulted in major environmental fluctuations as forests expanded during interglacials and receded during glacial periods (McGlone et al. 2001). Subsequent forest destruction caused by anthropogenic fires that began around 800 years ago allowed grasslands to expand in lowland and montane areas formerly occupied by forest (Rogers and Leathwick 1996; McGlone et al. 1997). Lloyd et al. (2003) suggested that for nonforest taxa such fluctuations would have resulted in evolutionary divergence toward one of two alternative strategies: (1) specialist—occupying habitats such as coastal rocks that never become forested even during interglacials, or (2) generalist—persisting in nonforest habitats during interglacials, but having traits (e.g., fast growth rate, wide environmental tolerance, strong dispersal) that allow expansion into periglacial areas that arise during periods of ice advance.

The endemic sect. *Aulaxyper*-related species appear to fit the specialist strategy, and largely remain restricted to sites that have lacked forest cover through several glacial/interglacial cycles. *Festuca multinodis* is the most widely distributed of these species (Fig. 3A). This species occurs on rocky coasts in central NZ and extends inland on cliffs and bluffs, mostly on limestone substrates (Connor 1998). *Festuca luciarum* is restricted to four small, geographically separate, eastern North Island mountain ranges (Fig. 3A) where it inhabits limestone rocks and cliffs, and adjacent grassland. *Festuca coxii* is coastal and restricted to the Chatham Island group, 870 km east of the NZ mainland, while *F. ultramafica* occurs in a limited area of ultramafic terrain in the northern South Island (Fig. 3A).

In contrast to the restricted distributions of the sect. *Aulaxyper*-related species, taxa from the ''southern endemic'' clade tend to have large geographic ranges and mostly fit the generalist strategy. They occur as subordinate species in tall-tussock (*Chionochloa*) grasslands, or as the dominants of short-tussock grasslands in dry inland basins and montane river valleys. Species from this clade were among the small grasses that pollen analysis has shown to have expanded rapidly following initial anthropogenic deforestation, before eventual domination by tall tussocks (*Chionochloa* spp.) in all but the driest areas (McGlone 2001). Subsequently, species of *Festuca* from the ''southern endemic'' clade have become dominant in tall-tussock grasslands that have been degraded by repeated burning and grazing during pastoral use (Wraight 1963; Connor 1964; Rose and Platt 1992).

Festuca deflexa occurs in the alpine zone on extensive areas of limestone topography in northwestern South Island (Fig. 3D). The four subspecies of *F. matthewsii* are widespread across the South Island, either in alpine areas or in montane valley floor grasslands (Fig. 3C). *Festuca novae-zelandiae* dominates short-tussock grasslands in dry inland basins and reaches the alpine zone as a component of tall-tussock grasslands. It is widespread in eastern grasslands and occurs on both the North and South islands (Fig. 3B). *Festuca actae* is the exception to these widespread grassland taxa, being limited to Banks Peninsula, a volcanic area at the coastal margin of extensive alluvial plains (Fig. 3D). Here *F. actae* occupies coastal rocks and extends upslope around rock outcrops on grassy hillsides. *Festuca madida* is a diminutive grass of wet places in alpine grasslands, and is the only species of endemic *Festuca* that is found on NZ's

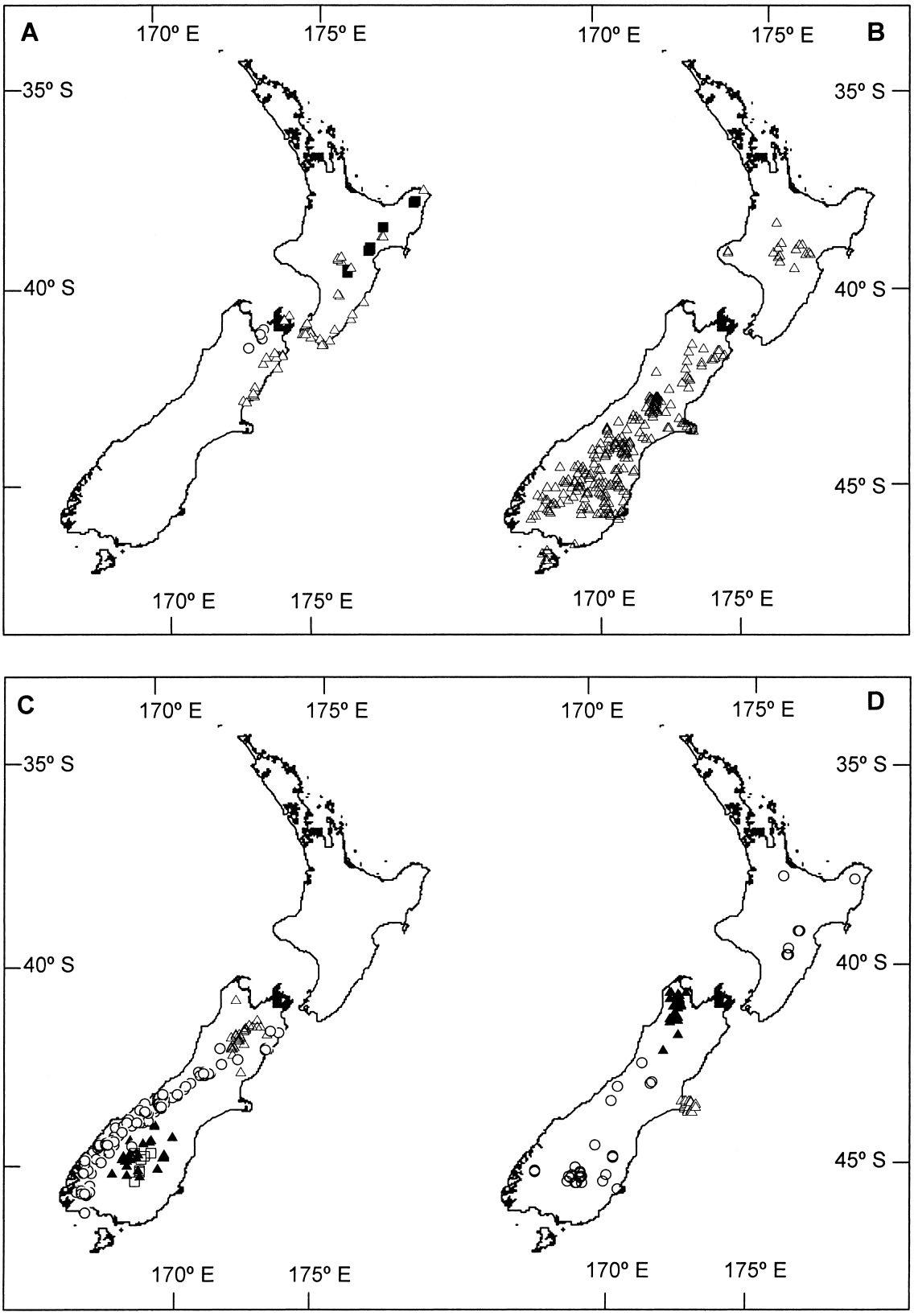

Fig. 3A–D.—Distributions of the endemic taxa of *Festuca* on the main islands of New Zealand. Maps were created using data from a range of sources, including major NZ herbaria (AKL, CANU, CHR, OTA, WELT, WELTU), unpublished species lists, records from the literature, and personal observations by one of us (KML). *Festuca coxii* and *F. contracta,* which are restricted to two outlying islands, were not mapped.—A. ■ = *F. luciarum,* △ = *F. multinodis,* ○ = *F. ultramfica.*—B. △ = *F. novae-zelandiae.*—C. *F. matthewsii,* △ = subsp. *aquilonia,* ○ = subsp. *matthewsii,* ▲ = subsp. *latifundii,* □ = subsp. *pisamontis.*—D. △ = *F. actae,* ▲ = *F. deflexa,* ○ = *F. madida* (also on Campbell Island, not shown).

main islands (Fig. 3D) as well as on a subantarctic island (Campbell Island, 52°30′S; Connor 1998).

Biogeography of Festuca *in New Zealand*

It has been suggested on the basis of ITS sequence data that the grass family arose in what is present-day South America and that the ancestors of the core pooid grasses of Eurasia (tribes Aveneae, Bromeae, Poeae, and Triticeae) migrated northward and diversified, with some lineages subsequently dispersing back southward (Hsiao et al. 1999). The restriction of presumably ancestral diploid taxa of *Festuca* to the Mediterranean area (Catalán et al. 2004) supports a northern origin for lineages of *Festuca* in the Southern Hemisphere, and a similar relationship is proposed for southern *Poa* on the basis of chloroplast restriction site information (Soreng 1990).

Our analyses have shown that the endemic NZ species of *Festuca* have affinities to taxa on the American continents. New Zealand shares many genera with South America and north temperate regions (Wardle et al. 2001), among the monocots including *Carex* L., *Eleocharis* R. Br., *Festuca, Juncus* L., *Poa, Puccinellia* Parl., and *Schoenoplectus* (Rchb.) Palla. The origin of *Festuca* in NZ is consistent with the movement of northern ancestors southward through the Americas before reaching high latitudes in the Southern Hemisphere. This hypothesis would account for the relatedness of some NZ and South American *Festuca* spp. to the northern red fescue clade (Fig. 1, 2) and a potential close relationship of *F. californica* to the ''southern endemic'' clade (Fig. 2).

Once in southern temperate latitudes, the westerly wind drift may have assisted movement of propagules to landmasses in the Southern Ocean and eventual dispersal to NZ. Wardle et al. (2001) suggested that formerly vegetated Antarctica may have been an important stepping-stone that assisted transoceanic dispersal of plants to NZ. The subantarctic islands lying to the south of the NZ landmass may also have assisted during these dispersal events. The presence of *Festuca madida* on Campbell Island may represent a trace of one such event. *Festuca madida* is well supported as a member of the ''southern endemic'' clade, but has morphological differences (discussed above) from the remaining members of the clade. Its wide yet disjunct geographical distribution (Fig. 3D) suggests a longer presence in NZ than the other endemic taxa, and could indicate an earlier dispersal event, in keeping with its basal position within the clade in the ITS tree (Fig. 1).

South America contains tetraploid, hexaploid, and octoploid lineages of *Festuca.* The NZ taxa are hexaploid or octoploid, supporting the hypothesis that increased ploidy levels have helped *Festuca* lineages to colonize new areas and diversify (Catalán et al. 2004). Rapid genomic change has been shown to occur in synthetic polyploids (Song et al. 1995), possibly because polyploidy can conserve novel genomic microstructural changes arising from unequal crossing over during meiosis (Bancroft 2001). New genetic variation resulting from polyploid formation in *Festuca* may have resulted in traits that allowed ancestral taxa to disperse from the Northern Hemisphere to South America and NZ, with subsequent diversification within these landmasses. In NZ, morphological variation apparent in geographically separated populations of *F. luciarum, F. multinodis,* and the four subspecies of *F. matthewsii* (Fig. 3; Connor 1998) reflects the ongoing nature of this diversification.

Molecular information from a wider sampling of the South American lineages would enable a stronger analysis of the origins of *Festuca* in NZ. The possibility of alternative dispersal routes to NZ through Melanesia and Australia should not be discounted, although Soreng (1990) suggested that this is a minor pathway in *Poa.* Neither should the potential role of NZ as a source of dispersing taxa be overlooked, with recent molecular evidence indicating dispersal in several directions from NZ to other Pacific basin landmasses (Winkworth et al. 2002). Besides greater sampling of South American taxa, future work should concentrate on taxa that occur on other southern landmasses, such as New Guinea, Australia, and southern Africa.

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LITERATURE CITED

AIKEN, S. G., M. J. DALLWITZ, C. L. MCJANNET, AND L. L. CONSAUL. 1996 onwards. *Festuca* of North America: descriptions, illustrations, identification, and information retrieval, vers. 12 Sep 2000. *http://biodiversity.uno.edu/delta/* [Now at: *http://delta-intkey.com/festuca/* Apr 2006.]

ALEXEEV, E. B. 1976. *Austrofestuca* (Tzvel.) E. Alexeev comb. nov.—a new genus of the family Poaceae from Australia. *Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol.* **81**: 55–60.

BANCROFT, I. 2001. Duplicate and diverge: the evolution of plant genome microstructure. *Trends Genet.* **17**: 89–93.

CAMPBELL, B. D., J. R. CARADUS, AND C. L. HUNT. 1999. Temperature responses and nuclear DNA amounts of seven white clover populations which differ in early spring growth rates. *New Zealand J. Agric. Res.* **42**: 9–17.

CARLSON, J. E., L. K. TULSIERAM, J. C. GLAUBITZ, V. M. K. LUK, AND C. R. R. KAUFFELDT. 1991. Segregation of random amplified DNA markers in F1 progeny of conifers. *Theor. Appl. Genet.* **83**: 194–200.

CATALÁN, P., P. TORRECILLA, J. A. LÓPEZ-RODRÍGUEZ, AND R. G. OLMSTEAD. 2004. Phylogeny of the festucoid grasses of subtribe Loliinae and allies (Poeae, Pooideae) inferred from ITS and *trn*L–F sequences. *Molec. Phylogen. Evol.* **31**: 517–541.

CHARMET, G., C. RAVEL, AND F. BALFOURIER. 1997. Phylogenetic analyses in the *Festuca-Lolium* complex using molecular markers and ITS rDNA. *Theor. Appl. Genet.* **94**: 1038–1046.

CONNOR, H. E. 1964. Tussock grassland communities in the MacKenzie country, South Canterbury, New Zealand. *New Zealand J. Bot.* **2**: 325–351.

———. 1998. *Festuca* (Poeae: Gramineae) in New Zealand. I. Indigenous taxa. *New Zealand J. Bot.* **36**: 329–367.

———, AND E. EDGAR. 1986. Australasian alpine grasses: diversification and specialization, pp. 413–434. *In* B. A. Barlow [ed.], Flora and fauna of alpine Australasia—ages and origins. CSIRO Publishing, Melbourne, Australia.

DARBYSHIRE, S. J., AND S. I. WARWICK. 1992. Phylogeny of North American *Festuca* (Poaceae) and related genera using chloroplast DNA restriction site variation. *Canad. J. Bot.* **70**: 2415–2429.

DE LANGE, P. J., AND B. G. MURRAY. 2002. Contributions to a chromosome atlas of the New Zealand flora—37. Miscellaneous families. *New Zealand J. Bot.* **40**: 1–23.

DUBCOVSKY, J. 1991. Cytotaxonomy of the *Festuca* spp. from Patagonia. *Canad. J. Bot.* **70**: 1134–1140.

———, AND A. MARTÍNEZ. 1988. Phenetic relationships in the *Festuca* spp. from Patagonia. *Canad. J. Bot.* **66**: 468–478.

EDGAR, E., AND H. E. CONNOR. 2000. Flora of New Zealand—the grasses, Vol. 5. Manaaki Whenua Press, Christchurch, New Zealand. 650 p.

GAUT, B. S., L. P. TREDWAY, C. KUBIK, R. L. GAUT, AND W. MEYER. 2000. Phylogenetic relationships and genetic diversity among members of the *Festuca-Lolium* complex (Poaceae) based on ITS sequence data. *Pl. Syst. Evol.* **224**: 33–53.

HARTLEY, W. 1961. Studies on the origin, evolution and distribution of the Gramineae 4: the genus *Poa*. *Austral. J. Bot.* **9**: 152–161.

———. 1973. Studies on the origin, evolution and distribution of the Gramineae 5: the subfamily Festucoideae. *Austral. J. Bot.* **21**: 201–234.

HICKMAN, J. C. (editor). 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley, USA. 1424 p.

HSIAO, C., N. J. CHATTERTON, K. H. ASAY, AND K. B. JENSEN. 1995. Phylogenetic relationships of the monogenomic species of the wheat tribe, Triticeae (Poaceae), inferred from nuclear rDNA (internal transcribed spacer) sequences. *Genome* **38**: 211–223.

———, S. W. L. JACOBS, N. J. CHATTERTON, AND K. H. ASAY. 1999. A molecular phylogeny of the grass family (Poaceae) based on the sequences of nuclear ribosomal DNA (ITS). *Austral. Syst. Bot.* **11**: 667–688.

HUFF, D. R., AND A. J. PALAZZO. 1998. Fine fescue species determination by laser flow cytometry. *Crop Sci. (Madison)* **38**: 445–450.

HUNTER, A. M., D. A. ORLOVICH, K. M. LLOYD, W. G. LEE, AND D. J. MURPHY. 2004. The generic position of *Austrofestuca littoralis* and the reinstatement of *Hookerochloa* and *Festucella* (Poaceae) based on evidence from nuclear (ITS) and chloroplast (*trn*L–*trn*F) DNA sequences. *New Zealand J. Bot.* **42**: 253–262.

LEE, D. A., W. G. LEE, AND N. MORTIMER. 2001. Where and why have all the flowers gone? Depletion and turnover in the New Zealand Cenozoic angiosperm flora in relation to paleogeography and climate. *Austral. J. Bot.* **49**: 341–356.

LLOYD, K. M., J. B. WILSON, AND W. G. LEE. 2003. Correlates of geographic range size in New Zealand *Chionochloa* (Poaceae) species. *J. Biogeogr.* **30**: 1751–1761.

MCGLONE, M. S. 2001. The origin of the indigenous grasslands of southeastern South Island in relation to pre-human woody ecosystems. *New Zealand J. Ecol.* **25**: 1–15.

———, R. P. DUNCAN, AND P. B. HEENAN. 2001. Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *J. Biogeog.* **28**: 199–216.

———, N. T. MOAR, AND C. D. MEURK. 1997. Growth and vegetation history of alpine mires on the Old Man Range, Central Otago, New Zealand. *Arctic Alpine Res.* **29**: 32–44.

MURRAY, B. G., I. E. WEIR, A. R. FERGUSON, AND P. J. DE LANGE. 2003. Variation in DNA C-value and haploid genome size in New Zealand native grasses. *New Zealand J. Bot.* **41**: 63–69.

RAMBAUT, A. 2002. Se-Al. Sequence alignment editor, vers. 2.0a11. *http://evolve.zoo.ox.ac.nz/* [Now at: *http://evolve.zoo.ox.ac.uk/software.html?name=Se-Al* Apr 2006.]

RAVEN, P. H. 1973. Evolution of subalpine and alpine plant groups in New Zealand. *New Zealand J. Bot.* **11**: 177–200.

ROGERS, G. M., AND J. R. LEATHWICK. 1996. North Island seral tussock grasslands. 3. The influence of heather (*Calluna vulgaris*) on rates of change from tussock grassland to shrubland. *New Zealand J. Bot.* **34**: 473–487.

ROSE, A. B., AND K. H. PLATT. 1992. Snow tussock (*Chionochloa*) population responses to removal of sheep and European hares, Canterbury, New Zealand. *New Zealand J. Bot.* **30**: 373–382.

SEAL, A. G. 1983. DNA variation in *Festuca*. *Heredity* **50**: 225–236.

SONG, K., P. LU, K. TANG, AND T. C. OSBORN. 1995. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. *Proc. Natl. Acad. Sci. U.S.A.* **92**: 7719–7723.

SORENG, R. J. 1990. Chloroplast-DNA phylogenetics and biogeography in a reticulating group: study in *Poa* (Poaceae). *Amer. J. Bot.* **77**: 1383–1400.

———, AND J. I. DAVIS. 1998. Phylogenetics and character evolution in the grass family (Poaceae): simultaneous analyses of morphological and chloroplast DNA restriction site character sets. *Bot. Rev. (Lancaster)* **64**: 1–85.

STAMMERS, M., J. HARRIS, G. M. EVANS, M. D. HAYWARD, AND J. W. FOSTER. 1995. Use of random PCR (RAPD) technology to analyse phylogenetic relationships in the *Lolium*/*Festuca* complex. *Heredity* **74**: 19–27.

STONEBERG HOLT, S. D., L. HOROVÁ, AND P. BUREŠ. 2004. Indel patterns of the plastid DNA *trn*L–*trn*F region within the genus *Poa* (Poaceae). *J. Pl. Res.* **117**: 393–407.

SWOFFORD, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), vers. 4.0. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

TABERLET, P., L. GIELLY, G. PAUTOU, AND J. BOUVET. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* **17**: 1005–1109.

THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAK, F. JEANMOUGIN, AND D. J. HIGGINS. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* **24**: 4876–4882.

TORRECILLA, P., AND P. CATALÁN. 2002. Phylogeny of broad-leaved and fine-leaved *Festuca* lineages (Poaceae) based on nuclear ITS sequences. *Syst. Bot.* **27**: 241–251.

———, J. A. LÓPEZ-RODRÍGUEZ, AND P. CATALÁN. 2004. Phylogenetic relationships of *Vulpia* and related genera (Poeae, Poaceae) based on analysis of ITS and *trn*L–F sequences. *Ann. Missouri Bot. Gard.* **91**: 124–158.

———, ———, D. STANCIK, AND P. CATALÁN. 2003. Systematics of *Festuca* L. sects. *Eskia* Willk., *Pseudatropis* Kriv., *Amphigenes* (Janka) Tzvel., *Pseudoscariosa* Kriv. and *Scariosae* Hack. based on analysis of morphological characters and DNA sequences. *Pl. Syst. Evol.* **239**: 113–139.

WALSH, R. A. 1994. *Festuca californica*. *In* Fire effects information system. Fire Sciences Laboratory, Rocky Mountain Research Station, Forest Service, U.S. Department of Agriculture. Available at: *http://www.fs.fed.us/database/feis/* (Apr 2006).

WARDLE, P., C. EZCURRA, C. RAMIREZ, AND S. WAGSTAFF. 2001. Comparison of the flora and vegetation of the southern Andes and New Zealand. *New Zealand J. Bot.* **39**: 69–108.

WHITE, T. J., T. BRUNS, S. LEE, AND J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, pp. 315–324. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White. [eds.], PCR protocols, a guide to methods and applications. Academic Press, San Diego, California, USA.

WINKWORTH, R. C., S. WAGSTAFF, D. GLENNY, AND P. J. LOCKHART. 2002. Plant dispersal NEWS from New Zealand. *Trends Ecol. Evol.* **17**: 514–520.

WRAIGHT, M. J. 1963. The alpine and upper montane grasslands of the Wairau River catchment, Marlbourough. *New Zealand J. Bot.* **1**: 351–376.