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The use of molecular techniques to resolve relationships among traditional weaving cultivars of *Phormium*

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Abstract Molecular techniques – sequencing of the nuclear internal transcribed spacer region (ITS), and fingerprinting of amplified fragment length polymorphisms (AFLP) and intersimple sequence repeats (ISSR) – were investigated for their potential to characterise genetic variation in harakeke (*Phormium* spp., family Hemerocallidaceae) in New Zealand. ITS showed no variation between species of *Phormium*. However, both AFLP and ISSR identified genome regions that were polymorphic among intraspecific accessions of *Phormium* obtained from the National New Zealand Flax Collection. When used as a direct sequencing marker, one AFLP fragment distinguished two major groups of nuclear haplotypes. The partitioning of taxa into these groups was supported by phylogenetic analysis of ISSR fingerprinting profiles. Split decomposition, a network-building technique, was shown to be useful in representing the sorts of relationships present in this study of intraspecific variation. Further sampling of natural populations is required to understand the origins and present-day distributions of these accessions; however, the present results show the usefulness of these methods for addressing such ethnobotanic questions.

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INTRODUCTION

Phormium is a widespread genus in New Zealand and contains great morphological diversity. Plants range from around 30 cm to over 2 m in height, with leaves from bright green to yellow or bronze. The genus may occupy a variety of habitats, but is presently largely restricted to highly disturbed or exposed areas, and those unsuitable for conversion to agriculture. Two species, *Phormium tenax* (harakeke) and *P. cookianum* (wharariki), are currently recognised (Moore & Edgar 1970); however, it has been demonstrated that these morphologically defined species can hybridise (Allan & Zotov 1937). Intraspecific variation within *P. cookianum* has been indicated (Wardle 1979). Largely based on the high diversity of leaf and fibre attributes for weaving and plaiting purposes, Maori selected, cultivated, and named many forms over generations (Scheele & Walls 1994; Harris & Woodcock-Sharp 2000). More than 70 accessions of these are held in trust in the National New Zealand Flax Collection (largely comprising cultivars donated by Rene Orchiston, Gisborne – the Orchiston Collection). Our purpose was to find the most appropriate technique for determining the origins and inter-relationships (whakapapa) of these culturally valued selections.

A difficulty with investigating human-directed selection and dispersal of plants is identifying DNA markers that will provide sufficient phylogenetic resolution to allow migratory paths to be detected or unravelled. The recent nature of the radiation (or diversification) of many of New Zealand's plant groups has meant that the morphological variation leading to the naming of different species or subspecies is often not reflected by a similar level of genetic variation in commonly studied DNA markers (Wagstaff & Dawson 2000; Winkworth et al. 2002). In contrast, DNA fingerprinting methods can be implemented to identify regions of the plant genome that differ between closely related taxa. For example, random amplified polymorphic DNA (RAPD) has been used successfully in the study of kumara cultivars (Harvey et al. 1997). Amplified fragment length polymorphisms (AFLP) and intersimple sequence repeats (ISSR) have also been applied to distinguish closely related taxa in natural populations of plants (e.g., Esselman et al. 1999; Perrie et al. 2000). Some concern has been raised over the use of fingerprinting methods for testing phylogenetic relationships (e.g., Swofford et al. 1996; Yang et al. 1996; McLenachan et al. 2000). For example, presence or absence of particular bands may be non-independent, and amplification of fragments may be influenced by quality and quantity of DNA template, rendering results unrepeatable. Nevertheless, fingerprinting methods can be used to reliably identify polymorphic genome regions on a relatively small number of accessions for which high-quality DNA can be obtained. From these profiles, PCR primers can then be designed for the rapid characterisation of homologous regions in DNA samples that may be of poor quality or small quantity. This approach was used here to search for sequence-characterised amplified regions (SCAR markers) that could be implemented to study phylogenetic variation in accessions of harakeke.

The nuclear internal transcribed spacer (ITS) region has been studied in hundreds of taxa, and the level of variability often allows comparison of sequences between recently evolved taxa.

The AFLP method, while typically used for obtaining DNA fingerprinting of plant genomes (Vos et al. 1995), can also be used to locate DNA fragments from genome regions that are variable in size or arrangement. Once identified, these regions can be amplified and characterised using PCR primers specific for these regions (Lockhart & McLenachan 1997; Shan et al. 1999). Often such regions show elevated substitution rates (Lockhart et al. 2001) and are useful for studying the relationships between closely related taxa (McLenachan et al. 2000). This implementation of AFLP was used in the present work.

ISSR is a PCR-based technique similar to RAPD methods, but which has been shown to be both more reliable and more variable (e.g., Esselman et al. 1999). The method uses short simple repeat sequences, sometimes anchored at one end, as primers to amplify genome regions between 300 bases and 3 kilobases.

The most commonly used phylogenetic methods are parsimony and maximum likelihood; however, bifurcating trees constructed by either of these may not be appropriate for representing the relationships of interest in an intraspecific study. Split decomposition is a method that is able to represent more complex relationships, such as those due to hybridisation or the presence of extant ancestors, as well as being useful for investigating the suitability of data for phylogenetic analysis. Thus, it may be particularly useful for largely intraspecific studies such as this. A detailed discussion on the calculation and biological interpretation of splits graphs has been given in Lockhart et al. (2001).

MATERIALS AND METHODS

Plant tissue and DNA extraction

All tissue of *Phormium* was obtained from the National New Zealand Flax Collection held by Landcare Research at Lincoln, Canterbury; numbering of samples refers to accessions within the living collection (Table 1). Voucher specimens were not available. Total DNA was extracted from a fresh sample of the youngest shoot using a modification of the technique of Doyle & Doyle (1987) allowing preparation in microcentrifuge tubes. A small amount of tissue was crushed in a tube using liquid nitrogen and a glass rod.

Molecular markers

ITS region

Extracted *Phormium* DNA was diluted 1:100 with sterile distilled water. Reactions were performed using Qiagen (Hilden, Germany) reagents in a total volume of 20 μl containing 1 × Q solution™ 1.5 m*M* $MgCl_2$ in 1 × PCR Buffer™, 250 μ*M* each dNTP, 10 ρ*M* each primer, 1 unit Taq polymerase, and 1 μl diluted genomic DNA. Reactions had an initial denaturing step at 94°C for 2 min, 34 cycles of

Table 1 Origin of accessions in this study. All *Phormium* from the New Zealand Botanical Region grown at and collected directly from the National New Zealand Flax Collection, Landcare Research, Lincoln. Only the accessions from Pitt Island, Norfolk Island, and Three Kings Islands are known to have come from natural populations. Accessions from the Wellington Botanic Gardens are thought to originate from Taranaki. All GenBank accession numbers refer to PhAFLP7 except where indicated.

No.	Cultivar	Source	GenBank
	Cordyline terminalis	GenBank sequences	U24016 (ITS1), U24031 (ITS2)
	Dianella nigra	MPN 25063 (Forest Hill Rd)	AY177600 (ITS)
1	Aohanga	Waiomatatini, East Coast, North Island	AY191019
2	Tupurupuru	Gisborne, East Coast, North Island	AY191048
3	Maeneene	Urewera District, North Island	AY191049
4	Tukura	Waihirere, East Coast, North Island	AY191057
5	Takirikau	Tauwharepare, East Coast, North Island	AY191064
6	Paoa	Muriwai, Gisborne District, East Coast, North Island	AY191038
7	Turingawari	Tauwharepare, East Coast, North Island	AY191059
8	Taniwha	Waiomatatini, East Coast, North Island	AY191022
9	Mawaru	Central North Island	AY191023
10	Paretaniwha	Rotoiti area, Central North Island	AY191069
11	Wharanui	Urewera District, North Island	AY191037
12	Atarau	Pipiriki, Whanganui River, North Island	AY191065
14	Ruawai	Near Mt Hikurangi, East Coast, North Island	AY191054, AY177602 (ITS)
15	Taeore	Opunake, Central North Island	AY191055
16	Kohunga	Waikato area, North Island	AY191076
17	Tutaewheke	Botanic Gardens, Wellington, North Island	AY191060
18	Tapamangu	Waiomatatini, East Coast, North Island	AY191024
19	Huhiroa	Botanic Gardens, Wellington, North Island	AY191066
20	Atewhiki	Botanic Gardens, Wellington, North Island	AY191075
21	Ate	Pipiriki, Whanganui River, North Island	AY191042
22	Parekoretawa	Waiomatatini, East Coast, North Island	AY191061
23	Kauhangaroa	Wairoa, Hawke's Bay, North Island	AY191070
25	Awahou	Eastern Bay of Plenty, North Island	AY191062
26	Oue	Gisborne area, North Island	AY191034
27	Tapoto	Hawke's Bay, North Island	AY191039
28	Tane-a-wai	Tikitiki, East Coast, North Island	AY191077
29	Ngaro	Moutoa Estate, Foxton, North Island	AY191035
30	Makaweroa	Torere, Eastern Bay of Plenty, North Island	AY191052
31	Moto-o-nui	Urewera District, North Island	AY191040
32	Rangiwaho	Wharerata Hills, Gisborne, East Coast, North Island	AY191031
33	Taumataua	Urewera District, North Island	AY191067
34	Te Tatua	Gisborne, East Coast, North Island	AY191071, AY177605 (ITS)
35	Raumoa	Botanic Gardens, Wellington, North Island	AY191041
36	Whareongaonga	Wharerata Hills, Gisborne, East Coast, North Island	AY191056
37	Matawai Taniwha	Matawai, near Waioeka Gorge, East Coast, North Island	AY191044
40	Tarere	Gisborne area, North Island	AY191072
41	Wharariki	Gisborne area, North Island	AY191047, AY177603 (ITS)
42	Arawa	Rotoiti area, Central North Island	AY191028, AY177604 (ITS)
43	Opiki	Piako District, North Island	AY191050
44	Whakaari	Urewera District, North Island	AY191068
45	Ruapani	Urewera District, North Island	AY191073
46	Ruahine	Urewera District, North Island	AY191020, AY177601 (ITS)
47	Unknown	Unknown	AY191021
48	Pango	Wharerata Hills, Gisborne, East Coast, North Island	AY191030
49	Takaiapu	Hawke's Bay, North Island	AY191063
50	Ngutunui	Waikato, North Island	AY191051
53	Te Mata	Hawke's Bay, North Island	AY191025
54	Ruawai B	East Coast, North Island	AY191027
60	Potaka	East Coast, North Island	AY191043

(*continued over page*)

Table 1 (*continued*)

No.	Cultivar	Source	GenBank
62	Wharariki	Unknown	AY191029, AY177599 (ITS)
63	Waihirere	Unknown	AY191078
Nka		Norfolk Island	AY191033
Nkb		Norfolk Island	AY191079
160		Camp Cove, Campbell Island (not endemic)	AY191026
161		Tucker Cove, Campbell Island (not endemic)	AY191053
165		Ranui Cove, Auckland Islands (not endemic)	AY191074
166		Raoul Island, Kermadec Islands (not endemic)	AY191045
167		Wharekauri, Chatham Islands (not endemic)	AY191032
168		Pitt Island, Chatham Islands	AY191036
177	Whakaari	Urewera District, North Island	AY191058
181		Three Kings Islands	AY191046

denaturing at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s, with a final step at 72°C for 5 min (to help fill in ends), then were held at 4°C until needed. A negative control (containing H_2O instead of DNA) was included in every polymerase chain reaction (PCR) experiment.

The PCR products were visualised under short-wave UV, cleaned using the Concert™ Rapid PCR Purification System to remove dNTPs and primers, and sequenced in both directions. Originally four accessions (25, 41, 42, 44) were sequenced using standard primers (ITS4 and ITS5; Table 2), but the sequence obtained was of poor quality, and we decided to clone the ITS products. Sequence was obtained from the cloned *Phormium* ITS region, and was found to differ from dicotyledonous plants at the standard priming sites. We then designed primers specific to the ITS region in *Phormium* (PhITS4 and PhITS5).

These primers were used to amplify and sequence the intervening region in a subset of the collection expected to contain much of the genetic variation (14, 37, 41, 42, 46, 62). In addition, sequence from the ITS region of representatives of two closely related native groups to *Phormium*, *Dianella nigra* (Hemerocallidaceae) and *Cordyline terminalis* (Laxmanniaceae), were used as outgroups in phylogenetic analyses. Outgroups have not been included in the fingerprinting techniques, as they are too genetically removed from *Phormium* (in comparison to the genetic distances among accessions) to be of use.

AFLP

Duplicate extractions of DNA were cut with MseI/EcoRI and PCR-amplified with selective primers, and the resulting fragments were visualised on polyacrylamide (PAA) gels. Bands displaying variability consistent between the duplicates were selected, cut from the gel, eluted in water, and PCR-amplified using the selective primers. The product was cloned and sequenced, and primers specific to the fragment were designed.

Seven combinations of selective primers were screened using duplicate extractions of a subset of the collection (25, 41, 42, 44). These were visualised on PAA gels, and showed high levels of polymorphism. Thirty bands were selected from the gels for investigation. Fifteen were cloned and sequenced. The sequence of each fragment was checked using BLAST against the GenBank database, and one (PhAFLP7) was found to have a high percentage of sequence identity with a truncated retrotransposon present in many taxa (e.g., GenBank accession AF350317). Retrotransposons often exhibit high mutation rates (Kumar & Bennetzen 1999; Bennetzen 2000). We speculated that PhAFLP7 would thus be highly variable and appropriate as a marker, and focused on this fragment in order to investigate the potential of the AFLP technique in locating useful sequence-specific markers. This region was amplified and sequenced for the entire collection.

To obtain sequence-quality template from this fragment, a nested pair of primers was designed and used in two rounds of amplifications (PhAFLP7o, PhAFLP7I; Table 2).

ISSR

Extracted DNA was diluted 1:100 with sterile distilled water. Reactions were performed using Qiagen (Hilden, Germany) reagents in a total volume of 20 µl containing 1 × Q solution ™, 1.25 m*M* $MgCl_2$ plus 1.5 m*M* $MgCl_2$ in 1 × PCR Buffer™,

250 µ*M* each dNTP, approximately 15 ρ*M* each primer, 0.5 units Taq polymerase, and 1 µl diluted genomic DNA. Reactions were denatured for 2 min at 94°C, followed by 40 cycles of 94°C for 45 s, annealing for 45 s, elongation at 72°C for 90 s, with a final step at 72°C for 5 min, and were held at 4°C until needed. The optimal annealing temperature was determined for each primer. Negative controls were used for all experiments.

Eleven primers were tested on a subset of the collection predicted to contain all variation within that collection, and ideally from which reasonable-quality DNA had been recently extracted (14, 37, 41, 42, 53, 62). Where available, duplicate extractions were used to determine the consistency of patterns.

Products were visualised on 2% agarose gels; several primers were also investigated on PAA gels. Co-migrating bands in the duplicate extractions were scored as present or absent for each of the accessions.

Cloning

DNA fragments were amplified by PCR and ligated at 4°C overnight into the PGem-T easy™ (Promega) vector. The ligation mix was transformed into Max-Efficiency Cells™ (Ecoli DH5α – Gibco Life Tech) and plated on LB/agar plates with 100 µg ml^{-1} ampicillin, 6 m*M* IPTG, and 3% (w/v) x-gal for blue/white selection. Single colonies were picked into 5 ml LB and 100 µg ml^{-1} ampicillin and grown at 37°C overnight. Plasmid DNA was extracted using the rapid boil method (Holmes & Quigley 1981).

Sequencing

Standard ABI sequencing reactions were performed on clean (dNTP and primer-free) DNA template using the Big Dye kit (PE-Biosystems Inc.). Unincorporated dyes were removed by precipitation with 95% ethanol in the presence of 3 *M* sodium acetate pH 5.2. Sequencing gels were performed by either MUSeq (Massey University DNA Analysis Service) or the Waikato DNA Sequencing Facility.

Phylogenetic analysis

ISSR and AFLP data were analysed using parsimony as implemented by PAUP* 4.0b1 (Swofford 1998), and by split decomposition (implemented in SplitsTree).

Table 2 Sequences of primers used. ITS4 and ITS5 are standard primers for amplifying the ITS region; primers 811–884 were obtained through UBC Biotechnology Laboratory (microsatellite primer set 9).

Primer	Sequence
ITS4(std)/	TCC TCC GCT TAT TGA TAT GC/
ITS5(std)	GGA AGT AAA AGT CGT AAC AAG G
PhITS4/	TTA AAC TCA GCG GGT GGC/
PhITS5	GGT GAA CCT GCG GAA GGA TCA T
PhAFLP7i	CTT GTT CAA CAT ACC TTT CTT GC/
	GTT ACA AAG TCA GAT TAG TTG TG
PhAFLP7o	TTA ACT GTG TGC ATG TTG AGA TTT TGC/
	GAA TTC ACA TGT TCG TTA CAA AGT C
811	GAG AGA GAG AGA GAG AC
813	CTC TCT CTC TCT CTC TT
827	ACA CAC ACA CAC ACA CG
835	AGA GAG AGA GAG AGA GYC
844	CTC TCT CTC TCT CTC TRC
845	CTC TCT CTC TCT CTC TRG
852	TCT CTC TCT CTC TCT CRA
859	TGT GTG TGT GTG TGT GRC
864	ATG ATG ATG ATG ATG ATG
868	GAA GAA GAA GAA GAA GAA
884	HBH AGA GAG AGA GAG AG

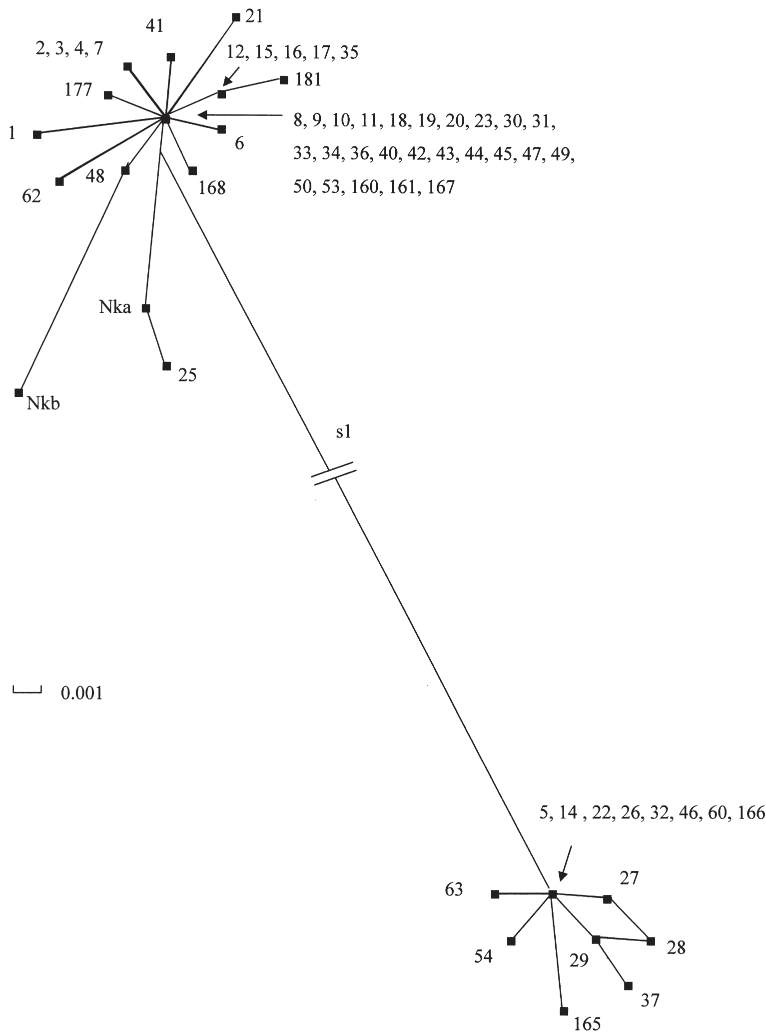

Fig. 1 SplitsTree of PhAFLP7 sequence data: 519 characters, fit = 100. Fit statistic = sum of distances between all pairs of taxa in splitsgraph / sum of distances between all pairs of taxa in distance matrix. s1 is a split present in analysis of both AFLP and ISSR data.

RESULTS

ITS

ITS sequences from morphologically and taxonomically diverse harakeke accessions were identical. The ITS region did not provide resolution between *P. tenax* and *P. cookianum*. When compared with *Cordyline* and *Dianella*, harakeke shared 70% and 92% similarity, respectively.

AFLP

AFLP identified distinct haplotypes within the collection. The AFLP-derived fragment produced from the primer pair PhAFLP7 was characterised at the level of primary sequence variation. A small size difference observed between accession 41 (*P. cookianum*) and the other samples corresponds to a deletion of 26 bases in 41. On sequencing the PhAFLP7 locus in the entire collection, another indel (of four bases) was found to distinguish a group of 15 accessions (5, 14, 22, 26, 27, 28, 29, 32, 37, 46, 54, 60, 63, 165, 166) from the remainder. Many substitution differences were observed between accessions in the collection, and over 30 site patterns supported the split suggested by the indel data (Fig. 1), emphasising the genetic differentiation between the two groups (represented by split s1 in Fig. 1). The data strongly separate accessions into these two clusters, and although relationships within clusters are unresolved in some cases, the data are largely tree-like. Closely related within one cluster are the accessions thought to have

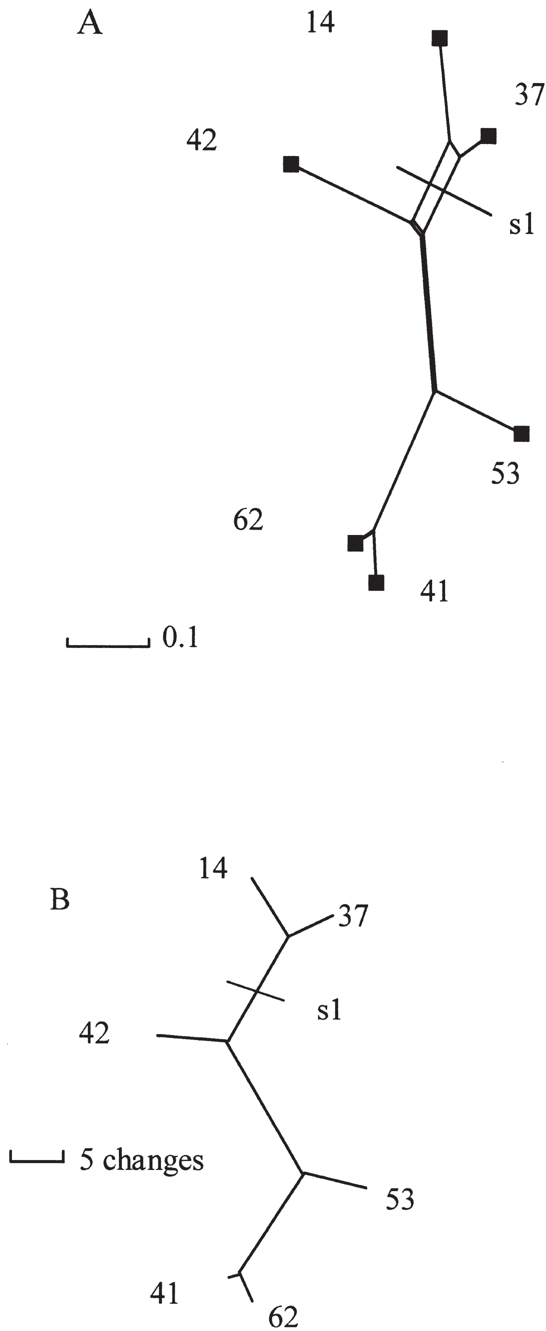

Fig. 2 **A**, SplitsTree of ISSR data: fit = 83.4; **B**, Unrooted phylogram. Accessions 41 and 62 are *P. cookianum*, the remainder are *P. tenax*. s1 is a split present in analysis of both AFLP and ISSR data.

been transported to New Zealand's subantarctic Auckland Islands (165) and to Raoul Island (Kermadec Islands; 166) by Maori or early European settlers in New Zealand.

ISSR

ISSR fingerprints yielded 32 reliable polymorphic bands to score. Even such a small sample provided enough information to distinguish distinct haplotypes in the collection (Fig. 2). Split decomposition analysis showed that these data are also tree-like, and are consistent with split s1 from the AFLP data. ISSR also resolves splits between some accessions that were unresolved in the splitsgraph built from the AFLP marker. In particular, analysis of the ISSR data indicates that accessions 41 and 62 (the only two *P. cookianum* sampled) are genetically similar and are distinct from the *P. tenax* accessions.

DISCUSSION

Interpretation of results is complicated because we do not know the original provenance, or natural location, of many of the plants. The majority of accessions were collected from the East Coast of the North Island, and although the locality at the time of collection is available in all but three cases, many are likely to have been taken there from other regions in the last 1000 years to be used by Maori. This is illustrated by the *P. tenax* accessions collected from the Urewera district, where the species does not naturally occur. In addition to Maori selection and growth of accessions, the period of commercial growing of *Phormium* for fibre (c. 1820s–1970s; Matheson 2000) will have further complicated the geographical association of accessions. Just as varieties selected by Maori were used for commercial production, it is possible that some commercial varieties were adopted by Maori and are now regarded as traditional selections. Biogeographic work in natural populations of harakeke has the potential to determine the geographic origins and relationships of accessions, and the molecular and analytical techniques indicated in this study can provide such genetic information.

The ITS results, with no genetic variation among harakeke accessions, indicate recent (i.e., Quaternary) morphological and presumably ecological diversification, a result consistent with findings for numerous other plant groups in New Zealand (Winkworth et al. 1999, 2002).

The AFLP data strongly separate the accessions into two clusters (Fig. 1), while the ISSR data show consistency with that split and show potential for further characterisation. Some of the groupings are consistent with morphology, such as the short distance between the two *P. cookianum* accessions (41 and 62) in the ISSR dataset, indicating a close relationship between these, in comparison with the other accessions. The grouping of 'Tupurupuru' (2), 'Maeneene' (3), 'Tukura' (4), and 'Turingawari' (7) in the AFLP tree is also consistent with morphology; these accessions are similar in form, markings, and weaving characteristics. Other groupings from the molecular data presented here are inconsistent with morphology, for example, 'Awahou' (25) and 'Oue' (26) are very similar and distinctive but are in the separate clusters of the AFLP tree.

All accessions in the smaller cluster of the AFLP tree were collected from the East Coast, with the exception of 'Ngaro' (29), and the Ranui Cove (Auckland Islands; 165) and Raoul Island (166) provenances. 'Ngaro' was collected from Moutoa Estate at Foxton, where *Phormium* was bred for commercial production. The Ranui Cove accession is quite distinct in its morphological characters from the other four plantings on the Auckland Islands and the three on Campbell Island (Walls 1996). It has similar form and colourings to the Raoul Island accession, but has more brittle fibre and the leaves are not so strong for plaiting (M. Murray pers. comm.). The Raoul Island accession has not been known to flower. Since both harakeke (165 and 166) were transported from the New Zealand mainland, it is interesting to reflect on whether they originated from the same geographical locality.

The eleven accessions examined by Harris & Woodcock-Sharps's (2000) study of *Phormium* fibre qualities are included in our tree based on the AFLP results, but only one accession from our ISSR tree is present in their study. When their cluster trees are compared with our AFLP tree, there is no obvious relationship. For example, 'Arawa' (42) and 'Tapamangu' (18) associate together based on both fibre characters and sequence from PhAFLP7, as do 'Paretaniwha' (10) and 'Takaiapu' (49). However, these two groups are identical based on PhAFLP7, while their fibre qualities are quite distinct; 'Arawa' and 'Tapamangu' have a high yield of easily extracted strong white fibre, whereas 'Paretaniwha' and 'Takaiapu' gave poor yields of brittle green fibre that became difficult to extract (Harris & Woodcock-Sharp 2000). Conversely, 'Ate' (21) and 'Potaka' (60) associate closely based on fibre characters but are widely separated in the AFLP tree.

This lack of concordance with morphology and weaving characters is not entirely unexpected. Our AFLP tree is based on one short segment of DNA. In order to adequately test the relationships, and provide resolution to the molecular tree, further regions of DNA would be examined. The results reported here show the methods we implemented to be useful for such a study.

To help us determine the origins and relationships of harakeke in the Collection, we need to characterise natural populations of *Phormium*. This study and an earlier one which reported on AFLP-derived SCAR markers for other New Zealand plants (McLenachan et al. 2000) suggest that standard conventional markers, such as the nuclear ITS region, provide limited resolution for characterising closely related selections of even morphologically diverse genera such as *Phormium*. Our results show that higher levels of resolution can be found using marker systems such as ISSR and AFLP.

However, care needs to be taken when implementing these methods on natural plant populations. The quality of DNA that was extracted from harakeke using CTAB protocols affected the banding pattern of both AFLP and ISSR primers, so for interpretation of fingerprints, dual DNA extractions were always carried out if possible. With this limitation, ISSR and AFLP fingerprinting methods appear best implemented on small numbers of taxa and/or for testing the phylogenetic groupings suggested by more widely sampled SCAR sequencing studies (the approach adopted in the present work with the PhAFLP7 locus). A further technical point is that, while agarose is commonly used by researchers to visualise AFLP and ISSR profiles (e.g., Lockhart & McLenachan 1997; Esselmann et al. 1999), in our experience with harakeke, the relative paucity of polymorphic bands resolved on agarose compared with PAA gels favoured the use of PAA gels.

The molecular methods we have investigated are not without their technical difficulties. However, the high resolution provided and consistency between the ISSR and the AFLP data indicate that the characterisation of natural populations using these methods, particularly as represented with a network-building technique such as split decomposition, will provide helpful insights into the whakapapa of valued weaving selections of harakeke. The resolution these methods achieved with such closely related and probably anciently hybrid accessions suggests that they will be useful in any such ethnobotanic study.

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