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Key words: Genetic variation; Pohutukawa; Metrosideros excelsa; Isozymes; Band patterns.

Heidi: another item for your literature review of Pohutukawa.

Genetic Variation in Local Populations of Pohutukawa (*Metrosideros excelsa*)

Written by D Hofstra
in consultation with KD Adam & J Bathgate.
Laboratory work by K Brown.

Funded by Project Crimson
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Abstract

The aim of this project is to investigate genetic differentiation in local populations of pohutukawa (*Metrosideros excelsa)* using starch gel electrophoresis of isozymes, with a view to enabling conservation managers to maintain natural patterns of genetic variation in pohutukawa planting programs.

Pohutukawa samples were collected from a total of fifteen populations from seven regions (Northland, Auckland, Kawhia, Tauranga, Lake Rotoiti, Whakatane and East Cape), which encompass the natural range of pohutukawa in New Zealand.

These samples were analysed for six enzyme systems: IDH, AAT, PGM, PGD, GPI and MDH. Two of these enzyme systems (IDH and AAT) seldom produced reliable band patterns for any of the pohutukawa populations. Four of these enzyme systems (PGM, PGD, GPI, and MDH), consistently yielded band patterns for all populations. These four enzyme systems were all polymorphic ie; they had more than one band pattern revealed in at least one of the regions. However the isozyme data reveal limited genetic variation between populations and even less variation within populations.

Introduction

Pohutukawa (*Metrosideros excelsa*) have been fondly mentioned in poetry, prose and folklore since the time that people first came to New Zealand. Today they are perhaps best known to many New Zealanders for their bright red flowers at Christmas time which makes them a prominent feature of northern shorelines. There are two species in New Zealand called pohutukawa. *Metrosideros kermadecensis* also called Kermadec pohutukawa, is found naturally only on Raoul Island in the Kermadec Islands, while *Metrosideros excelsa* naturally occurs on the northern shorelines of the North Island (Conly and Conly, 1988).

M.excelsa is distinguished by the generally larger size of all its parts, and its pointed-tipped leaves. *M. kermadecensis* is somewhat smaller and more compact, with rounded-tipped leaves. Although both may be flowering at once, the peak flowering for *M.kermadecensis* is slightly earlier (around November-December) than that of *M. excelsa* (around December-January). The pohutukawa are also closely related to the New Zealand rata and in fact are all placed in the same genus (*Metrosideros*). Indeed hybrids between the two species are common especially on Rangitoto (Cranwell, 1981). Northern bushmen recognised the status of such hybrids long ago, calling them 'bastard pohutukawa'. Like the parent plants hybrid pohutukawa flower freely and set good seed. Pohutukawa seeds are not hard to find, the average number of seeds in each capsule being about 850 (Conly and Conly, 1988). The dispersal of such a large quantity of fine seed enables quick colonisation of bare sites (Hancox ,1993).

Historically pohutukawa decorated the seaside north from about Gisborne on the east coast and Urenui on the west coast. Planting has extended the spread of the trees to places south to Wellington, even to the South Island, some of the more notable trees becoming conspicuous (Conly and Conly, 1988). However the present pohutukawa stands are only a fraction of the original pohutukawa resource, as a result of felling in the early settler day for shipbuilding and the more recent impact of feral animals. Pohutukawa timber is extremely strong and hard and has been used in the past for ready-made curves in shipbuilding. Thomas Kirk (1889) suggested that in view of the increasing scarcity of pohutukawa in the North Island it would be far wiser to conserve the small-leaved pohutukawa of the Kermadec Islands than to allow it to be destroyed merely to facilitate settlement (Conly and Conly, 1988).

In the Homunga Bay Block of the Orokawa Scenic Reserve "the presence of domestic farm stock and feral goats has had a profound influence on the plant community, with little regeneration of any tree or shrub species at ground level within the most heavily grazed areas" (Hosking and Hutcheson, 1993). In addition, possums are responsible for a large share of the damage to existing mature trees. Both crown thinning and crown contraction are related to severe and prolonged possum damage.

In 1990, Northland staff of the Department of Conservation and NZ Forest Products (now Carter Holt Harvey Forestry) decided pohutukawa needed help, and Project Crimson was born (Hancox, 1993). The aim of the Project Crimson Trust is to restore the coastal pohutukawa fringe of northern New Zealand through replanting and maintenance projects. However not just any pohutukawa will do. The trust has a policy not to risk genetic pollution by planting seedlings outside their area of origin (Simpson pers. com. 1994). Like humans, plants have DNA "fingerprints" which contain individual and species identity. Over millions of years plants have adapted to their particular surroundings, including factors such as climate, soils, pests and diseases. Genetic pollution, also called 'outbreeding depression', describes a situation where non-local and hence less well-adapted genetic material is brought into an area where it interbreeds with the local population, lowering the fitness of the latter. In the longer term the poorly adapted genetic material should be eliminated again by selection, but in the short term a reduced population fitness could threaten its chances of survival.

While the concept of outbreeding depression is a valid one, plant populations often experience dramatic shifts in their environments, not the least brought about very recently - in evolutionary terms - by human activity. This and other factors affecting their genetic structure, particularly related to their breeding system, means that in reality plant populations are not necessarily finely tuned and closely adapted to local environments in which we find them today.

In the genetic management of threatened plant species, it is sensible to try and maintain the 'genetic integrity' of local populations, provided that these populations are large and genetically variable enough to prevent 'inbreeding depression', the other far more serious scourge of conservation genetic management. Genetic integrity can be maintained by using local seed or vegetative material only for vegetative restoration. The aim of this project is to assess the amounts of genetic variation within, and the degree of genetic differentiation between local populations of pohutukawa in New Zealand with the help of isozyme genetic markers.

Materials and Methods

Collection of Samples

Populations of Pohutukawa were sampled from a wide distribution encompassing the known natural distribution of Pohutukawa on the North Island of New Zealand. Samples were collected from as far north as Whangarei, as far south as East Cape on the east coast of the North Island and Kawhia on the west coast of the North Island. The location of collection sites are given on Table 1 and Figure 1.

Table 1. **Pohutukawa Sample Sites**

Population Location	**Grid Reference** NZMS 260	**Latitude Longitude**	**Collector***
Northland			G Brackenberry
Whangarei	Q07	35°44'S 174°19'E	
Maunganui Bluff			
Area 1	O07 623 048	35°46'S 173°34'E	
Area 2	O07 600 068	35°45'S 173°33'E	
Area 3	O07 592 078	35°44'S 173°33'E	
Auckland			P Simpson
Rangitoto	R11	36°48'S 174°52'E	
Waitakere			
Karekare	Q11 418 675	36°59'S 174°29'E	
Pararaha	Q11 428 644	37°01'S 174°30'E	
Kawhia	R16 698 470	38°04'S 174°49'E	P Simpson
Lake Rotoiti	V15 107 441	38°04'S 176°49'E	S Smale
Tauranga			
Orokawa Reserve	U13 705 217	37°22'S 175°57'E	B Marhel
Whakatane	W15 &		
& Ohope	W13/14 630 545	38°57'S 177°01'E	D Boguslawski
East Cape			H Hovell
TomoTomo Ariki	Z14 782 900	37°34'S 178°19'E	
Te Koau	Z14 800 870	37°36'S 178°20'E	
Awatere Mouth	Z14 845 825	37°39'S 178°28'E	
Taumata	Z14 927 810	37°40'S 178°29'E	
Poretua	Z14 955 785	37°39'S 178°30'E	

* All collectors are DoC Staff (see Appendix) except D Boguslawski.
Site maps &/or Grid References were provided for the Manganui Bluff Lake Rotoiti, Orokawa and East Cape samples.

Twigs with young shoots and lateral buds were collected from each tree, with approximately forty trees in each locality being sampled. The shoot tips were kept moist after collection and once back at the laboratory were refrigerated prior to analysis by starch gel electrophoresis.

Figure 1. **Pohutukawa Sample Sites**

Isozyme Analysis

Each sample was analysed for the same six enzyme systems: AAT, IDH, PGM, PGD, GPI and MDH. Preliminary studies with pohutukawa had indicated these enzyme systems were likely to reveal distinct band patterns, which could be used to analyse populations on the basis of observed isozyme variation.

A sample of approximately 50mg of leaf bud or young leaf tissue was taken from each plant and ground with 50µl Walters extraction buffer (Walters et al, 1989). Samples were kept at a temperature of 4°C to 6°C throughout the extraction process. After grinding, the extracts were centrifuged for 3 minutes at 11000*g*. The extracts were loaded and run in horizontal starch gels, prepared from 11% (w/v) Connaught starch. Electrophoretic separation of isozymes was carried out using gel and tray buffer systems as described by Gottlieb (1981) and Adam et al (1987).

Following electrophoresis, gels were subjected to enzyme specific staining procedures based on those by Wendel and Weeden (1989) and Harris and Hopkins (1976). Enzyme systems stained for were aspartate aminotransferase (AAT, EC.2.6.1.1), isocitrate dehydrogenase (IDH, EC.1.1.1.42), phosphoglucomutase (PGM, EC.5.4.2.2), 6-phosphogluconate dehydrogenase (PGD (6-PGD), EC.1.1.1.44), glucose-6-phosphate isomerase (GPI (=PGI), EC.5.3.19), and malate dehydrogenase (MDH, EC.1.1.1.37). These are the enzyme systems which revealed bands of a consistent quality in preliminary experiments with pohutukawa extracts.

Upon development of the stains, those gels of suitable quality (with good activity) were photographed, and the individual banding patterns on all of the gels were recorded for later analysis.

Results

During the period of September to November 1994, fifteen populations of pohutukawa were sampled, from seven different regions. The regions were; Northland, Auckland, Kawhia, Lake Rotoiti, Tauranga, Whakatane and East Cape (Table 1). At least 40 samples were analysed from each of the different regions.

Every sample was stained for six enzyme systems, however two systems (IDH and AAT) did not consistently reveal bands for all populations. Therefore the results for IDH and AAT are not present for all populations analysed and can not be incorporated in a comparison of band patterns or band pattern (phenotype) frequencies between populations.

Of the other four enzyme systems (PGM, PGD, GPI and MDH) polymorphisms were revealed in at least one of the seven regions for each enzyme system (Table 2). PGM had variable activity. Only the top locus could consistently be scored. At this PGM locus three different band phenotypes were observed. The band phenotypes are in agreement with a simple genetic interpretation for a monomeric enzyme. The first and fastest allele occurs at the highest frequency in all populations and where the second allele is present it only occurs at a low frequency relative to the first allele.

PGD is monomorphic in all regions except Auckland. The Rangitoto samples include several individuals which have a band phenotype not seen in the other populations. Likewise GPI is monomorphic in all except one region. The samples from Whakatane and Ohope revealed the existence of a second allele present at very low frequency within the region (Table 2).

For the last enzyme system MDH, a second band pattern was observed in two populations from two different regions, Auckland and Kawhia. This second phenotype was present at lower frequencies in both of these populations than the first phenotype which is common to all regions (Table 2).

In general the level of variation observed within the pohutukawa populations analysed is low. There is limited variation between populations or regions, and even less within populations or regions.

Table 2. **Enzyme Systems & Phenotype Frequencies in Pohutukawa Populations**

Population	Enzyme System & Phenotype Frequencies			
	PGM	PGD	GPI	MDH
Northland				
Whangarei	13	17	17	17
Maunganui Bluff				
Area 1	9	7	10	10
Area 2	17	2	21	21
Area 3	2	5	8	8
Auckland				
Rangitoto	19	23 9	35	29
Waitakere	34 1	32	38	29 7
Karekare				
Pararaha				
Kawhia	28	28	28	22 6
Lake Rotoiti	36 4	40	40	40
Tauranga				
Orokawa Reserve	39 1	40	40	40
Whakatane				
& Ohope	31 3	39	31 1 3	36
East Cape				
TomoTomo Ariki	35	34	34	32
Te Koau	20 3	40	40	30
Awatere Mouth	19	29	29	25
Taumata	15 6	30	21	9
Poretua	32 5	38	21	38

Discussion

The aim of this project was to investigate genetic differentiation in local populations of pohutukawa using isozyme analysis. As a first look at pohutukawa, six to eight local populations (of approximately 40 samples each), which represented the entire current area of pohutukawa in New Zealand were analysed to estimate multilocus genotypes and allele frequencies at polymorphic loci.

This part of the project has been completed. The samples collected covered seven regions throughout the natural range of pohutukawa (Figure 1). As can be seen from Table 2, allele frequencies or phenotype frequencies for polymorphic loci do not differ significantly between populations. For example in the enzyme systems where the isozyme phenotypes can readily be described as allele frequencies (PGM and GPI), the occurrence of the second allele in those populations where it is revealed is at a very low frequency. The second PGM allele is observed in seven of the fifteen populations with frequencies that range from 0.14 - 0.01, in the Taumata and Waitakere populations respectively. The second GPI allele is only observed in the Whakatane region, at a frequency of 0.1.

Likewise although PGD and MDH are polymorphic enzyme systems in pohutukawa, the occurrence of the second phenotype in one population (Rangitoto) for PGD and in two populations (Waitakere and Kawhia) for MDH, is low compared with the first phenotype.

Although obvious differences have been reported in the physical appearance of different pohutukawa populations, such as the hybrids on Rangitoto (Cooper, 1954), and the recently discovered "genuine Taranaki Pohutukawa" (Gamble, 1995) the isozyme differences seen here are not sufficient to provide population mapping on a genetic differentiation basis. That is, the differences which have been observed through isozyme analysis are not significant and therefore do not enable mapping of populations on the basis of this variation. However, this is not to say that genetic differentiation between pohutukawa populations does not exist, only that this level of information is not revealed here where we are dealing with a limited number of enzyme systems.

In addition, some of the variation observed within populations may actually be due to spontaneous interspecific hybridisation. Hybrids are known to form readily between rata (*M. robusta*) and pohutukawa (*M. excelsa*) and between *M. excelsa* and

M.kermadecensis. The gradient in forms or appearance between hybrids and parents is not always obvious unless these plants are growing side by side, when distinctions may be apparent. Hence while due care was taken to sample *M. excelsa* only, we cannot rule out that some of the genetic variation observed was due to the inclusion of hybrid individuals, especially *M. excelsa x kermadecensis*, in some of our samples. It was not within the scope of this first and very limited investigation of the genetic structure of populations of *M excelsa* in New Zealand to analyse the isozyme patterns of rata spp. and Kermadec pohutukawa, and of confirmed hybrids between these species and *M. excelsa*, but future population genetic analyses are well advised to do so. Given the low levels of variability found at isozyme loci, future genetic analyses of pohutukawa in New Zealand should also include the use of other genetic markers, in particular DNA-level markers such as RAPDs (Random Amplified Polymorphic DNA), which are more abundant and likely to how more genetic variability.

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Appendix

List of Sample Collectors

S Smale	DOC, BOP conservancy PO Box 1146 Rotorua	PH 07 347 9179
B Marhel	DOC PO Box 1026 Tauranga	
P Simpson	DOC PO Box 10426 Wellington	PH 04 471 0726
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G Brackenberry	Whangarei	PH 09 438 0299
D Boguslawski	c/- D Hofstra Biological Science University of Waikato	PH 07 8562889 ext 8492