Genetic diversity of *Dactylanthus taylorii* in New Zealand

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CONTENTS

Abstract

Randomly amplified polymorphic DNA (RAPD) markers were used to investigate genetic variation amongst 17 populations (146 individuals) of the endangered parasitic plant *Dactylanthus taylorii*. The objective was to provide a means of identifying a set of populations that are representative of the full range of genetic diversity within the species, towards which conservation resources might be targeted. RAPDs produced clear, reproducible bands, and 84 polymorphic marker bands were identified. Analysis of the RAPD data, based on Nei's genetic distance, produced a dendrogram that grouped all individuals (bar one) into their expected populations. A similar analysis at the population level showed the grouping of populations was, to a significant extent, determined by geographical distribution. Two major clusters were evident, one containing populations close to and east of Lake Taupo, and the second consisting mainly of populations west of Lake Taupo. Little Barrier Island, the most isolated population, occupied a discrete branch within the second cluster. Further geographical ordering was evident within the major clusters, with neighbouring populations being grouped together. The populations at Little Barrier Island, Pirongia, Mamaku, and Waitaanga Forest were identified as being the most genetically distinct at the national level, and it is recommended that these are targeted for management. Overall, genetic groupings did not reflect conservancy boundaries. For this reason it is also recommended that conservancy management decisions regarding the allocation of resources to populations, or withdrawal thereof, should take into account the genetic status of those populations at the national level.

1. Introduction

1.1 BACKGROUND

Dactylanthus taylorii Hook. f. (Balanophoraceae) is an endemic, fully parasitic plant, and an ancient member of the New Zealand flora. The plant consists of a round, warty tuber of up to 50 cm diameter which attaches as a parasite to the root of a host tree or shrub (Ecroyd 1996). The tuber exists predominantly underground, with usually only the inflorescences of the plant exposed at the surface.

Isolated, often small (<20 tubers) populations of *D. taylorii* are scattered over a large area of the North Island. These populations are threatened, primarily, by the browsing of inflorescences by possums and rats (Ecroyd 1996). *Dactylanthus taylorii* is classified as an endangered species of national importance (Molloy & Davis 1994) and all North Island Department of Conservation (DOC) Conservancies (Northland and Wellington excepted) are

involved in managing populations. Management consists of both the caging of individual tubers and the use of poisoning, to prevent browsing (Ecroyd 1995).

A principal aim in the conservation of any endangered species is to preserve as much of its genetic diversity as possible. Where populations are widespread and scattered, as is the case for *D. taylorii*, the most financially and logistically realistic means of doing this is to concentrate efforts upon key populations that represent a maximum proportion of the species' genetic diversity (Maxted et al. 1997; Petit et al. 1998). However, in the absence of information on the genetic distinctness of *D. taylorii* populations, management efforts are currently spread over all populations. Significant resources have been committed to these efforts, especially in conservancies managing several small, scattered populations or a few, very large ones (Anon. 1995; Anon 1996).

The primary objective of this study was to obtain information about genetic variation amongst populations of *D. taylorii*, so that DOC resources, at both the conservancy and national level, might be targeted towards populations which best represent the overall genetic diversity of the species.

1.2 RANDOMLY AMPLIFIED POLYMORPHIC DNA MARKERS

Holzapfel (in press) carried out a preliminary investigation of genetic variation in *D. taylorii*, using randomly amplified polymorphic DNA markers (RAPDs). Based upon the success of that work, the current study also utilised RAPDs.

RAPDs form a DNA marker technique (Williams et al. 1990) which utilises the polymerase chain reaction (PCR). PCR allows the amplification of specific DNA sequences within a genome. Using this methodology, short DNA molecules (primers) are placed in a reaction mix with genomic DNA, and bind to sequence(s) within that DNA which are complementary to their own. If the two primer molecules bind within a distance of 2 kilobase pairs of one another, then the intervening sequence will be amplified (Hoelzel & Green 1998).

RAPDs utilise only a single primer per reaction. Because the primer is not designed to recognise one specific sequence, it may bind at multiple, unknown positions within the genome. Therefore RAPDs usually produce a number of DNA fragments of varying sizes, from each DNA sample being compared. The fragments are separated according to size on an agarose gel and then stained, resulting in a characteristic banding pattern for each individual sample. Bands which vary in terms of presence or absence across all samples (polymorphic bands) are identified as markers. The similarity of samples is then computed from the presence or absence of each marker in each sample.

2. Materials and methods

2.1 PLANT MATERIAL

Inflorescences were collected as buds, prior to or at the beginning of flowering, from populations at 17 North Island localities (Table 1, Figure 1) during 1998. The exception to this was the Mamaku population, for which samples collected in 1996 were used. Note that Tarawera Plot includes only a single sample, and the Egmont National Park population comprised individuals from six sub-populations. A peduncle section immediately below the inflorescence head was cleaned and excised as a block, then frozen in liquid nitrogen and stored at −80°C until DNA extraction could be performed. A reference collection of all samples is held by A.S. Holzapfel (DOC, Waikato Conservancy).

TABLE 1. LOCATION OF *Dactylanthus taylorii* POPULATIONS AND NUMBER OF INDIVIDUALS SAMPLED FOR GENETIC ANALYSIS.

LOCALITY	CONSERVANCY	NO. SAMPLED
Little Barrier Island	Auckland	10
Pirongia	Waikato	10
Pureora	Waikato	10
Paeroa Range	Bay of Plenty	10
Minginui	Bay of Plenty	10
Mamaku	Bay of Plenty	9
Opepe	Tongariro/Taupo	8
Whakaipo	Tongariro/Taupo	10
South Pihanga	Tongariro/Taupo	6
Ohakune	Tongariro/Taupo	4
Tarawera Plot	East Coast/Hawke's Bay	1
Te Araroa	East Coast/Hawke's Bay	10
Waikaremoana	East Coast/Hawke's Bay	10
Hawke's Bay	East Coast/Hawke's Bay	9
Waitaanga Forest	Wanganui	3
Egmont National Park	Wanganui	19
Mangaweka	Wanganui	7
Total		**146**

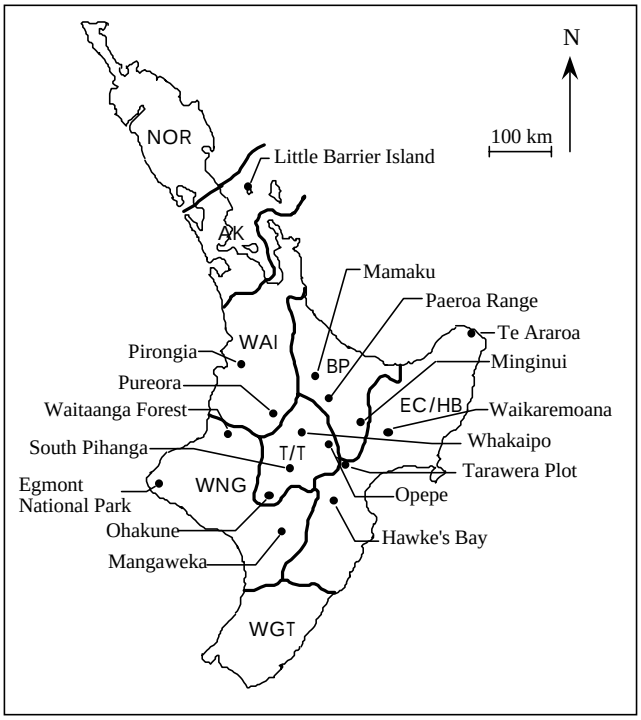

Figure 1. Map of the North Island of New Zealand, showing the location of the *Dactylanthus taylorii* populations used in the study and Conservancy boundaries (NOR Northland, AK Auckland, WAI Waikato, BP Bay of Plenty, EC/HB East Coast/Hawke's Bay, T/T Tongariro/Taupo, WNG Wanganui, WGT Wellington)

2.2 DNA EXTRACTION AND POLYMERASE CHAIN REACTION

DNA was extracted from c. 0.1 g of frozen tissue, using the CTAB method of Doyle & Doyle (1990), with some modifications (Appendix I). DNA quantity and quality were assessed using a spectrophotometer (Genequant, Pharmacia, USA).

DNA from all 146 individuals was amplified by PCR, using each of five different synthetic oligonucleotide primers (Operon Technologies, Inc., USA):

Primer	***Sequence***
OP-P02	5'-TCGGCACGCA-3'
OP-P10	5'-TCCCGCCTAC-3'
OP-P11	5'-AACGCGTCGG-3'
OP-P16	5'-CCAAGCTGCC-3'
OP-A20	5'-GTTGCGATCC-3'

PCR conditions are given in Appendix 1. Reproducibility between PCR runs was assessed by including a positive control sample with each set of reactions, and also by repeating PCR for selected individuals at a later date, for each primer used.

RAPD products were separated on a 1.5% Tris-borate-EDTA (TBE) agarose gel, and stained with ethidium bromide. For each primer, polymorphic bands were identified across all samples. Each individual was then scored for the presence or absence of each band, with 1 = present, 0 = absent and ? = missing data.

2.3 ANALYSIS OF GENETIC VARIABILITY

Using the population genetics analysis freeware POPGENE v.1.31 (Yeh et al. 1997), a UPGMA cluster analysis was performed on the data, based on Nei's genetic distance (Nei 1972). The higher the value of the genetic distance between samples, the more genetically distinct they are. Dendrograms were produced showing the relationships between all individuals, as well as between each population. Separate analyses were also made for populations at the conservancy level.

3. Results

The RAPDs method used resulted in clear, reproducible bands. Using the five primers listed above, a total of 84 polymorphic bands were identified amongst the 17 populations:

OP-P02, 20; OP-P10, 14; OP-P11, 14; OP-P16, 15; OP-A20, 21.

A further seven bands were identified that were monomorphic, and these were excluded from analysis. Of the markers found, nine were specific to certain populations: two to Little Barrier Island, two to Pirongia, and one each to Whakaipo, South Pihanga, Pureora, Waitaanga Forest, and Ohakune. The remainder varied in their presence or absence amongst all populations.

Banding patterns were relatively uniform within populations and more variable between populations (Fig. 2). This was reflected in a UPGMA analysis at the level of the individuals. This grouped all individuals into their correct popu-

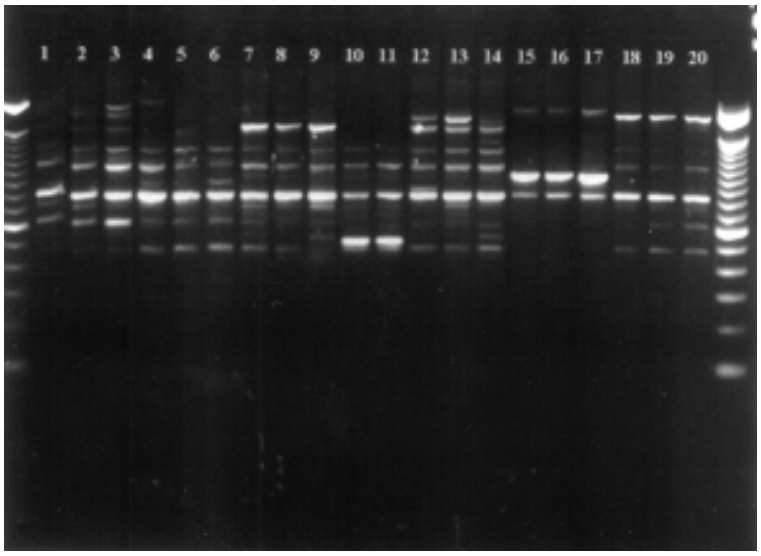

Figure 2. RAPD patterns of individuals from seven *Dactylanthus taylorii* populations, illustrating higher genetic variability between populations than within. Lanes 1-3 Little Barrier Island; lanes 4-6 Pirongia; lanes 7-9 Pureora; lanes 10-11 Waitaanga Forest; lanes 12-14 Egmont National Park; lanes 15-17 Opepe; lanes 18-20 Mamaku. The outside lanes contain 100 bp ladder (Life Technologies, USA).

lations (data not shown), with two exceptions. One Pureora sample, which had RAPD phenotypes that were unique amongst all sampled individuals, was placed on a branch outside of all other populations. It also lacked three marker bands that were otherwise monomorphic amongst the other samples (species markers). Based on the possibility that this DNA sample had been contaminated by an outside source of DNA, it was excluded from analysis at the population level. One Whakaipo individual was also grouped with individuals of the neighbouring Opepe population.

At the population level UPGMA analysis revealed groupings of populations that reflected to some extent their geographical distribution (Figs 1, 3). Two main clusters were evident. The first (uppermost in Fig. 3) consisted mainly of populations close to and/or east of Lake Taupo. The second major cluster mainly included populations located to the west of Lake Taupo, with the exception of Mamaku (Bay of Plenty). Within this cluster, Little Barrier Island, the most isolated population (Fig. 1), occupied a single, distinct branch, with the remaining populations clustering more closely. Within the major clusters, further geographical ordering was apparent. For example, neighbouring pairs of populations were often grouped together, e.g. Opepe and Whakaipo, Waikaremoana and Minginui, Hawke's Bay and Mangaweka. However, exceptions to this trend were also found, such as Pureora and Egmont NP, Paeroa Range and South Pihanga.

The population analysis showed that populations did not generally group naturally into conservancies on a genetic basis (Fig. 3) and UPGMA analyses of the populations within each conservancy corroborated this (Fig. 4). Populations that are both geographically close and within the same conservancy were closely grouped by the conservancy level analysis, but only the Opepe and Whakaipo populations fall into this category.

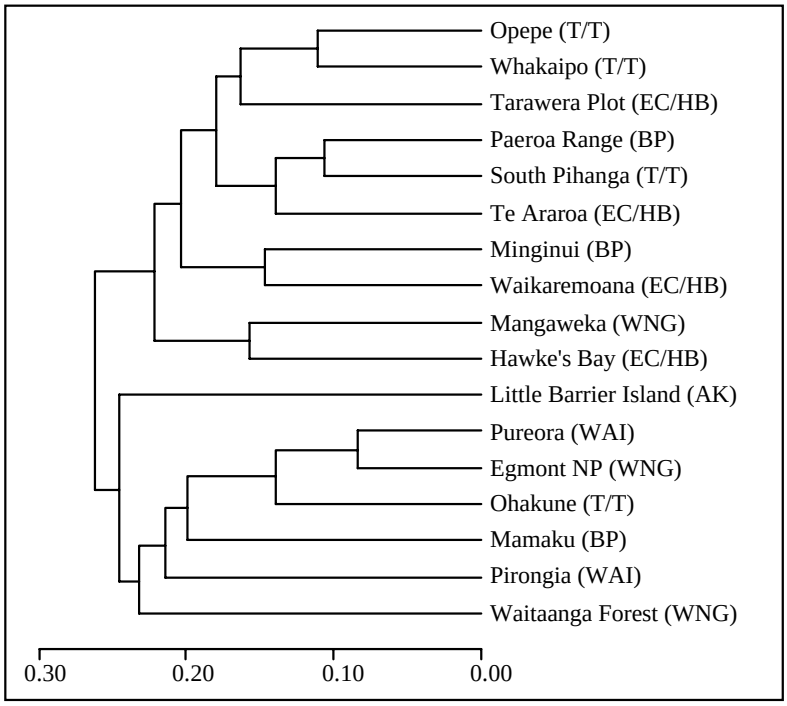

Figure 3. Dendrogram showing relationships between populations of *Dactylanthus taylorii*, based upon Nei's genetic distance (scale at bottom), produced by UPGMA cluster analysis. Conservancies are given in parentheses: T/T = Taupo/Tongariro, BP = Bay of Plenty, EC/HB = East Coast/Hawke's Bay, AK = Auckland, WAI = Waikato, and WNG = Wanganui.

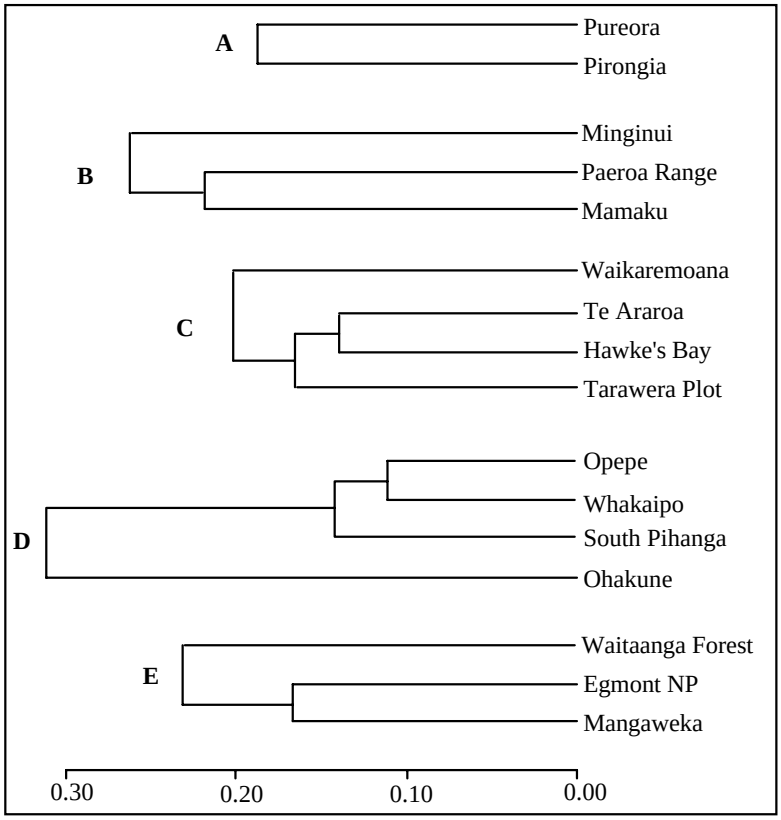

Figure 4. Dendrogram showing relationships between populations of *Dactylanthus taylorii* within each conservancy, based upon Nei's genetic distance (scale at bottom). A. Waikato; B. Bay of Plenty; C. East Coast/Hawke's Bay; D. Taupo/Tongariro; E. Wanganui. Note that the Auckland Conservancy contains only one population (Little Barrier Island), and is therefore not shown.

At the national level the Pureora and Pirongia populations occurred in different major clusters (Fig. 3), therefore within the Waikato Conservancy they are notably genetically distinct (Fig. 4A). Although the populations in the Bay of Plenty Conservancy (except Mamaku) occupied the same major cluster at national level, they were more similar to populations from other conservancies than each other (Fig. 3). Therefore, at the conservancy level these populations are genetically diverse (Fig. 4B). Similarly, genetic diversity amongst the widely-scattered populations of the East Coast/Hawke's Bay Conservancy is high (Fig. 4C). Although all four populations were within the same large cluster at national level, they were more closely associated with populations from other conservancies (Fig. 3). In the Taupo/Tongariro Conservancy the northern Lake Taupo populations, Opepe and Whakaipo, are genetically similar and both are also genetically close to the southern Lake Taupo population, South Pihanga (Fig. 4D). However, Ohakune occupied a different major cluster at national level and is significantly distinct from the other populations in this Conservancy. There is genetic dissimilarity amongst the populations of the Wanganui Conservancy (Fig. 4E). At national level, Mangaweka population occupied a different major cluster to Egmont National Park and Waitaanga Forest (Fig. 3) and the latter two are more closely associated with populations from other Conservancies. The Auckland Conservancy contains only the Little Barrier Island population.

4. Discussion

The purpose of this research was to investigate genetic variation amongst populations of *D. taylorii*, with the aim that populations that are genetically representative of the species as a whole might be identified. The RAPDs technique proved to be a successful means of achieving this, providing a set of 84 genetic markers from which a logical representation of the genetic similarity (measured as genetic distance) between populations was obtained. The information provided, primarily that in Fig. 3, is intended as a guide on which decisions regarding the targeting of certain key populations for management can be based.

We have shown that genetic similarity amongst populations of *D. taylorii* is influenced by their geographical distribution. Populations that are spatially close tend to be genetically similar whilst the more isolated populations are relatively distinct. The most genetically distinct populations identified at the national level are Little Barrier Island, the most isolated of the populations investigated, along with Pirongia, Mamaku, and Waitaanga Forest. As such, it is essential that these populations are amongst those targeted in any streamlined conservation strategy. The Tarawera Plot population was relatively dissimilar to others in its cluster, but it was represented only by a single plant and therefore its placement cannot be treated with certainty. The remaining populations grouped more closely in clusters of two or more, based largely upon their geographical association, and it is conceivable that, within these tighter

groupings, one or two representative populations could be selected for continued management, with the remainder to be excluded.

Decisions regarding the distribution of management resources to key *D. taylorii* populations will be made within each conservancy. The association between genetic similarity and geographical proximity has notable consequences at the level of the conservancy. Populations within each conservancy are generally widespread and, in some instances, their closest neighbour lies across a conservancy boundary (e.g. Minginui and Waikaremoana). The result of this is that populations do not group naturally into conservancies, upon a genetic basis, but tend to associate more closely with populations from other conservancies. It is therefore important that attention is not focused exclusively within the conservancy when selection of key populations is made, but that the national situation is also taken into account. By concentrating solely upon conserving the widest range of genetic diversity possible within the conservancy there is a risk that key populations at the national level could be excluded from management or, conversely, populations that constitute a less distinct population at the national level could be targeted for management efforts. This would lower the proportion of the species' overall genetic diversity that will receive management attention. Therefore, it is strongly recommended that management decisions made at the conservancy level should take into account the genetic status of each population at the national level.

5. Acknowledgements

We gratefully acknowledge the help received from staff at DOC who collected most of the samples and who provided details of the populations; Janet Oddy for assistance in the early stages of this study; Neil Fitzgerald for providing the Tarawera Plot sample; and the members of the *Dactylanthus* Recovery Group of DOC for their ongoing support of research into the genetic diversity of *D. taylorii*.

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Appendix 1. Details of methodology

DNA extraction

A block (c. 5 mm^3) of inflorescence tissue was submerged in liquid nitrogen and ground to a fine powder using a mortar and pestle. A volume equivalent to 100 µL powder (c. 0.1 g) was transferred to a 1.5 mL microcentrifuge tube and 600 µL pre-warmed (60°C) CTAB buffer (2% (w/v) CTAB, 1.4 M NaCl, 0.2% (w/v) β-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), 1% polyvinylpyrrolidine (MW 40 000)) and 30 µL proteinase K (1 mg/mL) were added. After briefly vortexing, an additional 1 µL β-mercaptoethanol was added, and the mixture incubated for at least 60 min at 60°C.

To extract DNA, 800 µL of chloroform:isoamyl alcohol (24:1) was added to the tube, the suspension inverted several times to mix, and then centrifuged for 5 min at 13 000 rpm in a microcentrifuge. The supernatant was removed to a new tube, the chloroform:isoamyl alcohol extraction repeated, and the supernatant was again transferred to a new tube. To precipitate DNA, 400 µL cold (−20°C) isopropanol was added to the extract, the suspension inverted several times to mix, and then left at −20°C for 90 min.

The DNA was pelleted by centrifuging at 13 000 rpm for 15 min in a microcentrifuge. The supernatant was discharged and the DNA resuspended in 500 µL NaCl, at 37°C. This suspension was centrifuged at 3000 rpm for 5 min to remove precipitated polysaccharides, and the supernatant removed to a fresh tube. This was then incubated at 95°C for 30 min.

The DNA was precipitated by adding 500 µL cold (−20°C) isopropanol, inverting to mix, and incubating at −20°C for 20 min. DNA was pelleted by centrifugation at 13 000 rpm for 15 min in a microcentrifuge, and the supernatant

discharged. The pellet was washed briefly in 500 µL 70% ethanol, followed by centrifugation at 13 000 rpm for 5 min in a microcentrifuge. The supernatant was discharged and the wash repeated with 500 µL 95% ethanol, followed by centrifugation.

The supernatant was removed and the pellet vacuum-dried before resuspension in 100 µL TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). 1 µL RNase (1 mg/mL) was added and the suspension incubated at 37°C for at least 60 min.

DNA samples were quantified using a low quantity spectrophotometer (Genequant, Pharmacia), and with the same device quality was assessed by the ratio of absorbances 260 nm:280 nm. Samples with a 260:280 value less than 1.7, and those with a quantity less than 30 ng *ds*DNA/µL, were discarded and later re-extracted. For polymerase chain reaction (PCR), extracts were diluted to a working concentration of 6 ng/µL.

PCR reaction mix and amplification conditions

A reaction volume of 25 µL was used, containing 1 µL 10 mM dNTPs (2.5 mM each of dATP, dCTP, dGTP and dTTP) (Boehringer Mannheim), 4 µL 5µM primer (Operon Technologies Ltd.), 2.5 µL 10x PCR buffer containing 1.5 mM $MgCl_2$ (Boehringer Mannheim), 0.2 µL Taq DNA polymerase (5 units/µL) (Boehringer Mannheim), 4 µL template DNA (6 ng/µL) and 13.3 µL sterile distilled water. Thin-walled 0.2 mL PCR tubes were used for the PCR reactions.

PCR was carried out using a Mastercycler gradient thermocycler (Eppendorf, USA). This thermocycler utilises a heated lid, so mineral oil was not used. Amplification conditions were: initial denaturation at 94°C for 2 min, followed by 34 cycles of denaturation at 94°C (1 min), annealing at 46°C (1 min) and elongation at 72°C (2 min). An additional 72°C elongation step (10 min) completed the programme.

Appendix 2. RAPD data for *Dactylanthus taylorii* populations

Populations: OP = Opepe, WH = Whakaipo, MIN = Minginui, WK = Waikaremoana, PR = Paeroa Range, LBI = Little Barrier Island, SP = South Pihanga, PUR = Pureora, OH = Ohakune, WF = Waitaanga Forest, EG = Egmont National Park, TA = Te Araroa, PI = Pirongia, MAN = Mangaweka, MAM = Mamaku, HB = Hawke's Bay, TPT = Tarawera Plot.

Population/ individual	**Primer**				
OP	OP-P16	OP-P10	OP-P11	OP-A20	OP-P02
1	0100010000010100	0000010000000000	0110111001000	0111110110110111101100	1110101100010100000000
2	0100010000010000	0000010000000000	0110111010000	????? 10?? 011001?0?100	1110101111001010000000
3	0100010000010000	0000010000000000	0110111111110001	1011010010010111101100	1110101111001010000000
4	0101010000010000	0000010000000000	0110111010000	0111010010010111101100	111010111?001010000000
5	0100010000010000	0000010000000000	0110111001001	0111010010010111101100	1110101111001010000000
6	0100010000010000	0000010000000000	0110111001001	???? 0100100101010101100	1110101111001010000000
7	0101010000010000	0000010000000000	0110111010001	1001010010010101101100	1110101111001010000000
8	0100010000010000	0000010000000000	0110111010001	0111010010010101101100	1110101111000001000000
WH					
1	0101010000000110	0000010000000000	0110111010000	1100?1101011011100000	1110101111011010000000
2	0101010000000110	0000010000000000	1110111010001	1111011010010111100000	1110101111011010000000
3	0101010000000110	0000010000000000	0110111010001	1111011010010101000000	1110101010011010000000
4	0100010000000110	0000010000000000	0110111010001	1110011010010101000000	1110101010110100000000
5	0101010000000110	0000010000000000	0110111010000	1110000101011010100000	1110100100000010000000
6	0101010000000??0	0000010000000000	0110111010001	1110011010110101000000	1110101111010010000000
7	0101010000000?10	0000010000000000	0110111111000	1111111010110111100100	1110101010110100000000
8	0100010000000?0	0000010000000000	1110111110000	1111010010010111101100	1110101111010010000000
9	0100010000000110	0000010000000000	0110111010001	1110011010110111100000	1110101111010010000000
10	0101010000000110	0000010000000000	0110111000000	1111?1101001011100100	1110101111001010000000
MIN					
1	0100010000001000	0110001000010	0110101110001	1111010012?01111100000	0110111111010010000000
2	0100010?0001000	0110001000010	0110101010001	1111010011011111100000	0110101100100100000000
3	0100010?0001010	0?1000?00000?0	0110101010001	1111000011010?? 100000	0110101100100100000000
4	0100010000001000	0110001000010	0110101110001	1111000011010011100000	0110111111010010000000
5	0100010000001000	0110001000000	0110101111001	1111000011011110100000	0110101100100100000000
6	0100010100001000	0110001000010	0?10101110001	1111000011010001000000	0110101111010010000000
7	0100010000010100	0110001000010	0001101010001	1111000011011111101000	0110101111010010000000
8	0100010000010?0	0110101000010	0110101010001	1111000011011110100000	01101?1110100100000000
9	0101010000010?0	0000101000010	0110101010001	1111000011011111100000	0110101111010010000000
10	0100010000001000	0100101000010	0110101110001	1111000011011110100000	0110111111010010000000

Population/ individual	Primer				
WK	OP-P16	OP-P10	OP-P11	OP-A20	OP-P02
1	0100010000000000	0000010000000001	0110111111110000	1101000011011111100000	0110111100100110000 0
2	0100010000000000	0000010000000001	0?10111111100000	0101000011011111100000	0110101100100110000 0
3	0101010000000000	0000010000000001	0010111111000 01	1111000011011111100000	0110101010100110000 0
4	0100010000000000	0000010000000001	0010111111000 01	11?1000011011111100000	0110101010100?100000
5	0100010000000000	0000010000000001	0010111111000 0?	???? 0000110111111001 00	0110101110100110000 0
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7	0100010000000000	0000010000000001	0010111111000 0?	1111000011011111100000	0110111000100110000 0
8	0100000000000000	0000010000000001	0000111111000 0?	1101000011011111100000	0100111110100110000 0
9	0100000000000000	0000010000000001	0110111111000 01	1111000010011111100000	0110101100100110000 0
10	0100?0000000000	0000010000000001	0010111111000 01	1111000011011111100000	0110101100100110000 0
PR					
1	0100010000000000	0000010000000000	0011001101000 0	1111010110111111101110	1100101110100100100 0
2	0101010100000000	0000000000000000	0110101111000 0	1111000010111101011?0	1100101000000000001000
3	0101010100000000	0110000000000001	1110100101010 0	1101010110111111101110	0100101000000000001000
4	0100010100000000	0000000010000000	0110100?110000	0111000010111111101110	0100101000000000001000
5	0100010000000000	0110000010000000	1110100011000 0	1101010110011101011110	0100101010000000001000
6	0100010000000000	0101100010000000	1110101101000 0	0111000110111111101010	1100101010000000011000
7	0100010?0000000	1100000010000000	1110101111000 0	1101010010011101011110	0100101010000000001000
8	0100010100000000	0010000000000000	1110101111010 0	1101010010011111101010	1100101010000010011000
9	0100010100000000	0101000010000000	1110101001000 0	1111010110111110101110	1100000101000000001000
10	0100010000000000	0000000000000000	1110101001000 0	1111000010111111101110	1100000110000001001000
LBI					
1	0101000000000000	0001101000000001	1010111111010 01	0101100010111111101000	0001010100100111111 00
2	0101000000000000	0001101000000001	1110110110000 01	0101100010011101010000	0111000110100110100 0
3	0101000000000000	0001101000000001	1110110110000 01	0101000010110111101000	0100000110100110100 0
4	0101000000000000	0101101100000001	0110111111000 01	0101000010110101000?000	0101000110100110101 0
5	0101000000000000	0001101000000011	1010111111000 01	0101100010111?101100	0100000110000011010 0 0
6	0101000000000000	0001101000000011	1110111111000 01	0101000010111111001100	0101000110100110100 0
7	0101000000000000	0001101000000011	1110110110010 1	0101000010110111100100	0101000110100110111 0
8	0101000000000000	0001101000000011	1110110110000 01	0101100010111111101100	0101000110100110101 0
9	0101000000000000	0001101000000011	1110111111001 01	0101000010111110101000	0100000110100110100 0
10	0101000000000000	0001101000000011	1110110110010 1	0101000010112101011100	0101000110000011010 0

Population/ individual	Primer				
SP	OP-P16	OP-P10	OP-P11	OP-A20	OP-P02
1	0100010000000000	0000010000000001	0010111110100000	1110000001001111101100	1100101100000?000000
2	0101010000000000	0000010000000001	0010111111100000	1100000001001111101000	1100101100000010000000
3	0101010000000000	0000000010000000	0010101101000000	1100000001001111101000	1100101100010100010000
4	0100010000000000	0000010000000011	0110011011000000	1100000001001111101000	1110101100100100000000
5	0101010000000000	0000010000000001	0010101101000000	1100000001011111101111	1100101100000010000000
6	0101010000000000	0000010000000001	0010101111100000	1111000001011111101111	1100101100000010000000
PUR					
1	0101010000000000	0100001000010 0	0011000010000000	1101000010001111101010	0100117100100110000000
2	0100010001010000	0100101000001 0 0	0110100100000000	1101000011001111101010	0100101100100110000000
3	0101010001010000	0100101000001 0 0	1110100110000000	1101000011001111101010	0100101110100110000000
4	0101010001000000	0000?0100000100	0010110110000000	0101000011001110101010	0100110100100110000000
5	0101010001000000	0000010100000100	0010010100000000	0101000?0000001010101010	0100111110100110000000
6	0100000010?0100	0001010100001 0 0	0000000011000000	0100000110101110101 0 0	1100110100010110000000
7	0101010101000000	0000?0110001 0 0	0010110110000000	0101000?0001111110 10 ??	0100101100100110000000
8	0100110100000000	0100001100000000	0010110110000000	1100000011000011101011	0100101100101110000000
9	0100110001010000	0100001000000000	0110110100000000	1111000110011111101010	0100100100100 1?00000
10	0101010000010000	0100001000000000	0110100100000000	0100000110011111010 ??	0100100100100100000000
OH					
1	0010000000000000	0100001?0000000	0010110100000000	0101100000011010101010	0100101100100100000000
2	0010000000000000	0100001100000000	0010010100000000	0101100000011101101010	0100111100100100000000
3	0010000000000000	0100001100000000	0010011100000000	0101100000011011101010	0100111100100100000000
4	0010000000000000	0100001100000000	0010000010000000	0101100000011011101010	0100101100100100000000
WF					
1	1101000000000000	0110000000000000	1110110110000000	0101100000001110101000	0100101107?0000000000
2	1101000000000000	0110000000000000	1110110110000000	0101100000001100101000	0100?0110100000000000
3	1101000000000000	0110000000000000	1110110110000000	0101100000001110101000	0100?0110?0000000000
EG					
1	0101110001100000	0000001000 1000	0010001100000000	1101000000000010001000	0100101100000010100000
2	0101010001100001	0000001000000000	0010010100000000	1101000000000101000 1000	0000101100000010100000
3	0101010001000001	0000001000000000	0010001100000000	0101000000000101000 1000	0000001100000010100000
4	01010?? 000100001	0000001100 1000	0110010100000000	0101000000000101000 1000	0000111100000010100000
5	010?011000000000	0000101000000000	0010010100000000	0101000000000101000 1000	0100101100000010100000

Population/ individual	Primer				
EG	OP-P16	OP-P10	OP-P11	OP-A20	OP-P02
6	0 1 0 ? 0 ? ? 0 0 0 0 0 0 0 0	0 0 0 0 1 0 0 0 0 0 0 0 0 0	0 0 1 0 0 1 0 1 1 1 0 0 0 0	1 1 0 1 0 0 0 0 0 0 1 1 0 1 0 0 0 1 0 0 0	0 1 0 0 1 1 1 1 0 0 0 0 1 1 0 1 0 0 0 0
7	0 1 0 1 0 1 1 0 0 1 1 0 0 0 1	0 0 0 0 1 0 1 1 0 0 0 0 0 0	0 0 1 0 0 1 0 1 0 0 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 0 0	0 ? 0 0 1 0 1 1 0 0 0 0 0 1 0 0 0 0 0 0
8	0 1 0 1 0 1 ? 0 0 0 0 0 0 0 ?	0 0 0 0 1 0 1 0 0 0 0 0 0 0	0 1 1 0 0 1 0 1 1 0 0 0 0 0	1 1 0 1 0 0 0 0 0 0 0 1 0 1 0 0 0 1 0 0 0	0 1 0 0 1 0 1 1 0 0 0 0 1 1 0 1 0 0 0 0
9	0 1 0 1 0 1 0 0 0 0 0 0 0 0	0 0 0 0 0 0 1 1 1 0 0 0 0 0	0 0 1 0 0 1 0 1 0 0 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 1 1 0 0 0 0 0 1 0 1 0 0 0 0
10	0 1 0 0 0 0 0 0 0 0 1 1 0 0 1	0 0 0 0 0 0 1 1 0 1 0 0 0 0	0 1 1 0 0 0 0 1 0 0 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 1 1 0 0 0 0 0 1 0 1 0 0 0 0
11	0 1 0 1 0 1 0 0 0 0 1 0 0 0 0	0 0 0 0 0 0 1 0 1 0 1 0 0 0	0 0 1 0 0 1 0 1 1 1 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 0 0 1 0 1 0 1 0 0 0	0 1 0 0 1 1 1 1 0 0 0 0 1 0 1 1 0 0 0 0
12	0 0 0 0 0 0 0 0 0 0 1 0 0 0 1	0 0 0 0 0 0 1 1 0 0 0 0 0 0	0 0 1 0 0 0 0 1 0 0 0 0 0 0	0 1 0 0 0 0 0 0 1 0 1 1 1 1 1 1 0 1 0 0 0	0 1 0 0 ? 0 1 1 0 0 1 0 0 1 0 1 0 0 0 0
13	0 1 0 0 0 1 0 0 0 0 1 0 0 0 ?	0 0 0 0 0 0 1 1 1 1 0 0 0 0	0 0 1 0 0 0 0 1 0 0 0 0 0 0	0 1 0 0 0 0 0 0 1 0 0 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 1 1 1 0 0 0 0 0 0 1 1 0 0 0 0
14	0 0 0 1 0 0 0 0 0 0 1 0 0 0 1	0 0 0 0 0 0 1 1 0 0 0 0 0 0	0 1 1 0 0 1 0 1 1 0 0 0 0 0	0 1 0 0 0 1 0 0 0 0 0 1 1 1 1 1 0 1 0 0 0	0 1 1 0 1 0 1 1 0 0 1 0 0 1 0 1 0 0 0 0
15	0 0 0 1 0 0 0 0 0 0 1 0 0 0 1	0 0 0 0 0 0 1 1 0 0 0 0 0 0	0 0 1 0 0 1 0 1 1 0 0 0 0 0	0 1 0 0 0 ? 0 0 1 0 0 1 1 1 ? 1 0 1 0 0 0	0 1 0 0 1 0 1 1 0 0 0 0 0 1 0 1 0 0 0 0
16	0 0 0 ? 0 1 0 0 1 1 1 0 0 0 1	0 0 0 0 0 0 1 1 0 0 0 0 0 0	0 1 1 0 1 0 0 1 0 0 0 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 1 1 0 0 0 0 0 1 0 1 0 0 0 0
17	0 1 0 1 0 0 0 0 1 1 1 0 0 0 1	0 0 0 0 0 0 1 1 0 0 0 0 0 0	0 0 1 0 0 0 0 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 0 0 1 0 1 1 0 0 0 0 0 0 0	0 1 0 0 1 1 1 1 0 0 1 0 0 1 0 1 0 0 0 0
18	0 1 0 1 0 1 0 0 ? 1 ? 0 0 0 0	0 0 0 0 0 0 ? ? 0 0 0 0 0 0	0 0 1 0 0 0 0 1 1 0 0 0 0 0	1 1 0 1 1 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0	0 1 0 0 1 ? 1 1 0 0 0 0 0 1 0 1 0 0 0 0
19	0 1 0 1 0 1 0 0 1 1 ? 0 0 0 0	0 0 0 0 0 0 1 1 1 0 1 0 0 0	0 1 1 0 0 1 0 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 0 1 0 1 0 1 0 0 0	0 1 0 0 1 1 1 1 0 0 0 0 0 0 0 0 1 0 0 0 0
TA					
1	0 1 0 0 0 1 0 0 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 1 0 0 0 0 1 1 1 1 1 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 1 1 1 1 0 1 1 0 0	0 1 1 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
2	0 1 0 0 0 1 0 0 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 1 0 0 0 0 1 1 1 1 1 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 1 1 1 1 0 1 1 0 0	0 1 1 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
3	0 1 0 0 0 1 ? 0 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 1 0 0 0 0 1 1 1 1 1 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 1 1 1 1 0 1 1 0 0	0 1 0 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
4	0 1 0 0 0 ? 0 0 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 ? 1 0 1 1 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 1 1 1 1 0 1 1 0 0	0 1 0 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
5	0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 0 0 1 1 0 1 1 1 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 1 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
6	0 1 0 1 0 0 0 1 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 1 1 1 1 1 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 1 1 1 1 0 1 1 0 0	0 1 1 0 1 0 1 1 0 1 0 0 0 ? 0 0 0 0 0 0
7	0 1 0 1 0 0 0 1 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 1 0 1 1 1 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 1 0 1 0 1 1 0 1 0 0 0 ? 0 0 0 0 0 0
8	0 1 0 1 0 0 0 1 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 1 0 1 1 1 0 0 0 1	1 1 0 1 1 0 0 0 1 0 1 0 1 1 1 1 0 1 1 0 0	0 1 0 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
9	0 1 0 1 0 0 0 1 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 1 0 1 1 1 0 0 0 1	1 1 0 1 1 0 0 0 1 0 1 0 1 1 1 1 0 1 ? 0 0	0 1 0 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
10	0 1 0 1 0 0 0 1 0 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 1 0 1 0 1 1 1 0 0 0 1	1 1 0 1 1 0 0 0 1 0 1 0 1 1 1 1 0 1 ? 0 0	0 1 1 0 1 0 1 1 0 0 1 0 0 ? 0 0 0 0 0 0
PI					
1	0 1 0 1 0 0 1 0 0 0 0 0 0 0 0	0 1 0 ? 0 0 1 0 1 0 0 0 0 0	0 0 1 0 0 0 0 1 1 0 0 0 0 1 0	0 1 0 1 1 0 0 0 1 0 0 1 1 0 0 0 1 0 0 1 0	0 1 0 0 0 0 0 1 0 0 0 0 0 0 1 1 0 0 0 0
2	0 1 0 1 0 1 1 0 1 0 0 0 0 0 0	0 1 0 ? 0 0 1 0 1 0 0 0 0 0	0 0 1 0 0 0 0 1 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 0 1 1 0 0 0 1 0 0 0 0	0 1 0 0 0 0 0 1 0 0 0 0 0 0 0 1 1 0 0 0 0
3	0 1 0 1 0 1 1 0 1 0 0 0 0 0 0	0 1 0 ? 0 0 1 0 1 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 0 1 1 0 0 0 1 0 0 0 0	0 1 0 0 1 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
4	0 1 0 1 0 1 1 0 1 0 0 0 0 0 0	0 1 0 1 0 0 1 0 1 0 0 0 0 0	0 0 1 0 1 0 1 ? 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 0 1 1 1 0 0 1 0 0 0 0	0 1 0 0 1 0 0 1 0 0 0 0 0 0 1 1 0 0 0 0 1
5	0 1 0 1 0 1 1 0 1 0 0 0 0 0 0	0 1 0 1 0 0 1 0 1 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 0 0 1 0	0 1 0 1 1 0 0 0 1 0 0 1 1 1 0 0 1 0 0 0 0	0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
6	0 1 0 1 0 ? ? 0 1 0 0 0 0 0 0	0 1 0 1 0 0 1 0 0 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 0 1 1 0 0 0 1 0 0 0 0	0 1 1 0 1 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
7	0 1 0 1 0 1 1 0 1 0 0 0 0 0 0	0 1 0 1 0 0 1 0 0 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 1 0 1 0	0 1 0 1 1 0 0 0 1 0 0 1 1 1 0 0 1 0 0 0 0	0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
8	0 1 0 1 0 ? ? 0 1 0 0 0 0 0 0	0 1 0 1 0 0 ? 0 ? 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 0 0 1 0	0 1 0 1 1 0 0 0 1 0 0 1 1 1 0 0 1 0 0 0 0	0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
9	0 1 0 1 0 0 0 0 1 0 0 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 0 0 0	0 0 1 0 1 0 0 1 0 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 0 1 1 1 0 0 1 0 0 1 0	0 1 0 0 1 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0
10	0 1 0 1 ? 0 0 0 1 0 0 0 0 0 0	0 1 0 1 0 0 0 0 0 0 0 0 0 0	0 0 1 0 1 0 1 1 0 0 0 0 1 0	0 1 0 1 1 0 0 0 1 0 0 1 1 0 0 0 1 0 0 1 0	0 1 0 0 1 0 0 1 0 0 1 0 0 0 1 1 0 0 0 0

Population/ individual	Primer				
MAN	OP-P16	OP-P10	OP-P11	OP-A20	OP-P02
1	0 1 0 ? ? ? 0 0 ? 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 0 0 1 1 1 1 0 1 0 0 0	1 1 1 1 1 0 0 0 1 0 1 1 0 0 1 0 0 1 0 0 0	0 1 1 0 1 1 0 1 0 0 1 0 0 1 0 0 0 0 0 0
2	0 1 0 0 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 0 1 0 0 1 1 1 1 0 1 0 0 0	1 1 0 1 1 0 0 0 0 1 0 1 1 0 0 1 0 0 1 0 0 0	0 1 1 0 1 1 ? 1 1 0 0 0 0 ? 0 0 0 0 0 0
3	0 1 0 0 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	1 0 0 0 0 1 0 1 1 0 0 0 0 0	1 1 0 1 1 0 0 0 0 1 0 1 1 0 0 1 1 0 1 0 0 0	0 1 ? 0 1 1 1 1 1 0 0 0 0 ? 0 0 0 0 0 0
4	0 1 0 0 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	1 0 1 0 0 0 0 1 1 1 0 1 0 0 0	0 1 0 1 0 1 0 0 1 0 0 1 0 0 1 1 0 0 0 0 0	0 1 1 0 1 1 0 1 0 0 1 0 0 ? 0 0 0 0 0 0
5	0 1 0 0 ? 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	1 0 1 0 0 0 0 1 1 1 0 1 0 0 0	1 1 1 1 1 1 0 0 1 1 0 1 0 0 1 1 0 1 0 0 0	0 1 0 0 1 1 1 1 1 1 0 1 0 0 ? 0 0 0 0 0 0
6	0 1 0 0 ? 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	1 0 1 0 0 0 0 0 1 1 0 1 0 0 0	1 1 0 1 1 1 0 0 1 0 0 1 0 0 1 1 0 1 0 0 0	0 1 1 0 1 1 1 1 1 0 0 1 0 0 1 0 0 0 0 0
7	0 1 0 0 1 1 1 0 1 0 0 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 1 1 0 0 0 0 1 1 0 0 0 0 0	1 1 0 1 0 0 0 0 1 0 0 1 0 0 0 1 0 1 0 0 0	0 1 1 0 1 1 1 1 1 0 0 1 0 0 1 0 0 0 0 0
MAM					
1	0 1 0 1 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 0 0 0 0 1 1 0 0 0 0 0	1 1 0 0 0 1 0 1 0 0 0 0 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 0 1 1 1 0 0 0 1 1 1 0 0 0 0
2	0 1 0 0 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 0 0 1 0 1 0 0 0 0 0 0	1 1 0 0 0 0 0 0 1 0 0 0 1 1 0 1 0 1 0 0 0	0 1 0 0 1 1 0 1 1 1 0 0 0 0 1 1 0 0 0 0
3	0 1 0 1 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 1 0 0 0 0 0 0 1	0 0 1 0 0 0 0 0 1 1 1 0 0 0 0	1 1 1 0 0 0 0 0 0 1 0 0 0 1 1 0 1 0 1 0 0 0	0 1 0 0 0 0 1 1 1 1 0 0 0 0 1 1 0 0 0 0
4	0 1 0 0 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 1 0 0 0 0 0 0 1	0 1 1 0 0 1 0 1 1 0 0 0 0 0	1 1 0 0 0 0 0 0 ? 0 0 0 1 1 0 1 0 1 0 0 0	0 1 0 0 1 1 0 1 1 1 0 0 0 0 1 1 0 0 0 0
5	0 1 0 1 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 1 1 0 0 1 0 0 0 0 0 0	1 1 1 0 0 0 0 1 1 0 0 1 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 1 1 1 1 0 0 0 0 1 1 0 0 0 0
6	0 1 0 1 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 0 0 0 0 1 1 1 0 0 0 1	1 1 0 1 0 0 0 1 1 0 0 1 1 1 1 0 1 0 1 0 0 0	0 1 0 0 1 1 0 1 1 1 0 0 0 0 1 ? 0 0 0 0
7	0 1 0 1 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 0 0 0 0 1 1 0 0 0 0 0	1 1 0 1 0 0 0 1 1 0 0 0 1 1 0 1 0 1 0 0 0	0 1 0 0 0 0 ? 1 1 1 0 0 0 0 1 ? 0 0 0 0
8	0 1 0 1 1 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 1 1 0 0 0 0 1 1 0 0 0 0 0	1 1 0 0 0 0 0 1 1 0 0 0 0 1 0 1 0 1 0 0 0	0 1 0 0 1 1 1 1 1 1 0 0 0 0 1 1 0 0 0 0
9	0 1 0 1 0 1 0 0 1 0 0 0 0 0 0	0 0 0 0 0 0 1 0 0 0 0 0 0 0 1	0 0 1 0 0 0 0 1 1 1 0 0 0 0	1 1 0 0 0 0 0 1 1 0 1 0 1 1 0 1 0 1 0 0 0	0 1 0 0 1 0 ? 1 1 1 0 1 0 0 1 1 0 0 0 0
HB					
1	0 1 0 1 1 0 1 0 1 1 1 0 1 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 1 0 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 0 1 0 0 1 1 1 1 0 1 0 0 ? 0 0 0 0 0 0
2	0 1 0 1 0 1 0 0 1 1 ? 0 ? 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 0 0 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 1 0 0 1 1 0 1 0 1 0 0 ? 0 0 0 0 0 0
3	0 1 0 1 1 0 1 0 1 1 1 0 1 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 1 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 1 0 0 1 1 1 0 0 1 0 0 0 0 0 0 0 0 0
4	0 1 0 1 1 0 1 0 1 ? 1 0 1 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 0 0 0 0 0	0 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 1 0 1 1 1 0 0 0 0 1 0 0 ? 0 0 0 0 0
5	0 1 0 1 0 0 0 0 1 1 ? 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 0	0 0 1 0 0 ? ? 1 1 0 0 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 0 1 1 1 0 1 1 0 0	0 1 0 0 0 1 1 0 0 0 0 0 0 ? 0 0 0 0 0 0
6	0 ? 0 ? ? ? ? 0 ? ? ? 0 ? 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 ?	0 0 1 1 0 1 0 1 1 0 0 0 0 0	? 1 0 ? ? 1 0 0 1 0 ? ? 0 0 ? ? 0 ? ? 0 0	0 1 0 0 ? 1 1 1 0 0 1 0 0 1 1 0 0 0 0
0					
7	0 1 0 1 0 0 0 0 ? 1 ? 0 0 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 1 0 0 0 0	1 1 0 1 1 0 0 0 1 0 1 1 0 0 0 1 0 1 ? 0 0	0 1 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0
8	0 1 0 1 0 0 0 0 1 1 ? 0 0 0 0	0 0 0 0 0 1 0 0 1 0 0 0 0 0	0 0 1 0 0 1 0 1 1 1 0 1 0 0	0 1 0 1 1 1 0 0 1 0 1 0 0 0 1 1 0 1 ? 0 0	0 ? 1 0 0 1 1 1 0 0 1 0 0 1 0 0 0 0 0 0
9	0 ? 0 ? ? ? ? 0 ? ? ? 0 ? 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0 1	0 0 1 0 0 1 0 1 1 1 0 0 0 0	1 1 0 1 1 0 0 0 1 0 0 1 0 0 1 1 0 1 ? 0 0	0 0 ? 0 0 1 1 0 0 0 0 0 0 0 0 1 0 0 0 0
TPT					
1	0 1 0 1 0 1 0 0 0 0 0 1 0 0	0 0 0 0 0 1 0 0 0 0 0 0 0 0	0 1 1 0 0 1 1 1 1 1 0 0 0 0	1 1 0 0 0 1 0 0 1 0 1 1 0 0 1 1 0 1 ? 0 0	0 1 1 0 1 1 1 1 0 0 0 0 0 ? 0 0 0 0 0 0