Conservation genetics of the endemic root holoparasite, *Dactylanthus taylorii*

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Plant Biology

At Massey University, Palmerston North,

New Zealand

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2011

Abstract

Dactylanthus taylorii is a New Zealand endemic root holoparasite in the family Balanophoraceae. The vegetative body is extremely reduced and the majority of the plant grows underground attached to its host root. Flowers are dioecious and are pollinated by the short tail bat; a native seed disperser is unknown. Pollen records indicate *D. taylorii* was formerly more widespread over the North Island of New Zealand but habitat fragmentation and browsing by introduced mammals is thought to have severely reduced population sizes. The species is classified as nationally vulnerable and conservation management is overseen by the New Zealand Department of Conservation. Here, a conservation genetics approach was taken in order to understand the genetic variation and structure of *D. taylorii* populations.

Previously, the genetic structure of 17 populations had been studied using randomly amplified polymorphic DNAs (RAPDs) and a broad geographical pattern was identified, but was not suitable for conservation management. Alternative molecular markers are microsatellites, which have many favourable attributes for use in conservation genetic studies. Microsatellites allow for estimates of allele frequencies and therefore can be used to determine heterozygosity and their high mutation rate can be used to detect more recent changes in the genetic structure of populations. Next-generation sequencing was used in order to develop microsatellite markers as this method does not require cloning. From the 62,000 sequences obtained, 4,000 microsatellites were identified and primers were able to be designed for 750 repeats. From this primer pool, 72 were chosen to be screened and ten microsatellite loci were found to be polymorphic and consistently amplifiable. These ten were used to genotype 241 *D. taylorii* individuals from 31 populations.

Across all populations a high number of alleles were identified, although a high percentage of these were private alleles. Within-population assessment of genetic variation indicated that many populations have low levels of genetic diversity and a high proportion of homozygotes. A high degree of genetic differentiation was detected and was found to be strongly correlated to geographic distance between populations. Also, populations grouped into two, three or eight clusters that were reflective of geography. Possible explanations for the geographic pattern observed include

volcanism, mountains as physical barriers to gene flow, habitat availability and gene flow mediated by the short tail bat.

This information suggests that although populations are secure from environmental risks such as habitat loss or herbivory, there is a genetic threat to extinction. In order to increase genetic variation within-populations, translocation of genetic variation (i.e. pollen or seed) is suggested between geographically adjacent populations.

Acknowledgements

Firstly, I would like to thank my supervisors Jennifer Tate and Vaughan Symonds for giving me the opportunity to do exactly what I wanted for my thesis. Your advice and support is hugely appreciated.

I am also grateful for the support given to me by Avi Holzapfel and Paul Cashmore from the Department of Conservation. Avi, thank you for your enthusiasm towards the project from its development, and for sharing your wealth of knowledge about *Dactylanthus*. Paul, without your support and the backing of the Recovery Group I never would have got close to collecting from as many populations as I have. I also have to thank countless DOC staff from all around the North Island for collecting samples for me and being welcoming when I tagged along during monitoring. I hope the results from this study aids conservation of *Dactylanthus* and the time you all put in worthwhile.

Without trying a new method of DNA extraction from the advice of Lara Shepherd, it's entirely possible I would still be trying to get something other than a brown sludge from my samples. Likewise, without the computer expertise of Patrick Biggs I would still be sitting at a computer trying to extract my 454 sequences from the Otago University server. Also, Chrissen Gemmill supplied the DNA for several populations collected from a previous study. Thank you all for your brief but extremely important help.

Thanks to all the members in LoST for undoubtedly at one stage or another hearing me express frustration at any one of DNA extraction, genotyping or plotting points on a map, and responding with a smile (or smirk). You guys are alright.

Thank you Mum, Dad, Jodie and Scott for letting me wax lyrical *ad nauseum* about *Dactylanthus* and pretending to know what I was talking about. Most importantly, thanks for being proud of me.

I would also like to thank Massey University for the Massey Masterate, J.P Skipworth and Coombs Memorial scholarships.

Abbreviations

A	-	number of alleles
AFLP	-	Amplified Fragment Length Polymorphism
BLAST	-	basic local alignment search tool
bp	-	base pairs
cpDNA	-	chloroplast DNA
CASS	-	cheaply amplified size standard
CTAB	-	hexa-decetylammonium bromide
DATA	-	*Dactylanthus taylorii* primer for microsatellite loci
DNA	-	deoxyribonucleic acid
dNTP	-	deoxyribonucleotide triphosphate
DOC	-	Department of Conservation (New Zealand)
EDTA	-	ethylene diamine tetra-acetic acid
ERMA	-	Environmental Risk Management Authority
EST	-	expressed sequence tag
F_{IS}	-	component of Wright's (1921) fixation index, used to define within population structure by calculating the average observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population it belongs to
F_{ST}	-	component of Wright's (1921) fixation index, used to define between population structure by comparing the expected heterozygosity of individuals within a subpopulation to the total expected heterozygosity of individuals across all populations
H_E	-	expected heterozygosity
H_O	-	observed heterozygosity
H_T	-	species-wide expected heterozygosity
HWE	-	Hardy-Weinberg equilbrium
IUCN	-	International Union for Conservation of Nature
N_A	-	number of alleles per locus within a population
N_E	-	effective number of alleles per locus within a population
Mb	-	mega base pairs (=1,000,000 bp)
mtDNA	-	mitochondrial DNA
PDL	-	primer designed locus

PCR	-	polymerase chain reaction
QTL	-	quantitative trait loci
RAPD	-	Randomly Amplified Polymorphic DNA
R_{ST}	-	derivation of F_{ST} that accounts for the high-rate stepwise mutation model of microsatellites (Slatkin 1995)
SNP	-	Single Nucleotide Polymorphism
SSR	-	simple sequence repeat (microsatellite)
STE	-	sucrose, TRIS, EDTA
Tris	-	tris(hydroxymethyl)aminomethane
TVZ	-	Taupo Volcanic Zone
UPGMA	-	Unweighted Pair Group Method with Arithmetic Mean
%P	-	percentage of polymorphic loci

Contents

Figures

1 Introduction

2 Microsatellite marker development for the endangered root parasite *Dactylanthus taylorii* using high-throughput sequencing

3 Conservation genetics of the endemic root holoparasite, *Dactylanthus taylorii*

Tables

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