

School of Environmental Biology

Variation and population genetic structure in the
Western Australian endemic genus
Geleznovia Turcz. (Rutaceae)

Linda Marie Broadhurst

This thesis is presented as part of the requirements for
the award of the Degree of Doctor of Philosophy

of the

Curtin University of Technology

November 1998

Declaration

I declare that all work presented in this thesis is that of myself alone unless otherwise acknowledged. The contents of this thesis have not been submitted previously, in whole or in part, in respect of any other academic award.

Linda Broadhurst

30 June 1998

Copyright

Under the conditions of the 1968 Copyright Act, no chapter or appendices of this thesis, in whole or in part, can be cited or reprinted without the prior permission of the author.

Linda Broadhurst

30 June 1998

Abstract

The endemic wildflower, *Geleznowia verrucosa* Turcz. (Rutaceae), is widely distributed as small disjunct populations throughout the sandplains of Western Australia (21° 50'S, 116° 12'E to 31° 12'S, 117° 02'E). Although the genus is supposedly monospecific, three morphological forms can be recognised in the field:

- (1) a small form (0.5-1 m) with small, often solitary flowers and small leaves;
- (2) a large form (1-2 m) with larger, more abundant flowers and larger leaves; and,
- (3) an intermediate form exhibiting mixed morphological characters.

The patterns of morphological, reproductive and genetic variation within and between populations of these three forms were investigated. Uni- and multivariate analyses of morphological traits found the large and intermediate forms to be closely allied, and distinguishable from the small form. Responses to controlled pollination experiments indicated that the small form favours selfing but maintains some level of outcrossing, while the large form exhibits a mixed mating system. The intermediate form displays a stronger self-pollination mechanism than the small form.

Patterns of genetic variation were analysed using both allozymes and randomly amplified polymorphic DNA (RAPDs). The allozyme analysis concurred with genetically depauperate expectations of endemic taxa (A , 1.4; P , 29.6%; H_o , 0.055; H_e , 0.097). Genetic diversity and patterns of allelic distribution, however, differed within the three forms. Lower levels of genetic diversity were found in the small form (H_T , 0.192) compared with the large form (H_T , 0.254), although both forms apportioned this diversity within populations (H_S , 0.122 and 0.164, respectively) rather than between (D_{ST} , 0.070 and 0.090, respectively). In contrast, populations of the intermediate form are highly divergent (G_{ST} , 54%), with genetic diversity apportioned between populations (D_{ST} , 0.121) rather than within (H_S ,

0.105). Whereas the morphometric analyses had indicated closer affinity between the large and intermediate forms, both allozyme and RAPD analyses suggested that the intermediate form is closer to the small form than to the large form.

There is circumstantial evidence to suggest that the intermediate form has arisen following hybridisation between the small and large forms. Its hybrid origin is supported by the high level of genetic diversity between the intermediate form populations, as well as its strong autogamous tendencies and mixed morphological characteristics. In a putative zone of hybridisation between the small and large forms, asymmetric introgression was observed, indicating gene exchange between the two forms can occur when they come into contact.

It is speculated that three major events have shaped the evolution of *Geleznovia verrucosa* and contributed to hybridisation between the small and large forms. Firstly, the small form is derived from the large form; associated with this speciation was a shift in reproductive strategy from outcrossing to selfing when the small form migrated into a harsher and more unpredictable environment. Secondly, recurrent Tertiary and Quaternary climatic perturbations have facilitated range expansion and contraction of these forms, generating opportunities for spatially and temporally distributed hybridisation events. Finally, more recent evolution has been driven by population disjunction, limited gene flow, and bottleneck and/or associated founder effects.

From this study it is apparent that the genus *Geleznovia* consists of at least two taxa and a series of hybrid derivatives. On this basis, formal systematic revision of the genus is now warranted. Systematic clarification will also assist with the conservation and management of this valuable natural resource, which is currently under threat.

Acknowledgements

I wish to sincerely thank my supervisors, Dr Beng Tan, Dr David Coates and Dr Michelle Waycott, for their patience, encouragement and advice during this research. Most of all, I thank them for their friendship, and for never letting me lose faith in this research, or in myself.

I also wish to thank my very good friends Leith, Janette, Helen, Jenny and Bev whose enduring friendship has supported me throughout this research. Special thanks to Leith for helping out during the many long and arduous field trips. To those who also spent time in the field with me - Adrienne, Leonie, Joe, Rob, Toni and Andrew - thanks also. Field sites were kindly provided by the Boase, Casley and Pleshkes families, the Department of Conservation and Land Management, the Shire of Coorow and the Town of Geraldton.

To my fellow students who made me laugh, fed my caffeine addiction and kept me going during the long nights and even longer weekends (especially John, Sandy, Reinier, Rachel, Mick, Florry, Lara, Match, Maria, Phil and Stu), thanks for everything. This thesis would not have been completed without the technical and administrative staff from the School of Environmental Biology, who cheerfully put up with my constant requests for printouts, money and technical support. To Lydia - special thanks for those difficult chromosomes !

And finally, I dedicate this work to my family, whose patience, encouragement and love have been unending. To my parents, Helen and Terry, who have travelled every step of this journey with me, always with support and encouragement - this work is as much yours as it is mine. I love you and thank you both very much. To Robyn, Larry, Cameron, Renee, Tony, Toni and Jared - thanks for being yourselves, and for being so understanding about not seeing much of me in the last few years. This work is also dedicated to my Grandparents who now travel elsewhere, but whose influences will always remain.

CONTENTS

Declaration	i
Abstract	ii
Acknowledgements	iv
Contents	v
List of Tables	viii
List of Figures	ix
CHAPTER 1 <u>General Introduction</u>	1
Identifying systematic divergence	3
Molecular markers and systematics	4
Diversity within the Western Australian flora	8
<i>Geleznovia verrucosa</i>	9
Aims of this investigation	12
Field sites	15
Organisation of this thesis	16
CHAPTER 2 <u>Morphological variation and chromosome number</u>	18
Introduction	18
Morphological variation	18
Chromosome number and polyploidy	20
Aims of morphometric and chromosomal analyses	21
Materials and methods	
Morphological variation	21
Cytology	25
Results	
Morphological variation	26
Cytology	34
Discussion	34
CHAPTER 3 <u>Reproductive biology and mating system</u>	38
Introduction	38
Aims of this investigation	40
Materials and methods	
Sampling strategy	41
Floral biology	41
Flowering phenology	41
Pollen viability	41
Stigma receptivity	42
Pollen tube growth	42
Pollination experiments	43
Pollinators	45
Mating system	45
Results	
Floral biology	46
Flowering phenology	49
Pollen viability	49
Stigma receptivity	50

Pollen tube growth	51
Pollination experiments	51
Pollination success within and between populations	52
Pollination success within and between forms	56
Pollinators	57
Mating system	57
Discussion	58
CHAPTER 4 <u>Allozyme variation and population genetic structure</u>	63
Introduction	63
Aims of this investigation	65
Materials and methods	
Sampling strategy	65
Electrophoresis	65
Genetic analysis	66
Results	67
Genetic variation within populations	67
Genetic differentiation between populations and forms	
Discussion	76
CHAPTER 5 <u>Variation identified by Randomly Amplified Polymorphic DNA (RAPDs)</u>	82
Introduction	82
Aims of this investigation	83
Materials and methods	
Sampling strategy	83
DNA extraction	84
Amplification	84
Results	86
Discussion	88
CHAPTER 6 <u>Variation across a zone of hybridisation</u>	91
Introduction	91
Aims of this investigation	93
Materials and methods	93
Sampling strategy	93
Morphometric analyses	94
Allozyme analysis	94
Results	95
Morphometric analyses	95
Allozyme analysis	98
Discussion	101
CHAPTER 7 <u>General Discussion</u>	105
REFERENCES	
Appendix 1: Root tip chromosome counts	114
Appendix 2: Manuscripts	136
	137

List of Tables

- Table 1.1: Location of sampled *G. verrucosa* populations.
- Table 2.1: Quantitative characters used for morphometric analyses.
- Table 2.2: Tukey's Compromise comparison of character means between populations.
- Table 2.3: Percentage of *G. verrucosa* plants correctly predicted following *a priori* classification by form.
- Table 3.1: Pollen source for the Xother pollination treatment.
- Table 3.2: Pollination treatments undertaken on *G. verrucosa* populations.
- Table 3.3: Flowering seasons for all *G. verrucosa* populations over two years.
- Table 3.4: Stigma receptivity for *G. verrucosa* buds and flowers.
- Table 3.5: Mean and percentage of potential seed set per flower for all pollination treatments.
- Table 3.6: Tukey's Compromise comparison of mean seed set per flower for each pollination.
- Table 3.7a: Tukey's Compromise comparison of mean seed set per flower for pollination treatments within populations.
- Table 3.7b: Tukey's Compromise comparison of mean seed set per flower for pollination treatments within populations, open-pollination treatment excluded.
- Table 3.8: Tukey's Compromise comparison of mean seed set per flower for pollination treatments between populations.
- Table 3.9: Index of self-incompatibility (ISI) for *G. verrucosa* populations.
- Table 4.1: Enzyme systems examined for allozyme electrophoresis.
- Table 4.2: Allele frequencies for the 19 *G. verrucosa* sites.
- Table 4.3a: Single locus diversity measures based on 16 loci at 19 *G. verrucosa* sites.
- Table 4.3b: Single locus diversity measures based on 16 loci pooled for *G. verrucosa* forms.
- Table 4.4: Gene diversity statistics for polymorphic loci for all *G. verrucosa* populations.
- Table 4.5: Gene diversity statistics over all loci unbiased for sample size and population number for *G. verrucosa* forms.
- Table 4.6: Average genetic distance (*D*) within and between forms of *G. verrucosa*.
- Table 5.1: *G. verrucosa* populations sampled for RAPD analysis
- Table 5.2: Primers used for PCR fingerprint analysis on six populations of *G. verrucosa*.
- Table 5.3: Multilocus genotype diversity statistics for the six *G. verrucosa* populations.
- Table 6.1: Morphological classification of individuals within the Transect at Pleshkes.

List of Figures

- Figure 1.1: Annual rainfall zones in the southwest of Western Australia.
- Figure 1.2: Distribution of *G. verrucosa* specimens lodged at the Western Australian Herbarium.
- Figure 1.3: Photograph of *G. verrucosa* flowers.
- Figure 1.4: Photographs of *Geleznovia* type specimens.
- Figure 1.5: Photographs of the three *G. verrucosa* forms.
- Figure 1.6: Location of the *G. verrucosa* field study sites.
- Figure 2.1: Scatterplot of canonical discriminant functions.
- Figure 2.2: Scatterplot of the first two principal component factors.
- Figure 2.3: Classification of *G. verrucosa* populations based on Euclidean distances and UPGMA algorithm.
- Figure 2.4: Nonmetric multidimensional scaling of *G. verrucosa* populations.
- Figure 2.5: Photographs and interpretations of mitotic chromosomes from root tips of two *G. verrucosa* forms.
- Figure 3.1: Flowers of the three *G. verrucosa* forms.
- Figure 3.2: Flowering stages in *G. verrucosa*.
- Figure 3.3: Mean percentage of viable pollen grains for *G. verrucosa* populations.
- Figure 3.4: Pooled seed set for each pollination treatment.
- Figure 3.5: Differences in mean seed set per flower between the *G. verrucosa* forms for the pollination treatments.
- Figure 3.6: Photograph and interpretation of *Lap* gels.
- Figure 4.1: Distribution of alleles for the *Aat-2* and *Aat-3* loci.
- Figure 4.2: Distribution of alleles for the *Aat-4* and *Lap-1* loci.
- Figure 4.3: Cluster analysis of *G. verrucosa* populations based on Nei's genetic distance (*D*) and UPGMA algorithm.
- Figure 5.1: Gel-electrophoresis of *G. verrucosa* RAPD bands.
- Figure 5.2: Cluster analysis of *G. verrucosa* populations based on Nei's genetic distance (*D*) and UPGMA algorithm.
- Figure 5.3: Nonmetric multidimensional scaling of *G. verrucosa* populations.
- Figure 6.1: Nonmetric multidimensional scaling of individuals at Pleshkes based on Euclidean distances and UPGMA algorithm.
- Figure 6.2: Comparison of allozyme and morphometric cluster analyses.
- Figure 6.3: Allelic distribution within the *Aat-1*, *Aat-2* and *Aat-3* loci at Pleshkes.
- Figure 6.4: Allelic distribution within the *Aat-4* and *Aat-5* loci at Pleshkes.