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# DETERMINING GENE FLOW, LINKAGE AND PARENTAL CONTRIBUTION IN *PINUS ELLIOTTII* X *PINUS CARIBAEA* PINE HYBRIDS.

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Submitted in fulfillment of the requirements for the degree

***Magister Scientiae***

In the faculty of Natural and Agricultural Sciences,

Department of Genetics at the University of Pretoria

PRETORIA

2001

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**Dedicated to**

**Chris**

# PREFACE

The need to understand how variations in survivorship, fertility and gene flow contribute to changes in allele frequencies within and amongst populations has been the driving force behind the research of many population biologists. The rapid development of molecular techniques has provided these population biologists with a new range of technical tools with which to approach a wide range of questions.

In this dissertation the results of a study, which was carried out in the Department of Genetics, University of Pretoria, Pretoria, under the supervision of Professor Anna-Maria Botha-Oberholster and the co-supervision of Professor Brenda D. Wingfield, are presented. The results are original and have not been submitted in any form to another University.

Chapter 1 focuses primarily on the background and history of *P. elliottii x P. caribaea* hybrid pine. The chapter addresses where the hybrid fits in and why it is of economic importance to the forestry industry. The chapter also focuses on molecular biology studies that have been done on conifers and more importantly on members of the family Pinaceae.

In chapter 2 the role of DNA fingerprinting using microsatellite markers was investigated. In this chapter microsatellite markers developed for use in related pine species were tested for their ability to produce fingerprints in representative populations of the *P. elliottii x P. caribaea* hybrid. The microsatellite markers were also tested for their ability to identify linkage, gene flow and parental contribution in the hybrid. The development of microsatellite markers that are more specific to the *P. elliottii x P. caribaea* hybrid pine was also addressed. Using Randomly Amplified Microsatellites (RAMs), primers specific to microsatellite loci distributed throughout the *P. elliottii* genome were designed. These microsatellite markers were then tested for their ability to detect polymorphisms in the *P. elliottii x P. caribaea* hybrid pine.

The final chapter, chapter 3, focuses on the viability and morphology of *P. caribaea* pollen. In this chapter the influence of storage and collection conditions on pollen viability was investigated. The chapter also investigates the correlation between germination rate, viability and pollen morphology.

Jacqueline H. Doyle

May 2001

# ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following people, organizations and institutions for their contribution towards the completion of this dissertation:

- Prof. Anna-Maria Oberholster for providing guidance, motivation and support.
- Prof. Brenda Wingfield for valuable practical input.
- Magriet van der Nest and fellow students associated with FABI for valuable advice and support.
- Dr. Chris van der Merwe of the Electron Microscopy Unit, University of Pretoria, and Prof. Rudi Verhoeven, Department of Botany and Genetics, University of Orange Free State, for their technical assistance with the Scanning Electron Microscope.
- Dr. Ben Eisenberg for providing statistical insight and solutions.
- SAFCOL, Cecilia Bester and John Mather for providing the pine material around which my entire project revolved.
- Department of Genetics, University of Pretoria for providing equipment and facilities.
- National Research Foundation and University of Pretoria for financial support.

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## CHAPTER 2

# INTRODUCTION

One of the driving factors behind forestry, as with any other commercial crop, is the optimization of the plant with its growing environment, thereby ensuring maximum yield per square meter. One of the major draw backs to forest tree improvement is the timespan involved from seedling to mature adult (anything from 20 to 40 years). This lengthy maturation period means that many years need to be invested in research and breeding and that progress towards the optimization of the forest trees with their environment is a slow and ongoing process.

One of the main foci in forest tree breeding is the creation of hybrids between promising parents with the objective of creating genetically superior offspring, which display hybrid vigor. An example of one such promising hybrid is the *Pinus elliottii x Pinus caribaea* hybrid. In 1955, the first crosses of the *P. elliottii x P. caribaea* hybrid were made in Australia (Hinze, 2000). The *P. elliottii x P. caribaea* hybrid, which displayed extraordinary growth characteristics (such as rapid growth combined with excellent wood quality), attracted widespread attention and extensive testing was initiated in South Africa during the late 1960's and early 1970's (Denison and Kietzka, 1993; Malan, 1995; Stanger *et al.*, 1999).

Molecular techniques offer many tools that can be employed by breeders to severely reduce the time scale of field trials. The aim of this study was to investigate the potential of microsatellite markers for determining gene flow, linkage and parental contribution in the *P. elliottii x P. caribaea* hybrid. To this end the cross-species amplification of microsatellite markers was investigated, as this technique holds important implications on a large scale in the forestry industry. The ability to use markers developed in closely related *Pinus* species would not only greatly reduce the time and costs involved in marker development, but also the time and costs involved during the nursery phase and the selection of superior clones for future plantations. For example, once superior *P. elliottii x P. caribaea* hybrids are identified, based on field trial performance, it would be possible to screen the seedlings produced in order to verify that they really are the progeny of the controlled pollination between the superior parents. This procedure would allow seedlings that do not conform to the microsatellite fingerprints expected for the hybrid to be discarded early on in the screening phase and ensure that only the correct genotypes enter into the bulking-up phase.

A number of obstacles have been encountered that complicate the large-scale planting of the *P. elliottii x P. caribaea* hybrid. Firstly, the *P. caribaea* pollen ripens approximately three months before the *P. elliottii* ovules are receptive (Mather, 2000); and secondly a degree of incompatibility seems to exist between *P. elliottii* and *P. caribaea,* which results in low seed set and a low number of viable hybrid seeds (SAFCOL, 1996).

The second aim of this study was therefore to investigate the association between *P. caribaea* pollen morphology and viability and to try and link this association to viability. The presence of a significant association would make it possible to incorporate pollen morphology as a screening step. This would be beneficial during the selection of the pollen parent by reducing the chances of inferior *P. caribaea* pollen parents from being used in crosses during hybrid production and ultimately from entering into the hybrid performance trials in general. The correct storage of the *P. caribaea* pollen would ensure the viability of the pollen used in the cross and therefore result in increased pollination that should in turn result in increased fertilization and thus higher seed set and ultimately more offspring.