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REPRODUCTIVE BIOLOGY OF POHUTUKAWA
(METROSIDEROS EXCELSA) (MYRTACEAE)

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A thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy.

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FRONTISPIECE

Flowering branch of pohutukawa (*Metrosideros excelsa* Sol. *ex* Gaertn.). Reproduction of a painting by Sydney Parkinson, draughtsman during Cook's first voyage to New Zealand (1769 - 70).

(from Conly and Conly 1988, with permission).

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ABSTRACT

The objective of this project is to describe and analyse the reproductive biology of pohutukawa, by integrating information from floral biology, breeding system and pollination biology.

New Zealand pohutukawa (*Metrosideros excelsa* Sol. *ex* Gaertn.), a member of the Myrtaceae, is a mass-flowering tree endemic to northern New Zealand coastlines. Compound inflorescences develop over a period of ten weeks in six morphologically distinct stages. Trees flower over a peak period of two weeks, and the inflorescences contain an average of 14.3 large, hermaphrodite, red 'brush' flowers that remain open for seven days. Most pollen is viable (93.6%) and the receptivity of the wet-papillate stigma extends for at least nine days. Each flower produces approximately 46 μL nectar per day, containing 18% (w/v) sucrose. Neither dichogamy nor herkogamy prevent pollen and stigma interference, and floral design and display are consistent with high levels of autogamous and geitonogamous self-pollination.

The stigmatic exudate of unpollinated pistils stains intensely for carbohydrates, lipids and proteins, but shows a notable decrease in lipids and proteins following pollination. The style has a solid transmitting tissue with large mucilage-filled intercellular spaces which stain weakly for polyanions and pectins irrespective of pollination. Although starch grains in the stylar cortex are depleted following pollination, stylar resources in general appear to be sparse and this results in a low speed of pollen tubes (2 mm / d) through the intercellular spaces of the transmitting tissue.

Seed capsules of pohutukawa contain a mixture of fertile (embryo-containing) and infertile (embryo-lacking) seeds. Fertile seeds weigh approximately 0.15 mg, stain positively with 1% tetrazolium chloride, and are randomly disposed on the placenta. Their germination rate exceeds 90% after up to one year of cold storage, but decreases rapidly when stored at room temperature.

Controlled pollinations with self- and cross-pollen from single donors and a pollen mixture from five unrelated parents showed that seven out of ten trees were self-incompatible, suggesting that natural populations may consist of a mosaic of self-incompatible and self-compatible individuals. Self-incompatibility is late-acting as pollen tubes from selfs and crosses reach the ovary simultaneously 10 - 15 d after pollination. In common with other Myrtaceae, the seed / ovule ratio in pohutukawa is low and not limited by the stigmatic pollen load. The pollen / ovule ratio of 462.5 (SE ±43.4) places the breeding system of pohutukawa between facultative selfing and facultative outcrossing.

Mainland populations of pohutukawa have been reduced to fragmented stands, and the original suite of bird pollinators has been largely replaced by introduced species. In contrast, the native pollinator fauna of several offshore islands remains intact, including the three species of the New Zealand honey eaters (Meliphagidae) and solitary bees. Using allozyme analyses, multilocus outcrossing rates were estimated for Little Barrier Island and Tiritiri Matangi Island and for three mainland populations in comparison. They were among the lowest in the Myrtaceae (t_m = 0.22 - 0.53) and the loss of native pollinators has no measurable effect on the mating system. Although there is no difference in the germination percentage of fertile seeds from self- and cross-pollination treatments (98.4%), 'selfed' seedlings show marked inbreeding depression in height after six months. Wright's fixation index is consistently higher for seedlings (F_s) than for mothers (F_m) in all populations, indicating that selection may eliminate selfed offspring from populations prior to reproductive maturity.

Exclusion experiments were undertaken on Little Barrier Island to assess the effect of native birds and bees on outcrossing and seed production. In bird exclusion experiments in the lower canopy (2 - 4 m) with flower access to bees only, estimated outcrossing rates were lower (t_m = 0.40) than in open pollination (t_m = 0.58), suggesting that bees effect more self-pollination than birds. The highest outcrossing rates (t_m = 0.71) were found for open pollination in the upper canopy (> 4 m). Numbers of fertile seeds per capsule were 45% higher after open pollination than in treatments with bee visitation only, and 28% higher than in treatments where all flower visitors were excluded. The results suggest that native bees visiting pohutukawa flowers reduce seed set and effect less outcrossing than birds, and that a large proportion of seeds arises from automatic self-pollination. In trees of a modified mainland population with predominantly introduced birds and a mixture of introduced and native bees there was no decrease in seed set for the treatment allowing flower access by bees only, indicating that - in contrast to native bees - honeybees did not reduce seed set in pohutukawa.

In conclusion, although the floral biology of pohutukawa permits geitonogamy, a combination of outcrossing (predominantly by bird pollinators), self-incompatibility, and inbreeding depression act to maintain heterozygosity and result in the production of sufficient offspring that will ensure the survival of the species.

GLOSSARY

Breeding system (mating system)	All aspects of sex expression in plants that affect the relative genetic contribution to the next generation of individuals within a species (Wyatt 1983).
Cymose inflorescence	Here: three-flowered structures terminating secondary axes of compound inflorescences (Dawson 1970a).
Compound inflorescence	Here: floral system of main (primary) axis terminating in a dormant vegetative bud and lateral (secondary) axes terminating in cymose inflorescences (Dawson 1970a).
Dichogamy	Separation of pollen and stigma presentation in time. Two types: protandry (male function before female) and protogyny (female function before male); generally reduces intraflower self-pollination (Lloyd and Webb 1986, Barrett 1998).
Floral design	Characteristics of individual flowers including their size, structure, colour, scent, nectar production and degree of herkogamy / dichogamy (Barrett 1998).

Floral display	Number of open flowers on a plant and their arrangement within and among inflorescences (Barrett 1998).
Geitonogamy	Pollination of flowers by pollen from other flowers within the same plant; genetically equivalent to self-pollination (de Jong et al. 1993).
Habitat fragmentation	Reduction of continuous habitat into several smaller spatially isolated remnants (Young et al. 1996).
Herkogamy	Separation of pollen and stigma presentation in space; generally reduces intraflower self-pollination (Webb and Lloyd 1986, Barrett 1998).
Inbreeding depression	Reduction in viability and fertility of inbred offspring in comparison to those from outcrossed matings; results primarily from homozygosity of deleterious recessive alleles (Barrett 1998).
Index of self-incompatibility (ISI)	Percentage of fertile seeds per capsule after self-pollination divided by their percentage after cross-pollination (Kenrick 1986).
Multilocus outcrossing rate (t_m)	Mating system parameter; estimated from genotype frequencies at multiple marker loci among parents and progeny of a given population (Ritland 1983, Brown et al. 1989).
Ovule	Reproductive structure containing female gametophyte with egg cell; develops into seed after fertilisation (Raven et al. 1986).

Ovulode	Sterile ovular structure; common in some Myrtaceae such as eucalypts (Carr and Carr 1962).
Pollen / ovule ratio	Ratio of the number of pollen grains and the number of ovules per flower (Cruden 1977).
Pre-emergent reproductive success (PERS)	Product of the fruit / flower ratio and the seed / ovule ratio (Wiens et al. 1987).
Seed / ovule ratio	Percentage of ovules developing into seeds (Wiens 1984).
Self-incompatibility	Inability of cosexual plant to set (abundant) seed following self-pollination; most common anti-selfing mechanism (Barrett 1998).
Transmitting tract	Secretory stylar tissue which exudes a mucilagenous extracellular (intercellular) matrix through which pollen tubes migrate (Herrero and Hormaza 1996).
Wright's fixation index *(F)* (inbreeding coefficient)	Measure of the effect of inbreeding based on the reduction of heterozygosity, when compared with random mating. $F = 0$, no inbreeding; $F = 1$, complete inbreeding (Hartl 1991).

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PREFACE

This thesis has been written in the format of individual papers, several of which have already been accepted for publication. This requires that the chapters form separate entities, and should be able to stand by themselves. However, they also represent different aspects of the reproductive biology of pohutukawa and thus cross-reference each other. While the link between individual chapters is not equally strong, they all rely on the information given in others to some extent. Each chapter introduces its particular topic separately and discusses the results in detail. In contrast, the general introduction and the conclusions both refer to the thesis as a whole.

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