Genetic Diversity and Interspecific Relationships
in Banksia L.f., (Proteaceae).

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Abstract

Banksias are amongst the best known Australian wild flowers. They are used in ornamental horticulture and last well as fresh cut flowers, or indefinitely as dried arrangements. Breeding and selection of new cultivars for the cut flower industry is currently underway. This thesis aims to increase knowledge essential for conservation biology and for focused and efficient breeding of banksias.

Pollen storage and viability testing are important adjuncts to a plant breeding program. *Banksia menziesii* pollen was stored at 20 °C, 4 °C, -20 °C, -80 °C and -196 °C and assessed using a semi solid medium of 1% agar, 15% sucrose, 0.01% boric acid, 0.03% calcium nitrate, 0.02% magnesium sulphate, 0.01% potassium nitrate, and an incubation temperature of 25 °C. Germination remained constant at around 70% in all treatments except room temperature 20 °C, which after six months had only 25% germination. Pollen viability was assessed using fluorescein diacetate (FDA), but the results did not reflect the loss of germinability at 20 °C. There was no effect of floret position on the inflorescence on germination; but pollen viability varied over the flowering period with maximum germination mid season.

Interspecific hybridisation is assessed as a potential breeding tool, and for the assessment of species relationships within the genus. Pollen tube growth was investigated using controlled hand pollination of the commercially significant species *Banksia coccinea*, to species of *Banksia*, and the related genus, *Dryandra*. Currently, the relationship between *B. coccinea* and the other species groups within *Banksia* is unclear. It has been found previously that success of pollen tube growth in the pistil following interspecific pollination was largely related to taxonomic distance between the species (Sedgley *et al.* 1994). Thus, interspecific hybridisation is a suitable technique to determine the compatibility relationships of the problematic species *B. coccinea*. Some species supported no germination of *B. coccinea* pollen. Others produced pollen tube
abnormalities including thickened walls, bulbous swellings, non-directional growth, burst tubes and branched tubes. Control of pollen tube growth in the pistil was imposed in the pollen presenter, a specialised region of the style for pollen presentation to foraging fauna, and in the upper style. There was no significant reciprocal effect on pollination success in the lower style. The results of pollen tube compatibility in the lower style indicated that *B. coccinea* had a closer affinity to the section *Oncostylis*, than to section *Banksia* where it is currently placed. Given the distinct morphology and close pollen-pistil relationship to section *Oncostylis*, it is proposed to move *B. coccinea* out of section *Banksia* to a new section *Coccinea*, the sister section to *Oncostylis*. Intergeneric crosses of *B. coccinea* with *Dryandra* species resulted in some compatibility, with one cross having low numbers of pollen tubes in the pollen presenter and upper style region. These results indicate a close relationship between *Banksia* and *Dryandra*, which are sister genera in the tribe *Banksiae*, family Proteaceae.

Species relationships within *Banksia* were also assessed using molecular techniques. Random amplified polymorphic DNA (RAPD) markers were assessed for their usefulness at various taxonomic levels within the genus. It was found that RAPDs are informative at the close species level, but not at more distant levels, such as between distantly related series, sections, and subgenera. In addition, species relationships at higher levels were investigated using direct polymerase chain reaction (PCR) sequencing of chloroplast DNA (cpDNA) spacer regions between the *trnL* and *trnF* exons. These regions are thought to be universal for plant species and informative at the intra and interspecific level of plants. Using the region between *trnL* and *trnF*, relationships within *Banksia*, and between *Banksia* and *Dryandra* were investigated. It was found that this region was conservative, with little variation between species. Section *Banksia* formed a group, section *Oncostylis* formed another group, and *B. coccinea* along with two *Dryandra* species was placed between the two sections. Resolution at this node however, was not complete. Subgenus *Isostylis* formed two groups away from the two sections in subgenus *Banksia*, with *B. illicifolia* and *D. formosa* together, while *B. cuneata* was
more distantly related. Based on DNA sequence and RAPD data, it appears that *Banksia* and *Dryandra* may be artificial genera, and that in the presence of each other, they cannot be separated using RAPD or *trnL* DNA sequence data.

Genetic variability within species of *Banksia* was investigated using RAPDs. Levels of genetic diversity were generally high, ranging from 0.59 - 0.90. This agrees with previous work using isozymes, pollen tube and fruit set data, showing that *Banksia* species are predominantly outcrossing. In particular, a detailed study was conducted on a geographically restricted, rare and endangered species, *B. cuneata*. Using RAPDs on all known populations, it was found that levels of genetic diversity were high, ranging from 0.65 - 0.74, and that there was no significant genetic differentiation between populations.

In conclusion, this study contributes to knowledge essential for further improvement and conservation of *Banksia* species, and raises questions regarding the currently accepted taxonomic relationships within *Banksia* and between *Banksia* and *Dryandra*. 