Breeding Systems and Pistil Structure in the Family Proteaceae

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Abstract

A considerable body of literature exists on the Australian Proteaceae. However, studies of the proteaceous breeding system have focussed upon a small range of genera, notably the genus *Banksia*. Although these studies are comprehensive, they highlight the need for a broadening of investigation to include other genera and specific aspects of the breeding system. One such aspect is the structure of the pistil which has been shown to potentially limit seed set. This limitation is of concern to plant breeders as it potentially hinders the commercial development of proteaceous species. The literature also highlights the unique features held by the Proteaceae, such as reduced quantities of transmitting tissue relative to the rest of the style and the presence of transfer tissue. To determine the significance of these features, comparison with other angiosperms is required.

Propagation of the Australian Proteaceae for commercial purposes primarily relies upon germination from seed. For some species such germination can be problematic. Thus investigation of methods designed to promote germination efficiency are required. Temperature has been shown to promote germination in some Australian Proteaceae but again research has concentrated upon ~~certain~~ a limited number of species.

Therefore it was the primary aim of this study to broaden our understanding of the breeding systems operating in the Australian Proteaceae and to provide a preliminary study of the optimum requirements for promotion of seed germination in two lesser researched genera which have horticultural potential.

In particular, the breeding systems of two species of *Dryandra* (Proteaceae); *D. quercifolia* and *D. formosa* were investigated. These species are currently sold internationally as cut flowers. The timing and pattern of stigma receptivity was determined using a combination of techniques. Hand pollinations were performed and pollen tubes counted to establish the time of peak pollen germination. In addition, changes at the stigma in terms of stigmatic groove openness and exudate production were assessed using an environmental scanning electron microscope. Results were combined to ascertain the physical and physiological timing of peak stigma receptivity. Stigmas of both species showed peak receptivity at two to six days post-anthesis. *Dryandra quercifolia* was receptive immediately after anthesis while *D. formosa* showed little germination until two days post-anthesis. A stigmatic exudate was produced by stigmas of both species, its production overlapping with the time of peak pollen germination and maximum groove openness. Each species showed a distinct pattern of exudate production and groove opening. For *D. quercifolia* these factors increased from day three post-anthesis until the end of the study period (day 12 post-anthesis). In contrast, maximum values for *D. formosa* were reached four days post-anthesis and decreased thereafter. These factors were consistently lower in *D. formosa* compared to *D. quercifolia*. In addition, exudate production was lower in pollinated than unpollinated pistils of *D. formosa*.

To determine the compatibility system operating in *D. quercifolia* and *D. formosa*, hand pollinations were performed (self, cross and open pollination) and the results assessed in terms of pollen tube counts and seed set. Both species showed a mixed breeding system; cross- and self-pollination was possible although self-pollination was less favoured and often resulted in post-zygotic abortion of at least one seed. *Dryandra formosa* was more self-compatible than *D. quercifolia*. Pollen tube counts of *D. quercifolia* pistils suggested a self-incompatibility mechanism operating in the upper style of this species. To complement the breeding system investigation of *Dryandra*, the temperature (5, 15, 25 or 35 °C) required for optimal seed germination was determined for three species of this genus, and for three species of the genus *Isopogon*. In addition, the rate and percentage emergence under glasshouse conditions (glasshouse; max/min. 27.9/20.1 °C) was determined for ten species of *Dryandra* and seven of *Isopogon*. Species selection was based on their current or potential use in the floriculture and amenity industries. These industries rely upon seed germination for propagation of many Australian species. Optimal percentage, and rates of germination for both genera were achieved after incubation at 15 °C. In addition, *Dryandra* species germinated at 25 °C, however germination was reduced and slower compared to the 15 °C treatment. Germination was inhibited at 5 °C and 35

°C. The emergence of seedlings in the glasshouse was slow for all species and numbers were low compared with germination at 15 °C. In some cases there was no emergence.

The family Proteaceae is characterised by a low fruit to flower ratio. To determine whether structural limitations within the flower, such as stigmatic cavity size and the amount of transmitting tissue within the style, contribute to this low fertility, pistil structure was investigated. An anatomical and morphological study of pollen presenters, styles and pollen was performed on species from the genera *Banksia, Dryandra, Hakea, Isopogon* and *Macadamia*. In particular, to determine whether the size of the stigmatic cavity restricted access of pollen grains to the stigmatic surface, pollen presenters were serially sectioned and cavity volume determined. In addition, the distribution and volume of tissues within the pollen presenter was quantified using image analysis software. A field emission scanning electron microscope and image analysis were used to calculate pollen grain volume, and in turn the maximum pollen grain holding capacity of the cavity. To assess the amount of transmitting tissue, pistils were transversely sectioned down their length and the number of transmitting tissue cells counted. There were three types of stigmatic cavity. A groove in which the stigmatic papillae were enclosed (*Banksia, Dryandra* and *Hakea*), a groove with protruding papillae (*Macadamia*) and a tube which enclosed the papillae (*Isopogon*). Anatomical studies revealed the pollen presenter to be internally complex, but similar in structure across species studied. Groupings could be formed based upon the presence or absence of transfer tissue and the presence or absence of sclerenchyma. These groups were not mutually exclusive. Transfer tissue was associated with transmitting tissue; the tissue through which pollen tubes grow, in the pollen presenter and upper style of all species except *Hakea bucculenta*. The presence of transfer tissue may contribute to the nutrition of the growing pollen tube. The physical dimensions of the cavity, namely its volume, length and diameter, restricted pollen grain access to the stigmatic papillae. The transmitting tissue tract narrowed significantly from the pollen presenter to the base of the style, at this point cell numbers were as few as eight in *Isopogon cuneatus*. There were three structural filters to pollen tube passage in the pistil. The first was at the stigma – a consequence of cavity dimensions – and the second and third related to a narrowing of the transmitting tissue tract within the pollen presenter, and to a lesser extent, within the lower style.

To determine whether reduced the quantities of transmitting tissue observed at the base of the proteaceous style were unusual, a comparative study incorporating seventeen species from nine angiosperm families, including one monocotyledon, was performed. Specifically, stylar anatomy was examined to determine whether structural limitations at this point influence pollen tube number, and thus seed set. Serial sections above the ovary were taken, and transmitting and stylar tissue quantified using image analysis software. A comparison was made of numbers of transmitting tissue cells, pollen tubes and ovules. Overall stylar structure and tissue quantities were consistent within families, but differed significantly between families. The proportion of transmitting tissue to the total style was very low in the Proteaceae compared to other families. Pollen tube number was related to transmitting tissue cell number and to ovule number. Species with multiple ovules (≥12) had the greatest area of transmitting tissue and highest number of pollen tubes. The ratio of transmitting tissue cells to pollen tubes was approximately 1:1 for these species, a ratio much lower than for species with few ovules (≤2). For all species, pollen tube number was similar to ovule number suggesting that ovule number may have a strong influence upon the number of pollen tubes reaching the base of the style. *Triticum aestivum* was the only exception to this, multiple pollen tubes reported at the base of the style to fertilise one ovule. Species with multiple ovules were potentially structurally limited by the amount of transmitting tissue in their style, however no firm conclusions could be drawn as pollen tube number was generally sufficient to fertilise the ovules present. The long styles of the Proteaceae may increase the number of transmitting tissue cells required to support the growth of one pollen tube in comparison to other families.

This study has addressed two areas of research into the Australian Proteaceae which needed attention. Firstly, the investigation of the breeding system of the commercial *Dryandra* species has provided a valuable study for comparison to the extensive work on *Banksia*, *Dryandra's* closest relative. It has also provided information which can be used to breed and improve these species for further commercialisation in the cut flower and garden industries. Secondly the study of pistil structure has further identified the potential of this structure to effect the capacity of a proteaceous flower to be fertilised. Aspects such as the morphology and size of the stigmatic cavity and the quantity of transmitting tissue in the style were highlighted as filters to pollen tube passage. It is noted that any study investigating low seed set in the family should consider pistil structure as a potential site for hindrance of pollen tube growth and thus seed set. The comparative study of the number of pollen tubes at the base of the style in the Proteaceae to other angiosperm species has confirmed the unusual structure of the pistil of the proteaceous flower. In particular, the reduced amount of transmitting tissue at this point relative to the rest of the style is a feature which distinguishes the Proteaceae. The study has shown that pollen tube number at the base of the style and ovule number appear to be related, and that species may differ in the amount of transmitting tissue required for the successful passage of a single pollen tube. Each of these results contribute to our general understanding of the angiosperm breeding system, and in particular the breeding system of the Australian Proteaceae.

The promotion of seed germination by exposure of seeds to controlled temperatures of 15 °C observed in this study is an important result, as it can be used to enhance the seed germination efficiency for commercial propagation of *Dryandra* and *Isopogon* species.

Each of these results contribute to, and potentially assist our ability to use species of the family Proteaceae for commercial cut flower and garden venture.