Limonium Hybrid Production and Post-Pollination Biology.

A thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Botany at Massey University.

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Abstract.

The aim of this thesis is to determine barriers to interspecific hybridisation in selected *Limonium* species. Production of new commercially viable hybrids is essential to the development of the *Limonium* cut flower market. Very few studies have been published with respect to pollination and post-pollination biology of this genus so this study contributes a better understanding of events leading to fertilization and some of the factors involved in interspecific hybrid production.

The thesis initially introduces the genus and its cultivation. Later chapters involve aspects of post-pollination biology and determination of normal development associated with fertilization, including pollen tube growth, ovary and ovule growth and embryo production. This information is used to judge the success, barriers and problem associated with new hybrid *Limonium* production.

Interspecific hybrids were not obtained, but the information provided serves to highlight difficulties in hybrid production and provides opportunities for more directed breeding programmes.

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1.

The Genus Limonium.

1. Taxonomy, Distribution, Phylogeny.

The genus *Limonium* (family Plumbaginaceae) contains 150 largely perennial and some annual species (Huxley *et al.* 1992). The plant is typified by entire or pinnatifid leaves arranged in basal rosettes or clustered at axils. Inflorescences are corymbose panicles of terminal spikelets. Flowers are 5-merous consisting of a coloured or hyaline calyx, often persistent after anthesis, surrounding a corolla of variable colour. The five stamens are epipetalous. Species are usually dimorphic for pollen and stigmas and homostylous, although heterostyly, pollen dimorphism-stigma monomorphism, stigma and pollen monomorphism, secondary monomorphism and male sterile apomicts do occur in some sections. Ovaries are unilocular and contain a single ovule, the fruit as a single seeded dehiscent capsule.

Present day *Limonium* taxonomy is based largely on Boissier's 1879 system (Baker, 1953). Several changes and additions have been made to Boissiers's 1879 classification. *Statice* was rejected as a *nomen ambiguum* (Baker, 1953 quoting Lawrence, 1940; 1947) and replaced with *Limonium*. Prior to this change Boissier's *Statice* contained both the sea lavenders and the thrifts, these two groups now separated to *Limonium* (Miller) and *Armeria* (Wildenow) respectively. In addition, the sections *Schizopetalum* (Boissier), *Pterolimon* (Hooker), and *Arthrolimon* (von Meuller) have been added to Boissier's original scheme (Baker, 1953) (Table 1.01).

Goniolimon, a genus closely related to Limonium (Fig. 1.03) was distinguished from Limonium by Boissier by the virtue of capitate stigmas (Baker, 1953) but before the discovery of the capitate condition in several Limonium species. Goniolimon, in addition to other qualities, can be distinguished from Limonium by the presence of free anthers. There is synonymy in several species of Limonium and Goniolimon.

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Section	Subsection	
1. Pteroclados	Odontolepidae	L.sinuatum, L.thouini
	Nobiles	L.perezii, L.macrophyllum
2. Ctenostachys	-	L.mucronatum, L.pectinatum
3. Plathymenium	Rhodantheae	L.flexuosum, L.congestum
	Chrysantheae	L.aureum, L.sinense. L.australe
4. Limonium	Genuinae	L.vulgare, L.gmelinii
	Densiflorae	
	Dissitiflorae	
	Steirocladae	L.cosyrense, L.dregeanum
	Hyalolepidae	L.latifolium, L.bellidifolium
	Sarcophyllae	L.axillare, L.arabicum
5. Sphaerostachys	-	L.globuliferum
6. Jovibarba	-	L.jovibarbum
7. Schyzhymenium	-	L.echoides
8. Circinaria	-	L.purpuratum, L.perigrinum
9. Polyarthrion	-	L.caesium , L.ornatum
10. Myriolepis	-	L.ferulaceum, L.diffusum
11. Syphonantha	-	L.tubiflorum
12. Psylliostachys	-	L.suworowii, L.spicatum
13. Schizopetalum	-	L.macrorhabdon, L.griffithii
14. Pterolimon	-	L.plumosum L.peruvianum
15. Arthrolimon	-	L.salicornaceum
16. Goniolimon	-	G.tataricum, G.dumosum

Table 1.01. *Limonium* Sections and Subsections (including *Goniolimon*) with examples (Baker, 1953).

Species inhabit coastal plains, semi arid, desert and saline areas in a broad band from Japan through China, India, the Middle East, and North Africa to Western Europe. Limonium also occurs in isolated groups on the South and North American continents, Australia, and the Canary and Cape Verde Islands (see Fig. 1.02). There are correlations between geographic distribution and reproductive method (Baker, 1953 b.) Certain subsections contain apomictic species ie Densiflorae and Dissitiflorae and these have probably arisen through lack of pollinating agents. Secondary monomorphism of pollen and stigmas has arisen from dimorphy in Genuinae, Chrysantheae and Steirocladae/Hyalolepidae. Secondary monomorphy found in species probably facilitates long distance dispersal, individual isolated genets being able to multiply by self fertilization. Baker (1953) suggests no separation between Steirocladae and Hyalolepidae. Species with continuous distributions are usually dimorphic for pollen and stigmas. Some Limonium sections, Circinaria, Pterolimon, Schizopetalum and Arthrolimon contain species with capitate stigmas. It is likely that this condition has developed through geographic isolation (Baker, 1953, 1966) rather than by derivation from dimorphism.

It is thought likely that the genus had its origins in the western Old World region, the present day centre of variability being the Mediterranean. During the Eocene, North Africa and South America were in contact and it is suggested by Baker (1953) that migration of the genus occurred from Africa to the American continents. From its new position in South America the genus then spread northwards by long distance dispersal and aided by secondary monomorphism. *L.humile* is thought to have originated in the North American Continent and then spread back to western Europe. The remainder of the genus radiated from the Old World to the Far East. The pattern of migration for the Asian Chrysantheae appears to follow that of Genuinae described above, the long distance dispersal of *L.australe* being aided by secondary monomorphism.

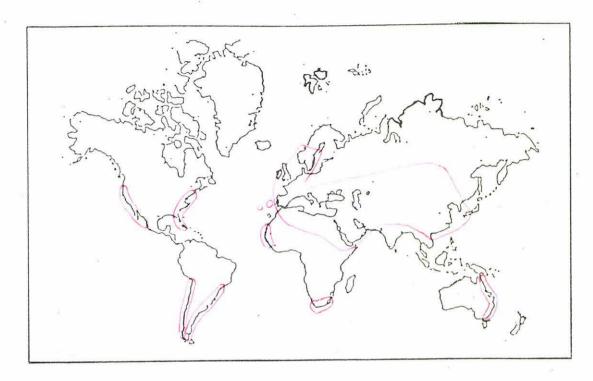


Fig. 1.01. Global distribution of *Limonium* (and *Goniolimon*) and sections. From Baker (1948).

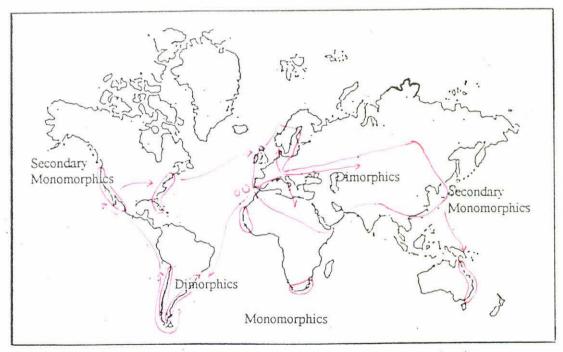


Fig. 1.02. Distribution patterns in *Limonium* with respect to reproductive biology according to Baker (1953 b.).

The following figure (Fig. 1.03) illustrates the phylogenetic relationship of *Limonium* and closely related genera (Baker, 1966).

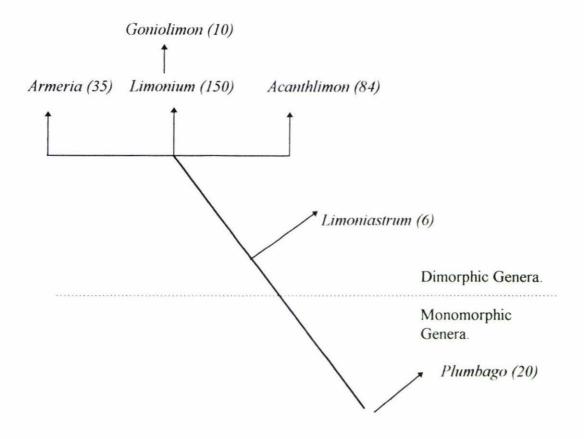


Fig. 1.03 Phylogenetic scheme for Plumbaginaceae (From Baker, 1966). The number of species in each genus is given in brackets.

Within the genus *Limonium*, phylogenetic derivation is postulated to have led through several stages culminating in comparatively rare apomicts and secondary monomorphs (monomorphy from dimorphy), possibly as a result of geographic isolation, and predominantly heterostylous dimorphic species (Baker, 1966)(See Table 1.02).

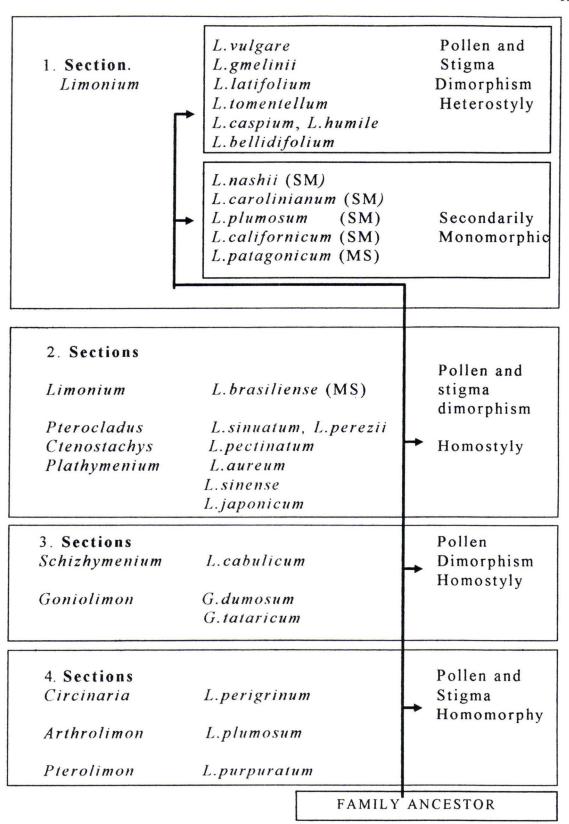


Table 1.02. Postulated phylogenetic sequence in heteromorphic incompatibility systems. (SM= secondary monomorphism MS= male sterile apomicts) (From Baker, 1966) (Modified).

2. Cultivation.

The wide global distribution of *Limonium* means considerable variation in form, physiology and habitat tolerance occurs between species. According to Harada (1992) *Limonium* tolerates a well drained soil with a pH of 6.5 and E.C (electrical conductance) of 0.5. Plants can be divided into type I and type II, seasonal and free flowering plants respectively. Type I plants must be well established and require a period of winter vernalisation to induce flowering the following summer while type II plants, if given suitable light and temperature (20-25 °C) will flower all year round (Harada, 1992).

The commercial success of *Limonium* species and hybrids as cut flowers is dependent largely on flower colour and form, vase life, and the persistence of calyxes on the inflorescence. Several hybrids and species are in commercial production. Some examples include *L.simuatum*, a species with a wide variety of colours in compact spikelets, especially valuable when dried and *L.perezii* with corymbs of persistent purple calyxes, *L.perigrinum* produces an open spike of conspicuous rose petals and calyxes; *L.latifolium* and *L.dumosum* (syn. *Goniolimon dumosum*) are valued for their long flowering stems but lack colourful calyxes. *Limonium* hybrids in commercial production include Saint Pierre and Bertlaard (*L.latifolium* X *L.caspium*), Charm Blue (*L.latifolium* X *L.gmelinii*), the Oceanic series (*L.latifolium* X *L.dumosum*) and Diacean (*L.latifolium* X *L.bellidifolium*).

Interspecific hybridisation has been used to transfer specific traits between species. A long stemmed form of *L.perigrinum* is a product of the cross between *L.perigrinum* and *L.purpuratum* (Morgan *et al.* 1995 (in press)). Embryo rescue is used to salvage often aborted interspecific hybrid embryos. Artificial cultivation and subsequent cloning is used to multiply such individuals. Transformation of *L.perigrinum* has been attempted using *Agrobacterium* but with little success. (E..Morgan *pers. comm.*)



Fig. 1.05. The colourful and persistent calyx of *L. simuatum* makes this species a valuable cut flower.

Commercially produced *Limonium* plants often originate from tissue culture, having distinct advantages over seed raised plants in that they are more uniform, and have a better stem length, flower colour and vase life (Harada, 1992).

The considerable range of *Limonium* species available for interspecific hybridisation attempts, and advances in embryo rescue techniques, suggest considerable potential for the development of superior and alternative commercially viable forms. Research into barriers to and possibilities for wide crosses within this genus continues.