

## Chapter 5 General Discussion and Conclusions

### 5.1 Introduction

Two species of *Nymphoides*, one with a distylous and the other a homostylous breeding system, were examined for variations in their pollination systems, the influence of these pollination systems on reproductive success, and the arrangement of male and female sex organs. Three approaches were taken: the reproductive biology of *Nymphoides montana* was studied to provide insight into the maintenance or evolutionary transition of distyly (Chapter 2); the possible causes and consequences of the loss of sex in the distylous *N. montana* species were examined (Chapter 3); and the benefits of homostyly under unfavourable pollination conditions were studied in *N. geminata* (Chapter 4). The major findings of this study suggest that, in distylous populations, pollinator activity and the presence of the two floral morphs (or mating partners) at an equal frequency are the most likely factors that assure successful sexual reproduction and maintenance of the breeding system. The only study population of *N. montana* found to be monomorphic for style length consists of a single genotype, producing no fruits. The absence of compatible mates and/or pollen for the monomorphic, self-incompatible plants could interfere with the functioning of distyly, resulting in the loss of sexual function. The homostylous congener *N. geminata* was found to be self-compatible, and the flowers with a stigma–anther separation value of near zero, self-pollinate autonomously. This floral morphology and breeding system would favour the persistence of the homostylous plants in ecologically marginal environments. Figure 5.1 summarises the key findings of this study and shows a hypothetical pathway to reach conditions that favour the maintenance of distyly, and two possible evolutionary transitions – evolution of asexuality and evolution of homostyly in the two species of *Nymphoides*.

## 5.2 What factors maintain distyly?

The reproductive biology of *Nymphoides montana* is particularly interesting since morphological and physiological components of distyly in other species of *Nymphoides* and its sister taxon, *Villarsia*, vary from the typical distylous syndrome. The results of the study show *N. montana* is indeed a truly distylous species because the populations have two floral morphs that differ reciprocally in stigma and anther heights in the flowers (Figure 2.1). The floral morphs also possess self- and intramorph incompatibility systems (Figure 2.14) and a set of ancillary dimorphic floral traits (Figure 2.11 and Figure 2.12). However, the reciprocity of anther position (Figure 2.10) and the strength of the incompatibility systems vary between the floral morphs. Although these atypical features of distyly are usually implicated in evolutionary transitions to, or from, this breeding system, several selective factors appear to assure the maintenance of distyly in *N. montana* (Figure 5.1).

In the distylous populations, with sex-organ reciprocity and incompatibility systems present, most mating should arise from cross-pollen transfer between flowers of the two morphs. Genetic and clonal diversity (Chapter 3), and the results of the open-pollinations, reveal that sexual reproduction following intermorph crosses and regular recruitment from seeds have played a significant role in population maintenance. In the populations with a 1:1 morph ratio, the two morphs had equal open-pollinated fruit set, indicating each morph can only mate with the opposite mating partner and has an equal fitness. In the biased population, however, fruit set of the more common morph declined. Provided the reproductive success of each mating type is dependent on the frequency of its compatible mating partner within populations, negative frequency-dependent selection should maintain distyly during disassortative mating (Charlesworth and Charlesworth 1979b; Lloyd and Webb 1992b). In negative frequency-dependent mating, the more rare morph has a mating advantage, and therefore a fitness advantage, over the common morph because successful reproduction only occurs after pollination between flowers of the different morphs. Under this form of mating, the biased population of *N. montana* should reach an equilibrium morph ratio of 1:1 over one generation due to segregation and recombination of recessive and dominant alleles at the locus controlling the mating system (Ganders 1979; Barrett 1992c). Since *N. montana* is a perennial clonal plant, the speed at which a 1:1 morph ratio is reached depends on other factors, including founder events, the extent of clonal growth and sexual recruitment (Eckert and Barrett 1995; Wang *et al.* 2005). Field experiments

involving the manipulation of morph ratios are required to determine if the reproductive success of the floral morphs is negatively frequency-dependent (Thompson *et al.* 2003; Wang *et al.* 2005; Brys *et al.* 2007).

The results of the study in Chapter 2 could not provide insights to the evolutionary transition to, or from, distyly. Regardless of whether distyly is in transition or maintained, what other potential factors could be responsible for the atypical features of the syndrome? One possible factor that could disturb the exact reciprocity of the sex organs would be the introduction of alien flower visitors into the study populations. *N. montana* is endemic to Australia and is assumed to have evolved parallel with its own pollinators. Therefore, it is natural to assume that the floral design and arrangement of the sex organs in this species are better adapted for pollination by native insects rather than introduced insects, such as the European honeybee, *Apis mellifera* L. The positions of the sex organs are possibly inaccurate for pollen donation and receipt by alien flower visitors, and in this case the selection to maintain the sex organs in perfect reciprocity would have begun to weaken. The relationship between the pollination efficiency of the native vs. introduced flower visitors and floral structure, e.g. the arrangement of the sex organs and the length of the floral tube, would be interesting to study.

### **5.3 What are the consequences of a loss of mating partners in a clonal distylous species?**

For distyly to function properly in clonal species with extensive clonal propagation, populations should contain two floral morphs that are spatially well-mixed (Shibayama and Kadono 2003; Wang *et al.* 2005; Brys *et al.* 2007). Large clones may otherwise suffer from inefficient cross-pollen transfer when pollinators transfer self-incompatible pollen among flowers of the same ramet or different ramets of the same genet, leading to reduced sexual reproduction. During population surveys of distylous *Nymphoides montana*, one population appeared to be monomorphic for style length; the consequences of the absence of mating partners on sexual and clonal reproduction were studied in Chapter 3. The results of the study indicated that sexual reproduction in the monomorphic population was impaired by the lack of compatible mating partners, providing conditions for the establishment and fixation of sterility mutations through extensive clonal propagation. The loss of sex in the

monomorphic population has resulted in asexual reproduction and stolons maintaining the population.

No fruits and seeds were detected following open-pollinations and controlled crosses in a glasshouse environment, indicating a genetic basis to the sterility of this population (Figure 3.4). To find out what factors reduced sexual reproduction in this population, inter-population crossings and chromosome counts were undertaken. The results showed the monomorphic population probably became sterile as the result of extreme limitation in the number of compatible mating partners rather than a change in ploidy level (e.g. triploidy in *Butomus umbellatus*, Eckert *et al.* 2000). Since, however, unfavourable environmental conditions are known to play a key role in limiting sexual reproduction in geographically peripheral populations (Dorken and Eckert 2001), a widespread sampling across the *N. montana* distribution range is warranted to consider the edge status and the frequency of monomorphic populations.

In the absence of seed production, the sexual traits that no longer function to increase fitness, such as the anthers, pollen and ovules, became degenerated in the monomorphic population, (Figure 3.6, Figure 3.7 and Figure 3.8). In addition, the functional distance between the stigma and anthers of the sterile flowers was near zero, whereas the distylous flowers showed a well-developed herkogamy (Figure 3.3). If stigma–anther separation is an adaptation to promote cross-pollination (Webb and Lloyd 1986), degeneration of such floral mechanisms in the sterile population appears to be corroborated by the loss of male and female function.

A clonal growth comparison between the sterile monomorphic and fertile distylous populations demonstrated that the sterile plants gained fitness through vegetative and clonal growth, compensating for their loss of sexual function. In this population, the loss of sexual reproduction was associated with enhanced clonal reproduction (Figure 3.9). Once genetic sterility has originated, it could spread into the population via resource reallocation or antagonistic pleiotropy; experimental removal of flower buds is needed to test these two hypotheses (Dorken *et al.* 2004).

ISSR genetic markers revealed a lack of genetic and clonal diversity in the sterile monomorphic population, whereas the distylous populations are genetically diverse (Table 3.6, Figure 3.10, Figure 3.11 and Figure 3.13). This would further confirm that in the

absence of seed production, sexual recruitment was extremely rare, and the sterile plants reproduced only through clonal propagation. Nei's genetic distance also showed that the sterile monomorphic population was genetically distanced from the two distylous populations, indicating that there has been enough evolutionary time for the loss of male and female functions since the origin of sterility in *Nymphoides montana* (Figure 3.12). Loss of sexual reproduction and its association with genetic uniformity has also been reported in two other distylous species of *Nymphoides*, *N. peltata* (Uesugi *et al.* 2004) and *N. indica* (Shibayama and Kadono 2007).

The relative importance of clonal vs. sexual reproduction for population maintenance could be explained in the following way (Figure 5.1). The monomorphic population is founded by a single genotype, e.g. a single seed or a broken leaf, or alternatively, several genotypes occur and all but one genotype is eliminated following a bottleneck. The single genotype flowers and spreads by stolons to form a single clone patch. In this situation, where a flower is surrounded either by flowers of the same ramet or different ramets of the same genotype, sexual reproduction involves self-fertilisation following auto- and/or geitonogamous pollination. In the absence of compatible mates and/or pollen, the self-incompatible ramets gradually lose their ability to produce viable seeds, only propagating clonally. The male (anther and pollen) and female (ovule) reproductive organs that no longer contribute to fitness via sexual reproduction are lost, i.e. the 'use it or lose it' hypothesis (Eckert 2002; Gross and Caddy 2006). Meanwhile, severely reduced sexual reproduction reinforces the lack of sexual recruitment in the population. Finally, clonal reproduction is favoured and the population expands further when the sterile plants divert resources from the inviable pollen and ovules to clonal propagation and vegetative growth. Clonal propagation, therefore, assures reproduction in the asexual population. However, a downside to the high level of clonal reproduction and recruitment is genetic uniformity within the population. Therefore, the loss of sex could have important implications for the long-term persistence of the asexual population. So, why does this asexual population persist? Probably, the benefit of reproductive assurance by spreading stolons is not only worth the loss of genetic diversity, it must be worth the cost of finding mates and sex.

#### 5.4 What features of homostyly allow persistence in temporary environments?

In ecological situations where opportunities for pollinator activity and outcross-pollination are limited, the ability to produce seed through self-fertilisation can provide reproductive assurance (Baker 1955; Jain 1976; Barrett *et al.* 1989; Ramsey and Vaughton 1996; Schoen *et al.* 1996; Fausto *et al.* 2001; Vaughton *et al.* 2007). In heterostylous plants, colonisation of ecologically or geographically marginal areas is associated with evolutionary changes in mating behaviour and floral biology in the direction of increased self-fertilisation and the development of homostyly (Ornduff 1972; Shore and Barrett 1985; Barrett and Shore 1987; Schoen *et al.* 1997; Guggisberg *et al.* 2006). This type of evolutionary change in the breeding system has probably occurred in homostylous *Nymphoides geminata*; this species occurs in the ecological margins of the range of its congener distylous *N. montana* (NE herbarium and personal observations). This prediction was tested in Chapter 4 by comparing floral traits, seed production without pollinators, pollinator activity and ploidy level between the homostylous and the distylous species. The results of the study show that the homostylous species has the ability to persist in temporary habitats because the plants produce seed autonomously, and have flowers adapted for self-pollination.

The homostylous species produces smaller flowers. Little or no stigma–anther separation (0.01–0.60 mm) was found, compared to the distylous species. As a result, mature self-pollen is brought into close contact with the stigma during pollen presentation, facilitating autonomous self-pollination. Modifications of floral traits that allow self-pollination in the absence of pollinators are associated with plants able to self-fertilise autonomously. Autonomous pollinations of *N. geminata* under glasshouse conditions (Figure 4.11) showed successful seed set, and floral visitor observations and open pollinations (Figure 4.12) showed the species is able to reproduce successfully despite floral visitor scarcity.

Since, homostyly is less common than distyly in *Nymphoides* (7 vs. 15 species; see section 1.4), the former is assumed to be a derived condition in this genus. On this basis, the development of homostyly in the species and its ability to persist in temporary or marginal habitats could be explained in the following way (Figure 5.1). Very early in distylous populations, a crossover in the *S* supergene results changes to male and female

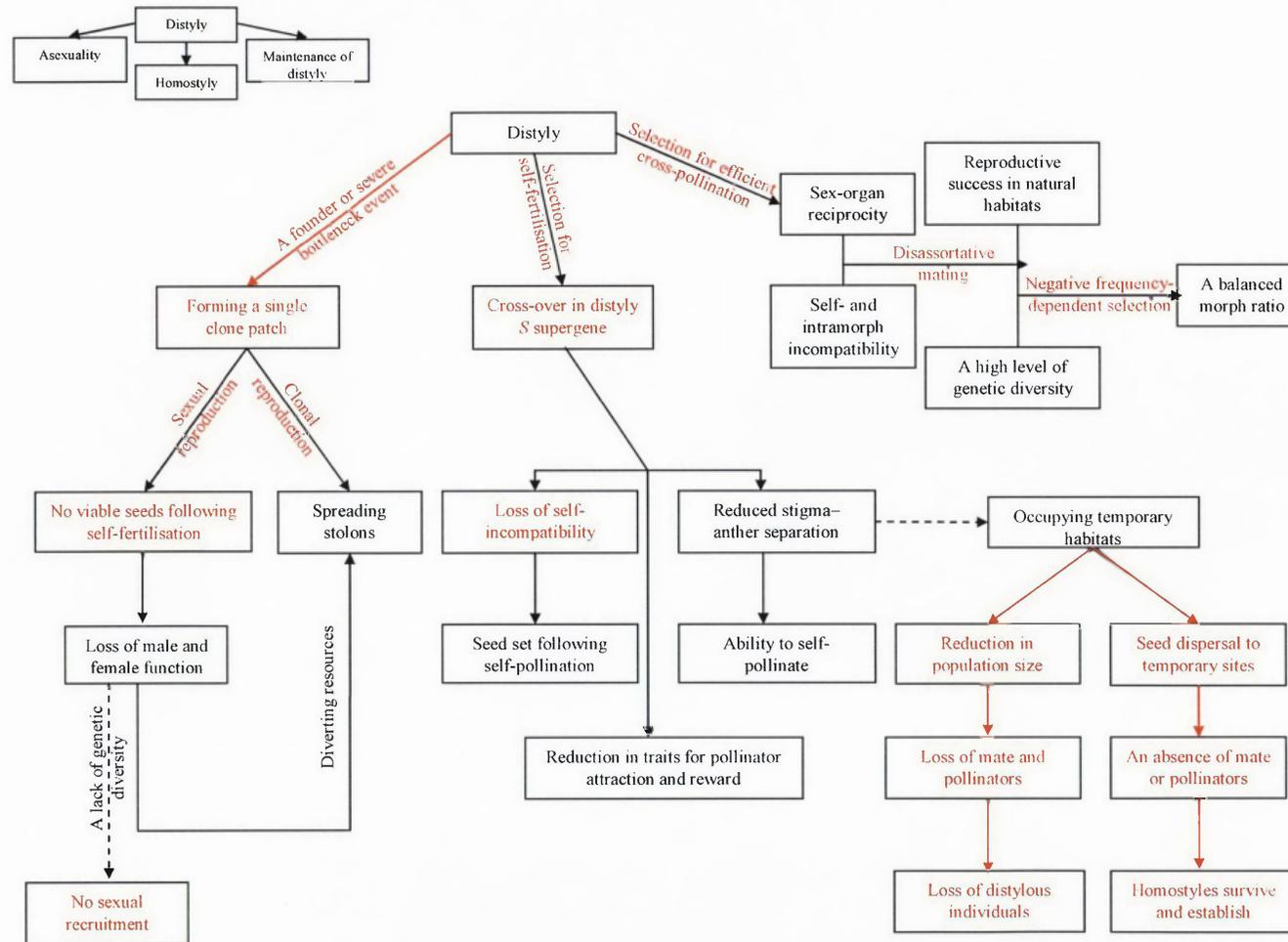
sex organs coinciding with a change in the incompatibility systems. Such genetic alteration, in one step, creates homostylous individuals with the ability to self-pollinate and self-fertilise. Fertilisation by self-pollen becomes possible with genetic modifications to the incompatibility systems of distyly. Concurrently, morphological traits that promote self-pollen deposition evolve, e.g. reduced stigma–anther separation. Meanwhile, the homostylous condition becomes favoured in two possible ways, with small population size and in temporary habitats with unreliable pollination conditions. Severe reductions in population size result in the loss of compatible mates and/or pollen, and hence the loss of distyles, whereas mate- and pollinator-independent homostyles are able to persist and reproduce sexually. The most likely scenario could be that following seed dispersal of homostylous and distylous plants to new and temporary habitats, only homostyles could survive and establish a sexually-reproducing population (Baker's theory). Following the evolution of autonomous self-pollination and self-compatibility, selection favours reduction in the floral traits that are no longer required for pollinator attraction and reward, e.g. smaller corolla size and lower pollen:ovule ratio.

Despite the selective advantage that homostyles encounter under unfavourable conditions for cross-fertilisation, in long-distance dispersal or in the colonisation of new environments, these species are reported to be evolutionarily short-lived (e.g. *Amsinckia* Schoen *et al.* 1997). Stebbins (1957) argued high rates of self-fertilisation usually limit genetic variability within populations due to the accumulation of deleterious genes and populations losing the ability to respond to environmental changes (reviewed in Takebayashi and Morrell 2001). Polyploidy in self-compatible species, however, provides a buffer against adverse effects of self-fertilisation and appears to be associated with a high level of genetic variability (Lande and Schemske 1985; for studies explaining higher inbreeding depression in polyploids see, however, Ronfort 1999 and ref. therein; Guggisberg *et al.* 2006). So, would the benefit of polyploidy in self-compatible species be out-weighed by the disadvantages of self-fertilisation, e.g. loss of genetic diversity and inbreeding depression?

**Conclusions**—Distylous plants can reproduce successfully in their natural habitats if there are compatible mating partners and pollinators. The self-incompatibility and flowers adapted for cross-pollination should enhance mating between plants of different genotypes and maintain a high level of genetic variability within populations. If the distylous plants

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do not receive enough compatible pollen to ensure seed set or fruit set, the plants can lose their sexual function and become male and female sterile. In the absence of sexual reproduction and recruitment, however, clonality allows the sterile population to persist. Alternatively, in response to the lack of mates or poor pollinator visitation, seed production can be assured through the development of homostyly and the ability to self-pollinate and fertilise autonomously. Although, clonality and self-fertility are believed to decrease genetic variation within populations, these modes of reproduction could be beneficial to the survival of populations under unfavourable pollination conditions. The microevolutionary studies reported here need to be supported with phylogenetic studies to determine the origin of different pollination systems and floral diversity, e.g. how frequent are the switches to small-flowered, self-pollinating plants from cross-pollinating plants with a reciprocal herkogamy?



**Figure 5.1.** Hypothetical pathways for the maintenance distyly, origin of homostyly and asexuality in the two species of *Nymphoides*. Black indicates the events supported by the results of this study. Red indicates the proposed theories. The broken lines indicate parallel events with the evolutionary processes.

After this study, I am tempted to say that each plant studied possesses its own personality. A distylous plant is happily sexual, a monomorphic plant rendered sexless, and a homostylous plant is selfishly independent. But the point is, after all, a suitable mate and sex do matter for a healthy and long-lasting life!

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