Floral Variation and Breeding System in Distylous and Homostylous Species of Clonal Aquatic *Nymphoides* (Menyanthaceae)

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Abstract

This study focuses on floral variation and the breeding system of two species of the clonal aquatic *Nymphoides* (Menyanthaceae), in particular, the phenomena of distyly, loss of sex and homostyly. Distyly is a floral design that promotes efficient pollen transfer while reducing self-interference and self-pollination. Populations of distylous species contain two floral morphs that differ in the reciprocal positioning of the sex organs; i.e. reciprocal herkogamy. Distylous species usually possess a dimorphic incompatibility system in which only pollination between flowers of different morphs produce seed. There are, however, atypical features of distyly, which provide excellent examples to study the maintenance or evolutionary transition of this breeding system. *Nymphoides montana* is a perennial clonal aquatic species, native to Australia, found in shallow water on the edges of wetlands. Selective factors that maintain distyly were sought by examining floral morphologies, compatibility relationships, morph frequencies and reproductive success in three *N. montana* populations. In two of these populations the two floral morphs are present in equal ratios (1:1), and natural pollination resulted in a high level of fruit and seed set (> 70%). The major morphological and mating features of the distyly in these populations were the reciprocal positioning of stigmas and anthers in the morphs and the presence of self- and intramorph incompatibility (0–26% seed set). Crosses between plants of the opposite morphs produced more than 95% seed set. A wide range of ancillary dimorphism in pollen, stigma and flower size accompanied the distyly. However, the reciprocity of anther position and the strength of the incompatibility systems varied between the floral morphs. Although these atypical features of distyly are usually implicated in evolutionary transitions to, or from, this breeding system, other lines of evidence appear to reflect the maintenance of distyly in *N. montana* populations.

During population surveys of distylous *Nymphoides montana*, one population appeared to be monomorphic for style length. A severely reduced, or an absence of, fertility is reported to be a common feature of monomorphic populations, so the consequences of an absence of a mating partner on sexual and clonal reproduction were studied for this monomorphic *N. montana* population. The monomorphic plants failed to produce any fruits and seeds following open-pollinations and controlled crosses in a glasshouse environment, indicating a genetic basis to the sterility of this population. The result of cross-pollinations between the sterile and conspecific fertile populations showed

an extreme limitation in the number of compatible mating partners could be responsible for the sexual sterility. Sterile polyploidy was ruled out as the possible cause of sexual infertility when both sterile and fertile populations were found to be at the same ploidy level ($6x = 54$). Observations of male sterility detected loss of male function following a failure in the mechanical release of pollen from anthers, together with a low proportion of pollen viability (0.05%) and a high proportion of malformed pollen (85%). Infertility in this population was also caused by the loss of female function; there was a very low level of seed set (< 4%) in the cross-pollination treatments using fertile pollen. A clonal growth experiment comparing vegetative growth and total plant dry mass between sterile and fertile plants revealed an association between sexual infertility and enhanced clonal propagation, indicating once genetic sterility has originated, it could spread into the population via resource reallocation or antagonistic pleiotropy. Comparisons of genetic and clonal diversity using ISSR markers between the sterile and fertile populations showed a strong link between the severely reduced seed production and the failure of sexual recruitment. Overall, the results suggest that following a severe bottleneck or founder event, the self-incompatible plants lost their ability to reproduce sexually in the absence of compatible mates and rely solely on extensive clonal propagation.

In the ecological or geographical margins of the range of distylous taxa, homostyly may evolve in response to a lack of mates and/or poor pollinator visitation. Evolution of homostyly is associated with the evolution of self-compatibility and floral adaptation to autonomous self-pollen deposition (or self-pollination without pollinators). This prediction was tested by comparing floral traits, seed production without pollinators, pollinator activity and ploidy level between the homostylous *Nymphoides geminata* and the distylous *N. montana*. The homostylous species has smaller flowers with little or no stigma–anther separation (0.01–0.60 mm) and lower pollen:ovule ratio than the distylous species, and produces many fruits and seeds autonomously (>70%). The result of floral visitor observations and open pollinations indicated the ability of homostylous plants to reproduce successfully despite pollinator scarcity. A study of chromosome number revealed that the origin of homostyly was not associated with a change in ploidy level. The ability of homostylous *N. geminata* to persist in temporary habitats with unreliable pollinator service is associated with the ability of plants to self-pollinate and self-fertilise autonomously.

Studies of distyly, loss of sex and homostyly in two species of the clonal aquatic *Nymphoides* provided an opportunity to answer some questions regarding the observed variation in floral morphology and reproductive systems. The self-incompatibility and flowers adapted for cross-pollination should enhance mating between plants of different genotypes and ensure a high level of genetic variability within populations. If the distylous plants do not receive enough compatible pollen, the plants can become 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.

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Glossary

Ancillary polymorphic traits	Variation in size and morphology of corolla, stigma and pollen between heterostylous floral morphs
Assortative mating	Mating of individuals with similar phenotypes
Autonomous self-fertilisation	Production of fruit and seed following a within-flower self-pollination in the absence of pollinators
Autonomous self-pollination	Pollination that occurs within a flower without an external pollinator
Bisexual	A flower with functional male and female sex organs
Clone	A group of genetically identical individuals produced by asexual propagation
Dichogamy	Separation of stamen dehiscence and stigma receptivity in time to reduce sexual interference
Dioecy	Male and female flowers are on separate individual plants
Disassortative mating	Mating in which one sex chooses the other in such a way that the offspring benefit from the diversity of parental genotypes
Geitonogamous self-pollination	Pollination of a flower by another flower of the same individual plant
Genet (clone)	A group of plants growing in tight proximity which are all genetically identical, originating vegetatively, and not sexually, from a single ancestor
Gynodioecy	Female and hermaphrodite flowers are on separate individual plants
Herkogamy	Separation of anther and stigma positions in space to reduce sexual interference
Hermaphrodite	An individual that possesses both male and female sex organs
Heterostyly	A sexual polymorphism in which populations are composed of two (distyly) or three (tristyly) floral morphs with reciprocal arrangements of stigmas and anthers

Homostyly	A floral monomorphism in which stigmas and anthers are positioned at a close proximity within a flower. Homostylous plants are a member of heterostylous genera/species, and usually have the ability to self-pollinate and self-fertilise autonomously (without pollinators).
Integration	Increased genetic and functional relationships among traits
Intermorph pollination	Pollination of a flower by another flower of the different floral morph, or pollen transfer between anthers and stigmas of reciprocal heights
Intramorph pollination	Pollination of a flower by another flower of the same floral morph, or pollen transfer between anthers and stigmas of non-reciprocal heights
Long style (approach herkogamy)	Presentation of stigma above the level of the anthers
Mid style	Presentation of stigma between two levels of the anthers
Monoecy	Male and female organs are found on all individuals, but in separate flowers
Pleiotropy	Capacity of an isolated allele to have more than one distinguishable effect
Ramet	An individual member of a clone that has been vegetatively reproduced from the original plant
Reciprocal herkogamy	A reciprocal arrangement in stigma and anther positions in the heterostylous floral morphs
Reproductive assurance	Reproduction is assured through self-pollination and/or fertilisation when conditions for outcrossing are unfavourable because of an absence of mates or pollinators
Self-incompatibility	Inability of a fertile bisexual flower to set seed following self-pollination
Short style (reverse herkogamy)	Presentation of stigma below the level of the anthers
Self-pollination	Pollination that occurs within a flower