

## Morph Ratio, Matting Pattern and Incipient Gender Specialization in *Nymphoides peltata* -an Invasive Species in Wular Lake of Kashmir (India)

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**Abstract:** *Nymphoides peltata* is a potential invasive species growing in the Wular lake of Kashmir-the largest freshwater lake of Asia. The species is distylous showing pin and thrum morphs in its populations. The present study revealed that the syndrome of heterostyly in the species is equipped with the biochemical barrier of self incompatibility. The self and intramorph pollinations resulted in zero reproductive success while as intermorph pollinations yielded high values of reproductive success. The species exhibits tremendous vegetative growth which interferes with the efficiency of sexual reproduction. The vegetative or clonal proliferation results in biased morph ratios as a result of which chances of mating are reduced. Populations where only pin morph individuals were encountered displayed low values of reproductive success while as populations which showed isoplethy with random distribution of individuals of the mating morphs showed peak values of reproductive success. Thus *Nymphoides peltata* shows negative frequency dependent reproductive success. The species displays remarkable incipient gender specialisation with thrum morph individuals showing more tendency towards maleness while as pin morph affiliated more to femaleness. Intermorph crosses where pin morph served as female partner showed higher seed set as compared to thrum which yielded low seed set of significant difference. The distyly in the species is preparing to evolve into dioecy with pin morph individuals converting into female and thrum morph individuals into male individuals.

**Key words:** Invasive species • Distyly • Incompatible • Heterostyly breakdown • Dioecy

### INTRODUCTION

*Nymphoides peltata* is an aquatic macrophytic species known for its invasive potential throughout the world. The species is a prolific invader of eutrophic lakes and water bodies. *Nymphoides peltata* is a distylous species showing sexual polymorphism where populations comprise of short styled thrum morphs and long styled pin morphs. Normally this sexual polymorphism is associated with a number of ancillary morphological and physiological characters. Among these characters the biochemical barrier of incompatibility attracts lots of attention. Heterostyly taxa associated with physiological incompatibility system exhibit disassortive mating where individuals of one morph can mate only with the individuals of opposite mating morph. This matting pattern drives populations to numerical equilibrium of isoplethy where pin and thrum morph individuals are

almost equal in number [1]. The maintenance of sexual polymorphism involves negative frequency dependent selection whereby a mating type's reproductive success depends on the frequency of the sexual morph with which it can mate [2]. Frequency dependent selection leads to an equal morph ratio at equilibrium. This numerical balance is having tremendous reproductive implications as it provides saturated mating chances and saturated mating environment for the two potential mating morphs. Therefore, as anticipated, at isoplethy (1:1morph ratio) the reproductive success of mating morphs is ideal and maximum.

Alternatively ratios with unequal frequencies result when morphs show clonal growth or differ in their ability to mate with each other. *Nymphoides peltata* witnesses prolific clonal proliferation [3]. The latter is anticipated to interfere with the normal sexual reproduction of the species, disturbing morph ratio, diluting compatible

pollens, reducing mating chances and fruit and seed set [4]. Under such stressful conditions where selection forces favoring this syndrome are rare or eliminated, the characters associated with heterostyly are believed to breakdown to dioecy or homostyly [5]. The present study was carried out to test presumed evolutionary changes from distyly to dioecy or homostyly in *Nymphoides peltata* in lakes of Kashmir (India) where till date no such study has been carried out.

## MATERIALS AND METHODS

**Study Species:** *Nymphoides peltata* (Gmel) O. kuntze (*Menyanthaceae*) native to Eurasia is growing abundantly in all the major lakes viz-Wullar, Dal, Nigeen and Mansbal lakes of Kashmir. *Nymphoides peltata* reproduces both vegetatively and sexually. Maturation of floral buds is underwater and on anthesis the buds are raised above the water level and flowering occurs. After a few hours the corolla withers, the pedicels deflect and subsequent fruit development is under water. The flowers are yellow and showy, consisting of a short basal tube and five spreading corolla lobes. In most lakes of Kashmir the species flowers from June to October. The species is distylous and flowers are visited by a wide range of insect pollinators mostly bees and flies. The flowers are brightly yellow, 5 petaloid and 3-4 cm in diameter. The flower edges are distinctly fringed (common name fringed water lily). The fruit is a capsule upto2.5 cm long containing numerous seeds.

**Morph Ratios in Natural Population:** We investigated 25 populations of *N.peltata* from all the major lakes of Kashmir. Each population was analyzed for floral morph ratio by randomly sampling flowering ramets. The populations displayed variation in size ranging from 15-350 flowering ramets. The populations were tested for pin thrum ratio and 1:1 isoplethy equilibrium.

**Controlled Pollinations:** We conducted controlled hand pollinations on plants from six populations in Wullar and Dal lakes. The experiments were conducted both in the field and transplanted populations in S.P College and Islamia College Srinagar. For each population sample we performed intermorph, intramorph and self pollinations shortly after the beginning of anthesis and harvested fruits 10-12 days after pollination. The flowers were selected randomly for carrying out manual pollinations of different classes.

**Morph Ratio and Fruit Set:** The effect of morph ratio on fruit set was calculated using different manipulated morph ratios. We used an experimental population of *N.peltata* growing in 3+3m pond at Islamia College. Flowers were removed from the population early in the morning to create different morph ratios. The treatments ranged from monomorphism to different biased ratios. The ratios were 0.1, 0.2, 0.3, 0.4 frequency of the minority morph. In one experiment the pin morph was maintained equal to thrum morph in numerical strength (0.5-isoplethic equilibrium).The ratios were created to investigate the effect of morph ratio on seed set and to determine frequency dependence of reproductive success if any in the species.

**Gender Specializations:** In order to analyze the gender potential or gender bias if any affiliated with either morph, reciprocal crosses were made between the thrum morph and pin morph individuals. In one exercise thrum morph served as male parent and pin as female parent while in the other, pin morph served as male and thrum morph as female. In both type of crosses seed set was analyzed and compared for the two morphs.

## RESULTS

**Variations in Floral Morph Ratios:** Floral morph ratios varied greatly among populations of *N.peltata* in the different lakes of Kashmir. Of the 25 populations studied 48% populations were monomorphic and 52% populations were dimorphic represented by both thrum and pin morphs. The dimorphic populations witnessed considerable heterogeneity with reference to morph frequencies. Few dimorphic populations exhibited isoplethy with 1:1 pin and thrum ratios while most of the dimorphic populations displayed a biased morph frequency as is evident from Fig. 1. Among 12 monomorphic populations 7 were of pin morph while the remaining five contained flowers of only short styled morph. During our survey we observed no homostyly or unisexual flowers in either dimorphic or monomorphic populations of *N.peltata* in the different lakes of Kashmir.

**Mating System:** A clear-cut evidence of the strong dimorphic incompatibility system in *N. peltata* comes from the manual pollinations from all the sites. Self and intramorph pollinations of the floral morphs resulted in no seed set whereas intermorph pollinations yielded a good seed set. The floral polymorphism in the species is thus equipped with a strong incompatibility barrier which restricts self and intramorph fertilizations as evidenced by our manual pollination studies.

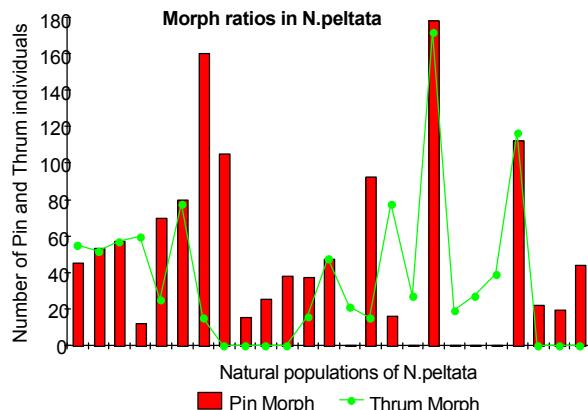


Fig. 1: Showing morph ratio in natural populations of *N.peltata*

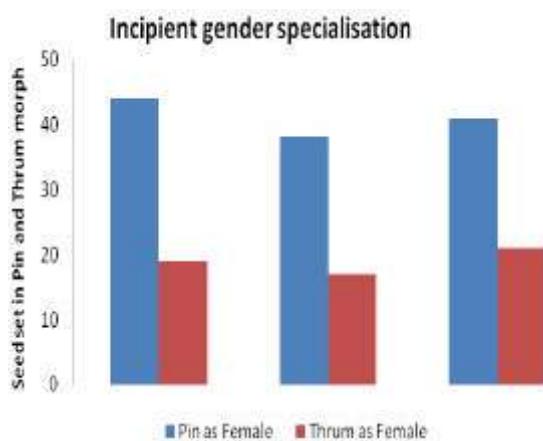


Fig. 2: Showing Incipient gender specialisation in *N. peltata* -pin morph acting as potential or functional female and thrum as potential or functional male in natural populations of Wullar Lake

**Floral Morph Ratio and Seed Set:** The variation in morph ratio is having a great influence on reproductive success. The populations with isoplethy i.e. 1:1 ratio showed abundant fruit/seed set. Populations with biased morph ratios showed a declining trend in seed set in common morphs. This decline in seed set witnessed lowest values in monomorphic populations where some populations displayed zero seed set disproving the earlier belief that *N.peltata* is having a weak incompatibility system. Fruit set in pin morph declined with decline in thrum morph frequency and vice versa. The above trend approves the fact that *N. peltata* displays negative frequency dependant reproductive success where the reproductive success of one mating morphs is determined by the frequency of other potential mating phenotype.

**Gender Specialisation:** The two morphs pin and thrum differ significantly as far as femaleness and maleness is concerned. In crossing experiments where pin served as female parent and thrum as male parent the seed set was more than double as compared to crosses where thrum served as female parent. The crossing experiments revealed that thrum morph serves as a potential male and pin as potential female. The seed set in the two morphs differed significantly with pin morphs producing almost double number of ovules as compared to thrum morph. Thus *N.peltata* shows distinct incipient gender specialization in its two morphs with thrum as potential male and pin as potential female.

## DISCUSSION

*Nymphoides peltata* is displaying heterostyly with reciprocal positioning of sex organs in its morphs. The species is distylous witnessing reciprocal herkogamy as also reported by earlier workers [5, 6]. The syndrome of heterostyly is equipped with a strong incompatibility barrier which usually characterizes heterostyly complex. In *N.peltata* self and intra morph pollinations failed to bring out fertilization and seed set while as only intermorph pollinations resulted in seed set. The strength of this biochemical barrier of incompatibility is witnessed in monomorphic populations which fail to produce fruits and seeds at all. Our results negate the earlier report of Orunduff [4] who envisages of a weak incompatibility system in the species but correspond to that of Wang *et al.* [7]. The species being heterostylosus requires the services of animal pollinator to execute successful pollinations. Bees and flies pursue the desired job with fidelity. In heterostylosus taxa with incompatibility complex the two mating morphs are obligatory for normal sexual reproduction. In such cases physiology determines mating patterns meaning that thrum can mate only with pin and vice versa. This negative frequency selection maintains the floral polymorphism and leads to isoplethic morph ratio [8-11]. The conservative index associated with heteromorphic incompatibility is isoplethy meaning that populations contain 1:1 ratio of individual morphs. However *N.peltata* resorts to profuse vegetative proliferation [3] which yields heterogeneous values of morph frequency [4, 12]. The clonal growth of the species results in biased morph ratios and wide monomorphic patches. The clonality thus interferes with the normal sexual reproduction which is mate and morph dependent. Thus seed set and fruit set is compromised at the altar of vegetative reproduction. Clonal monomorphic populations fail to set seeds as they experience mate deficiency [7]. In incompatible heterostylosus taxa

maximum fruit and seed set is achieved when the two morphs are mate saturated meaning that the morphs are at isoplethy-1:1 ratio. *N.peltata* being distylos with strong incompatibility also displayed the same trend. Maximum fruit and seed set were achieved when the morphs were at 1:1 ratio with normal random spatial dispersion of their individuals. Fruit and seed set declined with biased morph ratios with common morph failing to achieve expected fruit and seed set. The reproduction in the species is negative frequency dependent. Declining the ratio to 1:0, monomorphic populations failed to set any seed.

The stress of mate deficiency with clonality interfering with normal sexual reproduction has influenced the complex syndrome of heterostyly. The heterostyly syndrome struggles with fertility selection, the latter known to favor seed formation. During the present study reciprocal crosses between mating morphs yielded different values of seed set. Pin morph individuals produced almost double the number of seeds than thrum morphs while serving as female parent. The thrum produced half the number of seeds while serving as female parent. The pin morph serves as a potential female parent than thrum which acts better as male pollen producing parent than serving female functions. This is an initial event of gender specialization which in due course of time may lead to dioecy as interpreted by Ornduff in [4]. In several genera of angiosperms, distyly has evolved into dioecy [13-16] and in every case individuals bearing female flowers are evolutionary derived from long styled (pin) individuals, while male plants are derived from short styled (thrum) plants. Our results are a direct reflection of the above fact. Is *N.peltata* preparing to shift to dioecy from distyly, the incipient gender specializations certainly marks the beginning.

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#### REFERENCES

1. Finney, D.J., 1952. The equilibrium of self incompatible polymorphic species. *Genetica*, 26: 33-64.
2. Thompson, J.D., S.C.H. Barrett and A.M. Baker, 2003. Frequency dependent variation in reproductive success in *Narcissus*; implications for the maintenance of stigma -height dimorphism. *Proceedings of the Royal Society of London Series B*, 270: 949-953.
3. Van der Veld, G. And L.A. Van der Heijden, 1981. The floral biology and seed production of *Nymphoides peltata* (Gmel) O Kuntze (Menyanthaceae). *Aquatic Botany*, 10: 261-293.
4. Ornduff, R., 1966. The origin of dioecism from heterostyly in *Nymphoides* (Menyanthaceae). *Evolution*, 20: 309-314.
5. Ganders, F.R., 1979. The biology of heterostyly. *New Zealand J. Botany*, 17: 607-635.
6. Barrett, S.C.H. Ed., 1992. Evolution and function of Heterostyly. New York. NY USA. Springer Verlag, pp: 1-29.
7. Wang, Y., F.Q. Wang, H.Y. Guo and S.C.H. Barrett, 2005. Reproductive consequences of interactions between clonal growth and sexual reproduction in *Nymphoides peltata*-a distylos aquatic plant. *New Phytologist*, 165: 329-336.
8. Fisher, R.A., 1941. The theoretical consequences of polyploidy inheritance for the mid style form of *Lythrum salicaria*. *Ann. Eugen.*, 11: 31-38.
9. Charlesworth, D., 1979. The evolution and breakdown of tristyly. *Evolution*, 33: 486-498.
10. Heuch, I., 1979. Equilibrium populations of heterostylos plants. *Theor. Popul. Biol.*, 15: 43-57.
11. Barrett, S.C.H., 1993. The evolutionary biology of tristyly. In D Futuyma and J.Antonovics (eds) *Oxford surveys in evolutionary biology*. Vol. 9 Oxford University press Oxford, U.K.
12. Murai, H. And I. Waishnani, 1993. Heterostyly and seed production of *Nymphoides peltata* In: Lake Kasumigaura (In Japanese) *Syuseibutsugaku-Kenkyu*, 17: 59-63.
13. Baker, H.G., 1958. Studies in the reproductive biology of West African Rubiaeae. *J. West African Sci. Association* 4: 9-24.
14. missing
15. Vielleumuir, B.S., 1967. The origin and evolutionary development of heterostyly in angiosperms. *Evolution*, 21: 210-226.
16. Opler, P.A., H.G. Baker and G.W. Frankie, 1975. Reproductive biology of some Costa Rican *Cordia* species (Boraginaceae). *Biotropica*, 7: 234-247.
00. Lloyd, D.G., 1979. Evolution towards dioecy in heterostylos populations. *Plant Systematics and Evolution*, 131: 71-80.
00. Barrett, S.C.H., 1979. The evolutionary breakdown of tristyly in *Eichornia crassipes* (Mart.) Solms (water hyacinth). *Evolution*, 33: 499-510.
00. Barrett, S.C.H., 1980. Dimorphic incompatibility in *Nymphoides indica* (Menyanthaceae). *Canadian J. Botany*, 58: 1938-1942.