

Dimorphic incompatibility and gender in *Nymphoides indica* (Menyanthaceae)

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Received January 31, 1980

BARRETT, S. C. H. 1980. Dimorphic incompatibility and gender in *Nymphoides indica* (Menyanthaceae). Can. J. Bot. 58: 1938–1942.

Nymphoides indica (Menyanthaceae) is a distylous, aquatic perennial of pantropical distribution. Controlled field pollinations in a Lower Amazon rice field population demonstrated the presence of a strong self- and intramorph-incompatibility system in the 20 individuals tested. The average seed fertility of short-styled individuals was significantly greater than long-styled individuals, although significant variation among individuals within morphs was also evident. Fertility data obtained from experimental crosses are utilized for developing a new method for the estimation of gender in plants. The potential for gender specialization in *N. indica* populations and its significance to the evolution of dioecy from distyly in the genus *Nymphoides* is discussed.

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Nymphoides indica (Menyanthaceae) est un espèce pérennante aquatique, distylique, dont la distribution est pantropicale. Des pollinisations contrôlées dans une population d'une rizière de la région amazonienne inférieure démontrent chez les 20 plantes testées, la présence d'un système d'auto-incompatibilité et d'incompatibilité entre plantes de la même forme. La production moyenne de graines des individus brévistylés est significativement plus forte que celle des individus longistylés, bien qu'il existe une variation significative entre individus d'une même forme. Les données de fertilité obtenues à partir de croisements expérimentaux sont utilisées pour développer une nouvelle méthode d'estimer le sexe chez les plantes. L'auteur discute le potentiel pour la spécialisation sexuelle dans les populations de *N. indica*, ainsi que sa signification en rapport avec l'évolution de la dioécie vers la distylie dans le genre *Nymphoides*.

[Traduit par le journal]

Introduction

Nymphoides indica (Menyanthaceae) is a perennial, floating-leaved aquatic of pantropical distribution (Sculthorpe 1967; Ornduff 1969, 1970). It occurs at the periphery of streams and pools and as a weed of cultivated rice and artificial water bodies (Dirvan and Poerink 1955; Sculthorpe 1967; Bristow *et al.* 1972). Populations from the New and Old World were considered separate species (*N. humboldtiana* and *N. indica*, respectively). Ornduff (1969) has argued that since no consistent morphological characters separate populations from the two regions, *N. humboldtiana* is a synonym of *N. indica*.

In *Nymphoides*, individual species possess distylous, dioecious, or homostylous breeding systems (Darwin 1877; Ornduff 1966). Comparative studies suggest that dioecy and homostyly are derived from an ancestral distylous condition (Ornduff 1966). While the breakdown to homostyly is widely documented in heterostylous flowering plant families (Darwin 1877; Ernst 1955; Baker 1966; Barrett 1979), the evolutionary pathway from distyly to dioecy is less common. Elsewhere this derivation has been suggested in *Mussaenda* (Baker 1958), *Cordia* (Opler *et al.* 1975), and

perhaps in *Mitchella* (Keegan *et al.* 1979) and several tropical genera of *Rubiaceae* (Bawa 1980; Beach and Bawa 1980).

The evolution of dioecy from distyly involves gender specialization since the two floral morphs no longer make equal contributions to their offspring through pollen and ovules (Lloyd 1979a). This usually involves the long-styled morph becoming the ovule parent and the short-styled morph the pollen parent (Baker 1958; Ornduff 1966; Lloyd 1979a). However, it is not known to what extent populations in groups containing distylous and dioecious taxa have evolved in this direction since estimates of the effective gender of individual plants in distylous populations have not been undertaken (Lloyd 1979a).

This report presents the results of a crossing programme involving individuals in a colonizing weed population of *N. indica* in the Lower Amazon region of Brazil. The programme was originally designed to screen plants for self-compatibility and evidence of gender specialization. The model of the evolution of dioecy from distyly in *Nymphoides* involves a self-compatible stage (Ornduff 1966), and this condition is frequently associated with colonizing ability (Baker 1955; Allard 1965). Al-

though the crossing programme provides little evidence for incipient dioecy in *N. indica* the data are used for developing a novel method for the estimation of gender in plants.

Materials and methods

All experimental work was undertaken at Boca de Jari, Lower Amazon, Brazil. Twenty widely separated plants (probably different genotypes) of similar size were transplanted from a rice field population to a pollinator-free enclosure. The enclosure was constructed of wood and fine netting and was situated at the edge of the field. Differences between the environmental regimes in which pollinations occurred in field and those of test plants were probably minimal. Each plant was grown in a plastic bucket for a 3-month period in soil and water obtained from the rice field. During this time controlled hand pollinations were undertaken daily between 0800 and 1000 hours with the aid of fine forceps. All individuals were used in pollinations and an attempt was made to cross all long-styled individuals as egg parents with all short-styled individuals as pollen parents and vice versa. Self-pollinations and intraform (illegitimate) pollinations were also undertaken in order to detect self-incompatibility and cross incompatibility. Records of the egg and pollen parents of all crosses were made and data on fruit and seed production were obtained at harvest. Counts of the numbers of all long- and short-styled morphs in three populations at Boca de Jari were also made.

Individual plants were compared for seed fertility and pollen fertility following legitimate pollinations (interform) by two level, mixed model, nested ANOVA's, following F_{\max} tests for homogeneity of variance (Sokal and Rohlf 1969). Data from two long-styled plants were omitted from both analyses in order to equalize sample sizes for the ANOVA and because the individuals involved were not used in all crossing combinations. Their removal had little effect on the levels of significance obtained from the analyses. For example, inclusion of data from these plants in a one-way ANOVA comparing long- and short-styled seed fertility gave similar significance values ($F = 10.68(1, 18)$, $P < 0.005$) to those obtained from the nested ANOVA (see Table 3).

Average fertilities of the female (g_i) and male (a_i) component of individual plants were estimated from the crossing data in the following manner. The gynoecial fitness (g_i) is the average number of seeds produced per pollination when an individual is crossed as a maternal (egg) parent with all compatible plants. The androecial fitness (a_i) is the average number of seeds produced per pollination when an individual is crossed as a paternal (pollen) parent with all compatible plants. Gender (in this study, "potential femaleness" and "potential maleness") and total fitness of each plant were then calculated using equations developed by Lloyd (1979a). The femaleness of the plant is $G_i = g_i/g_i + a_i$ and the maleness $A_i = a_i/(g_i + a_i)$; the total fitness of individuals is the sum of their androecial and gynoecial fitnesses, i.e., $W_i = a_i + g_i$. These estimates are best described as "potential gender" since the data from which they are calculated involve controlled field pollinations, rather than open pollination. Only by controlled pollination can the pollen success of individuals be estimated since this requires information on the seed production of morphs and the sources of pollen which fertilized the ovules.

Results

Populations of *Nymphoides indica* at Boca de Jari exhibit a range of population structures ranging

TABLE 1. Representation of floral morphs in *Nymphoides indica* populations from Boca de Jari, Lower Amazon

Population No.	Source	No. of plants	% frequency	
			Long-styled form	Short-styled form
1	Rice field	150	0.51	0.49
2	Canal bank	40	0.65	0.35
3	Canal	15	0	1.00

from monomorphy to isoplethy (Table 1). All 20 individuals utilized in the controlled pollination programme exhibited strong self-incompatibility. Total seed production from self-pollinations and intraform pollinations was approximately 1% of that obtained from interform pollinations (Table 2).

There were significant differences among individuals in the amounts of seed produced following legitimate pollination. Short-styled plants exhibited significantly greater average seed fertility than long-styled plants (Table 3). In the ANOVA, 51.0% of the variation in average seed fertility could be accounted for by differences among the floral morphs, whereas 31.5% of the variation was the result of differences among individuals. The remaining variation was due to differences in the amount of seed produced by flowers on a single individual. Such variation probably results from developmental and environmental factors.

Variation in seed fertility between the floral morphs was not accompanied by differences in flowering propensity. There were no significant differences between style forms in average flower production during the 3-month study period (average no. of flowers per plant: long-styled form = 47.82, short-styled form = 42.89; $F = 0.48(1, 19)$, $P > 0.25$).

Seed and pollen fertilities of individual plants, together with estimates of their potential gender (femaleness and maleness) and total fitness are presented in Table 4. The data illustrate the significant variation both among individuals within morphs and between morphs in average seed fertility. This variation ranges from 5.4 (long 2) to 22.8 (short 15). The pattern of variation in average pollen fertility is strikingly different. Although there is a significant difference between the average pollen fertility of the morphs ($P < 0.001$); within morphs, differences among individuals are not significant (Table 3). In the ANOVA, 53.6% of the variation in pollen fertility can be accounted for by variation in the performance of pollen of single individuals when applied to the range of egg parents.

TABLE 2. Fruit and seed production following controlled pollinations of *Nymphoides indica*

Pollination	No. of parents		No. of pollinations	% fruit set	Total seeds produced	Mean no. of seeds per pollination
	♀	♂				
Long styled						
Self	11		148	7.4	16	0.1
Intraform	11	11	96	6.2	12	0.1
Interform	11	9	227	98.7	2163	9.5
Short styled						
Self	9		119	10.1	15	0.1
Intraform	7	8	28	14.3	18	0.6
Interform	9	11	229	100.0	3554	15.5

TABLE 3. Analysis of variance of seed fertility and pollen fertility of long- and short-styled individuals of *Nymphoides indica*

(A) Seed fertility

Source of variation	df	Sum of squares	Mean square	F
Among morphs	1	1209.806	1209.806	14.488*
Among individuals within morphs	16	1336.085	83.505	13.634**
Within individuals	108	661.452	6.124	
Total	125	3207.343		

* $P < 0.005$.
 ** $P < 0.001$.

(B) Pollen fertility

Source of variation	df	Sum of squares	Mean square	F
Among morphs	1	925.086	925.086	83.435**
Among individuals within morphs	16	177.399	11.087	0.662 NS
Within individuals	108	1808.734	16.747	
Total	125	2911.218		

NOTE: NS, not significant.
 ** $P < 0.001$.

Discussion

In *Nymphoides indica* floral dimorphism is associated with a strong self-incompatibility system. The results of this study confirm earlier demonstrations of dimorphic incompatibility in the species (Ornduff 1966; Reddy and Bahadur 1976). Despite the weedy nature of the population studied, all individuals examined were self-incompatible, indicating that there are exceptions to the frequent association between self-fertility and weediness (Baker 1965; Lloyd 1979b). Other tropical heterostylous weeds which are self-incompatible include *Pontederia rotundifolia* (Barrett 1977a) and taxa in the *Turnera ulmifolia* complex (Barrett 1978). It is noteworthy that these taxa are perennials and that the two aquatic species exhibit clonal propagation. Presumably longevity reduces the likelihood of reproductive failure and cloning al-

lows populations to persist where sexual reproduction is restricted by self-incompatibility and monomorphy.

In the majority of heterostylous taxa which have been examined experimentally, the seed fertility of floral morphs, following legitimate pollination, is similar. However, in several studies including the present one, the short-styled morph was significantly more fertile than the long-styled morph, e.g., *Primula* spp. (Dowrick 1956); *Eichhornia crassipes* (Barrett 1977b); *Turnera ulmifolia* (Barrett 1978). Dowrick (1956) demonstrated a smaller area of conducting tissue available for pollen-tube growth in long styles compared with short styles of *Primula obconica*. She suggested that this difference caused the observed variation in seed fertility between morphs. In legitimate pollinations of *N. indica* from India, Reddy and

TABLE 4. Average fertility of female and male function and potential gender of long- and short-styled individuals of *Nymphoides indica*

Individual plants	♀ seed fertility	♂ pollen fertility	Potential gender		Total fitness
			Femaleness	Maleness	
Long styled					
1	14.51	16.48	0.47	0.53	30.99
2	5.39	15.98	0.25	0.75	21.37
3	6.64	16.30	0.29	0.71	22.94
4	12.27	16.51	0.43	0.57	28.78
5	7.00	15.44	0.31	0.69	22.44
6	7.98	15.02	0.35	0.65	23.00
7	12.32	14.71	0.46	0.54	27.03
8	7.87	14.59	0.35	0.65	22.46
9	8.04	16.42	0.33	0.67	24.24
10	15.56	15.36	0.50	0.50	30.92
11	14.65	14.61	0.50	0.50	29.26
$\bar{x} \pm SD$	10.20 ± 3.7	15.58 ± 0.8	0.39 ± 0.9	0.61 ± 0.9	25.77 ± 3.7
Short styled					
12	13.07	10.40	0.56	0.44	23.47
13	12.71	9.77	0.56	0.44	22.48
14	12.48	7.39	0.63	0.37	19.87
15	22.76	9.38	0.71	0.29	32.14
16	16.39	10.76	0.60	0.40	27.15
17	17.15	10.64	0.62	0.38	27.79
18	12.43	10.34	0.55	0.45	22.79
19	15.86	9.37	0.63	0.37	25.23
20	15.75	8.74	0.64	0.36	24.49
$\bar{x} \pm SD$	15.40 ± 3.3	9.64 ± 1.1	0.61 ± 0.5	0.39 ± 0.5	25.04 ± 3.6

Bahadur (1976) reported that the long-styled morph was more fertile than the short-styled morph (average no. of seeds per pollination: long = 17.9, short = 9.9). Although no details of interplant variation are presented, these data suggest that differences in the amount of stilar conducting tissue are unlikely to be a significant factor in the regulation of seed production in *N. indica*. One possible explanation for the striking differences in seed fertility in *N. indica* populations involves genetic variation among individuals in ovule number. The relatively low variation in values of individual seed fertility compared to differences among individuals is consistent with this view. In the Lower Amazon population studied here the variation among individuals in seed fertility appears to be loosely associated with floral dimorphism. Whether or not this association has any biological significance is unclear. It is unlikely to have any effect on population structure and the functioning of heterostyly. The population from which the plants were obtained contained equal frequencies of the floral morphs. Isoplethy is probably maintained by complete disassortative mating guaranteed by strong self-incompatibility and through frequent episodes of sexual reproduction associated with rice field drainage and flooding (see Barrett 1977a).

The estimation of "potential gender" from experimental pollinations depends on obtaining seed set or "fertility" data in reciprocal crosses. If the same plants are used as egg and pollen parents with a range of mates, in all combinations, a common scale for paternal and maternal fitness results. This method can be used for nonheterostylous hermaphrodite plants but assumes that individuals produce equal numbers of flowers. In natural populations where phenotypic plasticity may affect stature and hence flower production, gender estimates involving this parameter are probably more realistic. Lloyd (1980) has recently documented gender patterns in natural populations of four species where variation arises from differences among individuals in the numbers of pollen and seed producing flowers. The genetic component of variation of this type is not known.

Although within the floral morphs of *N. indica* there were significant differences among individuals in average seed fertility, the same individuals varied little in their average pollen fertility. Ross (1977) and Horovitz (1978) have discussed the theoretical implications of variation in fertility in hermaphrodite plants. The inability to demonstrate significant variation in pollen fitness or "maleness" within morphs is probably due to the experimental

techniques employed to estimate this parameter. Differences in pollen fitness would result from variation among individuals in pollen production or pollen tube growth rates and would be manifested by differential fertilization and seed set. However in this study all pollen produced by a single flower was applied to stigmas at the beginning of anthesis and it is unlikely that pollen ever limited seed set. Under field conditions, where less efficient pollination may reduce fecundity, male fitness differences among individuals may be evident. However, it is difficult to devise experimental techniques to estimate the male fitness of individuals under field conditions unless genetic markers are available (see Horovitz and Harding 1972).

Where distyly and dioecy occur in a group of closely related species it appears to be the long-styled form which develops femaleness and the short-styled form which changes to the staminate condition. In *Mussaenda* and *Cordia*, transitional species occur; these may be best described as subdioecious (Baker 1958; Opler *et al.* 1975; Lloyd 1979a). In *Nymphoides*, Ornduff (1966) found no evidence of transitional populations exhibiting gender specialization. The taxa he described were either distylous or functionally dioecious. Data from *N. indica* suggest that a potential for gender specialization exists as a consequence of the great variation among individuals in seed fertility. Nevertheless, it seems unlikely that in the case of the Lower Amazon population examined here that this variation in gender is associated with selection for sexual dimorphism. All individuals tested were self-incompatible and gender specialization was the reverse of that expected in populations exhibiting incipient dioecy.

Acknowledgments

I thank S. W. Barrett and J. S. Shore for technical assistance, D. G. Lloyd and J. S. Shore for comments on the manuscript, and J. Wang for providing facilities at the International Rice Research Institute, Rice Experiment Station, Jarilandia. Research was funded in part by a grant from the Natural Sciences and Engineering Research Council of Canada.

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