Cytogenetics of Aquatic Ornamentals
VI. Evolutionary trends and relationships in the genus Nymphaea

P. P. Gupta

Department of Genetics, University of Liverpool, Liverpool 169 3BX, England

Received October 19, 1978

This genus is named after the Greek Goddess of springs, Nymphe. The members are perennial, rhizomatous, aquatic herbs widely distributed in tropical and temperate regions. The majority of members are cultivated for beautifying aquatic gardens. In many parts of Central America, Australia, West Africa and South Asia the seeds and tubers of locally growing species are used as food (Conard 1905).

The genus Nymphaea comprises about 40 species and numerous forms (van Royen 1962). In addition to many natural hybrids, a large number of artificially raised varieties (Grey 1900, Ames 1900 and Nutt 1967) have been increasing the list of nymphaeas. Due to the great variation in this genus, the taxonomy is complicated and no proper attention has been paid to elucidate the evolutionary patterns. The present research on chromosome number, their mitotic and meiotic behaviour and fertility of the nymphaeas growing in India was undertaken to understand the evolutionary trends and relationships in this genus.

Materials and methods

Cytological investigations were carried out on the following wild and cultivated forms of Nymphaea collected from the Uttar Pradesh, Bihar, Orissa, West Bengal, Assam and Sikkim, provinces of India. The details of localities of collection for the individual members have appeared in earlier publications (Gupta 1976, 1978a and 1978b).

i) N. lotus Linn.
ii) N. rubra Roxb.
iii) N. stellata Willd. var. cyanea (Roxb.) Hook. f. and T.
iv) N. stellata Willd. var. versicolor (Roxb.) Hook. f. and T.
v) N. stellata Willd. var. parviflora Hook, f. and T.
vi) N. sturtevanti Hort.
vii) N. pygmaea Ait.
viii) N. mexicana Zucc.
ix) N. caerulea Savigny.

1 This work was carried out at the Department of Botany, Banaras Hindu University, Varanasi and the National Botanical Research Institute, Lucknow, India.
Figs. 1a–h. Cytological abnormalities of evolutionary significance in nymphaeas.  
a, *N. bissetti*, root-tip metaphase showing syncyte of c. 672 chromosomes. ×725.  
b, *N. daubeniana*, polyploidy in two adjacent PMCs, ×400.  
c, *N. bissetti*, univalents at metaphase I. ×850.  
d, *N. daubeniana*, laggards and dividing univalents at anaphase I. ×1000.  
e, *N. dentatamagnifica*, failure of spindle at anaphase I causing nonsegregation of univalents. ×1000.  
f, *N. caerulea*, multipolar segregation of chromatids at anaphase II. ×850.  
g, *N. rubra* (8x), failure of cyto-
The studies of mitotic and meiotic chromosomes were undertaken following the usual aceto-orcein and iron acetocarmine methods, respectively.

**Observations**

The numerical data of chromosomes of all the above taxa were published earlier (Gupta 1972, 1976, 1978a and 1978b). The chromosome numbers varied from $2n=28$ to 112, each a multiple of the basic number fourteen (Table 1). Due to high number and minute size of the chromosomes, ranging between 0.5 $\mu$ and 2.0 $\mu$, it was difficult to undertake the karyotypic analysis.

**Mitotic behaviour of chromosomes**

Polysomaty was frequent in hexaploid *N. dentatamagnifica*, where the normal chromosome number $2n=84$ was recorded in only 64% of cells. In the remaining cells, other numbers such as 70, 105, 126 and 168 were observed.

*N. bissetii* was characterised by the presence of syncytes or large protoplasmic masses, which occurred among the normal cells of root apices. During the division of syncytes, the chromosome complements were found either partially intermingled or completely fused to form highly polyploid nuclei. The maximum chromosome number observed in these syncytes was about 672, which seems to have been formed by the fusion of 8 normal hexaploid cells (Fig. 1a).

**Meiotic behaviour of chromosomes**

In *N. daubeniana* meiosis showed normally 42 chromosomes, but some aberrant numbers such as 56, 70 and 84 were also noticed in 9% PMCs (Fig. 1b). Similarly, in *N. caerulea* besides the normal chromosome number 28, other $2n$ numbers like 48, 50 and 56 have also been encountered. These numerical alterations in the gametic cells found in some species of *Nymphaea* can be connected to the somatic abnormalities, which seem to persist in the sexual cells.

Most of the polyploid nymphaeas showed regular multivalent formation (Table 1). The triploid *N. daubeniana*, tetraploid *N. stellata* var. *versicolor*, hexaploid *N. lotus*, *N. bissetii*, *N. pygmaea*, *N. mexicana*, *N. dentatamagnifica* and octaploid *N. rubra* showed various multivalent configurations. The presence of multivalents in such polyploids was due either to residual homology in chromosomes originating from the same source or through the role played by interchange heterozygosity. In the other diploid, tetraploid and hexaploid members, *N. stellata* var. *cyanea*, *N. caerulea*, *N. sturtevantii*, *N. stellata* var. *parviflora* and *N. rubra* (6$x$), no multivalents were formed indicating that differentiation between the chromosomes was relatively high in them.

Out of twelve different taxa studied nine showed univalents in different kinesis resulting unreduced pollen grain. $\times$550. $h$, *N. rubra* (8$x$), size variation among fertile pollen grains. $\times$125.
Table 1. Chromosome numbers, association of meiotic chromosomes and pollen fertility of the investigated nymphaeas.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Chromosome number (2n)</th>
<th>Ploidy status</th>
<th>Average univalents per PMC at M I</th>
<th>Average chromosome association per PMC at M I</th>
<th>Pollen fertility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. lotus Linn.</td>
<td>84</td>
<td>6x</td>
<td>33.8</td>
<td>2.0</td>
<td>1.4</td>
</tr>
<tr>
<td>N. rubra Roxb. Cytotype I</td>
<td>84</td>
<td>6x</td>
<td>42.0</td>
<td>1.4</td>
<td>2.4</td>
</tr>
<tr>
<td>N. rubra Roxb. Cytotype II</td>
<td>112</td>
<td>8x</td>
<td>42.2</td>
<td>2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>N. stellata Willd. var. cyanea</td>
<td>28</td>
<td>2x</td>
<td>13.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(Roxb.) Hooker f. and T.</td>
<td></td>
<td></td>
<td>85.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. stellata Willd. var. versicolor</td>
<td>56</td>
<td>4x</td>
<td>24.4</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>(Roxb.) Hooker f. and T.</td>
<td></td>
<td></td>
<td>75.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. stellata Willd. var. parviflora</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hooker f. and T.</td>
<td>84</td>
<td>6x</td>
<td>42.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>N. sturtevantii Hort.</td>
<td>56</td>
<td>4x</td>
<td>28.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>N. caerulea Savigny.</td>
<td>28</td>
<td>2x</td>
<td>11.5</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>N. pygmaea Ait.</td>
<td>84</td>
<td>6x</td>
<td>26.7</td>
<td>1.7</td>
<td>2.3</td>
</tr>
<tr>
<td>N. mexicana Tunc.</td>
<td>84</td>
<td>6x</td>
<td>34.6</td>
<td>1.6</td>
<td>1.0</td>
</tr>
<tr>
<td>N. daubeniana Hort. ex. O. Th.</td>
<td>42</td>
<td>3x</td>
<td>13.3</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>N. bissetii Hort.</td>
<td>84</td>
<td>6x</td>
<td>21.3</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>N. dentatamagnifica Bisset.</td>
<td>84</td>
<td>6x</td>
<td>16.2</td>
<td>2.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>
proportions at metaphase I (Table 1). The average number of univalents per PMC was greatest in *N. dentatamagnifica* (39.8%) followed by *N. bissetii* and *N. daubeniana* (Fig. 1c). The fate of the univalents was uncertain. They either moved to the poles or wandered aimlessly in the cytoplasm and constituted laggards. These laggards ultimately led to the formation of micronuclei. In *N. daubeniana* and *N. bissetii*, some dividing univalents occurred during anaphase I. This precocious division of univalents were found to affect the normal segregation of chromosomes (Fig. 1d). Multipolar segregation of chromatids at second meiotic division was observed in *N. caerulea* (Fig. 1f).

In *N. dentatamagnifica* and *N. mexicana* restitution nuclei have been observed. The cause of formation of such nuclei was more likely to be the failure of spindle mechanism (Fig. 1e). Thus, disjunction was affected so that the chromosomes were unable to move to the poles and all of them were ultimately included within a single membrane. In case of *N. rubra* (8x) failure of cytokinesis led to polydiploid nuclei in some pollen grains (Figs. 1g and h).

**Pollen fertility and seed setting**

The percentage of pollen abortion was found to be high in various members of *Nymphaea* (Table 1). Varying number of univalents at metaphase I and their uncertain behaviour at subsequent stages caused unequal number of chromosomes in the telophase nuclei that finally led to pollen sterility (Gupta 1979).

In *N. mexicana* pollen sterility was high despite high bivalent frequency (Table 1). The meiotic anomaly was due to the failure in organization of spindle at second division, which prevented the formation of normal tetrads. Thus, the sterility in this taxon appeared to be due to some genic barrier.

*N. lotus*, *N. rubra* (6x), *N. stellata* var. *cyanea*, *N. stellata* var. *versicolor* and *N. stellata* var. *parviflora* showed normal seed setting, whereas the other investigated members were sterile. In the sterile members seedlessness could be explained by the presence of high percentage of pollen sterility. Yet in *N. sturtevantii*, despite full pollen fertility no seed was ever encountered. Evidently, this seedlessness might be the effect of foreign environment or more likely due to some hitherto undetectable genic causes.

**Discussion**

Polyploidy has been important for the origin of new nymphaeas. Out of nearly 40 species belonging to this genus, 22 worked out cytologically so far reveal more than 85% polyploids. The polyploid numbers ranged from 2x to 16x levels based on x=14. Besides these euploid forms, Lohammar (1942) in *N. candida*, Löve and Löve (1942) and Ehrenberg (1945) in *N. alba* and Wood (1959) in *N. tetragona* reported aneuploidy. During the present investigations, the unreduced or aberrant gametes have been observed frequently in various Indian populations of *Nymphaea*, so it can be assumed that at least some of the polyploid taxa have originated from such gametes during sexual reproduction. Apart from polyploidy originating sexually, the eusomaty and aneusomaty may also con-
tribute to polyploid cells, which on further development have the potentiality to grow into polyploid shoots and then to the polyploid plants by asexual mode of reproduction (cf. Sharma 1956).

The mitotic and meiotic studies show that structural differentiation of homologues have played an important role in the evolution of this genus. It is interesting to speculate as to how this heterozygosity has arisen in the various species of *Nymphaea*. Among majority of the species showing marked heterozygosity, *N. daubeniana* is a well known hybrid of *N. caerulea* and *N. micrantha*. Similarly, *N. dentatamagnifica* and *N. bissetti* are the crosses of *N. omarana* × *N. dentata* and *N. lotus* × *N. rubra*, respectively. In these cases, therefore, heterozygosity may have arisen due to crossing of different species. In *N. sturtevanti*, however, it appears only due to structural differentiation of the homologues as this species is derived directly from the seedlings of *N. devoniensis* (Conard 1905). Nothing definite is known regarding the interrelationships of the other species of *Nymphaea*. Under such circumstances, the heterozygosity in these cases may be the consequence of hybridization or simply due to structural differentiation of homologous chromosomes in wild populations. As most nymphaeas reproduce vegetatively, the structural changes of chromosomes have better chances of survival.

One of the most significant features of evolution in the genus *Nymphaea* is the weak development of isolating mechanisms. Most of its basal species hybridize freely among themselves naturally or by artificial means and thus swelling the list of nymphaeas as pointed out by Bhaduri and Desai (1962).

The role of gene mutations in the evolution of *Nymphaea* has earlier been studied. The findings on *N. rubra* clearly reveal the occurrence of gene mutations (Gupta 1977).

The present findings along with other published chromosomal data by earlier workers (Guignard 1898, Langlet and Soderberg 1927, Langlet 1936, Lohammar 1942, Løve and Løve 1942, Ehrenberg 1945, Harada 1952, Heslop-Harrison 1955, Janki Ammal 1956, Raghavan and Arora 1958, Wood 1959, Mitra and Datta 1967, Sen and Bhaduri 1971 and Gupta 1972, 1976, 1978a 1978b and 1979) are taken into consideration for the evolutionary relationships in *Nymphaea*. From Figure 2, which summarizes the chromosome data of different species of this genus, it appears that all the species together constitute a single phylogenetic complex. In the early evolution of *Nymphaea*, different taxa of the same or closely related family were probably involved naturally in giving rise to the diploid species. Its members represent various euploid forms, ranging from 2x to 16x, followed by aneuploidy in a few cases like *N. alba*, *N. tetragona* and *N. candida*. Among the euploid members the highest frequency is of hexaploids, which have possibly been evolved either by hybridization between diploid and tetraploid species followed by chromosome doubling or by intercrosses between tetraploid and octoploid species. Due to their different modes of origin, some hexaploid species are fertile with normal seed setting while others are highly sterile (Table 1). The origin of the tetraploid species might have taken place either directly from the diploids or through hybridization between diploid and hexaploid species.
Triploid *N. daubeniana* is a well known hybrid of diploid *N. caerulea* and tetraploid *N. micrantha*. The other members with 8x and 16x levels can be considered to have resulted due to numerically aberrant mitosis and meiosis in the lower polyploids. In diploid, tetraploid, hexaploid and octoploid categories of *Nymphaea*, structural changes of chromosomes and gene mutations also appear to have played important roles in speciation.
Summary

Cytogenetical investigations were carried out on the Indian populations, both wild and cultivated of the genus *Nymphaea*. The chromosome counts revealed a polyploid series ranging from diploid to octoploid levels based on $x = 14$. In majority of the members, various abnormalities in the mitotic and meiotic systems have been noticed. Pollen sterility and seedlessness were also found to be fairly high. The present findings along with earlier published work have been taken into consideration to trace the evolutionary trends and relationships in *Nymphaea*. Polyploidy, weak development of isolating mechanism, structural changes of chromosomes and gene mutations appear to have involved for the evolution in this genus.

References