A cytological study of the genus Nepenthes L. (Nepenthaceae)

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Abstract:
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Nepenthaceae are remarkably uniform in chromosome number with 2n = 80. There is good evidence that x = 5 (x = 10) is the basic chromosome number for the family. Chromosome size, condensing behaviour of chromatin and interphase nuclei structure are very similar to what is found in Droseraceae.

Zusammenfassung:
Die Nepenthaceae weisen mit 2n = 80 eine bemerkenswert einheitliche Chromosomenzahl auf. Als Basiszahl wird x = 5 (x = 10) für die Familie angenommen. Hinsichtlich der Chromosomengröße, der Chromatin-Kondensation und der Struktur im Interphasekern bestehen auffallende Übereinstimmungen mit den Droseraceae.

Phylogenetic relationships among carnivorous plant families are of special interest to systematic botanists. Till today the taxonomic position of Nepenthaceae has been difficult to determine, primarily because the characters of the taxa are predominantly highly derived. Whereas the species are so closely related to each other, that the separation is often difficult, the Nepenthaceae stand quite isolated among all other dicotyledonous families. Within the framework of current research on Nepenthaceae cytology has attracted our attention. The majority of studies in this family has been based on macroscopic observations and on anatomical-morphological features. In order to facilitate further progress, we consider it worthwhile to publish some observations and illustrations of chromosome numbers and karyotypes.

Nepenthaceae are a monogeneric family of approximately seventy species scattered throughout the Old World tropics. Most species are concentrated in South East Asia, especially on the islands of Borneo and Sumatra. From this main center of diversity the geographical distribution extends as far west as Sri Lanka, the Seychelles and Madagascar and eastwards to Northern Australia, New Guinea and New Caledonia.

The basic classification of the familiy was established by DANSER (1928) and modified by HARMS (1936), who devided the genus *Nepenthes* into 7 sections. Some authorities, however, relegate *Nepenthes pervillei* from the Seychelles to a separate genus, *Anourosperma*. In contrast to other carnivorous families (Sarraceniaceae, Droseraceae) only little work has been done on the cytology of Nepenthaceae. Except the study of LOWREY & TIMOTHY (1991) on some Malaysian and Singaporean *Nepenthes*, no reports or illustrations of chromosome numbers and karyotype structure are available. In view of the fragmentary cytological records, the chromosome numbers of 15 species of *Nepenthes* are reported and the cytological affinities to Droseraceae are discussed.

Materials and methods

All counts were made from samples taken from greenhouse-grown plants. The material used in this study came from the following sources: *N. madagascariensis, N. pervillei, N. truncata, N. tentaculata, N. clipeata, N. rafflesiana, N. veitchii* cultivated by P. Debbert (Munich), *N.distillatoria, N. albomarginata, N. gracilis, N. reinwardtiana, N. thorelii, N. eymei, N. stenophylla* (Botanical Garden Munich-Nymphenburg) and *N. khasiana* by A. Wistuba (Mannheim).

For cytological examination of somatic chromosomes, actively growing roots were pretreated with a solution of 0,002 M hydroxyquinoline for 4 hours at 4 °C, fixed in 1:3 acetic alcohol and then stored in 70% ethanol in a refrigerator. The fixed material was rehydrated for 5 minutes in distilled water, hydrolysed for 5 minutes in 1 N HCl at 60 °C and then treated with a 5% aqueous solution of pectinase/cellulase (1:1) for 10–15 minutes at room temperature prior to staining. The minute meristematic root tips were teased out onto a slide to obtain a suspension of cells and squashed in 2% orcein or stained in Feulgen for up to 2 hours. Counts were made under oil immersion (2000 ×) brightfield or phase-contrast optics on a Olympus BX 40 microscope.

Results and discussion

The chromosome number established for 15 species, selected taxa, which are of importance because of their taxonomic or geographical isolation, indicate that *Nepenthes* is remarkable constant cytologically. All the species investigated in this study exhibit a somatic chromosome number of 2n = 80. This conflicts with a previous report of meiotic number of n = 39 (2n = 78) (KONDO 1969) for *N. rafflesiana* and *N. thorelii*, which is probably erroneous. Our results are concordant with the data reported by LOWREY & TIMOTHY (1991). In their cytological study on seven Malaysian and Singaporean *Nepenthes* taxa they consistantly found a meiotic chromosome number of n = 40. On the basis of the data now safely established for many species with 2n = 80 (n = 40), the basic number of the genus appears to be x = 5 (x = 10?).

Species	2n	Origin
Nepenthes albomarginata	80	Sumatra
Nepenthes clipeata	80	Borneo
Nepenthes distillatoria	80	Sri Lanka
Nepenthes eymai	80	Sulawesi
Nepenthes gracilis	80	Malaysia
Nepenthes khasiana	80	India, Assam
Nepenthes madagascariensis	80	Madagascar
Nepenthes pervillei	80	Seychelles
Nepenthes rafflesiana	80	Borneo
Nepenthes reinwardtiana	80	Borneo
Nepenthes stenophylla	80	Borneo
Nepenthes tentaculata	80	Borneo
Nepenthes thorelii	80	Indochina
Nepenthes truncata	80	Phillippines
Nepenthes veitchii	80	Borneo

Table 1. Chromosome numbers of selected species of Nepenthaceae.

The chromosomes of all species investigated are very small and similar to each other in shape. The metaphasic karyotypes (fig. 1) show metacentric to submetacentric chromosomes of continuous decreasing length from 2 mm to 0.5 µm. Because of the small size of all chromosomes it is not possible to find any groupings within the karyotype. The interphase nuclei of all species show often reticulate, very pale staining euchromatin. The chromocentres stain darkly. They are small, but of variable size, mostly roundish and sometimes elongated. The centromeric region is only visible at mid-metaphase.

This cytological uniformity explains the lack of barriers to interspecific hybridisation, a common phenomenon in *Nepenthes*. Many species have been used for crossing experiments and the obvious genetic compatibility has been noted in the family for a long time.

In respect of a prevailing chromosome number of 2n = 80 and a basic number of x = 5 (x = 10?) in all species investigated, a high ploidy level (16x or 8x) seems to have been established throughout the genus. Apparently, diploid and taxa on lower ploidy levels have gone extinct. It is remarkable that isolated species (e.g. *N. madagascariensis, N. pervillei, N. distillatoria*) which have been suggested to be rather primitive within the genus, show the same ploidy level than derived taxa (e.g. *N. veitchii, N. stenophylla*). Furthermore the cytological data do not support the segregation of *N. pervillei* from the genus *Nepenthes* as proposed by HOOKER (1873) on the basis of morphological differences (seeds without appendages, tepals of the female flowers united).

From a study of isozyms LOWREY & TIMOTHY (1991) concluded that all enzymes exhibited isozyme numbers within the range of typical diploid seed plants. In respect of the lack of duplicated loci the authors assume that the Nepenthaceae are not of polyploid origin despite the high chromosome number.

A cytological comparison Nepenthaceae-Droseraceae

Recent molecular studies and cladistic analysis of rbcL sequence data (ALBERT et al. 1992; CHASE et al. 1993) indicate that Nepenthaceae may be surprisingly closely allied to Droseraceae and also to Caryophyllales. These affinities of Nepenthaceae and Droseraceae are strongly supported by the cytological data. Both families share common karyomorphological features. All chromosomes are generally very small in size and only in Droseraceae a classification in small, medium and large chromosomes (or karyotypes) is possible. The condensing behaviour, the chromatin and interphase nuclei structure is also very similar. In contrast to Droseraceae which probably lack a primary constriction (KONDO & LAVARACK 1984) centromeres have been observed in the larger chromosomes of Nepenthaceae .

In Droseraceae the most frequent basic number appears to be x = 5 (x = 10?), being found in many taxa, especially in *Drosera* subg. *Drosera*. In other sections of *Drosera* various chromosome numbers have been reported (KONDO 1966; 1969; 1970; 1971a, b; 1973; 1976) with primary basic numbers x = 6, 7, 8, 9 and perhaps also secondary basic numbers x = 11 and x = 13. Aneuploidy seems very common especially in some Australian groups of *Drosera*. For the related genera *Dionaea* and *Aldrovanda* x = 8 has been established and for the genus *Drosophyllum* the basic number x = 6 is accepted. It is remarkable that Droseraceae as well as Nepenthaceae have reached the same ploidy level (8x or 16x). Available data suggest that x = 5 or x = 10 may be ancestral for both families.

All available data show that Droseraceae exhibit an enormous spectrum of chromosome numbers. Hybridisation, parallel polyploidisation on different base numbers and numerical changes were probably involved in the evolutionary process. It is evident that in Droseraceae all ploidy levels are represented, whereas in *Nepenthes* diploids and lower polyploids have already gone extinct and the radiation was restricted to a high ploidy level. In view of the phylogenetic background one can assume that Nepenthaceae as palaeopolyploids with a

basisc number x = 20. The loss of taxa with lower ploidy levels, the high chromosome number, the isolated taxonomic position, the palaeotropic distribution, the uniformity in many characters and the reduced genetic variability support this assumption. On the other hand fossil record, the specialized morphology and recent molecular data are not in favour of this hypothesis.

In view of the molecular data (rbcL), palynological (tetrads), chemical (naphthoquinons) and morphological characters (fruit, embryo, ovules, onotogeny of digestive glands) and also from the cytological point of view a systematic position of the Nepenthaceae not to far away from the Droseraceae is proposed.

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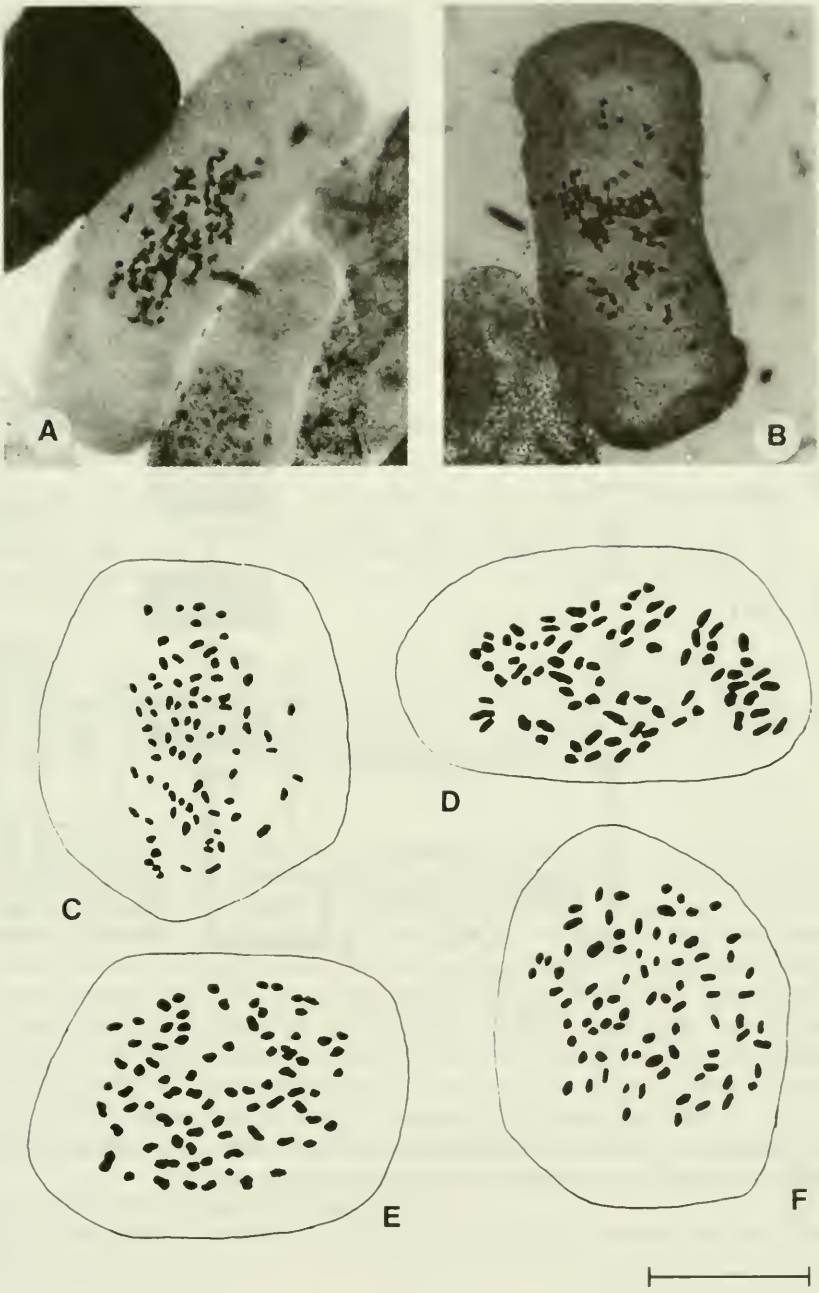

Fig. 1 Somatic, mid-metaphase chromosomes of 5 species of *Nepenthes*.
A: *N. madagascariensis*. B: *N. pervillei*. C: *N. clipeata*. D: *N. rafflesiana*. E: *N. distillatoria*.
Scale bar: 10 µm.

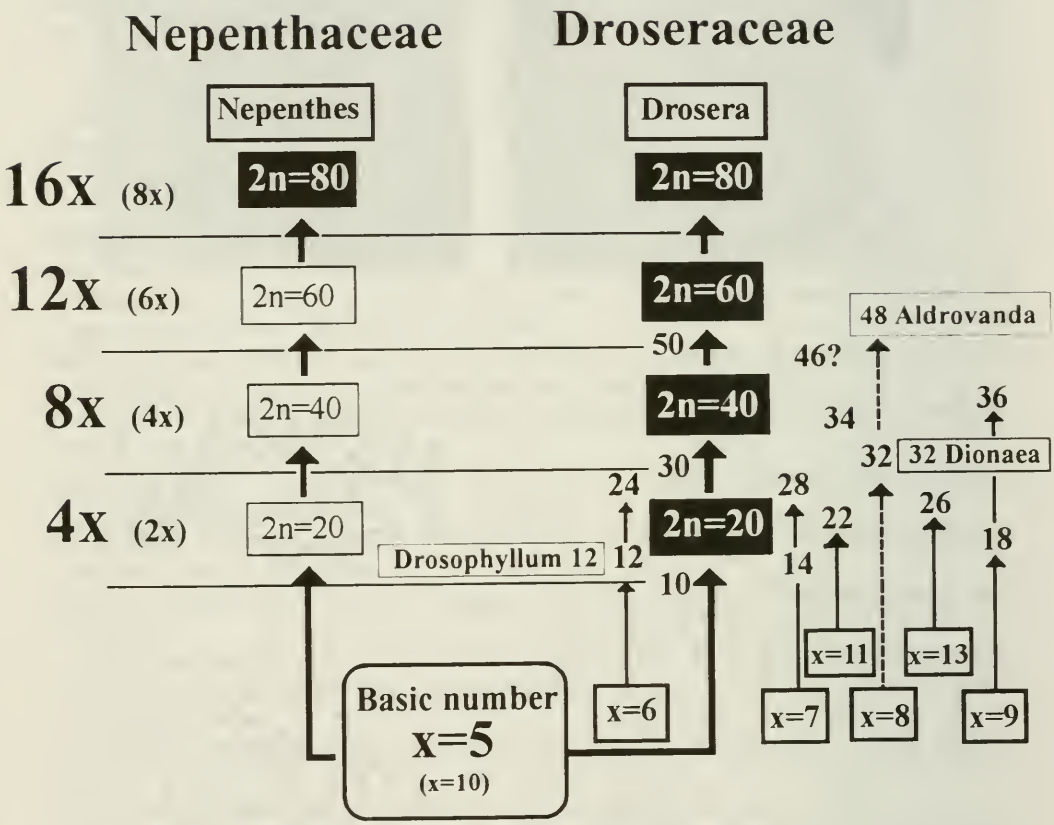

Fig. 2 Summary of chromosome numbers, ploidy levels and basic numbers ascertained for Nepenthaceae and Droseraceae. For further explanations see text.