Sub-haploid pollen in *Paphiopedilum insigne* (Orchidaceae)

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Abstract

Sub-haploid pollen grains with chromosome numbers ranging from n = 5–12 were seen in the cultivar 'Maulei' of *Paphiopedilum insigne* Pfitz. Many grains showed n = 5 and 6 (38%) while 3% showed a hyperploid number (n = 18, 20). It was also observed that pollen grains with the same chromosome number differed in karyotype.

The high percentage (81%) of sub-haploid pollen grains in the present case may be due to the high degree of segregational irregularities during meiosis or to split spindle or failure of wall formation. It may also be due to changes in the environment of the species which may influence the metabolic activity responsible for the initiation and subsequent development of the cell wall and the process of chromosome duplication.

Introduction

Pollen mitosis offers several advantages in the study of plant chromosomes (Khoshoo, 1966), especially in orchids where the use of root tips for cytological analysis is difficult. The roots are bulky and possess a velamen which interferes with the penetration of the pre-treating chemicals, often resulting in poor preparations and errors in determinations of chromosome number (Withner, 1974). As indicated by Singh (1980), pollen mitosis studies in orchids are very convenient as a large number of flowers are usually available at one time in vegetatively propagated clones. Furthermore the post-meiotic metaphases of pollen mitosis are very well synchronized and provide an excellent opportunity for haploid chromosome counting.

While studying pollen mitosis in different *Paphiopedilum* species the author came across cases of sub-haploid pollen in *Paphiopedilum insigne* 'Maulei' details of which are presented below.

Materials and methods

Different cultivars of *Paphiopedilum insigne* (Wall) Pfitz introduced from N.E. Himalayas and grown in the orchidarium of the Indian Institute of Horticultural Research, Hessaraghatta, Bangalore were used for the study.

Flowers were harvested 4–5 days before opening. The pollen grains were cultured in petri dishes containing a medium made up of 5 g sucrose, 1 g agar and 100 ml of distilled water, and the cultures were kept at a temperature of 25 ± 2 °C.

After 24 h a small portion of the cultured pollen was taken out and tested in a drop of 1% aceto orcein; if it showed the right stages of mitosis the remaining portion was immediately fixed in 1:1:2 95% ethanol:chloroform: acetic acid and squashed in 1% aceto orcein, to which a drop of Hoyer's medium was added (Beeks, 1955).

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Observations

Figure 1 shows chromosome numbers ranging from n = 5–13 in different pollen grains. In addition to these there were two cases where n = 18 and 20 were encountered. Considering n = 13 as the basic number, sub-haploid numbers were found in 81% of the pollen grains with 16% showing the normal number (13 chromosomes) and 3% showing higher than the basic number (18 and 20).

The normal pollen grain has n = 13 with only one satellite chromosome (Fig. 2, arrow), and this type was not found in sub-haploid grains (Figs. 3–7). A new satellited chromosome was however found in some of the other pollen grains (Fig. 5, arrow). This was totally different from the one seen in the normal complement. Furthermore, in sub-haploid grains there were relatively more medium and small chromosomes (Figs. 3–7), while in grains with 2n = 18 and 20 there was a tendency for small chromosomes to be represented more than once, indicating non-disjunction of smaller chromosomes during meiosis.

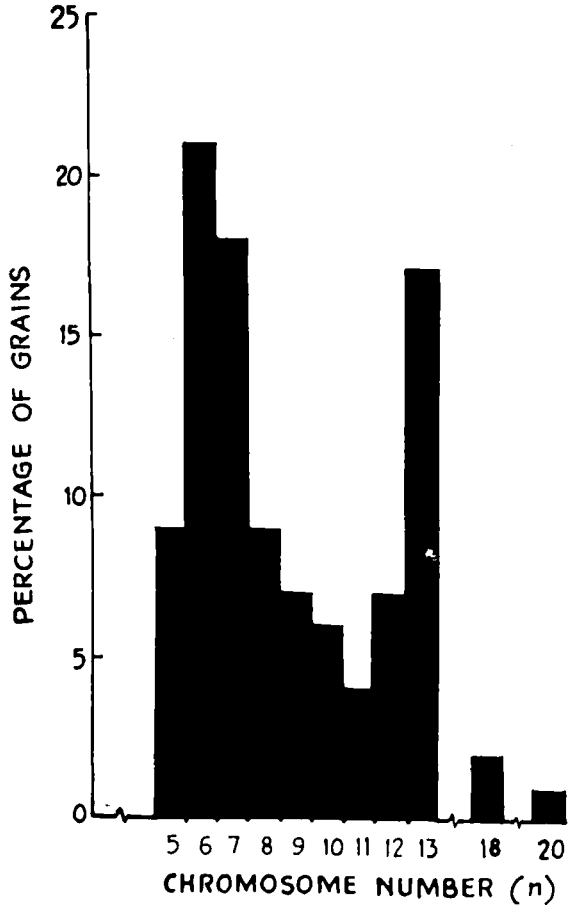

Fig. 1. Histogram showing the percentage of pollen grains with different chromosome numbers in *Paphiopedilum insigne* (2n = 26).

Analysis of different pollen grains with the same chromosome number also showed differences in the constitution of their karyotype.

Twenty seven grains were studied at anaphase of the first pollen mitosis. In 21 of these there was normal segregation, while 6 showed unequal disjunction, the pollen fertility in fully opened flowers ranged from 87 to 93%.

It was also observed that in spite of their chromosome deficiency haploid pollen grains have a perfect wall and conform in size and shape to the standard pollen grain. The rate of the development of pollen grains with different chromosome numbers was almost the same and was highly synchronous. All the chromosomes stained well, indicating a normal condensation cycle.

Discussion

Paphiopedilum insigne (2n = 26) is a highly variable complex with more than 90 described cultivars (Adams, 1954), most of which have been selected as horticulturally meritorious clones. *Paphiopedilum* represents the most primitive of the orchidaceous taxa on account of its flower structure, the formation of individual pollen grains and its cytologically symmetrical karyotype coupled with large chromosomes. The pollen is of the binucleate type (Brewbaker, 1967), the mature grains being held together by a viscous fluid.

Paphiopedilum insigne cultivars have been evaluated cytologically by Mehlquist (1947), Sasa and Torigata (1967), Tanaka and Aoyama (1974), Karasawa (1978), and several different chromosome numbers have been reported (Table 1).

Possibly some form of non-disjunction accounts for the chromosome number variation in the species. Belling (1924) described chromosome number detachment of elimination in pollen grain division in *Cypripedium acaule*. When non-disjunction occurs in the division either a hyperploid or a hypoploid generative cell results.

The difference in the constitution of karyotypes

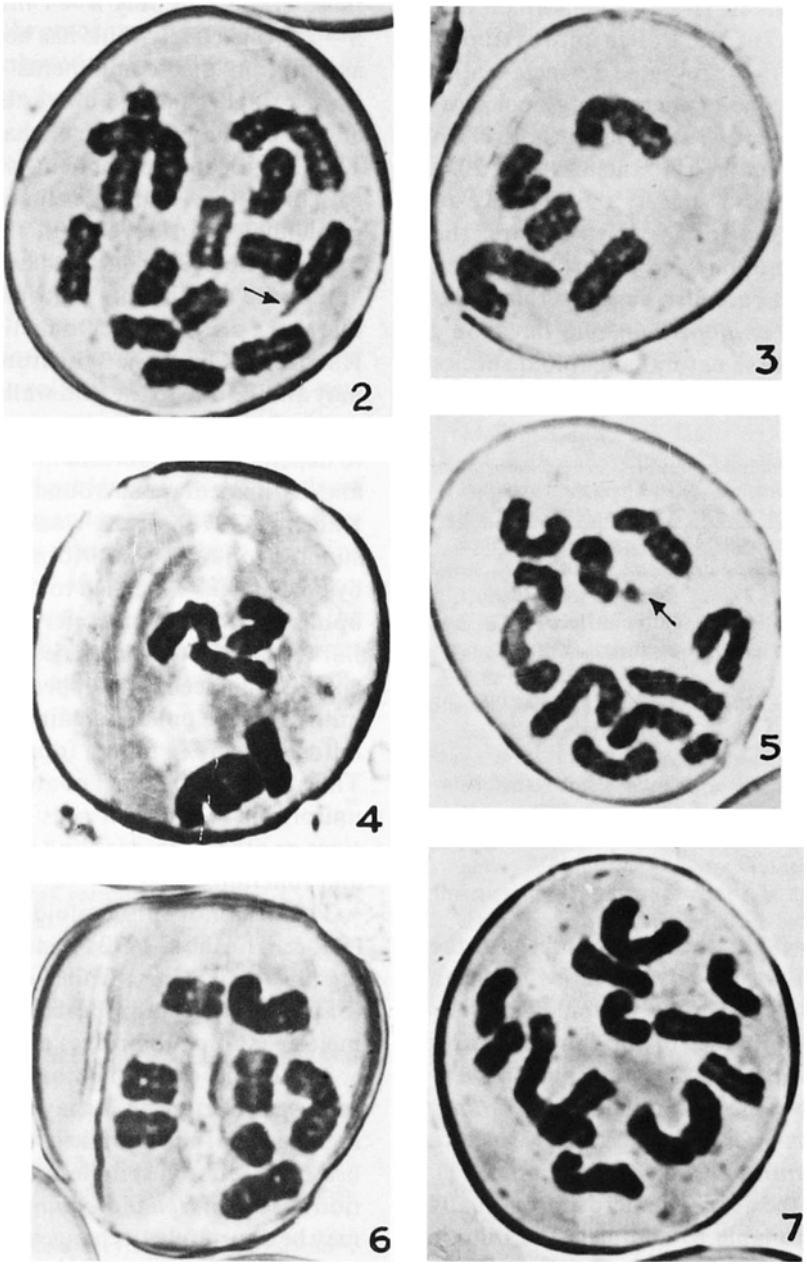

Fig. 2-7. Pollen grain mitosis (metaphase) in *Paphiopedilum insigne*: (2) n = 13 (Normal, note satellite); - (3) n = 5; - (4) n = 6; - (5) n = 11 (note satellite); - (6) n = 8; - (7) n = 12. × 1200.

in hypoploid pollen having the same chromosome number may be due to differences in the origin of these grains, or may be due to the recombination of structurally different chromosomes as reported earlier by Raina and Khoshoo (1971) in *Zephyranthes puertoricensis* and in *Allium chinense* by Gohil and Koul (1981). This can also be due to randomised chromosome elimination due to lagging.

Recent studies by Vij and Mehra (1974) and Karasawa (1978) have also reported 2n = 26 in *P.*

insigne except in cv. 'Harefield Hall', which is a natural triploid with 2n = 39. Pollen mitosis studies by Vij and Mehra (1974) revealed 13 metacentric chromosomes, only one of them being nucleolar. The earlier reports of n = 8, 9 (Sussenguth, 1921), n = 12 (Afzelius, 1916), n = 16 (Francini, 1931), 2n = 28 (Eftimiu-Heim, 1941) suggest the presence of aneuploid races in the species; furthermore, the occurrence of viable hypo- and hyperploid pollen grains in the present case also suggests that polyploidy in cultivated *Paphiopedilum* may have come through the use of these natural aneuploid species (Vij & Mehra, 1974).

Table 1. Chromosome number reports in *Paphiopedilum insigne*.

Cultivar	n	2n	References
P. insigne		26	Mehlquist, 1947
		28	Eftimiu-Heim, 1941
	16		Francini, 1931
	12		Duncan, 1959
		26	Sasa & Torigata, 1967
	8-9		Sussenguth, 1921
	13		Vij & Mehra, 1974
		26	Tanaka & Aoyama, 1974
'Harefield Hall'		39	Karasawa, 1978
'Sanderae'		26, 28	Tanaka & Aoyama, 1974
'Sanderianum'		26	Karasawa, 1978

Considering n = 13 as the basic number for the genus *Paphiopedilum* as advocated by Duncan (1959), the species *P. insigne* is diploid. The presence of such a high number of sub-haploid grains (81%) to enter mitosis is interesting but not very rare, since such cases have been reported in *Allium* (Ved Brat, 1967), *Amaranthus* (Pal & Khoshoo, 1972) and *Zephyranthes* (Raina & Khoshoo, 1971).

In higher plants the deficient microspores usually abort as they are unable to complete the mitotic division and the development necessary to form gametophytes. Even pollen grains with just one chromosome less then the haploid complement fail to develop (Sapre, 1975). In the present case, however, the deficient pollen grains are normal in size and grains with as low as n = 5, 6, 7, 8 proceed normally up to metaphase (Figs. 3, 4, 5). Similar cases where deficient pollen grains have developed normal walls and entered mitosis were reported earlier in *Amaranthus* (Pal & Khoshoo, 1972) and *Zephyranthes* (Raina & Khoshoo, 1971). Some of these grains had only n = 1 instead of n = 17 and n = 25 respectively. This has been explained on the assumption that components of one mother cell form a single balanced unit as they are held together, thereby having a free exchange of metabolites. The loss in one component is compensated by a gain in another, and under such circumstances deficient nuclei can develop at the same rate as the others. The synchronous development within an anther sac may be due to feeble wall formation or due to the presence of cytomictic channels (Heslop-Harrison, 1966). The synchrony of pollen mitosis may also be due to the thin walls of the cells in early stages. Later on, however, the development seems to depend on the coordination between the nucleus and its immediate surrounding cytoplasm (Pal & Khoshoo, 1972). These workers demonstrated the suppression of the wall formation in *Amaranthus* by cold shocks which led to the synchronous development of macro- and micro-pollen. According to Barber (1941) the incidence of aneuploidy in orchids is enhanced by their peculiar method of pollen mitosis where pollen remains in tetrads and pollen mitosis occurs in all the four cells simultaneously. Thus the physiological control over the process is uniform in all the four cells whether they are deficient or otherwise, enabling all four cells to survive and function.

The origin of sub-haploid grain in *Amaranthus* (Pal & Khoshoo, 1972) was reported to be due to lagging univalents while in *Aloe barbadensis* (Sapre, 1976) it was reported to be due to irregular meiosis rsulting in bridges and laggards.

The origin of sub-haploid grains in the present case is not very clear. It may be due to high levels of segregational and spindle irregularities during meiosis or due to split spindle and failure of wall formation as seen in *Helianthemum* by Snoad (1954), or it may be due purely to changes in the environment. It is a well known fact that the orchids are very selective and grow only under restricted environments. The shifting of *Paphiopedilum* from NE India to Bangalore may have influenced the metabolic activity responsible for the initiation and subsequent development of the cell wall and the process of chromosome duplication as has been reported in *Amaranthus* by Pal and Khoshoo (1972). The appearance of a new satellite chromosome type (Fig. 5, arrow) is very interesting and may indicate the mechanism of amphiplasty in the species, i.e.

the suppression of one form of satellite by other, or there may be new chromosome types which have arisen due to recombination of different types of chromosomes as has been seen earlier in *Allium* (Singh *et al.*, 1967).

The present study reveals the impact of the cytological polymorphism prevalent in cultivated orchid species like *P. insigne* and indicates the potentialities of creating an aneuploid pattern and new forms, assuming that the aneuploid pollen is viable enough to produce living progeny.

The role of variation in chromosome number and structure in somatic cells in the evolution of new forms, especially in vegetatively reproducing taxa, has been highlighted by Sharma (1973). It may be mentioned here that *Paphiopedilum*, although essentially a seed-setting genus, reproduces extensively through vegetative means. Further, since the changes in the number of chromosomes between different species are not prevalent it is only the changes within the complement which are responsible for directing the course of evolution in the genus.

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