

# CYTOLOGY OF POLLEN WITH PARTICULAR REFERENCE TO *IMPATIENS* AND *ALLIEAE*\*

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ON the basis of pollen cytology, the genera in angiosperms are classified according to the number of nuclei (or cells) present in the grain at the time of shedding. Thus there are two and/or three-nucleate genera. The essential difference between the two revolves round the time of the division of the generative nucleus, which occurs in the grain itself in the latter, while in the former it is delayed and occurs in the pollen tube. About 66% of the angiosperm families are with binucleate pollen grains. The remaining are either exclusively trinucleate or may contain both bi- and trinucleate genera. Trinucleate condition is believed to have evolved from the binucleate condition.

Pollen cytology does not end at finding the number of nuclei in the grain. The mitoses that occur inside the grain have been studied in a large number of angiosperms and also in some gymnosperms, and valuable cytological information has been gathered. There are indeed some distinct advantages of making cytological studies based on pollen mitoses. These are (i) availability of large number of mitoses at one time, (ii) chromosome number is half of what is found in root-tip and as such classification of chromosome types is easier. It eliminates the difficulty of sorting out homologous chromosomes as in root-tip mitoses, which is particularly true of high chromosome number with almost identical morphology, (iii) any variation in chromosome number, size and shape resulting after meiosis can be detected with ease immediately, (iv) investigations of the mechanism connected with the numerical increase of B chromosomes, and (v) exceedingly good information has been obtained on the effects of different forms of radiation and chemicals. Perhaps, the only limitation of basing conclusions exclusively on pollen mitoses is that, no idea can be obtained from it about the cytological mechanisms like structural hybridity, etc. For the understanding of such aspects, meiotic studies are very essential.

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\* Substance of the lecture delivered during the Palynology Symposium held in the Gardens.

In view of the advantages enumerated above, pollen grains are being increasingly used for cytological studies, particularly in the case of large samples of genetic material and for cytological studies of the progeny of hybrids between parents with different morphology and/or number of chromosomes. Some of the above points will be elucidated by a few examples which the writer has worked out incidental to his cytological work. The results obtained in 17 species of *Impatiens* and 3 species of *Allieae* are summarized in this paper.

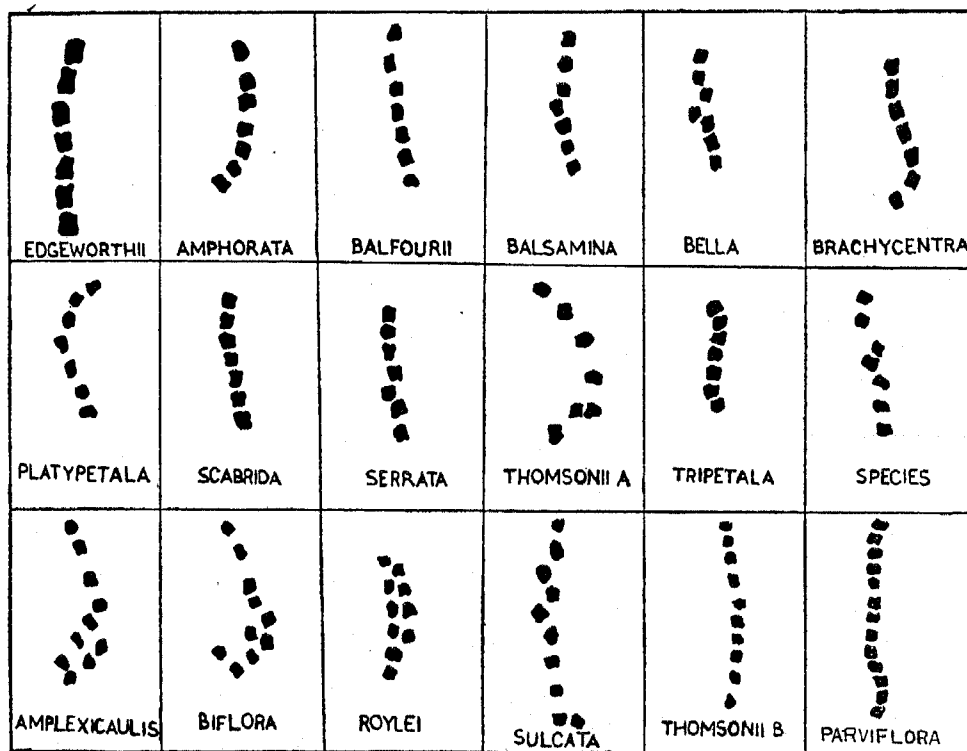
#### IMPATIENS

It is a very large genus containing about 550 species, out of which 241 grow in India alone. There is about 91% endemism. When making a cytological survey of such a large genus, the methods have to be simple and quick, and results have to be trustworthy, more so because it is not always possible to secure pollen mother-cell and root-tip materials for study as most of the species are localized in small ecogeographical pockets. However, the pollen of the genus possesses indeed some good characteristics, namely, the generative nucleus enters in mitosis before shedding of the grains. It remains so-to-say in "arrested" or "blocked" metaphase stage. At this stage the chromosomes can be counted with considerable ease and accuracy. Furthermore, there are a large number of grains available for study at any one time (Plate I, Fig. 1). The chromosomes arrange themselves in a linear row, often in line with the long axis of the grain (Plate I, Fig. 2). It was also possible to extend this study to the pollen of properly pressed herbarium specimens (Khoshoo, 1956). All these characteristics enabled the writer to sample widely each species and increase the chances of detection of intraspecific chromosomal diversity. The present results are tabulated below and drawn in Text-Fig. 1.

Haploid number	Name of species
6	<i>I. edgeworthii</i> Hook.
7	<i>amphorata</i> Edgew. <i>balfourii</i> Hook. f. <i>balsamina</i> Linn. <i>bella</i> Hook. f. and T. <i>brachycentra</i> Kar. and Kir. <i>platypetala</i> Lindl. <i>scabrida</i> DC. <i>serrata</i> Benth. <i>thomsoni</i> Hook. f. (Race A) <i>tripetala</i> Roxb. <i>species</i>

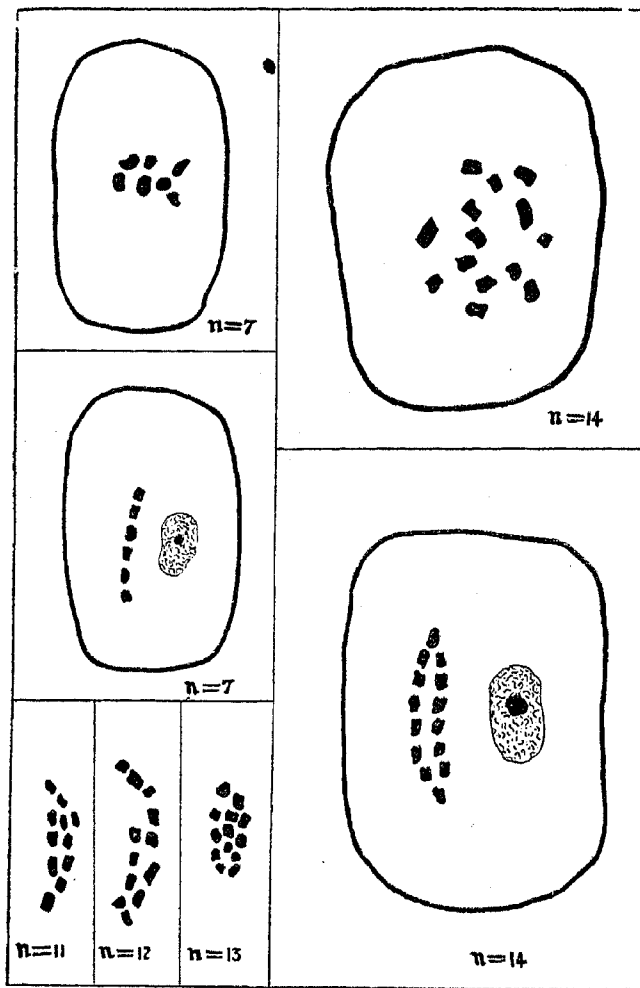
Haploid number	Name of species
10	<i>amplexicaulis</i> Edgew. <i>biflora</i> Walt. <i>roylei</i> Walp <i>sulcata</i> Wall. <i>thomsoni</i> Hook. f. (Race B)
13	<i>parviflora</i> DC.

In addition to the above 17 species, ten species have been studied by other authors (*cf.* Darlington and Wylie, 1955; Cave *et al.*, 1955-1962). All the data when taken together reveal the presence of a dysploid pattern in the genus,  $n = 6, 7, 9, 10$  and  $13$ . Furthermore, dysploidy is also found within the limits of three species. These are: *I. thomsoni* ( $n = 7$  and  $10$ ), *I. roylei* ( $n = 9$  and  $10$ ) and *I. parviflora* ( $n = 10, 12$  and  $13$ ).



TEXT-FIG. 1. Chromosomes from the generative nuclei of *Impatiens* species.

During the course of these studies, one plant of *I. balsamina* was found to produce normal, large and sterile pollen. The normal grains were found to be haploid ( $n = 7$ ) and the large grains were diploid or unreduced ( $n = 14$ ). Some grains were subdiploid in constitution, with numbers like  $n = 11$ , 12 and 13 (Text-Fig. 2).



TEXT-FIG. 2. Haploid, diploid and subdiploid pollen mitoses in an abnormal individual of *Impatiens balsamina*.

The evolutionary implications of the foregoing results appear to be very interesting, particularly when considered in the light of morphological and floral diversity, high endemism and the interesting distribution pattern of the genus (Khoshoo, 1957). While broad-based conclusions have to be drawn when this study is enlarged, however, at present one point seems obvious, that there is "abrupt" speciation in the genus which is associated with simultaneous origin of the genetic barriers. This probably also

explains not only the large size of the genus but also the high endemism. Most probably the species are youthful and have not had enough time to extend their ranges.

#### ALLIEAE

Pollen mitoses were studied in three interesting species. The results are discussed below:

##### *Nothoscordum fragrans* ( $n = 19$ )

Out of the 19 chromosomes, 13 are metacentric while 6 are telocentric. It is surmised that this species has arisen from an ancestor with 16 metacentric chromosomes, in which 3 chromosomes have fragmented across the centromere into 6 telocentrics. At meiosis the taxon forms rings and chains of 4-6 chromosomes involving both types of chromosomes. Anaphase is characterized by bridge-fragment configurations, lagging chromosomes, etc. The species is not only numerically different but is also a complex structural heterozygote. An examination of pollen mitoses (Text-Fig. 3) reveals that the most common numbers are 9 and 10 (41 and 43% respectively), followed by 8 and 11 (3 and 6% respectively) and 12 (7%). Furthermore, not only is there difference in the chromosome number in the grains (from  $n = 8$  to 12) but the composition of the complements also differs. An examination of the chromosomal polymorphism resulting after meiosis in this taxon (Text-Fig. 3) shows that some of the complementary numbers and karyotypes although theoretically expected, were not found, perhaps because they are subhaploid and therefore lethal. It is of significance to point out that it was possible to bring out the nature and extent of this polymorphism only after a study of pollen mitoses.

##### *Allium neapolitanum* ( $2n = 5x = 35$ )

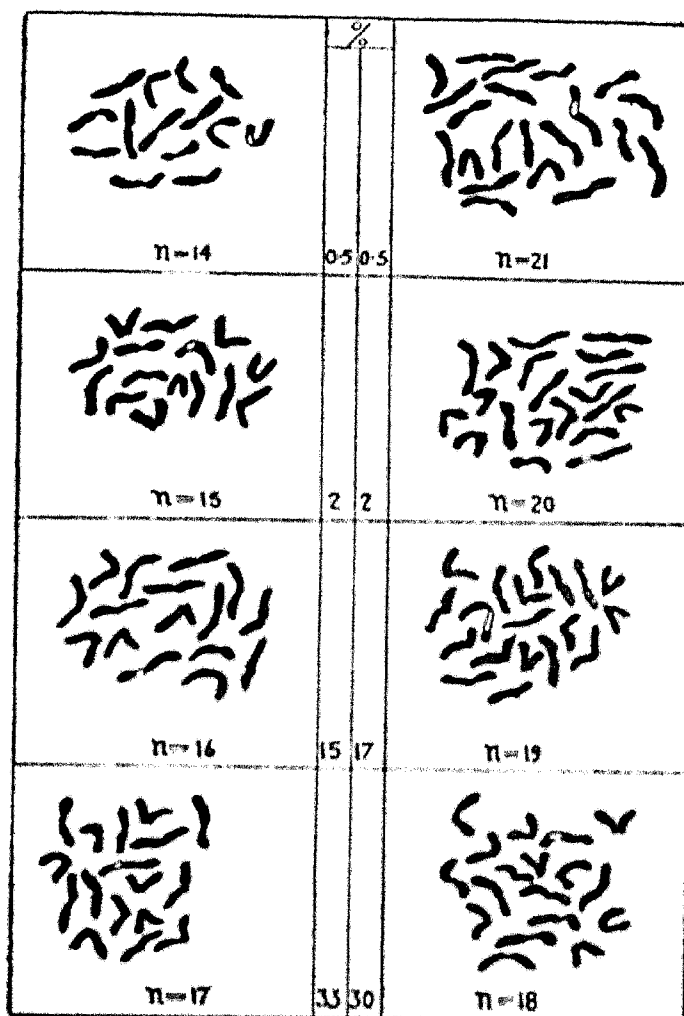
This taxon is a segmental allopolyploid based on  $x = 7$ . As expected, meiosis is characterized by multivalent formation, coupled with bridge-fragment configurations, misdivision, lagging chromosomes, etc., at anaphase. These characteristics result in unequal segregation and micronuclei, etc. The karyotype is characterized by the possession of a pair of chromosomes with an exaggerated centromere (*cf.* Khoshoo and Sharma, 1959 *b*; Text-Figs. 1-3).

Pollen mitosis was examined in a large number of grains in this species and the results are given in Text-Fig. 4. These results exclude several grains in which, in addition to the complement of chromosomes, there were small

Chromosome Number		MORPHOLOGY	%
DIPLOID	19	V V V V V V V V V	100
		V V V V V I I I I I I	
D	12	V V V V V V V V V I I	3
		V V V V V V V V V I I I	4
I	11	V V V V V V V V V I I	3
		V V V V V V V I I I I	3
L	10	V V V V V V V I I I	21
		V V V V V V I I I I	22
P	9	V V V V V V V I I	20
		V V V V V V I I I	21
A	8	V V V V V V I I	3
		V V V V I I I I	0
H	7	V V V V V I I	0
		V V V I I I I	0

TEXT-FIG. 3. Range of chromosomal polymorphism in pollen grains of *N. fragrans*.

chromatic fragments, etc. It is apparent that the chromosome number in the grains varies from 14 to 21, with the most common distribution as 17 and 18, followed by 16 and 19. This evidently confirms the earlier view of Khoshoo and Sharma (1959 *b*) that the taxon has genomic formula like  $AAA_1A_1B$  in which 28 chromosomes associate, in extreme cases, as 14 bivalents and the remaining 7 chromosomes as univalents (*see* Fig. 8, Khoshoo and Sharma, 1959 *b*). The complementary combination like 14–21 very probably arises from the normal segregation of 14 bivalents, with 7 univalents all passing to one pole. While this is the extreme, usual course is that there is regular and/or irregular disjunction of multivalents, but univalents segregate almost always irregularly. Thus various numbers are present at the poles. One fact is significant that each of the complements contains one chromosome with the exaggerated centromere,



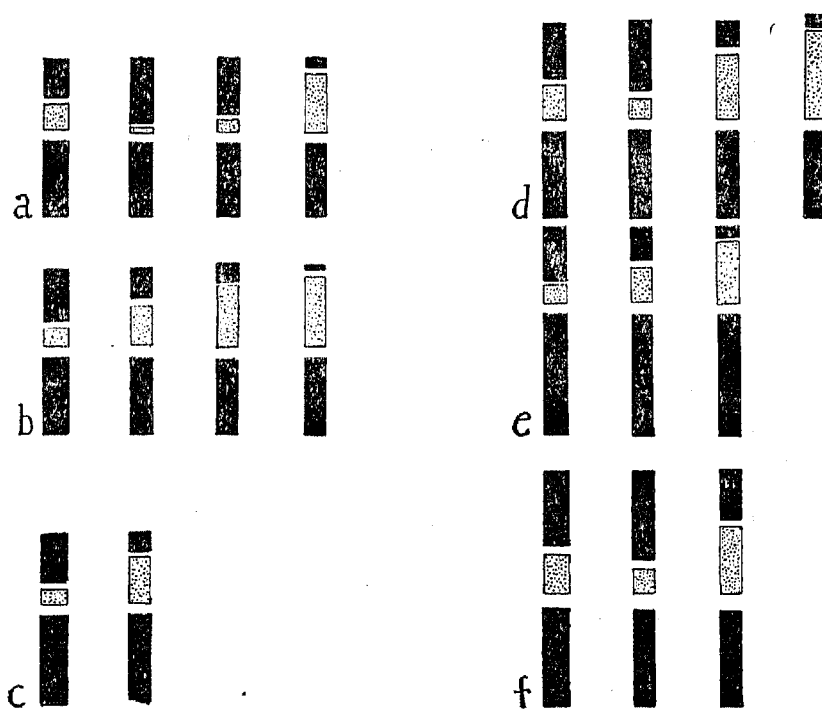
TEXT-FIG. 4. Range of chromosome number in pollen grains of *A. neapolitanum*.

*Allium rubellum* ( $2n = 3x = 24$ )

This species is an autotriploid (Khoshoo and Sharma, 1959 *a*) based on  $x = 8$ . The karyotype contains 6 secondarily constricted chromosomes which do not fall in three pairs, indicating that the autotriploid is possibly intervarietal in origin. The six chromosomes are distinguishable from one another on the basis of the total length, arm ratio and the position of the secondary constriction. The last affects the size of the middle segment of the chromosome.

Like *A. neapolitanum*, there is considerable diversity in chromosome number in the pollen grains (*see* Khoshoo and Sharma, 1959 *a*). However, a point of greater interest is the change in morphology of the marker chromosomes. At meiosis there is intergenomal pairing resulting in multi-

valent formation followed by other irregularities. It was interesting to examine in detail the six secondarily constricted chromosomes in 50 pollen mitoses. The results have been depicted in Text-Fig. 5. The first chromosome in each set indicates the morphology of the chromosome in the root-tip. The other figures are from pollen grains. It is clear that while the position of the centromere is nearly constant, the position of the secondary constriction changes in some grains. This can be appreciated better by comparing the length of the middle segment of the new chromosomes with their respective parental types. This shift in the secondary constriction is perhaps the result of pairing between morphologically dissimilar chromosomes and/or due to paracentric inversions.



TEXT-FIG. 5. Range of variation in the morphology of the 6 marker chromosomes of *A. rubellum* at pollen mitosis.

All the three species of *Allieae*, discussed above, show the desirability and also the usefulness of the study of pollen mitoses. In all these cases, from pollen mitosis alone, one can make an inference about the abnormal constitution of these taxa. They reveal reasonably clearly the way meiosis alters not only the chromosome morphology but also the number, particularly when the taxa involved are either numerically and structurally hybrid, or when they are triploids or pentaploids. In all such cases there are ample chances for not only irregular segregation, but also of reshuffling of chromosomes as a result of intergenomal pairing.



It is logical to surmise that with a single burst of sexual reproduction in each of the three species, there will arise not only individuals with new numbers but also with new morphology of chromosomes. For instance in  $5x$  *A. neapolitanum* the progeny is expected to have all numbers ranging from  $2x$  to  $6x$  in case the female meiosis is like the male one. However, neither *A. rubellum* nor *A. neapolitanum* reproduces by seeds, they propagate exclusively by vegetative means. *N. fragrans* no doubt reproduces both by seed and by vegetative means, but unpublished results show that in this species the seed is produced as a result of adventive embryony.

Cytological literature is replete with examples in which pollen cytology has been studied to some advantage to the understanding of cytogenetic and evolutionary processes. Among the gymnosperms, Mehra (1946) was the first to make karyotypic analysis based on pollen mitoses in *Ephedra*. Newcomer (1954) was able to identify two types of pollen in *Ginkgo*. The two types of karyotypes were aptly correlated by him with the dioecism in *Ginkgo*. Similarly, Abraham and Mathews (1962) discovered the same phenomenon in *Cycas pectinata*. Bhaduri and Majumdar (1955) made critical analysis of karyotype from pollen mitoses in wheat species and varieties. In this way, they have eliminated the difficulty of classification of chromosomes because the number is haploid in the grains. It also renders the task of discovering chromosomal biotypes easier.

## B CHROMOSOMES

A very important aspect in which pollen mitosis is of much use, is in the study of B chromosomes. Thus Müntzing (1946) was able to show that the numerical increase in the standard fragments in rye is due to non-disjunction at the first pollen mitosis. Furthermore, what is important, non-disjoined B chromosomes are often always included in the nucleus on the wall side, *i.e.*, the generative pole. Ever since this finding was made, the phenomenon has been found to be more widespread.

## EFFECT OF RADIATION AND CHEMICALS

The use of pollen grains and pollen mitosis is universally appreciated for the study of the immediate effects of chemicals and different forms of radiation. They provide rather simple and haploid radiation targets. Pollen grain is exceedingly useful in low penetration radiation. An impressive amount of data has accumulated on this subject, which has recently been reviewed by Brewbaker and Emery (1962) and would not be discussed here.

## LIMITATION

The only limitation of pollen mitosis is that in materials like *Rhoeo* and *Oenothera* which are complex structural hybrids with balanced lethals, pollen mitosis will not give any idea whatsoever of the nature and extent of the interesting cytogenetic mechanism prevailing in these taxa. Furthermore, even the small structural differences and hybridity cannot be inferred from pollen mitosis. For all such situations meiotic and karyotypic studies are very imperative.

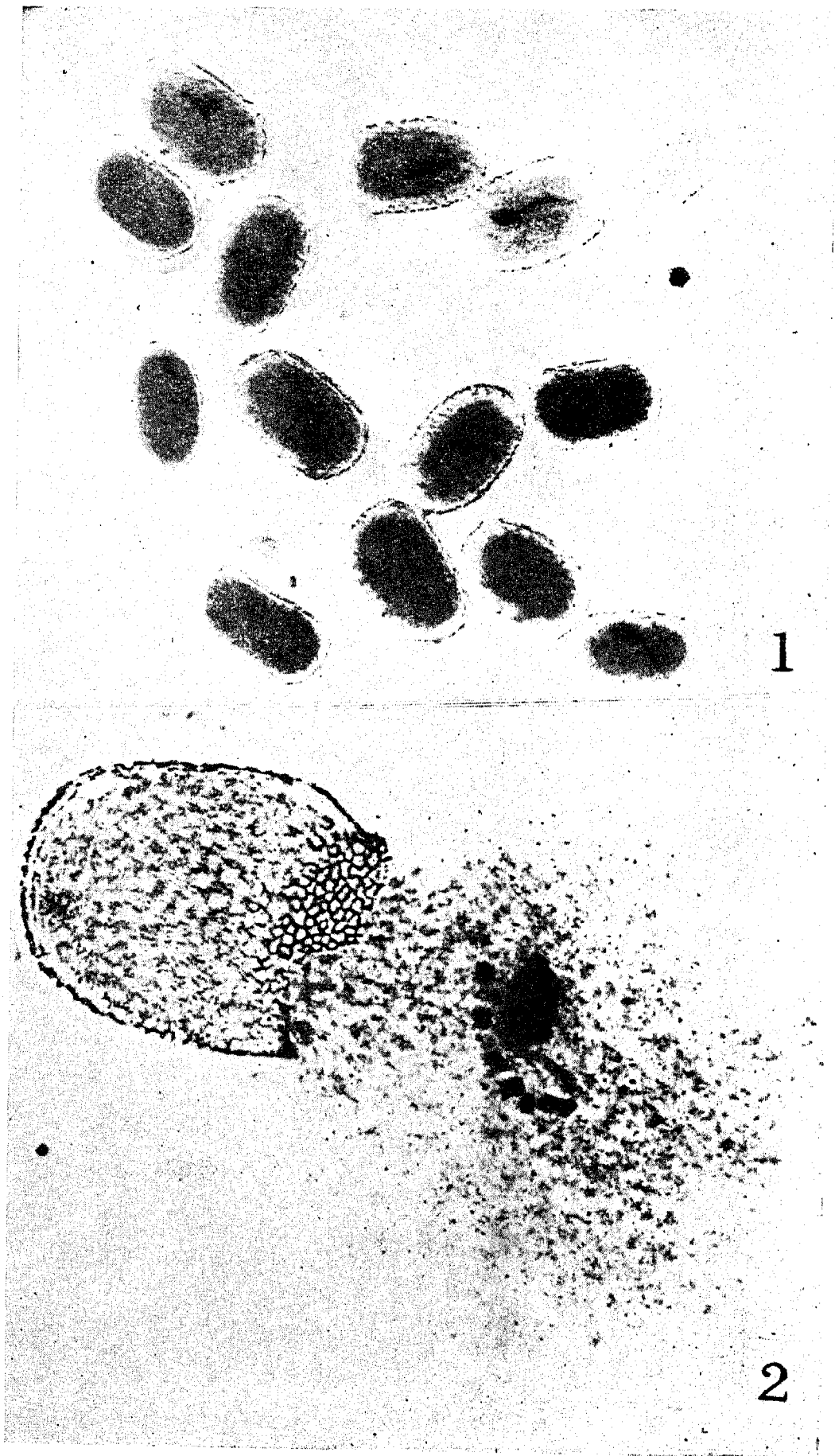
## CONCLUSIONS

From the foregoing accounts, a logical conclusion emerges, that while pollen mitosis cannot replace the cytological studies based on pollen mother-cells and root-tips, more often than not, it forms an excellent adjunct to them.

Apart from the study of pollen mitosis there are many other very interesting problems in pollen cytology, about which we have no clear understanding. These pertain mostly to the problems of cytoplasmic gradient and differentiation within the pollen grain.

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