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# On the relationship between the polyploidy and the occurrence of pollen-grains with an increased number of germinal apertures in the tribe *Brassiceae* *

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The morphological characteristics of the germinal furrows and pores in the pollen-grains are generally considered to be of phyletic, tending to be fixed throughout the family or still larger systematic group ; and moreover, the number and arrangement of such germination apparatus on each pollen-grain seem to be controlled to a certain extent by the internal environment, which is, in turn, determined by the number and arrangement of the sister microspores within each of pollen mother-cells. And therefore, it would be possible to detect various pollen-grains having different characteristics from the same anther (See Wodehouse, 1935). In certain aneuploid or polyploid plants in some species there could be found, besides the pollen-grains with the ordinary number of germinal apertures, several anomalous grains provided with the supernumerary apertures, and these facts have come to serve as a useful criterion in determining whether or not those plants under investigation are of aneuploid (Michaelis, 1951) or of polyploid (Fukes, 1956; Walther, 1961; Nagao and Takahashi, 1963) in their chromosome structures.

The authors, in the course of their cytogenetic studies in the tribe *Brassiceae*, have made examination upon the number of germinal apertures formed on each pollen-grain with some natural species and as well as with their certain polyploid forms induced artificially, and have succeeded to make clear that those anomalous pollen-grains provided with supernumerary apertures showed definite frequency occurrence with each of species used and also with each of artificially raised polyploid forms. A few noteworthy results obtained are described briefly in the following.

* Contribution from the Horticultural Laboratory, Faculty of Agriculture, Kyushu University.

Table 1. Frequency appearance of pollen-grains provided with various number of apertures in the natural species and the artificially induced polyploid forms in tribe *Brassiceae*.

| Species and forms | Chromosome number and genome constitution (2n) | | Number of germinal apertures 3 | 4 | 5 (%) | Number of pollen-grains examined |
|---|---|---|---|---|---|---|
| B. *nigra* 2x | 16 | *bb* | 100.0 | — | — | 2000 |
| 4x | 32 | *bbbb* | 54.9 | 45.1 | — | 2040 |
| B. *oleracea* 2x | 18 | *CC* | 100.0 | — | — | 2000 |
| *B. pekinensis 2x* | *20* | *aa* | 100.0 | — | — | 2000 |
| *4x* | *40* | *aaaa* | 50.3 | 49.7 | — | 2400 |
| *B. rapa 2x* | *20* | *an* | 99.9 | **0.1** | --- | 1200 |
| *B. trilocularis* 2x | 20 | *a′a′* | 100.0 | — | - | 2000 |
| B. *tournefortii* 2x | 20 | *TT* | 99.9 | 0.1 | — | 2003 |
| 4x | *40* | *TTTT* | 41.1 | 58.2 | 0.7 | 1366 |
| *S. alba* 2x | 24 | *??* | 100.0 | — | — | 2000 |
| S. *arvensis* 2x | 18 | *s s* | 100.0 | — | — | 2000 |
| *R. sativus* 2x | 18 | *RR* | 99.9 | 0.1 | — | 1700 |
| *4x* | *36* | *RRRR* | 65.8 | *34.2* | — | 1199 |
| *B. juncea (4x)* | *36* | *aabb* | 99.8 | *0.2* | — | 1200 |
| *B. cernua (4x)* | *36* | *aabb* | 94.5 | *5.5* | — | 3307 |
| *B. carinata (4x)* | *34* | *bbcc* | 88.6 | *11.4* | -- | 1800 |
| *B. napus (4x)* | 38 | *aacc* | 96.5 | *3.5* | -- | *4600* |
| *B. integrifolia* (4x) | *3 8* | *aacc* | 98.7 | *1.3* | — | *2539* |
| *C. abyssinica (6x)* | *90* | 333322 | 3.8 | *95.2* | 1.0 | *1500* |
| *Brassico-Raphanus 4x* | *36* | *RRcc* | 77.9 | 22.1 | — | 5504 |
| *Synthetic napus* (CO) 4x | 38± | *aacc* | 71.7 | 28.2 | 0.1 | 3529 |
| abc-amphidiploid 6x | 54 | *aabbcc* | 7.3 | 87.1 | 5.6 | 1197 |

N.B. 2x, *(4x)* and (6x): natural diploid, tetraploid and hexaploid forms, respectively. 4x and 6x: artificially induced tetraploid and hexaploid forms, respectively.

## Material and method

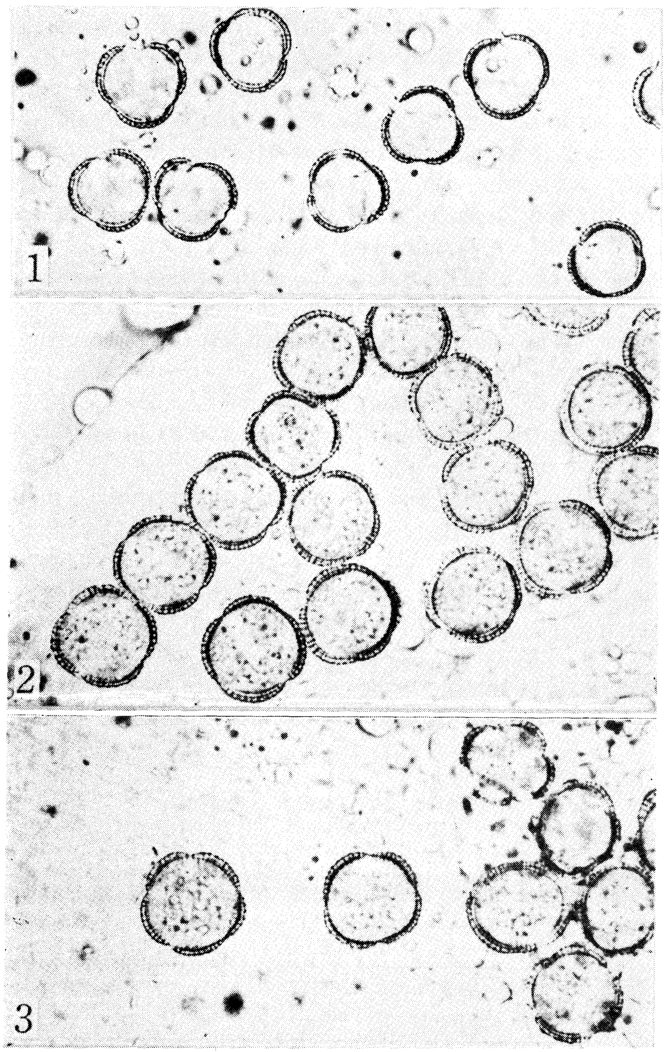
The plants used were obtained from the stock materials kept under the pedigree culture, their chromosome numbers being ascertained, at the Horticultural Laboratory, Kyushu University. As shown in Table 1, some material plants came from the natural species (each species was represented by 1, 2 or 3 horticultural varieties) and the rest from the artificial polyploid forms, i.e., 11 natural species of the genus *Brassica*, 2 of the *Sinapis*, 1 of the *Raphanus*, and 1 of the *Crambe*; and 4 kinds of the artificial autotetraploid forms, 2 of the artificial allotetraploid forms, and 1 of the artificial allohexaploid form. The detailed information of those artificial allopolyploid forms is given in the following; a synthetic B. *napus* plant (CO), whose genome constitution showing almost uniform convergence towards ***aacc***, was obtained in the hybrid progeny raised by a cross between *B. pekinensis* × *B. oleracea* (Hosoda, 1950); a *Brassico-Raphanus* hybrid plant, having *RRcc* in its genome constitution, which was raised in the hybrid progeny obtained from a cross between B. *oleracea* × ***R. sativus*** (Fukushima, 1945) ; a trigenomic hexaploid plant, having *aabbcc* in its genome constitution, raised by the chromosome-doubling of the $F_1$ hybrid, B. *carinafa* × *B. pekinensis* (Iwasa, 1951).

All these plants used as materials, without regard to their genome constitution, either natural or artificial, showed exclusively about 90 % or still higher of the morphological pollen-fertility, excepting only the trigenomic-hexaploid form, which showed only about 40 % fertility.

The flower-buds were collected and fixed in the Carnoy's solution and preserved for the occasional use. For the examination of pollen-grains the almost matured anthers were smeared on the slide glass in a mixture of conc. $H_2SO_4$ and 2 % acetic methylenblue, mixed in the ratio of 1 : 3, or in a mixture of aceto-carmine and glycerine, prepared in the ratio 10 : 1. Each of these two solutions proved equally effective for the present purpose. The pollen-grains thus prepared, amounting to ca. 200 to 500 grains with each of the anthers used in determining the frequency occurrence of the anomalous pollen-grains with supernumerary apertures. And the results were presented by the total sum of countings with each of the plant forms.

## Result and consideration

Table 1, in which whole the results of the observations are compiled, will show that the number of germinal apertures in each grain attained (i) to 3 as the general rule in most cases, and to 4 in some rare exceptional cases in several monogenomic species, e.g., 3/2003 (0.1 %) in B. *tournefortii*, 1/1200 (0.1%) in *B. rapa*, and 1/1700 (0.1 %) in *R. sativus*, respectively (Fig. 1) ; (ii) to 4 in 34.2～58.2 % of cases in the artificially raised autotetraploid forms of certain monogenomic species ; (iii) to 4 in 0.2～11.4 % of cases in the forms of certain digenomic species ; (iv) to 4 in 22.1~28.2 % of cases in the artificially synthesized allotetraploid forms; (v) to 4 in 95.2 % of cases and to either 3 or 5 in rare cases in C. ***abyssinica*** (Fig. 2), a plant considered to belong to a natural hexaploid form, having 90 somatic chromosomes (in this genus *Crambe* a polyploid series of 30, 60, 90, and ca. 120 somatic chromosomes are known (Manton, 1932), though their exact genome constitutions remain still unknown); (vi> to 4 in 87.1% of cases and to either 3 or 5 in the remaining cases in an




Figs. l-3. showing young pollen-grains.
1, 3-apertured pollen-grains of *Brassica pekinensis.*
*2,* 4-apertured pollen-grains of *Crambe sbyssinica.*
3, 4- and 5-apertured pollen-grains of artificial trigenomic hexaploid.

artificially synthesized trigenomic hexaploid form (Fig. 3).

Those data given the Table show, in brief, that in tribe *Brassiceae* the condition of polyploidy seems to be intimately related to the more or less usual occurrence of anomalous pollen-grains with supernumerary apertures. The following circumstances will be taken as the noteworthy facts in this connexion:

(1) An increase of the number of genomes is accompanied by a corresponding increase of the anomalous pollen-grains with supernumerary apertures in various forms of Brassiceae, being natural or artificial, and as well as of auto- or allopolyploid composition ; (2) the occurrence of the 4-apertured pollen-grains seems to be more frequent in the autotetraploid than in the allotetraploid form. And the latter circumstance will be taken as a noteworthy fact indicating that those two kinds of polyploid forms are quite different from each other in their respective structures of genotype.

Marked differences were, however, occasionally encountered among the data obtained with the same individual or strain. Moreover, the

Table 2. Frequency appearance of pollen-grains provided with supernumerary apertures in *B. napus*.

| Varieties | Plant No. | Flower No. | Anther No. | Number of germinal apertures (%) 3 | 4 | 5 | Number of pollen grains examined |
|---|---|---|---|---|---|---|---|
| "Suisai" | 1 | A | a | 99.0 | 1.0 | — | 300 |
| | | | b | 91.0 | 9.0 | — | 300 |
| | | B | a | 100.0 | — | — | 300 |
| "Undai" | 1 | A | a | 97.7 | 2.3 | — | 300 |
| | | B | a | 98.7 | 1.3 | — | 300 |
| | 2 | A | a | 93.3 | 6.7 | — | 300 |
| | | | b | 95.7 | 4.3 | — | 300 |
| | | B | a | 100.0 | — | — | 300 |
| | | | b | 98.0 | 2.0 | — | 300 |
| "Rutabaga" | 1 | A | a | 87.7 | 12.3 | — | 300 |
| | | B | a | 98.7 | 1.3 | — | 300 |
| | | | b | 100.0 | — | — | 300 |
| | 2 | A | a | 94.7 | 5.3 | — | 300 |
| | | | b | 92.7 | 7.3 | — | 300 |
| | | | c | 100.0 | — | — | 300 |
| Average and total | | | | 96.5 | 3.5 | — | 4600 |

data showed certain variation among the different anthers in the same flower. Table 2 is compiled with the data obtained with the anthers used individually in *B. napus* plants. It seems quite probable to consider that the different frequency occurrence of the pollen-grains with supernumerary apertures among the anthers examined can be attributed to certain environmental conditions. But the foregoing (1) and (2) circumstances would be verified without the influence of such environmental conditions.

The causal mechanism through which the number of germinal apertures in pollen-grains of some ***Brassiceae*** forms have increased with the progress of polyploidy in these plants can not yet be fully explained at present. As is generally known, in the dicotyledonous forms 4 daughter nuclei induced after two successive meiotic divisions in a PMC became to be oriented in the cell as if they have occupied 4 corners of a regular tetrahedron, and with the subsequent formation of cell walls 4 microspores thus formed became to be arranged tetrahedrally. If 4 spherical daughter cells placed together in such tetrahedral arrangement, each cell becomes to make contact at one point with each of its 3 neighbors, giving to each sphere of cell 3 equally spaced contact points, from which germinal apparatuses, i.e., furrows and pores, begin to be formed one at one point. This seems to suggest an explanation of the usual appearance of tricolpate character in the dicotyledonous pollen-grains. Some other kinds of arrangements such as rhomboidal or square one occur by some unknown reasons. Thus the change in the number and arrangement of spaced contact points will be produced. When the points of such contact were asymmetrical, the cells have supplementary furrows developed at the symmetrical positions with the contact points in them (Wodehouse, 1935). This unusual disposition of the cells is likely to result in the appearance of the abnormal 6-apertured pollen-grains? but no such result was obtained in the authors' observations. Whether or not there appear any 6-apertured pollen-grains in the artificially synthesized octoploid plants or in the natural octoploid ones in the genus *Crambe* (*C. cordifolia*, *C. grandifolia*, etc. ; Manton, 1935) is an interesting problem. In either cases, the typical processes of microsporogenesis taking place in the polyploid forms have been fully elucidated on the careful observations. In addition to the varying arrangement of the daughter cells in polyploid plants, more particularly in artificial polyploid ones, there could be induced the formation of the irregular-numbered daughter cells in a PMC at its tetrad stage. Therefore, such a complex arrangement of cells may be taken as a leading contribution to the occurrence of pollen-grains with an increased number of germinal apertures.

Table 3. Relationship between chromosome number and the occurrence of pollen-grains with supernumerary apertures in the genus *Rumex* and *Betula*.

| Species | Number of chromosomes (2n)* | Size of pollen-grains in diameter (μ) (Wodehouse, 1935) | Number of germinal apertures (Wodehouse, 1935) |
|---|---|---|---|
| *Rumex* | | | |
| *R. acetosa* | 14 (♀), 15 (♂) | 18.2 – 21.6 | Prevailingly 3, less frequently 4. |
| *R. acetocella* | 42 | 22.0 – 24.0 | Prevailingly 4, less frequently 3. |
| *R. scutatus* | 20 | 23.9 – 25.1 | Always 3. |
| *R. obtusifolius* | 40 | 26.4 – 31.0 | Prevailingly 4, less frequently 3, and rarely 5 or 6. |
| *Betula* | | | |
| *B. populifolia* | 28 | About 20 by 28.2 | Generally 3, occasionally 4. |
| *B. lenta* | 28 | About 25.9 by 21.6 | Always 3. |
| *B. utilis* | 28 | 36.0 – 40.0 | Always 3. |
| *B. lutea* | 84 | About 29.6 by 25.1 | Prevailingly 4, occasionally 5, 6, or 7. |

* According to Kihara and Ono (1928, 1930), Löve (1941, 1942), Takenaka (1941), Flovik (1940) and Woodworth (1929).

The 3-apertured pollen-grains, which show the usual occurrence in *Brassiceae*, are, moreover, of highly typical one in the majority of dicotyledonous forms. In addition to the above cases in ***Brassiceae*** the occurrence of pollen-grains with supernumerary apertures, i.e., 4- or 5-apertured pollen-grains, were observed in several polyploid forms in *Solanum*, *Antirrhinum*, *Trifolium*, and ***Nicotiana*** species by Fukes (1956), and in certain other polyploid forms in *Pyrus*, ***Hydrangea, Nicotiana, Verbena,*** *Solanum*, and ***Rosa*** species by the present authors (unpublished). Moreover, Wodehouse (1935) noticed that the pollen-grains with aberrant numbers of furrows constitute only a small proportion of the total in most cases, but in some cases, e.g., the sorrel dok (*Rumex*) and birch (*Betula*) plants, almost one-half of the pollen-grains have been provided with 4 germinal furrows. In ***Rumex*** and ***Betula*** the usual occurrence of polyploid forms have been reported by several workers (Kihara and Ono, 1926; Ono, 1930; Löve, 1941 and and 1942; Takenaka, 1941; Woodworth, 1929; etc.). And in fact, a quite intimate relationship between polyploidy and the occurrence of supernumerary apertured pollen-grains in some forms of ***Rumex*** and ***Betula*** is clearly shown in the Table 3.

The facts enumerated above will be duly taken to imply that the selection of a polyploid individual in those species provided with the 3-apertured pollen-grains may be quite easy from the detailed observation of the pollen-grains.

## Summary

The authors' examinations on the number of germinal apertures of pollen-grains in the tribe ***Brassiceae*** have disclosed the following facts :

1. As shown in Table 1, the number of germinal apertures is 3 in each of pollen-grains in the diploid forms, mainly 3 and in part 4 in tetraploid ones, and predominantly 4 and in a far small proportion 3 or 5 in hexaploid ones.

2. Frequency occurrence of the anomalous pollen-grains with supernumerary apertures in some polyploid forms showed certain variation among different anthers within the same flower (Table 2).

3. It was safely asserted, irrespective of the foregoing irregularities, that the germinal apertures in pollen-grains of certain polyploid forms tend to increase in their number in accordance with the degree of polyploidy of those forms concerned; and, moreover, the occurrence of 4-apertured pollen-grains becomes to be much frequent in the autotetraploid forms as compared with the allotetraploid ones. The latter fact is very interesting to show that there exist certain definite difference between the polyploid forms of different nature of ploidy.

4. The frequent occurrence of pollen-grains with the supernumerary germinal apertures in the polyploid plant may be ascribable to certain irregularities of meiotic divisions and of the subsequent processes in microsporogenesis, but the causal mechanism remains unidentified.

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