

## Microsporogenesis of *Betula oycoviensis* Bess. and of its progeny

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### Abstract

The crossing of two specimens of *Betula oycoviensis* Bess. described as a separate taxon of the subsection *Albae*, unexpectedly yielded a progeny among which three types were distinguished: *oycoviensis*, *verrucosa*, and *nova*, in the approximate ratio 1:2:1. The first two types were trees, whereas the third one was a small shrub. Moreover, the three types of birches differed from one another in a number of morphological and biological characters. Microsporogenesis was investigated in the parental specimens of *B. oycoviensis* and in several specimens of the progeny representing three types of birches. It was only one birch of the *oycoviensis* type, a mixoploid specimen, that exhibited disturbances in microsporogenesis. In the other examined birches of the *oycoviensis*, *verrucosa*, and *nova* types meiosis proceeded normally and resulted in normally developed tetrads and pollen grains. Therefore, *B. oycoviensis* belongs to hybrids of regular meiosis, its hybrid character being revealed only at crossing.

### INTRODUCTION

*Betula oycoviensis* from the Ojców region, described for the first time by Besser in 1809, had for many years been regarded as one of the taxons of the subsection *Albae* Regel (Winkler 1904). A detailed diagnosis of this species was given in 1921 by J. Jentys-Szaferowa, who next characterized it with an increasing precision on the basis of specimens from natural localities (Jentys-Szaferowa 1928, 1951, 1953) and undertook several experiments in the breeding of this birch (Jentys-Szaferowa 1967; Jentys-Szaferowa, Białobrzeska, Truchanowicz, Więckowska 1974). These investigations, carried out in the Department of Plant Variability of the Institute of Botany of the Polish Academy of Sciences in Kraków, were started in 1954 by sowing of the nutlets derived from open pollination and gathered from the specimens of *B. oycoviensis* growing wild in the villages of Karnio-

wice and Hamernia. The thus obtained seedlings segregated into two groups: *B. oycoviensis* and *B. verrucosa* in an approximate ratio 1:1 (Korczyk 1967a; Więckowska 1967). When planted in the experimental plot in the Botanic Gardens in Kraków, they were given the name of generation A. However, as a result of controlled crossings in 1957 one specimen of *B. oycoviensis* from generation A (No. 39), pollinated with the pollen of *B. oycoviensis* brought to the Botanic Gardens from Hamernia in 1920, was found to have unexpectedly yielded the progeny belonging to three types: *verrucosa*, *oycoviensis*, and *nova*, and amounting to 23, 48, and 13 specimens respectively, thus approximating to the ratio 1:2:1. This phenomenon recurred when in the following year another specimen of *B. oycoviensis* from generation A (No. 8) became pollinated with the pollen of the same *B. oycoviensis* as had been used in the first case. Again three types of birches were obtained, this time 15 specimens of the *verrucosa* type, 30 of the *oycoviensis* type, and 10 of the *nova* type. The progeny of *B. oycoviensis* No. 39 was called generation B, and that of the specimen No. 8 — generation C. With a lapse of a few years the seedlings of the *oycoviensis* and *verrucosa* types grew into large trees, whereas those of the *nova* type developed into small shrubs. These three types of birches varied from one another not only in height but also in several morphological and biological characters (Plate I; Jentys-Szaferowa 1967; Jentys-Szaferowa et al. 1974).

The *nova* type, given this provisional name by Jentys-Szaferowa (1967), was first obtained experimentally and then was found in a natural locality in the Kobylańska Valley (Korczyk 1967b). This locality was interesting inasmuch as around a solitary old tree of *B. oycoviensis* there grew out a coppice of young birches: *B. oycoviensis*, *B. verrucosa*, and *B. nova*, which most probably had germinated from the seeds of the old birch due to self-pollination. One of the specimens of *B. nova* has survived till now on the edge of the ravine and is easy to find.

The interesting fact of segregation of the progeny of *B. oycoviensis* × *B. oycoviensis* stimulated further various controlled pollinations and also made it necessary to perform a cytological analysis of the parental specimens as well as of their progeny representing three types of birches. This last task was undertaken by the present author. She carried out the investigations of pollen and karyology (Szwabowicz 1971, 1972), and, in the present study, of microsporogenesis in *Betula oycoviensis* and in its progeny comprising the *oycoviensis*, *verrucosa*, and *nova* types.

#### MATERIAL AND METHODS

The investigated material consisted of the male catkins of birches growing in an experimental plot in the Botanic Gardens in Kraków and of those growing wild in the Kobylańska Valley (Tables 1 and 2). For

Table 1

Investigated specimens of *Betula oycoviensis* Bess. and its progeny

Origin	Generation*	Species or Type**	Specimen
Hamernia	—	<i>oycoviensis</i>	<i>B. oycoviensis</i> Bot. Gardens
Hamernia <i>B. oycoviensis</i> open pollination	A	<i>oycoviensis</i>	No 39
<i>B. oycoviensis</i> No 39/A × <i>B. oycoviensis</i> Bot. Gardens	B	<i>oycoviensis</i> <i>oycoviensis</i> <i>oycoviensis</i> <i>verrucosa</i> <i>verrucosa</i> <i>nova</i> <i>nova</i> <i>nova</i> <i>nova</i>	No 19 No 62 No 81 No 5 No 43 No 30 No 46 No 65 No 84
<i>B. oycoviensis</i> No 8/A × <i>B. oycoviensis</i> Bot. Gardens	C	<i>oycoviensis</i> <i>verrucosa</i> <i>nova</i> <i>nova</i>	No 15 No 21 No 1 No 3
<i>B. oycoviensis</i> No 39/A self-pollination	H <sub>5</sub>	<i>oycoviensis</i> <i>oycoviensis</i>	No 401 No 463
<i>B. verrucosa</i> No 107/A × <i>B. nova</i> mixture of pollen/B	H <sub>6</sub>	<i>oycoviensis</i>	No 16
<i>B. nova</i> No 30/B × <i>B. verrucosa</i> Wolski Wood	H <sub>7</sub>	<i>oycoviensis</i>	No 141
<i>B. nova</i> No 65/B self-pollination	H <sub>9</sub>	<i>nova</i>	No 588
<i>B. nova</i> No 84/B self-pollination	H <sub>12</sub>	<i>nova</i>	No 800
<i>B. nova</i> No 84/B × <i>B. nova</i> mixture of pollen/B	H <sub>13</sub>	<i>nova</i>	No 715
<i>B. nova</i> No 36/B × <i>B. nova</i> No 84/B	H <sub>14</sub>	<i>nova</i> <i>nova</i> <i>nova</i>	No 627 No 661 No 671
Kobyłańska Valley <i>B. oycoviensis</i> <i>B. oycoviensis</i> self-pollination		<i>oycoviensis</i>   <i>nova</i>	<i>B. oycoviensis</i> young birch Kobyłańska Valley <i>B. nova</i> Kobyłańska Valley

\* Birch generations derived from controlled pollinations performed in particular years from 1954 onwards are denoted with successive letters of the alphabet. The term "generation H" covers the specimens obtained after several various pollinations executed in 1962.

\*\* The term "species" refers to the specimens from natural localities, and "type" to those from controlled pollination.

Table 2

Microsporogenesis in *Betula oycoviensis* Bess. and its progeny

Name of specimen and progeny	Species — Type	Observed stages							n	
		Division I			Division II			Tetrads		Pollen grains
		diakinesis	metaphase	ana — and telophase	prophase	metaphase	ana — telophase			
<i>B. oycoviensis</i> Bot. Gardens	<i>oycoviensis</i>					+		+		14
No. 39/A						+	+	+		14
No. 19/B								+		
No. 62/B			+	+		+	+	+		distur- bances
No. 81/B				+	+	+	+	+		14
No. 15/C				+				+	+	14
No. 401/H <sub>5</sub>								+		
No. 463/H <sub>5</sub>			+			+		+		14
No. 16/H <sub>6</sub>		+					+	+		14
No. 141/H <sub>7</sub>									+	
<i>B. oyc.</i> young Kobyl. Valley				+		+	+	+	14	
No. 5/B	<i>verrucosa</i>			+	+	+				14
No. 43/B								+	+	
No. 21/C								+		
No. 30/B	<i>nova</i>	+						+		14
No. 46/B			+			+		+		14
No. 65/B									+	
No. 84/B			+			+	+	+		14
No. 1/C								+		
No. 3/C									+	
No. 588/H <sub>9</sub>								+	+	
No. 800/H <sub>12</sub>		+	+					+		14
No. 715/H <sub>13</sub>			+		+			+		14
No. 627/H <sub>14</sub>								+		
No. 661/H <sub>14</sub>			+							
No. 671/H <sub>14</sub>								+		14
<i>B. nova</i> Kobyl. Valley			+	+	+		+	+	14	

the latter birches the names *Betula oycoviensis*, *Betula verrucosa*, and *Betula nova* were adopted, and the former were called the *oycoviensis* type, the *verrucosa* type, and the *nova* type respectively.

Out of a very ample material gathered between 1967 and 1974 only an insignificant part exhibited meiotic divisions.



August 9th was the earliest and September 16th the latest date of fixation of the catkins in which various stages of division and tetrads were observed. However, microsporogenesis proceeded most frequently in the latter half of August.

The meiotic divisions did not occur simultaneously in the whole catkin. One and the same catkin, and even the same flower was found to contain anthers in various stages of development: from PMC's, through certain phases of divisions I and II, to the tetrads. Very often both sacs of one anther contained cells in different stages of meiosis.

The birches did not yield themselves readily to cytological analysis. Besides the difficulty of fix male catkins of appropriate age, another difficulty arose from a small size of the chromosomes, so that even tiny granulations in the cytoplasm obscured the picture.

The material to be studied was usually fixed in acetic alcohol (3 : 1) or in a mixture of anhydrous ethanol, chloroform and glacial acetic acid (6 : 3 : 1), being sometimes pretreated with an 8-hydroxy-quinoline solution. While preparing microscopic samples, the author first executed permanent squashes by Murin's (1960) cellophane method, the material being previously softened for 10 minutes in a mixture of 96% ethanol and concentrated hydrochloric acid. Better results were obtained by embedding fragments of catkins in paraffin and subsequently cutting them into 10  $\mu$ m thick sections. Both the squashes and microtome sections were stained with gentian violet or with Merck's methyl violet by Newton's technique.

Observations were carried out on a Reichert microscope with the use of immersion lens 100  $\times$  combined with Zeiss eyepieces 20  $\times$ , 25  $\times$ , and 32  $\times$ . Microphotographs were taken with the Reichert eyepiece Plan 12  $\times$ .

## RESULTS

### Size of chromosomes

The length of the mitotic chromosomes of *B. oycoviensis*, *B. verrucosa*, and *B. nova*, calculated during karyological examinations, approximated to 0.4 — 1  $\mu$ m (S z w a b o w i c z 1972).

During metaphase I the bivalents of *B. oycoviensis* and *B. nova* were from c. 0.4 to 0.7  $\mu$ m long. *B. verrucosa* did not reveal the presence of metaphase I. The length of chromosomes during metaphase II ranged from c. 0.4 to 0.8  $\mu$ m for all three types of birches. Insignificant discrepancies between the lengths of chromosomes during mitosis in the somatic and reproductive cells might have been due to an error in calculations from the drawing.

## Meiotic divisions

*Betula oycoviensis* (*oycoviensis* type)

Various stages of microsporogenesis were observed in 10 specimens of *B. oycoviensis* derived from natural localities (*B. oycoviensis*) as well as from experimental breeding (*oycoviensis* type), two of them being found to contain tetrads only (Tables 1 and 2).

In the early stages of prophase I, following those of synizesis (Plate II a), the chromosomes of the studied birches were too poorly visible to lend themselves to observation (Plate II b).

Diakinesis was recorded in *B. oycoviensis* No. 16 from generation H<sub>6</sub> obtained together with other specimens of this generation by experimental pollination of the *verrucosa* type with the pollen of the *nova* type. In the majority of the examined nuclei the conjugation of chromosomes proceeded in a normal way so that 14 bivalents were observable in them (Fig. 1 a). A few nuclei exhibited a less distinct picture on account of the staining of some parts of the nuclear membrane, which, added to the small size of chromosomes made the observation difficult. However, it may be presumed with a high degree of probability that these meiocytes did not depart from a norm.

The subsequent meiotic stages were noted in a number of specimens of *B. oycoviensis* (Table 2). The course of these stages was normal in all the examined specimens except for the birch No. 62 from generation B. In metaphase I there occurred 14 bivalents (Fig. 1 b), and in telophase I — 14 chromosomes.

In the successive stage, two interkinetic nuclei were produced in the meiocytes but no cell wall was formed in these cells, although now and a temporary, very poorly visible cell plate could be discerned (Plate III a).

The interkinetic nuclei entered upon a fairly marked prophase of division II, in which the chromosomes could be counted.

Metaphase II as well as ana- and telophase II were quite normal. Both the two plates of metaphase II and four groups of ana-telophase II contained 14 chromosomes each (Fig. 2a, b; Plates III b and IV a).

The great majority of the tetrads of the examined specimens were normally developed (Plate IV b). The microcytes were encountered only sporadically. In *B. oycoviensis* No. 19 from generation B, for instance, one in 200 examined tetrads contained two smaller cells besides four cells of normal appearance. Likewise, in the case of a young *B. oycoviensis* from the Kobylańska Valley, among 500 tetrads the author discovered two microcytes and a tetrad with cells of different size: one cell was much smaller than the remaining three. The occurrence of a whole anther of the specimen No. 19 from generation B which contained tetrads with deformed cells should be regarded as one of major deviations from a norm.

It is difficult to establish what has brought about this kind of anomaly, nevertheless it does not seem likely to have been induced by a disturbance in the course of meiosis, since then the cells of the tetrads would differ

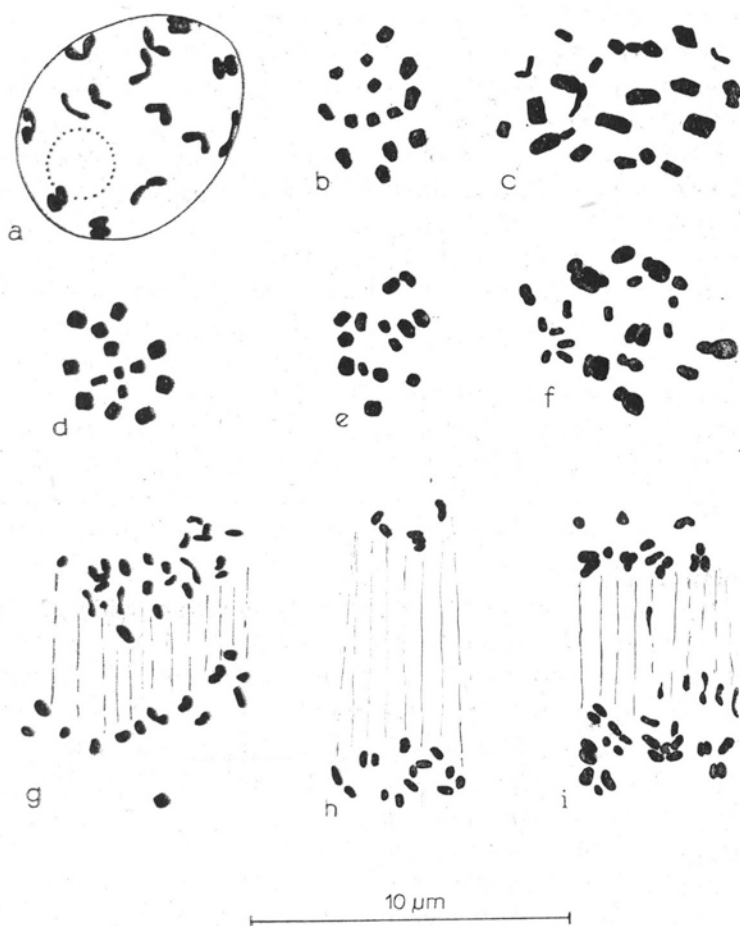


Fig. 1. Meiotic division I. a — diakinesis in specimen No. 16/H<sub>6</sub> of *oycoviensis* type; b — metaphase I in specimen No. 16/C of *oycoviensis* type; c, f — metaphase I disturbed in birch No. 62/B of *oycoviensis* type; g, h, i — anaphase I in birch No. 62/B; d — metaphase I in birch No. 84/B of *nova* type; e — metaphase I in *B. nova* from the Kobylańska Valley

from one another in size, besides, numerous microcytes would occur. Perhaps in such cases the deformation of the cells was evoked by external stimuli.

The only specimen exhibiting a marked deviation from a norm was the mixoploid birch No. 62 from generation B.

A lot of meiocytes of this birch showed a higher or lower degree of deformation. Some of pollen-sacs were filled with degenerated cells, whe-

reas others contained cells deformed in various ways, e.g. flattened or invaginated. Even the cells undergoing meiotic divisions were often deformed.

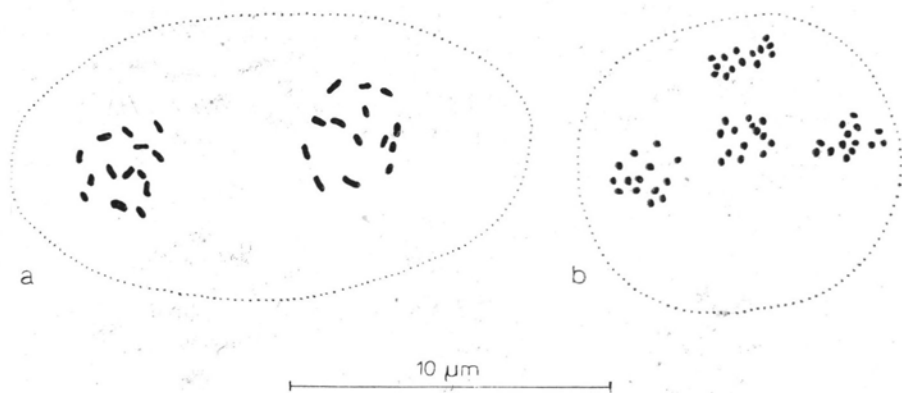


Fig. 2. Meiotic division II. a — metaphase II in *B. oycoviensis* from the Botanic Gardens; b — telephase II in *B. oycoviensis* No. 39/A.

In metaphase I, this birch was found to contain generally 21 to 24 configurations instead of 14 bivalents, and individual cells 13, 16, 17, 20 as well as 26 and 28 configurations, this being sometimes accompanied by the occurrence of tiny chromosome fragments. The term "configuration" was used because of the impossibility to determine whether these were uni-, bi-, or polyvalents (Table 3 a; Fig. 1 c and f; Plate III c).

Likewise, the course of anaphase and telophase I was highly abnormal. Among the major anomalies in these two stages were, above all, the migration towards the poles not only of individual chromosomes but from time to time also of indivisible bivalents or eventual polyvalents, a frequent occurrence of lagging chromosomes in the spindle, an occasional appearance in the cytoplasm of chromosomes or chromosome groups expelled from the spindle, and, finally, an uneven distribution of chromosomes at the two poles (Table 3 b; Fig. 1 g, h, i; Plate III d, e).

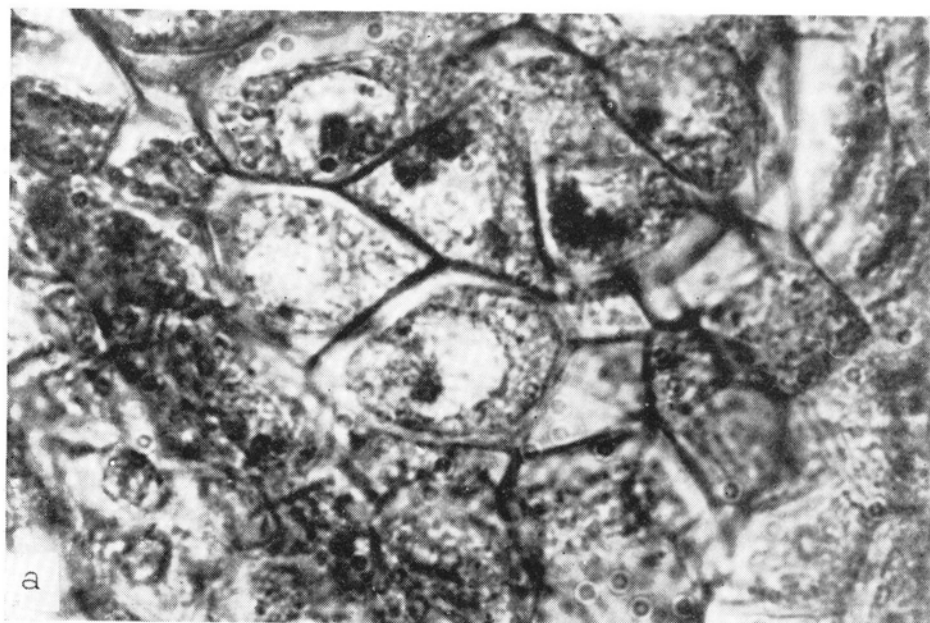
In metaphase II, the fact that the plates of the meiocyte were placed perpendicularly to each other prevented the counting of the chromosomes in both plates. Moreover, in several plates the chromosomes were connected on account of stickiness, which made impossible the establishment of their exact number. None the less, it was possible to discern that the two plates of numerous meiocytes differed from each other in the number of chromosomes and that in several plates the number of chromosomes exceeded 14. In metaphase II, much more frequently than in anaphase I, one or even two groups of chromosomes were found to have been expelled from the spindle (Table 3 c; Fig. 3 a, b).

Plate I



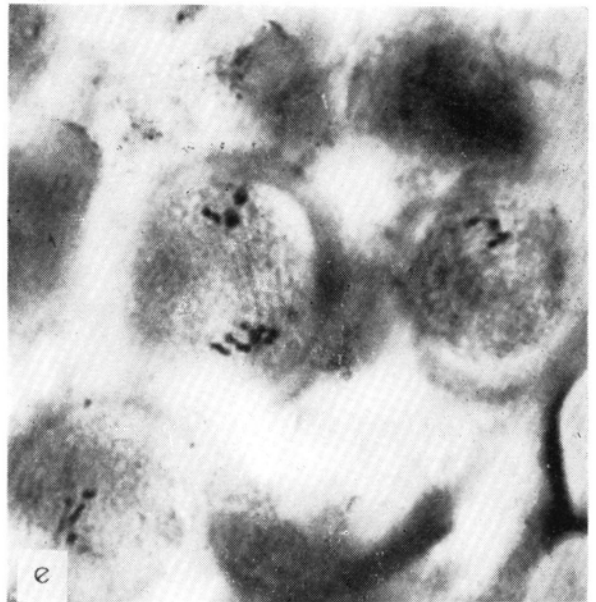
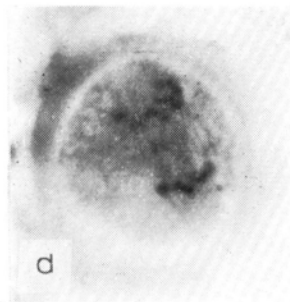
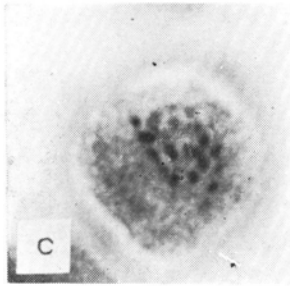
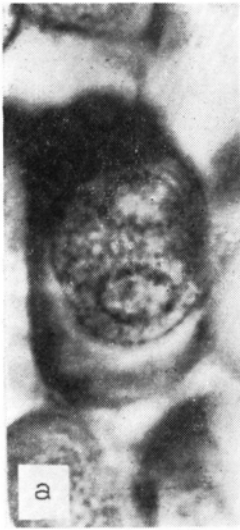
Leaves of three types of birches — progeny of *Betula oycoviensis* (after J. Jentys - Szaferowa 1967, 1974).

Plate II



PMC's in *Betula oycoviensis* No. 39/A. a — synizesis; b — early prophase.

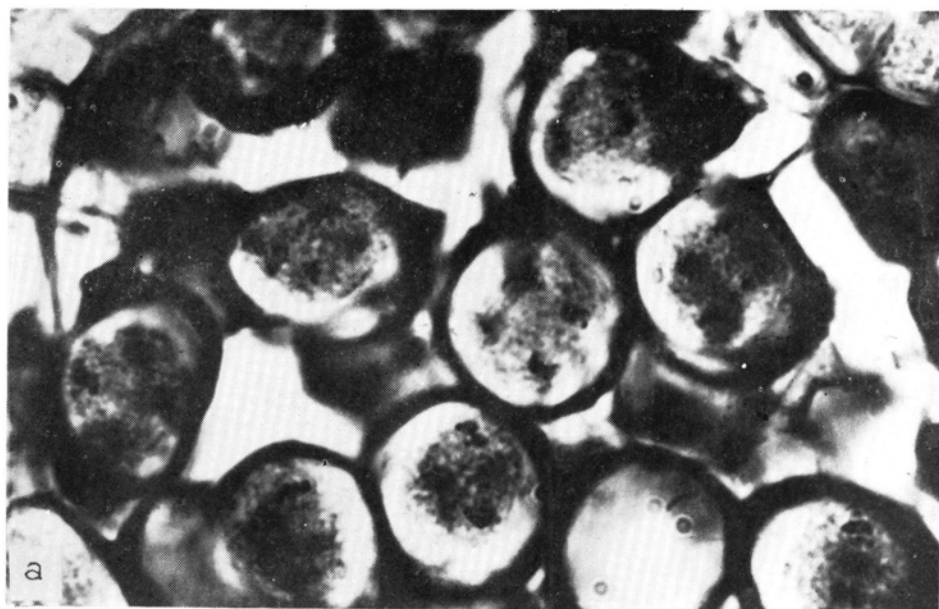
Plate III



a — interkinesis with cell plate formation; b — metaphase II in *B. oycoviensis* from the Botanic Gardens; c — metaphase I in specimen No. 62/B of *oycoviensis* type; d, e — anaphase I in specimen No. 62/B.



Plate IV



a — telophase II in *B. oycoviensis* No. 39/A; b — tetrads in No. 39/A.



Table 3

Microsporogenesis of specimen No 62/B (*oycoviensis* type)

## a. Metaphase I

Number of configurations	13	16	17	20	21	22	23	24	26	28
Number of cells	1	1	1	1	4	3	4	5	1	1

## Ana- and telophase I

Number of chromosomes or configurations\*

## b.

14+14	14+14	14+14 3 elimin.	14+ 5	14+18	14+min. 18
14+18	14+19	14+24? 3 elimin.	14+24*	16+20	18+min. 22
16+25	17+20	11+20 3 elimin.	18+20*	3+11	22+min. 19

## Metaphase II

## c.

14+ ?	14+ ?	14+ ?	14+ 7	14?+20 1 and 2 elim.	14+numerous	17+ ?
18+ ?	18+19	21+24	25+ ?	20+24 3—4 elimin.	26+min. 21	28+ ?

## Telophase II

Number of chromosomes

## d.

14?+7, 14+18 1 eliminated	14+14, 14?+14 6 eliminated	14+18, 14+0 4—5 eliminated	14+16, 14+20
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In telophase II, the chromosomes could be counted in only four cells, some chromosome groups, however, being very indistinct, hence the given values are but approximate, but they point to serious disturbances in the stage under observation. It was only in one of these cells that each of the four groups contained 14 chromosomes. One of the meiocytes lacked one of the four groups, only the middle of the cell being occupied by four chromosomes expelled from the spindle. Elimination of one or more chromosomes was detected in two other cells as well (Table 3d; Fig. 3c).

Owing to the above-described irregularities in the studied meiocytes, there was a tendency towards reduction of the number of chromosomes to  $n = 14$ , in both the ana- and telophase I, and also in meiotic division II.

Disturbances in the progress of microsporogenesis in the specimen No. 62 resulted in the abnormal development of the tetrads (Fig. 3d). Among 100 of the counted tetrads, 54 contained one, two, less frequently three, and in one case even four additional cells. These cells varied in size from tiny microcytes, through medium-size cells, to, exceptionally, almost as

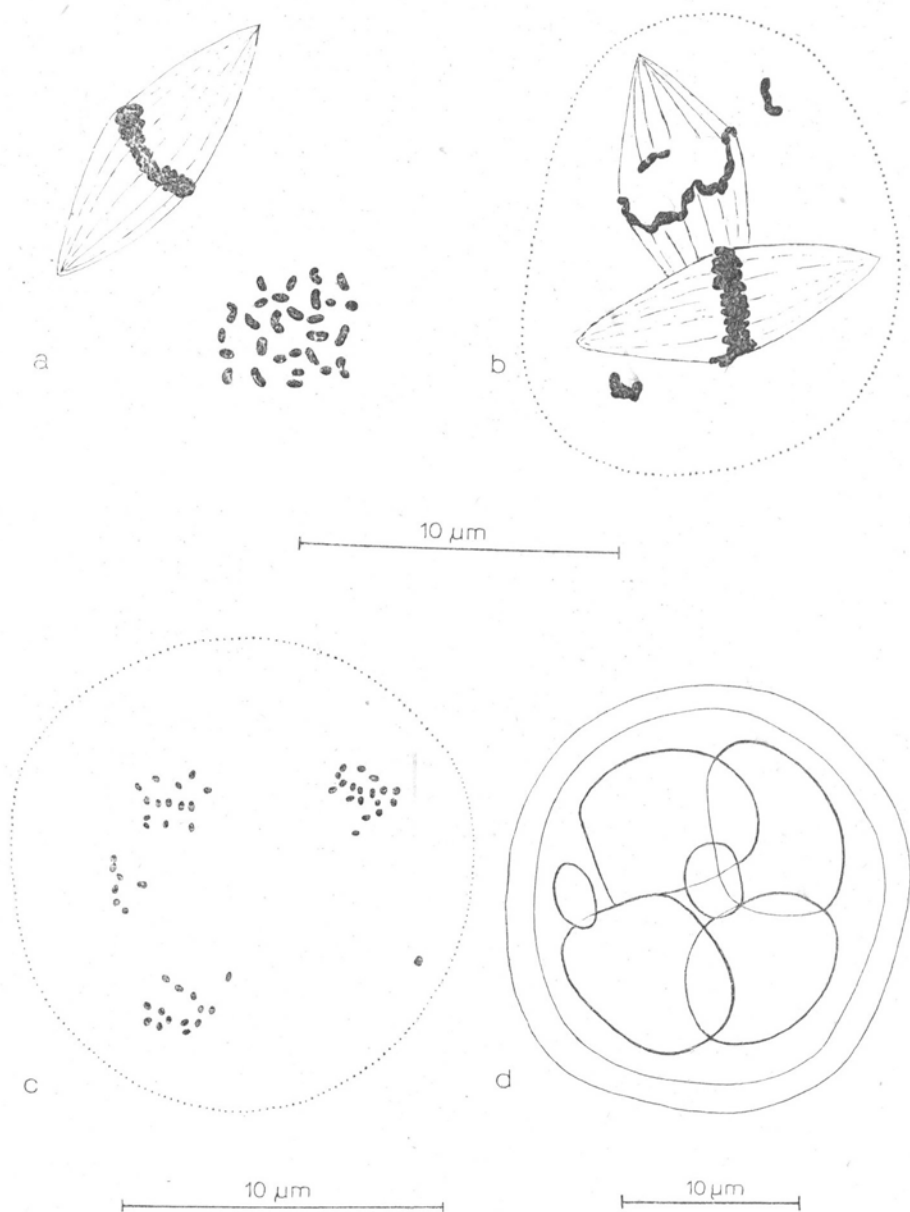


Fig. 3. Meiotic division II in birch No. 62/B of *oycoviensis* type. a, b — metaphase II; c — telophase II; d — tetrads

big as normal tetrad sporocytes. A varying size of the four cells of the tetrad was another deviation from a norm observed even in apparently well developed tetrads. All such anomalies were bound to lead to abnormal development of pollen. The present author's earlier studies (Szwabowicz 1971) on the pollen of the progeny of *B. oycoviensis* have shown that in 1965 the pollen of specimen No. 62 from generation B contained 44% of grains completely devoid of cellular content, while 7% of them were only partly filled with it. The number of empty grains increased in the following year of investigation. Moreover, degeneration of pollen was manifested by the occurrence, on the one hand, of microcytes and, on the other, of grains with thickened sporoderm and greater number of pores; grains with 4, 5 up to 8 pores were often encountered. The viable grains demonstrated a wide range of variability in size but all were rather large, on the average larger than in other specimens of this generation.

#### *Verrucosa* type

The verrucosa type obtained as one of three segregates by the crossing of two specimens of *B. oycoviensis*, was represented in the studied material far more modestly than the *oycoviensis* type (Table 2). Telophase I, prophase and metaphase II were recorded only in the specimen No. 5 from generation B. The majority of the plates were found to contain 14 chromosomes each except for one cell which was very weakly stained and which arouse some doubt that the number of chromosomes in it was slightly increased.

No tetrads were observed in this specimen. On the other hand, in the birch No. 43, also from generation B, and in *verrucosa* type No. 21 from generation C they were developed normally.

#### *Betula nova* (*nova* type)

The dwarf birch of the *nova* type which appeared unexpectedly among the progeny of *B. oycoviensis*, required a more detailed examination. To this end a number of self-pollinations and crossings of various specimens of this type from generation B were performed in the experimental plot (Table 1). It was also given more attention from the cytological point of view. In the ample fixed material 7 birches exhibited meiotic divisions, a few were found to contain only tetrads or already developed pollen grains, whereas the catkins of the remaining specimens turned out to be too young to be studied for meiosis.

All the examined specimens from experimental breeding and the birch from the Kobylańska Valley (Table 2) revealed a normal course of divi-

sions I and II. In diakinesis and metaphase I each meiocyte was found to contain 14 bivalents (Fig. 1 d, e), and in the plates of metaphase II and in groups of telophase II 14 chromosomes were ascertained. Deviations from a norm were very rare. In the birch No. 46 from generation B, for instance, a cell was encountered in which one plate contained 14 and the other over 20 chromosomes.

A great majority of the tetrads of birches of the *nova* type were developed quite normally, anomalies occurring only sporadically. Three specimens were noted to contain one microcyte each. Likewise three times a tetrad was found in which one cell was slightly smaller than the remaining three.

Other abnormalities such as invagination of the external tetrad wall or its excessive swelling, which could sometimes be observed in the analysed material, were probably not related with microsporogenesis. Besides, in two cases an anther with somewhat deformed tetrads was encountered. This phenomenon does not seem to have depended on meiotic divisions either.

### Tetrads and pollen grains

A great many birches were found to contain the anthers with tetrads (Table 2), probably owing to persistence of the tetrad stage, unlike meiotic divisions I and II, for a comparatively long time.

A characteristic appearance of the tetrads of the described types of birches was due to the intensely stained protective callose "special wall", whose formation had already commenced in the stage of prophase and whose thickness increased in the course of meiosis (Plates II b and III b; Feslop-Harrison, 1966). In the birch tetrads it was developed similarly as in *Helleborus foetidus* L. (Waterkeyn 1961, Figs 1—3), forming around the whole tetrad an uneven thickened wall and thinner walls around particular microspores (Plate IV b).

As has already been mentioned, all the examined birches save the specimen of the *oycoviensis* type No. 62, had most of their tetrads normally developed, this pointing to the regular course of meiotic divisions and conditioning a normal formation of pollen grains.

Each microspore in the tetrad and immediately after leaving it was c. 8  $\mu$  min diameter. In the oldest pollen grains observed in August and September, the diameter of 15  $\mu$ m prevailed (extreme values: 10.5 and 19.9  $\mu$ m). The maximum values were attained by the pollen of the specimen of the *oycoviensis* type No. 141 from generation H<sub>7</sub>, which had been obtained by pollination of the birch of the *nova* type (No. 30/B) with the pollen collected from several specimens of *B. verrucosa* in the Wolski Wood.

Only two microcytes were found in the analysed pollen grains, both of them in the *nova* type No. 3 from generation C, testifying to occasional disturbances in microsporogenesis.

The size of birch pollen found in the anthers in autumn was not normal yet. In all likelihood it was only in spring that the pollen of all three types attained its normal diameter of c. 23  $\mu\text{m}$ , and exceptionally c. 26  $\mu\text{m}$  in *B. oycoviensis* from generation H<sub>7</sub> (Szwabowicz 1971).

#### DISCUSSION

Microsporogenesis in both *B. oycoviensis* and 3 types of birches descending from it, i.e. *oycoviensis*, *verrucosa*, and *nova* types, did not exhibit any deviation from a norm save in one specimen of *B. oycoviensis* No. 62. It was only in isolated cases that the author observed anomalies in the course of meiotic divisions as well as some microcytes in the tetrads and among the already formed pollen grains.

Meiosis analysed, though not in all of its stages, in the specimen of the *verrucosa* type did not seem to vary from what had been ascertained by Anna Helms and C. A. Jørgensen (1925) for the species *B. verrucosa*. Thus, the *verrucosa* type, belonging to the progeny of *B. oycoviensis* as one of its segregates and morphologically identical with the species *B. verrucosa* (Jentys-Szaferowa 1967), showed slight or no abnormalities as regards meiotic divisions and pollen formation (Szwabowicz 1971).

The microsporogenesis of *B. nova*, investigated for the first time by the present author in a number of specimens, was regular. The pollen of this birch was also well developed as the number of viable grains ranged between 78.5 and 98% for individual specimens (Szwabowicz 1971); moreover, judging by a number of successful self-pollinations and crossings a high degree of fertility was revealed not only by its pollen but also by its female gametes (Table 1; Jentys-Szaferowa 1974).

*B. nova*, derived by experimental breeding and growing wild as one of the offspring of *B. oycoviensis*, has not till now been discovered outside the immediate vicinity of *B. oycoviensis*. It may well be that it did not attract anyone's attention, being regarded as a young *B. oycoviensis* or *B. verrucosa*, or that it does not exist any longer as an independent species in a natural state, the more so because it exhibits several lethal traits such as short life, excessive blossoming, and others.

From the genetic point of view, this birch, besides *B. verrucosa*, behaves like a parental species of *B. oycoviensis*. The reciprocal crossing of it with *B. verrucosa* yielded two very numerous, uniform generations of *B. oycoviensis* (H<sub>6</sub> and H<sub>7</sub>).

Finally, the meiotic divisions observed in 7 specimens of the species and type *oycoviensis*, except for the birch No. 62, proceeded normally.

The birch No. 62 demonstrated disturbances in meiosis and abnormally developed pollen, thus the traits usually attributed to hybrids (Sax 1935; Stebbins 1945). The earlier investigations on the karyology of birches have revealed a simultaneous presence in this specimen of some numbers of somatic chromosomes i.e. 28, c. 35, 36—38, 40, 42, 45—48, 52 and c. 56 (Szwabowicz 1971). Therefore, it should be regarded as an aberrant form with a markedly disturbed cytological balance. Morphologically, this birch exhibited a majority of the characters of *B. oycoviensis*, nevertheless, in the scatter diagram and in the diagram showing segregation of birches from generation B (Jentys-Szaferowa 1967, Figs 8, 12) it was found among *B. oycoviensis* birches which are most similar to *B. verrucosa*.

Extreme disturbances in microsporogenesis of this mixoploid birch resembled anomalies in the specimen of *B. pendula* Roth. (= *B. verrucosa* Ehrh.) from the Arnold Arboretum, most probably a natural hybrid, studied by Woodworth (1929), and even more the anomalies in one allo- and one autotriploid specimen of *B. verrucosa* studied by Johnsson (1944, Fig. 6), of which the first came from the crossing of *B. verrucosa* with *B. pubescens*. Similar abnormalities were also detected by Johnsson (1949, Figs 7—10) in the meiosis of the hybrids *B. pubescens* × *B. papyrifera*.

Unlike the specimen No. 62, the other investigated *B. oycoviensis* birches demonstrated a normal course of microsporogenesis. Likewise the pollen of these birches was well developed. However, these facts do not rule out the possibility that the species *B. oycoviensis* is a hybrid but may only point to a high degree of homology between the chromosome complexes of the parental species. Similarly, the hybrids *B. verrucosa* × *B. japonica*, and *B. verrucosa* × *B. papyrifera*, described by Johnsson (1949, Figs 5, 6) did not deviate much from a norm in either the course of meiosis or pollen formation. Stebbins (1945), who fully accorded with Sax's opinion (1935) that hybrids are characterized by irregular meiotic divisions and pollen sterility, at the same time reported that only 11 genera of the hybrids examined in the period between 1935 and 1945 had been found to contain at least two univalents, and as many as 41 had exhibited a complete chromosome conjugation. As regards fertility of interspecific hybrids, it may range from absolute sterility to complete fertility, sterility affecting principally the pollen grains (Dillemann 1954).

From the cytological point of view then, *B. oycoviensis* Bess. belongs to this kind of hybrids which does not manifest any irregularity in meiosis and possesses a well developed pollen. Numerous successful pollinations have also borne out its fertility as far as female gametes are concerned (Table 1; Jentys-Szaferowa 1967, 1975). A hybrid character of this

birch which for many years passed for a species, has been revealed only by segregation of its progeny. The crossing of two specimens of *B. oycoviensis* carried out in experimental breeding, at full fertility of the hybrid i.e. *B. oycoviensis*, produced such a result as can be observed in  $F_2$  i.e. segregation according to Mendel's law (Dillemann 1954). The same process also took place in the Kobylańska Valley where a solitary specimen of *B. oycoviensis* became self-pollinated.

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*Mikrosporogeneza u brzozy ojcowskiej i jej potomstwa*

## Streszczenie

Brzoza ojcowska, uważana przez długie lata za gatunek, po przeprowadzeniu krzyżowania *Betula oycoviensis* × *B. oycoviensis*, dała nieoczekiwane potomstwo dzielące się na trzy typy brzoź: typ *verrucosa*, typ *oycoviensis* i typ *nova*, mniej więcej w stosunku 1:2:1. Dwa pierwsze typy były to drzewa, trzeci zaś okazał się małym krzewem. Ponadto wszystkie trzy typy różniły się szeregiem cech morfologicznych i biologicznych.

Przebadano mikrosporogenezę u okazów *B. oycoviensis*, będących osobnikami rodzicielskimi w wymienionej krzyżówce, oraz u szeregu okazów potomnych, należących do trzech typów brzoź. Tylko u jednej brzozy typu *oycoviensis*, będącej okazem miksploidalnym (Szwabowicz 1972), miały miejsce zakłócenia w mikrosporogenezie, podobne do zaburzeń występujących u triploidów lub u niektórych mieszańców międzygatunkowych. Mejoza u innych przebadanych brzoź typu *oycoviensis*, *verrucosa* i *nova* miała przebieg prawidłowy i prowadziła do normalnie wykształconych tetrad oraz ziarn pyłku.

*B. oycoviensis* należy zatem do mieszańców, o regularnej mejozie, której charakter mieszańcowy ujawnia się przy krzyżowaniu.