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*Analiza zbiorowego gatunku **Betula alba** L. na podstawie pomiarów liści. — Analysis of the collective species **Betula alba** L., on the basis of leaf measurements.*

*Część III. **Betula oycoviensis** Bess. i **Betula obscura** Kotula. Określanie na podstawie jednego liścia. — Part III. **Betula oycoviensis** Bess. and **Betula obscura** Kotula. Determination on the basis of a single leaf.*

Mémoire

de Mme ***J. JENTYS-SZAFEROWA***

présenté le 10 Juin 1950, par M. W. Szafer m. t. et. M. B. Pawłowski m. c.

Contents:

Contrary to the problem of *Betula tortuosa* and *B. carpatica*, which is of general European importance, *Betula oycoviensis* and *B. obscura* have but local significance. The fact is that both of the latter have been hitherto known exclusively from Polish territory. Nonetheless, both these birches are very interesting. The author has elaborated them by the same method which she has described in Parts I and II of her work.

1. Betula oycoviensis Bess.

Betula oycoviensis Bess. considered by some botanists to be a small-leafed variety of *B. verrucosa*, is a well characterized and easily distinguishable species. The author gave a detailed description of this species in a paper printed in 1928 in the annuals of the Polish Dendrological Society. To the data given therein, the author wishes to add now that the station of *B. oycoviensis* quoted by Schur (1866) from Transylvania has not been so far rediscovered. On the other hand, Prof. J. Walas (1934) discovered two young specimens belonging to this species in the Bolechowice Valley (near Krakow), six kilometres in a straight line from the station of *B. oycoviensis* in the Ojców Valley. He rightly supposes that they are probably newcomers in the Bolechowice Valley. What we have here, therefore, is an endemic plant of very small, purely local range. Moreover, to the characteristic given in 1928, the author wishes to add that a detailed study of the station of *B. oycoviensis* at Hamernia in the Ojców Valley (*locus classicus*, Fig. 2 A.), as well as observations on cultivated specimens, have shown that indeed the species does occur most frequently in a bushy form, but it can also develop into a tree, especially when growing in a thicket. Such trees differ from afar from specimens of *B. verrucosa* by the thick bushy ramification of their crowns. The reason therefore lies in the fact that *B. verrucosa* has on each vegetative dwarf shoot (i. e., on those not terminated by a female catkin) only one bud from which a summer shoot can develop. In *B. oycoviensis* such dwarf shoots are much longer, with two, three, or four buds. From each of the latter can in summer a long shoot develop; this produces a completely different manner of ramification.

The author's present problem was to discover whether the leaves of *B. oycoviensis* have a characteristic shape by means of which they would be distinguishable from the leaves of *B. verrucosa*. Of the leaves of *B. oycoviensis* the author had written in 1928 only that they are small and have five, exceptionally six, pairs of lateral nerves (in contradistinction to *B. verrucosa* v. *microphylla*, which has much denser nervation). The greatest difficulty consisted in collecting a sample which would be analogical to the samples of the other investigated trees, i. e., collecting 50 vegetative dwarf shoots from 50 shrubs or trees of *B. oycoviensis*. At the place where this birch occurs and where at present a reservation has been created, the author discovered after a detailed search only 30 specimens. However, she supplemented these with leaves from old herbarium specimens, originating from Hamernia and collected from birch-trees which at present are already undoubtedly non-existent, and from specimens growing in the Krakow Botanical Garden, cultivated from seeds brought from Hamernia. A sample thus composed of 100 leaves from 50 trees, possessing the value of «a sample from the whole distributional area», is compared by the author in Fig. 1 with a sample of *B. verrucosa* from the whole European distributional area. The manner of comparing is described by the author in Part I of her work (page 182). It consists in comparing for each character the ratio of the arithmetic mean of the investigated sample to the arithmetic mean of another sample which is considered to be a comparative unit and which is expressed graphically by means of a straight line with the designation «1». The points which show by how much the arithmetic means of the investigated sample are greater or smaller than the arithmetic means in the comparative unit, are connected by means of straight lines, and thus a polygonal line is obtained. The mutual relationship of the straight line and the polygonal one constitutes the mutual relationship of leaf shape in the two investigated samples. The figures placed at the left-hand side of Fig. 1, and of other analogical figures, denote the following characters:

1. Petiole length.
2. Blade length.
3. Blade width.
4. Number of pairs of lateral nerves.
5. Distance of first tooth from blade base.
6. Distance between tips of second and third nerve.
7. Number of teeth between tips of second and third nerve.
8. Ratio of blade length to petiole length.
9. Ratio of blade length to blade width.
10. Mean distance of nerves.
11. Ratio of blade length to distance of first tooth.
12. Position of widest part of blade.
13. Axil of second nerve.
14. Base angle.
15. Apex angle.
16. Number of leaves on dwarf shoot.

Characters 1—7 are characters of size, or quantitative ones; characters 8—15 are characters of shape, or qualitative ones. Character 16 does not pertain to leaves but to shoots from which leaves were collected for the measurements.

In Fig. 1 the comparative unit (the straight line) is a sample of 100 leaves from 50 trees of *B. verrucosa* from its whole European

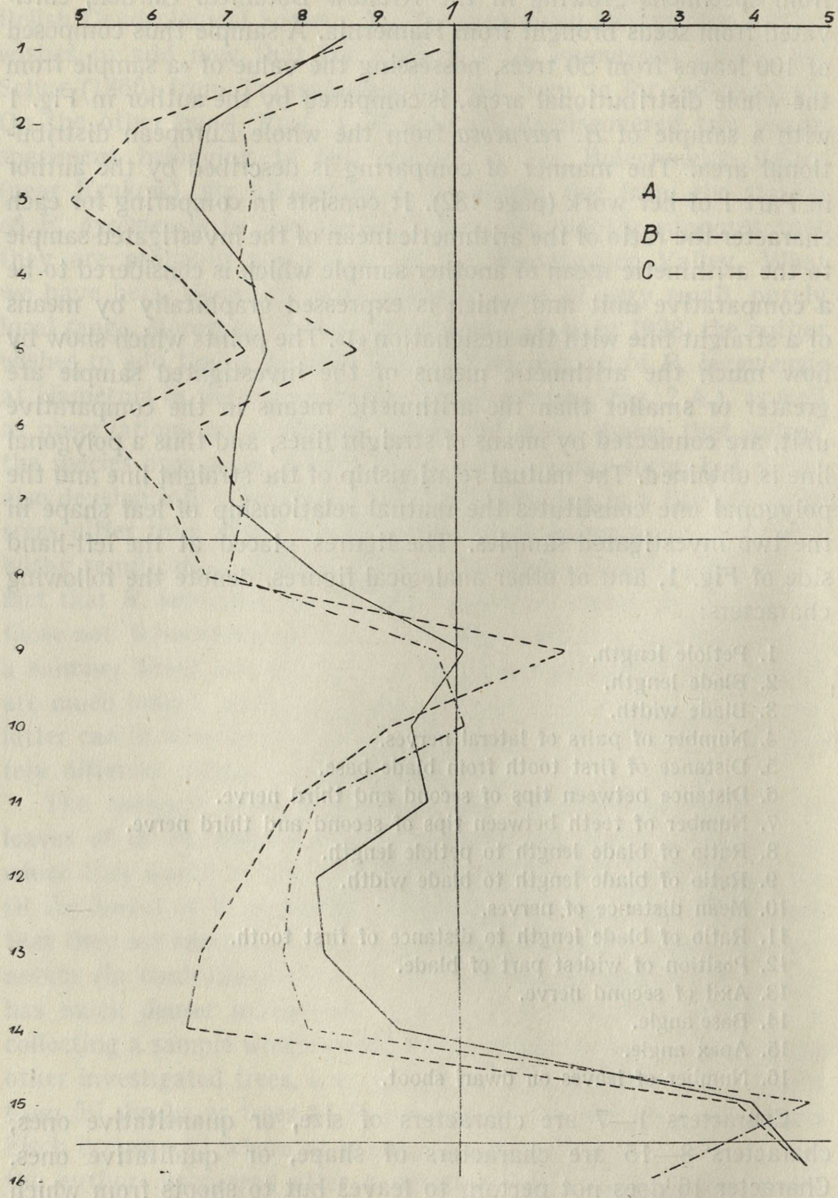

Fig. 1. Comparison of leaves of *B. oycoviensis* Bess. with *B. verrucosa* Ehrh (straight line). The polygonal lines are *B. oycoviensis*: A. sample of 100 leaves from 50 trees, B—C two samples, each containing 100 leaves from one tree.

distributional area. The continual polygonal line gives a picture of the sample of *B. oycoviensis* from the classical place of its occurence in the Ojców Valley. We see there that not only does *B. oycoviensis* have smaller leaves (characters No 1—3), but that they differ from *B. verrucosa* in having petioles which are markedly longer in relation to blade length (character No. 8), a small number of pairs of lateral nerves (character No. 4) and comparatively scarce nervation (character No 10), on account of the fact that these small blades have almost the same mean distances of lateral nerves as the large leaves of *B. verrucosa.* Furthermore, they have on an average, a more acute base angle (14), the position of their widest part is higher (inasmuch as its distance from the base is contained less times within the blade length) (12) and they posses a wider apex angle (15), i. e., more obtuse apexes. The number of leaves on dwarf shoots is here also higher than in all the investigated trees from the group *alba* (16).

In the same figure the author has given the lines of size and shape for two single specimens of *B. oycoviensis,* drawn on the basis of measurements published in the first part of her biometric studies on the collective species *B. alba* L. (1937). Although what we have here is individual variability, the leaves of these trees have an almost identical shape with the one in the sample with 50 specimens, this being proof that the leaves of *B. oycoviensis* differ decidedly and distinctly from the leaves of *B. verrucosa.* Consequently, to the characteristic of *B. oycoviensis* published in 1928, there is now added one more good character concerning leaf shape.

2. Betula obscura Kotula

B. obscura is the species first described by Fiek (1887) on the basis of specimens found by Kotula, a notary from Cieszyn. Particulars pertaining to this species are given by Stecki, Ślósarz and Wiertelak (1928), and by Kobendza (1935). The distribution of this birch is shown on the map in Fig. 2.

For the purpose of studying the leaf shape of this species, the author had at her disposal two local samples. One of them was from the Kampinos Forest, from a station discovered and decribed in 1935 by Kobendza, and the other from the Carpathian foreland, from Dobra near Limanowa. The author encountered difficulties in preparing a sample of 50 trees from the whole distributional area, inasmuch as she had not enough material from Silesia or Great Poland. She only succeeded in preparing a sample composed of 50 leaves collected from 25 trees, and consequently incomplete. However, although the author's sample had only one-half of the normal number of leaves and did not include the whole distributional area of *B. obscura*, the line of shape of its leaves, drawn in Diagram I of Fig. 3, shows that these leaves do not differ in any detail from the leaves of *B. verrucosa* apart from the fact that they are slightly smaller (characters No 1—6). This is in accordance with the observation made by Stecki and described in his above mentioned work. Very interesting are the lines of size and shape of two local samples shown in Diagram II of Fig. 3. They are almost identical and, furthermore, their relationship to the comparative unit is the same as that of local samples of *B. verrucosa* possessing leaves with a wide base angle, described in Part I of this work (p. 190—193, Fig. 5). This gives the impression that *B. obscura* has the same local variability as *B. verrucosa*, and that it is to be expected when a larger number of samples is investigated that one will also discover samples with an acute base angle, analogical to

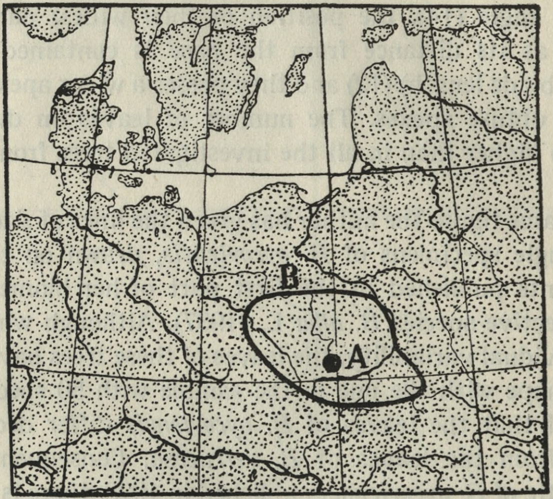

Fig. 2. A. Locality of *B. oycoviensis* Bess in the Ojców Valley near Kraków. B. The area of distribution of *B. obscura* Kot. in Poland.

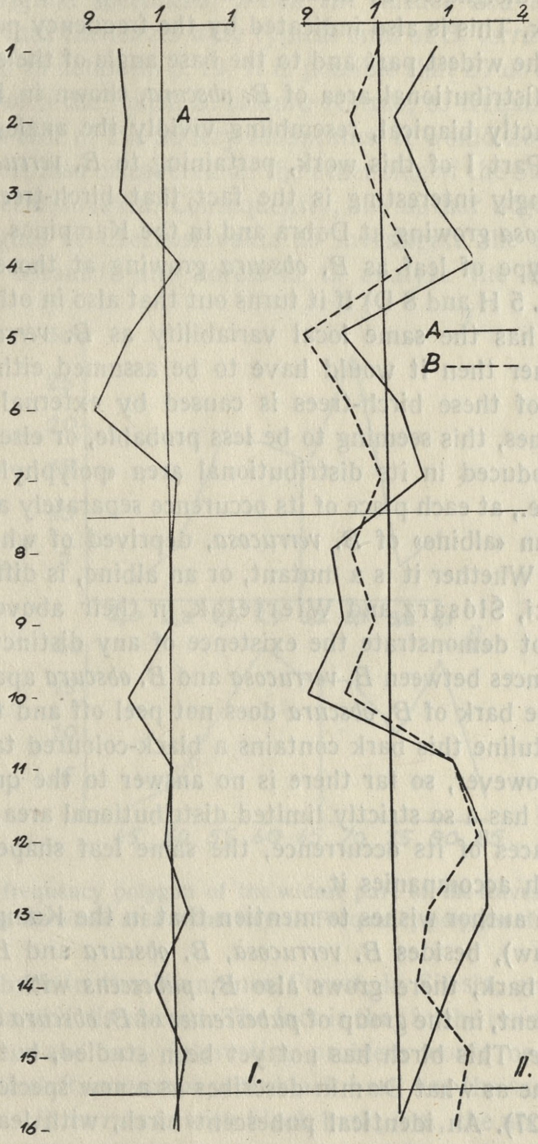

Fig. 3. I. Comparison of the sample of *B. obscura* from its whole distributional area (polygonal line) with the sample of *B. verrucosa* from Europe (straight line). II. A—B lines of size and shape of leaves of two local samples of *B. obscura* in comparison with *B. verrucosa*.

the samples disignated by means of thicker lines in Fig. 5, in Part I of this work. This is also indicated by the frequency polygons pertaining to the widest part and to the base angle of the sample from the whole distributional area of *B. obscura*, shown in Fig. 4, both being distinctly biapical, resembling vividly the analogical Figs 9 and 10 in Part I of this work, pertaining to *B. verrucosa*.

Exceedingly interesting is the fact that birch-trees belonging to *B. verrucosa* growing at Dobra and in the Kampinos Forest have the same type of leaf as *B. obscura* growing at those place (cf. Part I, Figs. 5 H and 8 D) If it turns out that also in other localities *B. obscura* has the same local variability as *B. verrucosa* which grow together then it would have to be assumed either that the leaf shape of these birch-trees is caused by external conditions, ecological ones, this seeming to be less probable, or else that *B. obscura* is produced in its distributional area «polyphyletically», so to speak, i. e., at each place of its occurence separately as a mutant, or else as an «albino» of *B. verrucosa*, deprived of white pigment in its bark. Whether it is a mutant, or an albino, is difficult to decide. Stecki, Ślósarz and Wiertelak in their above-mentioned work did not demonstrate the existence of any distinct morphological differences between *B. verrucosa* and *B. obscura* apart from the fact that the bark of *B. obscura* does not peel off and that instead of white betuline this bark contains a black-coloured tannin, phlobaphene. However, so far there is no answer to the question why this mutant has a so strictly limited distributional area, and at the separate places of its occurrence, the same leaf shape as *B. verrucosa* which accompanies it.

Here the author wishes to mention that in the Kampinos Forest (near Warsaw), besides *B. verrucosa*, *B. obscura* and *B. pubescens* with white bark, there grows also *B. pubescens* with black bark, i. e., equivalent, in the group of *pubescentes* of *B. obscura* in the group of *verrucosae*. This birch has not yet been studied, but in no case is it the same as what Domin describes as a new species: *B. atrata* Domin (1927). An identical pubescent birch, with leaves typical of *B. pubescens* but with black bark, has been collected by Wróblewski in Silesia. The species *B. atrata* Domin must be regarded critically. From the description and latin diagnosis given by Domin it follows that this birch has nude branchlets and that it differs from *B. verrucosa* only in the shape of its leaves. However, on

Domin's original specimens, which the author had at hand she ascertained that the leaves were typical ones of *B. verrucosa*, a matter discussed in detail on p. 19. It is possible that *B. atrata* Domin, is identical with the Polish *B. obscura* Kotula, so frequent in Silesia to the north-east of the Sudety Mountains. It would not be strange at all if it were also discovered on the other side of the Sudety Mountains, in Czechoslovakia. Consequently, the author urges her botanical colleagues in Czechoslovakia to investigate the matter, and also Polish botanists and foresters to analyze the *B. pubescens* with black bark in the Kampinos Forest, in Silesia, and also elsewhere in Poland if discovered. The fact is that in the issue concerning birches a new and very interesting problem would be added if it turned out that *B. pubescens* with black bark has the same distributional area as *B. verrucosa* with black bark, i. e., the above-desribed *B. obscura* Kotula, and that it also contains phlobaphene in its bark.

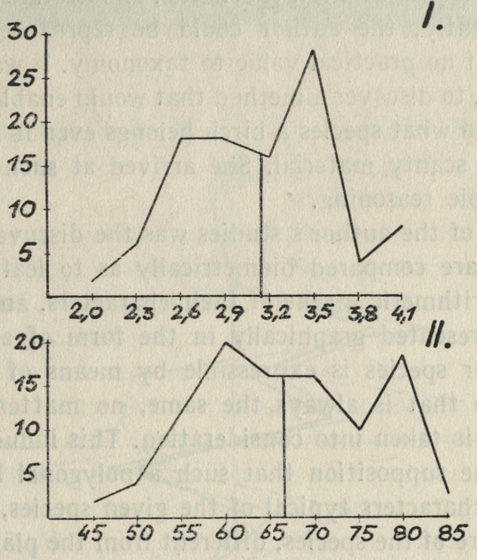

Fig. 4. I. The frequency polygon of the widest part of the leaves of *B. obscura* in the sample of the whole area of this tree. II. Frequency polygon of its base angle.

As far as the taxonomic rank of *B. obscura* Kotula is concerned, the author is of the opinion that in the present state of our knowledge both those who consider it to be a small species, and those who regard it as a variety of the species *B. verrucosa* Ehrh. are right.

3. Determination on the basis of a single leaf

The analysis of the collective species *B. alba* L., described in Parts I, II, and III of the author's work was based on abundant material. Indeed, the author mostly utilized samples composed of 100 leaves from 50 trees and from the largest number of localities possible. Such procedure is the proper one in a monographic elaboration of certain genera or species. However, it is impossible to adopt it in everyday taxonomic practice, when one must make decisions on the basis of a few specimens, or sometimes even a single one. Consequently, the author could be reproached that works like hers are of no practical value to taxonomy. It was the author's aim, therefore, to discover a method that would enable a taxonomist to determine to what species a birch belongs even in cases in which he deals with scanty material. She arrived at such a method by means of simple reasoning.

The result of the author's studies was the discovery that if two birch species are compared biometrically as to leaf shape on the basis of the arithmetic means of their characters, and one of these species is represented graphically in the form of a straight line, then the other species is expressible by means of a chacteristic polygonal line that is always the same, no matter what sample of the species is taken into consideration. This induced the author to advance the supposition that such a polygonal line represente a relation of characters typical of the given species, i. e., the plan of leaf structure of the species, different from the plan of leaf structure of the other species which served here as a comparative unit. Consequently, this line is termed by the author the line of size and shape of leaves of species A in comparison with species B.

If this is so, and if the line of shape of leaves of a given species is a graphic representation of the plan of their structure, then each leaf must be built according to this plan. Therefore, not only on the basis of the arithemtic means from 100 leaves, but also on the basis of measurements of any average leaf should we be able to draw a similar line of shape. And actually, such is the case. This will be demonstrated by means of several examples.

In the upper part of Fig. 5 there is a photograph of three leaves of *B. verrucosa*, all three of them taken from vegetative dwarf shoots. The middle leaf is taken at random from among frequently

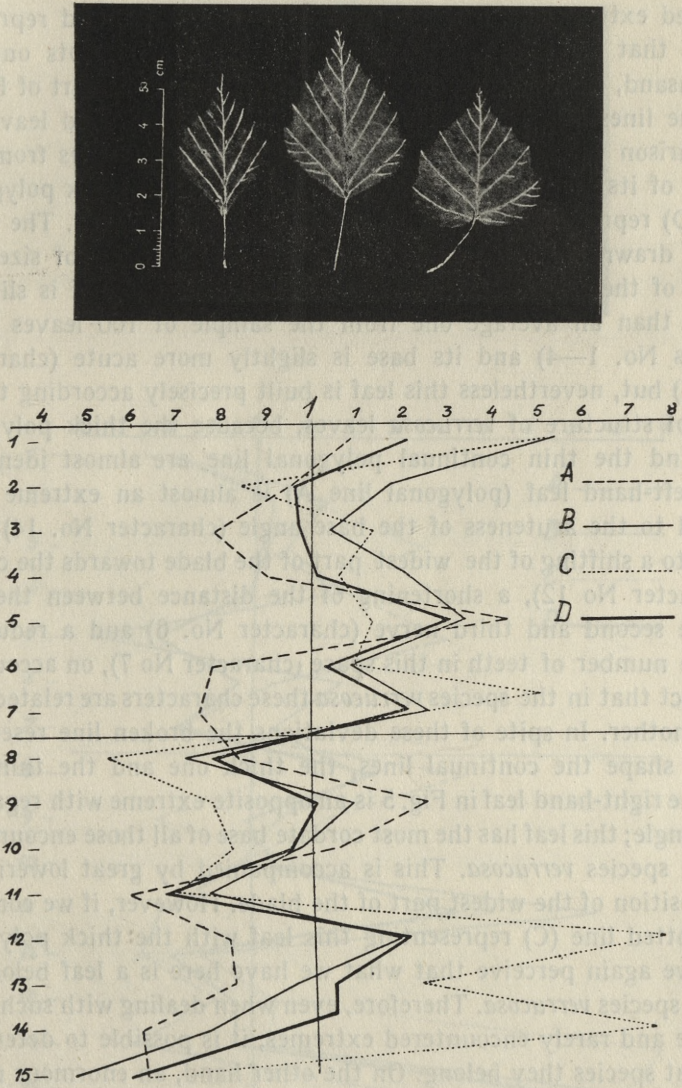

Fig. 5. Above: 3 leaves of *B. verrucosa*; the middle one is frequently encountered, the left and right ones are extremes. Below: the lines of size and shape of these leaves in comparison with *B. pubescens* (straight line): A. the left leaf), B. the middle leaf, C. the right leaf, D. line of size and shape of leaves of *B. verrucosa* from the whole of Europe.

encountered leaves. The two leaves at the sides are intentionally selected extremes with regard to certain characters and represent leaves that are encountered on vegetative dwarf shoots once in a thousand, or perhaps even more rarely. In the lower part of Fig. 5 are the lines of size and shape of the above-mentioned leaves in comparison with a sample of 100 leaves of *B. pubescens* from the whole of its distributional area (straight line.) The thick polygonal line (D) represents a sample of 100 trees of *B. verrucosa.* The more finely drawn continual polygonal line (B) is the line of size and shape of the middle leaf. It is noticeable that this leaf is slightly larger than an average one from the sample of 100 leaves (characters No. 1—4) and its base is slightly more acute (character No 14) but, nevertheless this leaf is built precisely according to the plan of structure of *verrucosa* leaves, because the thick polygonal line and the thin continual polygonal line are almost identical. The left-hand leaf (polygonal line A) is almost an extreme with regard to the acuteness of the base angle (character No. 14); this leads to a shifting of the widest part of the blade towards the centre (character No 12), a shortening of the distance between the tips of the second and third nerve (character No. 6) and a reduction of the number of teeth in this space (character No 7), on account of the fact that in the species *verrucosa* these characters are related with one another. In spite of these deviations the broken line resembles in its shape the continual lines, the thick one and the thin one.

The right-hand leaf in Fig. 5 is an opposite extreme with regard to base angle; this leaf has the most cordate base of all those encountered in the species *verrucosa.* This is accompanied by great lowering of the position of the widest part of the blade. However, if we compare the dotted line (C) representing this leaf with the thick polygonal line, we again perceive that what we have here is a leaf belonging to the species *verrucosa.* Therefore, even when dealing with such most remote and rarely encountered extremes, it is possible to determine to what species they belong. On the other hand, an enormous majority of leaves of *B. verrucosa* will give lines of shape approximately the same as the thick polygonal line in Fig. 5. This applies both to leaves on dwarf shoots without catkins and to those on other shoots.

Fig. 6 has been prepared in the same manner. The middle leaf is a frequently encountered leaf type of *B. pubescens.* The leaves

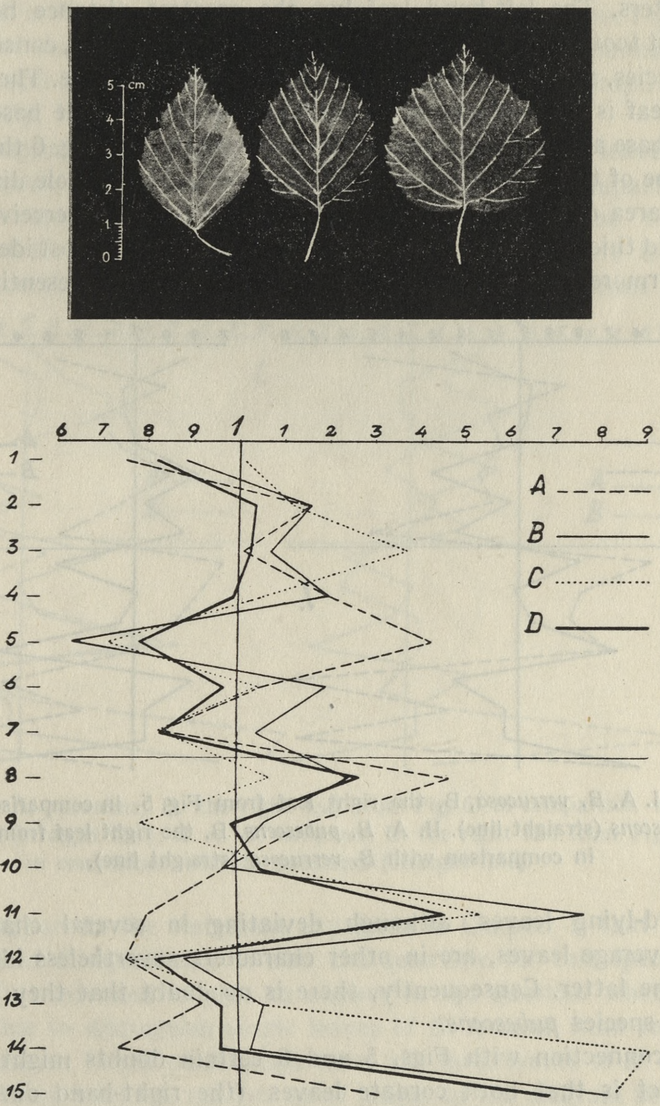

Fig. 6. Above: 3 leaves of *B. pubescens*; the middle one is frequently encountered, the left and right ones are extremes. Below: the lines of size and shape of these leaves in comparison with *B. verrucosa*: A. the left leaf, B. the middle leaf, C. the right leaf, D. line of size and shape of leaves of B. *pubescens* from the whole of Europe.

on the sides are rarely encountered extremes with regard to various characters. The left-hand leaf has the greatest distance between the first tooth and base encountered in *B. pubescens*, and, considering the species, a low position of the widest part of the blade. The right-hand leaf is an example of a broad leaf with a cordate base (maximal base angle). If we compare in the lower part of Fig. 6 the lines of shape of these three leaves with the line from the whole distributional area of *B. pubescens* (thick polygonal line) we perceive that the solid thick line (D) and the thin solid line (B) are almost identical. Furthermore, the broken line and the dotted one, representing the outward-lying leaves, although deviating in several characters from average leaves, are in other characters nevertheless identical with the latter. Consequently, there is no doubt that they belong to the species *pubescens*.

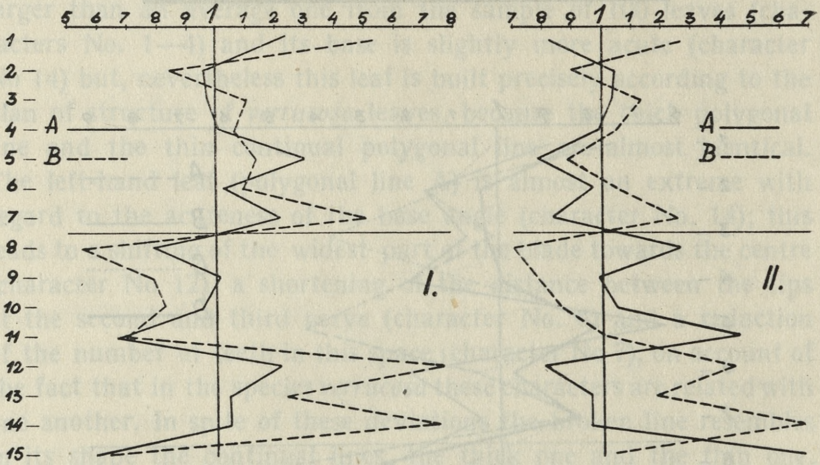

Fig. 7. I. A. *B. verrucosa*, B. the right leaf from Fig. 5. in comparison with *B. pubescens* (straight line). II. A. *B. pubescens*, B. the right leaf from Fig. 5. in comparison with *B. verrucosa* (straight line).

In connection with Figs. 5 and 6 certain doubts might arise. The fact is that both cordate leaves (the right-hand ones) are similar to each other. It might be suspected, therefore, that their lines of shape approximate those of both species: *verrucosa* and *pubescens*. These doubts are eliminated by Figs. 7 and 8. In Fig. 7 we notice that the right-hand leaf from Fig. 5 has a line of shape similar to that of *B. verrucosa* (Graph I), and not to that of *B. pu-*

bescens (Graph II); consequently, it is a leaf of *B. verrucosa*. On the other hand, in Fig. 8 we perceive that the right-hand leaf from Fig. 6 has a line of shape which is the same as that of the species *pubescens* (Graph I), and different from that of the species *verrucosa* (Graph II); consequently, it is certainly a leaf of *B. pubescens*.

In the upper part of Fig. 9 there is a photograph of three leaves of *B. oycoviensis*. Their lines of shape, drawn below, in comparison with *B. verrucosa* (straight line) are so similar to the line from 50 trees of *B. oycoviensis* (thick polygonal line) that there is no doubt concerning the species to which they belong. This lends additional support to the statement expressed in Chapter 1, that the leaf shape of *B. oycoviensis* is also characteristic for this species.

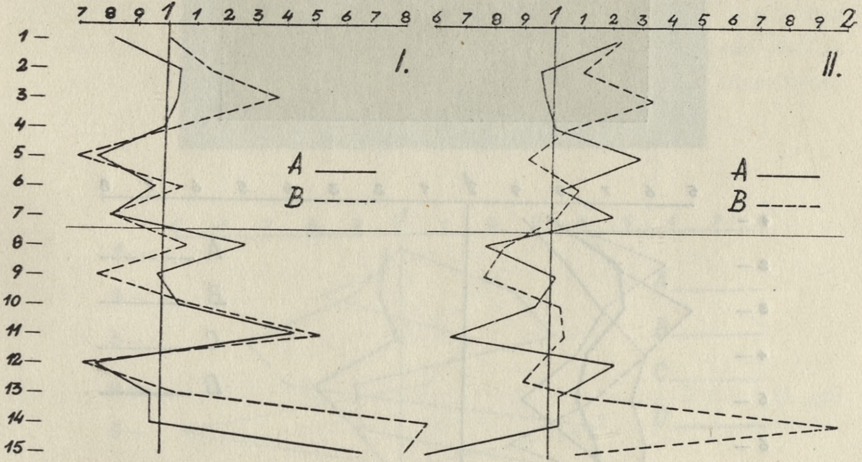

Fig. 8. I. A. *B. pubescens*, B. the right leaf from Fig. 6. in comparison with *B. verrucosa* (straight line); II. A. *B. verrucosa*, B. the right leaf from Fig. 6. in comparison with *B. pubescens* (straight line).

Fig. 10 is intended to give an answer to the question whether it is possible to distinguish single leaves of *B. tortuosa* from those of *B. pubescens*. In the photograph there are three leaves of *B. tortuosa*, differing from one another as to shape. In the diagram below the photograph are the lines of shape of these leaves (polygonal lines) in comparison with *B. pubescens* from all of its distributional area (straight line). In characters No. 1—10 we notice nothing characteristic apart from the fact that two leaves have a smaller number of pairs of lateral nerves than the mean figure in *B. pubescens*.

In characters No. 11—15, however, there becomes distinctly noticeable an acute axil of the second nerve (character No 13), so characteristic of *B. tortuosa*, and a comparatively wide apex angle (character No. 15). Such a shape of the polygonal line is possessed by c. 80% of leaves of *B. tortuosa* when compared with *B. pubescens*.

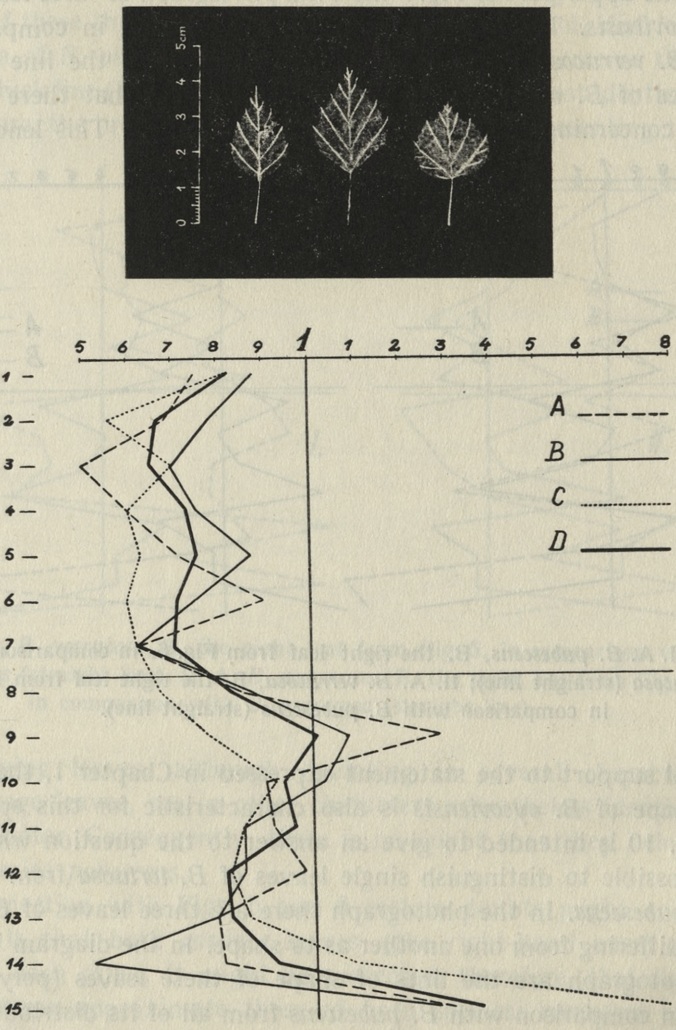

Fig. 9. Above: 3 different leaves of *B. oycoviensis* Bess. Below: A. B. C. their line of shape, D. the line of shape of *B. oycoviensis* from its whole area. The straight line represents *B. verrucosa*.

Consequently, having at one's disposal herbarium specimens of birches, it is possible in a large percentage of cases to determine on the basis of the above-mentioned characters that they belong to the species *B. tortuosa*. A small number of pairs of lateral nerves inde-

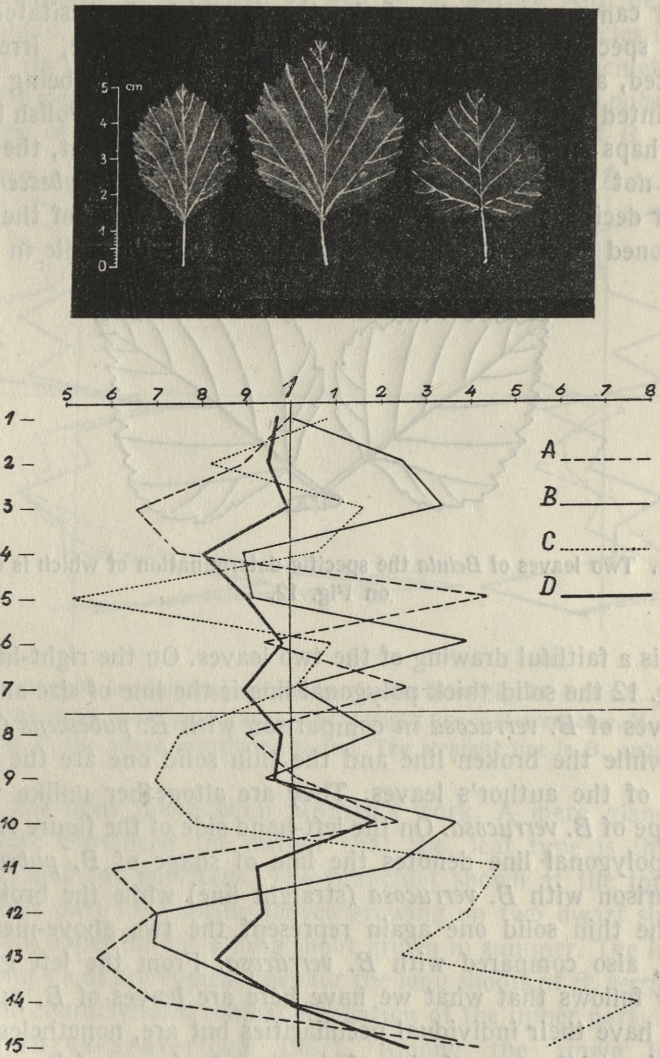

Fig. 10. Above: 3 different leaves of *B. tortuosa*. Below: A. B. C. their line of shape, D. the line of shape of *B. tortuosa* from its whole area. The straight line represents *B. pubescens.*

pendent of leaf size is also a good indication in such cases (cf. Fig. 14 in Part II of this work).

In Figs 11 and 12 we have an example of practical application of the line of shape for the purpose of determining the birch species. Sorting the birch leaves in her herbarium material the author came across a dwarf shoot over which she hesitated as to what species it belonged. The leaves were nude, irregularly dentated, and their shape was such that, in spite of being so well acquainted with the leaf variability of the common Polish birches, or perhaps on account of being well acquainted with it, the author could not determine whether it was *verrucosa* or *pubescens*. The author decided, therefore, to draw the lines of shape of the above-mentioned leaves. This graph is shown in Fig. 12, while in Fig. 11 there is a faithful drawing of the two leaves. On the right-hand side of Fig. 12 the solid thick polygonal line is the line of size and shape of leaves of *B. verrucosa* in comparison with *B. pubescens* (straight line) while the broken line and the thin solid one are the lines of shape of the author's leaves. They are altogether unlike the line of shape of *B. verrucosa*. On the left-hand side of the figure the thick solid polygonal line denotes the line of shape of *B. pubescens* in comparison with *B. verrucosa* (straight line) while the broken line and the thin solid one again represent the two above-mentioned leaves, also compared with *B. verrucosa*. From the left graph it clearly follows that what we have here are leaves of *B. pubescens* which have their individual peculiarities but are, nonetheless, built according to the general plan of structure for leaves of *B. pubescens*, inasmuch as the solid polygonal line, the broken one, and the thin solid one are very much alike.

Fig. 11. Two leaves of *Betula* the specific determination of which is explained on Fig. 12.

Another example of practical application concernes the species *B. atrata* Domin (see page 9). The author of this new species, Domin (1927), writes of this birch, discovered on the Bohemian-Moravian upland and distinguished by its dark bark, that it very much resembles *B. verrucosa*, as well as *B. obscura* Kot. desribed from Poland, but that it differs from the two latter species in the shape of its leaves. On this basis, in spite of nude branchlets and leaves, Domin includes it in the broadly conceived species *pubescens*, as a tribe of the latter. Fig. 13, showing the leaves of *B. atrata* Domin. has been taken from Domin's work. A mere glance at this picture convinced the author that the leaf type is that of *verrucosa*, and not *pubescens*. The branchlet shown at the top has in its lower part four spring leaves growing on two dwarf shoots, while in its upper part is a long shoot grown in summer. The leaves on the long shoot are typical ones for the long shoots of *B. verrucosa*, with their characteristic conical elongation of the upper part of the blade (Jentys-Szaferowa 1937). Among the single leaves shown in the lower part of Fig. 13, with regard to which it is known from what shoots they were taken, there is not one which could not be encountered in the species *verrucosa*. Thanks to Prof. Do-

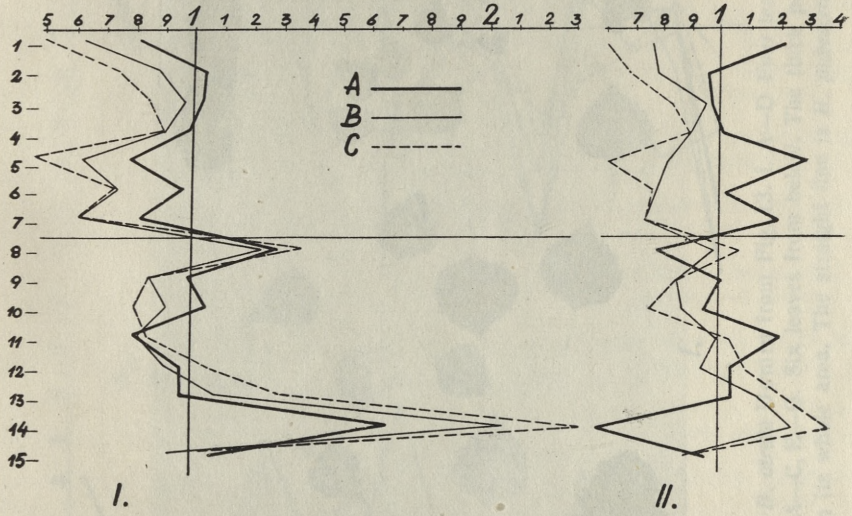

Fig. 12. The lines of size and shape of the two leaves from Fig. 11. I. A : *B. pubescens*, B. C. the above mentioned leaves. The straight line is *B. verrucosa*. II. *A* : *B. verrucosa*, B. C. the above mentioned leaves. The straight line is *B. pubescens*.

min's kindness the author received from Prague an original specimen, a photograph of which is shown in Fig. 13. Consequently, the author was able to measure the leaves on the specimen and prepare their lines of shape, adopting *B. pubescens* as a comparative unit. On the left-hand side of Fig. 14 are the lines of size and shape of four leaves from spring dwarf shoots of the upper branchlet shown on Fig. 13. Comparing them with the line of shape of leaves of *B. verrucosa* (thick polygonal line), it is clearly noticeable that they are small leaves of the species *verrucosa* and that their most important individual character is in two cases a relatively short petiole (characters No. 1 and 8). The graph on the right-hand side of Fig. 14

Fig. 13. *B. atrata* Domin. The original specimen from the Herbarium of prof. Domin.

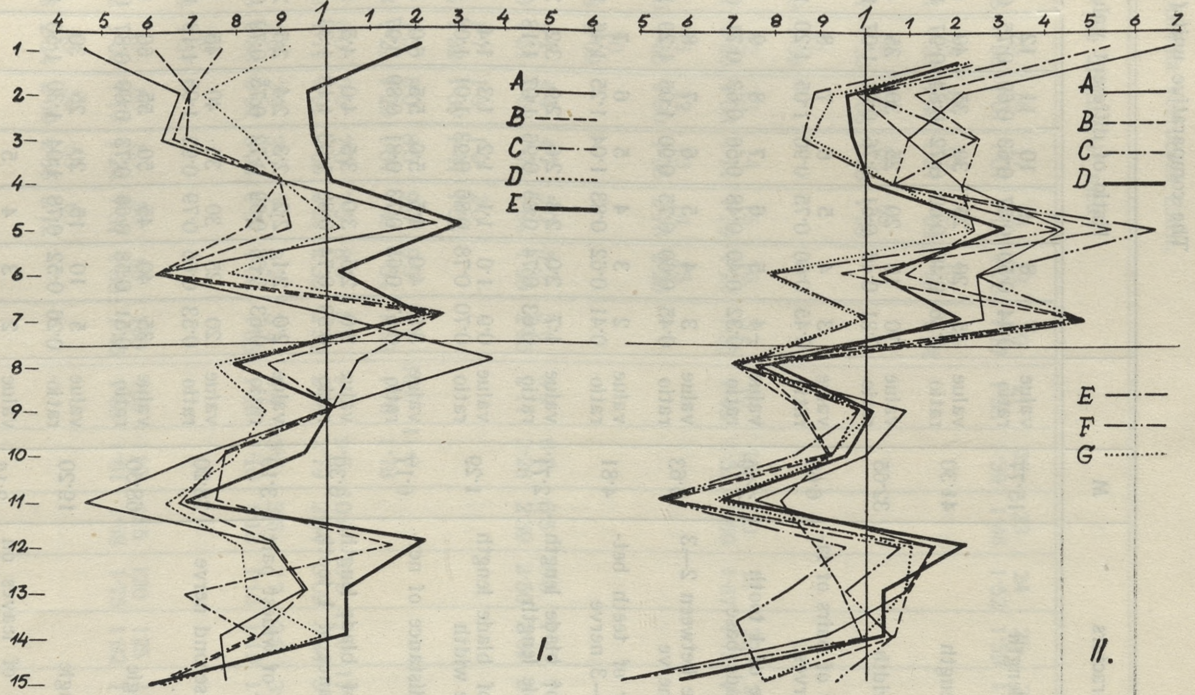

Fig. 14. Lines of shape of leaves of *B. atrata* Domin from Fig. 13. I. A—D Four leaves from the spring dwarf shoots from the upper branch, II. A—C, E—G. Six leaves from below. The thick polygonal line represents *B. verrucosa* from its whole area. The straight line is *B. pubescens.*

TA

The comparative unit (M)

Characters	M		Ratio of different values						
1. Petiole length	15·77	value ratio	7 0·44	8 0·50	9 0·57	10 0·63	11 0·69	12 0·75	13 0·82
2. Blade length	41·30	value ratio	15 0·36	20 0·48	25 0·60	30 0·72	35 0·84	40 0·97	45 1·09
3. Blade width	32·65	value ratio	10 0·31	15 0·46	20 0·61	25 0·76	30 0·91	35 1·07	40 1·22
4. Number of pairs of lateral nerves	6·68	value ratio	3 0·45	4 0·60	5 0·75	6 0·90	7 1·05	8 1·20	9 1·35
5. Distance of 1 tooth from blade base	12·59	value ratio	4 0·32	5 0·40	6 0·48	7 0·56	8 0·63	9 0·71	10 0·79
6. Distance between 2—3 lateral nerve	6·63	value ratio	3 0·45	4 0·60	5 0·75	6 0·90	7 1·05	8 1·20	9 1·35
7. Number of teeth between 2—3 nerve	4·81	value ratio	2 0·41	3 0·62	4 0·83	5 1·04	6 1·25	7 1·46	8 1·67
8. Ratio of blade length to petiole length	2·71	value ratio	1·7 0·63	2·0 0·74	2·3 0·85	2·6 0·96	2·9 1·07	3·2 1·18	3·5 1·29
9. Ratio of blade length to blade width	1·29	value ratio	0·9 0·70	1·0 0·78	1·1 0·86	1·2 0·93	1·3 1·01	1·4 1·08	1·5 1·16
10. Mean distance of nerves	6·17	value ratio	3·5 0·57	4·0 0·65	4·5 0·73	5·0 0·81	5·5 0·89	6·0 0·97	6·5 1·05
11. Ratio of blade length to distance of 1 tooth	3·39	value ratio	2·0 0·59	2·5 0·74	3·0 0·88	3·5 1·03	4·0 1·18	4·5 1·33	5·0 1·47
12. Position of widest part	3·19	value ratio	2·0 0·63	2·1 0·66	2·2 0·69	2·3 0·72	2·4 0·75	2·5 0·79	2·6 0·82
13. Axil of second nerve	38·20	value ratio	20 0·53	25 0·66	30 0·79	35 0·92	40 1·05	45 1·18	50 1·31
14. Base angle	68·50	value ratio	35 0·51	40 0·58	45 0·66	50 0·73	55 0·80	60 0·87	65 0·95
15. Apex angle	19·20	value ratio	5 0·26	10 0·52	15 0·78	20 1·04	25 1·30	30 1·56	35 1·82
16. Number of leaves on dwarf shoot	2·16	value ratio	2 0·92	3 1·36	4 1·85	5 2·31			

BLE I

is Betula verrucosa Ehrh.

to the arithmetic mean														
14 0·88	15 0·95	16 1·01	17 1·07	18 1·14	19 1·20	20 1·27	21 1·33	22 1·39	23 1·46	24 1·52	25 1·59	26 1·65	27 1·71	28 1·78
50 1·21	55 1·33	60 1·45	65 1·57	70 1·69	75 1·81	80 1·94								
45 1·37	50 1·53	55 1·68	60 1·83	65 1·99										
10 1·50	11 1·65													
11 0·87	12 0·95	13 1·03	14 1·11	15 1·19	16 1·27	17 1·35	18 1·43	19 1·51	20 1·59	21 1·67	22 1·75	23 1·83	24 1·91	25 1·99
10 1·50	11 1·65	12 1·80	13 1·95	14 2·10	15 2·25									
9 1·88	10 2·09													
3·8 1·40	4·1 1·51	4·4 1·62	4·7 1·73	5·0 1·84	5·3 1·95	5·6 2·06	5·9 2·17	6·2 2·28	6·5 2·39	6·8 2·50	7·1 2·61	7·4 2·72		
1·6 1·24	1·7 1·32	1·8 1·39	1·9 1·47	2·0 1·55										
7·0 1·13	7·5 1·21	8·0 1·29	8·5 1·37	9·0 1·45	9·5 1·54	10·0 1·62	10·5 1·70	11·0 1·78						
5·5 1·62	6·0 1·77	6·5 1·91	7·0 2·06	7·5 2·21	8·0 2·35	8·5 2·50	9·0 2·65	9·5 2·79	10·0 2·94	10·5 3·09	11·0 3·24	11·5 3·38	12·0 3·53	12·5 3·68
2·7 0·85	2·8 0·88	2·9 0·91	3·0 0·94	3·1 0·97	3·2 1·00	3·3 1·03	3·4 1·06	3·5 1·10	3·6 1·13	3 7 1·16	3·8 1·19	3·9 1·22	4·0 1·25	4·1 1·28
55 1·44	60 1·57													
70 1·02	75 1·09	80 1·17	85 1·24	90 1·31	95 1·38	100 1·46	105 1·53	110 1·61	115 1·68	120 1·75	125 1·82	130 1·90		
40 2·08	45 2·34	50 2·60	55 2·86	60 3·12										

TA

The comparative unit M

Characters	M		Ratio of different values								
1. Petiole length	12·98	value ratio	4 0·31	5 0·39	6 0·46	7 0·54	8 0·62	9 0·69	10 0·77	11 0·85	12 0·92
2. Blade length	42·90	value ratio	15 0·35	20 0·46	25 0·58	30 0·70	35 0·81	40 0·93	45 1·05	50 1·17	55 1·28
3. Blade width	33·55	value ratio	10 0·30	15 0·45	20 0·60	25 0·75	30 0·89	35 1·04	40 1·19	45 1·34	50 1·49
4. Number of pairs of lateral nerves	6·63	value ratio	3 0·45	4 0·60	5 0·75	6 0·90	7 1·06	8 1·21	9 1·36	10 1·51	11 1·66
5. Distance of 1 tooth from blade base	9·71	value ratio	2 0·21	3 0·31	4 0·41	5 0·51	6 0·62	7 0·72	8 0·82	9 0·92	10 1·03
6. Distance between 2—3 lateral nerve	6·41	value ratio	2 0·31	3 0·47	4 0·62	5 0·78	6 0·94	7 1·09	8 1·25	9 1·40	10 1·56
7. Number of teeth between 2—3 nerve	3·98	value ratio	2 0·50	3 0·75	4 1·01	5 1·26	6 1·51	7 1·76	8 2·01	9 2·26	10 2·51
8. Ratio of blade length to petiole length	3·44	value ratio	1·7 0·49	2·0 0·58	2·3 0·67	2·6 0·75	2·9 0·84	3·2 0·93	3·5 1·02	3·8 1·10	4·1 1·19
9. Ratio of blade length to blade width	1·28	value ratio	0·8 0·63	0·9 0·70	1·0 0·78	1·1 0·86	1·2 0·94	1·3 1·01	1·4 1·09	1·5 1·17	1·6 1·25
10. Mean distance of nerves	6·51	value ratio	3·5 0·54	4·0 0·61	4·5 0·69	5·0 0·77	5·5 0·85	6·0 0·92	6·5 1·00	7·0 1·08	7·5 1·15
11. Ratio of blade length to distance of 1 tooth	4·96	value ratio	2·0 0·40	2·5 0·50	3·0 0·61	3·5 0·71	4·0 0·81	4·5 0·91	5·0 1·01	5·5 1·11	6·0 1·21
12. Position of widest part	2·60	value ratio	1·7 0·65	1·8 0·69	1·9 0·73	2·0 0·77	2·1 0·81	2·2 0·84	2·3 0·88	2·4 0·92	2·5 0·96
13. Axil of second nerve	36·65	value ratio	20 0·55	25 0·68	30 0·82	35 0·95	40 1·09	45 1·23	50 1·36	55 1·50	60 1·64
14. Base angle	65·75	value ratio	35 0·53	40 0·61	45 0·68	50 0·76	55 0·84	60 0·91	65 0·99	70 1·06	75 1·14
15. Apex angle	32·30	value ratio	5 0·15	10 0·31	15 0·46	20 0·62	25 0·77	30 0·93	35 1·09	40 1·24	45 1·39
16. Number of leaves of dwarf shoot	2·28	value ratio	2 0·88	3 1·32	4 1·75	5 2·19					

BLE II

is Betula pubescens Ehrh.

to the arithmetic mean

13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1·00	1·08	1·15	1·23	1·31	1·38	1·46	1·54	1·62	1·69	1·77	1·85	1·92	2·00	2·08	2·15
60	65	70	75	80											
1·40	1·51	1·63	1·75	1·87											
55	60	65	70												
1·64	1·79	1·94	2·09												
11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1·13	1·24	1·34	1·44	1·55	1·65	1·75	1·85	1·96	2·06	2·16	2·26	2·36	2·47	2·57	2·67
11	12	13													
1·72	1·87	2·03													
4·4	4·7	5·0	5·3	5·6	5·9	6·2	6·5	6·8	7·1	7·4	7·7	8·0	8·3	8·6	
1·28	1·37	1·45	1·54	1·63	1·71	1·80	1·89	1·97	2·06	2·15	2·24	2·33	2·41	2·50	
1·7	1·8	1·9	2·0												
1·33	1·41	1·48	1·56												
8·0	8·5	9·0	9·5	10·0	10·5	11·0									
1·23	1·31	1·39	1·46	1·54	1·62	1·69									
6·5	7·0	7·5	8·0	8·5	9·0	9·5	10·0	10·5	11·0	11·5	12·0	12·5	13·0	13·5	
1·31	1·41	1·51	1·61	1·71	1·81	1·92	2·02	2·12	2·22	2·32	2·42	2·52	2·62	2·72	
2·6	2·7	2·8	2·9	3·0	3·1	3·2	3·3	3·4	3·5	3·6	3·7	3·8	3·9	4·0	4·1
1·00	1·04	1·08	1·11	1·15	1·19	1·23	1·27	1·30	1·34	1·38	1·42	1·46	1·49	1·53	1·57
80	85	90	95	100	105	110	115	120	125	130	135				
1·22	1·29	1·37	1·44	1·52	1·60	1·67	1·75	1·82	1·90	1·97	2·05				
50	55	60													
1·55	1·71	1·86													

shows the lines of size and shape of six single leaves from Fig. 13. Here also there is no doubt whathever that these leaves are built according to the same plan of structure as the leaves of *B. verrucosa* (thick polygonal line), although each leaf has some individual character of its own as, for instance, relatively great width, high position of the widest part, etc. This is proof, together with the remarks in the previous chapter, that inclusion of the birch with dark bark from the Bohemian-Moravian upland on the basis of leaf shape in the broadly conceived species *pubescens* is incorrect. Therewith eliminated are grounds for regarding it as separate and new species.

It must be added here, that Fiek (1887), desribing the original specimens of *B. obscura* found by Kotula in Silesia writes: «Blätter rundlich bis rombisch-eiförmig, kurz zugespitzt, an den Seitenecken abgerundet, doppelt gesägt mit kurzen Zähnen zweiter Ordnung, völlig kahl... Habituell unterscheidet sich diese Art von den baumartigen Birken nicht, sie stimmt in der Form der Blätter mit *B. pubescens*, in dem Mangel jeglicher Bekleidung auch der jungen Zweige und Blätter mit *B. verrucosa* überein...» It agrees with the above mentioned description of Domin. But as *B. obscura* has in other parts of its area in Poland no roundish leaves, it is perhaps a local form characteristic of Silesia. We have here one more problem to be solved concerning *B. obscura*.

For the purpose of facilitating the drawing of the line of shape of single birch-leaves the author has prepared two tables. Table I is employed when we wish to compare any leaves with the average ones of *B. verrucosa*, and Table II, when the comparative unit is *B. pubescens*. In these tables is shown the relationship displayed by the measurements of single leaves, with regard to various characters, to the arithmetic means of samples of 100 leaves from 50 trees from the European distributional areas of *B. verrucosa* and *B. pubescens*. For instance, if a certain leaf, whose line of shape we wish to draw, has a petiole length of 16 mm, blade length of 30 mm, and blade width of 28 mm, and we wish to compare this leaf with *B. verrucosa*, then we read from Table I for the petiole: 1·01, for blade length: 0·72, for blade width: 0·85. The latter figure is calculated by dividing the difference between 0·76 (width 25 mm) and 0·91 (width 30 mm) by 5 and adding three-fifths of this difference to 0·76. Reading in the manner described above the ratio of all the characters of our leaf to the comparative unit, we at once draw, doing so best on millimetre paper, its line of shape by means of the method by which are prepared all of the author's graphs (see Part I: Table I and Fig. 1). We do the same determining the relationship of our leaf to *B. pubescens* on the basis of Table II. Comparing the lines of shape thus obtained with the lines of shape of the species *verrucosa* and *pubescens* (solid polygonal lines in Fig. 7) we immediately discover to what species the leaf belongs. The drawing of the lines of shape of single birch-leaves with the assistance of Tables I and II is done quickly, and the execution of such a determination on the basis of any fresh or herbarium material will convince one better as to the value of the author's method than the examples quoted by her above.

4. Application of the lines of shape to taxonomic purposes

At the conclusion of her studies the author presents Figs 15—17 which are an attempt to apply the lines of shape to taxonomic units that are higher than species. In Fig. 15 and 16 the two species most common in Europe: *verrucosa* and *pubescens* are adopted by the author as comparative units. In Fig. 15. we perceive that the first three species — *oycoviensis*, *obscura* and *verrucosa* — although their leaves (particularly those of *B. oycoviensis* and *B. verrucosa*) differ from one another by their shape in a decided manner, constitute nonetheless in comparison with the species *pubescens* a similar, leaf type, characteristic of the group *verrucosae*. On the other hand we see in Fig. 16, that the three remaining species, also differing from one another, constitute in comparison with *B. verrucosa* another leaf type, characteristic of the group *pubescentes*. However, if, all these birch species, which according to their leaf shapes may be divided into two distinct groups, are compared with a species from another section, viz., *B. nana*, then the differences between them disappear. This is observable in Fig. 17. We have there five lines of shape, (*B. obscura* is omitted), completely similar to one another for which the author introduces the general term: the line of shape of the collective species *B. alba* L. Moreover, the author supposes that if, instead of taking the species *B. nana* as a comparative unit, we took the mean leaf shape from the whole section *nanae* and if we drew in relation to it the lines of shape of all the species at present

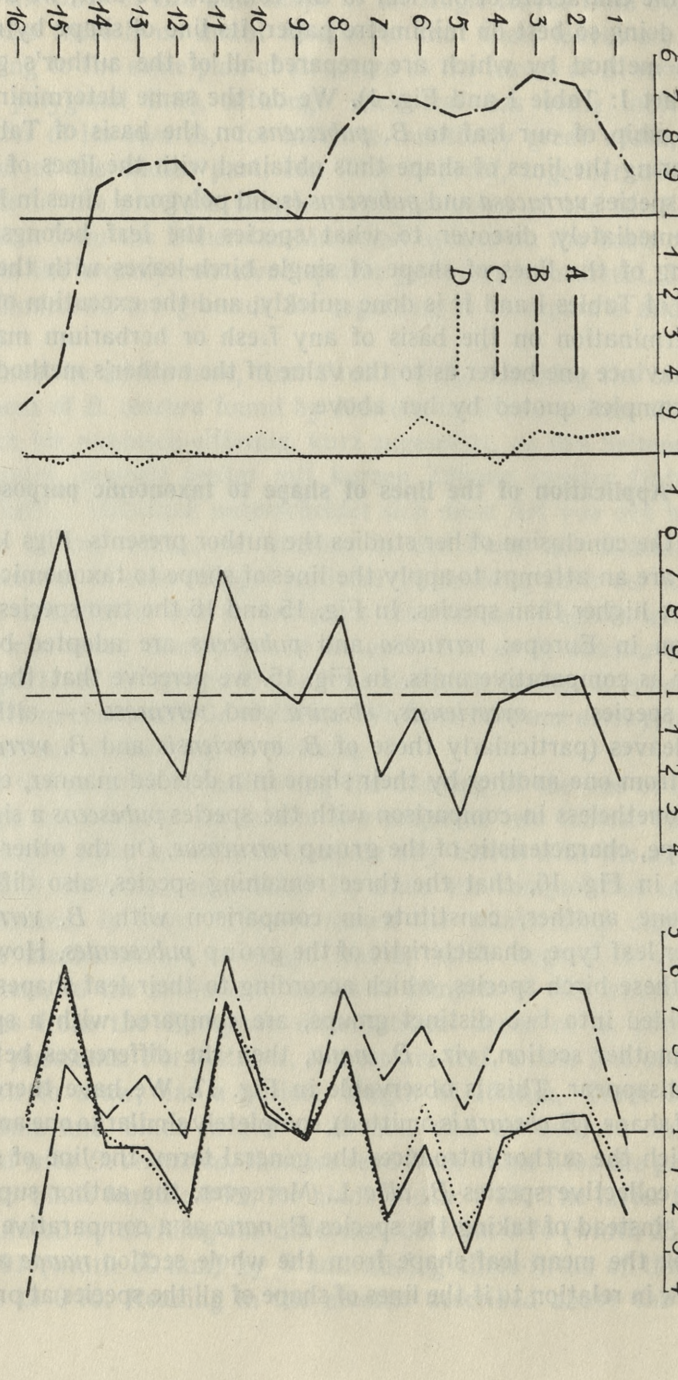

Fig. 15. Lines of size and shape of the group *verrucosae*: A — *verrucosa*, B — *pubescens*, C — *oycoviensis*, D. — *obscura*.

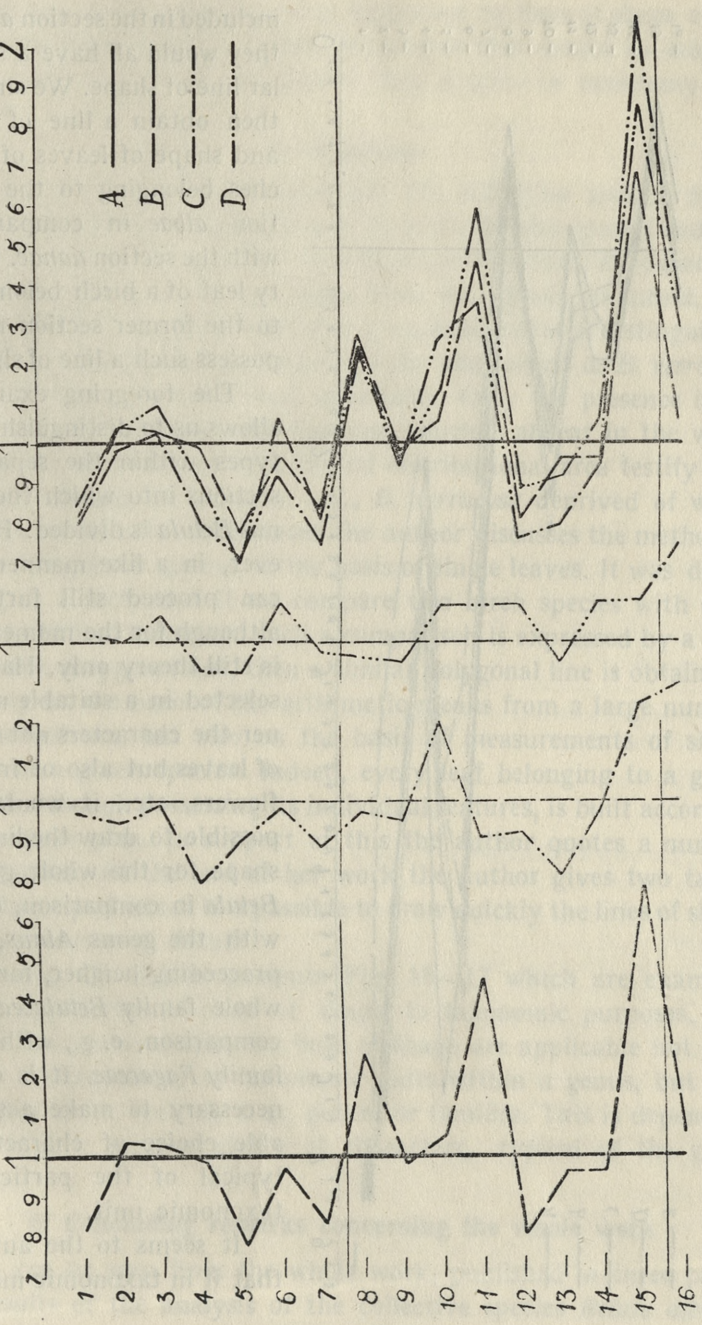

Fig. 16. Lines of size and shape of the group *pubescentes*: A. — *verrucosa*, B. — *pubescens*, C. — *tortuosa*, D. — *carpatica.*

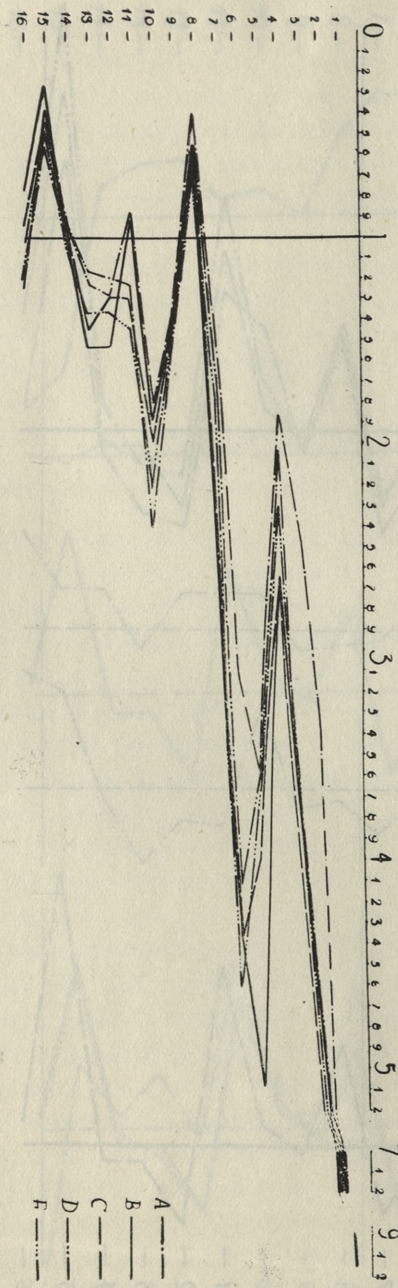

Fig. 17. Lines of size and shape of the leaves of *Betula alba* L. A. — *oycoviensis*, B. — *verrucosa*, C. — *pubescens*, D. — *tortuosa*, E. — *carpatica*. The straight line is *Betula nana*.

included in the section *albae*, they would all have a similar line of shape. We should then obtain a line of size and shape of leaves of birches belonging to the section *albae* in comparison with the section *nanae*. Every leaf of a birch belonging to the former section must possess such a line of shape.

The foregoing example allows us to distinguish leaf types within the separate sections into which the genus *Betula* is divided. However, in a like manner we can proceed still further, although for the moment it is still theory only. Having selected in a suitable manner the characters not only of leaves but also of fruits, flowers, etc., it would be possible to draw the line of shape for the whole genus *Betula* in comparison, e. g., with the genus *Alnus*, or, proceeding heigher, for the whole family *Betulaceae* in comparison, e. g., with the family *Fagaceae*. It is only necessary to make a suitable choice of characters, typical of the particular taxonomic unit.

It seems to the author that if in taxonomic monographs the Latin diagnoses were supported by lines of shape of the given species in relation to another species that would be selected as a comparative unit, we should then acquire in taxonomy new values.

5. Summary

Part III of the work concerning the collective species *Betula alba* L. contains a characteristic of the leaves of two species endemic in Poland: *B. oycoviensis* Bess. and *B. obscura* Kotula. *B. oycoviensis* distinctly differs in its leaf shape from the species *verrucosa*, this being an additional proof that it is a separate and well distinguished species. *B. obscura* Kot. has leaves of the same shape as *B. verrucosa* and the same enigmatic local variability. Only the presence in its bark of a black tannin, phlobaphene, which is absent in the white bark of *B. verrucosa*, and its limited distributional area testify that it is not simply an «albino», i. e., *B. verrucosa* deprived of white betuline in its bark. Afterwards the author discusses the method of determining birch species on the basis of single leaves. It was discovered, as a fact, that if we compare two birch species with each other as to leaf shape and such a comparison is expressed by a characteristic polygonal line, then a similar polygonal line is obtainable not only on the basis of the arithmetic means from a large number of observations, but also on the basis of measurements of single leaves of a given species. Indeed, every leaf belonging to a given species, although possessing its individual features, is built according to the same plan. In support of this the author quotes a number of examples. At the end of her work the author gives two tables with the help of which it is possible to draw quickly the lines of shape of single leaves of *Betula*.

Finally, the author presents Figs 15—17 which are examples of the application of lines of shape to taxonomic purposes. The author is convinced that the lines of shape are applicable not only to the distinguishing of taxonomic units within a genus, but also to higher units, such as, e. g., genera or families. This is dependent only upon a suitable choice of characters, typical of the given unit.

6. Concluding remarks concerning the whole work

As can be seen from the whole work, published in three parts, the results of the analysis of the collective species *Betula alba* L.

on the basis of leaf measurements have a twofold importance. First, the shedding of some light on the problem of the collective species *Betula alba* L., which — in relation to all the existing taxonomic problems — is a quite small one. Secondly, the elaboration of a method of carrying out a comparison of shapes with regard to many characters simultaneously, and of drawing the so-called «lines of shape»; this method may have a broader application. Apart from taxonomy it might be attempted, indeed, to employ this method wherever it is necessary to compare shapes, e. g. in morphology or embryology. Above all, however, this method is applicable in palaeontology when determining the remnants of fossil plants.

The work closes with a tabulation of the numerical results of the most important measurements executed by the author. The more detailled statistics are at the disposal of the specialists in the Botanical Institute of the Jagellonian University in Kraków. The fact is, as the author wrote in Part I of her work, that interpretation is always a discretionary procedure, while measurements are facts which at present the author interprets in the manner presented above, though in the future someone else may interpret them differently.

Botanical Institute of the Jagellonian University, Kraków.
Director: Professor W. **Szafer**.

7. Bibliography

1) **Domin** C.: *Betula atrata* Domin, nova briza z Českomoravské vysociny. Rozpr. České Akad. Tř. II. 36, Praha 1927. — 2) **Fiek** E.: Für das Gebiet neue Arten und Formen. Jahresber. Schles. Gesell. vaterl. Kultur, 314, Wrocław 1888. — 3) **Kobendza** R.: Przyczynek do poznania brzozy czarnej w Polsce. Beitrag zur Kenntnis der Schwarzbirke (*Betula obscura* Kot) in Polen. Annal. Soc. Dendr. Pol. 6, 99—110, Lwów 1935. — 4) **Schur** I. F.: Enumeratio Plantarum Transsilvaniae. Vindobona 1866. — 5) **Stecki** K., **Ślósarz** Z., **Wiertelak** J.: Studia nad brzozą czarną (*Betula obscura* Kot) w Polsce. Studien über *Betula obscura* Kot. in Polen. Roczn. Nauk Roln. Leśn. 19, Poznań 1928. — 6) **Szaferowa** J.: Brzoza ojcowska. *Betula oycoviensis* Bess. Histoire et characteristique de l'espèce. Annal. Soc. Dendr. Pol. 2, 69—81, Lwów 1928. — 7) **Szaferowa** J.: Biometrical studies of the collective species *Betula alba* L. I. Polymorphism of the leaves of birches. Travaux Inst. Forêt. Dom. Pol. Serie A. Nr 40. Warszawa 1937. — 8) **Walas** J.: Drugie stanowisko brzozy ojcowskiej w Polsce. Zweiter Fundort von *Betula oycoviensis* in Polen. Ann. Soc. Dendr. Pol. 6. 93—98, Lwów 1935.

8. Statistics

1. Petiole length

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	8—27	14	15·77±0·44	±4·43
,,	,, ,,	500	1000	5—30	14	15·71±0·11	±3·39
pubesces	,, ,,	50	100	7—22	11	12·98±0·37	±3·67
,,	,, ,,	500	1000	4—29	14	13·85±0·13	±4·21
tortuosa	Whole area	350	700	2—29	11	12·65±0·15	±4·06
carpatica	,, ,,	500	1000	4—27	14	13·22±0·11	±3·59
oycoviensis	,, ,,	50	100	5—21	11	13·10±0·36	±3·56
obscura	,, ,,	25	50	7—23	14	14·78±0·49	±3·48
nana	,, ,,	50	100	1—5	1	1·72±0·09	±0·85
humilis	,, ,,	50	100	2—8	3	3·74±0·13	±1·27

2. Blade length

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	22—70	35 and 40	41·30±0·85	± 8·46
,,	,, ,,	500	1000	19—70	40	41·50±0·21	± 6·98
pubesces	,, ,,	50	100	27—74	40	42·90±0·86	± 8·60
,,	,, ,,	500	1000	20—80	45	45·15±0·33	±10·23
tortuosa	Whole area	350	700	14—71	40	40·80±0·37	± 9·84
carpatica	,, ,,	500	1000	15—73	40	42·79±0·27	± 8·66
oycoviensis	,, ,,	50	100	15—42	25	27·85±0·57	± 5·72
obcura	,, ,,	25	50	22—53	35	39·20±1·11	± 7·78
nana	,, ,,	50	100	4—14	8	8·23±0·22	± 2·23
humilis	,, ,,	50	100	10—39	17	19·85±0·55	± 5·56

3. Blade with

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	22—62	35	32·65±0·66	±6·61
,,	,, ,,	500	1000	13—62	35	33·15±0·17	±5·89
pubescens	,, ,,	50	500	21—55	30	33·55±0·68	±6·76
,,	,, ,,	500	1000	10—60	30	34·65±0·25	±7·89
tortuosa	Whole area	350	700	10—60	35	33·40±0·30	±8·01
carpatica	,, ,,	500	1000	11—58	35	35·43±0·26	±8·12
oycoviensis	,, ,,	50	100	8—30	20	21·10±0·45	±4·50
obscura	,, ,,	25	50	17—43	30	30·70±0·85	±6·00
nana	,, ,,	50	100	4—15	10	8·70±0·23	±2·29
humilis	,, ,,	50	100	7—26	14	14·18±0·40	±4·02

4. Number of pairs of lateral nerves

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	5—10	7	6·68±0·09	±0·88
,,	,, ,,	500	1000	4—10	7	6·96±0·03	±0·89
pubescens	,, ,,	50	100	5—9	7	6·63±0·09	±0·95
,,	,, ,,	500	1000	4—11	7	7·17±0·03	±1·03
tortuosa	Whole area	350	700	3—8	5	5·35±0·03	±0·86
carpatica	,, ,,	500	1000	4—8	6	6·11±0·03	±0·87
oycoviensis	,, ,,	50	100	3—7	5	4·87±0·07	±0·73
obscura	,, ,,	25	50	4—9	7	6·82±0·16	±1·16
nana	,, ,,	50	100	1—4	3	2·65±0·06	±0·56
humilis	,, ,,	50	100	3—7	5	4·98±0·07	±0·72

5. Distance of first tooth from blade base

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	7—21	11	12·59±0·03	±3·04
,,	,, ,,	500	1000	4—23	14	12·86±0·10	±3·19
pubescens	,, ,,	50	100	4—22	11	9·71±0·34	±3·41
,,	,, ,,	500	1000	2—26	11	10·05±0·12	±3·70
tortuosa	Whole area	350	700	2—28	8	8·82±0·12	±3·29
carpatica	,, ,,	500	1000	2—23	8	8·83±0·07	±2·91
oycoviensis	,, ,,	50	100	4—14	11	9·11±0·26	±2·56
obscura	,, ,,	25	50	5—23	11	12·02±0·44	±3·10
nana	,, ,,	50	100	1—5	2	2·53±0·10	±0·99
humilis	,, ,,	50	100	1—9	5	4·24±0·15	±1·47

6. Distance between tips of second and third nerve

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	4—13	7	6·63±0·15	±1·51
,,	,, ,,	500	1000	3—13	6	6·45±0·04	±1·40
pubescens	,, ,,	50	100	3—10	6	6·41±0·16	±1·60
,,	,, ,,	500	1000	3—13	6	6·68±0·05	±1·67
tortuosa	Whole area	350	700	2—15	6—7	6·29±0·07	±1·87
carpatica	,, ,,	500	1000	2—15	7	7·01±0·06	±1·80
oycoviensis	,, ,,	50	100	2—7	5	4·69±0·09	±0·93
obscura	,, ,,	25	50	3—9	5—6	5·98±0·17	±1·32
nana	,, ,,	50	100	1—4	1	1·53±0·08	±0·70
humilis	,, ,,	50	100	2—8	3	4·02±0·12	±1·18

7. Number of teeth between tips of second and third nerve

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	3—7	5	4·81±0·08	±0·81
,,	,, ,,	500	1000	3—9	5	4·85±0·03	±0·86
pubescens	,, ,,	50	100	3—6	4	3·98±0·03	±0·62
,,	,, ,,	500	1000	2—7	4	4·23±0·02	±0·69
tortuosa	Whole area	350	700	2—6	3	3·60±0·03	±0·75
carpatica	,, ,,	500	1000	2—7	4	3·91±0·02	±0·77
oycoviensis	,, ,,	50	100	2—5	3	3·38±0·06	±0·60
obscura	,, ,,	25	50	2—7	5	4·88±0·12	±0·84
nana	,, ,,	50	100	2—3	2	2·03±0·02	±0·17
humilis	,, ,,	50	100	3—5	3—4	3·64±0·06	±0·64

8. Ratio of blade length to petiole length

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	1·5— 4·5	2·6	2·71±0·05	±0·53
,,	,, ,,	500	1000	1·4— 4·5	2·6	2·67±0·01	±0·43
pubescens	,, ,,	50	100	2·0— 6·4	3·2	3·44±0·08	±0·75
,,	,, ,,	500	1000	1·7— 7·4	3·2	3·38±0·02	±0·78
tortuosa	Whole area	350	700	1·7— 8·6	3·2	3·39±0·03	±0·78
carpatica	,, ,,	500	1000	1·4— 7·1	3·2	3·35±0·02	±0·69
oycoviensis	,, ,,	50	100	1·4— 4·2	2·0	2·21±0·05	±0·49
obscura	,, ,,	25	50	1·7— 3·6	2·6	2·72±0·07	±0·47
nana	,, ,,	50	100	2·3—11·0	5·0	5·45±0·23	±2·33
humilis	,, ,,	50	100	3·0—11·3	4·5	5·61±0·13	±1·33

9. Ratio of blade length to blade width

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	0·9—2·0	1·3	1·29±0·02	±0·15
,,	,, ,,	500	1000	0·9—2·0	1·2	1·27±0·01	±0·16
pubescens	,, ,,	50	100	0·9—1·7	1·3	1·28±0·02	±0·16
,,	,, ,,	500	1000	0·9—1·9	1·3	1·31±0·01	±0·17
tortuosa	Whole area	350	700	0·8—2·0	1·2	1·23±0·01	±0·17
carpatica	,, ,,	500	1000	0·7—2·1	1·2	1·22±0·05	±0·16
oycoviensis	,, ,,	50	100	0·8—2·0	1·3	1·30±0·02	±0·23
obscura	,, ,,	25	50	1·1—1·7	1·3	1·29±0·02	±0·13
nana	,, ,,	50	100	0·6—1·3	1·0	0·94±0·01	±0·14
humilis	,, ,,	50	100	1·0—1·9	1·3	1·43±0·02	±0·20

10. Mean distance of nerves

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	4·0— 9·0	5·5	6·17±0·10	±0·97
,,	,, ,,	500	1000	3·5— 9·0	5·5	5·99±0·01	±0·93
pubescens	,, ,,	50	100	4·3—11·2	6·5	6·51±0·13	±1·27
,,	,, ,,	500	1000	3·5—11·2	6·5	6·30±0·04	±1·19
tortuosa	Whole area	350	700	3·0—13·0	7·0	7·67±0·05	±1·52
carpatica	,, ,,	500	1000	3·0—11·0	6·5	7·05±0·04	±1·25
oycoviensis	,, ,,	50	100	3·8— 8·1	5·5	5·79±0·09	±0·94
obscura	,, ,,	25	50	3·4— 8·0	5·5	5·83±0·14	±1·02
nana	,, ,,	50	100	1·5— 6·0	2·5	3·19±0·09	±0·95
humilis	,, ,,	50	100	2·2— 6·4	3·5	4·01±0·09	±0·88

11. Ratio of blade length to distance of first tooth

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	2·3— 6·6	3·0	3·39±0·07	±0·73
,,	,, ,,	500	1000	1·5— 9·5	3·0	3·39±0·03	±0·87
pubescens	,, ,,	50	100	2·5—11·5	4·0	4·96±0·06	±0·57
,,	,, ,,	500	1000	2·0—13·5	4·0	4·79±0·06	±1·87
tortuosa	Whole area	350	700	1·5—12·5	4·5	4·57±0·06	±1·67
carpatica	,, ,,	500	1000	2·0—17·5	4·5	5·34±0·06	±1·83
oycoviensis	,, ,,	50	100	1·9— 6·4	3·0	3·26±0·08	±0·81
obscura	,, ,,	25	50	2·2— 5·8	3·5	3·38±0·11	±0·74
nana	,, ,,	50	100	1·3—11·0	2·5	3·71±0·15	±1·55
humilis	,, ,,	50	100	2·3—17·1	3·5	5·22±0·54	±5·37

12. Position of the widest part

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	2·0—5·8	2·9, 3·5	3·24±0·07	±0·65
,,	,, ,,	500	1000	2·0—8·3	2·6	3·12±0·01	±0·71
pubescens	,, ,,	50	100	1·9—4·0	2·6	2·60±0·04	±0·39
,,	,, ,,	500	1000	1·7—5·3	2·6	2·63±0·01	±0·45
tortuosa	Whole area	350	700	1·4—4·7	2·3	2·42±0·02	±0·43
carpatica	,, ,,	500	1000	1·4—5·3	2·6	2·80±0·02	±0·57
oycoviensis	,, ,,	50	100	1·2—5·0	2·6	2·62±0·06	±0·62
obscura	,, ,,	25	50	2·1—4·1	3·5	3·14±0·07	±0·51
nana	,, ,,	50	100	1·5—3·0	2·1	2·04±0·03	±0·32
humilis	,, ,,	50	100	1·2—2·8	2·0	2·11±0·03	±0·27

13. Axil of second nerve

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	25—50	40	38·20±0·51	±5·07
,,	,, ,,	500	1000	25—60	40	38·90±0·20	±6·52
pubescens	,, ,,	50	100	30—50	40	36·65±0·52	±5·15
,,	,, ,,	500	1000	20—60	40	36·90±0·20	±6·38
tortuosa	Whole area	350	700	15—50	30	30·20±0·22	±5·83
carpatica	,, ,,	500	1000	20—60	30	34·77±0·20	±6·32
oycoviensis	,, ,,	50	100	20—50	30	31·20±0·54	±5·43
obscura	,, ,,	25	50	25—50	40	39·10±0·88	±6·22
nana	,, ,,	50	100	15—40	25	25·35±0·53	±5·31
humilis	,, ,,	50	100	25—75	40	43·10±0·86	±8·60

14. Base angle

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	50—115	60	68·50±1·14	±11·43
,,	,, ,,	500	1000	35—120	60, 70	67·15±0·45	±14·40
pubescens	,, ,,	50	100	40—115	70	65·75±1·25	±12·46
,,	,, ,,	500	1000	35—130	60	66·20±0·49	±15·64
tortuosa	Whole area	350	700	30—110	60	64·35±0·53	±14·02
carpatica	,, ,,	500	1000	30—130	70	71·76±0·46	±14·62
oycoviensis	,, ,,	50	100	45— 90	50	63·20±1·05	±10·52
obscura	,, ,,	25	50	45— 85	60	66·70±1·38	± 9·68
nana	,, ,,	50	100	45— 90	60, 70	67·90±1·12	±11·19
humilis	,, ,,	50	100	35— 90	55	56·45±1·18	±11·82

15. Apex angle

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	5—40	20	10·20±0·64	±6·38
,,	,, ,,	500	1000	5—40	20	19·40±0·19	±6·06
pubescens	,, ,,	50	100	15—60	30	32·30±0·76	±7·63
,,	,, ,,	500	1000	15—70	30	30·75±0·23	±7·24
tortuosa	Whole area	350	700	20—70	40	39·98±0·30	±7·98
carpatica	,, ,,	500	1000	15—60	40	35·39±0·24	±7·71
oycoviensis	,, ,,	50	100	10—40	25	26·80±0·56	±5·64
obscura	,, ,,	25	50	10—35	20	19·40±0·76	±5·36
nana	,, ,,	50	100	50—90	70	71·15±0·84	±8·39
humilis	,, ,,	50	100	30—75	50	51·35±0·90	±9·05

16. Number of leaves on dwarf shoot

Species	Locality	Number of trees	Number of leaves	Extreme values	Mo	M±m	σ
verrucosa	Whole Europe	50	100	2—3	2	2·16±0·05	±0·37
,,	,, ,,	500	1000	2—4	2	2·11±0·01	±0·33
pubescens	,, ,,	50	100	2—4	2	2·28±0·08	±0·57
,,	,, ,,	500	1000	2—4	2	2·51±0·03	±0·64
tortuosa	Whole area	350	700	2—5	3	2·93±0·04	±0·74
carpatica	,, ,,	500	1000	2—5	3	2·80±0·03	±0·73
oycoviensis	,, ,,	50	100	2—6	3	3·20±0·14	±0·96
obscura	,, ,,	25	50	2—3	2	2·12±0·07	±0·33
nana	,, ,,	50	100	2—4	3	2·72±0·09	±0·66
humilis	,, ,,	50	100	2—4	3	2·64±0·09	±0·62

Problem Heterozji. VI. Różne typy szeregów rozdzielczych drugiego pokolenia mieszańców. — The problem of Heterosis. VI. Different shapes of the F_2 frequency distributions.

Mémoire

de ***M. E. MALINOWSKI*** m. t.

présenté le 10 Juin 1950, par M. E. Malinowski m. t. et
M. M. Korczewski m. c.

Contents:

1. «True» and «hypothetical» heterosis

There is a voluminous literature on heterosis in which numerous observations and valuable experimental data are reported. But as far as I know the F_2 frequency distributions, where heterosis is shown in F_1, has so far not been analysed with sufficient accuracy. There are many more papers discussing the nature of heterosis than those giving the F_2 frequency distributions in the case of vigorous F_1. Such an analysis of the F_2 frequency distributions may enable us to throw light on the nature of heterosis by genetical methods. As far as the plant material is concerned the F_2 frequency distributions are given in the papers by Emerson and East (1913), Hayes (1912), Baur (1914), Malinowski (1932, 1950), Ware (1930) and Müntzing (1930). The first two publications dealing with maize and tobacco, refer mostly to such cases in which the F_1 generation is intermediate in the characters studied, being as a rule larger than the average of the parents. The remainder of the above mentioned papers refer to the cases in which the characters of the hybrid are superior to the larger parent.

In the former cases the F_2 frequency distributions are symmetrical and in the latter they are skew. From the above it appears that we may distinguish two kinds of heterosis: one — the «true» heterosis — in which the hybrids exceed the larger parent and the other — the «hypothetical» heterorosis — in which the hybrids do not exceed the larger parent. The «true» heterosis presents no doubts whatsoever. As to the «hypothetical» heterosis we infer its existence from the behaviour of the corresponding F_2 generation.

2. The F_2 frequency distributions in maize and tobacco

Let us dwell more at length on the phenomena of heterosis described in the paper by Emerson and East (1913). This is an important publication. It is upon the statistical data of this paper that Jones (1917) based his considerations when bringing forward his well known therory of dominance of linked factors as a means of accounting for heterosis.

Considering the cross of two varieties of maize namely Tom Thumb Pop and Black Mexican Sweet, Emerson and East argued as follows: «If the F_1 generation was exactly intermediate between the two parents, its modal class would be about 52 inches. In reality, however, it is about 70 inches. In other words it has been pushed up about six classes above the intermediate position. This phenomenon is not to be regarded as an expression of dominance. It is due to the icreased vigor (largely internodal) that appears when many gametic factors are in the heterozygous condition».

With reference to the cross of Missouri Dent with California Pop Emerson and East (1913) write as follows: «The F_1 lots of both years were almost as tall as the tall parent»... «That the tallness of the F_1 plants of the cross under consideration was not due to dominance of long over short internodes but rather to the increased vigor accompanying heterozygosis of many characters is indicated by the fact that the mean height of the F_2 fraternities was practically the mid-parental height — partial heterozygosis having occurred in F_2 — and also by the fact that the F_2 frequency distributions were not so pronouncedly skewed as they would have been if great internode length were dominant».

The above mentioned authors came to the following general conclusions: «A peculiarity of these crosses is that in three of them the F_1 plants were almost as tall as the tall parent and in the fourth were considerably taller than the mean of the two parents. That this increase in height of F_1 plants over the mid-parental height is in no case ascribable to dominance of tallness over shortness, but due rather to increased vigor accompanying heterozygosis, is indicated by the fact that in every case the mean height of the F_2 plants is about half-way between the heights of the parents and is also shown by the lack of skewness in the F_2 frequency distributions».

From the foregoing considerations it appears that we have to deal in maize, in the cases just cited, with what we have called hypothetical heterosis and that the F_2 frequency distributions are symmetrical. On the whole such a conclusion is correct. But when examining in more detail the data reported in the paper by Emerson and East (1913) we find interesting deviations from typical cases.

Let us first consider one of such typical cases. The F_2 frequency distribution in height of maize presented in Table I may be looked upon as such an illustrative example. The mean value of one parent in this cross is 228·5 cm (calculated for 5 lines grown in 1910) and that of the other parent — 84·7 cm. The mean value of F_1 is 175·0 cm while the mid-parental value is 156·6 cm. The height of F_1 exceeds but little the mid-parental value. The F_2 frequency distributions are symmetrical as is seen from Table I (F_2 1127 and F_2 11 28).

In other examples reported on in Emerson and East's paper the mean values of the F_1 generation came nearer to the values of the larger parental type, being sometimes almost equal to them. In such cases the F_2 frequency distributions displayed a strong tendency towards a negative asymmetry. Such examples are given in Tables II, III, IV, V, VI and VII.

Fisher, Immer and Tedin (1932) showed that some of the F_2 frequency distributions looked upon by Emerson and East as symmetrical are in reality negatively skew. These authors write as follows: «In the summary of the paper by Emerson and East we find a statement relative to the «lack of skewness in the F_2

TABLE I

Frequency distributions of heights of plants in cross between Tom Thumb Pop and Missouri Dent (After Emerson and East, 1913)

Designation	Year	Class centres in decimeters for height of plants																										Mean	n	g_1	S. E. g_1
		5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
Missouri dent	1909																	←						→							
Tom Thumb	1909				←			→																							
(177) F_1	1909										←							→													
Missouri dent	1910																	2	4	9	9	6	11	11	2	1		24·93 ± ·17			
„ „	1910												1	1	2	1	3	1	6	2	4	1						21·27 ± ·35			
„ „	1910													2	1	2	6	2	4	3	3							20·91 ± ·29			
„ „	1910														1		4	2		2	3	3	2	2	1	2		23·86 ± ·45			
„ „	1910															2	1	1	5	4	1	3	3	1	1			23·32 ± ·35			
Tom Thumb	1910			2	6	5	2																					8·47 ± ·15			
(509) F_1	1910											1	3	5	7	4												17·50 ± ·17			
(510) F_2	1910			2			2	8	6	25	26	29	30	32	28	22	6	4	1	2								15·86 ± ·12	223	—0·305	±0·163
Missouri dent	1911															2		5	3	4	2	3	1					22·50 ± ·29			
„ „	1911													1	1			2	2	4	4	4	1	1				23·10 ± ·36			
„ „	1911															2	1	2	4	11	10	6	3	1				23·40 ± ·19			
Tom Thumb	1911	2	9	7	1	0																						6·37 ± ·12			
(1127) F_2	1911					1	1	2	3	5	4	1	6	11	3	6	13	11	4	3	3							17·97 ± ·28	77	—0·518	±0·274
(1128) F_2	1911					1	1		3	3	5	11	7	4	8	12	10	5	5	2	3	1	1					17·87 ± 26	82	—0·160	±0·266

TABLE II

Frequency distribution of heights of plants in cross Tom Thumb Pop × Black Mexican sweet
(After Emerson and East, 1913)

Designation	Gen.	Class centers in inches for heights of plants																								*n*	g_1	S. E. g_1
		22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91			
54 (Black Mexican)	P													2	6	10	17	24	26	21	14	14	7	3	1			
60 (Tom Thumb)	P	←						→																				
(60 — 3×54)	F_1												←											→				
(60 — 3×54) — 1	F_2						1	5	2	2	9	24	12	29	10	14	7	4	1							120	—0·301	±0·221
(60 — 3×54) — 5	F_2						2	5	7	24	19	33	30	26	13	19	4	3	2							187	+0·136	±0·178
(60 — 3×54) — 6	F_2						2	5	4	6	3	11	18	17	11	14	3	6	2							102	—0·355	±0·239
(60 — 3×54) — total	F_2						5	15	13	32	31	68	60	72	34	47	14	13	5							409	—0·107	±0·121

TABLE III

Frequency distribution of heights of plants in cross Tom Thumb Pop × Black Mexican sweet
(After Emerson and East, 1913)

Designation	Gen.	Class centers in inches for heights of plants																								*n*	g_1	S. E. g_1
		22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91			
54 (Black Mexican)	P													2	6	10	17	24	26	21	14	14	7	3	1			
60 (Tom Thumb)	P	←						→																				
(60 — 5×54)	F_1														←								→					
(60 — 5×54) — 2	F_2						1			1	4		3	15	31	37	18	10	12	9	1					142	—0·497	±0·203
(60 — 5×54) — 6	F_2								1	2	6	10	16	21	27	23	19	10	3			1				139	—0·082	±0·206
(60 — 5×54) — 8	F_2									2	2	9	8	22	19	24	12	4	3	3						108	+0·044	±0·223
(60 — 5×54) — 11	F_2							2		2	3	3	12	20	28	24	17	7	4							122	—0·796	±0·219
(60 — 5×54) — 12	F_2						1			1	3	4	5	17	12	18	26	28	9	10	2	2				138	—0·606	±0·206
(60 — 5×54) — total	F_2						2	2	1	8	18	26	44	95	117	126	92	59	31	22	3	3				649	—0·260	±0·096

TABLE IV

Frequency distribution of heights of plants in cross Tom Thumb Pop × Black Mexican sweet
(After Emerson and East, 1913)

Designation	Gen.	Class centers in inches for heights of plants																						n	g_1	S. E. g_1
		22	25	28	31	34	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	88	91			
54	P													2	6	10	17	24	26	21	14	14	7	3	1			
60	P	←						→																				
(60 — 8×54)	F_1											←											→					
(60 — 8×54) — 1	F_2						1			4	5	8	9	23	17	21	11	13	9	5	2	1		1		130	+0·063	±0·212
(60 — 8×54) — 8	F_2								1	1	1	5	4	12	12	15	16	8	11	4	4	3	1			98	—0·003	±0.244
(60 — 8×54) — total	F_2						1		1	5	6	13	13	35	29	36	27	21	20	9	6	4	1	1		228	—0·175	±0·161

TABLE V

Frequency distribution of heights of plants in cross smal flint (N 5) × large dent (N 6)
(After Emerson and East, 1913)

No	Class centers in inches for heights of plants																					$\bar{x}$	n	g_1	S. E. g_1
	51	54	57	60	63	66	69	72	75	78	81	84	87	90	93	96	99	102	105	108	111				
5 (small flint)	3	2	4	5	11	17	17	18	10	6	4											68·22 ± ·41			
6 (large dent)													1	2	2	13	14	16	14	8	3	101·18 ± ·40			
(5×6) F_1											5	4	3	9	9	6	5	4	4	7		94·53 ± ·74			
(5×6) — 1 F_2	2	1	3	6	3	5	10	10	22	16	10	7	11	13	6	10	8	4	4	2		81·00 ± ·69	153	—0·001	±0·196
(5×6) — 2 F_2				2		2	4	2	6	5	12	8	12	6	5	8	3	10	8	2	2	88·33 ± ·80	97	—0·133	±0·245
(5×6) — 8 F_2	2				4	4	12	7	9	13	12	15	10	8	8	1	1	1				79·46 ± ·62	107	—0·360	±0·234
(5×6) — 14 F_2		1	2	4	2	7	8	17	21	11	27	24	16	9	11	10	5	4	2			81·24 ± ·51	181	—0·040	±0·181

TABLE VI

Frequency distribution of height of plants in cross between California Pop and Missouri Dent
(After Emerson and East, 1913)

	Year	Class centers in decimeters for height of plants																				$\bar{x}$	n	g_1	S. E. g_1
		6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25				
Missouri dent	1910											1	1	2	1	3	1	6	2	4	1	21·27 ± ·35			
California pop	1910				1	2	7	8	1													11·32 ± ·14			
F_1 (502)	1910											1	2	1	5	13	5	1				19·64 ± ·17			
F_1 (505)	1910												1	1	4	6	8	1	1			20·18 ± ·19			
Missouri dent	1911									1	5	11	16	22	13	11	4	1	0	1		18·00 ± ·13			
,, ,,	1911									1	1		4	5	5	2	1					18·05 ± ·25			
California pop	1911			1	9	18	16	7														10·37 ± ·09			
,, ,,	1911	1	2	11	24	14	2															9·00 ± ·09			
F_1 (836)	1911							1	0	1	2	2	6	14	12	3						17·80 ± ·17			
F_1 (837)	1911										2	6	9	9	3	1						17·27 ± ·15			
F_2 (829)	1911					1	1	4	10	13	14	13	20	8	8	2	1					15·75 ± ·15	95	—0·088	±0·247
F_2 (830)	1911		1		2	3	9	12	12	19	22	10	3	2								13·65 ± ·14	95	—0·523	±0·247
F_2 (831)	1911			2	0	5	6	13	14	29	14	7	6									13·54 ± ·13	96	—0·496	±0·246
F_2 (832)	1911					2	5	11	17	12	12	15	9	7	5	1						14·69 ± ·16	96	+0·168	±0·246
F_2 (839)	1911				1	1	2	7	8	13	17	14	9	2								14·58 ± ·15	74	—0·595	±0·279
F_2 (840)	1911			1	1	0	2	2	7	9	17	19	6	7	3							15·19 ± ·16	74	—0·842	±0·279

TABLE VII

Frequency distribution of internode length of plants in cross between California Pop and Missouri Dent
(After Emerson and East, 1913)

	Year	Class centers in millimeters for internode lengths										$\bar{x}$	n	g_1	S. E. g_1
		44·5	54·5	64·5	74·5	84·5	94·5	104·5	114·5	124·5	134·5				
Missouri dent	1910					3	4	4	5	3		105·03±2·04			
California pop	1910			4	14	1						72·92± ·76			
F_1 (502)	1910						1	10	11	6		112·36±1·04			
F_1 (505)	1910						1	6	10	4	1	113·59±1·29			
Missouri dent	1910				5	25	24	28	3			94·38± ·73			
,, ,,	1910				4	7	5	3				88·18±1·52			
California pop	1910	3	13	21	11	3						64·11± ·92			
,, ,,	1910	4	28	18	3							58·27± ·66			
F_1 (836)	1910			1	1	6	7	23	3			98·89±1·12			
F_1 (837)	1910				2	3	8	14	3			98·83±1·26			
F_2 (829)	1910		3	8	22	37	8	17				83·97± ·89	95	+0·010	±0·247
F_2 (830)	1910	1	13	19	31	22	8	1				73·76± ·85	95	—0·034	±0·247
F_2 (831)	1910	2	11	17	43	17	3	3				73·15± ·81	96	+0·123	±0·246
F_2 (832)	1910		4	27	33	21	8	2	1			75·75± ·80	96	+0·719	±0·246
F_2 (839)	1910	1	2	7	25	27	8	4				80·04± ·89	74	—0·242	±0·279
F_2 (840)	1910		2	5	11	27	12	14	3			87·47±1·07	74	—0·143	±0·279

frequency distributions» for the crosses employed in studies on inheritance of height of plants. Taking the total distributions for F_2 in Tables 25, 26, 27, 28 and 30 and the same for the F_2 in 1911 in Table 29 we may determine statistically whether these distributions were or were not symmetrical. We find that $(k_3 k_2^{-\frac{3}{2}})$ was $-0{\cdot}105 \pm 0{\cdot}120$, $-0{\cdot}254 \pm 0{\cdot}096$, $+0{\cdot}028 \pm 0{\cdot}161$, $+0{\cdot}012 \pm$ $\pm 0{\cdot}105$, $-0{\cdot}355 \pm 0{\cdot}192$ and $-0{\cdot}161 \pm 0{\cdot}106$ in Tables 25 to 30, respectively. A negative bias is indicated in four of the six crosses tested; although in the second alone is the skewness statistically significant, yet two other negative values are suggestively large».

I reproduce here all Tables mentioned in this quotation in order to consider particular F_2 frequency distributions separately. Tables II, III, IV, V and VI of the present paper correspond respectively to the Tables 25, 26, 27, 28 and 30 of the publication by Emerson and East. Table I of our text corresponds to Table 29 of Emerson and East. I calculated R. A. Fisher's statistics g_1 and S. E. g_1 for all F_2 frequency distributions. In 8 cases the negative skewness proved to be significant. In Table III such a skewness has been shown in the F_2 frequency distributions No 2 and No 12. The total of all F_2 frequency distributions of this Table showed also significantly negative skewness (in this last case $g_1 = -0{\cdot}260$ and S. E. $g_1 = \pm 0{\cdot}096$.

In Table V significantly negative skewness has been found in F_2 No 8 and in Table VI — in the F_2 generations No 830, No 831, No 839 and No 840. As the F_1 generation moves from intermediate classes to those of the larger parental form the F_2 frequency distribution becomes negatively skew instead of being symmetrical. When the range of variation of the larger parent is surpassed by the F_1 generation the sign of skewness of F_2 changes from negative to positive. In crosses between California Pop and Missouri Dent a slight surpassing of the larger parent has been sometimes observed with respect to the length of internodes (Table VII, F_1 No 502, 505, 836 and 837) and in the F_2 generation of this cross positive skewness of the F_2 frequency distribution has been frequent. In some cases this skewness proved significant (Table VII, F_2 No 832).

A more pronounced shift beyond the larger parent has been observed in respect to the total length of stalks in crosses between Tom Thumb Pop and Missouri Dent (Tables VIII and IX). In these crosses all of the F_2 frequency distributions were more or less

TABLE VIII

Frequency distribution of total length of stalks in cross between California Pop and Missouri Dent (After Emerson and East, 1913)

	Year	Class centers in decimeters for total length of stalks											$\bar{x}$	n	g_1	S. E. g_1
			15	25	35	45	55	65	75	85	95	105				
Missouri dent	1911		58	17	6	4							19·82± ·60			
” ”	1911		3	3	7	2	2	1	1				37·11±2·46			
California pop	1911		1	7	15	15	9	4					42·06±1·13			
” ”	1911	1	2	7	14	16	11	2					40·66±1·17			
F_1 (836)	1911		2			5	7	9	7	7	4		66·46±2·00			
F_1 (837)	1911				2	8	7	8	3	1	1		58·00±1·72			
F_2 (830)	1911		18	20	24	19	12	2					34·26± ·94	95	+0·199	0·247
F_2 (839)	1911		25	11	14	16	8						31·08±1·11	74	+0·245	0·279
F_2 (840)	1911	1	19	15	14	13	8	4					32·97±1·22	74	+0·389	0·279
F_2 (829)	1911		8	14	21	26	16	7	2	1			41·53±1·04	95	+0·101	0·247
F_2 (831)	1911	1	10	12	14	25	14	13	6		1		44·06±1·24	96	+0·092	0·246
F_2 (832)	1911		4	13	25	22	15	12	3	1		1	44·48±1·12	96	+0·706	0·246

TABLE IX

Frequency distributions of total length of stalks in cross between Tom Tumb pop and Missouri dent (After Emerson and East, 1913)

	Year	Class centers in decimeters for total length of stalks											$\bar{x}$	n	g_1	S. E. g_1
		4·5	14·5	24·5	34·5	44·5	54·5	64·5	74·5	84·5	94·5	104·5				
Missouri dent	1911		2	36		2							25·00 ± ·53			
,, ,,	1911		1	10	2	4	2		1				34·50 ± 2·19			
,, ,,	1911		1	10	2	4	1	2					34·50 ± 2·13			
Tom Thumb	1911	15	4										6·61 ± ·63			
F_2 (1127)	1911		12	27	18	7	6	2	3	1	1		34·11 ± 1·34	77	+1·434	0·274
F_2 (1128)	1911		12	18	16	9	11	6	6	1		3	41·45 ± 1·65	82	+0·976	0·266

TABLE X

Added vigour in F_1 as compared with the average between the parental forms in the crosses of tobacco varieties Tabulation made after Hayes's data of 1912

Characters of plants	Cross	Mean values of the parental varietes	Average of the parents	Mean values of F_1
Number of leaves per plant	Sumatra × Broadleaf Havana × Cuban	19·2 ± 0·053 × 28·2 ± 0·082 19·8 ± 0·076 × 19·9 ± 0·082	23·7 19·8	23·6 ± 0·072 19·8 ± 0·067
Height of plants in inches	Sumatra × Broadleaf Havana × Cuban	55·0 ± 0·212 × 76·1 ± 0·251 56·5 ± 0·218 × 65·4 ± 0·264	65·5 60·9	70·8 ± 0·250 65·5 ± 0·270
Area of leaves in sq. dcms	Sumatra × Broadleaf Havana × Cuban	3·23 ± 0·031 × 8·65 ± 0·093 5·26 ± 0·048 × 6·73 ± 0·063	5·94 5·99	6·35 ± 0·062 6·46 ± 0·066
Lenght of leaves in cm	Sumatra × Broadleaf Havana × Cuban	28·8 ± 0·125 × 48·7 ± 0·258 36·1 ± 0·104 × 45·2 ± 0·183	38·7 40·6	40·0 ± 0·187 42·2 ± 0·191
Width of leaves in cm	Sumatra × Broadleaf Havana × Cuban	15·4 ± 0·111 × 23·8 ± 0·164 20·1 ± 0·109 × 20·3 ± 0·118	19·6 20·2	20·9 ± 0·138 20·8 ± 0·120

positively skew, and some of them showed significantly positive skewness (Table VIII, F_2 No 832, Table IX, F_2 No 1127 and 1128).

It is important to note that in the just mentioned cases of positive skewness the differences between the parental forms were not large. Typical pisitive skewness of the F_2 generation which I found in *Phaseolus* and *Petunia* occurs in such cases in which the parental forms do not differ much between themselves and in which the F_1 generation is superior to the stronger parent.

Another paper cited by Jones (1917) as evidence in favour of his theory of dominance of linked factors is that published by Hayes (1912) concerning hybrid vigour in tobacco. Certainly all the F_2 frequency distributions given in this paper are symmetrical with positive or negative bias. But we should also take into account the fact that all the data which interest us in this paper concern «hypothetical» heterosis. The data in question are given in our Table X in a concise form. They refer to the hybrids between varieties of Nicotiana Tabacum, namely to crosses: Sumatra × Broadleaf and Havana × Cuban. It is evident from this Table that the F_1 generation is always intermediate in all characters studied, being as a rule somewhat larger than the average of the parents. All characters studied except the number of leaves per plant showed such an added vigour.

3. Heterosis in particular characters of the plants investigated

Some characters are much more affected by heterosis than others. According to Jones (1918) heterosis in maize is particularly noticeable in the height of the plants, the diameter of the stalk, root development, the length of the ear and the productiveness of grain. From the tables given in Jones's publication of 1918 it is seen that the yield of the crosses is increased by 180 per cent, height by 27 per cent. and length of ear by 29 per cent over the average of their parental lines. On the other hand, the number of nodes per plant and number of rows of grain on the ear is increased only by 6 and 5 per cent, respectively.

Analogous phenomena were observed in other plants. Veach (1930), for instance, found that in soy beans there was an average percentage increase of hybrids over the average of parents in every character studied. These characters were: number of pods, average seed weight, seed number, time of flowering, average internode length, number of nodes, total stem and branch length, plant height, straw-grain ratio, plant weight, number of seeds per pod. The largest increase was observed in plant weight, total stem and branch length, seed weight, seed number and pod number. The better parents were exceeded in F_1 only in some of the characters studied, namely: number of pods, seed number, seed weight, number of nodes, total stem and branch length, plant height and plant weight.

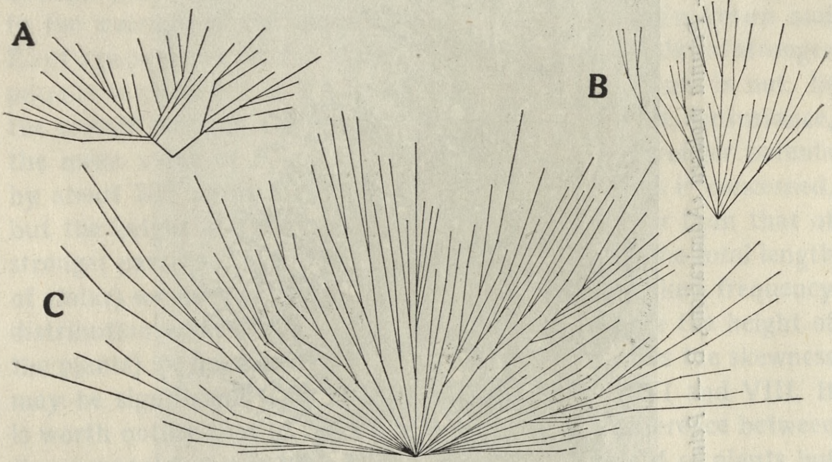

Fig. 1. Drawings showing diagrammatically all internodes of two varieties of *Petunia violacea* and of F_1, namely: A — «Admiration», B — Vilmorin's *Petunia*, C — F_1.

In *Phaseolus* and *Petunia* all characters investigated showed vigorous growth exceeding that of the superior parent. In *Phaseolus* the height of plants in F_1 increased by 136% over the higher parental form, the number of internodes increased by 260% as compared with the parental form, the total length of all internodes increased by 351%, the weight of dry vines by 370%, the number of leaves by 114%, the average length of internodes by 45% and the length of inflorescenses by 31%. The hybrid vigour in *Petunia* is also very distinct and in all cases the size of the stronger parental variety is exceeded in F_1. The height of plants, for example, increased by 46% over the better parent, the weight of fresh plants by 659%, the total length of all internodes by 1251%, the average length

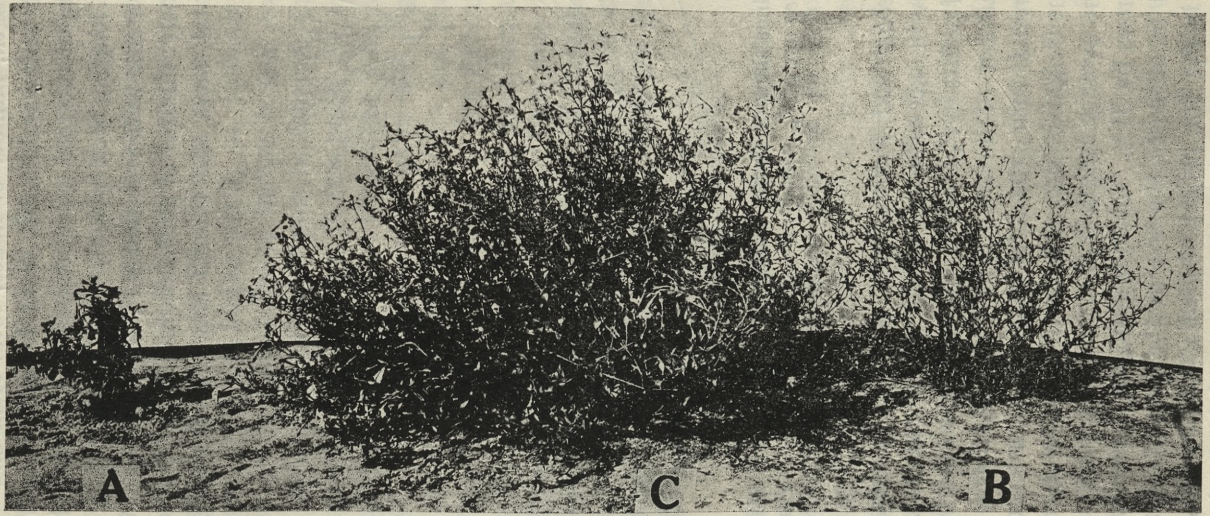

Fig. 2. A — *Petunia violacea* «Admiration», B — *Petunia Nyctaginiflora*, C — F_1.

of internodes by 27% and the average length of leaves by 16%. The greatest vigour manifested itself in the total length of all internodes (1251% in *Petunia* and 351% in *Phaseolus*). Fig. 1 shows all internodes of two parental individuals and of the F_1 plant of *Petunia*. As is seen from this drawing the height of the plants increases but slightly in F_1 in comparison with the total length of internodes. The same may be seen in Fig. 2 showing a photograph of two other parental forms of *Petunia* and of their hybrid.

In some characters the hybrids may significantly exceed the stronger parents, and in others they may be only somewhat superior to the average of the parents. The data given in Emerson and East's paper show that in maize the total length of stalks of stronger parents is exceeded in F_1 but the height of the plants is not. In the crosses between California Pop and Missouri Dent, for instance, the mean value of F_1 exceeds the mean value of stronger parents by about 200 cm as far as the total length of stalks is concerned, but the height of the F_1 plants is about 10 cm lower than that of stronger parents. Thus in one character (for instance the total length of stalks) we have in the F_2 generation a positively skew frequency distribution while in the other character (for instance the height of the plants) we have a negative skewness. In both cases the skewness may be significant. This is seen from our Tables VI and VIII. It is worth noting that in the cases in question the difference between the parental forms is large with respect to the height of plants but it is rather small with respect to the total length of stalks.

4. D. F. Jones's theory of dominance of linked factors

This well known theory tries to explain the phenomena of heterosis. In his paper Jones (1917) gives a diagrammatic illustration of the way in which heterosis may be brought about. This author assumes: 1) that in the case of hybrid vigour the F_2 frequency distributions are symmetrical, 2) that both parental forms are of about the same size and 3) that the F_1 generation is superior to the stronger parent.

As to the first asumption Jones writes as follows: «In the vast amount of data accumulated upon the inheritance of quantitative characters no such tendencies toward an asymmetrical distribution is evident in the majority of cases recorded. In Emerson and

East's paper (1913), referred to, dealing with quantitative characters in maize and Hayes's publication (1912) dealing with the same type of characters in tobacco, the distributions in F_2, where heterosis is shown in F_1, are all considered to be of the type of normal frequency distributions». Under normal frequency distribution a symmetrical distribution is understood.

As to the second assumption Jones's theory (see diagram I, page 473 and Table 2, page 474 of the paper by Jones 1917) takes as a basis of departure that the parental forms are of about the same size and that they have similar development (in the diagram they have development of six units).

As to the third assumption, Jones's theory postulates that F_1 is larger than either parent. In the diagram cited the F_1 generation has the development of twelve units.

Such assumptions of Jones's theory are at variance with the statistical data of Emerson and East's paper and also with the data of Hayes's publication, both of which have been taken by Jones as a basis for his theory. The following facts given in the two just mentioned papers are not consistent with the fundamental assumptions of Jones's theory:

1. Only some of the F_2 frequency distributions are symmetrical (a fact consistent with Jones's theory), the other being negatively or positively skew (facts not consistent with Jones's theory).

2. The examples cited in the paper by Emerson and East as well as in the paper by Hayes refer to such cases in which the parental forms differ markedly from one another (facts not consistent with Jones's diagrammatic scheme). Only in the case of the total length of stalks are the parental forms rather similar (a fact consistent with Jones's diagrams).

3. The F_1 in the examples of Emerson, East and Hayes is never twice as large as the larger parent and even a slight transgression of the stronger parent by the F_1 generation is recorded only once. In the majority of cases the F_1 is somewhat larger than the midparental value but it is smaller than the better parent (facts not consistent with Jones's diagrams).

It appears from the above that Jones's theory is not applicable to the case of «hypothetical» heterosis. The question arises if it holds for «true» heterosis. True heterosis, as we have assumed in this paper, is a manifestation of hybrid vigour which exceeds that of the stronger parental form. In such cases the parental forms are more or less similar in their vigour and the F_1 generation exceeds (sometimes considerably) both parents. In some cases, as for example in the case of the total length of internodes in *Petunia*, the vigour of F_1 is more than 20 times as great as that of the stronger parental form.

In connection with the phenomena of «true» heterosis Jones's theory should account for positive skewness of the F_2 generation. Such a skewness is a characteristic feature of true heterosis. But Jones's theory applies only to the cases of symmetrical shapes of the F_2 frequency distributions. I do not see any possibility of accounting for heterosis by means of the theory of dominance of linked factors.

5. The F_2 frequency distributions in Phaseolus, Petunia, Antirrhinum and Gossypium

In the third paper of this series I demonstrated that in *Phaseolus* and *Petunia* we have to deal in F_2 with positively skew frequency distributions. This is a general phenomenon in *Phaselus* crosses showing hybrid vigour. This increased vigour manifests itself in all vegetative parts of the plants investigated. The same holds for our *Petunia* crosses in which we found also positive skewness in the F_2 frequency distributions.

Both in *Phaseolus* and in *Petunia* crosses the F_1 plants are larger than the better parent and the parental forms do not differ to any extent. In other words in *Phaseolus* and in *Petunia* we deal with the phenomena of «true» heterosis. In the above cited paper (Malinowski 1950 III) I presented data concerning this problem and in the present paper I give some diagrams (Figs 3—9) which illustrate the phenomena in question. The diagrams concerning *Phaseolus* and *Petunia* are based upon the data of the just mentioned third paper of this series, and those concerning *Antirrhinum* and *Gossypium* are founded upon the papers by Baur (1914) and Ware (1930) respectively.

The following are the characteristic features of the diagrams (Fig. 3—6) concerning kidney bean crosses: 1) The parental forms are similar as to their sizes, 2) The F_1 generation is for the most part much larger than the stronger parental form, 3) The F_2 frequency distribution is positively skew. Its modal value corresponds

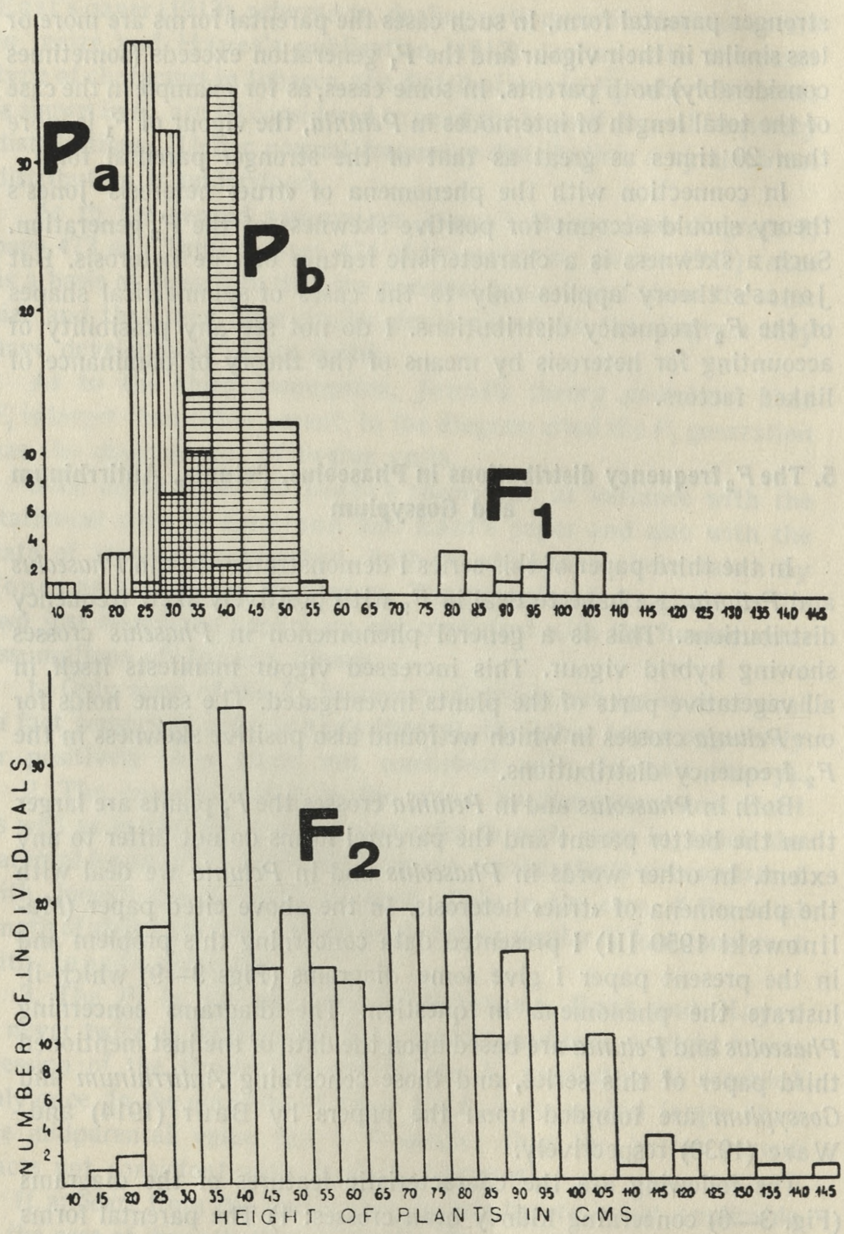

Fig. 3. Frequency polygons illustrating the distribution in height of the parental forms (*Phaseolus vulgaris melleus* and Inexhaustible bean), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

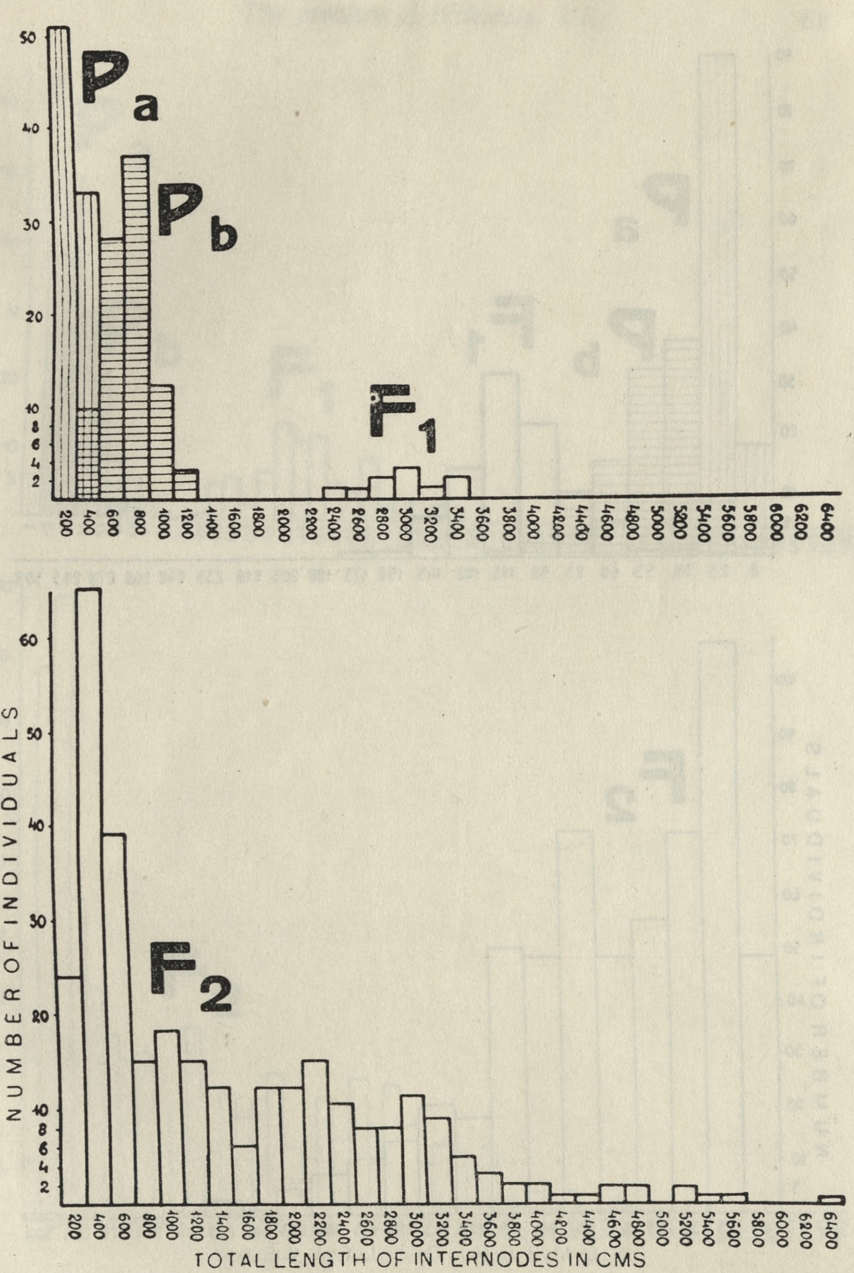

Fig. 4. Frequency polygons illustrating the distribution in total length of internodes of the parental forms (*Phaseolus vulgaris melleus* and Inexhaustible bean), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

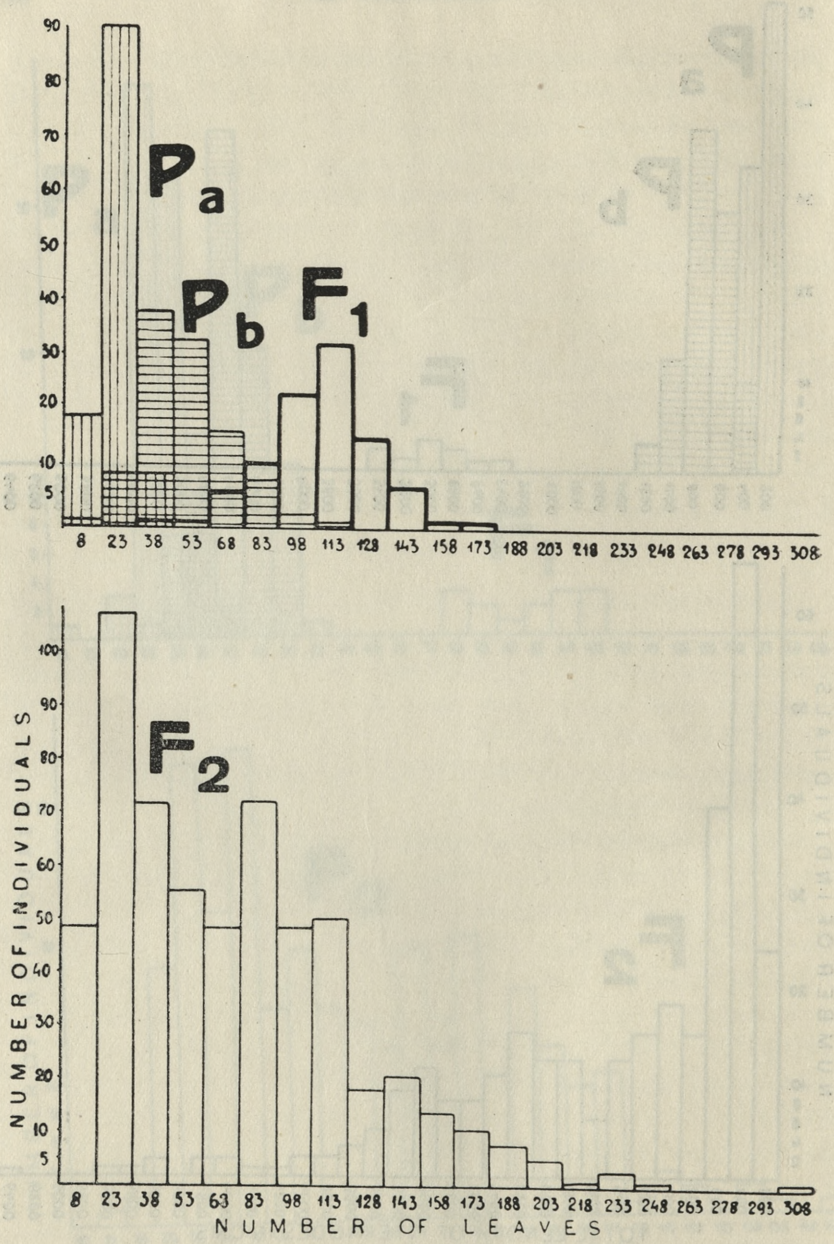

Fig. 5. Frequency polygons illustrating the distribution in the number of leaves of the parental forms (*Phaseolus vulg. melleus* and Inexhaustible bean), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

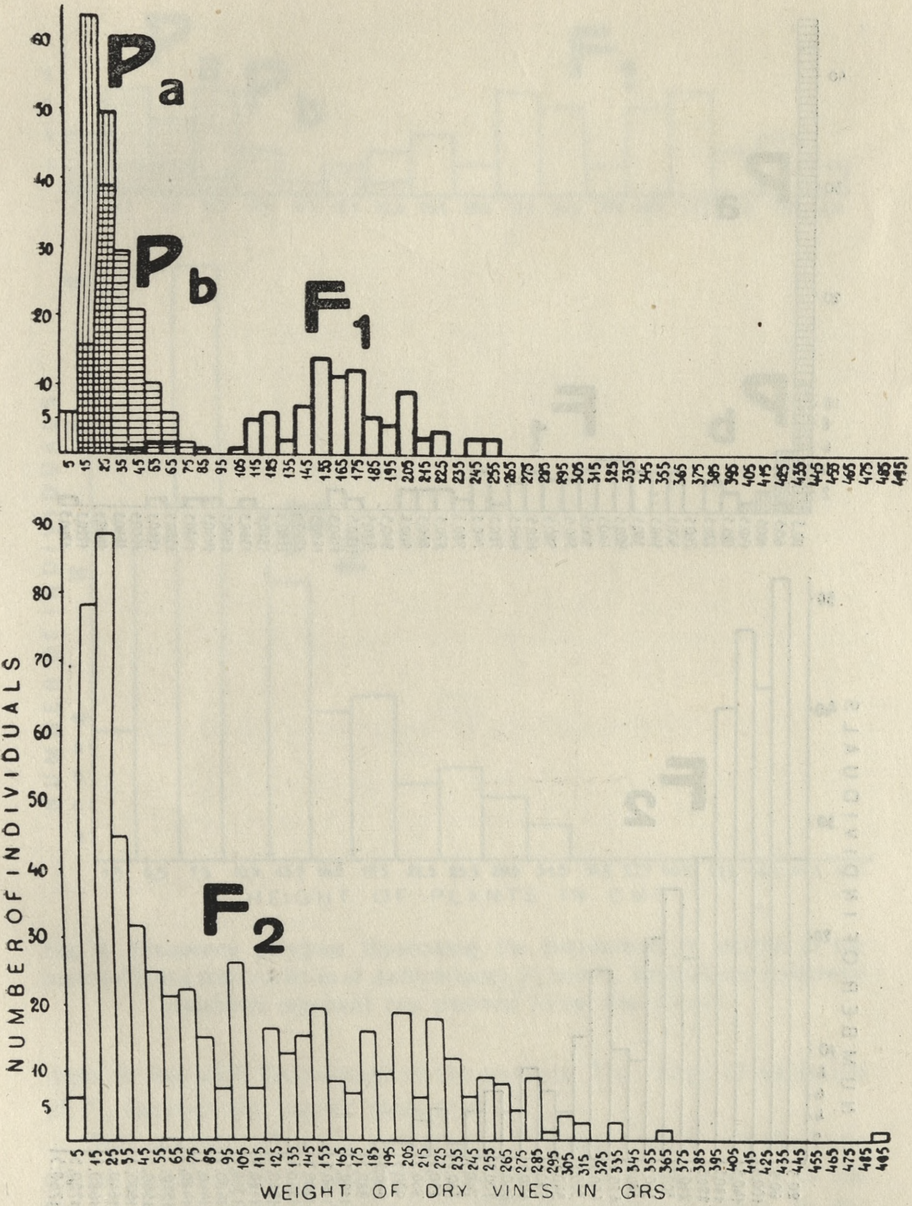

Fig. 6. Frequency polygons illustrating the distribution in the weight of dry vines of the parental forms (*Phaseolus vulg. melleus* and Inexhaustible bean), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

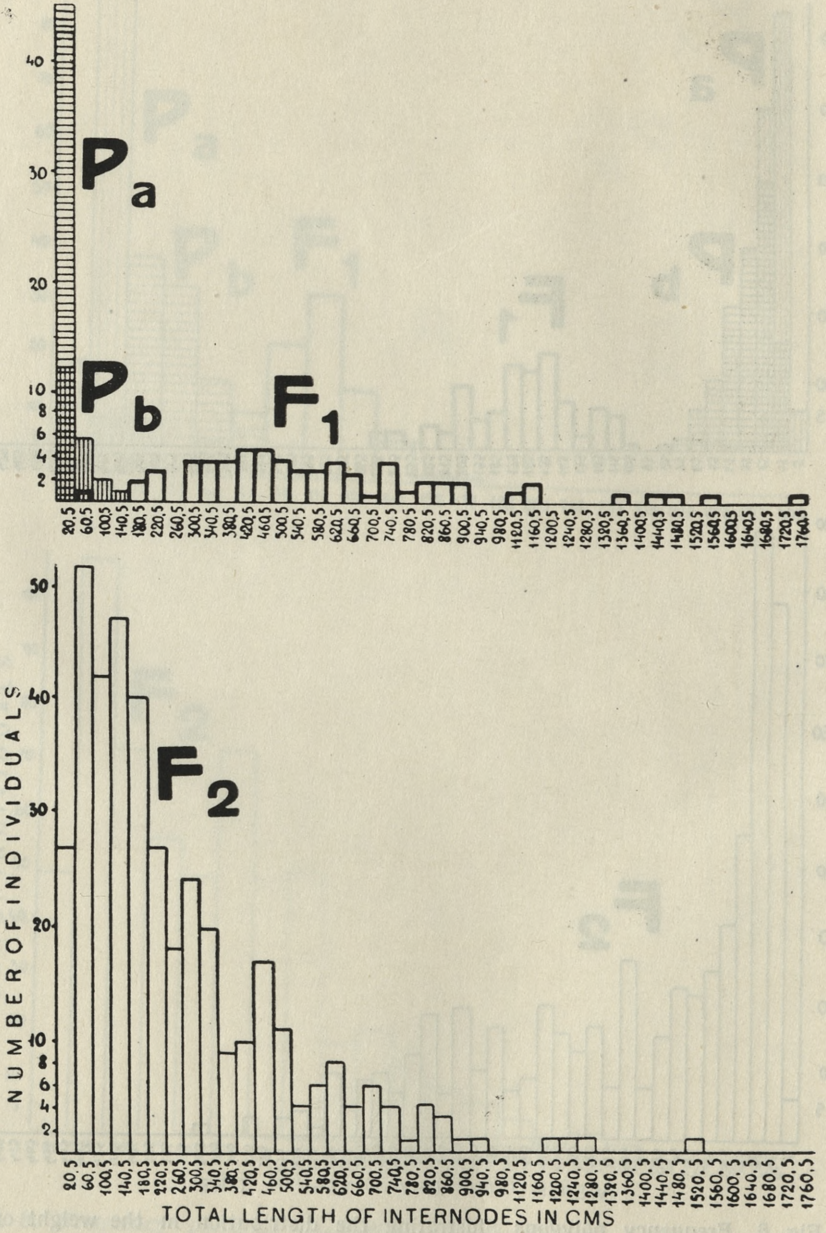

Fig. 7. Frequency polygons illustrating the distribution in total length of internodes of the parental forms (two varieties of *Petunia violacea*), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

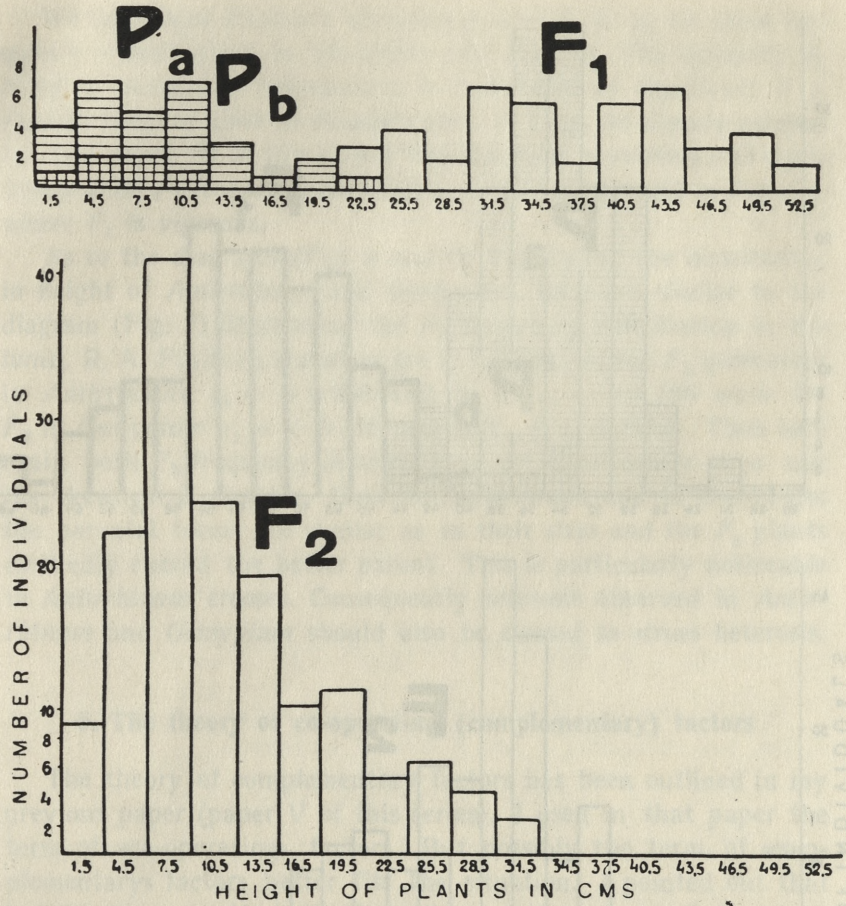

Fig. 8. Frequency polygons illustrating the distribution in height of the parental forms (two varieties of *Antirrhinum*), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

more or less with the average of the parents. The range of variation of F_2 exceeds the higher classes of F_1.

The diagram (Fig. 7) concerning *Petunia* crosses has the same characteristic features as the diagrams (Figs 3—6) relating to the kidney bean. There is however a difference between them. In *Petunia*, contrary to what is observed in *Phaseolus*, the range of variation of F_2 does not exceed the higher classes of F_1.

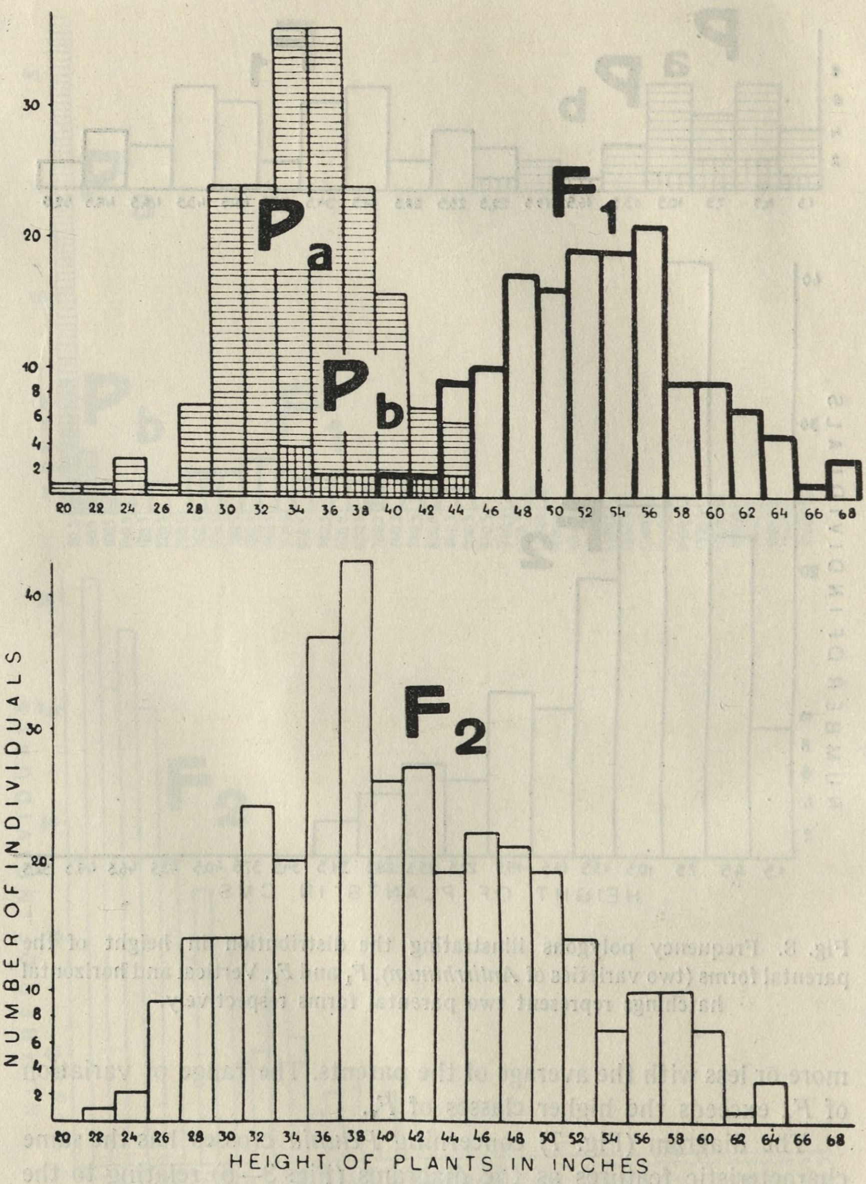

Fig. 9. Frequency polygons illustrating the distribution in height of the parental forms (two varieties of *Gossypium*), F_1 and F_2. Vertical and horizontal hatchings represent two parental forms respectively.

We calculated Fisher's statistics g_1 and S. E. g_1 for skew frequency distributions in *Phaseolus* and *Petunia*. The quantity g_1 being a measure of asymmetry, is considered as significant if it exceeds twice or more its standard error S. E. g_1. As already pointed out (see paper III of this series) this skewness is positive and significant in all F_2 frequency distributions in *Phaseolus* and in *Petunia*, where F_1 is vigorous.

As to the diagrams (Figs 8 and 9) illustrating the distribution in height of *Antirrhinum* and *Gossypium*, they are similar to the diagram (Fig. 7) illustrating the F_2 frequency distribution in *Petunia*. R. A. Fisher's statistics are as follows: for the F_2 generation in *Antirrhinum* $g_1 = +0{\cdot}956$ and S. E. $g_1 = \pm 0.196$ while for F_2 in *Gossypium* $g_1 = +0{\cdot}376$ and S. E. $g_1 = \pm 0{\cdot}131$. Thus here again both F_2 frequency distributions are significantly skew and the skewness is positive. Both in *Antirrhinum* and in *Gossypium* the parental forms are similar as to their sizes and the F_1 plants markedly exceed the better parent. This is particularly noticeable in *Antirrhinum* crosses. Consequently heterosis observed in *Antirrhinum* and *Gossypium* should also be classed as «true» heterosis.

6. The theory of co-operating (complementary) factors

The theory of complementary factors has been outlined in my previous paper (paper V of this series). I used in that paper the term of «co-operating» factors. But possibly the term of «complementary» factors better fits the situation. I pointed out that hybrid vigour manifests itself in all vegetative organs of plants and I assumed that such a simultaneous increase in all vegetative parts is a manifestation of a set of complementary factors. A simultaneous presence of all complementary factors is needed to bring about heterosis. The complementary factors are separated in the parental forms and they unite in F_1. I compared this case to a well known example of coloured sweat peas obtained in F_1 from a cross of two white varieties. In our case however the number of factors necessary to bring about vigorous growth is greater than the one concerning the factors responsible for the coloured sweat peas.

Let us suppose that there are n complementary factors

$$A_1, A_2, A_3 \ldots A_n.$$

With regard to these factors all individuals of the F_2 generation may be divided into two broad categories α and β.

An individual belongs to the category α if all complementary factors are present either in the homozygous or in the heterozygous condition. The remaining individuals form the category β. Now it is easy to calculate the probability of an individual belonging to the category α.

Let p denote this probability.

Consider first A_1, one of the complementary factors. If the individual belongs to the category α, its genetical constitution with regard to the factors A_1 and a_1 must be either A_1A_1 or A_1a_1. The probability of such combinations is 3/4. The same is obviously true with regard to any other of the complementary factors. From which it follows that

$$p=\left(\frac{3}{4}\right)^n$$

Let us denote by q the probability of an individual belonging to the category β. We shall have

$$q=1-p=1-\left(\frac{3}{4}\right)^n=\frac{4^n-3^n}{4^n}$$

Now the theoretical ratio of the numbers of individuals belonging to the two categories α and β will be as follows:

$$p:q=3^n:(4^n-3^n)=l:m \text{ (say).}$$

The Table XI gives the value of l, m and the ratio $\frac{l}{m}$ corresponding to the increasing values of n.

If two pairs of complementary factors came into play there would be in F_2 more vigorous than non-vigorous plants, the ratio being 9 : 7. But then, beginning with the case of three complementary factors, necessary to bring about hybrid vigour, we should expect reverse relations, namely the numerical prevalence of non-vigorous plants over vigorous ones. In the case of three complementary factors the ratio between non-vigorous and vigorous individuals would be 37 : 27, in the case of four factors this ratio would be 175 : 81 and so on (see Table XI).

We assumed (Malinowski, V, 1950) that our population with positive skewness is combined of two populations of different sizes. One of these populations includes the size of both parental forms and the other corresponds to the size of F_1. The second frequency distribution may be looked upon as a modification of the first one. This modification would be due to the complementary factors. If we shift the first frequency distribution from the lower classes, corresponding to those of the parental forms, to the higher classes, corresponding to the classes of F_1, we shall get the second frequency distribution. These two frequency distributions make up together the general skew frequency distribution characteristic of «true» heterosis.

In the case of such a true heterosis the population corresponding to F_1 is always less numerous than the one corresponding to the parents. The number of complementary factors in such cases is larger than 2. I tried to divide one of our F_2 populations in *Phaseolus vulgaris* into two components and I obtained about 286 non-vigorous and 117 vigorous individuals. Then the ratio $\frac{l}{m}$ would be 0·4 (see Table XI) which would indicate that the number of complementary factors responsible for vigorous growth is 4. Of course such a conclusion as the to actual number of complementary factors is rather difficult to defend on the basis of our experimental data.

TABLE XI

n	4^n	$l = 3^n$	$m = 4^n - 3^n$	$\frac{l}{m}$
2	16	9	7	1·28571
3	64	27	37	0·72973
4	256	81	175	0·46286
5	1024	243	781	0·31114
6	4096	729	3367	0·21651
7	16384	2187	14197	0·15405
8	65536	6561	58975	0·11125
9	262144	19683	242461	0·08118
10	1048576	59049	989527	0·05967

The theory of complementary factors presented in this paper in a rather simplified form fits the facts characteristic of true heterosis, namely the facts found in *Phaseolus*, *Petunia*, *Antirrhinum* and *Gossypium*. The question arises if it fits also the facts found in maize.

I shall try to explain the phenomena of the so-called hypothetical heterosis in maize (desribed in Emerson and East's paper) in terms of this theory.

We may assume that in the case of maize there are also two populations in F_2 one with the mode corresponding to the mid-parental value and the other with the mode shifted to the higher classes of the range of variation. This second population is less numerous than the first one. The individuals of this population show added vigour due to complementary factors, which in the case of maize does not exceed the vigour of the better parent. Of course the general F_2 frequency distribution has its mode shifted also to the higher classes as compared with the mid-parental value and it displays a negative bias. Depending upon the degree of added vigour the shift will be more or less pronounced and in this connection the negative bias will be more or less marked.

In Fig. 10 four diagrams (A—D) are shown representing the ranges of the frequency distributions of the parental forms, F_1 and F_2 in each of the 4 maize crosses referred to above. Marks of cross (M) in these diagrams represent position of respective modal values. Diagram A represents cases of nearly symmetrical F_2 frequency distribution. In such case the mode of F_1 is shifted a little to the higher classes as compared with the mid-parental value. Diagram B refers to such cases in which F_2 frequency distribution shows a noticeable negative bias and its mode is greater than the mid-parental value. The F_1 is nearer the larger parent than it was in the preceding case. Diagram C represents such cases in which the F_2 frequency distributions show significant negative skewness and its mode approaches closer the larger parent. The F_1 proved equal to the larger parent.

In the cases represented by the diagrams A, B, and C the range of variation of the general F_2 frequency distribution does not exceed the range of the larger parental form.

All cases of heterosis found in maize and represented in the diagrams A, B and C, may be explained in terms of the theory of co-operating factors. Diagram A refers to such cases in which added vigour charcteristic of F_1 is very small and the negative bias of the F_2 frequency distribution is insignificant. The complementary factors increase very slightly the vigour of F_1 as compared with the mid-parental value. Diagram B refers to the instances in which the added vigour in F_1 is greater and the modal value of the population of vigorous plants of the F_2 generation is more distant from the mid-parental value than in the preceding case. In accordance with our theory this population of vigorous F_2 plants is less numerous than

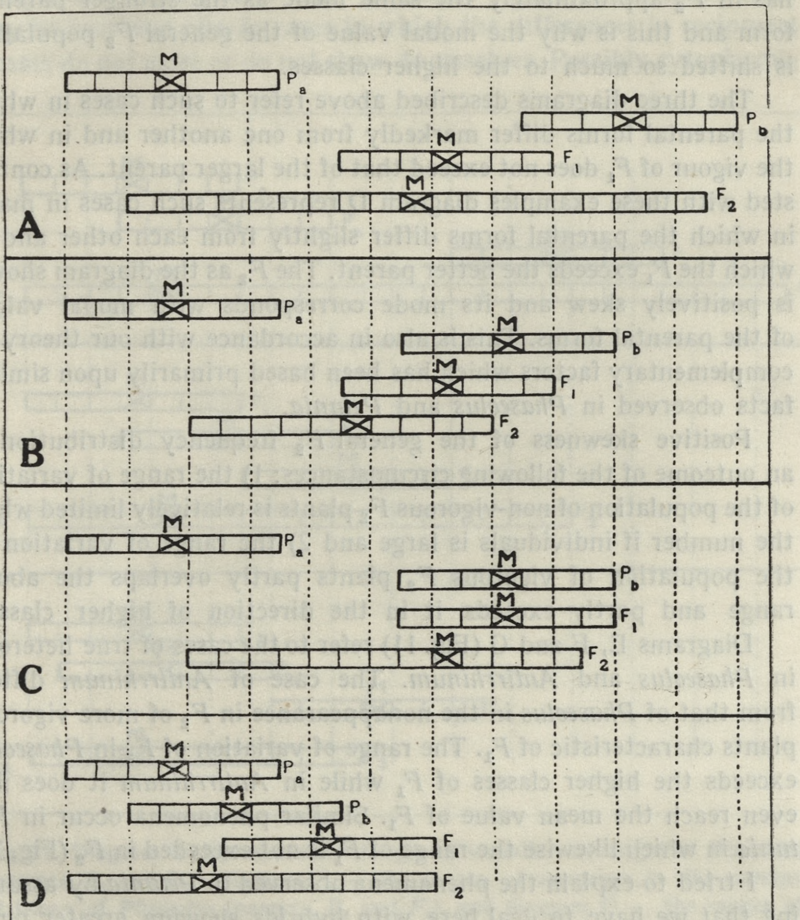

Fig. 10. Diagrams (A, B, C, D) represent the ranges of variation of the parental forms, F_1 and F_2 in 4 maize crosses. M — position of modal value.

that of non-vigorous F_2 plants. Owing to the fact that the population of vigorous F_2 plants in this case is more distant from the mid-parental value the modal value of the general F_2 population is shifted more in the direction of higher classes than in the preceding case. Diagram C refers to the cases in which complementary factors increase vigour still more, so that the F_1 generation approaches the size of the stronger parental form. The population of vigorous plants has in F_2 approximately the same mode as the stronger parental form and this is why the modal value of the general F_2 population is shifted so much to the higher classes.

The three diagrams described above refer to such cases in which the parental forms differ markedly from one another and in which the vigour of F_1 does not exceed that of the larger parent. As contrasted with these examples diagram D represents such cases in maize in which the parental forms differ slightly from each other and in which the F_1 exceeds the better parent. The F_2 as the diagram shows, is positively skew and its mode corresponds with modal values of the parental forms. This is also in accordance with our theory of complementary factors which has been based primarily upon similar facts observed in *Phaseolus* and *Petunia*.

Positive skewness of the general F_2 frequency distribution is an outcome of the following circumstances: 1) the range of variation of the population of non-vigorous F_2 plants is relatively limited while the number if individuals is large and 2) the range of variation of the population of vigorous F_2 plants partly overlaps the above range and partly exceeds it in the direction of higher classes.

Diagrams E, F and G (Fig. 11) refer to the cases of true heterosis in *Phaseolus* and *Antirrhinum*. The case of *Antirrhinum* differs from that of *Phaseolus* in the nonappearance in F_2 of more vigorous plants characteristic of F_1. The range of variation of F_2 in *Phaseolus* exceeds the higher classes of F_1 while in *Antirrhinum* it does not even reach the mean value of F_1. Similar phenomena occur in *Petunia* in which likewise the range of F_1 is not exceeded in F_2 (Fig. 7).

I tried to explain the phenomena observed in *Petunia* by assuming that we have to deal here with hybrids showing greater physiological effectivness due to heterogeneity of the protoplasmic material. The stimulating effect of this heterogeneity diminishes in F_2 and probably disappears in the subsequent generations. In favour of such an assumption I cited the fact of the occurence of hybrids of different sizes in reciprocal crosses in *Petunia*. Two years ago I repeated these experimets taking for each reciprocal crossing only two individuals. Each of such individuals belonged to a different inbred and degenerate line and of course to a different variety. Investigations concerning this problem are still in progress and will be published later.

Differences in reciprocal hybrids can be taken as evidence of cytoplasmic inheritance. But of course cytoplasmic inheritance may be involved also in cases in which the differences in reciprocal crosses do not exist or do not show themselves. Possibly cytoplasmic inheritance takes place also in maize with respect to vigorous growth independently of complementary factors determining heterosis.

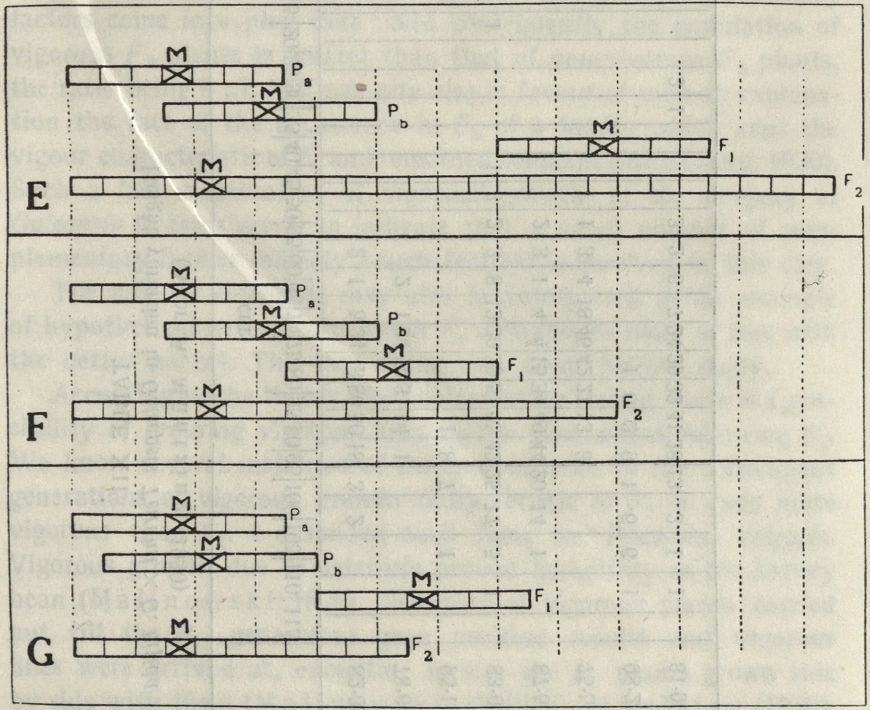

Fig. 11. Diagram E represents the ranges of variation in height of ***Phaseolus*** (parents, F_1 and F_2); diagram F — the ranges of variarion in the number of leaves of *Phaseolus* (parents, F_1 and F_2) and diagram G — the ranges of variation in height of *Antirrhinum* (parents, F_1 and F_2).

I should like to discuss further the phenomena of heterosis in *Galeopsis* described by Müntzing (1930). Table XII reproduced here after Müntzing's monograph shows two F_2 frequency distributions. In both cases frequency distributions are negatively skew and

TABLE XII

Height variation in *Galeopsis Tetrahit* × *G. bifida*
(After A. Müntzing, 1930)

		cm																					$\bar{x}$	*n*	g_1	S. E. g_1
		15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115				
Tetrahit	1928									2	1	5	24	48	80	50	18	3	2				82·05 ± 0·45	233		
bifida	1928										2	1	24	59	31	14	1						78·65 ± 0·44	132		
F_1	1928															1	8	7		1			95·15(± 1·07)	17		
F_2	1928	1					1	3	2	3	8	12	30	39	51	55	42	25	17	5	1		83·60 ± 0·72	295	—0·953	±0·142
Tetrahit	1929								2	3	1	4	4	15	34	49	44	20	14	1			87·55 ± 0·69	191		
bifida	1929							1	1	3	4	18	46	42	21	13	3						75·50 ± 0·61	152		
F_1	1929													1	0	2	9	1	6	6	1		98·25 ± 1·66	26		
F_2	1929			2	1				3	2	6	7	24	29	32	41	28	13	3	1	1		81·90 ± 0·88	193	—1·371	±0·175

the skewness is significant. The parental forms are similar as to their vigour. The F_1 plants are higher than both parents and typically luxuriant. But this hybrid vigour is rather small. The modal value of the F_2 frequency distribution is equal to or a little higher than the mode of the better parent.

Negative skewness of the general F_2 frequency distribution may be accounted for in assuming that only two complementary factors come into play here and consequently the population of vigorous F_2 plants is greater than that of non-vigorous F_2 plants, the ratio being 9 : 7. We may cite also in favour of such an explanation the fact of the occurrence in F_3 of a family which kept the vigour characteristic of F_1 and remained constant (Müntzing, 1930). Such a fast stabilisation of vigorous growth in the progeny of *Galeopsis* hybrids seems to indicate that a small number of complementary factors (namely 2 such factors) is involved in this case.

The case of *Galeopsis* may also be interpreted as an example of hypothetical heterosis in which F_1 corresponds more or less with the better parent. This interesting case needs further study.

According to the theory of complementary factors there is a possibility of securing vigorous lines in the generations following F_1. We know several instances of the maintenance in the subsequent generations of vigorous growth characteristic of F_1 or even more vigorous than F_1. I described such facts for *Phaseolus vulgaris*. Vigorous growth due to heterosis proved hereditary in the kidney bean (Malinowski 1928). Selection of vigorous plants carried out till the F_{11} generation gave positive results and vigorous lines were arrived at, exceeding in size the F_1 plants grown side by side with them (Malinowski, IV 1930). Müntzing (1930), as I already pointed out, obtained in *Galeopsis* constant segregates equally or more vigorous than F_1. The mother plants of the F_3 families were in part selected from among the most vigorous F_2 plants. Ten such mother plants were more than 100 cm high. Of the resulting progenies 9 showed a decrease varying between 1,3 and 30,0%; one family, however, kept the vigour and remained constant. The mother plant was 107 cm high, the progeny (n = 51) had the average value 109,45 ± 0,96 cm. At the same time the height variation was low. This F_3 family was also remarkably uniform in leaf shape and other morphological characters. The relative height values for the parents, F_1 and F_2

were 100, 121 and 100 respectively. This F_3 family will have the value 134. Veatch (1930) obtained in F_2 of soy-bean crosses hybrid vigour greater than that of the F_1 generation. Rave (1934) observed the maintenance in the F_4 generation of the F_1 increase in yield in three out of seven crosses between German varieties of tobacco. Kosmodemjjanski (1941) observed a transgression in the F_1 and subsequent generations of tobacco crosses. In some crosses a marked transgression occurred in the F_2 in most families and some families in the F_3 and F_4. In the fourth generation three progenies were no longer segregating and still showed transgression for both height and number of leaves.

The question arises whether the inbred and degenerate lines of *Petunia* which were used for our crossing experiments represent genotypes differing from the initial varieties. We may ask the question if such inbred lines of *Petunia* may be compared to the varieties of *Phaseolus vulgaris* used for our crosses in which F_1 was vigorous (Malinowski, I and II, 1950). The varieties of *Petunia* are cross-fertilizing ones while those of *Phaseolus vulgaris* are self-fertilizing. In *Petunia* we observe a marked reduction in size of individuals after 10—12 years of self-fertilization[1]. In *Phaseolus vulgaris* such a reduction in size does not occur or perhaps it is very small and not visible. The reduction in size of inbred and apparently degenerate lines of *Petunia* may be caused either by lack of stimulation in the inbred homozygotes or by isolation of genotypes which differ from the original cross-fertilizing varieties. Both causes may of course be operative simultaneously. The inbred lines of *Petunia*, when crossed between themselves, show heterosis in F_1. In F_2 we observe a segregation into two populations, one composed of vigorous plants and the other including small individuals similar to the degenerate parental forms (Fig. 7). The occurrence of such a segregation seems to indicate that inbreeding degeneration, in part at least, is a Mendelian phenomenon.

Summary

The author distinguishes two kinds of heterosis: true heterosis in which the characters of the hybrid are superior to the better

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[1] The flowers were self-pollinated in buds.

parent and hypothetical heterosis in which the F_1 generation is intermediate in the characters studied, being as a rule more or less larger than the average of the parents.

In the case of true heterosis the F_2 frequency distribution is positively skew and its mode corresponds to the average of the parents. In the case of hypothetical heterosis the F_2 frequency distribution is symmetrical or negatively skew. There are also intermediate types of heterosis.

The author attempts to explain all these various types of heterosis by means of the theory of complementary factors.

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Bibliography

1) Baur E.: Einführung in die experimentelle Vererbungslehre. Berlin 1914. — 2) Emerson R. A. and East E. M.: The inheritance of quantitative characters in maize. Nebraska Agr. Exp. Station. Research. Bull. 2. 1913.—3) Fisher R. A., Immer F. R., and Tedin O.: The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. Genetics. 17, 1932. — 4) Hayes H. K.: Correlation and inheritance in *Nicotiana Tabacum*. Connecticut Agr. Exp. Station. Bull. 171, 1912. — 5) Jones D. F.: Dominance of linked factors as a means of accounting for heterosis. Genetics. 2, No 5, 1917. — 6) Jones D. F.: The effects of inbreeding and crossbreeding upon development. Connecticut Agr. Exp. Station. Bulletin 207. 1918. — 7) Kosmodemjjanski V. N.: Transgression in different generations of hybrids of *Nicotiana Tabacum*. Wsiesojuznyj Nauczno Izsledowatelskij Institut Tabacznoj i Machorocznoj Promyszlennosti im. Mikojana 1941. — 8) Malinowski E.: A peculiar case of heterosis in *Phaseolus vulgaris*. V Int. Congress of Genetics. Zeit. f. ind. Abstamm. u. Vererbungslehre. Berlin 1928. — 9) Malinowski E.: Phénomènes de l'accroissement de la vigueur chez les hybrides de *Phaseolus vulgaris* C. R. Séances. Soc. Biol. III. 1035, Paris 1932. — 10) Malinowski E.: The problem of Heterosis. I. Heterosis in intervarietal crosses of *Phaseolus vulgaris*. Bull. Acad. Polon. Scie. Cracovie 1950. — 11) Malinowski E.: The problem of Heterosis. II. Heterosis in interspecific and intervarietal crosses of *Petunia*. Bull. Acad. Polon. Sc. Cracovie. 1950. — 12) Malinowski E.: The problem of Heterosis. III. Positive skewness of the F_2 frequency distributions. Bull. Acad. Polon. Sc. Cracovie 1950. — 13) Malinowski E.: The problem of Heterosis. IV. Inheritance of vigorous growth. Bull. Acad. Polon. Sci. Cracovie 1950. — 14) Malinowski E.: The problem of Heterosis, V. The hypothesis of co-operating (complementary) factors. Bull. Acad. Polon. Sci. Cracovie 1950. — 15) Müntzing A.: Outlines to a genetic monograph of the genus *Galeopsis*. Hereditas 13, 1930. — 16) Rave L.: Heterosis beim Tabak. Züchter 1934. — 17) Sprague G. F.: The experimental basis for hybrid maize. Biol.

Reviews. 21, 1946. — 18) Veatch C.: Vigor in soybeans as affected by hybridity. Journ. Amer. Soc. Agronomy 22, No 4, 1930. — 19) Ware J. O.: Hybrid intensification of plant height in cotton and the relationship of node number and internodal length to the phenomenon. Journ. Amer. Soc. Agronomy 22, No 9, 1930.

CORRIGENDA

In paper IV of this series:

Page 261, line 3 from below, for «Plates 2 and 3» read «Plates 11 and 12».

Page 282, line 7 from below, for «(Fig. 4)» read «(Fig. 5)».

Page 285, line 5 from below, for «Fig. 9. Drawing of a vigorous F_6 plant» read «Fig. 9. Drawing of a vigorous F_7 plant».

Page 287, line 1 from below, for «was equal to 0·19 cm» read «was equal to 0·18 cm».

Page 292, line 2 from above, for «the difference being 3·48 cm» read «the difference being 3·50 cm».

In paper V of this series:

Page 148, line 5 from below, for «not exceeded in F_2» should read «is not exceeded in F_2».

Problem Heterozji. VII. Bujny wzrost i tendencja do wicia się u fasoli karłowej. — The problem of Heterosis. VII. Vigorous growth and twining tendency in bush beans.

Mémoire

de M. ***E. MALINOWSKI*** m. t.

présenté le 10 Juin 1950 par M. E. Malinowski m. t. et M. M. Korczewski m. c.

In our studies on heterosis in *Phaseolus vulgaris* two varieties of bush beans were used primarily, namely: *Phaseolus vulgaris melleus* and Inexhaustible bean (Malinowski, I, 1950). The first of these parental forms never shows any twining tendency. We grew this variety in poor and rich soil, in the field and in the greenhouse but we never found even one internode showing cirmcumnutation. The Inexhaustible bean, on the contrary, shows a twining tendency when grown in rich soil, in the greenhouse. In Figs 1 and 2 we see diagrams of the Inexhaustible bean grown in 1950 in the field (Fig. 1) and in pots in compost soil in a greenhouse (Fig. 2). In these diagrams all internodes are drawn to the same scale. Some twining internodes were observed only in compost soil in the green-

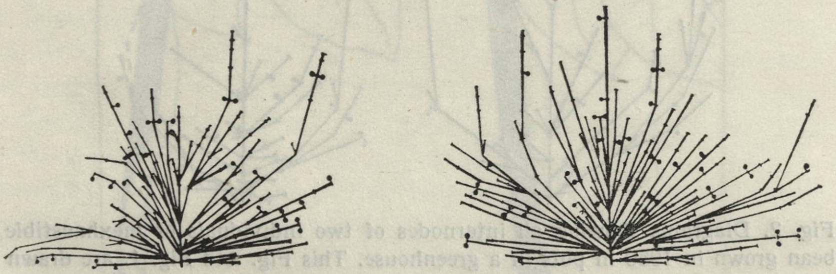

Fig. 1. Diagrams showing all internodes of two individuals of Inexhaustible bean grown in 1950 in the field. This Fig. and Fig. 2 are drawn to the same scale.

house. Under such conditions the individuals of the Inexhaustible bean grew higher, they were less branched and showed a slight twining tendency. In the first plant, represented diagrammatically in Fig. 2, we see 3 twining internodes (shown with broken line) and in the second one— 2 twining internodes (one on the main axis and the second on a lateral shoot).

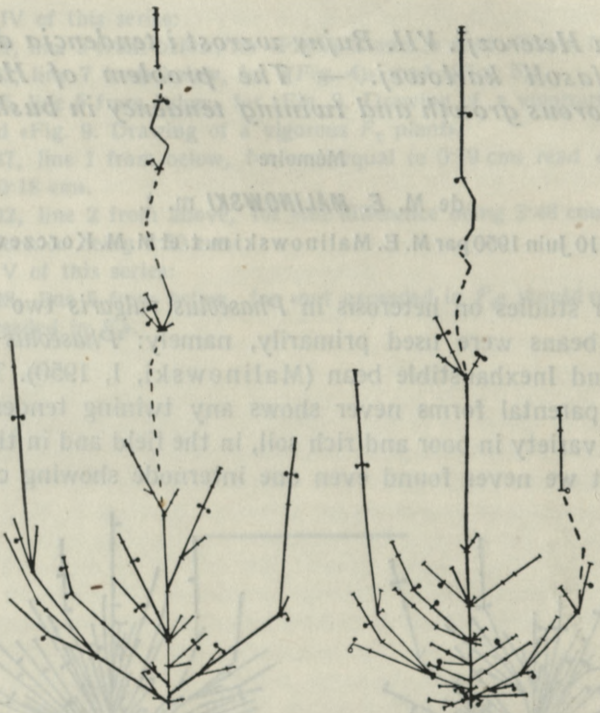

Fig. 2. Diagrams showing all internodes of two individuals of Inexhaustible bean grown in 1950 in pots in a greenhouse. This Fig. and Fig. 1. are drawn to the same scale.

The plants of the F_1 generation from the cross *Phaseolus vulgaris melleus* × Inexhaustible bean never showed any twinnig tendency. They remained bush beans. Not one case of twining round the support was observed in this generation. In spite of a careful examination of the F_1 plants I did not find even one internode showing circumnutation.

As concerns the F_2 plants they may develop circumnutation consisting in a twining of one or two internodes around the support.

This phenomenon was observed several times in the F_2 generation grown in the field in a rather poor soil, the distance apart between particular plants being 1 m in both directions. Neither *Phaseolus vulgaris melleus* nor the Inexhaustible bean, grown side by side in alternate rows with the F_2 generation, showed any circumnutation.

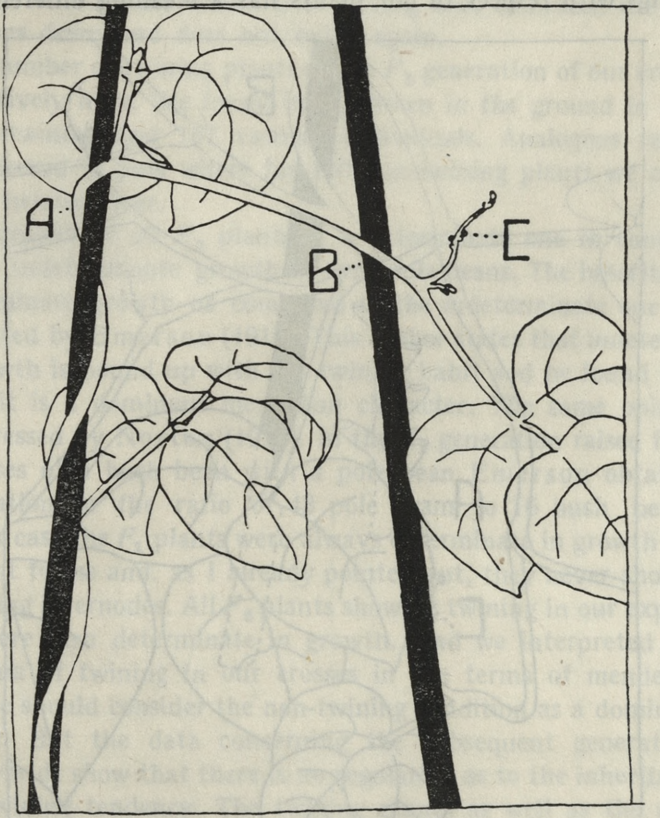

Fig. 3. Twining of an individual of the F_2 generation.

The Inexhaustible bean under such conditions had a compact habit shown in Fig. 1. Twining observed in bush beans (and all our F_2 plants are bush beans) cannot, however, be compared to the twining habit of beans. Our F_2 plants do not climb on poles. When one or two of their internodes turn round the pole this does not suffice to support the whole plant. Without an artificial fastening with string to the support many branches would droop and lie loosely on the ground. To say of our plants that they have a twining habit would be an exaggeration. The mature F_2 plants do not look at all like pole beans and it is difficult sometimes to find a twining internode among the numerous non-twining branches. The term «twining» with respect to our plants has a meaning different from that which it has when we refer to the real pole beans. The twining branches turn, in our case, usually once or twice around the supporting pole. There are rare instances when a vine twines three times but I have never seen a branch turning more than three times around the pole, and in general such twining branches are rare. Sometimes one can see a long and slender branch which has to be

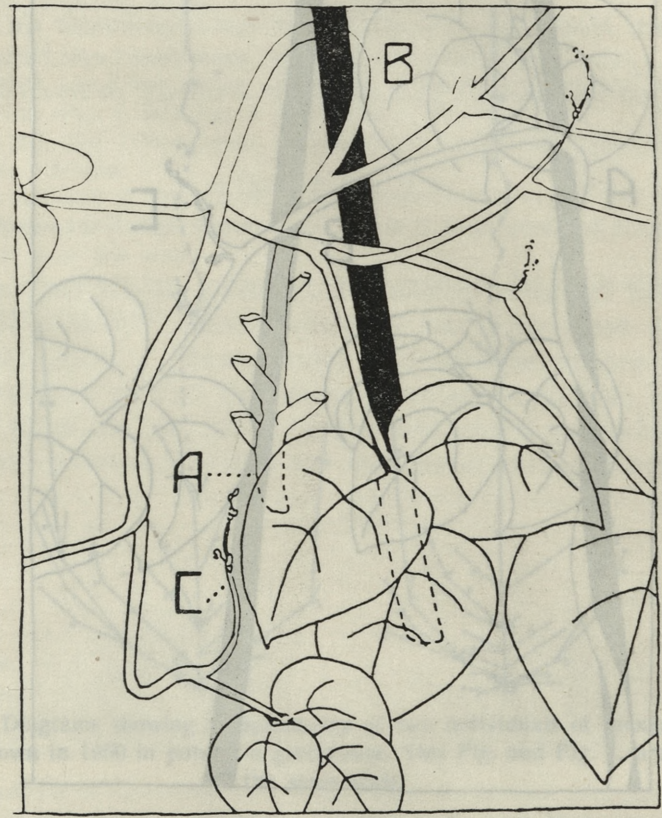

Fig. 4. Twining of a plant of the F_2 generation.

tied up to the support but which does not take hold of it and does not show any circumnutation.

The drawings shown in Figs 3 and 4 represent the mode of twining of some F_2 plants. In Fig. 3 the branch turns twice and afterwards remains straight in spite of two poles touching it. In Fig. 4 we see a branch which after having once embraced the support drops down and does not twine again.

The number of twining plants in the F_2 generation of our crosses was relatively high. We found in F_2 grown in the ground in 1925 223 non-twining and 167 twining individuals. Analogous results were observed in pots where for 140 non-twining plants we counted 112 twining ones.

The growth of all F_2 plants is a determinate one in contrast with the indeterminate growth of true pole beans. The inheritance of determinate growth as compared to the indeterminate one was investigated by Emerson (1916). This author states that indeterminate growth is bound up with the twining habit and he found that this habit is a dominant mendelian character. The same opinion was expressed by Norton (1915). In the F_2 generation raised from the crosses of a bush bean with a pole bean Emerson obtained a segregation in the ratio of 48 pole beans to 16 bush beans.

In our case the F_1 plants were always determinate in growth like the parent forms and, as I already pointed out, they never showed any twining internodes. All F_2 plants showing twining in our experiments were also determinate in growth. Had we interpreted the phenomena of twining in our crosses in the terms of mendelian factors we should consider the non-twining condition as a dominant character. But the data concerning the subsequent generations of our hybrids show that there is no regularity as to the inheritance of the twining tendency. The twining plants as well as the non-twining ones may «segregate» in their progeny into twining and non-twining individuals, the number of non-twining being greater in both cases. From Tables I and II we see that in the progeny of non-twining plants there were in the field 12·00% of twining ones and in the progeny of twining individuals the percentage of twining plants was nearly the same, namely 10·50%. It is difficult to say on the basis of such data that there is a genetic difference between twining and non-twining plants in our case.

TABLE I
Progeny of non-twining plants

Nº of line	Number of individuals			
	in the field		in pots	
	non-twining	twining	non-twining	twining
129 — 1927	3	—	7	1
349 ,,	29	—	12	—
499 ,,	23	1	18	—
463 — 1928	16	—	2	4
494 ,,	—	—	4	5
117 ,,	14	—	5	1
174 ,,	33	—	9	—
544 ,,	6	1	5	1
211 ,,	33	—	—	—
205 ,,	20	—	—	—
174 ,,	33	—	—	—
547 ,,	11	—	—	—
117 ,,	13	—	—	—
463 ,,	16	—	—	—
486 — 1929	34	10	—	—
461 ,,	37	1	—	—
525 ,,	54	8	—	—
257 ,,	14	—	—	—
Total	399	21	62	12
% of twining plants in all lines	95·00%	5·00%	83·79%	16·21%
% of twining plants in the lines segregating either in the field or in pots or in both	88·00%	12·00%	65·72%	34·28%
% of twining plants in particular segregating lines taken as a whole	92·50%	7·50%	84·62%	15·38%

TABLE II

Progeny of twining plants

Nº of line	Number of individuals			
	in the field		in pots	
	non-twining	twining	non-twining	twining
506 — 1926	—	—	4	9
22	68	8	—	—
93	110	8	—	—
232	116	10	21	7
216	3	1	6	5
808	20	1	31	6
843	22	1	35	4
211	52	—	15	3
184	41	12	11	19
160	52	—	8	—
292 — 1927	30	—	8	8
592	27	1	12	7
134	38	2	11	5
938	42	9	16	11
901	14	—	5	5
437	—	—	16	13
131 — 1928	13	3	6	2
132	9	3	7	2
216	17	—	—	—
278 — 1929	3	2	—	—
293	25	2	—	—
Total	702	63	220	110
% of twining plants in all lines	91·77%	8·23%	66·67%	33·33%
% of twining plants in the lines segregating either in the field or in pots or in both	89·50%	10·50%	65·81%	34·19%
% of twining plants in particular segregating lines taken as a whole	90·95%	9·05%	65·81%	34·19%

We may possibly search for the cause of the twining tendency of our plants in their vigorous growth. Emerson (1916) holds that under ordinary conditions bush beans rarely show prominent circumnutation, but if forced into a very vigorous growth the long upper internodes develop pronounced circumnutation. This author is of the opinion that the twining habit is not a distinguishing characteristic of pole beans. Its absence from bush beans is incidental to the fact that their main axis is equivalent to the first four-eight internodes of pole beans, in which twining is little developed, particularly if growth is slow.

In favour of such an opinion I may cite the results of our experiments carried out in pots. In our pots the percentage of twining plants proved to be much higher than in the field. From Tables I and II we see that in pots in the progeny of non-twining plants there were 34·28% of twining ones and in the progeny of twining individuals we obtained 34·19% of twining plants. There is little difference, if any, between the progeny of twining and non-twining plants as far as the percentage of individuals showing the twining tendency is concerned, and with respect to this problem a close similarity exists between the plants grown in pots and those grown in the field. The important point is that the percentage of twining plants (regardless of whether they descended from twining or from non-twining individuals) was greater in pots (34·19%—34·28%) than in the field (10·50—12·00%%). Such a great difference shows that the character in question is influenced to a very high degree by the environmental conditions, stimulating an increased growth of plants.

If we compare the height of plants of particular lines grown in the field to that of the ones grown in pots we find that on an average the plants in pots are higher. This applies to the majority of lines descending from one individual of the preceding year. Each year we have sown a part of the seeds of a given individual in the field and the other part in pots. So, for instance, the line Nr 184 grown in the field in 1926 exhibited an average height $\overline{x} = 70{\cdot}94$cm while its average height in pots in the same year was $\overline{x} = 99{\cdot}50$ cm.

All lines, we experimented with, regardles of the generation of hybrids to which they belonged, behaved in the same manner. Table III shows that the average height of the plants was always greater in pots than in the field. For the parental varieties it was the same (Table III).

Two other features characterizing vigour, namely the length of the terminal leaflets and the weight of the vines, behave in a rather different way.

TABLE III

Arithmetic means concerning the height of plants, the length of terminal leaflets and the weight of vines in F_3 and F_4 lines and in the parental varieties grown in the field and in pots

		Height of plants in cm		Length of leaflets in cm		Weight of vines in g	
		in field	in pots	in field	in pots	in field	in pots
F_3 lines (1926):	808	47·86	72·76	12·67	15·60	38·25	43·02
	843	49·78	68·71	12·43	16·05	44·13	49·48
	211	43·34	66·38	13·98	16·22	44·81	40·00
	184	70·94	99·50	14·87	16·17	63·43	58·34
	160	70·20	82·50	14·42	15·00	67·74	55·00
	232	68·73	83·40	13·57	14·93	56·55	44·28
	216	71·25	66·82	17·50	16·36	53·75	50·00
F_4 lines (1927):	292	61·00	108·82	12·50	14·82	64·33	56·47
	592	71·43	114·73	13·21	15·10	102·50	58·94
	134	63·75	109·33	15·05	16·86	104·50	73·33
	129	64·62	91·00	14·08	16·20	107·69	70·00
	938	82·94	106·29	16·00	15·48	157·45	65·92
	901	75·00	103·00	15·29	15·00	147·14	57·00
	349	46·21	48·33	13·14	12·83	85·86	39·16
	499	54·58	63·88	15·00	14·50	106·25	52·22
Phas. vulgaris melleus	1925	31·82	35·00	10·60	12·00	27·73	24·00
Inexhaustible bean	1925	51·67	56·00	8·50	11·10	36·92	37·00
Phas. vulgaris melleus	1926	24·28	42·77	9·90	14·89	16·66	28·33
Inexhaustible bean	1926	39·44	58·57	9·11	12·43	27·22	36·42
Phas. vulgaris melleus	1927	35·00	42·00	11·09	13·20	40·00	32·00
Inexhaustible bean	1927	60·00	65·00	12·17	11·50	72·78	40·00

In the majority of lines (namely in 15 out of 21) the terminal leaflets were larger in pots than in the field (Table III) but as to the weight of vines the reverse phenomenon took place, for in 16 lines out of 21 the weight of the vines was smaller in pots than in the field (Table III).

We do not take into consideration here the F_5 lines grown in pots in 1928 because, as we have already pointed out (Malinowski, IV, 1950) during such a year the plants in pots suffered greatly from the red spider and therefore their vigour was smaller than it would have been under normal conditions.

The conclusion we came to as to the percentage of twining individuals may lead to some objections if we base ourselves upon the ground that when calculating this percentage we did not take into consideration some non-segregating lines. So, for instance, we omitted the lines Nr 129 (from 1927) and Nr 463 (from 1928) which did not segregate in the field in spite of their segregation into twining and non-twining plants in pots (Table II). If those lines had been taken into consideration the results regarding the percentage of twining plants would be a little different. Namely in the progeny of non-twining plants we would obtain fewer twining individuals than in the progeny of twining ones. In the former case the percentage of twining individuals would be 7·50% in the field and 15·38% in pots and in the latter case it would be 9·05% and 34·19% respectively. Thus, the percentage of twining plants in pots proved, as it was formerly, much greater than in the field.

Some lines enumerated in Tables I and II did not segregate at all, neither in the field nor in pots, for instance the lines Nr 211 (1928), Nr 205 (1928), Nr 174 (1928), Nr 547 (1928), Nr 117 (1928), Nr 463 (1928), Nr 257 (1929), Nr 160 (1926), Nr 216 (1928). All those lines comprised non-twining individuals only.

We never came across an entirely twining progeny. Not one line in our experiments, even the least numerous in individuals, was composed of twining plants only. These facts seem to indicate that the peculiar twining habit about which we speak is not a constant character and that its occurrence depends, to a large extent, upon the degree of vigour of a given plant.

Such a view is supported also by the fact that in F_2 of our crosses the average height of plants, the average weight of vines and the average length of terminal leaflets are greater in twining plants than in non-twining ones.

The average height of non-twining F_2 plants grown in the field in 1925 was 58·96 cm and that of the twining ones — 93·71 cm. In pots the average height of non-twining plants was 56·28 cm and of twining ones — 80·08 cm. Analogous differences were observed as to the length of terminal leaflets and the weight of the vines.

The average length of terminal leaflets of the non-twining F_2 plants grown in the field was 11·18 cm and that of the twining ones — 13·93 cm. In pots the averages were 11·92 cm and 14·04 cm respectively.

For the average weight of vines we obtained the following values: 41·50 g for the non-twining F_2 plants grown in the field, 92·55 g for the twining F_2 plants grown in the field, 35·50 g for the non-twining F_2 plants grown in pots and 50·48 g for the twining F_2 plants grown in pots.

It is perhaps worth noting that during the unusually cold season of 1933 the twining was hardly discernible. We had in this year over 1000 F_2 plants derived from 3 F_1 individuals and only a very few of those plants showed a slight tendency to twine. I found, namely, in 4 plants only some twining internodes, but these internodes did not make even one complete circumnutation around the supporting pole.

Heterosis affects all vegetative characters. Hybrid vigour concerns the plant as a whole. East (1936) writes that its effect «is comparable to the effect on a plant of the addition of a balanced fertilizer to the soil, or to feeding a more adequate and more chemically complete diet to the animal». Addition of fertilizers to the soil brings about a circumnutation of internodes in some bush varieties. Heterosis as we have demonstrated above produces a similar effect.

Summary

Many plants of the F_2 generation show a twining tendency. Some internodes of such plants circumnutate and turn round the support Such F_2 plants do not, however, climb to poles. When one or two of their internodes turn round the pole this does not suffice to support the whole plant. Individuals showing such a tendency are more vigorous than the ones not showing any circumnutation at

all. Heterosis, like fertilizer added to the soil, seems to stimulate in bush beans circumnutation of internodes.

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Bibliography

1) East E. M.: Heterosis. Genetics. 21, 1936. — 2) Emerson R. A.: A genetic study of plant height in *Phaseolus vulgaris*. Bull. Agr. Exp. Station, Nebraska 1916. — 3) Malinowski E.: The Problem of heterosis. I. Heterosis in intervarietal crosses of *Phaseolus vulgaris*. Bull. Acad. Sc. Cracovie B. I 1950. — 4) Malinowski E.: The Problem of heterosis. IV. Inheritance of vigorous growth. Bull. Ac. Sc. Cracovie B. I. 1950. — 5) Norton J. B.: Inheritance of habit in the common bean. American Naturalist. 1915.

Studia cytologiczne nad Cochlearia Tatrae Borb.
Cytological studies on Cochlearia Tatrae Borb.

Mémoire

de M. ***A. BAJER***

présenté dans la séance le 22 Janvier 1951 par M^{me} M. Skalińska m. c. et
M. B. Pawłowski m. c.

(Plates 1—2)

Contents:

Introduction

The genus *Cochlearia* is represented in Poland by two endemic species: *C. polonica* Fröhl and *C. Tatrae* Borb. The natural stands of *C. polonica* lie between the town Olkusz and the Błędowska Desert; cytological studies dealing with this species were the subject of a previous paper (Bajer 1950). *C. Tatrae* grows in the Tatra mountains, in the alpine layer on the slopes of Męguszowiecki above Morskie Oko, in the Męguszowiecki glacial cirque (about 1900 m. o. s. l.) and on the Męguszowiecki Pass pod Chłopkiem (2311 m. o. s. l.). It grows there in streams and on wet screes. These two stands are found on northern slopes; snow lies there till very late in spring and even till early summer. Often snow falls occur there also in summer. This happened in 1948, the first year of my observations; the higher stand was covered with snow in June and July. The next year both stands were covered by a thick layer of snow and ice in August (Fig. 45).

On these stands C. *Tatrae* grows abundantly; this is especially interesting since, according to the results obtained it does not represent a perennial but a biennial plant which spreads exclusively by seeds.

The purpose of this work was: 1) to establish the somatic chromosome number, 2) to investigate the meiosis in P. M. C.'s in normal conditions and to compare it with the course of meiosis in plants long covered by snow, to find out whether there is any connection between external conditions and irregularities in meiosis.

Material and methods

Plants for investigations were collected in the Mięguszowiecki Pass pod Chłopkiem and in the Mięguszowiecki glacial cirque.

Root tips were fixed exclusively in the laboratory. For the study of meiosis buds were fixed both in natural stands and in the laboratory. In order however to have comparable material the course of meiosis has been studied exclusively on material fixed in nature. P. M. C's with an irregular course of meiosis were fixed on Mięguszowiecki Pass pod Chłopkiem on July 21-st, 1948 in a temperature several degrees below 0°C. Some plants were covered by snow and others were so coated with ice and frozen, that when I touched them their stalks and leaves broke. P. M. C.'s with a regular course of meiosis were mostly fixed on July 24, 1949 on Mięguszowiecki Pass pod Chłopkiem at a temperature of +7°C. and in Mięguszowiecki glacial cirque at a temperature of +12°C, after a long spell of fine weather in the mountains. As a fixative only the Navashin fluid was used. Microtome sections 12 μ thick were stained with Newton gentian violet; most of the slides were differentiated in xylene-phenol (3 : 1) instead of clove oil.

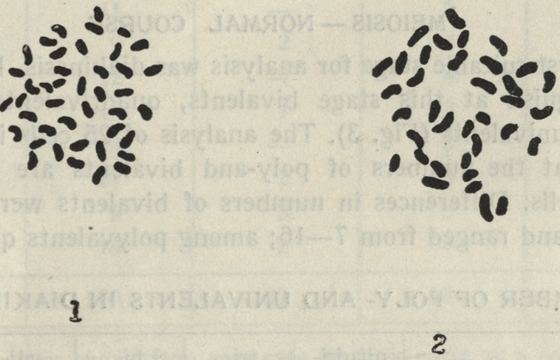
Drawings were made with a Leitz oil immersion lens 100 x N. A. 1·30 and Zeiss' compensating eyepiece 20 x (Fig. 1—42) and 15 x (Fig. 43). Abbè's camera lucida of Zeiss was used. Magnification of Figs. 1—42 is 3000 x and Fig. 43, — 2000 x. Microphotos were made with the aid of Zeiss' apochromate oil immersion lens

90 x N. A. 1·30, Zeiss' compensating eypiece 15 x and Makam of Leitz. Their magnification is 2850 x.

Cytological studies

MITOSIS

In all plants studied (15 individuals) the chromosome number in root tips was 2n = 42. This number is higher than that found in *C. polonica* Fröhl. (Bajer 1950) which has 2n = 36. Contrary to *C. polonica* which shows a differentiation of the chromosomes into smaller and larger, in *C. Tatrae* all chromosomes have approximately the same length i. e. 1·2—1·5 μ. The chromosome complexes of *C. Tatrae* and *C. polonica* are represented on Figs. 1—2. Previous investigations of chromosome numbers of some other species within this genus have shown that there are two basic numbers: 7 and 6. Accordingly *C. Tatrae* is a hexaploid with the basic number 7 while the autohexaploid *C. polonica* has the basic number 6.



Figs. 1—2. 1. Somatic plate of *C. Tatrae*. 2. Somatic plate of *C. polonica* differentiation in smaller and larger chromosomes. (Figs. 46—47).

Chromosome numbers of previously investigated species of this genus are given below:

C. arctica Schlechtend. (Greenland)	14	(unpubl. data of Sörensen from Löve and Löve 1948)
C. officinalis L. var. groenlandica Geleit. (Spitzbergen)	14	(Flovik 1940)
C. officinalis L. var. alpina (Färöer Islands)	24	(Böcher 1938)

. officinalis L. (Dennmark)	24, 24+1—4 ff	(unpubl. data of **Sörensen** from **Löve** and **Löve** 1948)
C. officinalis (Coast of S. W. Wales)	28	(**Crane** and **Gairdner** 1923)
C. micacea	36—34	(**Crane** and **Gairdner** 1923)
C. polonica Fröhl. (Surrounding of Olkusz)	36	(**Bajer** 1950)
C. danica	42	(**Crane** and **Gairdner** 1923)
C. anglica (Hayling Island Hant's)	49—50	(**Crane** and **Gairdner** 1923)
C. anglica (Dennmark)	48+2 ff	(unpubl. data of **Sörensen** from **Löve** and **Löve** 1948)

MEIOSIS — NORMAL COURSE

The first suitable stage for analysis was diakinesis. It is possible to distinguish at this stage bivalents, quadrivalents, trivalents and also univalents (Fig. 3). The analysis of 25 cells in this stage shows that the numbers of poly-and bivalents are different in various cells. Differences in numbers of bivalents were very considerable and ranged from 7—16; among polyvalents quadrivalents were observed most frequently. In cells with trivalents usually a corresponding number of univalents was found; this suggests that these univalents originated as a consequence of the separation of quadrivalents (Table I). Sometimes however univalents originated from bivalents. In most cases in diakinesis there are 3—5 quadrivalents and 1—2 trivalents with a corresponding number of univa-

NUMBER OF POLY- AND UNIVALENTS IN DIAKINESIS

Types of association	quadrivalents	trivalents	bivalents	univalents	fragments
Min. and max. number in one cell	0—6	0—3	7—16	0—4	0—5

Types of quadrivalents in diakinesis	rod	ring	Y	Total
Number observed	49	41	5	95

lents. Presumably these differences in configuration are caused by the variable conjugation in the hexaploid chromosome complex and by the well known fact that in plants with small chromosomes some associations may often separate in diakinesis as a consequence of chiasmas terminalization. Among polyvalents the most often observed were the rod and ring type. It should be added that, contrary to *C. polonica*, both in diakinesis and in heterotypic me-

TABLE I
Analysis of 25 cells in diakinesis

Type of association of chromosomes				Number of cells
I	II	III	IV	
—	9	—	6	2
2	7	2	5	3
1	9	1	5	1
3	8	1	5	1
—	11	—	5	1
2	9	2	4	3
2	12	—	4	3
1	13	1	3	3
2	14	—	3	2
2	11	2	3	2
3	9	3	3	2
4	16	2	—	1
43	270	29	95	25

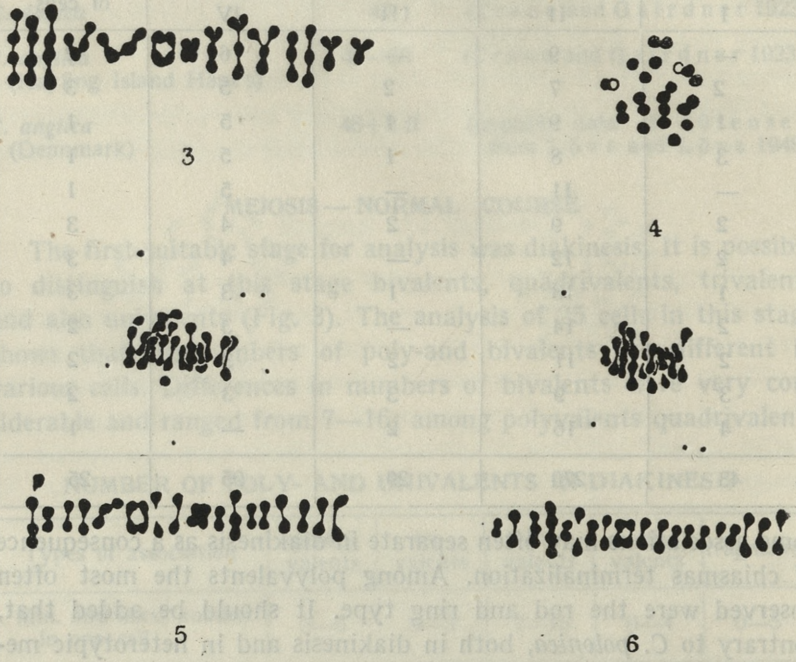
NUMBER OF POLY- AND UNIVALENTS IN METAPHASE I

Type of association	quadrivalents	trivalents	bivalents	univalents	fragments
Min. and max. number in one cell	1—5	0—2	9—11	0—6	0—8

Type of quadrivalents in metaphase I	rod	ring	Y	Total
Number observed	27	44	4	75

taphase, associations higher than quadrivalents were not found. This is especially interesting in view of the fact that *C. Tatrae* is also a hexaploid.

In the first metaphase the plates have a regular appearance; only in a few cells in the cytoplasm 1—2 chromosomes were observed. In this stage a remarkable crowding of the chromosomes occurs in the plate. It is best observable in side view of the spindles



Figs. 3—6, 3. Types of quadri- and trivalents in diakinesis. 4. Top view of metaphase I: «secondary pairing» (Fig. 53). 5—6. Side view of metaphase I spindles; below chromosomes drawn separately.

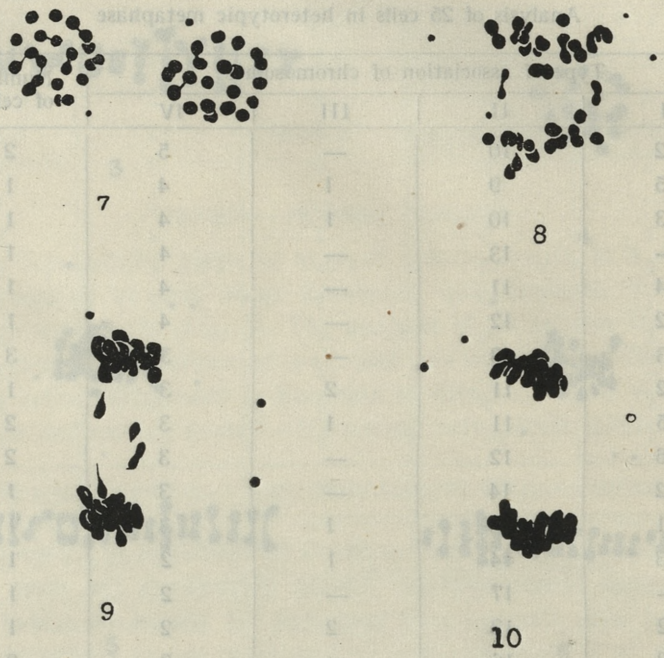
(Figs. 5—6). A full analysis of the metaphase configurations is very difficult. A top view of metaphase plates (Fig. 4) shows that bivalents and polyvalents are very close to each other and often touch themselves. A similar congression of bivalents was reported as a «secondary pairing» (Lawrence 1931, Meurman 1933, Müntzing 1933 and others). The results of analysis of 25 cells in metaphase are shown on Table II. In comparison to diakinesis the number of tri- and quadrivalents diminishes and a parallel increase of uni- and bivalents is observed. Among polyvalents the number of rods decreases. Pairs and univalents are probably derived from them. On the other hand the number of the rare configurations Y is maintained. In the heterotypic metaphase the number of ring quadrivalents seems to be higher than in diakinesis. Probably this is due to an observation error, as it is extremely difficult to distinguish the rings from two bivalents if they are very near to each other. Also in this configuration the chromosomes are more closely connected than in rod quadrivalents.

TABLE II

Analysis of 25 cells in heterotypic metaphase

Type of association of chromosomes				Number of cells
I	II	III	IV	
2	10	—	5	2
5	9	1	4	1
3	10	1	4	1
—	13	—	4	1
4	11	—	4	1
2	12	—	4	1
3	13	—	3	3
2	11	2	3	1
5	11	1	3	2
6	12	—	3	2
2	14	—	3	1
1	13	1	3	1
3	14	1	2	1
—	17	—	2	1
2	13	2	2	1
2	16	—	2	2
1	15	1	2	1
4	15	—	2	1
5	15	1	1	1
76	319	12	75	25

In heterotypic anaphase the top view of the two plates (Fig. 7) is very similar to what was observed in the first metaphase. Some of the chromosomes are in close contact. The analysis of the first anaphase (Table III) shows that in some cases the cell division is quite regular while in others it is more or less disturbed. The elimination of some anaphasic chromosomes as well as their unequal distribution belongs to the most frequent irregularities. The number of retarded chromosomes is variable and one to four chromosomes



Figs. 7—10, 7. Two plates in anaphase I, «secondary pairing». 8. Side view of anaphase I, dividing of laggards (Fig. 48). 9—10. Side view of telophase I, laggards (Fig. 49).

may be found in the spindle. Some of the laggards divide in later stages of anaphase (Fig. 8). In some cases in the cytoplasm 1—2 chromosomes were found.

Unequal distribution of chromosomes was less frequent than the elimination of some of them; the first irregularities were found in 14% of cells (7 cells) while the latter in 38% (19 cells.) In spite of such irregularities 66% of cells had the normal number (21) of chromosomes on both poles.

The elimination of the chromosomes and their behaviour in the spindle is best observable in late heterotypic telophase (Fig. 9—10). The results of the analysis of 100 P. M. C.'s. in this stage are found on Table IV.

In 45% of cells neither laggards were found in the spindle nor there were any chromosomes in the cytoplasm. Most probably however some of such divisions were not quite normal, as in the heterotypic anaphase there is often an unequal distribution without elimination of chromosomes. In consequence in some of these cells unequal numbers of chromosomes are present on the poles.

TABLE III

Distribution of chromosomes in heterotypic anaphase
(50 cells, number of cells in brackets)

	Classes of distribution				
	21+21 (24) 20+22 (5) 19+23 (3)	20+21 (11)	19+21 (3) 20+20 (1)	18+21 (2)	17+21 (2)
Total	32	11	4	2	2
Eliminated chromosomes	0	1	2	3	4

Chromosome numbers on poles of 50 cells

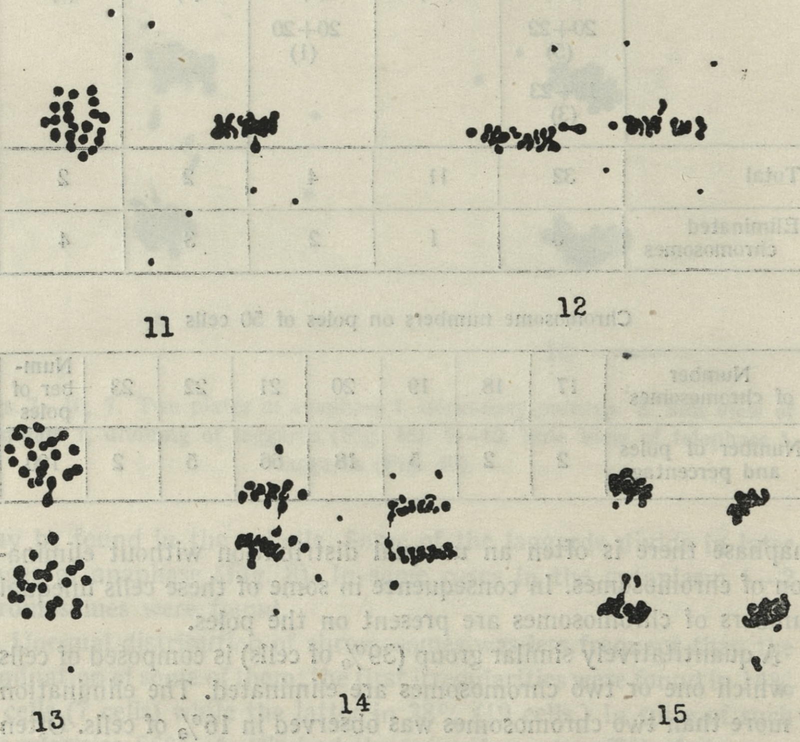
Number of chromosomes	17	18	19	20	21	22	23	Number of poles
Number of poles and percentage	2	2	5	18	66	5	2	100

A quantitatively similar group (39% of cells) is composed of cells in which one or two chromosomes are eliminated. The elimination of more than two chromosomes was observed in 16% of cells. Often 2—3 of the laggards divide and in consequence in some cells the number of chromosomes is higher than 42. The chromosomes which

TABLE IV
The behaviour of retarded and eliminated chromosomes in heterotypic telophase (100 P. M. Cs, in brackets number of dividing chromosomes)

Number of eliminated chromosomes	4	3	2	1	0	Total
Number of chromosomes in cytoplasm			1 (0), 2 (0)	1 (0)		
Number of chromosomes between poles	4 (3)	3 (3), 3 (2)	2 (2), 2 (1), 2 (0), 1 (0) 0	1 (1). 1 (0). 0	0	
Sum of cells	5	6 5	7 5 3 1 2	11 6 4	45	
Sum of cells in classes and percentage	5	11	18	21	45	100

resulted from this process are probably resorbed by the cytoplasm during interkinesis and in the homeotypic division. Tiny fragments were visible throughout the cycle of meiosis. In some cells during the



Figs. 11—15, 11—13. Metaphase II. 12. Metaphase spindles near each other (Fig. 51). 14—15. Anaphase and telophase II.

heterotypic telophase the dividing of these fragments was observed.

Interkinesis and homeotypic division (Figs. 11—15) are nearly normal. The observed irregularities are: retarding of chromosomes and in some cases the presence of chromosomes in the cytoplasm. It seems probable that at least some of these chromosomes have arisen from the division of univalents in the spindle during the heterotypic division.

In homeotypic metaphase the two spindles are orientated parellelly or perpendicularly and frequently very near to each other (Fig. 12); owing to this they may fuse in homeotypic anaphase leading to the formation of dyads, this was however not observed.

In general the meiotic cycle is characterised by: the lack of synchronism in P. M. C.'s in the same anther and by irregularities usually found in meiosis of most polyploids (i. e. retarding and elimination of chromosomes).

After the second division, a variable number of microspores degenerate and in old anthers the percentage of sterile pollen varies (8 to 100%). It seems probable that the percentage of sterile pollen depends in a high degree from external conditions. This may be inferred from the fact that after a long spell of fine weather the average percentage of sterile pollen was 8 (end of July 1949) while after prolonged covering by snow (July 1948) it was found to be 43.

FORMATION OF FRUITS AND SEEDS, AND LIFE-FORM

The formation of seeds and pollen sterility are closely connected. The siliculae in lower parts of the stalks, formed just after the melting of snow are small, badly formed (Fig. 44) and seedless. The development of the seeds may be inhibited by the lack of pollination. It also may be checked by disturbances induced by the low temperature. Siliculae formed later, when there is no snow or frost, are well developed and contain 4—8 seeds.

It is interesting to note that on natural stands *C. Tatrae* grows abundantly despite its life-form: according to my observations it is a biennial although hitherto it was regarded as a perennial (Szafer, Pawłowski i Kulczyński 1924, Kulczyński, Paczoski i Pawłowski 1927). It spreads exclusively by seeds. During the first year the rosette of leaves is formed, and in the second the plant flowers, develops seeds and perishes soon afterwards. This has been proved by autumn observations on natural stands where only dead stalks and roots were found of those plants which had produced seeds. Plants growing in exceptionally difficult conditions seem to finish their development in their third year.

IRREGULAR COURSE OF MEIOSIS

A parallel series of observations was carried out on buds exposed for a long time to low temperatures during the summer in the natural habitat. The disturbances are considerable and lead to the formation of two, three, five and even more cells instead of typical tetrads. Such irregularities were found in P. M. C.'s of almost all buds which were fixed during snow falls in summer.

As compared with the normal course the duration of meiosis is probably considerably prolonged. The occurrence of meioses in almost all P. M. C.'s in buds fixed in a suitable stage strongly supports this assumption.

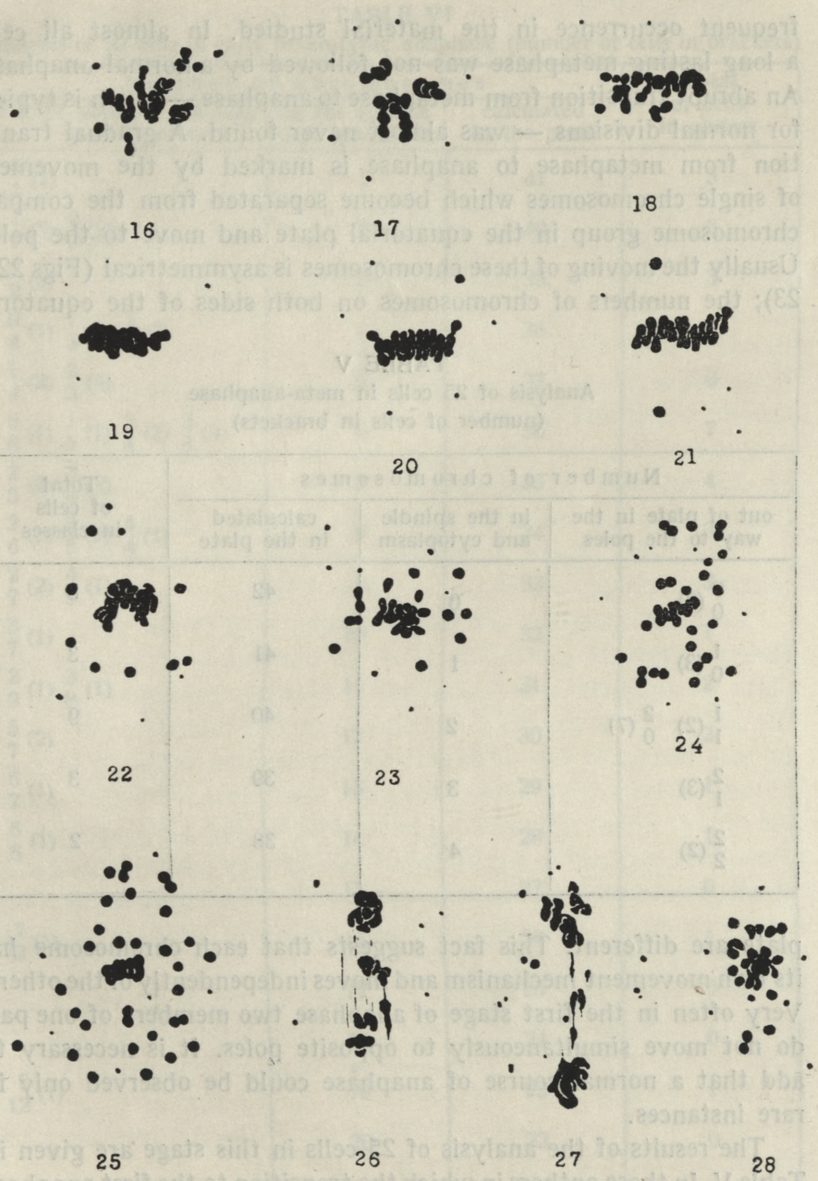
In diakinesis no cells suitable for a complete analysis were found. A partial analysis however was possible in many cells.

NUMBER OF POLY-, UNIVALENTS AND FRAGMENTS IN DIAKINESIS OF 25 CELLS

Type of association	quadrivalents	trivalents	bivalents	univalents	fragments
Min. and max. number in one cell	0—2	0—2	10—19	2—8	0—8

It is evident that deviations from a normal conjugation are slight. They manifest themselves in a formation of a lower number of quadri-, and trivalents; in consequence the number of bi-, and univalents increases. This fact leads to the assumption that the conjugation is weakened.

In metakinesis the chromosomes move towards the equatorial plate; in this stage they are strongly crowded (Figs. 18—19). In the heterotypic metaphase the plate is rather regular (Fig. 21). The chromosomes however are far more crowded than in normal divisions. No efforts were made to analyse their configurations. The first metaphase lasts very long; this may be inferred from its



Figs. 16—28. Irregular division. 16. Metakinese (Fig. 50.) 17. Top view of metaphase I (Fig. 52). 18—20. More or less regular metaphase I. 21. Meta-anaphase, rare symmetry. 22—23. Asymmetry in early anaphase (Figs. 54—55). 24—25. Late anaphase I (24, Figs. 57a—57b). 26—28. Three, two and one groups of chromosomes in late telephase (27, Fig. 56).

frequent occurrence in the material studied. In almost all cells a long lasting metaphase was not followed by a normal anaphase. An abrupt transition from metaphase to anaphase — which is typical for normal divisions — was almost never found. A gradual transition from metaphase to anaphase is marked by the movement of single chromosomes which become separated from the compact chromosome group in the equatorial plate and move to the poles. Usually the moving of these chromosomes is asymmetrical (Figs 22—23); the numbers of chromosomes on both sides of the equatorial plate are different. This fact suggests that each chromosome has its own movement mechanism and moves independently of the others. Very often in the first stage of anaphase two members of one pair do not move simultaneously to opposite poles. It is necessary to add that a normal course of anaphase could be observed only in rare instances.

TABLE V
Analysis of 25 cells in meta-anaphase
(number of cells in brackets)

Number of chromosomes			Total of cells in classes
out of plate in the way to the poles	in the spindle and cytoplasm	calculated in the plate	
$\substack{0\\0}$ (8)	0	42	8
$\substack{1\\0}$ (3)	1	41	3
$\substack{1\\1}$ (2) $\substack{2\\0}$ (7)	2	40	9
$\substack{2\\1}$ (3)	3	39	3
$\substack{2\\2}$ (2)	4	38	2

The results of the analysis of 25 cells in this stage are given in Table V. In those anthers in which the transition to the first anaphase was observed, about 33% of all cells had no chromosomes outside the plate either in the spindle or in the cytoplasm; these cells were still in the stage of first metaphase. In 4 cells only out of 25 analysed a symmetrical separation of chromosomes was observed.

TABLE VI

Analysis of 50 cells in early heterotypic anaphase (number of cells in brackets)

Number of chromosomes			Total of cells in classes
out of plate in the way to the poles	in the spindle and cytoplasm	calculated in the plate	
$\genfrac{}{}{0pt}{}{0}{1}$ (1)	1	41	3
$\genfrac{}{}{0pt}{}{1}{1}$ (3) $\genfrac{}{}{0pt}{}{2}{0}$ (2)	2	40	5
$\genfrac{}{}{0pt}{}{1}{2}$ (3)	3	39	3
$\genfrac{}{}{0pt}{}{0}{4}$ (1) $\genfrac{}{}{0pt}{}{1}{3}$ (3) $\genfrac{}{}{0pt}{}{2}{2}$ (2)	4	38	6
$\genfrac{}{}{0pt}{}{1}{4}$ (2) $\genfrac{}{}{0pt}{}{2}{3}$ (4)	5	37	6
$\genfrac{}{}{0pt}{}{0}{6}$ (1) $\genfrac{}{}{0pt}{}{1}{5}$ (1) $\genfrac{}{}{0pt}{}{2}{4}$ (2) $\genfrac{}{}{0pt}{}{3}{3}$ (3)	6	36	7
$\genfrac{}{}{0pt}{}{2}{5}$ (2) $\genfrac{}{}{0pt}{}{3}{4}$ (2)	7	35	4
$\genfrac{}{}{0pt}{}{2}{6}$ (1) $\genfrac{}{}{0pt}{}{3}{5}$ (1) $\genfrac{}{}{0pt}{}{4}{4}$ (1)	8	34	3
$\genfrac{}{}{0pt}{}{2}{7}$ (2) $\genfrac{}{}{0pt}{}{4}{5}$ (1)	9	33	3
$\genfrac{}{}{0pt}{}{3}{7}$ (1)	10	32	1
$\genfrac{}{}{0pt}{}{2}{9}$ (1) $\genfrac{}{}{0pt}{}{3}{8}$ (1)	11	31	2
$\genfrac{}{}{0pt}{}{5}{7}$ (2)	12	30	2
$\genfrac{}{}{0pt}{}{6}{7}$ (1)	13	29	1
$\genfrac{}{}{0pt}{}{6}{8}$ (1)	14	28	1
	15	27	0
$\genfrac{}{}{0pt}{}{7}{9}$ (1)	16	26	1
	17	25	0
	18	24	0
$\genfrac{}{}{0pt}{}{7}{12}$ (1)	19	23	1
	20	22	0
	21	21	0
	22	20	0
$\genfrac{}{}{0pt}{}{8}{15}$ (1)	23	19	1

The results of analysis of a later stage corresponding to early anaphase are found in Table VI.

In all analysed cells chromosomes were found along the spindles. In most cells their number is small (4—7); there were however cells in which 19 and even 23 chromosomes were out of the plate. In 18% of cells the distribution of chromosomes is symmetrical. In some cells however the number of chromosomes between the plate and the poles is almost twice as large on one side of the plate as on the other. In extreme cases two chromosomes were found on one side of the plate while on the other nine were present. In the spindle and in the cytoplasm not only single chromosomes but — though it seems strange — also bivalents were observed. Along the spindle sometimes tri- and quadrivalents were discerned. The arrangement of some chromosomes in the equatorial plate (Figs 22, 23, 25) suggests the possibility of a transverse movement of chromosomes in the spindle (Gajewski 1949, Östergren 1949).

The results of the analysis of the stage corresponding to the middle anaphase, are found in Table VII.

More chromosomes are found in their way to the poles than in earlier stages. In most cells 11 to 19 chromosomes are out of the plate the numbers on both sides of the plate are not equal but usually the difference is smaller (1—2 chromosomes) than in earlier stages. In rare instances the difference in distribution is 3—10 or 3—11. In most cells on both sides of the plate 9—10 or 13—15 chromosomes are found. Equal numbers on both sides of the plate are rare and were observed only in 5 cells out of 108. In very few cells the number of chromosomes in the spindle exceeds 30. These cells are in a more advanced stage (late anaphase) and preced in their division other cells in the same anther.

In all the material studied I did not succed in finding anthers with the majority of P. M. C.'s in this stage. A later stage charactetrised by the clumping of chromosomes in plain groups points on the existence of this stage, which though hardly similar to a normal telophase yet corresponds to it. The mode of the chromosome grouping in 105 cells is represented in Table VIII.

It appears from the Table that in this stage one, two groups or three are formed (Figs 28, 27, 26); only in a few cells (5 out of 105) the chromosomes are scattered along the spindle.

TABLE VII

Analysis of 108 cells in late heterotypic anaphase
(number of cells in brackets)

Number of chromosomes			Total of cells in classes
out of plate in the way to the poles	in the spindle and cytoplasm	calculated in the plate	
$\frac{1}{3}$ (1)	4	38	1
$\frac{1}{4}$ (1) $\frac{2}{3}$ (2)	5	37	3
$\frac{2}{4}$ (1)	6	36	1
$\frac{2}{5}$ (1) $\frac{3}{4}$ (3)	7	35	4
$\frac{2}{6}$ (1) $\frac{3}{5}$ (2) $\frac{4}{4}$ (1)	8	34	5
$\frac{3}{6}$ (2) $\frac{4}{5}$ (1)	9	33	3
$\frac{4}{6}$ (3)	10	32	3
$\frac{4}{7}$ (2) $\frac{5}{6}$ (5)	11	31	7
$\frac{4}{8}$ (2) $\frac{5}{7}$ (3) $\frac{6}{6}$ (1)	12	30	6
$\frac{3}{10}$ (1) $\frac{5}{8}$ (2) $\frac{6}{7}$ (3)	13	29	6
$\frac{3}{11}$ (1) $\frac{4}{10}$ (2) $\frac{5}{9}$ (2) $\frac{6}{8}$ (3) $\frac{7}{7}$ (1)	14	28	9
$\frac{5}{10}$ (2) $\frac{6}{9}$ (3) $\frac{7}{8}$ (4)	15	27	9
$\frac{6}{10}$ (2) $\frac{7}{9}$ (5) $\frac{8}{8}$ (2)	16	26	9
$\frac{6}{11}$ (2) $\frac{7}{10}$ (2) $\frac{8}{9}$ (4)	17	25	8
$\frac{4}{14}$ (1) $\frac{5}{13}$ (1) $\frac{6}{12}$ (2) $\frac{8}{10}$ (3)	18	24	7
$\frac{7}{12}$ (2) $\frac{8}{11}$ (2) $\frac{9}{10}$ (3)	19	23	7
	20	22	0
$\frac{10}{11}$ (1)	21	21	1
$\frac{9}{13}$ (1) $\frac{10}{12}$ (1)	22	20	2

TABLE VII (Continued)

Number of chromosomes			Total of cells in classes
out of plate in the way to the poles	in the spindle and cytoplasm	calculated in the plate	
$\frac{7}{16}$ (1) $\frac{10}{13}$ (1)	23	19	2
$\frac{8}{16}$ (1) $\frac{9}{15}$ (1) $\frac{12}{12}$ (1)	24	18	3
	25	17	0
$\frac{11}{15}$ (1)	26	16	1
$\frac{11}{16}$ (1) $\frac{9}{18}$ (1)	27	15	2
$\frac{12}{16}$ (1) $\frac{13}{15}$ (1)	28	14	2
$\frac{13}{16}$ (1)	29	13	1
$\frac{12}{18}$ (1)	30	12	1
	31	11	0
$\frac{14}{18}$ (1)	32	10	1
	33	9	0
$\frac{13}{21}$ (1)	34	8	1
$\frac{16}{19}$ (1)	35	7	1
$\frac{16}{20}$ (1) $\frac{17}{19}$ (1)	36	6	2
	37	5	0
$\frac{18}{20}$ (1)	38	4	1

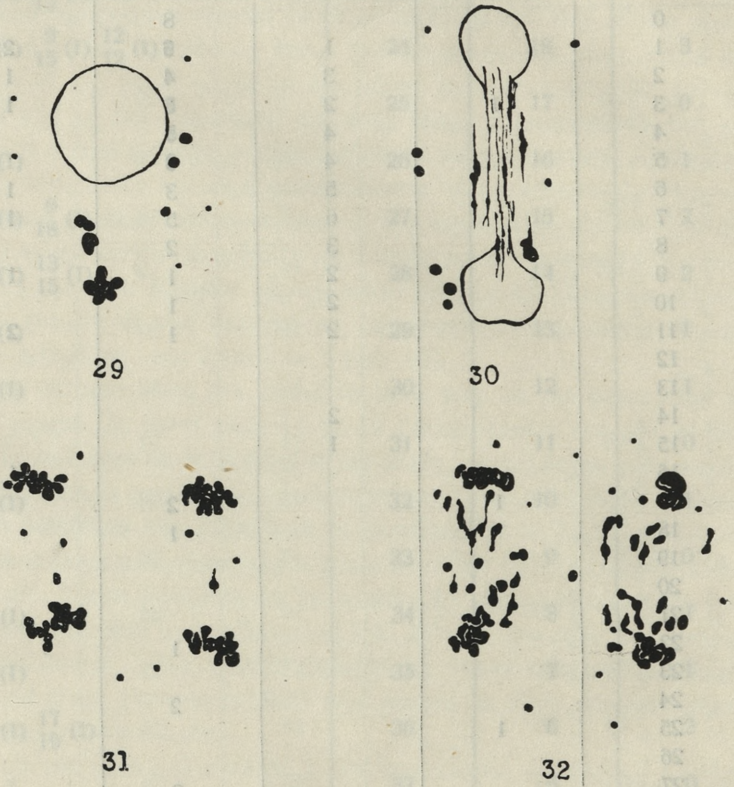
The presence of two groups which give rise to two nuclei proves that in spite of a varied degree of disturbances, the distribution to the poles is regular. At interkinesis 50% of cells with two nuclei were found. Only in nearly 8% (8 out of 105 observed) of cells the course of division has been most probably normal and without elimination even of a single chromosome. In some of these cells

TABLE VIII

Analysis of 105 cells in heterotypic telophase

Number of				
chromosomes not included into groups	chromosome groups and cells in each group			
	0	I	II	III
0			8	
1		1	6	2
2		3	4	1
3	1	2	6	1
4		4	5	
5		4	3	
6		5	3	1
7		6	5	1
8		3	2	
9		2	1	1
10		2	1	
11		2	1	2
12				
13				
14		2		
15		1		
16				1
17	1		2	
18			1	
19				
20				
21				
22			1	
23				
24			2	
25	1			
26				
27			2	
28				
29				
30	1			
31				
32				
33				
34				
35	1			
Total	5	37	53	10

an unequal distribution was noted. In 20% of cells the distribution to the poles was connected with the retardation and elimination of 1 to 4 chromosomes. These disturbances are of the same type as the disturbances in normal meiosis (Table III). In almost 23% of cells the numbers of chromosomes not included into the nuclei is low and does not exceed 4.



Figs. 29—32. Irregular division. 29. Interkinesis, some chromosomes not included into restitution nucleus. 30. Two interkinesis nuclei connected by a chromosomal bridge. 31. Normal telophase II after irregular first division. 32. Telophase II, retardation of chromosomes (Fig. 58).

The rare cells with three chromosome groups (Fig. 26) are in an unbalanced stage. Their further development may have a different course; some of them after the separation of the chromosomes left in the plate might give two-nucleate cells, and others form restitution nuclei.

Three-nucleate cells were not found in interkinesis. A frequent formation of restitution nuclei could be observed in this material. As it is visible from Table VIII, in the course of the first telophase cells in which the chromosomes are clumped in a single group are numerous. The presence of one-nucleate cells in interkinesis proves that they have restitution nuclei (Rosenberg 1926, 1926/27). They were found in 35% of cells. Usually in this process some of the chromosomes are not included into the nuclei. This fact is best observable in interkinesis (Fig. 30). According to Table VIII the number of eliminated chromosomes usually does not exceed 11 but exceptionally may reach 15. It is possible that a part of these chromosomes will be included later on into the nucleus in view of the lack of synchronism between different cells. The formed nuclei are large. It was not observed whether at this stage a membrane is formed around small groups of chromosomes outside the nucleus.

In interkinesis also one nucleus is often present and outside it small groups of single chromosomes are scattered (Fig. 29). Usually in interkinesis two nuclei differ in volume; this suggests that their chromosome numbers are different. If there are two nuclei they often are connected by chromosomal bridges (Fig. 30). In interkinesis frequently single chromosomes or even small groups of them are discernible along the spindle and in the cytoplasm.

After a first division with such great irregularities the course of homeotypic division may be either regular or more or less disturbed. The results of the analysis of this stage are found in Table IX.

In cells with two nuclei the course of homeotypic division may be normal and in consequence a normal tetrad is formed. This was observed in 74 cells out of 109 (approx. 67%). In other cells two types of disturbances were observed: 1) the elimination of retarded chromosomes in second anaphase, 2) the fusion of spindles. The latter was a result of their near and paralell or oblique orientation in cells.

A varied number of retarded and eliminated chromosomes has been observed (Figs 31, 32). If only a few chromosomes remain between the two separating groups they might be probably resorbed by the cytoplasm in later stage, since no pentads resulted from a normal division, though often retarded chromosomes were observed. On the other hand if the number of the retarded chromosomes is high (10—20 chromosomes, Fig. 32) they form accessory nuclei;

in consequence «tetrads» instead of having 4, have 5, 6 or more nuclei.

In homeotypic anaphase or telophase the spindles may fuse. If in this process two groups of chromosomes moving to the poles unite (Fig. 33), after division binucleate cells will be formed. On the other hand as a consequence of an oblique orientation of the two spindles, two groups of the chromosomes unite, three-nucleate cells will be formed. In the latter case (21% of cells) tryads (Figs 34, 35) with three instead of four nuclei are derived.

TABLE IX

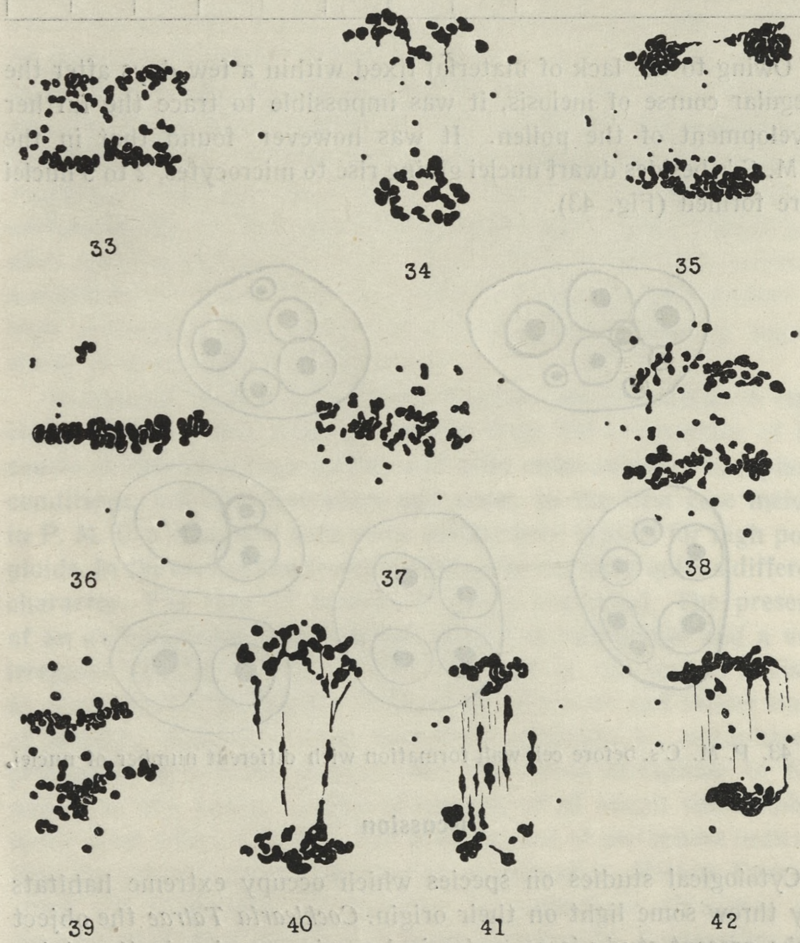
Analysis of 212 cells in homeotypic telophase

2-nucleate: 109 cells			1-nucleate: 103 cells
Normal division	fusion of plates on poles		
	two	one	
74 67%	23 21%	12 12%	
% calculat. for all 212 cells 34	11	6	49

The homeotypic division in cells with restitution nuclei (Figs 37—42) was observed in 103 cells, which corresponds approximately to 49%. In these cells usually the course of the homeotypic division was irregular.

The distribution of the chromosomes is characterized by a lack of symmetry in their movement to the poles, in a lesser degree however than in the heterotypic division preceding the formation of the restitution nucleus. Usually some chromosomes are eliminated. As a result of such division two or three nuclei different in size are formed. This shows that their chromosome numbers are different.

In the early tetrad stage (before formation of cell walls) 2 to 9 nuclei may be found (Table X). Most frequently however cells with 4 or 5 nuclei were observed. On the other hand the number of two-nucleate cells was smaller than anticipated on the basis of the analysis of the second telophases (approx. 50%, Table IX). According to expectation 49% of two-nucleate cells would originate from restitution nuclei and 11% from fusion of two chromosome groups on the poles. Actually they were found only in 22% (Table X). Probably this may be explained by an irregular homeotypic division in restitution nuclei; lagging chromosomes not included into the telophasic nuclei may form a third nucleus. The increased number (from 6 to 11%) of the three-nucleate cells observed in this stage seems to confirm this assumption.



Figs. 33—42. Irregular division: 33. Fusion of four plates on two poles. 34—35. Fusion of two plates on one pole (34, Fig. 59). 36. Metaphase II after formation of restitution nucleus in first division (Fig. 61). 37, 39. Anaphase II after restitution nucleus formation in first division (39, Fig. 62). 40—42. Telophase II after restitution nucleus formation in first division (Figs 60, 63).

TABLE X

Analysis of 120 cells in tetrad stage before formation of cell walls in microspores

Number of nuclei in 1 cell	2	3	4	5	6	7	8	9
Number of cells	26	14	23	31	15	6	4	1
%	22	11	20	26	12	5	3	1

Owing to the lack of material fixed within a few days after the irregular course of meiosis, it was impossible to trace the further development of the pollen. It was however found that in the P. M. C.'s besides dwarf nuclei giving rise to microcytes, 2 to 9 nuclei were formed (Fig. 43).

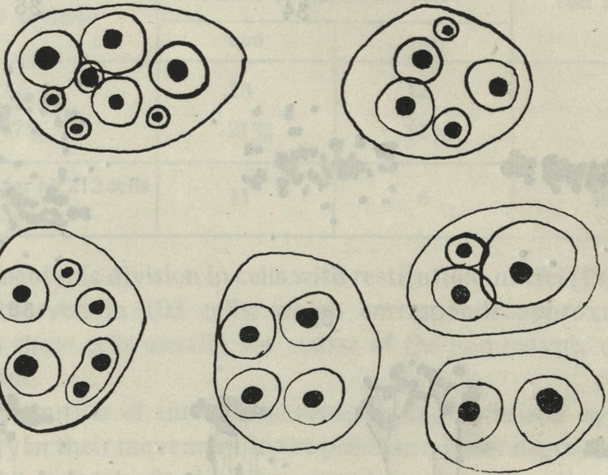


Fig. 43. P. M. C's. before cell wall formation with different number of nuclei.

Discussion

Cytological studies on species which occupy extreme habitats may throw some light on their origin. *Cochlearia Tatrae* the object of the present study is an endemical species growing in the alpine layer of Tatra mountains.

The relatively high chromosome number ($2n = 42$) shows that it is a polyploid. The comparison of its chromosome number with the chromosome numbers of other species of this genus leads to the conclusion that it should be considered as a hexaploid with the basic number 7.

Recent cytological studies are often connected with observations concerning natural stands, life-form and the mode of reproduction. According to Gustafsson (1947, 1948) high alpine plants are frequently diploids and in high mountains the percentage of polyploids is rather low. He points also to the fact that alpine layers are occupied almost exclusively (in 99% Gustafsson 1947 p. 281) — by perennial plants. *C. Tatrae* represents a rare exception in respect to its chromosome number as well as its life-form: it is a hexaploid and a biennial not a perennial. In spite of the fact that in high polyploids the sexual reproduction is often replaced by apomixis and vegetative spreading (Gustafsson 1947), *C. Tatrae* reproduces exclusively by seeds. In laboratory conditions it formed seeds only after artificial pollination. It is necessary to add that high mountain conditions do not favour the vegetative spreading; therefore in high mountains the percentage of rock-plants spreading vegetatively is lower than in the plain.

In natural stands of *C. Tatrae* however seed formation is especially difficult. This may be inferred from the comparison of the course of meiosis in fine weather and after unfavourable atmospheric conditions, fall of temperature and snow. In the first case meiosis in P. M. C.'s is normal with some disturbance typical for high polyploids. In the second the occurring disturbances have quite a different character. The time of meiosis is much prolonged. The presence of an exceptionally high number of cells in metaphase and a very irregular anaphase, both in the first and in the second division as compared to the number of those in diakinesis and earlier stages of meiosis, shows that the time from prophase to metaphase is prolonged in a lesser degree than that from metaphase to telophase. In the normal course of division of all stages the anaphase is the most difficult to find. The prolongation of metaphase indicates that the mechanism of chromosome movement is affected. Probably there are some tries of anaphase in metaphase (Hughes and Swann 1948) and only last of them is successful. Contrasting with fairly synchronised normal anaphases here no synchronisation of the chromosome movement is observed; this is especially observable in the first stages of such anaphase: single chromosomes move to the poles independently of each other. As the chromosomes are distributed at random, the asymmetry decreases in later stages, thus most probably similar chromosome numbers are found on both poles. The desynchronisation of the anaphase is similar to the desynchronisation of the kinetochores and chromosome movement in normal prophases of certain mantids (Hughes-Schrader 1943). In my opinion the independent movement of the chromosomes seems to indicate that each of them has its own movement mechanism. Most probably such irregular movement can be explained by the „strain-theory" («Zugfasertheorie», Schmidt 1937, 1939) or any way by fiber action, shown by Cornmann (1944). In consequence of such irregular courses of meiosis, numerous restitution nuclei are formed.

Irregular divisions as a result of low temperature were observed by a number of authors. Sakamura and Stow (1926) observed in *Gagea lutea* disturbances as a consequence of a rise of temperature in greenhouse conditions whereas the normal temperature for meiosis ranges from —2·5° to +2°C. Meiotic disturbances in *Epilobium* (Michaelis 1926) were caused by low temperatures (—3° to —5°C). They are similar to the irregularities observed in *C. Tatrae*. In P. M. C.'s Michaelis found restitution nuclei, fusion of spindles and microcytes. It is interesting that contrary to *Epilobium* and other plants investigated later (Barber 1942) the conjugation of the chromosomes in *C. Tatrae* is only slightly affected.

Frost occurring often immediately after snow melting may cause in the development of *C. Tatrae* various disturbances. As a rule at this time siliculae are abortive and seedless. The greatest probability of development of a high percentage of fertile pollen exists in summer (*C. Tatrae* flowers from mid July till mid August; single plants may flower in September). Frost and snow occurring at this time may cause meiotic disturbances resulting in 100% pollen sterility. The occurrence of a periodical sterility has been proved by finding of groups of abortive siliculae in close proximity of each other. Their simultaneous occurrence in many plants suggests the existence of a common cause of sterility, which is probably the irregular course of divisions and difficulties of pollination in unfavourable natural conditions.

Chromosome numbers of *C. Tatrae* and of the previously investigated *C. polonica* are different. *C. polonica*, the second Polish endemic species has the number $2n = 36$; it is an autohexaploid with the basic number 6 and not 7 as *C. Tatrae*.

In some instances the change of the basic number may be connected with the fragmentation of one or more chromosomes (Sweschnikowa 1929, Darlington 1937).

A different way of increasing the chromosome set is represented by the case of *Datura stramonium* (Blakeslee 1934): two homologous extra-chromosomes have been added to the normal diploid complement leading to the production of a balanced type with 26 chromosomes.

Studies of chromosome configurations in meiosis of the two *Cochlearia* species show yet another difference between these hexaploids. In *C. polonica* hexavalents were often observed, while in *C. Tatrae* the highest associations were represented by quadrivalents. This fact seems to indicate that *C. polonica* is autohexaploid, wheras *C. Tatrae* has only four homologous chromosome sets. Such a species could have originated from the hybridization of a diploid and an autotetraploid species. The formation of a triploid might have been followed by the doubling of chromosomes either in early embryo stages (*Nicotiana*, Goodspeed and Clausen 1925, *Aquilegia*, Skalińska 1935) or in the growing points of the shoots (*Primula Kewensis*, Newton and Pellew 1929, *Valeriana*, Skalińska 1947). In this way hexaploid with four sets of one species and two of the other may have originated. In such types quadrivalents would represent the highest association at meiosis.

The two species show at meiosis disturbances (chromosome elimination, lack of synchronism) which are common in polyploids. In autoploids meiosis is usually more disturbed than in alloploids. The course of meiosis is less regular in *C. polonica* than in *C. Tatrae* — this is in agreement with the assumption that it represents an autoalloploid.

According to Clausen, Keck and Hiesey (1945) alloploids have usually a greater capacity for expansion than autoploids which often are endemic. Accordingly *C. Tatrae*, an endemic species growing in the Tatra mountains, mainly in High Tatra in Czecho-Slovakia, has a much larger area thas the autohexaploid endemic species *C. polonica*, which occurs very locally only between the town Olkusz and the Błędowska Desert.

This study as well as the data obtained from the literature concerning the chromosome numbers of the genus *Cochlearia* suggest that in this genus there were two main ways of evolution: 1) the formation of two basic numbers, 2) a polyploid differentiation sometimes resulting from crossing. Manton (1932) expressed the opinion that in the family *Cruciferae* the appearance of different basic numbers has given origin to new genera, while a species formation within the genera was the result of polyploidy. Numerous genera within the family however have several basic numbers; *Cochlearia* is one of them.

Summary

Cochlearia Tatrae Borb. an endemic species growing mainly in the alpine layer of the Tatra mountains is a hexaploid with the basic number 7 and the somatic number 2n = 42.

It is a biennial, not a perennial and it reproduces only by seeds.

In natural stands during fine weather the course of meiosis in P. M. C.'s is normal with irregularities typical for high polyploids. The lack of polyvalents higher than quadrivalents leads to the assumption that it is an auto-alloploid.

In P. M. C.'s fixed in natural stands after summer snow fall other irregularities probably induced by low temperature were found. These disturbances (viz.: the prolonged duration of meiosis and a desynchronised and asymmetrical chromosomes distribution) resulted in the formation of numerous restitution nuclei.

The percentage of sterile pollen varies from 8 to 100.

This work was carried out in the Institute of Plant Anatomy and Cytology of the Jagellonian University in Kraków. The author wishes to express his sincere gratitude to the Head of the Institute Prof. Dr M. Skalińska for advice, interest and valuable criticism.

The grant for field-studies of the Polish Academy of Sciences in 1949 is gratefully acknowledged.

References

1) Bajer A.: Cytological studies on the *Coehlearia polonica* Fröhl. Acta Soc. Bot. Pol. 1950. — 2) Barber H. N.: Experimental control of chromosome pairing in *Fritillaria*. Journ. Gen. 43, 359—374, 1942. — 3) Blakeslee A. F.: New Jimson Weeds from old chromosomes. Journ. Hered 25, 81—108. 1934. — 4) Blackburn K. B.: On the relation between geographical races and polyploidy in *Silene ciliata* Pourr. Genetica 15, 49—66, 1933. — 5) Böcher T. W.: Zur Zytologie einiger arktischen und borealen Blütenpflanzen. Svensk. Bot. Tidskr. 32, 346—361, 1938. — 6) Clausen J., Keck D. D., Hiesey W. M.:

Experimental studies on the nature of species. Part II. Evolution through amphiploidy and autoploidy with examples from the *Madiinae*. Carnegie Institution of Washington 1945. — 7) Cornmann I.: A summary of evidence in favour of the traction fiber in mitosis. Amer. Natur. 78, 410—422 1944. —8) Crane M. B. and Gairdner A. E.: Species crosses in *Cochlearia* with a preliminary account of their cytology. Journ. Gen. 13, 187—200, 1923. — 9) Darlington C. D.: Recent advances in cytology. London 1937. — 10) Flovik K.: Chromosome numbers and polyploidy within the flora of Spitzbergen. Hereditas 26, 230—240, 1940. — 11) Fröhlich E.: Systematische Studien über polnische Esslöffel (*Cochlearia* L.) unter Berücksichtigung der verwandten Arten. Bull. l'Acad. Pol. Sc. et Lettres. Ser. B. 129—146, 1937. — 12) Gajewski W.: The description of meiosis with special references to the behaviour of univalents. Hereditas 35, 221—241, 1949. — 13) Gustafsson Å.: Apomixis in higher plants. Part. III Lund 1947. — 14) Gustafsson Å.: Polyploidy, life-form and vegetative reprodution. Hereditas 34, 2—22, 1948. — 15) Hughes-Schrader S.: Polarisation, kinetochore movements, and bivalent structure in meiosis of male mantids. Biol. Bull. 85, 265—300, 1943. — 16) Hughes A. F.: and Swann M. M.: Anaphase movement in living cell. Journ. exp. Biol. 25, 45—70, 1948. — 17) Lawrence W. J. C.: The secondry association of chromosomes. Cytologia 2, 352—384, 1931. — 18) Löve A. and Löve D.: Chromosome numbers of northern plant species. Reykjawik 1948. — 19) Kulczyński S., Paczoski J., Pawłowski B.: Flora Polska. Vol. III, 1927. — 20) Manton I.: Introduction to the general cytology of *Cruciferae*. Ann. Bot. 46, 509—536, 1932. — 21) Meurmann O.: Chromosome morphology, somatic doubling and secondary association in *Acer platanoides* L. Hereditas 18, 145—173, 1929. — 22) Michaelis P.: Über den Einfluss der Kälte auf die Reduktionsteilung vom *Epilobium*. Planta, 1 569—582. 1926. — 23) Müntzing A.: Studies on meiosis in diploid and triploid *Solanum tuberosum* L. Hereditas 18, 1933. — 24) Östergren G.: *Luzula* and the mechanism of chromosome movements. Hereditas 35, 444—468, 1949. — 25) Rosenberg O.: Über die Verdoppelung der Chromosomenzahl nach Bastardierung. Ber. d. Bot. Ges. 44, 455—460, 1926. — 26) Rosenberg O.: Die semiheterotypische Teilung und ihre Bedeutung für die Enstehung verdoppelter Chromosomenzahlen. Hereditas 8, 305—338, 1926/27. — 27) Sakamura T. and Stow J.: Ueber die experimentell veranlasste Entstehung von keimfähigen Pollenkörner mit abweichenden Chromosomenzahlen. Jap. Journ. Bot. 3, 1926. — 28) Schmidt W. J.: Doppelbrechung von Chromosomen und Kernspindel und ihre Bedeutung für das kausale Verständnis der Mitose. Arch. exp. Zellf. 19, 352—360, 1937. — 29) Schmidt W. J.: Doppelbrechung der Kernspindel und Zugfasertheorie der Chromosomenbewegung. Chromosoma 1, 253—264, 1939. — 30) Skalińska M.: Cytogenetic investigations of an allotetraploid *Aquilegia*. Bull. l'Acad. Pol. Sc. et Letters. Ser. B. 33—63, 1935. 31) Skalińska M.: Polyploidy in *Valeriana officinalis* Linn. in relation to its ecology and distribution. Linn. Soc. Bot. 53, 159—186, 1947. — 32) Sweschnikowa J.: Die Genese des Kerns in Genus *Vicia*. Verh. V. Kongr. Vererb. 1415—1421, 1928. — 33) Szafer W., Pawłowski B., Kulczyński B.: Rośliny Polskie. Warszawa 1924.

Explanation of plates

Fig. 44. *C. Tatrae* from natural stand: Mięguszowiecki Pass pod Chłopkiem (2311 m. o. s. 1.) showing both degenerated and well formed siliculae.

Fig. 45. Stand of *C. Tatrae* in the Mięguszowiecki glacial cirque (ca 1900 m o. s. 1.) on August 21-st 1949, after snow-fall.

Fig. 46. Somatic plate of *C. Tatrae.*

Fig. 47. Somatic plate of *C. polonica.*

Fig. 48. Normal division. Anaphase I — division of retarded chromosomes.

Fig. 49. Normal division. Telophase I. Retardation of chromosomes.

Fig. 50. Irregular division. Metakinese.

Fig. 51. Normal division. Near placed spindles in second division.

Fig. 52. Irregular division. Metaphase I — top view.

Fig. 53. Normal division. Metaphase I — top view.

Fig. 54—55. Early anaphase. Irregular division.

Fig. 56. Telophase I — two groups of chromosomes.

Figs. 57a—57b. One cell. Late anaphase I.

Fig. 58. Irregular telophase II after regular first division.

Fig. 59. Fusion of two plates on one pole.

Figs. 60, 63. Telophase II in uni-nucleate cells after formation of restitution nucleus in first division.

Fig. 61. Metaphase II after formation of restitution nucleus in first division.

Fig. 62. Anaphase II after formation of restitution nucleus in first division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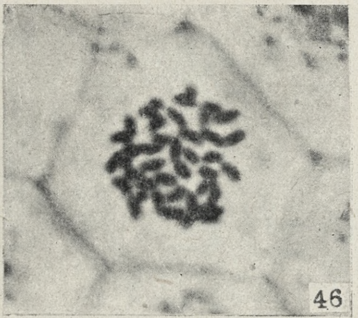
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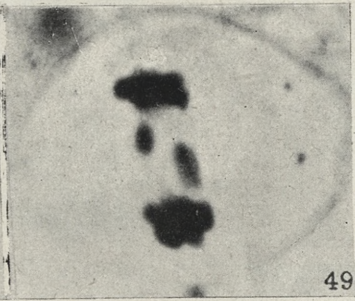
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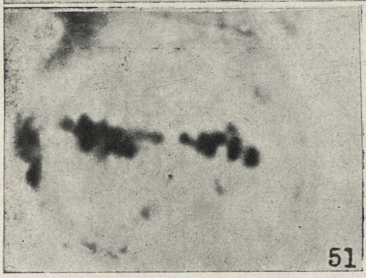
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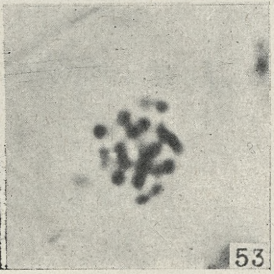
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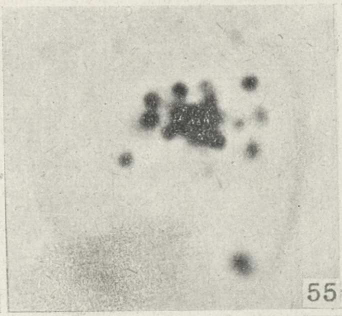
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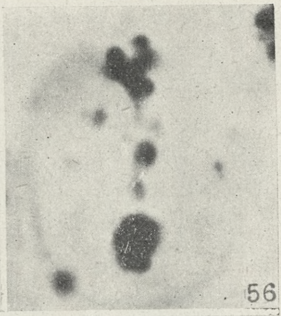
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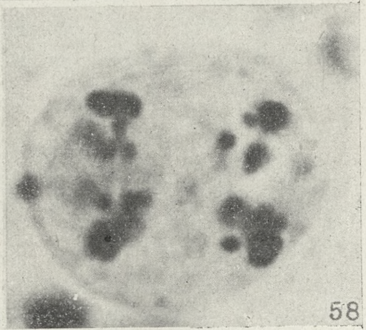
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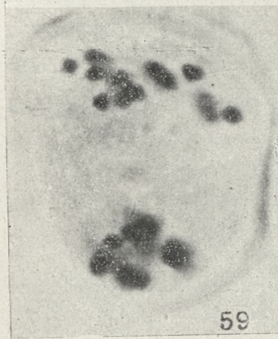
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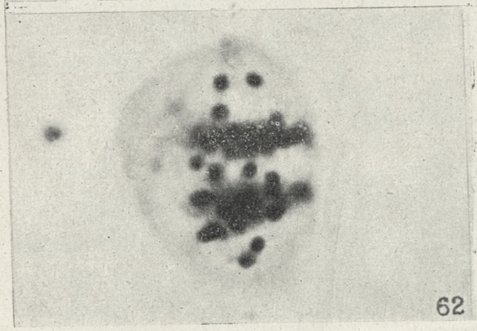
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A. Bajer

Badania cytologiczne nad gatunkami rodzaju Gentiana z Tatr i Pienin. — Cytological studies in Gentiana-species from the Tatra and Pieniny Mts.

Mémoire

de M^{me} ***M. SKALIŃSKA*** m. c.

présenté dans la séance le 22 Janvier 1951 par M^{me} M. Skalińska m. c. et M. B. Pawłowski m. c.

(Plate 3)

Contents:

Introduction

The purpose of this study was to give a contribution towards the elucidation of the problem how far the classification of the large genus *Gentiana* finds its expression in the cytological differentiation of its species. The studies have been undertaken as a part of the research work of the Institute of Plant Anatomy and Cytology of the University of Kraków dealing with the cyto-taxonomy, cyto-geography and cyto-ecology of plants from the Tatra and Pieniny Mts. The investigations were started in spring 1947. At that time only very few data concerning chromosome numbers of *Gentiana* were published (*G. lutea*, Stolt 1921, *G. makinoi*, Sakai 1934, a few species from Pamir and Altai mountains studied by Sokolovskaja and Strelkova 1938, and G. *pneumonanthe*, Scheerer 1939). Recently however two important papers on this subject have appeared: the paper of Favarger (1949) dealing with species from Switzerland, and that of Rork (1949) giving chromosome numbers of *Gentianaceae* chiefly native in America. The paper of Favarger is particularly interesting for our studies: it deals with chromosome numbers of 12 species of *Gentiana* native in the Alps and in the Swiss Jura; a number of these species occur also in the mountains of Southern Poland and are involved in the present investigations; this gives the possibility of comparing the results obtained from studies of various specimens originating from different and rather remote points of a large or discontinuous area of distribution. On the other hand in the material studied by Favarger we find some species which do not occur in Poland; they are however representatives of the same sections as some of the species from Poland investigated in the course of the present work; therefore the present studies supplement the previous results and give a contribution to the knowledge of chromosome numbers within some sections of the genus.

Material and methods

The present study deals with seven species of *Gentiana* native in the mountains of Southern Poland: the Tatra and Pieniny Mts. The following species have been investigated: *Gentiana punctata* L., *G. asclepiadea* L., *G. frigida* Haenke, *G. cruciata* L., *G. clusii* Perr. et Song., *G. verna* L. and *G. praecox* Kern. Specimens for studies have been collected in a variety of natural habitats in the Tatra and Pieniny Mts; in addition one specimen of *G. asclepiadea* has been brought from the Carpathians near Sącz.

As already pointed out by authors who made extensive studies in *Gentiana* (Favarger, Rork) there exist considerable technical difficulties in obtaining a suitable material for cytological investigations. Any fixation of root tips in natural habitats proved impossible. Most plants brought from the mountains did not survive transplanting and the few which remained alive have shown a very poor development of new roots, despite a very careful treatment. The fixation of the flower buds for meioses also presents serious difficulties in view of the fact that early in spring most alpine species are inaccessible in their natural mountain habitats. It should be added that the seeds are extremely difficult to germinate in laboratory conditions.

In view of the above difficulties, a number of special methods of rooting the plants have been elaborated in the course of the present work, in accordance with the different edaphic requirements of the particular species studied. It should be emphasized however that it is essential for obtaining good results to start all kinds of treatment very early in the season; only specimens without flower buds ought to be selected for this purpose.

The various kinds of treatment may be briefly summarized as follows:

For species native in the granitic part of the Tatra Mts in habitats with a rather high degree of acidity of the soil (*G. punctata* and the calcifuge species *G. frigida*) the best results have been obtained by placing the underground parts of the plants in garden pots loosely filled with moss kept moist by moderate watering from the bottom. This treatment resulted in the development in nearly all plants of a limited number of new rootlets which appeared on older parts of the rhizomes. This method allowed us to keep the plants alive for only a few weeks; after fixation of the newly developed root tips the plants invariably perished, the material however proved valuable since relatively numerous mitoses were found in the root tips.

For calcicolous species like *G. clusii*, as well as those which show a preference for calcareous soils *(G. cruciata*, *G. verna*, *G. praecox)* the best results were obtained in the following ways:

G. clusii: flat lumps of soil with rooted plants were placed over Petri dishes filled with limestone debris brought from natural habitats of this species; a water layer of about 3 mm on the bottom of the Petri dishes kept moist the limestone debris. In these conditions vigorous long and straight roots developed abundantly in the spaces between the limestone fragments.

G. verna: the same treatment as that described for *G. clusii* failed to give favourable results with *G. verna*: rooting was rather unsatisfactory. A development of new roots however could be stimulated by treatment of the rhizomes with very weak solutions (0·0002%) of growth promoting substances (α naphtalene acetic acid) for a period of 48 hours. After this treatment one series of the plants was planted in small garden pots with sand, and a second with a mixture of soil and sand. In both series fragments of limestone rocks were inserted on the bottom of the pots which were watered exclusively from the bottom. Two weeks after this treatment abundant rooting could be observed.

For *G. cruciata* as well as the tall and dwarf forms of the hapaxanthic species *G. praecox* the main difficulty consisted in finding and collecting very young plants early in the season before the flower buds were formed. Rooting was obtained after planting the specimens in small pots filled with soil from their natural habitats and watered exclusively from the bottom. This method has given satisfactory results also with *G. asclepiadea*.

Root tips were fixed chiefly in diluted Navashin fixative which gave better results than Levitsky's fixative (5 : 5). Unfortunately, for some species *(G. verna, G, asclepiadea)* the usual ways of fixation failed to give satisfactory results: the chromosomes were crowded and extremely difficult to count in the plates available. Plates with better spaced chromosomes have been obtained by cooling the root tips on blocks of ice immediately before fixation. Microtome sections were 10 μ thick; the slides were stained with Newton's gentian violet. Buds of *G. asclepiadea* have been investigated on aceto-carmine smears, with a pre-treatment in acetic alcohol (1 : 3).

Drawings were made with the aid of a Zeiss-Abbè camera lucida; for the somatic plates a Leitz oil immersion lens (x 100) and a Zeiss compensating eyepiece (x 30) was used; for meiotic chromosomes (Fig. 3) the same lens was combined with a Zeiss compensating eypiece x 15. The drawings have been reduced to 3/4 of their original size and have a magnification of roughly 2700, except Fig. 3 which has a magnification of about 1500.

The estimation of the vertical distribution in the Tatra Mts of the species studied has been given according to Szafer (1929) who distinguished the following layers or vertical regions of the mountain flora:

1. Submountain layer (from 300 to 600 m); 2. Lower mountain layer (from 600 to 950 m); 3. Higher mountain layer (from 950 to 1250 m); 4. Layer of subalpine forests (from 1250 to 1600 m); 5. Layer of *Pinus montana* (from 1600 to 1900 m); 6 Alpine layer (from 1900 to 2250 m).

In collecting the materials for the present study specimens from habitats situated in various layers have been selected for species with a wider vertical distribution, in order to compare the results obtained for plants originating from different altitudes.

The species studied and their cytology

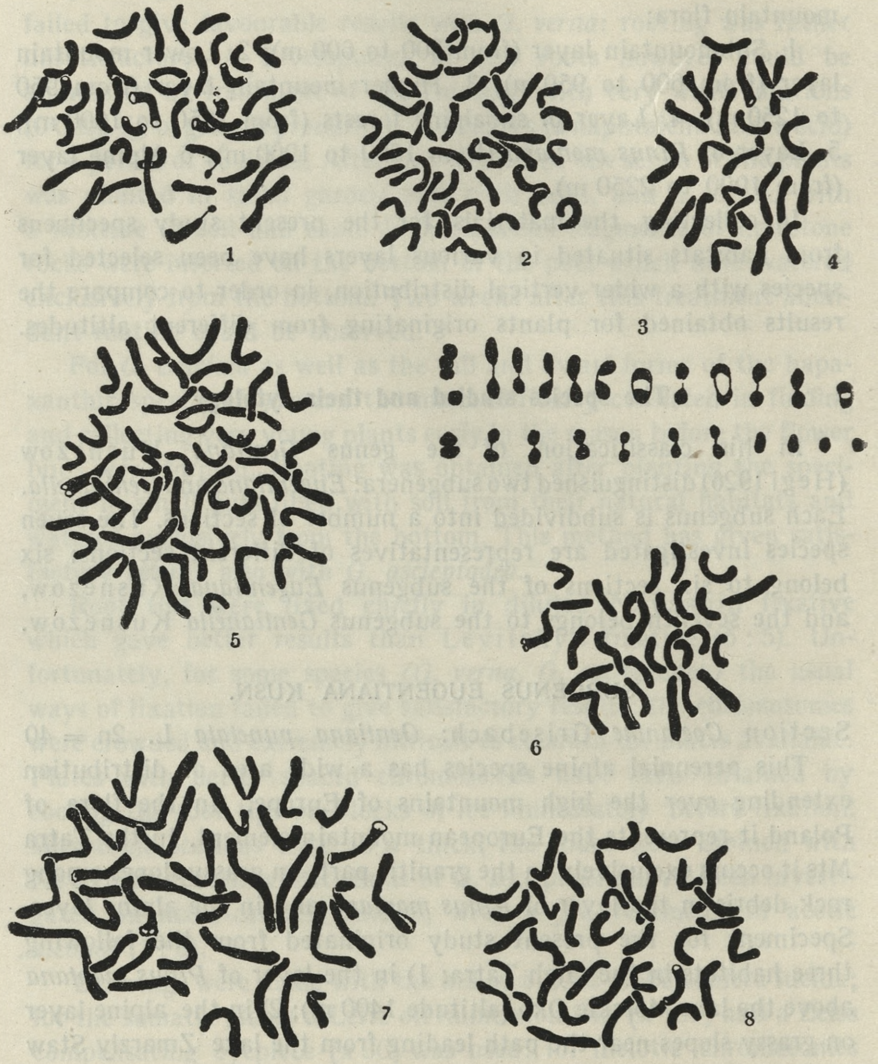
In his classification of the genus *Gentiana* Kusnezow (Hegi 1926) distinguished two subgenera: *Eugentiana* and *Gentianella*. Each subgenus is subdivided into a number of sections. The seven species investigated are representatives of different sections: six belong to six sections of the subgenus *Eugentiana* Kusnezow, and the seventh belongs to the subgenus *Gentianella* Kusnezow.

SUBGENUS EUGENTIANA KUSN.

Section *Coelanthe* Grisebach: *Gentiana punctata* L. $2n = 40$

This perennial alpine species has a wide area of distribution extending over the high mountains of Europe. In the flora of Poland it represents the European mountain element. In the Tatra Mts it occurs exclusively in the granitic part, on grassy slopes among rock debris in the layer of *Pinus montana* and in the alpine layer. Specimens for the present study originated from the following three habitats in the High Tatra: 1) in the layer of *Pinus montana* above the lake Morskie Oko (altitude 1400 m); 2) in the alpine layer on grassy slopes near the path leading from the lake Zmarzły Staw to the pass Zawrat (altitude about 2000 m); 3) in the alpine layer on slopes of Mięguszowiecki above the lake Czarny Staw (about 2000 m).

Studies of root tip mitoses permit to establish the number 40 (Figs. 1, 9 and 10). In view of the relatively small size of the chromosomes and their frequent crowding in the somatic plates exact counts are rather difficult. In a preliminary report (1950) the number



Figs 1—8. 1. — *Gentiana punctata* (2n = 40). 2. — *G. asclepiadea* (2 = 44). 3. — *G. asclepiadea*, I Metaphase with chromosomes drawn separately: 1 trivalent, 18 bivalents and 5 univalents. 4 — *G. frigida* (2n = 24). 5 — *G. cruciata* (2n = 52). 6 — *G. verna* (2n = 28). 7 — *G. clusii* (2n = 36). 8 — *G. praecox*, dwarf form (2n = 36). — Magnification of the somatic plates is c. 2700, that of Fig. 3 is c. 1500 ×.

36 has been given for this species. In a renewed study however the number 40 could be established with certainty. The length of the individual chromosomes of this species ranges from 1·5 to 3·5 μ (Table 1). Two chromosomes with trabants could be observed.

Two other species of the same section *Coelanthe*, viz. *G. lutea* L. and *G. purpurea* L. have been investigated by Favarger (1949) on plants from Switzerland. Neither of these species does grow in the Tatra Mts. Their chromosome numbers (n = 20) established by this author from studies of meioses agree with the number found in *G. punctata*. The study of somatic plates of *G. lutea* undertaken by this author has revealed the occurrence of two chromosomes with trabants, observable likewise in *G. punctata* (Fig. 1). The presence in the Alps of hybrids between *G. punctata* and *lutea*, as well as *G. punctata* and *purpurea* (according to Weber and Kranz 1879 and Hegi, p. 2046) proves that intercrossing within the section is possible. Evidently it is favoured by the fact that the three species have the same chromosome numbers.

Section *Pneumonanthe* Necker: *G. asclepiadea* L. 2n = 44

This perennial species has a relatively large ecological range which is combined with a wide general area of distribution (Central and Southern Europe chiefly in mountain regions and the Caucasus — Hegi). In Poland *G. asclepiadea* occurs only in the south: it is very common in the Tatra Mts where it is found both on limestone and on granit; its vertical distribution extends there from the higher mountain layer where it is common in mixed forests and on borders of woods in valleys upwards to the layer of subalpine forests; sometimes it ascends even higher to the layer of *Pinus montana*. In the Carpathians and the Holy Cross Mts it occurs on lower altitudes: in the submountain layer and the lower mountain layer.

The present investigation dealt with three groups of plants: 1) in the limestone part of the Tatra Mts: from a shady slope above the valley Strążyska at about 1100 m (higher mountain layer); 2) in the granitic part of the Tatra Mts: from the border of a mixed wood near the valley of the lake Morskie Oko at about 1400 m (layer of subalpine forests); 3) In the Carpathians near Sącz (lower mountain layer).

Studies of somatic plates from root tips of plants from the three above mentioned habitats have led to establish the number 44 in accordance with the previous results of Favarger and of Rork (Figs. 2, 12 and 13). The analysis of the somatic plates however presents considerable difficulties in view of the frequently occurring crowding of the chromosomes which are relatively thick and short; they are shorter than those of the other species studied (Table 1). Their length ranges from 1·5 to 3 μ and the smaller chromosomes are relatively numerous. In many instances two short chromosomes lying in close contact give the appearance of a single biarmed chromosome; therefore in a preliminary study the chromosome number of this species has been estimated too low.

The material for the study of meioses has been collected in the end of July 1948 from the first habitat in the form of cut shoots with young flower buds. The shoots were placed in jars with water and the buds examined in short time intervals in order to find the suitable stages for fixation. Subsequently meioses were studied on permanent aceto-carmine smears. The occurrence of a variable number of univalents in the P. M. C.'s was possibly due to laboratory conditions. A reinvestigation of the slides in 1950 permit to confirm the results obtained from somatic plates. Fig. 3 shows the configuration of the chromosomes at I Metaphase in a pollen mother cell: in addition to 18 bivalents, one trivalent and 5 univalents have been found.

Section *Frigida* Kusnezow: *G. frigida* Haenke $2n = 24$

This dwarf perennial species has a small and discontinuous area of distribution: it is restricted to the Alps of Stiria and Transsilvania and has also an isolated centre in the High Tatra. It represents a strictly calcifuge species and in view of its edaphic requirements it occurs only in the granitic part of the Tatra Mts. Its vertical distribution in the High Tatra is confined to the alpine layer.

The specimens for the present studies have been collected in the following four habitats: 1) On the northern slope of Mały Kozi Wierch, below the pass Zawrat at elevations ranging from about 1900 m to 2000 m; the plants grow there abundantly chiefly in tufts of *Sphagnum*; 2) on the northern slopes of Miedziane at about 2000 m; 3) below the pass Owcza Przełęcz in humus in cracks of granitic rocks; 4) on the north-western slope of Granaty at the elevation of about 2000 m, on cliffs of granitic rocks.

The chromosome number of this species (2n) is 24 (Fig. 4). Kusnezow (cited according to Hegi, p. 2003) regards *G. frigida* as a geographic race of the North-Asiatic and North-American species *G. algida* Pall. It should be added however that according to Sokolovskaya and Strelkova (1938) the species *G. algida* has 26 somatic chromosomes whereas for *G. frigida* the correct somatic number seems to be 24, in accordance with our preliminary report (1950); this number has been recently controlled on new materials. This result is interesting in view of the fact that the number 24 has not been found hitherto in any other *Gentiana* species.

The length of the somatic chromosomes of *G. frigida* ranges from 2·5 to 3·5 μ (Table I), thus its chromosomes are larger than those of the two preceeding species and the size differences within the set are less pronounced than in *G. punctata*. Some of the chromosomes are approximately isobranchial, others are heterobranchial with notable differences between the length of the two arms (Fig. 4).

Section *Aptera* Kusn: *G. cruciata* L. 2n = 52

According to Hegi, *G. cruciata* is the only European representative of the chiefly Central-Asiatic section *Aptera*. The general area of distribution of this perennial species extends over Southern and Central Europe, Asia Minor, the Caucasus, Turkestan and West-Siberia. Ecologically, it shows a preference for dry and rather warm habitats.

The specimens for the present studies originated from three separate habitats in the Pieniny Mts where this species occurs on calcareous soils: 1) Over Krościenko, in the lower part of the road leading to the peak Trzy Korony, on grass verges along the border of a young spruce wood (altitude about 600 m); 2) Higher part of the same road (about 800 m) in a mountain meadow with high vegetation 3) In a dry meadow with low vegetation, near the bank of river Dunajec, between Szczawnica and Krościenko (450 m).

Owing to good rooting of young plants numerous somatic plates with well spaced chromosomes have been obtained. Despite the relatively high number of chromosomes (2n = 52) the cytological material proved suitable for studies (Figs 5 and 15). The somatic number 52 is the same as that previously established by Favarger (1949) for specimens from the Botanical Garden of Neuchatel and by Rork (1949) for cultivated specimens of this species. The chromosomes of *G. cruciata* show distinct size differences within the set, their length ranging from 2 to 5 μ (Table I).

Section *Thylacites* Ren. *G. clusii* Perr. et Song. 2n = 36

According to Hegi (map p. 2010) the main centre of distribution of this dwarf perennial species is confined to mountains in central Southern Europe (limestone parts of the Alps, Jura and Schwarzwald); in addition it has also a small isolated centre in the Tatra Mts. *G. clusii* represents a strictly calcicolous species; in the Tatra Mts it occurs on soils rich in lime as well as on cliffs of limestone rocks. Its vertical distribution extends from the higher mountain layer upwards to the alpine layer. Specimens from the following four habitats have been studied: 1) The valley Strążyska (higher mountain layer); 2) The valley Jaworzynka (higher mountain layer); 3) Higher parts of the slopes of Kopa Magóry on cliffs of limestone rocks (layer of subalpine forests); 4) Higher parts of Skupniów Upłaz in the layer of *Pinus montana*.

The somatic number of chromosomes (2n = 36) found in plants from the above habitats, has been already reported in our preliminary account (1950). It supplements the number n = 18 given for this species by Favarger (1949) on the basis of pollen grain mitoses of plants from the Alps. Concerning the size of the chromosomes it should be added that *G. clusii* has notably larger somatic chromosomes than all the other species investigated in the course of the present study (Figs 7 and 16). The length of the chromosomes of this species ranges from 3 to 7 μ, medium size chromosomes being the most frequent (Table I). The three largest pairs are represented by one pair of isobranchial chromosomes and two pairs of chromosomes with distinct differences in the length of the two arms. The occurrence of two large chromosomes has been already observed by Favarger in haploid sets of this species. The length of the chromosomes ranges according to this author, from 2·5 to 4·3 μ. Since these values concern the length of chromosomes in pollen grain mitoses, whereas our measurements were done on somatic plates in large cells of the dermatogen (Figs 7 and 16), as well as those of the periblem in root tips, the results obtained are not directly comparable.

It is worth while mentioning that in one of the plants of *G. clusii* collected in July 1949 on Skupniów Upłaz a small sector with a doubled chromosome number has been detected (Figs 17 and 18) in a root tip. This sector had notably larger cells of the dermatogen and the periblem; one of the latter was found at metaphase; its chromosome number roughly corresponds to the double set of *G. clusii* (c. 70). Presumably this somatic mutation was induced by external conditions: snow and a sudden fall of temperature. Similar phenomena have been observed in the same season also in some other plants from the Tatra Mts, studied in our Institute: *Ranunculus glacialis* (Z. Bauer, unpublished), *Cardamine Opizii* (E. Banach, in the press).

G. clusii is in the flora of the Tatra the only representative of the section *Thylacites*. According to Favarger (1949) also two other species of this section viz. *G. kochiana* Perr. et Song. and *G. alpina* Vill. (which are closely related to *G. clusii* although they have a different geographic and ecological range) have the same chromosome numbers ($2n = 36$). According to these results, the section *Thylacites* seems to be cytologically uniform. Therefore the occurrence of any intra-specific differentiation concerning chromosome numbers could be hardly expected: in *G. clusii*, a species with a discontinuous area of distribution, plants from the isolated centre in the Tatra Mts where they grow at various altitudes, have the same chromosome number as the specimens from the Alps.

Section *Cyclostigma* Grisebach: *G. verna* L. $2n = 28$

G. verna is a dwarf mountain perennial species with a very wide area of distribution. According to Hegi, it extends over Europe and large territories of Asia. Therefore in the flora of Poland it is considered as a representative of the European-Asiatic mountain element (Szafer 1949). The vertical distribution of this species in the Tatra Mts extends from the higher mountain layer to the alpine layer with the main centre in the layer of subalpine forests and the layer of *Pinus montana* (Szafer 1929); occasionally however it may be found also at lower altitudes. Although this species is not strictly calcicolous it shows a preference for soils containing lime and therefore in the Tatra Mts it occurs almost exclusively in the limestone part where it is very common. Specimens for our studies originated from the following habitats: in the valleys Strążyska, Kościeliska, Jaworzynka (higher mountain layer); slopes of Kopa Magóry (layer of subalpine forests),

Difficulties in obtaining somatic plates with well spaced chromosomes have been already mentioned. The improved technique of fixation (on ice blocks) permit to obtain better slides on which the number 2n = 28 (not 26) could be definitely established for this species (Fig. 6 and 11). This number is in agreement with n = 14 recently reported by Favarger (1949) who studied meiotic chromosomes of *G. verna* from the Alps.

The size of the somatic chromosomes ranges from 2 to 4μ (Table I). In the set there are four somewhat larger pairs (3 to 4μ). In the plants from Switzerland, Favarger has observed at meiosis similar size differences between the bivalents.

SUBGENUS *GENTIANELLA* KUSN.

Section *Endotricha* Froelich (*Ameralla* Grisebach): *G. praecox* Kern. 2n = 36

G. praecox is the only hapaxanthic species studied in the course of the present work. The plants develop either as annuals or as biennials. This species manifests a high degree of diversity both in morphology and ecology. According to Hegi (p. 2043) its general area of distribution is limited to mountains of South-Central Europe. In Poland it occurs in the Carpathians and in the limestone part of the Tatra Mts. Its vertical distribution extends from the higher mountain layer to the upper limit of the layer of subalpine forests, however dwarf forms may ascend higher and are found also in the layer of *Pinus montana* on sunny grassy slopes as well as on cliffs of limestone rocks.

In view of great difficulties in collecting the specimens in their natural habitats before the buds are developed in early spring, only two groups of plants have been investigated: the first group is represented by minute plants about 3 cm high originating from cliffs of limestone rocks and grassy slopes in a sunny situation on Skupniów Upłaz (layer of *Pinus montana*); the second group consisted of taller plants about 25 cm high with rather long internodes and developing many flowers; these specimens have been collected on woody slopes of Łysanki at the altitude of about 1300 m (layer of subalpine forests).

After overcoming the considerable difficulties in rooting specimens of this species, in the two groups of plants very good and well spaced metaphase plates have been obtained. The two distinctly different forms proved to have the same chromosome numbers, 2n = 36 (Figs 8 and 14). The size of the chromosomes of the two forms ranges from 2 to 4 μ. In the second group (tall plants which flower later in the season) also the first pollen grain mitosis has been studied on aceto-carmine smears and the number n = 18 could be established in agreement with the number 36 found in somatic plates in root tips.

It is noteworthy that, according to the results obtained, *G. praecox* seems to represent a species which lacks any cytological differentiation in spite of a sharply marked morphological differentiation into a number of distinct forms.

Favarger who has studied another representative of the section *Endotricha*, *G. campestris* L. from the Alps and the Swiss Jura, has found in meioses and pollen grain mitoses of this species n = 18. Although hitherto only two species of this section have been investigated, the results obtained suggest that this section may be cytologically uniform with the somatic number 36.

TABLE I

Length of somatic chromosomes in seven species of *Gentiana*

Species	2 n:	Length classes in microns											
		1·5	2·0	2·5	3·0	3·5	4·0	4·5	5·0	5·5	6·0	6·5	7·0
G. punctata	40	6	14	8	8	4	—	—	—	—	—	—	—
G. asclepiadea	44	8	22	8	6	—	—	—	—	—	—	—	—
G. frigida	24	—	—	8	10	4	2	—	—	—	—	—	—
G. cruciata	52	—	12	16	14	6	2	—	2	—	—	—	—
G. clusii	36	—	—	—	4	2	2	14	4	4	2	2	2
G. verna	28	—	8	12	4	2	2	—	—	—	—	—	—
G. praecox dwarf form	36	—	4	4	14	8	6	—	—	—	—	—	—
G. praecox tall form	36	—	6	14	10	2	4	—	—	—	—	—	—

Note. — The data in this table give a comparison of size differences of the different species and an approximate estimation of the various lengths within each set. Very exact measurements are impossible owing to the frequent curling upwards of the chromosome arms.

Discussion

According to the opinion of taxonomists the differentiation of the numerous groups within the genus *Gentiana* has been achieved at a rather early stage of evolution and the various sections represent ancient and sharply delimited groups. Recent cytological investigations concerning a number of species, chiefly those of Favarger (1949) and of Rork (1949), as well as the present results have shown that the distinct differences between the numerous sections are not limited to purely morphological features but are linked with well marked differences in karyological structure.

Previous investigations have revealed the existence of the following chromosome numbers: $n = 5$, 7 (Favarger), 13 (Scheerer, 1939, Sokolovskaya and Strelkova 1938, Sakai 1934), 14 (Favarger), 18 (Favarger, Rork), 20 (Favarger), 21 (Rork), 22, 26 (Favarger, Rork), 39 (Rork). In the present study the numbers $n = 14$, 18, 22 and 26 have been also found in four species already investigated by the previous authors *(G. verna*, *G. clusii*, *G. asclepiadea* and *G. cruciata)*. In addition, new numbers have been established for three species not investigated hitherto: $n = 12$ *(G. frigida)*, $n = 18$ *(G. praecox)* and $n = 20$ *(G. punctata)*. The numbers of the two latter species supplement the results of Favarger concerning the sections *Endotricha* and *Coelanthe*: *G. campestris* of the section *Endotricha* has the same chromosome number as *G. praecox* and the number of *G. punctata* is exactly the same as that of *G. lutea* and *G. purpurea* of the section *Coelanthe*. On the other hand, the results obtained for *G. frigida* add to the numbers quoted above a new number not recorded by the previous authors: $n = 12$.

The above results show clearly that the genus *Gentiana* has several basic numbers. Already Favarger discussed in his paper the putative origin of the numbers found in the various species and suggested the occurrence of a series of basic numbers. In addition to the very low numbers $x = 5$ and 7 established by him for two diploid annuals, and $x = 13$ frequently occurring in the sections *Pneumonanthe* and *Aptera*, he gives also $x = 9$ and 11 as putative basic numbers of presumably tetraploid species with 36 and 44 somatic chromosomes.

Concerning the somatic number 26, it should be added that, according to Favarger, it might also be possibly regarded as a tetraploid one formed from $2n = 14$ by the fusion of one pair followed by doubling of the chromosome set. The origin of this number however may be explained in a different way in the light of the present results obtained for *G. frigida*. They suggest the existence of the basic number 6 in addition to those given by Favarger. Accordingly, the number 13 might have arisen by allopolyploidy from types with 6 and 7 chromosomes. In the same way the putative basic number 11 could have been produced in the course of evolution from types with 5 and 6 chromosomes.

It is worth while mentioning that some other genera of the family *Gentianaceae* have the same chromosome numbers as those found within the genus *Gentiana*: the number $n = 12$ has been reported by Woycicki (1937) for *Swertia perennis* L. var. *typica*; *Blackstonia perfoliata* Huds. has, according to Maude (1939) 44 somatic chromosomes, thus, the same number as that found in some *Gentiana* species. On the other hand Sakai (1935) has established for *Swertia perennis*, var. *cuspidata* the somatic number 18 which seems to be more primitive than the number 36 found in several *Gentiana* species. In view of the fact however that within the large genus *Gentiana* only a limited number of species have been studied cytologically till now, there exists still the possibility of finding new somatic numbers in the course of future investigations.

Owing to the occurrence of several basic numbers and of polyploid series (multiples of 13: $2n = 26$, 52, 78) the cytological differentiation within the genus *Gentiana* seems to be very complex. To some extent it is comparable with that occurring in the genus *Primula* (Bruun 1932) and *Viola* (J. Clausen 1931) and — according to recent investigations (Roy Clausen 1949) also in *Nicotiana*. In the latter genus, in addition to the numbers 9, 10 12, 16 and their multiples, the numbers 19, 21 and 22, evidently of allopolyploid origin have been found in some species. In *Gentiana*, as pointed out by Favarger, some numbers might have resulted both from autopolyploidy and allopolyploidy; therefore great caution is advisable in interpreting their putative origin. In fact, all somatic numbers found in its species might be deduced from the four basic numbers 5, 6, 7, 9.

It is well known that differences in chromosome numbers may have a value for taxonomy chiefly on the species level within a genus. In addition to the numbers, also other details, the chromosome length and their morphology may contribute to the distinction of species. As pointed out by the previous investigators (Favarger, Rork), in all *Gentiana*-species length differences are observable within each set, the largest chromosomes having approximately twice the length of the smallest; a range of intermediate sizes is always found between the extremes (Favarger, 1949, p. 78). In the present study we attempted at a more exact estimation of these size differences. The results obtained from measurements of the somatic chromosomes of the seven species studied are presented on Table I. It is evident that the species show well marked differences in the length of their chromosomes. However the range of variability of the chromosomes length does not express adequately the differences in the somatic sets of the seven species studied; therefore in Table I the chromosomes of each species have been grouped in the various length classes according to their frequency in the respective somatic set. Such a groupment allows us to estimate more precisely the differences between the species. It shows that the karyological differences are not limited to chromosome numbers. We find within the genus some species with relatively large chromosomes and others with notably smaller chromosomes. The extremes are represented by *G. clusii* with the largest and *G. asclepiadea* with the smallest chromosomes. In the former the sizes range from 3 to 7 μ and the medium sizes (c. 4·5 μ) are relatively frequent. In the latter the sizes attain only from 1·5 to 3 μ and there is a high frequency in length classes with chromosomes not exceeding 2 μ. It should be added that *G. clusii* with its remarkably large chromosomes is cytologically distinct from *G. praecox* in spite of the same numbers of chromosomes; the two species are representatives of different subgenera: these two remote species differ distinctly in their external morphology as well as in details of their karyological structure.

In a recent paper Turrill (1950) expressed the opinion that a classification based on cytology is a special, not a general classification (p. 5). In the case of *Gentiana* the results of cytological investigations give — on the whole — a support to the opinion of taxonomists concerning the classification within the genus. They have shown also the occurrence of a very complex cytological differentiation which could have been attained only in a long process of evolution. Therefore the results of cytological investigations have added some further evidence in favour of the opinion of taxonomists that the sections within the genus are of ancient origin and represent sharply delimited groups.

Summary

The present study deals with the cytology of seven species of *Gentiana* native in the Tatra and Pieniny Mts. In four species studied the chromosome numbers agree with those found previously for plants of a different origin: *G. verna*: $2n = 28$, *G. clusii*: $2n = 36$, *G. asclepiadea*: $2n = 44$ and *G. cruciata*: $2n = 52$. New chromosme numbers have been established for three species: *G. frigida*: $2n = 24$, *G. praecox* (two forms): $2n = 36$ and *G. punctata* $2n = 40$. The numbers of the two latter species agree with those of other representatives of the sections *Endotricha* and *Coelanthe*, whereas in *G. frigida* a new number, not recorded previously has been established.

This result suggests the existence of the basic number 6 besides the series given by Favarger (1949) for the genus *Gentiana* ($x = 5, 7, 9, 11, 13$). The presumable occurrence of the basic number 6 in addition to this series suggests that the numbers 11 and 13 could have arisen by allopolyploidy from types with 5 and 6, or with 6 and 7 chromosomes.

A comparison of the length of the somatic chromosomes of the seven species (Table I) shows that the cytological differences between the species are not limited to the numbers of chromosomes. The results have added some further evidence in favour of the opinion of taxomists that the sections into which the genus is subdivided are of ancient origin and represent sharply delimited groups.

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References

1) Bruun H. G.: Cytological studies in *Primula*. Symb. Bot. Upsalianse 1, 1—239, 1932. (cited from Darlington and Janaki-Ammal, 1945). — 2) Clausen Roy E.: The primary cytogenetic classes of *Nicotiana*. Portug. Acta Biol. (A), R. B. Goldschmidt Vol. 137—145, 1949. — 3) Darlington C. and Janaki-Ammal: Chromosome atlas of cultivated plants. Allen and Unwin, London 1945. — 4) Favarger C.: Contribution à l'étude caryologique et biologi-

que des *Gentianacèes.* Bull. Soc. Botanique Suisse 59, 62—86, 1949. — 5) Hegi G.: Illustrierte Flora von Mitteleuropa, V, 3. 1926 — 6) Maude P. F.: The Merton Catalogue. A list of the chromosome numerals of species of British flowering plants. New Phytologist 38, 1—31, 1939. — 7) Rork C. L.: Cytological studies in the *Gentianaceae.* Am. Journ. Bot. 36, 687—701, 1949. — 8) Sakai K.: Studies on the chromosome numbers in alpine plants. Jap. Journ. of Gen. 9, 226—230, 1934. — 9) Sakai K.: Studies on the chromosome numbers of alpine plants II. Jap. Journ. of Gen. 11, 68—72, 1935. — 10) Scheerer H.: Chromosomenzahlen aus der Schleswig-Hollsteiner Flora I. Planta 29, 636—642, 1939. — 11) Skalińska M.: Studies in chromosome numbers of Polish Angiosperms. Acta Soc. Bot. Pol. 20, 45—68, 1950. — 12) Sokolovskaya and Strelkova: Polyploidy in the high mountain regions of Pamir and Altai. C. R. Acad. Sc. U. S. S. R. 21. 1938 (cited from Rork, 1949). — 13) Stolt H.: Zur Embryologie der Gentianaceen und Menyanthaceen. K. Svensk. Vet. Ak. Hand. 61, No 14, 1921. — 14) Szafer W.: The mountain element in the flora of the Polish plain. Bull. Int. Acad. Pol. des Sciences et des Lettres (B) 343—381, 1929. — 15) Szafer W.: Zarys geografii roślin Warszawa 1949. — 16) Turrill W. B.: Modern trends in the classification of plants. The Advancement of Science 26, 1—16, 1950. — 17) Weber J. C. and Kranz C. A. Die Alpen-Pflanzen Deutschlands und der Schweiz. Vol. 3, 1879. — 18) Woycicki Z.: L'aspect et nombre des chromosomes meiotiques chez *Swertia perennis.* Cytologia, Fuji Jub. Vol. II, 1094—1100, 1937.

Explanation of plate 3

Microphotos of root tip metaphases of *Gentiana* species

The microphotos have been taken with Leitz Makam combined with the Practiflex camera. For all photos, except Fig. 17, a Leitz oil immersion lens × 100 and a Leitz periplan eyepiece × 10 have been used. Their magnification is about 1000 ×. Fig. 17 has been taken with the use of a Zeiss apochromatic oil immersion lens × 60 and a Zeiss compensating eypiece × 5. Its magnification is c. 200 ×.

Figs. 9, 10. — *Gentiana punctata* (plate taken in two planes, the same as Fig. 1 Periblem cell.

Fig. 11. — *G. verna* (the same plate as Fig 6). Dermatogen cell.

Fig. 12. — *G. asclepiadea* from Carpathians (the same as Fig. 2). Dermatogen cell.

Fig. 13. — *G. asclepiadea* from the Tatra Mts. Periblem cell.

Fig. 14. — *G. praecox*, tall form. Periblem cell.

Fig. 15. — *G. cruciata* (the plate on the right is that one drawn in text Fig. 5) periblem cells.

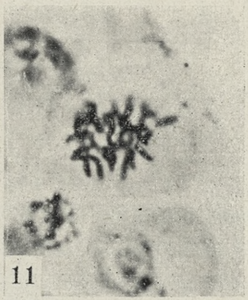
Fig. 16. — *G. clusii* from Kopa Magóry (the same as Fig. 7). Dermatogen cell.

Fig. 17.—*G. clusii* from Skupniów Upłaz: section of root tip with a polyploid sector. Top left — group of giant dermatogen cells; centre — group of giant periblem cells with one at metaphase.

Fig. 18. — *G. clusii*: detail of Fig. 17 showing the cell at metaphase in higher magnification; c. 70 chromosomes.


9


10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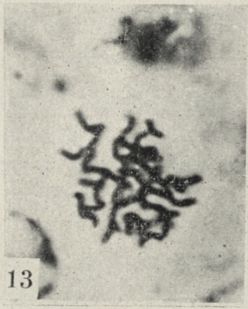
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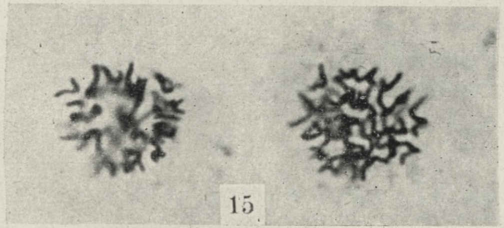
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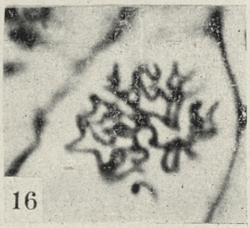
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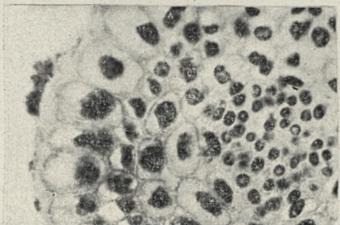
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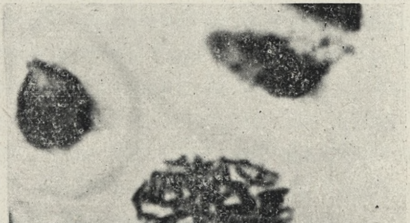

18

M. Skalińska

Badania nad szczepieniem ziemniaków. I. Wpływ podkładki pomidorowej na kwitnienie i płodność ziemniaków.

Potato grafting experiments: I. The effect of tomato stock on the flowering and fertility of potato.

Mémoire

de MM. ***E. MALINOWSKI***, ***J. BERNADOWSKI*** and ***M. ZAMOYSKA***

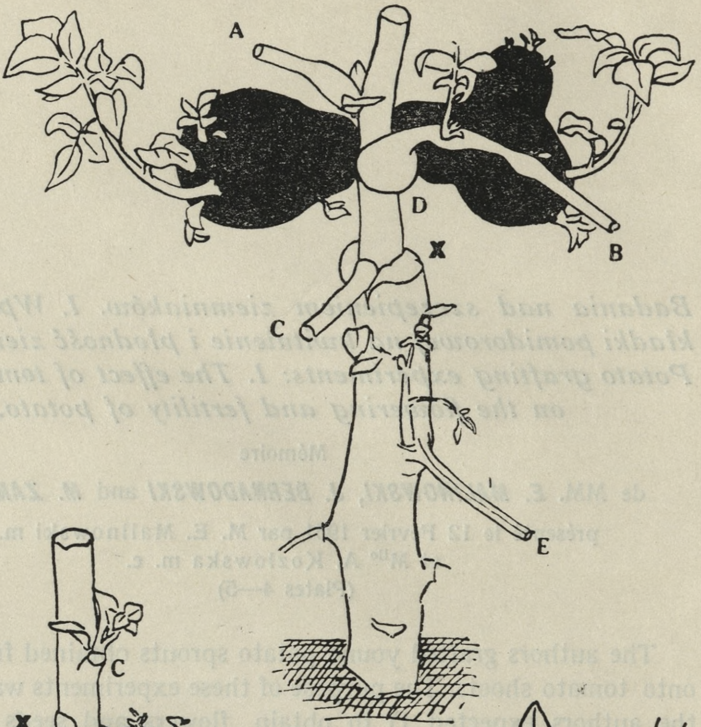
présenté le 12 Fevrier 1951 par M. E. Malinowski m. t.
et M^{lle} A. Kozłowska m. c.
(Plates 4—5)

The authors grafted young potato sprouts obtained from tubers onto tomato shoots. The purpose of these experiments was twofold: the authors expected 1) to obtain flowers and seeds in potato varieties which under normal conditions do not bloom at all and 2) to cause self-incompatible potato varieties to become self-compatible.

1. The effect of tomato stock upon the flowering of potato scion

We used for grafting experiments two varieties of potato which do not bloom in our country under normal conditions, namely the varieties: 1) Ulster Chieftain and 2) Erstling (Eersteling). Both varieties belong to the species *Solanum tuberosum*. We grew in 1950 100 plants of Ulster Chieftain in the field and we observed only one flowering plant with 3 flowers. These flowers remained 1 day on the plant and dropped off afterwards together with calyx. The variety Erstling flowers a little better in the field than Ulster Chieftain. Out of 100 plants grown in 1950 6 individuals came to flower. These individuals developed poor inflorescences, one on each plant, with 4—5 flowers each. The flowers dropped off before the opening of the petals or immediately afterwards. The plants flowered on the average one day.

1



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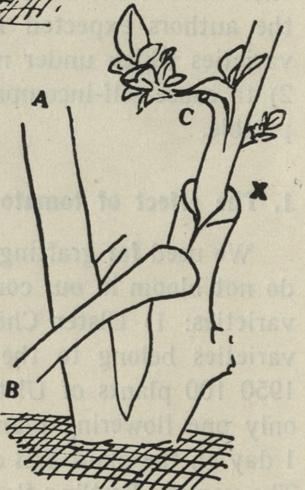

Fig. 1. Potato variety Ulster Chieftain grafted onto tomato stock. X — region of union between stock and scion. A, B, C — shoots of the scion. D — thickened base of a lateral shoot of the scion. E — leaf of the tomato stock. The stock is deprived of its lateral shoots. Aerial tubers are shown in solid black.
Fig. 2. Potato variety Ulster Chieftain grafted onto tomato stock. X — region of union between stock and scion. A, B — big lateral shoots of the tomato

stock (the leaves of these shoots are not shown in the picture). C — leaf scar and a small shoot above it. Small aerial tuber at the base of scion is shown in solid black. Fig. 3. Potato variety Erstling (Eersteling) grafted onto tomato stock. X — region of union between stock and scion. A — lateral shoot of the tomato stock (the leaves are not shown in the picture). B — leaf of the tomato stock. C — leaf scar of the potato scion (above it a shoot with thickened base is seen).

We conducted our experiments in 1949 and 1950. At the end of March 1949 the tomato variety Golden Jubilee was sown in greenhouse. On April 13-th the tomatoes were transplanted into pots and remained in the greenhouse where they were used as stocks for grafting potatoes. On March 28-th potato tubers of the varieties Ulster Chieftain and Erstling were put into pots. The first sprouts were observed on April 10-th. Grafting was performed on the first days of May. The tomato shoots were cut off at the 2-d or 3-d node and the young potato sprouts were grafted onto these stocks. The grafted plants were kept at a temperature of +26°C. in shade during 3—5 days and afterwards they were grown in the greenhouse.

Control plants of the variety Ulster Chieftain did not flower at all. When grafted onto tomato stocks the shoots of this variety flowered abundantly and also produced sometimes fruits and seeds.

In cases in which the tomato stock was deprived of lateral shoots the scion flowered abundantly but the flowers dropped off and consequently did not produce seeds. On such shoots numerous aerial tubers were observed (Fig. 1). In cases in which the tomato stock had 2—4 lateral shoots with young leaves the scion produced sometimes fruits and seeds but did not develop any aerial tubers. Exceptionally we found in such cases single and very small tubers at the base of the scion (Fig. 2).

In 1950 we repeated our experiments and obtained analogous results. In cases in which the tomato stock had 2—4 lateral shoots the scion displayed a tendency towards the formation of fruits. The flowers remained for about 7 days on the plants and afterwards the petals dropped off (but not the calyx) and the ovary became larger in volume. The fruits however did not mature; they dryed up and dropped off. In cases in which the tomato stock had no lateral shoots or when such shoots were old the flowers of the scion dropped off together with calyx. We call old those shoots which bear flowers or flower buds.

Tables I and II show the correlation between the number of lateral shoots left on the tomato stock and the number of aerial tubers developed on the potato scion. We obtained the following results for 46 plants of Ulster Chieftain (Table I) grafted onto the tomato:

1. In 8 plants without any lateral shoots on the stock the scion developed aerial tubers.
2. In 8 plants in which the tomato stock had old lateral shoots the scions also developed aerial tubers.
3. In 7 plants in which the tomato stock had more than 4 lateral shoots with young leaves the scions also developed aerial tubers.
4. In 23 plants in which the tomato stock had 2—4 lateral shoots with young leaves the scions did not produce any aerial tubers. In these plants we observed a tendency towards the production of fruits.

Analogous results were obtained for the variety Erstling (Table II and Fig. 3) and for *Solanum Rybini*. Scions of *Sol. Rybini* grafted onto a tomato stock with a few young lateral shoots produced fruits but did not develop any aerial tubers. On the other hand the scions of the same species grafted onto tomato stocks with old lateral shoots did not produce any fruits but developed aerial tubers (Table III).

2. The effect of tomato stock on the germination of pollen grains of the potato scion

We found that pollen grains of the potato scions grafted onto tomato stocks without lateral shoots germinate slowly and develop short tubes like those of the control plants. The same occurs when tomato stocks have many (more than 4) lateral shoots or when the lateral shoots are old. However in cases in which the tomato stock has 2—4 young lateral shoots the pollen tubes of the potato scions grow faster and become much longer. We found such differences in the following potato species: *Solanum saltense*, *S. Rybini*, *S. stenotomum*, *S. andigenum*, *S. malinchense* (Tables IV and V).

Pollen grains were germinated in artificial media (agar with sacharose and diastase). Immediately after collection the pollen grains were sown in a hanging drop kept at +24°C for 12 hours and examined under the microscope. We found that the pollen grains

TABLE I

Correlation between the number of lateral shoots of the tomato stock and the number of aerial tubers of the potato scion (of the variety Ulster Chieftain)

Ulster Chieftain (potato scion)	Golden Jubilee (tomato stock)	Number of old lateral shoots of the tomato stock	Number of young lateral shoots of the tomato stock	Number of aerial tubers of the potato scion	Ulster Chieftain (potato scion)	Golden Jubilee (tomato stock)	Number of old lateral shoots of the tomato stock	Number of young lateral shoots of the tomato stock	Number of aerial tubers of the potato scion
No	No				No	No			
8·35	7·11	0	0	15	8·95	7·21	0	1	0
8·43	7·15	0	0	12	8·114	7·29	0	3	0
1·54	7·6	0	0	4	8·69	7·17	0	3	0
2·60	7·—	0	0	9	8·82	7·18	0	3	0
8·3	7·2	0	0	11	1·53	7·8	0	2	0
1·59	7·23	0	0	17	1·58	7·12	0	1—2	0
8·70	7·19	0	0	39	8·37	7·11	0	2	0
8·29	7·8	0	0	9	8·71	7·18	0	2	0
2·63	7·—	3	0	3	1·56	7·27	0	1—3	0
8·34	7·10	2	0	1	2·114	7·34	0	2	0
2·62	7·—	3	0	8	2·115	7·35	0	1—2	0
2·64	7·—	3	0	8	2·116	7·35	0	1—2	0
8·31	7·9	3	0	2	2·61	7·—	0	2—3	0
8·41	7·13	3	0	7	2·119	7·38	0	1—3	0
8·101	7·22	2	0	4	8·73	7·19	0	2	0
8·42	7·14	1	0	2	8·96	7·21	0	2	0
2·120	7·39	0	4	1	1·28	7·33	0	2	0
2·117	7·36	0	5	5	8·2	7·2	0	2	0
5·56	7·—	0	5	2	1·33	7·37	0	2	0
2·124	7·41	0	6	5	8·112	7·27	0	1—2	0
8·5	7·3	0	7	1	8·33	7·10	0	3—4	0
8·78	7·20	0	12	11	2·65	7·—	0	2	0
2·57	7·—	0	8	2	2·58	7·—	0	3	0

TABLE II

Correlation between the number of lateral shoots of the tomato stock and the number of aerial tubers of the potato scion (of the variety Erstling)

Erstling (potato scion)	Golden Jubilee (tomato stock)	Number of old lateral shoots of the tomato stock	Number of young lateral shoots of the tomato stock	Number of aerial tubers of the potato scion	Erstling (potato scion)	Golden Jubilee (tomato stock)	Number of old lateral shoots of the tomato stock	Number of young lateral shoots of the tomato stock	Number of aerial tubers of the potato scion
No	No				No	No			
8·19	34·4	0	0	6	2·48	34·41	0	2	0
8·62	34·13	0	0	4	2·45	34·29	0	3	0
8·27	34·7	0	0	3	1·41	34·38	0	2	0
8·48	34·9	0	0	3	8·55	34·11	0	3	0
8·56	34·4	0	0	9	2·31	34·35	0	3	0
8·127	34·16	0	0	3	8·124	34·16	0	1—2	0
8·45	34·8	0	0	1	1·45	34·28	0	3	0
2·35	34·37	2	0	4	2·49	34·41	0	2	0
2·55	34·44	3	0	5	2·50	34·42	0	3	0
8·126	34·16	0	2	0	2·54	34·44	0	2	0
8·147	34·22	0	2	0	1·40	34·27	0	2—3	0
1·44	34·28	0	3	0	1·41	34·27	0	2	0
1·39	34·26	0	3	0	1·50	34·31	0	2—3	0
1·47	34·29	0	2	0	1·60	34·33	0	2	0
2·39	34·38	0	3	0	2·113	34·50	0	2	0
1·42	34·27	0	2	0	2·109	34·46	0	3	0
1·62	34·33	0	3	0	2·52	34·42	0	2	0
2·34	34·36	0	3	0	1·63	34·33	0	3	0
2·30	34·35	0	2	0	2·44	34·39	0	2	0
2·36	34·37	0	3	0	8·121	34·15	0	1	0

TABLE III

Correlation between the age of lateral shoots of the tomato stock and the number of fruits and aerial tubers of *Solanum Rybini* scion

Solanum Rybini (scion) No	*Solanum lycopersicum* (stock) No	Number of young lateral shoots of the tomato stock	Number of old lateral shoots of the tomato stock	Number of fruits of the potato scion	Number of aerial tubers of the potato scion
8·160	087·2	3	—	1	0
8·162	087·3	1	—	1	0
8·86	087·1	—	1	—	23
8·161	087·2	—	2	—	8

of *Solanum Rybini* do not germinate on the stigma of the same flower. This species is self-incompatible. Neither do pollen grains of *S. Rybini* germinate in an artificial medium. But the scions of *S. Rybini* grafted onto tomato stocks produce pollen grains which germinate on the stigma of the same flower and also germinate in agar with the addition of sacharose and diastase.

The pollen grains of other potato species examined by the authors germinate in an artificial medium but the tubes are always longer when these species are grafted onto the tomato stocks. The pollen grain tubes were on an average longer in scions grafted onto the tomato stocks with 2—4 young latral shoots than in scions grafted onto stocks without lateral shoots. Some results concerning the influence of the tomato stocks upon the germination of the pollen grains of the potato scions are given in Tables IV and V.

We also found that the percentage of good pollen grains is greater in potato scions grafted onto tomato stocks than in the control potato plants. The percentage of good pollen grains was estimated by means de the aceto-carmine method.

Summary

The influence of the tomato stocks on the potato scions is manifold. It manifests itself in the following characters: 1) in the flowering of potato varieties, 2) in the production of aerial tubers, 3) in the fertility of pollen grains.

The effect produced by tomato stocks depends on the number and the quality of lateral shoots of the stock in question. Tomato stocks without lateral shoots bring about the flowering of varieties of potatoes which under normal conditions do not flower at all. Such varieties do not however produce any fruits. At the base of potato scions under such conditions appear many aerial tubers.

Tomato stocks with many lateral shoots (more than 4) produce an analogous effect upon the potato scions. In both cases the pollen grains behave in the same way as in the control plants.

TABLE IV

Length of pollen grain tubes in potato scions grafted onto tomato stocks and in the control potato plants

Potato scions of:	Date of measuring in 1950	Length of pollen grain tube in scale divisions of the eyepiece of the microscope			
		in control potato plants	in potato plants grafted onto tomato stocks		
			tomato stock without lateral shoots	stock with 2—4 young lateral shoots	stock with old lateral shoots
Solanum saltense	31. V	5	7	10	5
	6. VI	35	30	41	30
	12. VI	40	22·5	25	—
	4. VII	50	43	60	—
	12. VII	15	27·5	60	—
	average	29	26	39	—
Solanum Rybini	6. VI	0	7·5	41·6	—
	12. VI	0	20	30	—
	20. VI	0	5	7·5	—
	27. VI	0	8	39	—
	4. VII	0	23	50	—
	average	0	13	36	—

TABLE V

Length of pollen grain tubes in potato scions grafted onto tomato stocks and in the control potato plants

Potato scions of:	Date of measuring in 1950	Length of pollen grain tube in scale divisions of the eyepiece of the microscope				
		in contrcl potato plants	in potato plants grafted onto tomato stocks			
			tomato stock without lateral shoots	stock with 2—4 young lateral shoots	stock with old lateral shoots	stock with more than 4 lateral shoots
Solanum stenotomum	4. VII	3	7	60	30	—
	12. VII	20	10	35	10	5
	20. VII	0	5	7	5	—
	average	7	7	34	15	—
Solanum andigenum	12. VI	10	27·5	22·5	—	—
	4. VII	—	20	30	—	—
	average	10	24	26	—	—
Solanum malinchense	31. V	5	5	10	—	—
	6. VI	15	23	37	—	—
	12. VII	10	20	33·5	—	—
	average	10	16	27	—	—

Quite different is the effect produced by the tomato stocks when they have from 2 to 4 young lateral shoots. In such cases the potato varieties which did not flower in the field under normal conditions produce not only flowers but frequently also fruits and seeds. Such potato scions do not however develop any aerial tubers.

As to the fertility of pollen grains we found that the pollen grain tubes of such scions germinate faster and produce longer tubes. Even in self-incompatible species like *Solanum Rybini* pollen grains of the scion germinate on the stigma of the same flower.

Explanation of plates

Fig. 4. Potato variety Ulster Chieftain grafted onto tomato stock. The tomato stock is deprived of lateral shoots. Numerous aerial tubers are developed at the base of the potato scion.

Fig. 5. Potato variety Ulster Chieftain grafted onto tomato stock. The tomato stock has two lateral shoots left. The aerial tubers do not develop. The scion produced one fruit.

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Badania cytologiczne i embriologiczne nad rodzajem Doronicum L. — Cytological and embryological studies in Doronicum L.

Mémoire

de Mlle ***H. WCISŁO***

présenté dans la séance du 16 mars 1951 par M^{me} M. Skalińska m. c. et M. W. Szafer m. t.

(Plates 6—7)

Contents:

Introduction

The present paper deals with the cytology of representatives of the genus *Doronicum* in Poland. Two species only occur in the Polish flora: *Doronicum austriacum* Jacq. and *Doronicum Clusii* All. *D. austriacum* is the only representative of the subsection *Austriaca*. In Poland this perennial species is common chiefly in lower mountain regions of the Tatra Mts and of the Western Carpathians; it is also found in a few isolated habitats in the southern part of the Polish plain (near the springs of river Biała in the vicinity of the Błędowska desert — Piech 1924, and in light forests near Kielce — Szafer 1930). According to Szafer (1930, 1949) *D. austriacum* represents a mountain and climatic relict. It belongs to the group of the so called complete relicts, since its present centre of distribution is localized in mountainous regions, with a well marked lower limit (ca 500 m o. s. l.), while habitats in the plain represent isolated spots (Szafer 1930, 1949). With reference to its age it is regarded as one of the subarctic relicts of scrubs and forests, which in decline of Diluvium entered the forestless tundra, together with pioneer tree species (Szafer 1930). *Doronicum Clusii* All. belongs to the subsection *Grandiflora*. It represents an alpine perennial with a distribution in mountains of Central Europe (Pawłowski 1929). In Poland it is found in the granitic part of Tatra Mts; its vertical distribution extends from the layer of *Pinus montana* upwards to the highest mountain peaks. Its maximal elevation in the Tatra Mts attains the altitude of 2663 m o. s. l. on Garłuch (Pawłowski 1930). Historically *D. Clusii* is considered in the flora of Central Europe as a tertiary element (Kulczyński 1923).

The first cytological data concerning this genus have been published recently by Favarger (1949). He found in *Doronicum grandiflorum* Lam. $n = 30$. In the following year (1950) at the time when a preliminary report of the present work was already in the press (Wcisło in Skalińska 1950), Lindqvist published his paper dealing with the cytology of *Doronicum*. He studied three species of this genus and established the following chromosome numbers: for *D. cordatum* Schultz. and *D. pardalianches* L. $n = 30$, for *D. plantagineum* L. $n = 60$.

The chromosome numbers established in the course of the present work for the two Polish species (*D. austriacum* $2n = 60$, *D. Clusii* $2n = 120$) agree with those found by Favarger and Lindqvist for other species of the genus.

Materials and methods

The materials for cytological and embryological studies were collected from a variety of natural habitats in Poland. The studied specimens of *D. austriacum* originated from habitats in the Tatra Mts. in the Western Carpathians (Babia Góra) and in addition from the plain in a habitat near the springs of river Biała in the vicinity of Błędowska desert (Table I).

All specimens of *D. Clusii* were collected in the granitic part of the Tatra Mts (Table II).

The material for cytological and embryological studies (root tips and flower buds) has been fixed in nature; the respective plants have been transplanted into the experimental field in order to investigate the mode of their propagation. The root tip material was fixed with the Navashin fixative as well as its modifications according to Delaunay and to Manton (1942). The flower buds were fixed in the same way after pretreatment with Carnoy. Sections 8 μ thick were stained with Newton gentian violet by replacing the clove oil by a solution of phenol in xylene (1 : 3).

TABLE I

Doronicum austriacum.

Localities and habitats of the specimens studied.

Field No	2*n*	Locality and habitat
1.	60	Błędowska desert (springs of river Biała — wet part of a scrub).
2.	,,	Western Carpathians — Babia Góra (near the path «taternicka perć» ca 1150 m o. s. l. — higher mountain layer).
3.	,,	Tatra Mts — A road from Zakopane to Morskie Oko; bank of a stream near the common «Waksmundzka Polana», ca 1200 m o. s. l. — higher mountain layer.
4.	,,	Tatra Mts — The high road from Zakopane to the lake Morskie Oko; border of a spruce forest, ca 1450 m o. s. l. — layer of subalpine forests.
5.	,,	Tatra Mts — Valley of Morskie Oko, a forest on the slopes of Żabie, ca 1450 m o. s. l. — layer of subalpine forests.
6.	,,	Limestone part of the Tatra Mts — peak of Łysanki; in a scrub ca 1450 m o. s. l. — layer of subalpine forests.
7.	,,	Tatra Mts — Path to the lake Czarny Staw over Morskie Oko: among rock debris; ca 1450 m o. s. l. — layer of subalpine forests.
8.	,,	Tatra Mts — lower part of the road from the Valley of Morskie Oko to the Szpiglasowa pass; bank of a stream, ca 1500 m o. s. l. — layer of subalpine forests.
9.	,,	Tatra Mts — higher part of the same road; border of a stream, among *Pinus montana*, ca 1700 m o. s. l. — layer of *Pinus montana*.

The cytological drawings have been done with the Reichert imersion lens 100 x N. A. 1, 25 in combination with Zeiss compensating eyepiece 20 x and the Zeiss -Abbé camera-lucida. The magnification of these drawings is ca 2500 x.

The microphotographs have been done with the Zeiss apochromatic oil imersion lens 90 x N. A. 1, 30, Zeiss compensating eyepiece 10 x with the help of Leitz Makam. combined with the Practiflex photographic camera. The microphotographs are magnified ca 1500 x.

Somatic chromosome numbers

The somatic chromosome numbers of *D. austriacum* and *D. Clusii* have been established on the basis of studies of roots tip mitoses.

TABLE II

Doronicum Clusii

Localities and habitats of the specimens studied.

Field No	2*n*	Locality and habitat
1.	120	Path to the lake Czarny Staw over Morskie Oko, ca 1450 m o. s. l. — layer of subalpine forests.
2.	,,	Border of the lake Czarny Staw over Morskie Oko, ca 1580 m o. s. l. — layer of *Pinus montana.*
3.	,,	Slopes of Żabie above the lake Czarny Staw over Morskie Oko, ca 1650 m o. s. l. — layer of *Pinus montana.*
4.	,,	Slopes of Mięguszowiecki above the lake Czarny Staw over Morskie Oko, ca 1680 m o. s. l. — layer of *Pinus montana.*
5.	,,	Slopes of Opalone near the path from the Valley of Five Polish Lakes to the Valley of Morskie Oko, ca 1700 m o. s. l. — layer of *Pinus montana.*
6.	,,	Western slope of Kościelec, ca 1700 m o. s. l. — layer of *Pinus montana.*
7.	,,	Valley behind Mnich (Dolina za Mnichem), ca 1780 m o. s. l. — layer of *Pinus montana.*
8.	,,	The surroundings of the lake Zmarzły Staw below Zawrat, ca 1780 m o. s. l. — layer of *Pinus montana.*
9.	,,	Lower part of the path from the lake Czarny Staw to Granaty, ca 1800 m o. s. l. — layer of *Pinus montana.*
10.	,,	Path from the Szpiglasowa pass to the Valley of Five Polish Lakes, ca 1900 m o. s. l. — layer of *Pinus montana.*
11.	,,	The mount Kasprowy Wierch, ca 1900 m o. s. l. — layer of *Pinus montana.*
12.	,,	The mount Beskid, ca 2000 m o. s. l. — alpine layer.
13.	,,	The pass Wrota Chałubińskiego, ca 2000 m o. s. l. — alpine layer.
14.	,,	Świnicka pass, ca 2050 m o. s. l. — alpine layer.
15.	,,	Szpiglasowa pass, ca 2100 m o. s. l. — alpine layer.
16.	,,	The pass Zawrat, ca 2150 m o. s. l. — alpine layer.

TABLE III

Results of cytological investigations in the genus *Doronicum*, sectio: *Doronicastrum*

Subsectio acc. to Cavillier	species	chromosomes numbers $2n$	author	geographical distribution	source of the material investigated
Austriaca	*D. austriacum* Jacq.	60	Wcisło 1950	Pyrenees, Eastern Alps, Sudetes, Carpathians, Balkans and northern parts of the European plain	9 habitats in the Tatra Mts, Western Carpathians and the Polish plain
Cardiophylla	*D. cordatum* Schultz	60	Lindqvist 1950	South-eastern Europe and Asia Minor (cf. Cavillier)	Botanical garden Berlin—Dahlem
Pardalianchia	*D. pardalianches* L.	60	Lindqvist 1950	Western Europe (cf. Cavillier)	Botanical garden Praha
Plantaginea	*D. plantagineum* L.	120	Lindqvist 1950	Pyrenees, Alps, Great Britain, France (cf. Cavillier)	Botanical garden Lund
Grandiflora	*D. grandiflorum* Lam.	60	Favarger 1949	Pyrenees, Alps, Corse, calcicolous species (cf. Hegi)	col du Balme ca 2200 m o. s. l.
	D. Clusii All.	120	Wcisło 1950	Pyrenees, Alps, Carpathians-calcifuge species (cf. Hegi)	16 habitats in the granitic part of the Tatra Mts.

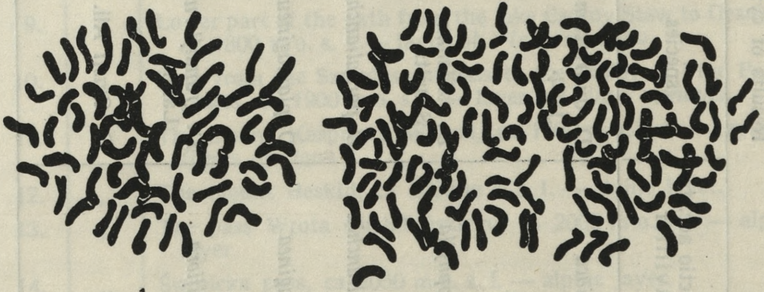
The following chromosome numbers were found: *D. austriacum* $2n = 60$, (Figs 1, 15), *D. Clusii* $2n = 120$ (Figs 2, 16). The fact that the above chromosome numbers have been found in plants originating from various habitats, permits us to assume that the two investigated species are karyologically uniform. *Doronicum* represents a rather difficult cytological object in view of the high numbers of chromosomes and their relatively small sizes.

The results of the previous investigations supplemented by those obtained in the course of the present work are summarized on Table III. It is noteworthy that the two representatives of the subsection *Grandiflora* have different chromosome numbers.

The high chromosome numbers of both investigated species suggest that they represent polyploids. In view of a frequent occurrence of apomixis in polyploid plants (Gustafsson 1946, 47) the mode of reproduction of the two species has been studied. This problem was attacked in the course of the present work in two different ways: 1) — by experiments in seed development from emasculated and bagged flowers: 2) — by studies of meioses in P. M. C's and E. M. C's, as well as of further stages of the development of the embryosac and the embryo.

Experiments in seed development

In spring 1950, 25 inflorescences of the two species transplanted into the experimental field, have been emasculated and bagged to prevent pollination by insects. Not a single seed has been obtained from these experiments. The above results suggest that both *D. austriacum* and *D. Clusii* have a normal sexual reproduction in



1. 2.

Fig. 1. *Doronicum austriacum*, Fig. 2 — *D. Clusii* — somatic plates in root tips.

spite of their high chromosome numbers, and that pollination is indispensable for the development of seeds.

Sporogenesis in Doronicum austriacum ($2n = 60$)

MICROSPOROGENESIS

The P. M. C's of *D. austriacum* represent a favourable material for the study of meiosis. This is chiefly due to the fact that various stages of meiosis may be frequently found within a single flower and sometimes even within a single loculus. Thus a well marked lack of synchronismus is observable in the development of the individual P. M. C's. The early stages of prophase, leptotene, zygotene, pachytene (Fig. 18) show a normal course. More detailed studies have been undertaken beginning with the diplotene. In this stage several (2—3) chiasmata may be discerned in each pair of the strongly contracted chromosomes. In the stage of diakinesis which is attained after a further linear contraction the paired chromosomes are distributed rather regularly near the periphery of the nucleus. The nucleolus is still visible (Fig. 19). Pairing of the chromosomes is almost complete; of the various associations bivalents show a distinct prevalence. On the other hand, multivalent associations which frequently occur in high polyploid, are relatively rare (Table IV).

TABLE IV

Doronicum austriacum

Incomplete analysis of chromosome associations in diakinesis of 300 P. M. C's.

Types of associations:	I	II	III	IV	V	VI	VII	VIII
Frequency	51	5070	19	909	—	6	1	8

The analysis of 300 P. M. C's in the stage of diakinesis permits us to establish a great regularity in chromosome pairing which is rather unexpected in high polyploids. The majority of the chromosomes form bivalents. Besides those quadrivalents are rather frequent. By contrast, hexa- and heptavalents may be observed only occasionally. The univalents and trivalents are rare; they have probably arisen by a precocious break of their chiasmata. Observations concerning the position of chiasmata in diakinesis carried out on 65 P. M. C's have shown the occurrence of only terminal chiasmata in this stage (Table V).

TABLE V

Doronicum austriacum

Incomplete analysis of chromosome configurations at diakinesis in 65 P. M. C's.

Types of configurations	II rod	II ring	III rod	IV rod	IV ring	IV cross	IV Y	other confi-gurations
Frequency	129	145	3	34	47	3	1	34

Among bivalents small rings with two terminal chiasmata are frequent. Loosely paired chromosomes with one terminal chiasma (rods) show approximately the same frequency. Among polyvalents, the most frequent types are rings and rods whereas other configurations are rare.

Metaphase I. This stage has been found in numerous loculi: however the high chromosome number and their rather small sizes render very difficult the analysis of the configurations found in the metaphase plates (Fig. 3, 4, 5, 19). The difficulties are increased by the frequent crowding of the chromosomes in the equatorial plates, thus their complete analysis is possible only in rare instances. The metaphase plates in side view have a typical appearance (Fig. 19). A distinct prevalence of bivalents may be frequently observed, like in diakinesis (Fig. 5). Occasionally some irregularities may be observed (viz: chromosomes outside the equatorial plate or beyond the spindle — Fig. 5, Table VI).

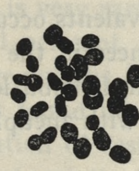
TABLE VI

Doronicum austriacum

I Metaphase in 924 P. M. C's

	No of chromosomes outside the equatorial plate or beyond the spindle										
	0	1	2	3	4	5	6	7	8	9	10
Frequency	786	41	48	26	10	2	4	—	7	—	—
Percentage	85·05	4·55	5·1	2·95	1	0·2	0·4	—	0·75	—	—

Of 924 analysed metaphase plates, 786 (85·05%) have a normal appearance whereas in 138 plates (14·95%) the above mentioned disturbances have been found. The most frequent number of chromosomes scattered in the cytoplasm or lying in the spindle outside



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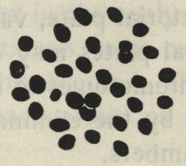

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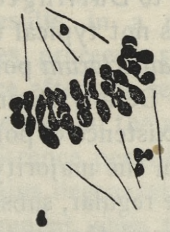

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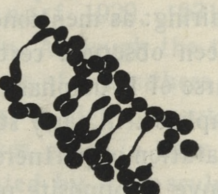

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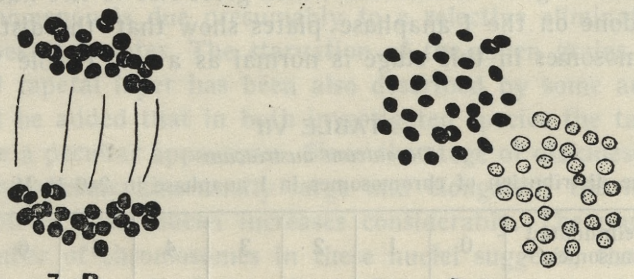


7. B

7. A

Figs. 3—7. *D. austriacum*, meiosis in P. M. C's. Fig. 3 — Metaphase plate in polar view (30_{II}), Fig. 4 — I Metaphase plate in polar view (28_{II} 4_{I}), Fig. 5 — I Metaphase plate in side view; some chromosomes outside the equatorial plate, Fig. 6 — I anaphase, Fig. 7 — I telophase, two cells: A — in polar view, B — in side view.

the equatorial plate, varies from 1 to 3. Univalents occurring in the equatorial plates may cause some disturbances in the distribution of the chromosomes to the poles. An irregular distribution is usually followed by the elimination of microspores with aneuploid chromosome numbers.

A peculiar phenomenon in the I Metaphase is a close approximation of some bivalents, giving the impression of a frequent occurrence of polyvalents. This phenomenon already observed by Lindqvist (1950) in meiosis of the above mentioned three species of the genus *Doronicum*, was interpreted by him as secondary pairing. It should be reminded however that according to Darlington (1932) the occurrence of polyvalents in diakinesis is not typical of secondary pairing: as mentioned previously in *D. austriacum* polyvalents have been observed certainly at diakinesis. Details observable in the course of I Anaphase likewise prove the existence of polyvalents in metaphase. In early stages of I anaphase in the majority of cells the separation of partners in bivalents is quite regular, subsequently they move to opposite poles. On the other hand the separation of the chromosomes associated in quadrivalents or higher polyvalents is always slower; in view of a delayed break of their chiasmata, their connection is still maintained while other chromosomes have already reached the poles (Figs 6, 20). On the whole, the anaphase has a normal course; it is followed by the telophase (Fig. 7a, b) and by the stage of interkinesis which gives rise to two nuclei. The counts done on the I anaphase plates show that the distribution of chromosomes in this stage is normal as a rule (Table VII).

TABLE VII

Doronicum austriacum

The distribution of chromosomes in I anaphase in 246 P. M. C's

No of eliminated chromosomes	0	1	2	3	4	5	6	7
Frequency	208	6	18	—	9	—	5	—

In the 208 out of 246 P. N. C's the distribution of chromosomes is normal; in the remaining 39 cells a variable chromosome number is eliminated (1—6). An important irregularity in the course of the anaphase is the formation of restitution nuclei (Fig. 21). This phenomenon is very rare; it has been observed in the analysed cells in less than 1 per cent. In the stage of interkinesis, some cells with only one large nucleus instead of two could be observed. The giant pollen grains found occasionally probably have arisen as a result of the formation of restitution nuclei.

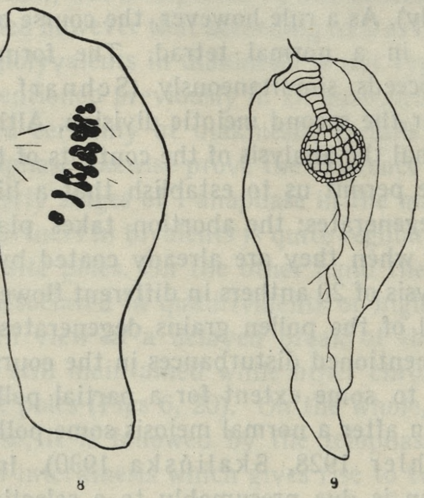
The second meiotic division has a normal course in the majority of the investigated cells (Fig. 22). Occasionally chromosomes scattered in the cytoplasm have been observed; in few cases II metaphase spindles were orientated parallelly; their fusion may also lead to the formation of nuclei with somatic chromosome numbers (syndiploidy). As a rule however, the course of the microsporogenesis results in a normal tetrad. The formation of cellular membranes proceeds simultaneously (Schnarf 1929, 1931) and takes place after the second meiotic division. Although the tetrads seem to be normal the analysis of the contents of the anthers before their dehiscence permit us to establish that a high percentage of pollen grains degenerates; the abortion takes place in later stages of development when they are already coated by their sculptured exine. The analysis of 20 anthers in different flowers has shown that about one third of the pollen grains degenerates.

The above mentioned disturbances in the course of meiosis may be responsible to some extent for a partial pollen sterility. It is known that even after a normal meiosis some pollen grains may be abortive (Tischler 1928, Skalińska 1930). In some instances this phenomenon is due presumably to a selective elimination of some types of gametes. The starvation of the pollen grains by an abnormal tapetal layer has been also described by some authors. It should be added that in both investigated species the tapetum cells have a peculiar appearence. Since the stage of diakinesis they frequently become abnormally large and elongated; the number of nucleoli in their nuclei increases considerably. The unusually high number of chromosomes in these nuclei suggests, that they have arisen as a result of endomitosis. Details of their development will be published separetely.

MACROSPOROGENESIS

The first meiotic division of the embryosac mother cell takes place, as usually later than in P. M. C's and its time corresponds to the stage when in anthers primary pollen grains are observable.

The prophase stages seem to have a normal course. In the I metaphase the spindle may be orientated parallelly to the long axis of the E. M. C's but frequently it lays somewhat obliquely. The only associations of the chromosomes found in I metaphase are represented by bivalents with one terminal chiasma (Figs 8, 23). In the I anaphase the chromosomes are regularly distributed to the poles. Two sister nuclei formed are subsequently separated by a cellular membrane. The second meiotic division which occurs simultaneously in the two daughter cells gives rise to the tetrad. It is the chalazal cell of the tetrad which gives origin to the eight-nucleate embryosac. The degeneration of the three sister macrospores begins from the micropylar pole. The development of the secondary embryosac is quite normal. After fertilization the egg cell passes through a resting stage; in this time the endosperm of the cellular type begins its development. The investigated ovules contained embryos in different stages of development ranging from very young 2 cellular embryos to those which were built of numerous cells and were surrounded by the partially depleted endosperm tissue. In ovules with very young embryos in some instances the presence of pollen tubes could be observed. In later stages of embryo development a typical suspensor



Figs. 8, 9 — *D. austriacum*, Fig. 8 — I Metaphase in E. M. C's in side view, Fig. 9 — the embryo with typically formed suspensor.

becomes differentiated; this gives evidence that the embryos have arisen from the egg cell (Fig. 9).

The following details established in the course of the present work prove that *D. austriacum* has a quite normal sexual reproduction in spite of its high chromosome number:

1) a normal course of meioses in P. M. C's and E. M. C's, 2) the presence of typical suspensors in the embryos which show their origin from the egg cell, 3) the occurrence of pollen tubes in ovules with young embryos which gives evidence of the accomplished fertilization, 4) entirely negative results of experiments on seed formation of emasculated and bagged flowers.

Sporogenesis in *Doronicum Clusii* ($2n = 120$)

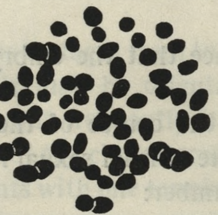
MICROSPOROGENESIS

Observations concerning the course of microsporogenesis in *D. Clusii* have given similar results as in *D. austriacum*. It can be seen in diakinesis nuclei that pairing of chromosomes is complete with a distinct prevalence of bivalents. Associations higher than quadrivalents were not observed. In I metaphase (Figs. 10, 11) some chromosome may be eliminated (Table VIII).

TABLE VIII
Doronicum Clusii
Number of eliminated chromosomes in 1000 P. M. C's

Chromos. elim.	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Frequency	806	50	61	34	12	14	15	—	4	1	2	—	1	—

In comparison with *D. austriacum* no essential differences could be noticed in the course of meiosis in *D. Clusii*. Likewise bivalents with one terminal chiasma are most frequent. The chromosomes are strongly crowded in metaphase plates; therefore they were discernible only at the periphery of the spindles or in parts of the spindles cut off by the microtome knife (Fig. 11). A closely approximation of bivalents in metaphase plates reminds a similar phenomenon observed in *D. austriacum*. The disturbances in I anaphase differ from those in *D. austriacum* only in their frequency. In the bivalents the separation of the partners is more precocious than in the quadri-



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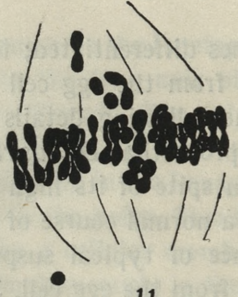

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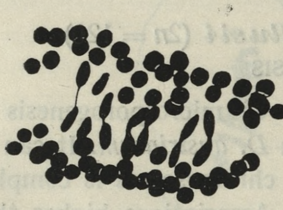

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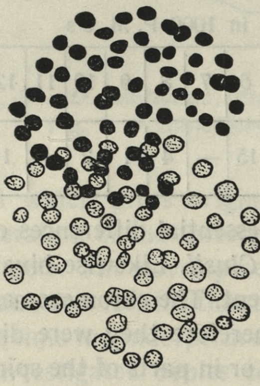
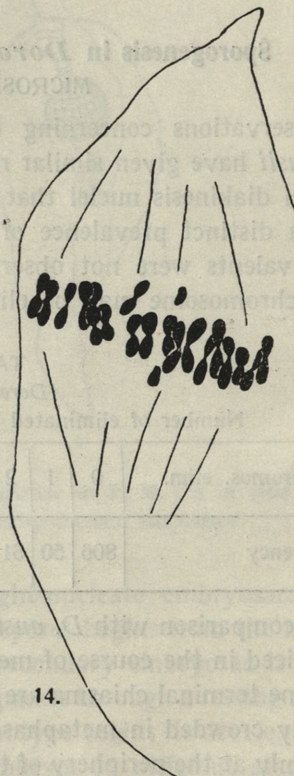


13.



14.

Figs. 10—13, *D. Clusii* meiosis in P. M. C's: Fig. 10 — I Metapnase plate in polar view (60_{II}), Fig. 11 — I Metaphase plate in side view; some chromosomes outside the equatorial plate, Fig. 12 — I Anaphase in side view, Fig. 13 — I Anaphase in polar view. Fig. 14. *D. Clusii*, I Metaphase in E. M. C. in side view.

valents. In these last associations the connections are longer maintained, and sometimes they are still observable in the number of 2 to 8 when the remaining chromosomes are already on their way to the poles (Fig. 12). Owing to very high chromosome number of *D. Clusii* only in few anaphase plates exact counts could be carried out (Fig. 13). Thus in this stage the degree of irregularity could be estimated only from the number of lagging chromosomes or those scattered in the cytoplasm (Table IX).

TABLE IX

Doronicum Clusii

Distribution of chromosomes in I anaphase in 250 P. M. C's

	Number of eliminated chromosomes											
Chromos. elim.	0	1	2	3	4	5	6	7	8	9	10	11
Frequency:	212	17	9	5	2	3	1	—	1	—	—	—

The distribution of the chromosomes on the two poles is normal as a rule. Disturbances resulting from the delay of breaking of chiasmata and affecting a regular distribution of the chromosomes to the poles seem to be less frequent in *D. Clusii* than in *D. austriacum*. Occasionally in this stage some abnormally shaped P. M. C's could be found. These cells are remarkably narrow and elongated; in II anaphase their two spindles are orientated along the axis of the cell and lie in one line.

As a rule the two meiotic divisions in P. M. C's give rise to tetrads. The study of the tetrads has revealed the existence of size differences among the primary pollen grains. In rare instances some abnormal tetrads could be observed in which two of the four nuclei failed to undergo a complete separation and were still connected by thin bridges. Such abnormal tetrads have probably resulted from some disturbances during the II anaphase. The analysis of the contents of the anthers shows a high percentage of degenerated pollen grains; they reach 50% in the majority of the loculi.

MACROSPOROGENESIS

The ovules of *D. Clusii* are similar to those of *D. austriacum*. The first metaphase spindle in all analysed E. M C's was orientated parallelly to the long axis of the cell. Only bivalents are formed

(Figs 14, 24). The anaphase and telophase have a regular course. The tetrad stage was not found in investigated material; therefore it was impossible to establish which one of the tetrad's cells gives rise to the secondary embryosac. The eight-nucleate embryosac has a typical appearance. According to observations in subsequent stages the endosperm of the cellular type precedes the embryo in its development. All older embryos show a typical suspensor.

The regular course of meioses in *D. Clusii* is especially interesting in view of the very high chromosome number of this species.

Discussion

The chromosome numbers of *D. austriacum* and *D. Clusii* agree with those recently found by Favarger (1949) and Lindqvist (1950) for four other species of the genus; they show that all these species are polyploid. The following facts established in the course of the present study give evidence that the two species investigated have a normal sexual reproduction: 1) the regular course of meioses in P. M. C's and E. M. C's, 2) the occurrence in older embryos of typical suspensors which prove that they have arisen from the egg cell, 3) the presence of pollen tubes in ovules with very young embryos giving evidence of the accomplished fertilization, and finally 4) the entirely negative results of experiments on seed formation of emasculated and bagged flowers.

The normal sexual reproduction occurring in species with such high chromosome numbers is particularly interesting since in numerous polyploids apomixis or vegetative propagation frequently replaces a normal sexual reproduction. Such facts were already observed by Strasburger (1905) and then by Rosenberg (1907). According to Gustafsson (1935) in some genera *(Taraxacum, Hieracium)*, diploid species show a normal sexual reproduction whereas polyploids are apomictic. On the other hand apomixis in diploids is less frequent. According to Bergman (1935) it occurs in *Leontodon hispidus* ($2n = 14$) and *Picris hieracioides* ($2n = 10$). In comparison with the above results, a normal sexual reproduction in high polyploid species of *Doronicum* is rather unexpected.

The basic number of the genus *Doronicum* is difficult to estimate. On the basis of previous investigations on the genus *Senecio* which belongs together with *Doronicum* to the tribe *Senecioneae*, the following chromosome numbers have been inferred: $n = 5$, 6 (Löve and Löve 1948), $n = 10$, 11, 12, 23 (Darlington and Janaki-Ammal 1945). Presumably, the higher numbers could have arisen by amphidiploidy from the lowest numbers, therefore it is difficult indeed to estimate on these observations the basic number of the related genus *Senecio*. Likewise the basic number of *Doronicum* remains undetermined. Details observed in the course of meioses of the two species studied (a low frequency of polyvalents) suggest that they represent allopolyploids. As well known polyploids of hybrid origin show in meioses in general a regular bivalents formation and a regular distribution of chromosomes to the poles in anaphase.

Both *D. austriacum* and *D. Clusii* are representatives of the mountain flora. According to the investigations of Gustafsson (1947) in the alpine flora the percentage of polyploids is rather low in spite of the prevalence of perennial species in which polyploidy is more frequent than in annuals. Karyological studies in Angiosperms of the Polish Tatra Mts (Skalińska 1950) have also revealed a rather low percentage of polyploids. In higher polyploids occurring there the high chromosome numbers are often associated with the phenomenon of vivipary (*Polygonum viviparum* $2n =$ ca 132, *Poa granitica* $2n =$ ca 80). Contrasting with these plants, the investigated *Doronicum* species have maintained the capacity of sexual reproduction in spite of their high chromosome numbers.

The Polish representatives of the genus *Doronicum* are common in their natural habitats in Poland especially in the Tatra Mts. The two species differ in their vertical distribution. *D. Clusii* represents a typical oreophyt with its main centre of distribution in the alpine layer. Lower it becomes rarer and finally it disappears entirely below the limit of the layer of *Pinus montana* at the altitude of ca 1450 m o. s. l. On the other hand *D. austriacum* occurs in the Tatra Mts chiefly in lower situations, it ascends however to the layer of *Pinus montana* (ca 1700 m o. s. l.). Thus the areas of the two species overlap partially in the lowest part of the range of *D. Clusii* which corresponds to the highest part of that of *D. austriacum*. It should be emphasized however that in spite of the lack of ecological isolation and the partial overlapping of the flowering time of the two species, hybrid forms in the Tatra Mts were never observed. This may be due to incompatibility.

In the literature we find a few data concerning putative hybrids within the genus. Cavillier (1910) considers *D. scorpioides* Widd. as a hybrid between *D. plantagineum* ($2n = 120$) and *D. pardalianches* ($2n = 60$). In view of the existing difference in the chromosome numbers of these two species only a cytological study could throw light upon the real value of *D. scorpioides*. In 1948 Widder also reported a putative hybrid between *D. Columnae (D. cordatum)* and *D. grandiflorum*, two species with 60 somatic chromosomes. The occurrence of hybrids in nature would afford an additional proof of the typical sexual reproduction of the species belonging to the genus *Doronicum*.

Summary

The present investigations deal with two species of the genus *Doronicum* L. native in the Tatra Mts: the first *Doronicum austriacum* Jacq. is a plant occurring there in lower situations up to the layer of *Pinus montana* and in addition found in a few isolated habitats in the southern part of the Polish plain, where it is regarded as a mountain relict. The second species *Doronicum Clusii* All. is a characteristic component of the alpine layer in the granitic part of the Tatra Mts.

The somatic number of chromosomes established for *D. austriacum* in the course of the present work is 60; this suggests that this species is polyploid. A still higher number ($2n = 120$) has been found in *D. Clusii*, which represents a species with a very high degree of polyploidy.

A normal course of meiosis in P. M. C's has been observed in both studied species in spite of their high chromosome numbers. The occurring disturbances (the occasional formation of polyvalents, the elimination of some chromosomes) are common in high polyploids. Occasionally in *D. austriacum* restitution nuclei could be observed, a phenomenon leading to the formation of giant pollen grains.

The tapetum cells in the anthers of both species investigated have nuclei with an unusually high number of chromosomes; they have possibly arisen as a result of endomitosis.

The course of meioses in E. M. C's and the development of the secondary embryosac are also quite normal both in *D. austriacum*

and in *D. Clusii*. The occurrence of typical suspensors in later stages of embryo development gives evidence of their origin from the egg cell.

Experiments on emasculated and bagged flowers have shown that pollination is indispensable for seed formation.

The above results prove that the two species investigated have a quite normal sexual reproduction, in contrast to a large number of high polyploids in which apomixis or vegetative propagation frequently replaces a normal course of sexual reproduction.

The present study has been carried out in the Institute of Plant Anatomy and Cytology of the Jagiellonian University of Kraków. I am glad to have the opportunity to express here my deeply felt gratitude to Professor M. Skalińska Head of the Institute for valuable guidance and never-failing interest throughout the course of my work.

My thanks are also due to all persons who helped me in collecting the plant specimens for investigations and to dr A. Bajer for the microphotos. The research grant of the Polish Academy of Sciences in 1949 and 1950 is gratefully acknowledged.

References

1) Afzelius K.: Embryologische und zytologische Studien in *Senecio* und verwandten Gattungen. Acta Horti Berg. 8, 123—218, 1924. — 2) Bergman B.: Zytologische Studien über die Fortpflanzung bei den Gattungen *Leontodon* und *Picris*. Sv. Bot. Tids. 29, 155—301, 1935. — 3) Cavillier F.: Nouvelles études sur le genre *Doronicum*. Ann. Cons. et du Jard. Bot. de Genève 13—14, 195—368, 1909—1911. — 4) Darlington C. D.: Recent advances in cytology. London Churchill, 1932. — 5) Darlington C. & Janaki-Ammal: Chromosome atlas of cultivated plants. Allen & Unwin, London, 1945. — 6) Favarger C.: Notes de caryologie alpine. Bul. Soc. Neuchat. de Sc. Nat. 72, 15—22, 1949. — 7) Gustafsson A.: Apomixis in higher plants. Lund Univ. Arsskr. 43, 1946—47. — 8) Juel H.: Untersuchungen über die Auflösung der Tapetenzellen in den Pollensäcken der Angiospermen. Jahrb. wissenschaftl. Bot. 56, 337—364, 1915. — 9) Kulczyński S.: Das boreale und arktisch-alpine Element in der mitteleuropäischen Flora. Bull. Acad. Polon. Sc. Ser. B, 128—214, 1923. — 10) Lindqvist K.: Some results of a cytological investigation of *Doronicum*. Hereditas 36, 94—102, 1950. — 11) Löve A. & Löve D.: Chromosome numbers in northern plant species. Reykjavik 1948. — 12) Manton I.: A note on the cytology of *Psilotum* with special reference to vascular prothalli from Rangitoto Island. Ann. Bot. N. S. 6, 283—292, 1942. — 13) Müntzing A.: The evolutionary significance of autopolyploidy. Hereditas 21, 263—378, 1936. — 14) Nygren A.: The genesis of some Scandinavian species of

Calamagrostis. Hereditas 32, 131—261, 1946. — 15) Nygren A.: Cytological and embryological study of *Antennaria Posildii*. Hereditas 36, 483—486, 1950.— 16) Pawłowski B.: Elementy geograficzne i pochodzenie Flory tatrzańskiego piętra turniowego. P. A. U. t. 65, 1—64, 1928. — 17) Pawłowski B.: Maksima wysokościowe kilkudziesięciu roślin tatrzańskich. Spr. Kom. Fizj. P. A. U. 15, 153—158, 1930. — 18) Piech K.: *Doronicum austriacum* Jacq. i *Cochlearia officinalis* L. w okolicy Olkusza. Act. Soc. Bot. Pol. 2, 1924. — 19) Rosenberg O.: Cytological studies on the apogamy in *Hieracium*. Sv. Bot. Tidskr. 11, 145—206, 1907. — 20) Schnarf K.: Embryologie der Angiospermen. Berlin, Gebr. Borntraeger. 10/2 1929. — 21) Schnarf K.: Vergleichende Embryologie der Angiospermen. Berlin, Gebr. Borntraeger 1931. — 22) Skalińska M.: A new case of reciprocal hybrids in *Aquilegia*. Proc. the Fifth Internal Bot. Congr. Cambridge, 1930. — 23) Skalińska M.: Cytogenetic studies in triploid hybrids in *Aquilegia*. Jour. Genetics 47, 88—110, 1945. — 24) Skalińska M.: Studies in cyto-ecology, geographic distribution and evolution of *Valeriana* L. Bull. Int. Acad. Cracovie 149—177, 1950. — 25) Skalińska M.: Studies in chromosome numbers of Polish Angiosperms. Acta Soc. Bot. Pol. 20, 45—68, 1950. — 26) Szafer W.: Element górski we Florze niżu polskiego. P. A. U. 69, ser. 3, 29, 1—112. 1930. — 27) Szafer W.: Zarys geografii roślin. Warszawa 1949. — 28) Tischler G.: Über eigenartige Chromosomenbindung bei *Ribes Gordonianum (R. sanguineum x R. aureum)*. Verhandl. internat. Kongress für Vererbungswissenschaft. Berlin, 1488—1494, 1928. — 29) White H. J. D.: Animal cytology and evolution. Cambridge, University press 1945. — 20) Widder F.: Der neue Bastard *Doronicum Columnae* x *grandiflorum* — *Doronicum Prenni* Widder. Phyton 1. 1948. — 31) Witkus R. E.: Endomitotic tapetal cell division in *Spinacia*. Am. Jour. Bot. 326—330, 1945.

Explanation of Plates 6—7

Fig. 15. *D. austriacum* — somatic plate (the same as Fig. 1).

Fig. 16. *D. Clusii* — somatic plate (the same as Fig. 2).

Fig. 17. *D. austriacum*, anther with P. M. C's in early prophase (a quadrivalent visible in a cell on the left).

Fig. 18. *D. austriacum*, anther with P. M. C's in diakinesis.

Fig. 19. *D. austriacum*, I Metaphase in polar view.

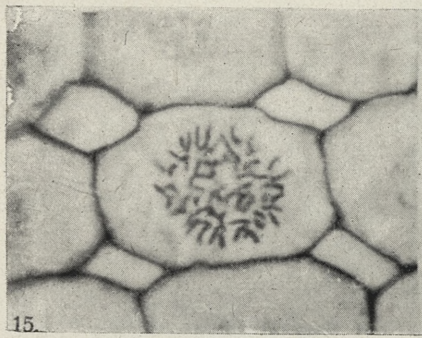
Fig. 20. *D. austriacum*, anther with P. M. C's in I Anaphase and Telophase.

Fig. 21. *D. austriacum*, interkinesis; two binucleate cells and one with a restitution nucleus.

Fig. 22. *D. austriacum*, II Telephase; note the peculiar appearance of the tapetum cell.

Fig. 23. *D. austriacum*, I Metaphase in E. M. C. in side view.

Fig. 24. *D. Clusii*, I Metaphase in E. M. C. in side view.



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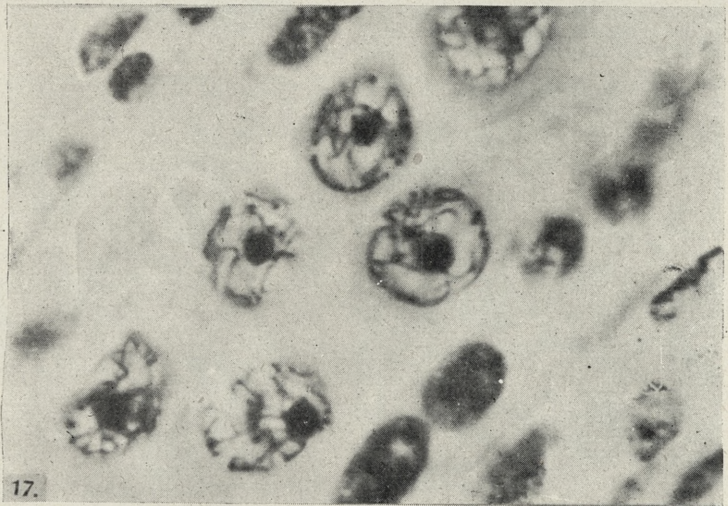

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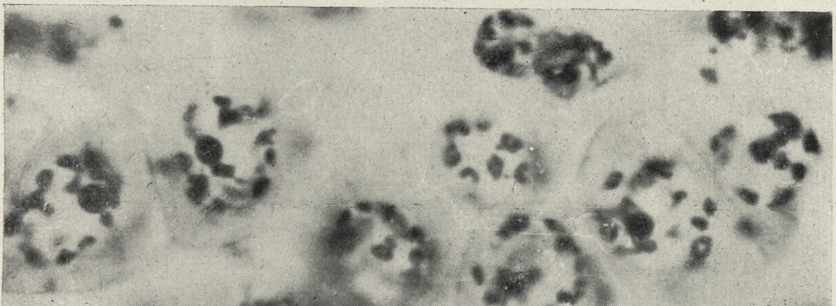

18.

H. Wcisło

BULLETIN INTERNATIONAL DE L'ACADÉMIE POLONAISE DES SCIENCES ET DES LETTRES

CLASSE DES SCIENCES MATHÉMATIQUES ET NATURELLES

SÉRIE *B*: SCIENCES NATURELLES

DERNIERS MÉMOIRES PARUS

Nº 7—10 B I, 1949.

Jentys-Szaferowa J. Analysis of the collective species *Betula alba* L. on the basis of leaf measurements. Part I: Aim and method of the work on the example of *Betula verrucosa* Ehrh.

Kozłowska A. Investigations on the strains of potato virus X in ultraviolet light (Plate 9).

Miczyński K. Genetic studies in the *genus Aegilops*. IV. The inheritance of some characters in the intervarietal crosses of *Aegilops ventricosa* Tausch., *Ae. triuncialis* L. and *Ae. ovata* L. (Plate 10).

Malinowski E. The problem of Heterosis. IV. Inheritance of vigorous growth.

Nº 1—3 B I, 1950.

Jentys-Szaferowa J. Analysis of the collective species *Betula alba* L. on the basis of leaf measurements. Part II: *Betula pubescens* Ehrh., *B. tortuosa* Ledeb., *B. carpatica* Waldst. et Kit.

Kozłowska A. Investigation of masked virus X in potatoes by complement fixation test.

Kłaput A. Recherches sur les sols des associations végétales rocheuses du Jura de Cracovie.

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Sawicki J. Studies on the structure of the aleurone layer in varieties of the cultivated barley *Hordeum sativum* Jess. (Plates 1—2).

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