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Mémoire

de M. *J. SAWICKI*

présenté le 16 Juin 1950 par M^lle^ A. Kozłowska m. c.
et M. B. Pawłowski m. c.

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I. Introduction

The development and progress in cereal breeding, constantly affording new varieties for farming needs, necessitate the distinction of these varieties. This is very important, as the different cultivated varieties belong not only to the same species or subspecies, but very often to the same botanical variety *(varietas)* and subvariety *(subvarietas)*. These commercial varieties, although often morphologically similar, differ in practical characters (producti-

vity and quality) and consequently in their economic significance as well as in their usefulness for special purposes.

In such cases it may occur that the characters considered in the systematics, or even in more detailed monographs elaborated for a given kind of the cereal, prove insufficient for the differentiation of these varieties. Therefore we have to look for other, more subtle methods, which would allow the detection of the apparently small but essential and from the practical point of view valuable differences between the varieties. For this purpose in the classification of cereals several auxiliary methods, e. g., staining with phenol, examination of fluorescence in ultraviolet light, etc., are often applied.

The anatomical characters both of the vegetative organs and of the seeds seem to possess great possibilities of application in the classification of cultivated plants. The application of the anatomical characters of seeds may have considerable significance, especially in cases when we are compelled to establish the identity of the variety on the basis of the examination of the grain alone.

It is the purpose of this study to examine the possibilities of employing the differences in the structure of the aleurone layer of the grain in different varieties of spring barley in order to classify them.

II. Review of the literature

It is well known that the aleurone layer in a grain of barley is composed of a greater number of cells than in other cereals belonging to the family *Gramineae*.

Harz (1885) states that the aleurone layer in cultivated barleys consists of 2—4 rows of cells, emphasizing at the same time the differences in the number of cells existing between the different species and the botanical varieties.

Lauck (1906, 1907) states, on the basis of his studies, that the aleurone layer of the barley grain consists of 2, sometimes 3 and 4, or even 6 cell rows. At the same time he draws attention to the relation existing between the protein content in the grain of barley and to its anatomical structure.

Quante (1913), dealing in his manual «Die Gerste» with the anatomical structure of the barley grain, states that the outer part of the endosperm adherent to the testa is formed by a layer

consisting of mostly 3 or, more seldom, 2 or 4 rows of cells. He also deals with the question of the protein content, which Lauck treats identically.

Maurizio (1903) also points out that the outer part of the barley endosperm is composed of 2—4 cells containing protein.

Moeller and Griebel (1928) also state that the endosperm of the barley grain differs from that of other cereals in so far as the aleurone layer is composed of 2—4 cell rows. This author does not however give any more detailed data on the differences appearing within the one species.

Schnegg (1921), referring to the anatomical structure of a grain of brewing barley observes that the aleurone layer is usually composed of quadrangular cells with thick walls, strongly refracting light, arranged in 2 or 3 rows one above the other. Under this layer lie the starch cells, which are smaller when located immediately beneath it, but in the second and third layer they grow larger and in the further layers towards the middle of the grain they become more elongated. Schnegg also points out that in varieties with a flinty grain 2 or 3 rows of cells underlying the aleurone layer are chiefly filled with reserve albumen, the amount decreasing towards the middle of the grain. In the varieties with a mealy grain and low protein content, the amount of this reserve albumen is smaller than in the varieties with a high protein content, which allows to a certain degree the distinction of varieties with a low from those with a high protein content by means of anatomical sections.

The relation between the protein content and the anatomical structure of the aleurone layer is also pointed out by Lindner (1918) and Delbrück (quoted by Lauck (1906).

The taxonomic value of the anatomical characters of seeds has been discussed by several authors. It will be sufficient to mention the work of: Sempołowski (1874), Harz (1885), Krauze (1919), Jegorowa (1922) and Kamieńskij (1935), who applied the anatomical characters of seeds for the determination of species.

After the establishment of the different centres of origin of cultivated plants by Vavilov (1926), special attention has been paid to the anatomical differences inside the species and to the possibility of their application in the classification and selection of cultivated plants. Several authors have examined the ana-

tomical structure of different geographical forms within the limits of a species. One of the first investigators who drew attention to this matter was Percival (1921, 1927), who separated different anatomical coleoptile types in wheat. Among other researches there should be mentioned the paper of Melnikow (quoted from Aleksandrow & Aleksandrowa, 1935), dealing with the anatomy of the pericarp in *Triticum durum*. He found some differentiation in the anatomical structure of the pericarp in wheat originating from different centres of origin. A similar purpose inspired the work of Pisarew and Kardiumowa (1929) on flax *Linum usitarissimum*, the work of Aleksandrow and Aleksandrowa (1935) on peas *Pisum sativum*, the papers of Jakowlew and Nikołajenko (1931) and of Miczyński (1937) on the wheat coleoptile, as well as the study of Jakowlew (1937) on the coleoptile of barley and oats. All these papers support the thesis of Vaviłov concerning the different centres of origin of cultivated plants.

The work of Melnikow (1929), presented in the form of a short summary in the reports from the Genetical Congress held in 1929 in Leningrad, which was intended to establish a comparison of the anatomy of the wild and cultivated barley grains, had also the same purpose. He does not present, however, any data on the differentiation of the number of cell rows in the aleurone layer. On the basis of his studies he confirms the supposition of Vaviłov concerning the Asiatic and African centres of origin of the cultivated barley.

The distinct differences in the anatomical structure of the aleurone layer between the barleys originating from two different centres were established for the first time by Orlov (1931). According to this author: «the aleurone layer in the grain of the geographical form *abyssinicum* is composed of only two rows of thin and long cells, whereas in the case of the barleys originating from other regions, the aleurone layer consists always of 3—4 thick, short cells». In another paper Orlov (1934) states: «The Arabian barleys with the exception of the forms of ecological *abyssinicum* type include — as was proved by our examinations — also the forms of barleys of the *indicum* ecological type. The aleurone layer in some Arabian barleys consists of 2 to 3 thin rows of cells. The same anatomical structure of the grain is seen in the barley varieties originating from India. The aleurone layer in the grain of barleys originating from Abyssinia and Erytrea is composed of only two

thin cell rows». Orlov (1936) distinguishes within the limits of the same botanical variety different geographical types of barleys and differentiates them (among others) on the basis of the number of cell rows in the aleurone layer. Thus within the limits of var. *glabriparallelum* Orl. (subsp. *vulgare*) he distinguishes: the Abyssinian geographical type with a double layer of aleurone cells and the Jugoslavian (European) geographical type, in which the aleurone layer is composed of 3—4 rows of thick cells. Within the limits of: var. *nigrescens* Körn. (subsp. *distichum*) he distinguishes: subvarietas α as the Abyssinian type with a double layer and subvarietas β *(anatolicum)* with the aleurone layer composed of 3—4 rows of cells. In the same work Orlov enumerates several varieties of the botanical Abyssinian geographical type (subproles *abyssinicum*) e. g. from 4-rowed barleys: var *glabrigracilius* Orl., var. *eurylepis* Körn., var. *griseinigrum* Vav. and Orl., from the 2-rowed varieties: var. *Brauni* Körn., var. *contractum* Körn. and var. *melanocritum* Körn., stating that the aleurone layer is here always composed of 2 rows of cells. On page 249 we find two figures representing the section of the naked barley grain, one of which (var. *coeleste* L. from Abyssinia) has the aleurone layer composed of 2 rows of cells and the second (var. *violaceum* Körn.) originating from Mongolia has the aleurone layer composed of 3—4 cell rows.

The above-mentioned data of Orlov as well as the reproduction of the illustrations mentioned are given by Isenbeck and Hoffmann (1942).

In the Polish plant breeding literature the single reference to the differences in the structure of the aleurone layer in barley can be found in the work of Bezradecki (1930) who, referring to the anatomical structure of the naked barley of Asiatic origin, emphasizes that its aleurone layer is composed of 3 rows of cells, and not of 2 rows as in the case of the «Puławski» brewing barley. He illustrates this differences with figures of the median transverse sections of grains belonging to the two studied varieties.

III. The problem, material and methods

The above-mentioned facts, as described in the agro-botanical literature allowed the supposition that in the anatomical structure of the aleurone layer there are distinct varietal differences, which could be used in the classification and breeding of barley.

Taking into account that all the present data were based on sporadical observations or have been published without quoting the material investigated i. e. the measurements, it was necessar to examine this problem on a larger series of varieties, which would enable more certain, practical conclusions to be drawn. Thus it was necessary to examine the differences in the structure of the aleurone layer on the one hand between various distant botanical varieties originating from different geographical centres, and on the other the anatomical differentiation between various cultivated varieties belonging to the same botanical variety and at last to study the variability of the character investigated and its transmission to the offspring on the material from several harvest years.

The primary purpose of this work was only the solution of the problem whether the number of cells in the aleurone layer in the grain of barley is a constant varietal character and, if so, whether it can be used for the classification of barley varieties. During the preliminary examinations, conducted on a small series of varieties over a period of three years, it was shown that apart from the number of cells, the thickness of the aleurone layer may also constitute a characteristic varietal feature. Consequently further investigations have been carried out in both directions.

The material for the above-mentioned investigations consisted of a collection of spring barley grown by the Department of Plant Breeding and Agricultural Experimentation of the Jagiellonian University in 1937, 1938 and 1939 on the Plant Breeding Farm in Polanowice, and since 1945 in the Experimental Farm of the Jagiellonian University in Mydlniki. From this collection 103 varieties representing all the subspecies *Hordeum sativum* Jess., among these several varieties originating from different countries and different parts of the world, were chosen. Some of them have been studied on material deriving from 3—4 years.

For want of a uniform and generally obligatory classification of the species *Hordeum sativum* Jess. (*Hordeum vulgare* Körn.) the studied varieties in Table I have been arranged according to the classification of Körnicke (1885), while in the 2-rowed barleys a fourth subspecies: *Hordeum decipiens* Steud. with lateral rudimentary spikelets *(deficiens)* has been additionally introduced after Beaven (1947). Consequently we have in Table I the following subspecies:

1. Subsp. *Hordeum polystichum* Döll.
   a) *Hordeum hexastichum* L.
   b) *Hordeum vulgare* L. (*H. tetrastichum* Körn.)
2. Subsp. *Hordeum intermedium* Körn.
3. Subsp. *Hordeum distichum* L.
4. Subsp. *Hordeum decipiens* Steud.

Within the limits of subsp. *Hordeum polystichum* there were separated: *Hordeum hexastichum* and *Hordeum vulgare* on the basis of the morphology of the ear alone. Within the limits of the subsp. *Hordeum distichum*, which in the present work is represented very richly, the following botanical varieties (after the nomenclature of Körnicke) were separated: var. *nutans*, var. *zeocrithum*, var. *inerme* and var. *nudum*.

The varieties, itemized in Table I have been examined on material from the years 1937, 1938, 1939 and 1947; thus advantage was taken of the long space of time in order to point out the differentiation of the climatic conditions, as well as of the culture and soil conditions, taking into account that the examined material was sown out in different years and in different localities.

40 cultivated varieties, which were statistically worked out on material deriving from the years 1937, 1938 and 1947, have been separated out of the total number of 103 varieties. While the present work was being carried out 28 varieties of cultivated barley obtained from the Plant Breeding Institute in Cambridge (harvest year 1944) were included in the material examined.

Before the investigations of the above-mentioned material could be udertaken it was necessary to establish the variability of the number of cells and of the thickness of the aleurone layer within the limits of a single plant and within one ear. For this purpose both characteristics have been studied in 6 varieties, on individual ears of the same plant. In each variety the variability in the ear has been examined on transverse sections of the grains taken from the median spikelets and from every second internode, starting from the base of the ear. In both cases, neither within the limits of a single plant nor within the ear could any essential variation of the value of both characters be confirmed.

The measurements of the characters of the aleurone layer studied were carried out on microscopic preparations cut with a razor and stained with iodine in potassium iodide. For each variety

and each crop year several sections were made out of a considerable number of grains, taken at random from a bunch of barley ears. The sections were always cut at the same height (in the middle) of the grain, and a segment from among three middle nerves of the lemma of the flowering glume was taken for examination. The cell countings and measurements of the thickness of the layer were made on at least three different preparations. In the case of an insignificant variability of the cell number in preparations from a given year and variety, only several score of measurements were made, If, however, more pronounced differences between the preparations, or greater variation in the number of cells or in the thickness of the layer could be observed within the limit of a variety, about 100 or more measurements were made. They were carried out in such a way that in the same place of the aleurone layer where the number of cells was counted the thickness of the layer was simultaneously measured by means of an eyepiece micrometr. The thickness of the layer was determined by the number of segments of the eyepiece scale, while the actual value of one segment amounted to 5·4 μ.

The results of measurements are presented in the form of annual means for each variety and each harvest year (M) with the standard error ($\pm m$) of the mean, the standard deviation ($\pm \sigma$) and the variability coefficient (v). In Table I the number of cells is additionally shown in percentage of the total number of observations. The general mean for a variety has been calculated from the annual means in order to establish the characteristic number for each variety.

A more detailed «analysis of variance» for both the characters examined has been carried out on the base of a series of 40 varieties grown in the years 1937, 1938 and 1947.

Moreover, in order to examine the influence of climatic conditions on the stability of the features investigated a comparison was made between the material of 28 varieties obtained from Cambridge and the same material grown afterwards on the experimental ground in Mydlniki.

Finally, having no certitude whether the varieties from the collection were quite homogenous, further investigation was carried out in order to prove the varietal differences and their inheritance

on 15 pedigree lines separated from the collection in 1947 and sown out later on in 1948 in pot cultures in the vegetation room in Kraków, and in 1949 on the experimental ground in Mydlniki.

IV. Investigation of a collection of 103 barley varieties

The results of the examination of 103 varieties of spring barley have been presented in Table I with regard to the number of cells in the aleurone layer as well as to the thickness of the latter. The number of measurements in different varieties and crop years is unequal, but sufficient to characterize the value of both characters in the varieties examined by means of arithmetical means. This is proved by the comparatively small standard errors of the mean of the average number of cell rows and of the average thickness of the aleurone layer.

The number of cell rows in the varieties examined is shown in Table I in the form of the percentage of each of the variants (1, 2, 3, 4 rows) for each variety and each crop year. The average value of this character is given in the form of arithmetical means.

The number of cell rows in the aleurone layer of the same grain is of course not everywhere equal. In microscopic sections we often find, in the same preparation, fragments composed of 1 and 2, or 1, 2 and 3 and finally of 2, 3 and 4 cell rows placed alternately in groups close to one another. Within the limits of a preparation, however, there generally prevails a certain number of cells.

The number of cells in the aleurone layer — as shown in Table I — varies in the varieties of spring barley examined from 1 to 4. During the examinations no variety in which the aleurone layer consisted of more than 4 cell rows has been found. In a few cases, where 5 cell rows were observed in some preparations, it was found that the increase of the cell number is due to an improper, oblique cutting of the preparations. In properly made preparations the cells are symmetrical, similar in size, having an almost square or rectangular shape and slightly rounded edges. The individual cells are arranged one above the other, any longer cells which may occur among them, being generally twice as long as their neighbours. This is probably due to the fact that one parent cell has not divided into two daughter cells. In the calculations a cell of double length was always considered as one single cell. It should be remarked that in the material examined, in three cases only did the cell num-

TABLE I

showing the results of measurements in 103 varieties

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer: Percentage within the preparations examined 1	2	3	4	Annual means from different years $M \pm m$	Mean for the variety	Thickness of the aleurone layer in μ: Annual means from different years $M \pm m$	Mean for the variety
				Subsp. *Hordeum polystichum*							
				1. *Hordeum hexastichum* L.							
1.	a six rang de Chine	1938	126	—	20·6	76·9	2·5	2·82 ± 0·04		84·34 ± 0·76	
		1947	34	—	32·3	64·7	3·0	2·71 ± 0·09	2·76	93·07 ± 1·66	88·70
2.	Emilio Mariani	1938	46	—	41·3	52·1	6·6	2·65 ± 0·09		87·57 ± 1·89	
		1947	38	—	2·6	94·8	2·6	3·00 ± 0·04	2·82	87·11 ± 1·35	87·34
3.	Pyramidatum Körn.	1938	51	—	15·7	76·4	7·9	2·92 ± 0·07		85·76 ± 0·97	
		1947	80	—	15·0	85·0	—	2·80 ± 0·04	2·86	87·00 ± 0·76	86·38
4.	Pyramidatum W. C.	1938	88	11·3	68·0	18·0	2·7	2·11 ± 0·07		60·00 ± 1·24	
		1939	81	2·4	64·2	33·4	—	2·31 ± 0·06	2·21	63·46 ± 1·02	61·73
				2. *Hordeum vulgare* L. (*H. tetrastichum* Körn.)							
5.	American 6-row. Nr. 166	1938	43	—	7·0	93·0	—	2·93 ± 0·04		76·46 ± 0·51	
	(H. furcatum)	1939	104	—	30·0	70·0	—	2·69 ± 0·05		73·58 ± 0·91	
		1947	30	—	3·3	96·7	—	2·97 ± 0·03	2·86	71·36 ± 1·19	73·80
6.	American 6-row. Nr. 169	1938	63	—	29·0	68·0	3·0	2·75 ± 0·07		72·17 ± 1·03	
	(H. furcatum)	1947	30	—	3·3	96·7	—	2·97 ± 0·03	2·86	76·50 ± 1·60	74·33

7.	American 6-row. Nr. 175 *(H. furcatum)*	1938	40	—	20·0	80·0	—	2·80 ± 0·06		73·71 ± 0·70	
		1947	52	—	11·5	88·5	—	2·88 ± 0·04	2·84	76·95 ± 1·35	75·33
8.	American 6-row. Nr. 115	1938	47	—	17·0	83·0	—	2·83 ± 0·07		81·80 ± 0·92	
		1947	44	—	13·0	87·0	—	2·82 ± 0·06	2·82	74·80 ± 0·97	78·30
9.	American 6-row. Nr. 131	1938	106	—	32·0	68·0	—	2·68 ± 0·05		82·24 ± 0·65	
		1939	130	—	27·7	72·3	—	2·72 ± 0·02		80·51 ± 0·82	
		1947	113	—	34·5	65·5	—	2·65 ± 0·02	2·68	76·30 ± 0·86	79·68
10.	Californian 6-row.	1938	42	—	40·5	59·5	—	2·60 ± 0·08		71·27 ± 1·03	
		1939	122	—	40·1	59·9	—	2·60 ± 0·04		71·30 ± 0·81	
		1947	27	—	48·1	51·9	—	2·52 ± 0·09	2·57	66·99 ± 1·73	69·85
11.	Scotch 6-row. «Bere»	1938	36	—	22·2	69·4	8·4	2·86 ± 0·05		74·84 ± 1·56	
		1947	52	—	19·2	78·8	2·0	2·83 ± 0·06	2·85	76·95 ± 0·86	75·89
12.	Irish 6-row.	1938	51	—	17·7	82·3	—	2·82 ± 0·05		79·20 ± 1·13	
		1947	43	—	32·3	62·8	4·9	2·72 ± 0·08	2·77	73·33 ± 1·46	76·26
13.	Covra (Tunis)	1938	41	—	24·4	75·6	—	2·76 ± 0·07		88·64 ± 1·13	
		1939	83	—	57·8	42·2	—	2·41 ± 0·05		77·36 ± 0·76	
		1947	48	—	33·3	66·7	—	2·67 ± 0·07	2·61	76·16 ± 0·97	80·72
14.	Dłużewski	1937	99	—	90·1	9·9	—	2·09 ± 0·03		68·74 ± 0·86	
		1938	106	10·4	55·6	34·0	—	2·24 ± 0·06		68·74 ± 0·92	
		1939	64	—	93·8	6·2	—	2·06 ± 0·03		70·03 ± 0·86	
		1947	68	—	67·7	32·3	—	2·34 ± 0·06	2·18	67·23 ± 1·18	68·68
15.	Marchijski	1938	111	—	67·5	32·5	—	2·32 ± 0·04		63·99 ± 0·81	
		1939	80	3·7	91·3	5·0	—	2·01 ± 0·03		78·64 ± 0·86	
		1947	49	2·0	79·6	18·4	—	2·16 ± 0·06	2·16	65·55 ± 0·97	69·46
16.	Première à barbes lisses	1938	106	2·8	50·0	47·2	—	2·44 ± 0·05		77·22 ± 0·86	
		1939	129	—	57·3	42·7	—	2·43 ± 0·04		70·45 ± 0·97	
		1947	72	—	62·5	37·5	—	2·38 ± 0·06	2·41	75·22 ± 1·35	74·29

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
17.	Abed July	1937	49	—	30·6	69·4	—	2·69 ± 0·07		83·64 ± 0·92	
		1938	15	—	—	100·0	—	3·00 ± 0·00		86·75 ± 1·62	
		1947	27	—	48·0	52·0	—	2·52 ± 0·09	2·74	74·00 ± 2·27	81·46
18.	Lapin II	1937	121	—	40·5	59·5	—	2·60 ± 0·04		69·48 ± 0·70	
		1938	30	—	33·3	66·7	—	2·67 ± 0·07		77·76 ± 1·67	
		1947	82	3·7	30·5	65·8	—	2·62 ± 0·06	2·63	73·82 ± 0·86	73·68
19.	Pertu	1938	14	—	—	100·0	—	3·00 ± 0·00		85·61 ± 2·07	
		1939	27	—	33·3	66·7	—	2·67 ± 0·09		72·99 ± 0·86	
		1947	52	—	13·6	86·5	—	2·87 ± 0·05	2·84	78·30 ± 0·91	78·96
20.	O. A. C. 21 — C. A. N. 1086	1937	176	—	44·9	55·1	—	2·55 ± 0·04		71·36 ± 0·81	
		1938	70	4·3	61·4	34·3	—	2·30 ± 0·04		65·17 ± 1·02	
		1939	108	—	53·7	46·3	—	2·46 ± 0·05		74·05 ± 0·97	
		1947	81	3·6	48·2	48·2	—	2·44 ± 0·03	2·44	75·53 ± 0·70	71·52
21.	Velvet C. A. N. 155	1938	110	—	31·8	68·2	—	2·68 ± 0·04		71·42 ± 0·92	
		1939	135	—	47·4	52·6	—	2·53 ± 0·04		71·59 ± 0·81	
		1947	56	—	23·2	76·8	—	2·77 ± 0·06	2·66	71·55 ± 0·99	71·52
22.	Heines vierzeilige	1937	107	2·8	78·5	18·7	—	2·16 ± 0·04		68·73 ± 0·75	
		1938	88	6·9	63·6	29·5	—	2·23 ± 0·06		69·03 ± 0·98	
		1939	125	3·2	79·2	17·6	—	2·14 ± 0·04		73·87 ± 0·86	
		1947	74	8·1	83·8	8·1	—	2·00 ± 0·07	2·13	59·32 ± 0·86	67·73

23.	Hohenfinover 4-zlg.	1937	143	0·7	83·2	16·1	—	2·15 ± 0·03		80·20 ± 0·81	
		1938	101	2·1	64·3	33·6	—	2·32 ± 0·05		78·16 ± 1·08	
		1939	107	12·2	75·6	12·2	—	2·00 ± 0·05		75·90 ± 0·92	
		1947	70	2·9	61·4	35·7	—	2·33 ± 0·06	2·20	69·19 ± 0·22	75·86
24.	Pallidum 10371 U. S. S. R.	1938	84	—	13·1	79·8	7·1	2·94 ± 0·05		90·82 ± 1·13	
		1947	81	—	30·8	69·2	—	2·69 ± 0·05	2·82	81·13 ± 1·35	85·97
25.	Pallidum 3732 U. S. S. R.	1937	143	—	33·5	66·5	—	2·66 ± 0·04		75·55 ± 0·59	
		1938	71	—	35·2	64·8	—	2·65 ± 0·06		77·80 ± 1·08	
		1947	66	—	10·7	89·3	—	2·89 ± 0·04	2·73	78·78 ± 0·81	77·37
26.	Pallidum 10342 U. S. S. R.	1937	61	4·9	83·6	11·5	—	2·07 ± 0·05		66·65 ± 0·92	
		1938	70	4·1	71·6	24·3	—	2·20 ± 0·06		65·49 ± 0·70	
		1939	108	8·4	78·7	12·9	—	2·05 ± 0·04		61·75 ± 0·86	
		1947	48	23·0	62·5	14·5	—	2·15 ± 0·09	2·11	63·22 ± 1·45	64·27
27.	Pallidum 10343 U. S. S. R.	1937	107	11·2	79·4	9·4	—	1·99 ± 0·04		64·49 ± 0·81	
		1938	36	8·3	86·1	5·6	—	1·97 ± 0·06		63·45 ± 1·13	
		1939	97	3·1	72·2	24·7	—	2·22 ± 0·05		62·96 ± 0·92	
		1947	85	9·5	67·0	23·5	—	2·14 ± 0·06	2·08	62·40 ± 0·92	63·32
28.	Pallidum from Urkujsk	1937	118	—	41·5	58·5	—	2·58 ± 0·05		76·92 ± 0·70	
		1938	40	—	7·5	85·0	7·5	3·00 ± 0·06		81·97 ± 1·18	
		1939	178	—	24·7	72·5	2·8	2·78 ± 0·04		73·95 ± 0·70	
		1947	89	—	19·1	80·9	—	2·81 ± 0·04	2·87	78·99 ± 0·59	77·71
29.	Nr. 14 — Tunis	1937	86	—	68·6	31·4	—	2·31 ± 0·05		79·48 ± 1·13	
		1938	147	2·8	72·7	24·5	—	2·22 ± 0·04		74·60 ± 1·24	
		1939	136	—	71·3	28·7	—	2·29 ± 0·04		84·77 ± 0·76	
		1947	79	—	31·8	68·2	—	2·68 ± 0·05	2·37	74·78 ± 1·08	78·20
30.	Nr. 4. A. Tunis	1937	108	—	25·9	74·1	—	2·74 ± 0·04		83·74 ± 0·59	
		1938	70	—	34·3	65·7	—	2·66 ± 0·06		80·46 ± 0·92	
		1939	95	—	28·5	71·5	—	2·72 ± 0·05		92·82 ± 0·92	
		1947	91	—	26·4	70·3	3·3	2·77 ± 0·05	2·72	81·41 ± 1·13	84·61

No	Variety	Year	Number of measurements	Number of cells in the aulerone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
31.	Erhardt Frederiksen	1938	63	—	14·3	85·7	—	2·86 ± 0·04		88·28 ± 1·24	
		1947	60	—	—	100·0	—	3·00 ± 0·00	2·93	70·60 ± 0·97	79·44
32.	Pusa — Type 21.	1938	62	1·7	87·0	11·3	—	2·09 ± 0·04		54·69 ± 0·65	
		1939	129	19·4	60·5	20·1	—	2·01 ± 0·06		61·95 ± 0·97	
		1947	125	7·2	82·4	10·4	—	2·00 ± 0·04	2·03	56·16 ± 0·55	57·60
33.	Pusa — Hybride I—92.	1938	52	7·7	65·4	26·9	—	2·19 ± 0·08		65·41 ± 1·51	
		1947	106	1·9	70·8	27·3	—	2·25—0·05	2·22	67·80 ± 0·76	66·60
34.	Pusa — R. 1.	1939	113	1·8	69·9	28·3	—	2·27 ± 0·05		69·54 ± 0·81	
		1947	106	—	47·1	52·9	—	2·53 ± 0·05	2·40	72·28 ± 0·70	70·91
35.	Abyssinian 5336	1938	43	11·6	88·4	—	—	1·88 ± 0·05		53·87 ± 1·13	
		1939	87	13·8	86·2	—	—	1·86 ± 0·04		58·27 ± 0·81	
		1947	63	6·4	80·9	12·7	—	2·06 ± 0·05	1·93	61·03 ± 0·81	57·72
36.	Big Wheat	1938	117	—	40·9	59·1	—	2·59 ± 0·03		96·79 ± 0·97	
		1939	128	—	50·0	50·0	—	2·50 ± 0·04		79·05 ± 0·92	
		1947	122	—	41·9	58·1	—	2·58 ± 0·04	2·56	86·66 ± 1·24	87·50
37.	Nigrum 10345	1937	122	—	46·7	52·4	0·9	2·54 ± 0·05		71·88 ± 0·76	
		1938	26	—	23·1	73·0	3·9	2·81 ± 0·09		78·30 ± 1·67	
		1939	81	—	49·4	50·6	—	2·51 ± 0·06		86·00 ± 1·37	
		1947	135	—	18·5	78·6	2·9	2·84 ± 0·04	2·67	91·59 ± 1·19	81·94

38.	Nudum Nr. 150 from Podhale	1937	75	—	1·4	98·6	—	2·99 ± 0·01		82·72 ± 0·58	
		1939	91	—	18·7	81·3	—	2·81 ± 0·04		70·55 ± 0·70	
		1947	68	—	8·8	91·2	—	2·91 ± 0·01	2·90	73·21 ± 0·81	75·49
39.	Nudum No. 151 from Podhale	1937	154	—	8·4	83·7	7·9	2·99 ± 0·03		98·12 ± 1·13	
		1938	88	—	12·5	87·5	—	2·86 ± 0·04		83·33 ± 1·13	
		1947	194	—	8·2	91·8	—	2·92 ± 0·02	2·93	104·10 ± 0·86	99·18
40.	Nudum No. 154 from Podhale	1938	26	—	—	100·00	—	3·00 ± 0·00		70·40 ± 0·70	
		1939	70	—	15·7	84·3	—	2·84 ± 0·04		77·06 ± 0·81	
		1947	83	—	—	100·00	—	3·00 ± 0·00	2·95	79·24 ± 0·65	75·57
	Subsp. *Hordeum intermedium* Körn.										
41.	Transiens Körn.	1938	141	—	80·8	19·2	—	2·19 ± 0·03		79·27 ± 0·70	
		1939	122	—	82·8	17·2	—	2·17 ± 0·03		79·76 ± 0·76	
		1947	140	6·5	57·1	36·4	—	2·30 ± 0·05	2·22	76·18 ± 1·03	78·40
42.	Anderson Intermediate	1938	33	—	27·3	72·7	—	2·73 ± 0·08		68·89 ± 1·35	
		1939	131	—	38·9	60·3	0·8	2·62 ± 0·04		67·93 ± 0·70	
		1947	75	—	4·7	64·0	31·3	3·33 ± 0·06	2·89	85·46 ± 1·03	74·09
	Subsp. *Hordeum distichum* var. *nutans* Schübl.										
43.	Borzymowicki	1937	95	12·6	77·8	9·6	—	1·97 ± 0·07		62·98 ± 0·70	
		1938	92	5·4	90·2	4·4	—	2·01 ± 0·04		62·52 ± 0·70	
		1947	108	—	89·8	10·2	—	2·10 ± 0·03	2·03	62·14 ± 0·59	62·54
44.	Elka	1937	94	—	5·3	92·5	2·2	2·97 ± 0·03		77·26 ± 0·59	
		1938	100	—	21·0	77·0	2·0	2·81 ± 6·05		72·84 ± 1·03	
		1947	122	—	21·3	77·0	1·7	2·80 ± 0·05	2·86	72·90 ± 0·70	74·33
45.	Hanna Gambrinus	1937	125	—	41·6	58·4	—	2·58 ± 0·04		73·74 ± 0·70	
		1938	106	—	37·7	62·3	—	2·62 ± 0·05		79·87 ± 0·81	
		1947	107	—	19·3	80·7	—	2·81 ± 0·04	2·67	75·08 ± 0·76	76·23

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
46.	Hanna z Goli	1937	138	—	54·3	45·7	—	2·46 ± 0·04		71·37 ± 0·81	
		1938	124	—	27·5	72·5	—	2·73 ± 0·04		73·15 ± 0·65	
		1939	187	—	60·9	39·1	—	2·39 ± 0·04		66·35 ± 0·59	
		1947	125	—	38·4	61·6	—	2·62 ± 0·04	2·55	72·18 ± 0·59	70·76
47.	Kazimierski	1937	140	—	38·6	61·4	—	2·61 ± 0·04		73·67 ± 0·70	
		1938	175	—	51·4	48·6	—	2·49 ± 0·04		70·87 ± 0·48	
		1947	135	—	31·8	64·4	3·8	2·72 ± 0·05	2·61	79·81 ± 0·54	74·78
48.	Kujawski	1937	159	—	30·2	67·2	2·6	2·72 ± 0·04		84·39 ± 0·86	
		1938	141	—	35·4	61·7	2·9	2·67 ± 0·04		83·25 ± 0·70	
		1947	126	—	31·8	68·2	—	2·68 ± 0·04	2·69	86·27 ± 1·03	84·63
49.	Kutnowski 08	1937	114	—	43·0	57·0	—	2·57 ± 0·05		85·07 ± 1·08	
		1938	115	—	35·7	64·3	—	2·64 ± 0·04		78·69 ± 0·76	
		1947	129	—	45·0	55·0	—	2·55 ± 0·04	2·58	77·56 ± 0·86	80·44
50.	Puławski	1937	145	1·5	42·0	55·1	1·4	2·57 ± 0·03		79·95 ± 0·76	
		1938	196	1·5	45·4	53·1	—	2·53 ± 0·04		79·61 ± 0·92	
		1939	81	—	37·1	62·9	—	2·63 ± 0·05		84·32 ± 1·24	
		1947	95	—	37·8	51·5	10·7	2·73 ± 0·04	2·61	92·80 ± 1·19	84·17
51.	Przeworski	1937	127	2·4	27·4	68·5	1·7	2·69 ± 0·05		82·00 ± 0·86	
		1938	128	—	34·4	56·6	—	2·66 ± 0·04		86·94 ± 0·86	
		1939	162	—	17·2	82·1	0·7	2·83 ± 0·03		88·06 ± 0·76	
		1947	129	—	23·3	76·7	—	2·77 ± 0·04	2·74	83·80 ± 0·59	85·20

52.	Hanna Kargyn	1937	117	—	42·7	57·3	—	2·57 ± 0·05		68·63 ± 0·70	
		1938	158	—	40·5	59·5	—	2·59 ± 0·04		72·38 ± 0·76	
		1939	101	—	29·8	70·2	—	2·70 ± 0·05		71·22 ± 0·81	
		1947	115	—	48·7	51·3	—	2·51 ± 0·05	2·59	66·91 ± 0·97	69·78
53.	Diosecky 496	1937	130	3·1	80·0	16·9	—	2·14 ± 0·04		67·83 ± 0·59	
		1938	153	1·4	64·0	34·6	—	2·33 ± 0·04		66·35 ± 0·59	
		1939	104	—	84·6	15·4	—	2·15 ± 0·04		62·72 ± 0·70	
		1947	135	0·9	72·5	26·6	—	2·26 ± 0·04	2·22	64·80 ± 0·70	65·42
54.	Hanna Proskovetza	1938	138	—	23·9	76·1	—	2·76 ± 0·04		73·40 ± 0·48	
		1947	119	—	38·7	61·3	—	2·61 ± 0·04	2·69	69·47 ± 0·59	71·43
55.	Kneifluv P. 13.	1937	121	—	27·3	72·7	—	2·73 ± 0·04		87·64 ± 0·97	
		1938	123	—	29·0	71·0	—	2·71 ± 0·04		83·21 ± 0·81	
		1947	120	—	7·5	92·5	—	2·93 ± 0·02	2·79	94·50 ± 0·76	88·45
56.	Nolcs Moravia	1937	98	—	17·3	80·6	2·1	2·85 ± 0·04		81·33 ± 0·85	
		1938	150	—	24·6	74·6	0·8	2·76 ± 0·04		84·45 ± 0·59	
		1947	97	—	31·9	63·9	4·2	2·70 ± 0·05	2·77	83·28 ± 0·81	83·02
57.	Perbete 4	1937	123	13·0	85·4	1·6	—	1·89 ± 0·03		58·60 ± 0·76	
		1938	136	8·8	86·8	4·4	—	1·96 ± 0·03		61·06 ± 0·76	
		1947	162	1·3	87·0	11·7	—	2·10 ± 0·03	1·98	66·16 ± 0·65	61·97
58.	Postoloprtsky	1937	179	—	59·7	40·3	—	2·40 ± 0·04		71·79 ± 0·59	
		1938	153	—	66·6	33·4	—	2·33 ± 0·04		71·85 ± 0·65	
		1939	106	—	56·6	43·4	—	2·43 ± 0·05		70·45 ± 0·92	
		1947	144	—	36·8	63·2	—	2·63 ± 0·04	2·45	74·99 ± 0·65	72·27
59.	Abed Binder	1937	167	4·3	71·8	23·9	—	2·20 ± 0·04		69·06 ± 0·76	
		1938	160	1·3	56·2	42·5	—	2·41 ± 0·04		72·42 ± 0·70	
		1947	110	—	48·1	51·9	—	2·52 ± 0·05	2·38	74·32 ± 0·76	71·70

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
60.	Abed Opal	1937	132	3·9	50·7	45·4	—	2·42 ± 0·05		74·20 ± 0·96	
		1938	125	4·8	55·2	40·0	—	2·35 ± 0·05		70·80 ± 0·92	
		1939	166	5·6	67·9	26·5	—	2·18 ± 0·04		72·60 ± 0·96	
		1947	134	0·5	77·1	22·4	—	2·22 ± 0·04	2·29	73·26 ± 0·96	72·71
61.	Halliko	1937	102	—	7·9	82·3	9·8	3·02 ± 0·04		88·99 ± 0·70	
		1938	127	—	20·9	74·2	4·9	2·84 ± 0·04		82·95 ± 0·70	
		1939	113	—	14·1	84·0	1·9	2·88 ± 0·03		88·93 ± 0·70	
		1947	78	—	24·4	75·6	—	2·76 ± 0·05	2·87	81·75 ± 0·86	85·65
62.	Louhi	1937	81	—	13·6	86·4	—	2·86 ± 0·04		89·79 ± 1·03	
		1938	118	—	5·1	92·3	2·6	2·97 ± 0·03		89·83 ± 0·76	
		1947	134	—	14·2	85·8	—	2·86—0·03	2·90	94·09 ± 0·86	91·24
63.	Hanna Skrzeszowicka	1937	144	—	34·0	66·0	—	2·66 ± 0·04		91·16 ± 0·81	
		1939	96	—	37·5	62·5	—	2·63 ± 0·05		74·02 ± 0·81	
		1947	75	—	14·7	85·3	—	2·85 ± 0·04	2·71	88·05 ± 0·76	84·41
64.	Chevalier race Francaise	1938	116	—	36·2	63·8	—	2·64 ± 0·04		76·48 ± 0·54	
		1939	99	—	45·4	54·6	—	2·55 ± 0·05		79·84 ± 0·70	
		1947	133	—	36·1	63·9	—	2·64 ± 0·04	2·61	78·07 ± 0·76	77·89
65.	De Moravie	1937	109	—	27·6	72·4	—	2·72 ± 0·04		78·12 ± 0·60	
		1938	72	—	34·7	65·3	—	2·65 ± 0·06		73·57 ± 0·76	
		1939	127	—	71·6	28·4	—	2·28 ± 0·04		74·15 ± 0·70	
		1947	66	—	78·7	21·3	—	2·21 ± 0·05	2·47	73·71 ± 0·92	74·88

66.	Ackermanns Bavaria	1937	121	—	52·0	48·0	—	2·48 ± 0·05		76·17 ± 0·59	
		1938	90	—	53·3	46·7	—	2·47 ± 0·05		71·03 ± 0·65	
		1939	137	1·4	59·9	38·7	—	2·37 ± 0·04		76·54 ± 0·70	
		1947	106	2·0	52·6	47·4	—	2·47 ± 0·05	2·45	81·76 ± 0·92	76·37
67.	Ackermanns Danubia	1937	135	2·3	69·6	28·1	—	2·26 ± 0·04		72·44 ± 0·81	
		1947	161	4·4	60·2	35·4	—	2·43 ± 0·04	2·35	73·68 ± 0·65	76·06
68.	Ackermanns Isaria	1938	104	—	75·0	25·0	—	2·25 ± 0·04		73·09 ± 0·70	
		1947	125	8·0	76·8	15·2	—	2·07 ± 0·04	2·16	68·60 ± 0·76	70·87
69.	Bethges u. Oelzes XIII	1937	123	2·5	75·6	21·9	—	2·20 ± 0·04	—	69·41 ± 0·65	
		1938	123	5·7	51·2	43·1	—	2·37 ± 0·05		70·07 ± 0·81	
		1947	137	3·6	67·9	28 5	—	2 25 ± 0 04	2·27	66 69 ± 0 70	68 72
70.	Dornburger Heils Franken	1937	136	11·1	73·5	15·4	—	2·04 ± 0·25		64·12 ± 0·70	
		1938	130	12·3	85·6	3·1	—	1·90 ± 0·03		65·92 ± 0·70	
		1947	112	3·6	87·5	8·9	—	2·05 ± 0·03	1·99	63·06 ± 0·70	64·37
71.	Hadostreng	1937	136	11·1	73·5	15·4	—	2·04 ± 0·03		73·49 ± 0·70	
		1938	130	12·3	84·6	3·1	—	1·90 ± 0·03		78·19 ± 0·54	
		1947	112	3·6	87·5	8·9	—	2·05 ± 0·04	2·00	80·14 ± 0·65	77·27
72.	Mahndorfer Victoria	1937	177	1·2	54·2	44·6	—	2·44 ± 0·04		78·80 ± 0·86	
		1938	147	—	40·8	59·2	—	2·59 ± 0·04		80·59 ± 0·65	
		1947	146	—	48·7	51·3	—	2·51 ± 0·04	2·51	82·99 ± 1·03	80·79
73.	Strengs Frankengerste	1937	180	—	66·6	33·4	—	2·33 ± 0·03		78·54 ± 0·76	
		1938	215	—	54·4	45·6	—	2·46 ± 0·03		75·72 ± 0·59	
		1947	215	4·6	67·5	27·9	—	2·23 ± 0·03	2·34	74·24 ± 0·93	76·17
74.	Svalöfs Guldgerste	1947	107	1·9	41·1	57·0	—	2·55 ± 0·05		84·13 ± 0·13	
		1938	136	1·5	50·0	48·5	—	2·47 ± 0·05		73·85 ± 1·10	
		1947	135	3·7	51·1	45·2	—	2·41 ± 0·05	2·48	81·67 ± 0·92	79·88

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
75.	Siegesgerste	1937	101	3·0	39·6	57·4	—	2·54 ± 0·06		72·50 ± 0·86	
		1938	111	—	39·6	60·4	—	2·60 ± 0·05		77·50 ± 0·82	
		1939	141	0·7	54·6	44·7	—	2·44 ± 0·04		73·19 ± 0·70	
		1947	123	—	50·4	49·6	—	2·50 ± 0·05	2·52	73·01 ± 0·86	74·05
76.	Russia I	1938	83	—	7·2	85·5	7·3	3·00 ± 0·04		81·39 ± 1·03	
		1947	107	—	1·0	99·0	—	2·99 ± 0·01	3·00	76·75 ± 0·48	79·07
77.	Beavens Archer	1938	147	—	17·7	82·3	—	2·82 ± 0·03		92·53 ± 0·70	
		1939	119	—	31·7	67·4	0·9	2·69 ± 0·04		84·95 ± 0·59	
		1947	122	—	26·3	73·7	—	2·74 ± 0·04	2·75	92·33 ± 0·92	89·94
78.	Chevalier II	1938	117	—	11·2	88·8	—	2·86 ± 0·03		93·32 ± 0·65	
		1939	104	—	29·8	68·8	1·4	2·71 ± 0·05		84·37 ± 0·70	
		1947	119	—	6·7	93·3	—	2·93 ± 0·02	2·83	86·67 ± 0·49	88·12
79.	Hallets Chevalier	1938	114	—	21·8	76·3	1·9	2·80 ± 0·04		88·44 ± 0·54	
		1939	83	—	14·4	84·3	1·3	2·87 ± 0·04		89·46 ± 0·86	
		1947	143	—	11·2	88·8	—	2·89 ± 0·03	2·85	91·08 ± 0·59	89·66
80.	Laschkego Tybetański	1938	113	—	1·9	95·5	2·6	3·01 ± 0·02		90·70 ± 0·86	
		1939	98	—	6·1	93·9	—	2·94 ± 0·02		92·57 ± 0·86	
		1947	132	—	9·1	90·9	—	2·91 ± 0·02	2·95	88·32 ± 0·86	90·53
	Subsp. *Hordeum distichum* var. *erectum* Schübl.										
81.	Album Invincible	1937	125	—	5·6	77·6	16·8	3·11 ± 0·02		110·16 ± 0·97	
		1938	156	—	7·7	90·4	1·9	2·94 ± 0·01		79·80 ± 0·81	
		1947	107	—	—	98·1	1·9	3·02 ± 0·01	3·02	98·99 ± 0·81	96·32

82.	Plumage Archer	1937	141	—	59·6	40·4	—	2·40 ± 0·04		78·31 ± 0·59	
		1938	185	—	34·1	65·9	—	2·65 ± 0·04		85·16 ± 0·43	
		1947	118	—	33·9	66·1	—	2·66 ± 0·04	2·57	84·84 ± 0·86	82·77
83.	Landsorte aus Tirol »Rotholz«	1937	150	—	69·3	30·7	—	2·31 ± 0·04		82·40 ± 0·76	
		1938	79	—	65·8	34·2	—	2·34 ± 0·05		69·58 ± 0·92	
		1939	133	8·3	45·1	46·6	—	2·38 ± 0·05		73·65 ± 0·86	
		1947	80	1·2	57·5	41·3	—	2·40 ± 0·06	2·38	72·96 ± 0·97	74·65
84.	Dubasquier	1937	189	0·5	40·2	59·3	—	2·59 ± 0·04		85·31 ± 0·70	
		1938	120	—	30·0	70·0	—	2·70 ± 0·04		79·29 ± 0·65	
		1947	84	2·4	21·4	76·2	—	2·74 ± 0·05	2·68	79·39 ± 0·76	81·33
85.	St. Croix	1938	97	—	56·6	43·4	—	2·43 ± 0·05		71·98 ± 0·92	
		1947	70	2·9	88·5	8·6	—	2·06 ± 0·04	2·24	67·65 ± 1·03	69·81
86.	v. Webskys Silesia	1937	165	—	21·2	78·8	—	2·79 ± 0·03		97·20 ± 0·75	
		1938	125	—	12·8	81·6	5·6	2·93 ± 0·04		100·26 ± 0·81	
		1947	165	—	4·3	79·4	16·3	3·12 ± 0·03	2·95	93·99 ± 0·92	97·15
87.	Gartons 1917	1938	120	—	33·3	66·7	—	2·67 ± 0·04		84·24 ± 0·70	
		1939	145	—	26·2	73·8	—	2·74 ± 0·04		77·61 ± 0·54	
		1947	54	—	31·5	68·5	—	2·69 ± 0·06	2·70	84·79 ± 0·86	82·21
88.	Arch.-Goldthorpe	1938	115	1·9	36·5	61·6	—	2·62 ± 0·05		81·44 ± 0·81	
		1947	108	1·9	42·6	55·5	—	2·54 ± 0·05	2·58	86·39 ± 0·97	83·93
89.	Golden Pheasant	1938	144	—	45·2	54·8	—	2·55 ± 0·04		81·86 ± 0·75	
		1939	136	3·0	36·0	61·0	—	2·58 ± 0·02		81·03 ± 1·19	
		1947	112	—	43·7	56·3	—	2·56 ± 0·05	2·56	85·72 ± 0·97	82·87
90.	Malster	1938	196	—	85·7	14·3	—	2·14 ± 0·03		75·57 ± 0·48	
		1939	58	1·8	81·0	17·2	—	2·16 ± 0·05		70·01 ± 0·97	
		1947	134	3·8	87·3	8·9	—	2·05 ± 0·03	2·12	62·94 ± 0·49	72·87

No	Variety	Year	Number of measurements	Number of cells in the aleurone layer						Thickness of the aleurone layer in μ	
				Percentage within the preparations examined				Annual means from different years	Mean for the variety	Annual means from different years	Mean for the variety
				1	2	3	4	$M \pm m$		$M \pm m$	
91.	Peacock	1938	143	0·8	71·3	27·9	—	2·27 ± 0·04		77·32 ± 0·79	
		1939	105	—	77·1	22·9	—	2·23 ± 0·04		76·88 ± 0·65	
		1947	110	2·8	90·9	6·3	—	2·04 ± 0·03	2·18	65·73 ± 0·86	73·31
92.	Svalöf Primus II	1938	149	—	53·7	46·3	—	2·46 ± 0·04		77·37 ± 0·81	
		1947	83	—	65·0	35·0	—	2·35 ± 0·05	2·41	77·61 ± 0·70	77·99
93.	Swonneck	1938	154	—	40·3	59·7	—	2·60 ± 0·04		88·63 ± 0·76	
		1939	114	—	20·1	77·2	2·7	2·82 ± 0·04		87·67 ± 0·76	
		1947	112	—	13·4	83·0	3·6	2·90 ± 0·04	2·77	84·89 ± 0·92	87·06
	Subsp. *Hordeum distichum* var. *zeocrithum* Körn.										
94.	H. zeocrithum album	1937	109	11·9	77·1	11·0	—	1·99 ± 0·03		65·35 ± 0·92	
		1938	17	—	100·0	—	—	2·00 ± 0·00		58·14 ± 0·32	
		1947	56	3·5	93·0	3·5	—	1·98 ± 0·03	1·99	63·35 ± 0·86	61·28
	Subsp. *Hordeum distichum* var. *inerme* Körn.										
95.	H. distichum inerme album	1938	33	—	3·0	93·0	3·0	3·00 ± 0·04		79·20 ± 1·67	
		1947	46	—	—	100·0	—	3·00 ± 0·00	3·00	80·41 ± 1·11	79·80

Subsp. *Hordeum decipiens* Steud.

96.	Deficiens A.	1939	117	24·7	75·3	—	—	1·75 ± 0·04		63·39 ± 1·09	
		1947	40	7·5	92·5	—	—	1·93 ± 0·04	1·84	67·23 ± 1·03	65·31
97.	Deficiens 11	1938	32	6·3	93·7	—	—	1·94 ± 0·04		65·48 ± 0·70	
		1939	30	6·6	93·4	—	—	1·93 ± 0·04		61·56 ± 0·97	
		1947	28	3·5	93·0	3·5	—	2·00 ± 0·04	1·96	66·73 ± 1·53	64·59
98.	Deficiens 16	1938	30	20·0	80·0	—	—	1·80 ± 0·07		62·10 ± 1·72	
		1947	58	—	100·0	—	—	2·00 ± 0·00	1·90	68·99 ± 0·86	65·54
99.	var. Steudelii	1937	100	—	76·0	14·0	—	1·94 ± 0·07		77·87 ± 0·80	
		1947	77	6·5	62·4	31·1	—	2·25 ± 0·06	2·09	70·76 ± 1·12	74·31
100.	var. macrolepis nigrum	1937	52	17·3	76·9	5·8	—	1·88 ± 0·06		54·70 ± 0·97	
		1939	106	17·0	79·2	3·8	—	1·87 ± 0·04		53·51 ± 0·65	
		1947	42	11·9	81·0	7·1	—	1·95 ± 0·07	1·90	58·64 ± 1·18	55·61
101.	var. abyssinicum Ser.	1939	116	3·4	82·7	13·9	—	2·10 ± 0·04		60·43 ± 0·70	
		1947	61	6·5	73·8	19·7	—	2·13 ± 0·06	2·12	58·97 ± 1·13	59·70
102.	var. gymnospermum Körn. (furc. nigronudum)	1938	58	—	13·8	86·2	—	2·86 ± 0·05		80·62 ± 1·29	
		1947	35	—	17·2	82·8	—	2·83 ± 0·06	2·85	84·08 ± 2·19	82·35
103.	var. copticum Vav.	1938	23	21·7	78·3	—	—	1·78 ± 0·09		49·54 ± 0·60	
		1947	92	35·8	64·2	—	—	1·64 ± 0·05	1·71	52·59 ± 0·05	51·06

ber in a single variety vary within the limits of the whole scale, i. e. from 1 to 4 (No.: 4, 50, 51).

The average number of cell rows in the aleurone layer of the different barley varieties is from 1·64 to 3·11.

The thickness of the aleurone layer in the varieties examined varies from 51 to 110 μ.

On the basis of Table I we can state further that there is a distinct differentiation between the varieties studied in regard to the thickness of the aleurone layer, as well as of the number of cell rows. Between the extreme groups of varieties there are, however, transitory groups, and consequently the material of all varieties, considered as a whole, presents a continual variability in respect of both characters.

The standard deviation indicates the degree of variability of the characters examined within one variety in one year. The average standard deviation calculated from the individual standard deviations for the varieties and crop years amounts for the number of cells to $\pm 0{\cdot}325$ of cell, and for the thickness of the aleurone layer to $\pm 9{\cdot}196\,\mu$.

The means from individual years for the same variety are not identical, and frequently greater or smaller differences in both characters occur between different years. These variations, with the exception of a few varietes, are not very pronounced. They generally do not exceed 1/3 of the cell and 15 μ of the thickness of the layer. On this account all varietal differences exceeding 1/3 of the cell and 15 μ can be considered with a high probability as significant. Exceptionally great variations of the mean values of the examined characters in different years have been ascertained in the number of cells in varieties Nos. 17, 28, 29 and 65; and in the thickness of the aleurone layer in varieties Nos. 31, 36, 37, 39, 42, 63 and 81. Presumably the material of these varieties was not uniform and in the course of different years several biotypes were taken for examination.

V. The variability of the anatomical features studied on the material of 40 varieties and the analysis of variance

The results presented in Table I show that between many varieties of barley are distinct quantitative differences in the anatomical structure of the aleurone layer, which considerably exceed

the differences due to annul variations. A more detailed analysis of variability of both the characters examined was carried out, by means of statistical methods, on a series of 40 varieties on the basis of data given in Tables II and III for 1937, 1938 and 1947. The analysis of variance and the calculation of the half-confidence interval were carried out after Fisher (1936) by a method similar to that of Student, in which the different crop years were treated as single repetitions (blocks).

In the following analysis of variance the different kinds of variability have been presented in %% of the total variability.

a) Analysis of variance in the number of cell rows:

Variability due to differences between the varieties ...	93·00%
Variability due to differences between the years	0·60,,
Variability due to error.....	6·40,,
Total variability	100·00%

As we see from the above analysis almost the whole variability is due to varietal differences and a minimal part, of about 0·60%, to the variability caused by differences between the years. The differences between the materials obtained from various years have obviously a causal character, and consequently the seasonal variability for the whole material is expressed by a very small figure, while there is a relatively greater variability due to error. The diagram on Fig. 1, in which the varieties have been arranged according to the increasing number of cells, illustrates the differences of the average number of cells between the varieties and the relatively small variations within the limits of a variety between different years. The varieties No. 36 and 39 exhibit considerable variations between the years.

b) Analysis of variance in the thickness of the aleurone layer:

Variability due to differences between the varieties ...	85·60%
Variability due to differences between the years	0,49,,
Variability due to error.....	13·91,,
Total variability	100·00%

As in the previous case, the variability due to difference between the varieties constitutes more than 85% of the total varias

TABLE II

Average number of cells in the aleurone layer in 40 commercial varieties for the years 1937, 1938, 1947, and the mean for 3 years

Nº	Variety	Average number of cells in the aleurone layer			Mean for 3 years
		1937	1938	1947	
1.	Perbete 4	1·89	1·95	2·10	1·98
2.	H. zeocrithum album	1·99	2·00	1·98	1·99
3.	Dornburger Heils Franken	2·04	1·90	2·05	1·99
4.	Borzymowicki	1·97	2·01	2·10	2·02
5.	Pallidum 10345	1·99	1·97	2·14	2·03
6.	Heines vierzeilige	2·16	2·22	2·00	2·12
7.	Pallidum 10342	2·06	2·20	2·14	2·13
8.	Dłużewski	2·09	2·23	2·34	2·22
9.	Diosecky 496	2·14	2·33	2·26	2·24
10.	Hadostreng	2·15	2·19	2·42	2·25
11.	Hohenfinover vierzeilige	2·15	2·31	2·32	2·26
12.	Bethges u. Oelzes XIII	2·20	2·37	2·24	2·27
13.	Abed Opal	2·41	2·35	2·21	2·32
14.	Strengs Frankengerste	2·33	2·45	2·23	2·34
15.	Landsorte aus Tirol »Rotholz«	2·30	2·34	2·40	2·34
16.	O. A. C. 21 — C. A. N. 1086	2·55	2·30	2·44	2·43
17.	Postoloprtsky 21	2·40	2·33	2·63	2·45
18.	Ackermanns Bavaria	2·47	2·46	2·47	2·47
19.	Siegesgerste	2·54	2·60	2·49	2·54
20.	Hanna Kargyn	2·57	2·59	2·51	2·56
21.	Kutnowski 08	2·57	2·64	2·55	2·58
22.	Hanna z Goli	2·45	2·72	2·61	2·59
23.	Kazimierski	2·61	2·48	2·71	2·60
24.	Lapin II	2·59	2·66	2·62	2·62
25.	Hanna Gambrinus	2·58	2·62	2·80	2·67
26.	Dubasquier	2·58	2·70	2·73	2·67
27.	Kujawski	2·72	2·67	2·68	2·69
28.	Przeworski	2·69	2·65	2·76	2·70
29.	Nr. 4 A. — Tunis	2·74	2·65	2·77	2·72
30.	Nigrum 10345	2·54	2·81	2·84	2·73
31.	Pallidum 3732	2·66	2·64	2·89	2·73
32.	Nolcs Moravia	2·84	2·76	2·70	2·76
33.	Kneifluv P. 13	2·72	2·71	2·92	2·78
34.	Elka	2·96	2·81	2·80	2·86
35.	Halliko	3·02	2·87	2·75	2·87
36.	Pallidum from Urkujsk	2·58	3·30	2·80	2·80
37.	Louhi	2·86	2·97	2·85	2·89
38.	Nudum Nr. 151 from Podhale	2·91	2·86	2·91	2·92
39.	v. Webskys Silesia	2·78	2·92	3·12	2·94
40.	Album Invincible	3·11	2·94	3·01	3·02

The standard error of the difference $\mu = \pm 0{\cdot}0794$ of the cell. The half-confidence interval calculated for the confidence coefficient $P = 0{\cdot}95$ amounts to: 0·159 of the cell.

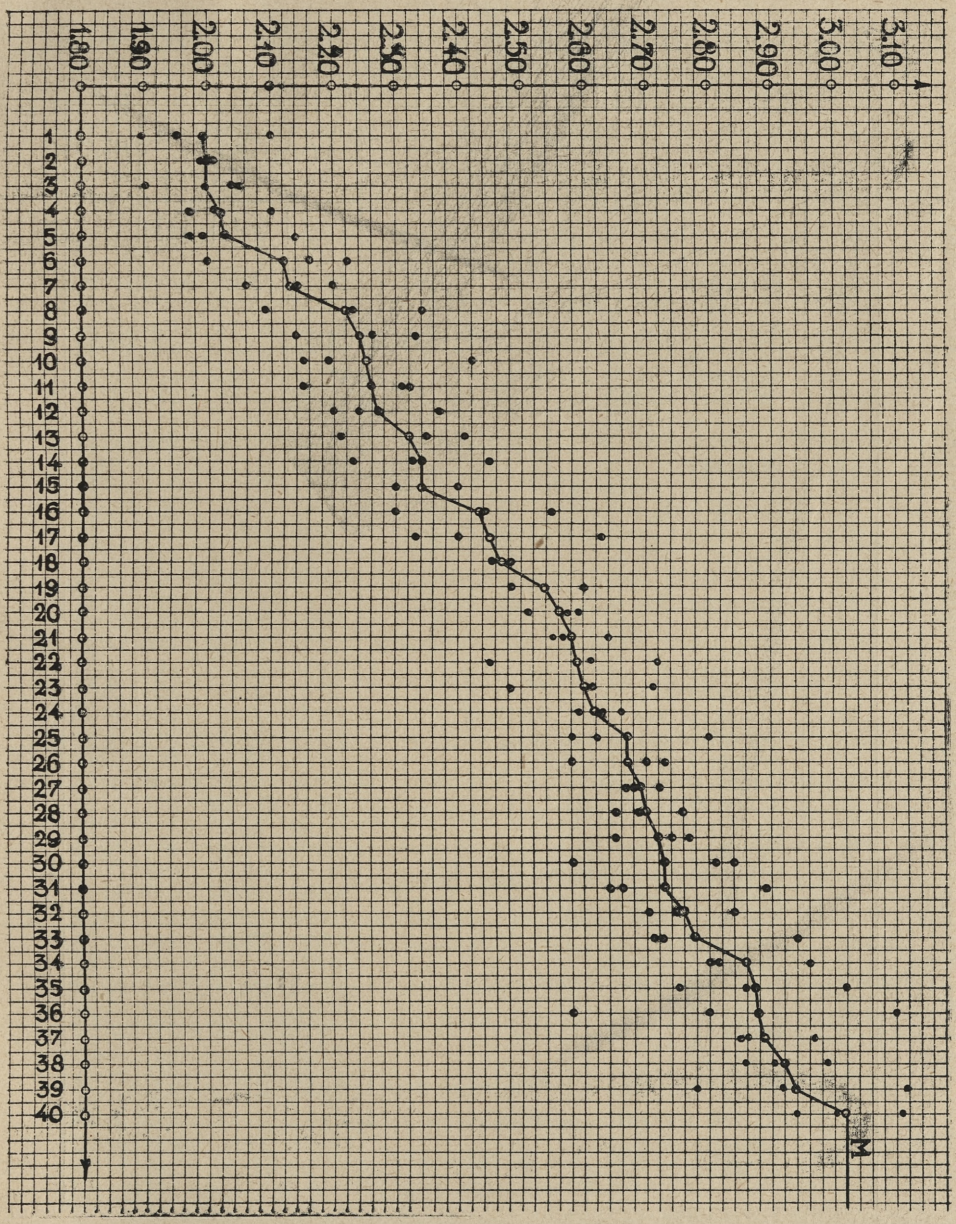

Fig. 1. Average number of cells in the aleurone layer in 40 varieties in the years 1937, 1938, 1947. Ordinates: the number of cells, abscissae: the No. of variety according to Table II.

TABLE III

Average thickness of the aleurone layer in 40 commercial varieties for the years 1937, 1938, 1947, and the mean for 3 years

N°	Variety	Average thickness of the aleurone layer in microns			Mean for 3 years
		1937	1938	1947	
1.	Perbete 4	58·60	61·06	66·16	61·94
2.	H. zeocrithum album	64·35	58·14	63·35	61·94
3.	Borzymowicki	62·98	62·52	62·14	62·54
4.	Pallidum 10343	64·49	63·45	62·40	63·44
5.	Dornburger Heils Franken	64·12	65·92	63·06	64·37
6.	Pallidum 10342	66·65	65·49	63·22	65·12
7.	Heines vierzeilige	68·73	69·03	59·32	65·69
8.	Diosecky 496	67·83	66·35	64·80	66·32
9.	Dłużewski	68·74	68·74	67·23	68·23
10.	Bethges u. Oelzes XIII	69·41	70·07	66·69	68·72
11.	Hanna Kargyn	68·63	72·38	66·91	69·30
12.	O. A. C. 21 — C. A. N. 1086	71·36	65·17	75·53	70·68
13.	Hanna z Goli	71·37	73·15	72·18	72·23
14.	Abed Opal	74·20	70·80	73·26	72·75
15.	Postoloprtsky	71·79	71·85	74·99	72 87
16.	Lapin II	69·48	77·76	73·82	73·68
17.	Elka	77·26	72·84	72·90	74·33
18.	Siegesgerste	72·50	77·50	73·01	74·34
19.	Kazimierski	73·67	70·87	79·81	74·88
20.	Landsorte aus Tirol „Rotholz“	82·40	69·58	72·96	74·98
21.	Hohenfinover vierzeilige	80·20	78·16	69·19	75·85
22.	Strengs Frankengerste	78·54	75·72	74·24	76·17
23.	Hanna Gambrinus	73·74	79·87	75·08	76·23
24.	Ackermanns Bavaria	76·17	71·03	81·76	76·32
25.	Hadostreng	73·49	78·19	80·14	77·27
26.	Pallidum 3732	75·55	77·80	78·78	77·37
27.	Pallidum from Urkujsk	76·92	81·97	78·99	77·71
28.	Kutnowski 08	85·07	78·69	77·56	80·44
29.	Nigrum 10345	71·88	78·30	91·59	80·59
30.	Dubasquier	85·31	79·29	79·39	81·33
31.	Nr. 4 A. — Tunis	83·74	80·46	81·41	81·87
32.	Nolcs Moravia	81·33	84·45	83·28	83·02
33.	Przeworski	82·00	86·94	83·80	84·24
34.	Halliko	88·99	82·95	81·75	84·56
35.	Kujawski	84·39	83·25	86·27	84·63
36.	Kneifluv P. 13	87·64	83·21	94·50	88·45
37.	Louhi	89·79	89·83	94·09	91·24
38.	Nudum Nr. 151 from Podhale	98·12	83·33	104·10	95·18
39.	Album Invincible	110·16	79·80	98·99	96·32
40.	v. Webskys Silesia	97·20	100·26	93·99	97·15

The standard error of the difference $\mu = \pm 3{\cdot}775$ micr. The half-confidence interval calculated for the confidence coefficient $P = 0{\cdot}95$ amounts to: 7·55 micr.

bility. In this analysis the part of variability due to error is in percentage more than twice as great as that of the number of cells. It is probably due to the fact that during the measurements made with the eyepiece micrometer, the thickness of the layer was always expressed in full segments of the micrometer scale, which after recounting in microns must have increased the measurement error. The diagram on Fig. 2 illustrates the differences in the thickness of the aleurone layer between the varieties, and small variations of individual varieties within the period of three years. Varieties Nos. 29, 38 and 39 may be regarded as exceptions in this respect.

For the same series of 40 varieties, the standard error of differences for both characters, as well as the half-confidence intervals, were then calculated by means of a simplified Wishart's method. (Barbacki 1935) — accepting after Fisher the value for $t = 2$ (strictly $t = 1{\cdot}95996$), $P = 0{\cdot}95$ and $n = 78$. On the basis of the latter values the differences between individual varieties and their significance have been calculated in respect of both characters. For the comparison of differences there were taken the general means, calculated for each variety from the annual means from three years.

The standard error of the difference for the average number of cell rows is $\mu = 0{\cdot}0794$ of the cell, and hence the half-confidence interval is $2\mu = 0{\cdot}159$ of the cell. On the basis of the half-confidence interval calculated in this way, a difference in the number of cells between the compared varieties smaller than 0·159 has been considered as not significant. For the total number of 780 possible combinations in 212 cases the difference between the compared varieties appeared to be insignificant, and in the remaining 568 cases, we have to deal with significant differences between the varieties.

The standard error of the difference for the average thicknesses of the layer amounts to $\mu = \pm 3{\cdot}775\,\mu$ and the half-confidence interval to $2\mu = 7{\cdot}55\,\mu$. In comparing the thickness of the aleurone layer between the varieties it was ascertained that for the total number of 780 possible combinations, significant differences appeared in 452 cases, whereas in 328 cases they were insignificant. It has been demonstrated that the number of cells is a character of a better taxonomic value than the thickness of the aleurone layer.

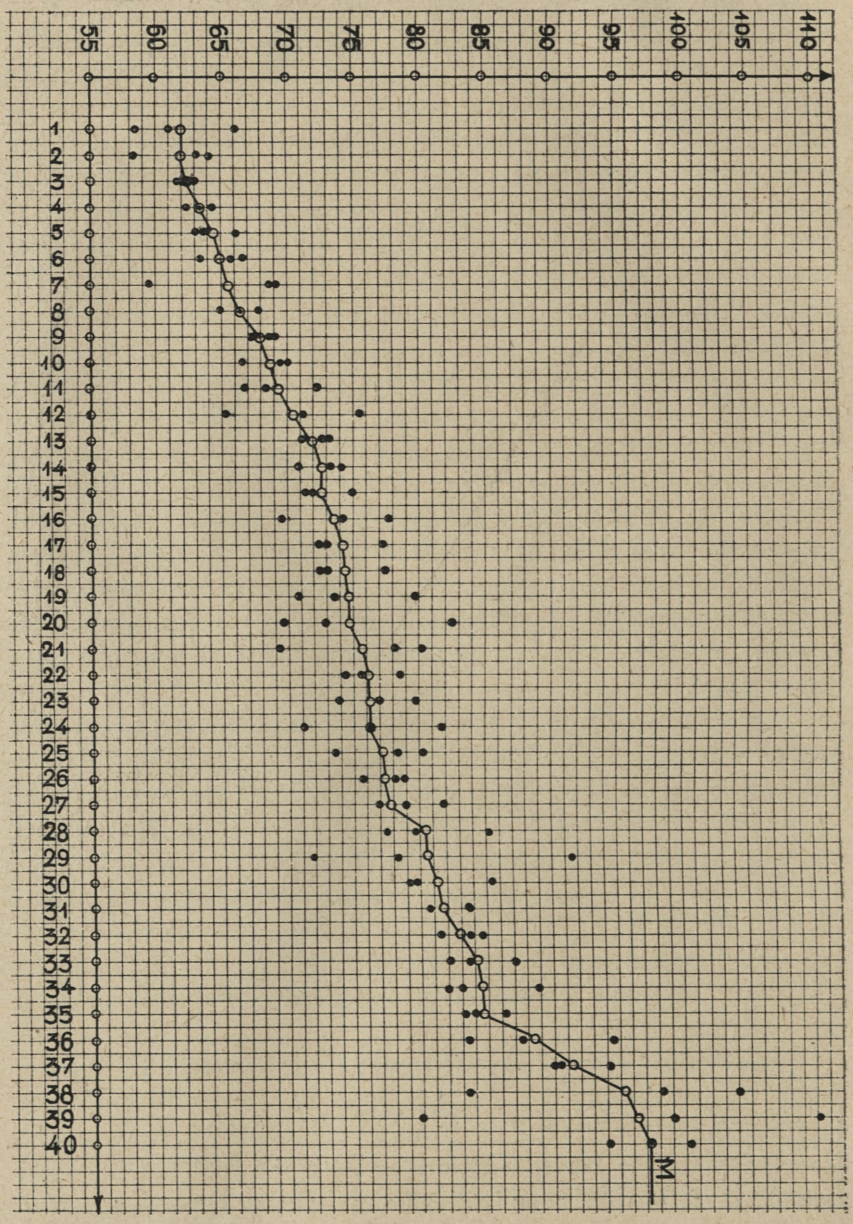

Fig. 2. Average thickness of the aleurone layer in 40 varieties in the years 1937, 1938, 1947. Ordinates: the thickness of the aleurone layer, abscissae: the No. of variety according to Table III.

The above-calculated half-confidence intervals may be considered as a criterion of the significance of differences between the varieties when the mean values obtained from the material of three years are compared. In practice, however, is it not always possible to have data from a period of three years; most often we have to compare the varieties on the basis of the data obtained from one year. In such case we must adopt another criterion calculated on the basis of the standard error of a single observation ($\pm\sigma$). In our example the standard error of a single observation calculated for 40 varieties from the analysis of variability amounts to:

1. for the number of cells $\sigma = \pm 0{\cdot}0097$ of the cell, hence the standard error of the difference between two varieties: $\mu = 0{\cdot}137$.
2. for the thickness of the layer $\sigma = \pm 4{\cdot}623\,\mu$, hence the standard error of the difference between two varieties is $\mu = 6{\cdot}53$.

In comparing the annual data we shall consider as significant such differences between the varieties as are greater than the double value ($t = 2$) of the half-confidence interval, i. e.:

1. for the number of cells — 0·274 or roundly 0·3 of cell,
2. for the thickness of the layer — 13·06 or roundly 13 microns.

The simple observation of the differences between the means from different years for 103 examined varieties has brought us to similar conclusions.

VI. A comparison of materials grown in different climatic conditions

As it was supposed that the frequently observed differences of results for the same variety occurring between individual years may be due to climatic factors, it was decided to find out whether stronger climatic differences might cause greater variations in the value of the characters studied. For this purpose a part of the material obtained from Cambridge in 1946 has been used. After examining the anatomy of the aleurone layer in 28 varieties of the spring barley harvested in Cambridge in 1944, the rest of the grain was grown in 1948 on the experimental ground in Mydlniki and after the harvest the grain obtained was examined in a similar manner. The results are shown in Table IV and V and in the diagrams, Figs. 3 and 4. It can be seen that the differences in the number of cells and in the thickness of the aleurone layer between the

grain harvested in Cambridge and its reproduction from Mydlniki are not at all greater than similar variations appearing in the same varieties grown in different years in Poland. In comparing the sum of differences between Cambridge and Mydlniki in 21 varieties with the sum of maximal differences which appeared in the same varieties cultivated in Poland in 1937, 1938 and 1947, we can observe that the latter is even greater:

Sum of differences	Number of cells	Thickness of the aleurone layer in microns
Between Cambridge and Mydlniki	2·20	88·52
Maxim. differences between the material grown in Poland in 1937, 1938 and 1947	3·55	141·28

VII. The varietal differences and their inheritance in pedigree lines

As it had not been established whether the examined varietal material was uniform, there were separated in 1947 pedigree lines from 15 varieties on which again the varietal differences and the stability of inheritance of both characters were examined. The material of measurements obtained from the 3 harvest years is presented in Table VI.

The analysis of variance of the average number of cell rows and the thickness of the aleurone layer was made, and the values thus obtained were compared with the corresponding values calculated for the previously described series of 40 varieties.

a) Analysis of variance of the number of cell rows:

Variability of the cell number	Varieties	Pedigree lines
Varietal	93·00%	95·24%
Due to differences between the years	0·60%	1·29%
Due to error	6·40%	3·47%

b) Analysis of variance for the thickness of the layer:

Variability of the thickness of the layer	Varieties	Pedigree lines
Varietal	85·60%	89·97%
Due to differences between the years	0·49%	0·84%
Due to error	13·91%	9·19%

TABLE IV

Average number of cells in the aleurone layer in the same material of varieties grown in Cambridge and Mydlniki

N⁰	Variety	Average number of cells in the aleurone layer		Difference between Cambridge and Mydlniki	Maximal differences between the harvest years 1937, 1938, 1947 in Poland
		1944 Cambridge	1948 Mydlniki		
1.	Perbete 4	1·85	1·90	0·05	0·22
2.	Ackermanns Isaria	2·12	1·94	0·18	—
3.	Nr. 14 — Tunis	2·16	2·05	0·11	—
4.	Pallidum 10343	2·07	2·20	0·13	0·15
5.	Mahndorfer Victoria	2·14	2·23	0·09	—
6.	Dornburger Heils Franken	2·12	2·34	0·22	0·15
7.	Heines vierzeilige	2·24	2·22	0·02	0·22
8.	Strengs Frankengerste	2·23	2·34	0·11	0·22
9.	Landsorte aus Tirol »Rotholz«	2·28	2·31	0·03	0·10
10.	Dłużewski	2·22	2·38	0·16	0·24
11.	Bethges u. Oelzes XIII	2·21	2·43	0·22	0·17
12.	Ackermanns Danubia	2·29	2·37	0·08	—
13.	Ackermanns Bavaria	2·47	2·28	0·19	0·01
14.	Marchijski	2·47	2·33	0·14	—
15.	Hanna Kargyn	2·50	2·47	0·03	0·08
16.	Premiere a barbes lisses	2·51	2·49	0·02	—
17.	Hanna z Goli	2·48	2·54	0·06	0·27
18.	Postoloprtsky	2·52	2·61	0·09	0·30
19.	O. A. C. 21 — C. A. N. 1086	2·48	2·66	0·18	0·25
20.	Hanna Gambrinus	2·57	2·61	0·04	0·22
21.	Kujawski	2·72	2·72	0·00	0·05
22.	Hanna Skrzeszowicka	2·69	2·76	0·07	—
23.	Kutnowski 08	2·75	2·79	0·04	0·09
24.	Louhi	2·88	2·85	0·03	0·12
25.	Dubasquier	2·95	2·79	0·16	0·15
26.	v. Webskys Silesia	2·98	2·92	0·06	0·20
27.	Nudum Nr. 151 from Podhale	2·81	3·11	0·30	0·13
28.	Kneifluv P. 13.	3·04	2·95	0·09	0·21

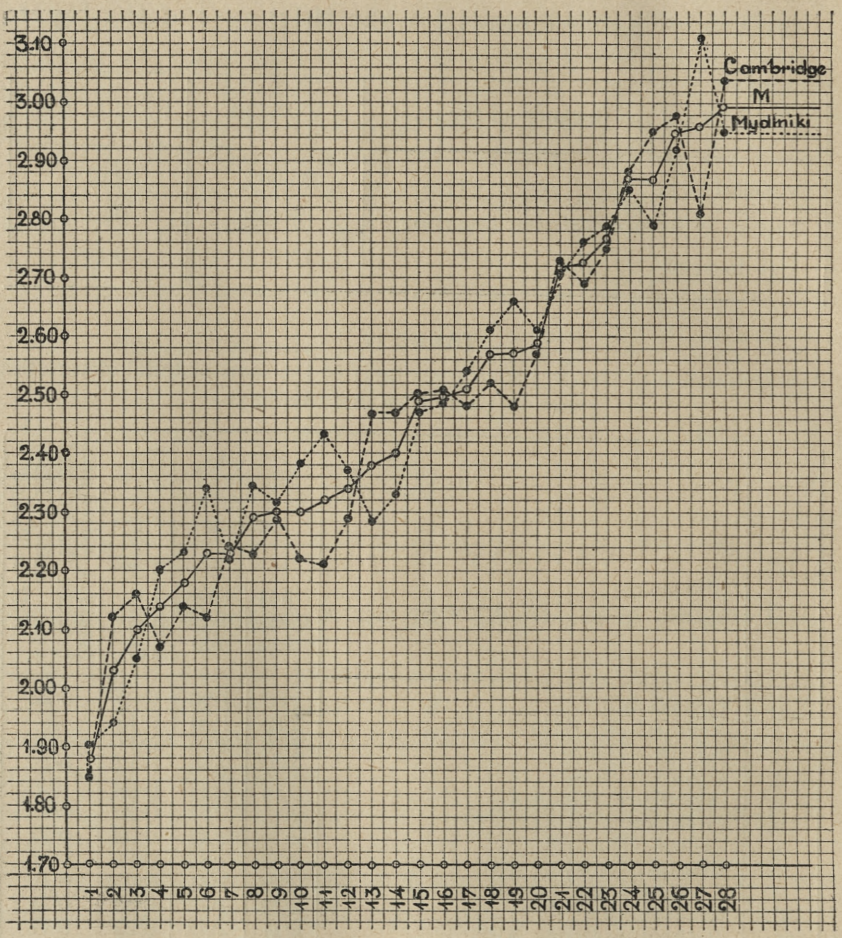

Fig. 3. Average number of cells in the aleurone layer in the same material of 28 varieties grown in Cambridge and Mydlniki. Ordinates: the number of cells, abscissae: the No. of variety according to Table IV.

The comparison of the analysis of variance of the varieties and pedigree lines permits the statement that between both materials there were no significant differences. Therefore the varieties were rather uniform and the results obtained on the basis of examination of the varietal material should be regarded as generally correct.

TABLE V

Average thickness of the aleurone layer in the same material of varieties grown in Cambridge and Mydlniki

N°	Variety	Average thickness of the aleurone layer in microns		Difference between Cambridge and Mydlniki	Maximal differences between the harvest years 1937, 1938, 1947 in Poland
		1944 Cambridge	1948 Mydlniki		
1.	Hanna Kargyn	60·07	66·52	6·45	5·47
2.	Perbete 4	60·23	67·21	6·98	7·56
3.	Pallidum 10343	64·59	65·35	0·76	2·09
4.	Ackermanns Isaria	65·45	64·49	0·96	—
5.	Bethges u. Oelzes XIII	70·20	69·51	0·69	3·38
6.	Ackermanns Danubia	69·15	71·31	2·16	—
7.	Hanna Gambrinus	71·62	71·31	0·31	6·13
8.	Dornburger Heils Franken	67·41	75·91	8·50	2·86
9.	Landsorte aus Tirol »Rotholz«	77·32	67·75	9·57	12·82
10.	Nr. 14 — Tunis	72·84	72·58	0·26	—
11.	Heines vierzeilige	72·25	74·14	1·89	9 71
12.	Dłużewski	71·82	74·92	3·10	1·51
13.	Mahndorfer Victoria	70·72	77·38	6·66	—
14.	Marchijski	76·62	71·71	4·91	—
15.	Strengs Frankengerste	74·24	75·32	1·08	4·30
16.	Postoloprtsky	75·65	74·96	0·69	3·20
17.	O. A. C. 21 — C. A. N. 1086	75·79	77·19	2·40	10·36
18.	Ackermanns Bavaria	81·76	75·57	6·19	10·78
19.	Premiere a barbes lisses	74·77	83·70	8·93	—
20.	Kujawski	84·39	85·31	0·92	3·02
21.	Hanna Skrzeszowicka	85·10	86·94	1·84	—
22.	Hanna z Goli	84·81	88·24	3·43	1·98
23.	Dubasquier	83·33	89·94	6·61	6·02
24.	Louhi	86·03	90·06	4·03	4·30
25.	Kutnowski 08	88·53	89·15	0·62	7·51
26.	Kneifluv P. 13	91·25	100·50	9·25	11·29
27.	v. Webskys Silesia	102·41	90·59	11·82	6·27
28.	Nudum Nr. 151 from Podhale	97·29	100·52	3·23	20·77

VIII. An attempt to group barley varieties according to the anatomical structure of the aleurone layer and practical conclusions

During the whole course of the present study both the characters, i. e. the number of cells of the aleurone layer and the thickness of the latter, have always been jointly treated. It is interesting to compare their variability and to study their inter-relation.

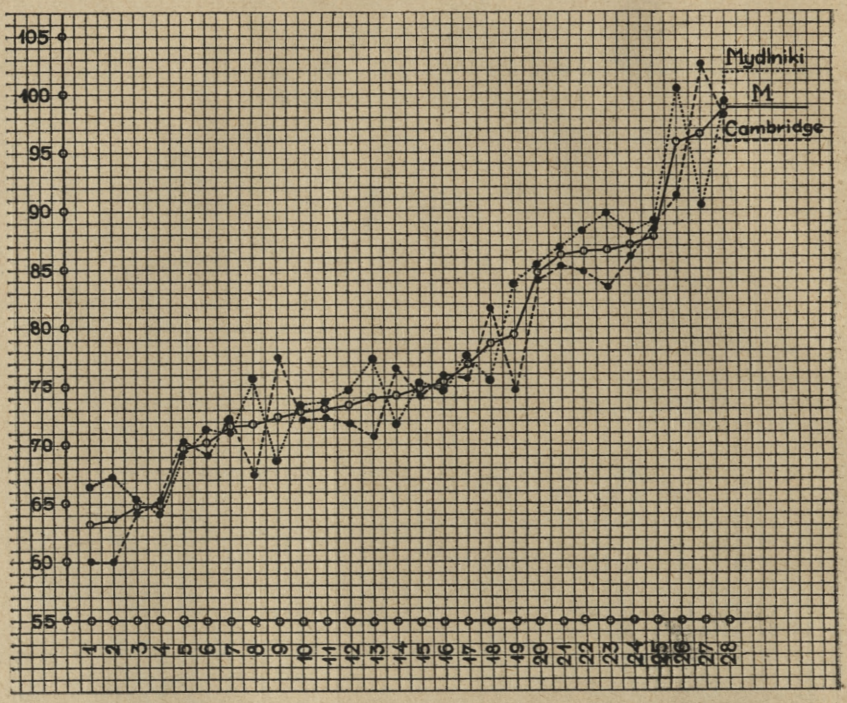

Fig. 4. Average thickness of the aleurone layer in the same material of 28 varieties grown in Cambridge and Mydlniki. Ordinates: the thickness of the aleurone layer in μ, abscissae: the No. of variety according to Table V.

The average coefficient of variability, calculated on the material of 103 varieties, amounts for a variety in one year:

for the number of cells to ..... 17·2
for the thickness of the layer to ... 11·2

As we see the variability of the cell number within one year is greater than the variability of the thickness of the layer.

Similarly — though within markedly narrower limits — the seasonal variability for both characters varies. The coefficient of seasonal variability calculated on the material of 40 varieties amounts:

for the number of cells to ..... 7·44
for the thickness of the layer to ... 7·17

TABLE VI

Showing the average number of cells in the aleurone layer in 15 pedigree lines for the years 1947, 1948, 1949. The average thickness of the aleurone layer in microns in the same material is given below

N⁰ of var.	Variety	Average number of cells in the aleurone layer			Mean for 3 years
		1947	1948	1949	
1.	a six rang de Chine	2·82	2·81	2·91	2·84
2.	Pyramidatum Körn.	2·80	2·77	2·96	2·84
3.	Emilio Mariani	2·56	2·73	2·94	2·75
4.	No. 14 — Tunis	2·63	2·57	2·57	2·59
5.	Big Wheat	2·59	2·60	2·86	2·68
6.	Pusa Type 21	2·06	2·16	2·25	2·16
7.	Nigrum 10345 from Manchuria	2·28	2·39	2·53	2·40
8.	Nudum No. 151 from Podhale	2·92	3·11	2·94	2·99
9.	Perbete 4	1·85	1·90	2·06	1·94
10.	v. Webskys Silesia	2·98	2·92	2·82	2·91
11.	Hord. zeocrithum album	1·99	2·07	2·16	2·07
12.	Hord. deficiens erect. nigr.	1·79	1·74	1·92	1·81
13.	Hord. macrolepis album	2·03	2·16	2·05	2·08
14.	Deficiens 11	1·89	2·03	1·80	1·90
15.	Selec. from Dificiens 16[1]	2·30	2·25	2·35	2·30

N⁰ of var.	Variety	Average thickness of the aleurone layer in microns			Mean for 3 years
		1947	1948	1949	
1.	a six rang de Chine	84·34	98·34	85·76	89·48
2.	Pyramidatum Körn.	87·00	90·39	86·91	88·10
3.	Emilio Mariani	87·16	93·88	77·66	86·23
4.	No. 14 — Tunis	80·04	83·43	94·90	86·12
5.	Big Wheat	96·79	84·80	97·56	93·05
6.	Pusa Type 21	62·97	68·02	69·09	66·96
7.	Nigrum 10345 from Manchuria	81·00	77·81	88·53	82·45
8.	Nudum No. 151 from Podhale	104·10	100·52	99·14	101·25
9.	Perbete 4	60·23	67·21	69·76	65·75
10.	v. Webskys Silesia	102·41	90·59	105·73	99·58
11.	Hord. zeocrithum album	64·35	67·32	68·99	66·89
12.	Hord. deficiens erect. nigr.	60·27	58·56	62·27	60·36
13.	Hord. macrolepis album	60·93	62·48	65·49	62·96
14.	Deficiens 11	66·22	74·34	64·43	68·33
15.	Selec. from Deficiens 16[1]	70·04	69·23	77·81	72·37

[1] The variety No. 15 has been selected from the variety Deficiens 16 (*H. decipiens* Steud.), but in regard to the morphology of the ear it actually belongs to var. *nutans*. Presumably the variety Deficiens 16 was not a pe digree line.

A superficial observation shows that the thickness of the aleurone layer depends upon the number of cell rows, and that consequently there is a positive correlation between both characters. Table VII shows that this correlation is rather strong.

The correlation coefficient calculated for 103 barley varieties after the Bravais formula (Johannsen 1913).

$$r = +0{\cdot}776 \pm 0{\cdot}039$$

Very interesting for the whole problem is the distribution of the varieties in the correlation table. We see here namely that in field «*A*» (number of cells: 1·7—2·4, thickness of the layer: 50—74 μ) the botanical varieties, which according to Vavilov (1926) are endemic in the Abyssinian geographical centre, are concentrated. We find here var. *africanum* Vav. *(deficiens* A*)*, var. *copticum* Vav. *(deficiens erectum nigrum)*, var. *abyssinicum* Ser. *(deficiens macrolepis album)*, var. *macrolepis* A. Br. *(deficiens macrolepis nigrum)* and var. *Steudelii* Körn. *(deficiens nutans nigrum)* in the neighbouring zero class. From the other botanical varieties we find here var. *pyramidatum (Pyramidatum W. C.)*, which differs, however, markedly in number of cells and in thickness of the layer from the variety *pyramidatum* Körn. (No. 3), and var. *zeocrithum*. Besides the types, mentioned, in field «*A*» there are several cultivated varieties belonging to *H. vulgare*, *H. distichum nutans* and *H. distichum erectum*.

In the opposite field «*C*» together with the neighbouring middle class of the thickness of the layer (number of cell rows: 2·5—3·1, thickness of the layer: 74—98 μ), there are collected 6-rowed varieties endemic in the Asiatic centre of origin i. e. short-awned (No. 1 and 2), long-awned (Non. 3), awnless (No. 95), the varieties of 2-rowed and 4-rowed naked barleys (No.: 38, 39, 40, 80) and the hooded varieties of *H. furcatum* type as for instance var. *gymnospermum* Körn. *(furcatum deficiens nigronudum)* and var. *trifurcatum* Körn. In field «*C*» we also find several European cultivated varieties from the group *H. vulgare*, *H. distichum nutans* and *H. distichum erectum*.

On figures 5 and 6 are presented microscopic drawings of cross-sections through the outer layers of the grain, made by means of Abbé's apparatus. Fig. 5 represents the two varieties which are typical of the Abyssinian geographical centre (I), with the aleurone

TABLE VII

Correlation between the average number of cells and the average thickness of the aleurone layer in 103 varieties of spring barley

Number of cells in the aleurone layer		Thickness of the aleurone layer in microns																								Frequency
		50—52	52—54	54—56	56—58	58—60	60—62	62—64	64—66	66—68	68—70	70—72	72—74	74—76	76—78	78—80	80—82	82—84	84—86	86—88	88—90	90—92	92—94	94—96	96—98	
		—12	—11	—10	—9	—8	—7	—6	—5	—4	—3	—2	—1	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11	
1·7—1·8	—7	103 *d*																								1
1·8—1·9	—6								96 *d*																	1
1·9—2·0	—5			100 *d*	35 *v*		57 *n* 94 *z*		70 *n* 97 *d* 98 *d*						71 *n*											8
2·0—2·1	—4				32 *v*	A		27 *v* 43 *n*						99 *d*					B							4
2·1—2·2	—3					101 *d*			26 *v*	22 *v*	14 *v* 15 *v*	68 *n*	90 *e* 91 *e*	23 *v*												9
2·2—2·3	—2						4 *hp*		53 *n*	33 *v*	69 *n* 85 *e*		60 *n*			41 *i*										7
2·3—2·4	—1											34 *v* 59 *n*	67 *n*	83 *e*	73 *n*	29 *v*										6
2·4—2·5	0											20 *v*	58 *n*	16 *v* 65 *n*	64 *n* 92 *e*	74 *n*										7
2·5—2·6	+1										10 *v* 52 *n*	46 *n*		75 *n*			49 *n* 72 *n*	82 *e* 88 *e* 89 *e*		36 *v*						10
2·6—2·7	+2					D						21 *v* 54 *n*	18 *v*	47 *n*	45 *n* 64 *n*	9 *v*	13 *v* 84 *e*	37 *v* 87 *e*	48 *n* 50 *n*	C						13
2·7—2·8	+3														12 *v* 25 *v*		17 *v*	56 *n*	30 *v* 51 *n* 63 *n*	1 *h* 93 *e*	77 *n* 55 *n*						11
2·8—2·9	+4												5 *vf*	61 *f*, 42 *i* 7 *vf* 11 *v* 14 *n*	28 *v*	8 *v* 19 *v*			102 *df* 24 *v* 61 *n*	2 *h* 3 *hp*	78 *n*	62 *n* 79 *n*					17
2·9—3·0	+5													38 *vn* 40 *vn*		31 *v* 76 *n*						80 *n*		39 *vn*	86 *e*	7
3·0—3·1	+6															95 *in*									81 *e*	2
Frequency		1	0	1	2	1	3	2	6	2	6	7	7	14	9	9	5	6	8	5	3	3	0	1	2	103

Numbers given in the correlation table correspond to N⁰ of varieties according to Table I; marks close by = subspecies or botanical variety.

h = *H. hexastichum*, *hp* = *H. hexastichum* v. *pyramidatum*, *v* = *H. vulgare*, *vf* = *H. vulgare furcatum*, *vn* = *H. vulgare nudum*, *i* = *H. intermedium*, *n* = *H. nutans*, *nn* = *H. nutans nudum*, *e* = *H. erectum*, *z* = *H. zeocrithum*, *in* = *H. inerme*, *d* = *H. decipiens*.

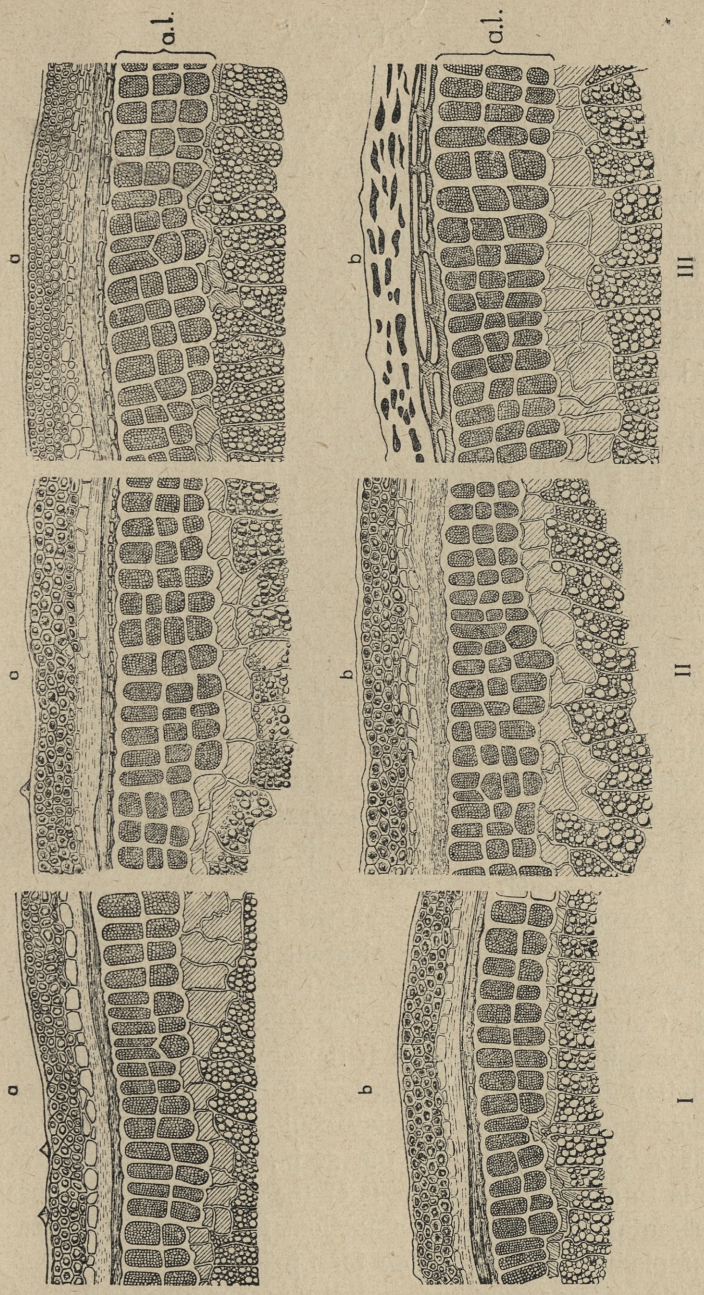

Fig. 5. Median transerve sections through grain from dorsal side in varieties of:
I. Abyssinian origin: a) *H. decipiens* Steud. — Deficiens A. b) *H. decipiens* Steud. — var. *copticum* Vav.
II. Asiatic origin: a) *H. hexastichum* — a six rang de Chine, b) *H. vulgare furcatum* — American 6-row. No. 175.
III. Asiatic origin: a) var. *pyramidatum* Körn. b) *H. valgare nudum*.
(a. l. = aleurone layer).

layer composed of 2 cell rows as well as four varieties of Asiatic origin (II, III), with a thick aleurone layer composed of three cell rows. The ears of the same varieties are presented in Fig. 7 (I, II, III).

Figure 6 presents differences in the anatomical structure of the aleurone layer between pairs of morphologically similar commercial varieties. It shows two varieties Elka and Borzymowicki belonging to var. *nutans* (I); two varieties Album Invincible and Peacock belonging to var. *erectum* (II), and finally two different varieties, Pallidum 10371 from Manchuria and Dłużewski belonging to *H. vulgare* (III). In all these three cases as shown in Fig. 8 (I, II, III) the ears of each pair of the demonstrated varieties are very similar in the morphological respect, but the different structure of the aleurone layer allows a distinction to be made quite certainly.

On the anatomical cross-sections of the grain are also seen the previously mentioned differences between the varieties in respect of the development of the layer of cells filled with reserve albumen. On the drawings they have been marked by cross-hatching.

The distribution of different commercial varieties belonging to the same subspecies and botanical varieties, between the two opposite fields of the correlation table, which include the varieties endemic in two different centres of origin of barley, permits on one hand to make a more accurate distinction between these varieties, and on the other hand it suggests the explanation of their primary origin.

The anatomical structure of the barley grain and especially of the strongly differentiated aleurone layer is probably correlated with other characters, which are important from the economic point of view (Orlov 1929 — data concerning barleys originating from Abyssinia and Erytrea). If it were possible to establish such correlation in the cultivated barleys, valuable indications for cross-breeding and selection of barley could be obtained by means of microscopic examination of the grain. The plant breeder would be able to obtain, on the bassis of the anatomical examination of the parental forms, certain indications for a rational choice of the parent plants for crossing and for further selection.

Taking into account the general means calculated for both characters studied for a period of several years as well as the distribution of varieties in the correlation table, the author divides the varieties examined into 3 groups. This division is, of course,

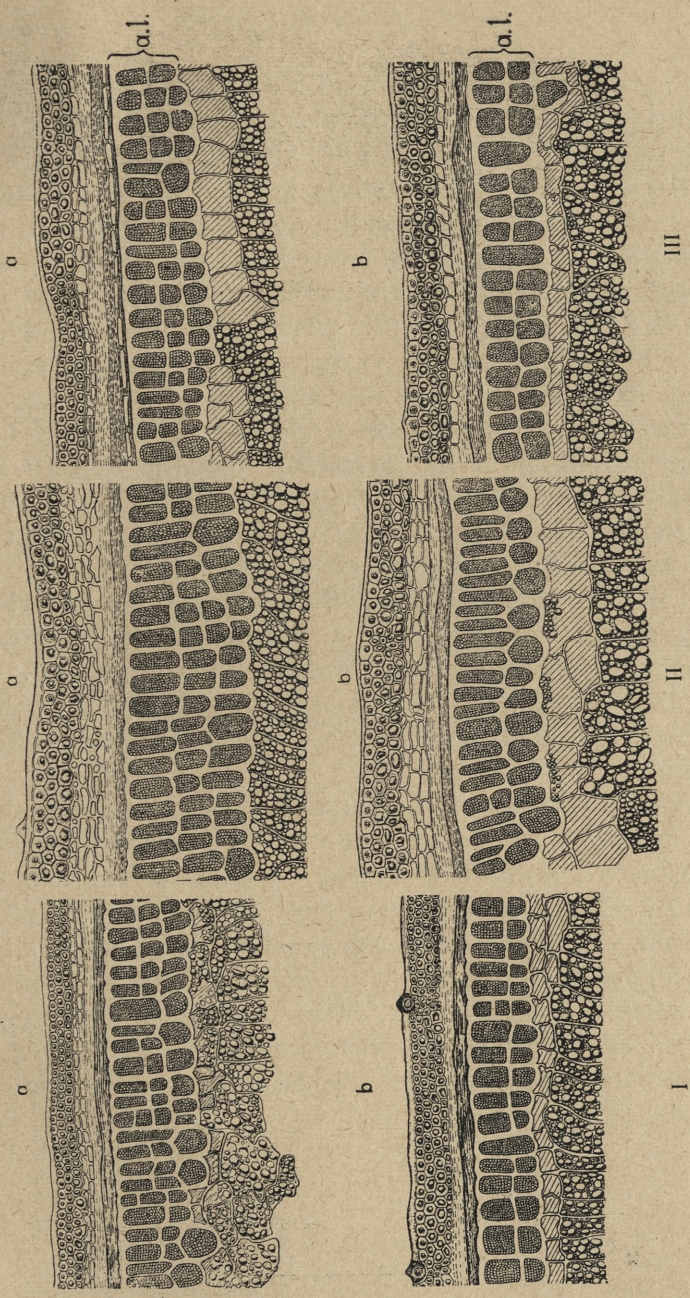

Fig. 6. Differences in the anatomical structure of the aleurone layer between the commercial varieties of:
I. *nutans* type: a) Elka b) Borzymowicki.
II. *erectum* type: a) Album Invincible b) Peacock.
III. *vulgare* type: a) Pallidum 10371 from Manchuria b) Dłużewski.
(a. l. = aleurone layer)

arbitrary — for we are dealing with a continual scale of variation. The first group includes varieties with a thin aleurone layer ($<80\,\mu$) and a low average number of cells, varying about 2 ($<2{\cdot}30$). The varieties with a thick aleurone layer ($<72\,\mu$) and an average number of cell rows ($>2{\cdot}70$) constitute the second group. The third group embraces intermediate forms in respect of the number of cell rows (2·30—2·70) in the aleurone layer as well as in respect of its thickness (68—88 μ). This grouping is shown in Table VIII, in which the varieties are arranged according to the increasing average number of cell rows in the aleurone layer.

As can be seen from the above table, distinct differences in the number of cells and in the thickness of the aleurone layer appear not only between various botanical types of barley, endemic in different centres of origin, but also among the Europeen commercial varieties, belonging to the 2- and 4-rowed type.

So, for instance, among *H. distichum nutans* the following varieties chiefly possess a 2-rowed aleurone layer: Borzymowicki, Hadostreng, Perbete 4, Heils Franken, Isaria, Bethges u. Oelzes XIII, Diosecky 495. The predominance of 3 rows of cells is exhibited by Przeworski, Elka, Beavens Archer, Chevalier, Halliko, Russia I and others.

Within the limits of *H. distichum erectum* a 2-rowed aleurone layer appears in the varieties Malster, Peacock and St. Croix, and Invincible and Silesia have usually 3 rows of cells.

In the 4-rowed barleys *(H. vulgare)*, the predominance of 2 rows of cells in the aleurone layer can be found in the varieties Heine's 4-rowed, Marchijski, Dłużewski, Hohenfinover 4-rowed; and 3 rows in Erhardt Frederiksen, Bere, Pertu and Irish 6-rowed.

The majority of the European varieties, however, belong to the intermediate group, in which the aleurone layer is built partly of 2 and partly of 3 cell rows.

TABLE VIII

The division of the 103 varieties examined into 3 groups, made on the basis of the distribution of varieties in the correlation table and of the average number of cell rows and of the average thickness of the aleurone layer, counted over a period of several years

No. of variety according to Table I	Variety (subspecies, bot. variety)	Average number of cells	Average thickness of the layer in microns	Group
103	var. *copticum* Vav. *(H. decipiens)*	1·71	51·06	I
96	*Deficiens* A. *(H. decipiens)*	1·84	65·31	I
98	*Deficiens* 16 *(H. dicipiens)*	1·90	65·54	I
100	var. *macrolepis* A. Br. *(H. decipiens)*	1·90	55·61	I
35	Abyssinian 5336 *(H. vulgare)*	1·93	57·72	I
97	*Deficiens* 11 *(H. decipiens)*	1·96	64·59	I
57	Perbete 4 *(H. nutans)*	1·98	61·94	I
70	Dornburger Heils Franken *(H. nutans)*	1·99	64·37	I
94	Hord. *zeocrithum album* (var. *zeocrithum*)	1·99	61·28	I
71	Hadostreng *(H. nutans)*	2·00	77·27	I
43	Borzymowicki *(H. nutans)*	2·03	62·54	I
32	Pusa Type 21 *(H. vulgare)*	2·03	57·60	I
27	*Pallidum* 10343 from Manchuria *(H. vulgare)*	2·08	63·32	I
99	var. *Steudelii (H. decipiens)*	2·09	74·31	I
26	*Pallidum* 10342 from Manchuria *(H. vulgare)*	2·11	64·27	I
90	Malster *(H. erectum)*	2·12	72·84	I
101	var. *abyssinicum* Körn. *(H. decipiens)*	2·12	59·70	I
22	Heines vierzeilige *(H. vulgare)*	2·13	67·73	I
68	Ackermanns Isaria *(H. nutans)*	2·16	70·84	I
15	Marchijski *(H. vulgare)*	2·16	69·46	I
91	Peacock *(H. erectum)*	2·18	73·31	I
14	Dłużewski *(H. vulgare)*	2·18	68·68	I
23	Hohenfinover vierzeilige *(H. vulgare)*	2·20	75·86	I
4	*Pyramidatum* W. C. *(H. hexastichum)*	2·21	61·73	I
41	*Transiens* Körn. *(H. intermedium)*	2·22	78·40	I
53	Diosecky 496 *(H. nutans)*	2·22	65·42	I
33	Pusa Hybride 1—92 *(H. vulgare)*	2·22	66·60	I
85	St. Croix *(H. erectum)*	2·25	69·81	I
69	Bethges u. Oelzes XIII *(H. nutans)*	2·27	68·72	I
60	Abed Opal *(H. nutans)*	2·29	72·71	I
73	Strengs Frankengerste *(H. nutans)*	2·34	76·17	III
67	Ackermanns Danubia *(H. nutans)*	2·35	73·06	III
29	No. 14 Tunis *(H. vulgare)*	2·37	78·20	III
83	Landsorte from Tirol »Rotholz« *(H. erect.)*	2·38	74·65	III
59	Abed Binder *(H. nutans)*	2·38	71·70	III *m*[1]
34	Pusa R-1 *(H. vulgare)*	2·40	70·91	III *m*
92	Svalöf Primus II *(H. erectum)*	2·41	77·99	III
16	Première a barbes lisses *(H. vulgare)*	2·41	74·29	III
20	O. A. C. 21 C. A. N. 1086 *(H. vulgare)*	2·43	71·52	III
66	Ackermanns Bavaria *(H. nutans)*	2·45	76·37	III

[1] *m* indicates a variety, which in different years gave unequal results and is presumably a mixed variety.

No of variety according to Table I	Variety (subspecies, bot. variety)	Average number of cells	Average thickness of the layer in microns	Group
58	Postoloprtsky *(H. nutans)*	2·45	72·27	III
65	de Moravie *(H. nutans)*	2·47	74·88	III
74	Svalöfs Goldgerste *(H. nutans)*	2·48	79·88	III
72	Mahndorfer Victoria *(H. nutans)*	2·51	80·79	III
75	Siegesgerste *(H. nutans)*	2·52	74·05	III
46	Hanna z Goli *(H. nutans)*	2·55	70·76	III *m*
36	Big Wheat *(H. vulgare)*	2·56	87·50	III
89	Golden Pheasant *(H. erectum)*	2·56	82·87	III
10	Californian *(H. vulgare)*	2·57	69·85	III
82	Plumage Archer *(H. erectum)*	2·57	82·77	III
88	Arch-Goldthorpe *(H. erectum)*	2·58	83·93	III
49	Kutnowski 08 *(H. nutans)*	2·58	80·44	III
52	Hanna Kargyn *(H. nutans)*	2·59	69·78	III
47	Kazimierski *(H. nutans)*	2·61	74·78	III
64	Chevalier race Francaise *(H. nutans)*	2·61	77·89	III
13	Covra *(H. vulgare)*	2·61	80·72	III *m*
50	Puławski *(H. nutans)*	2·61	84·17	III
18	Lapin II *(H. vulgare)*	2·63	73·68	III
21	Velvet *(H. vulgare)*	2·66	71·52	III
45	Hanna Gambrinus *(H. nutans)*	2·67	76·23	III
37	*Nigrum* 10345 from Manchuria *(H. vulgare)*	2·67	81·94	III *m*
84	Dubasquier *(H. erectum)*	2·68	81·33	III
9	American 6-row. No. 131 *(H. vulgare)*	2·68	79·68	III
54	Hanna Proskovetza *(H. nutans)*	2·69	71·43	III
48	Kujawski *(H. nutans)*	2·69	84·63	III
87	Gartons 1917 *(H. erectum)*	2·70	82·21	III
63	Hanna Skrzeszowicka *(H. nutans)*	2·71	84·41	II
30	No. 4 A — Tunis *(H. vulgare)*	2·72	84·61	II
25	*Pallidum* 3732 from Manchuria *(H. vulgare)*	2·73	77·37	II
17	Abed July *(H. vulgare)*	2·74	81·46	II
51	Przeworski *(H. nutans)*	2·74	85·20	II
77	Beavens Archer *(H. nutans)*	2·75	89·94	II
1	a six rang de Chine *(H. hexastichum)*	2·76	88·70	II
93	Svonneck *(H. erectum)*	2·77	87·06	II
56	Nolcs Moravia *(H. nutans)*	2·77	83·02	II
12	Irish 6-row *(H. vulgare)*	2·77	76·26	II
55	Kneifluv P. 13 *(H. nutans)*	2·79	88·45	II
24	Pallidum 10371 from Manchuria *(H. vulgare)*	2·82	85·97	II
8	American 6-row. No. 115 *(H. vulgare)*	2·82	78·30	II
2	Emilio Mariani *(H. hexastichum)*	2·82	87·34	II
78	Chevalier II *(H. nutans)*	2·83	88·12	II
7	American 6-row. No. 175 *(H. vulgare furc.)*	2·84	75·33	II
19	Pertu *(H. vulgare)*	2·84	78·96	II
102	var. *gymnospermum* Körn. *(H. dist. furc.)*	2·85	82·35	II
79	Hallet's Chevalier *(H. nutans)*	2·85	89·66	II
11	Scoth 6-row. »Bere« *(H. vulgare)*	2·85	75·89	II
6	American 6-row. No. 169 *(H. vulg. furc.)*	2·86	74·33	II
44	Elka *(H. nutans)*	2·86	74·33	II
3	var. *pyramidatum* Körn. *(H. hexastichum)*	2·86	86·38	II
5	American 6-row. No. 166 *(H. vulg. furc.)*	2·86	73·80	II
28	Pallidum from Urkujsk *(H. vulgare)*	2·87	77·71	II

No of variety according to Table I	Variety (subspecies, bot. variety)	Average number of cells	Average thickness of the layer in microns	Group
61	Halliko *(H. nutans)*	2·87	85·65	II
42	Anderson Intermediate *(H. intermedium)*	2·89	74·09	II
62	Louhi *(H. nutans)*	2·90	91·24	II
38	Nudum No. 158 from Podhale *(H. vulg. nudum)*	2·90	75·49	II
39	Nudum No. 151 from Podhale *(H. vulg. nudum)*	2·93	95·18	II
31	Erhardt Frederiksen *(H. vulgare)*	2·93	79·44	II
86	v. Webskys Silesia *(H. erectum)*	2·95	97·15	II
40	*Nudum* No. 154 from Podhale *(H. vulg. nudum)*	2·95	75·57	II
80	Laschkego Tybetański *(H. dist. nudum)*	2·95	90·53	II
76	Russia I *(H. nutans)*	3·00	79·07	II
95	*Hord. distichum* var. *inerme*	3·00	79·80	II
81	Album Invincible *(H. erectum)*	3·02	96·32	II

IX. Summary

Taking into consideration the sporadic observations and generalized data found in the agro-botanical literature, according to which some varieties of barley differ in the anatomical structure of the aleurone layer of the grain, the author has undertaken a detailed investigation of the number of cell rows in the aleurone layer, and of the thickness of the latter in different spring varieties of cultivated barley. At the same time he has examined the range of variability of these characters in order to establish whether they may be employed in the classification of barleys.

A collection of 103 barley varieties was examined; these were representative of all the subspecies of *Hordeum sativum* Jess. as well as of different countries and parts of the world. There were investigated the differences in the anatomical structure of the aleurone layer between distant botanical races originating from different geographical centres, the same differences between European varieties belonging to the same botanical type, and the transmission of the characters named to the offspring studied on material grown on the experimental ground during the years 1937, 1938 and 1947. The behaviour of both the characteristics was also examined on the material of 15 pedigree lines in the three consecutive years 1947, 1948 and 1949. Moreover, in order to con-

trol the possible influence of different climatic conditions, both the characteristics were investigated in the grain material of 28 varieties obtained in 1946 from Cambridge and its reproduction grown at the Experimental Farm of the University (Mydlniki) in 1948.

It has been ascertained that the varieties examined show distinct differences in regard to both the characteristics studied. The mean number of cell rows in the aleurone layer of the different barley varieties amounts to 1·64—3·11, and the mean thickness of the aleurone layer from 49·94 to 110·16 microns.

The observation of the two characteristics in question on material from several years and from different climatic conditions showed that they exhibit a comparatively small variability due to environmental conditions. A detailed analysis of variance performed by means of the statistical methods of Fisher on a material of 40 varieties from 1937, 1938 and 1947 showed that as regards the number of the cell rows the varietal differences are responsible for 93·00% of the total variation, and for 85·60% as regards thickness of the aleurone layer. The variation due to seasonal differences is very small; in the first case 0·60%, and the in second 0·40% of the total variation. The remaining 6·40% resp. 13·91% of variation are due to unknown causes (environmental changes). Similar results were obtained by analysis of the variation of the pedigree lines from 1947, 1948 and 1949, which shows that there are no essential differences between the pedigree lines and the material of commercial varieties examined.

Considering the small individual variation within the limits of the same variety and the small seasonal variation between the yields of different years, the mean number of cell rows as well as the thickness of the aleurone layer must be regarded as comparatively constant characteristics of a given variety, and as such they may be employed in the classification of barleys.

It was found that differences between varieties exceeding 0·3 cell layer and 13 microns of layer thickness may be regarded as sufficiently significant if materials from only one year are compared.

There exists a strong positive correlation between the characteristics described, expressed by the correlation coefficient $r = +0{\cdot}776$, $\pm 0{\cdot}039$. On this account they should be treated jointly in the varietal classification.

Within the limits of the same botanical variety, e. g. *Hordeum distichum nutans*, *Hordeum distichum erectum*, *Hordeum vulgare* etc. there were found distinct differences between the commercial varieties as regards the development of the aleurone layer, its thickness, and the number of cell rows. These differences appear among others also in European varieties of cultivated barley and may be used as taxonomical characteristics in the classification of these varieties.

The results of these investigations fully corroborate the earlier data of Orlov, who found that barley endemic in the Asiatic centre of origin as a rule posses 3 rows, and those of the African centre 2 rows of cells in the aleurone layer only. As the European parieties of cultivated barley show a similar differentiation, the vossibility exists in some cases of explaining their primary origin.

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I am indebted to the Director of the Department, Professor Dr Kazimierz Miczyński for his valuable suggestions offered during the elaboration of the material.

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Explanation of figures

7. Ears of the varieties, transverse sections through grain of which are shown in figure 5:

I. a) Deficiens A. b) var. *copticum* Vav.
II. a) a six rang de Chine b) American 6-row. No. 175.
III. a) var. *pyramidatum* Körn. b) *H. vulgare nudum.*

8. Ears of the commercial varieties, sections through grain of which are shown in figure 6:

I. a) Elka b) Borzymowicki
II. a) Album Invincible b) Peacock
III. a) Pallidum 10371 from Manchuria b) Dłużewski.

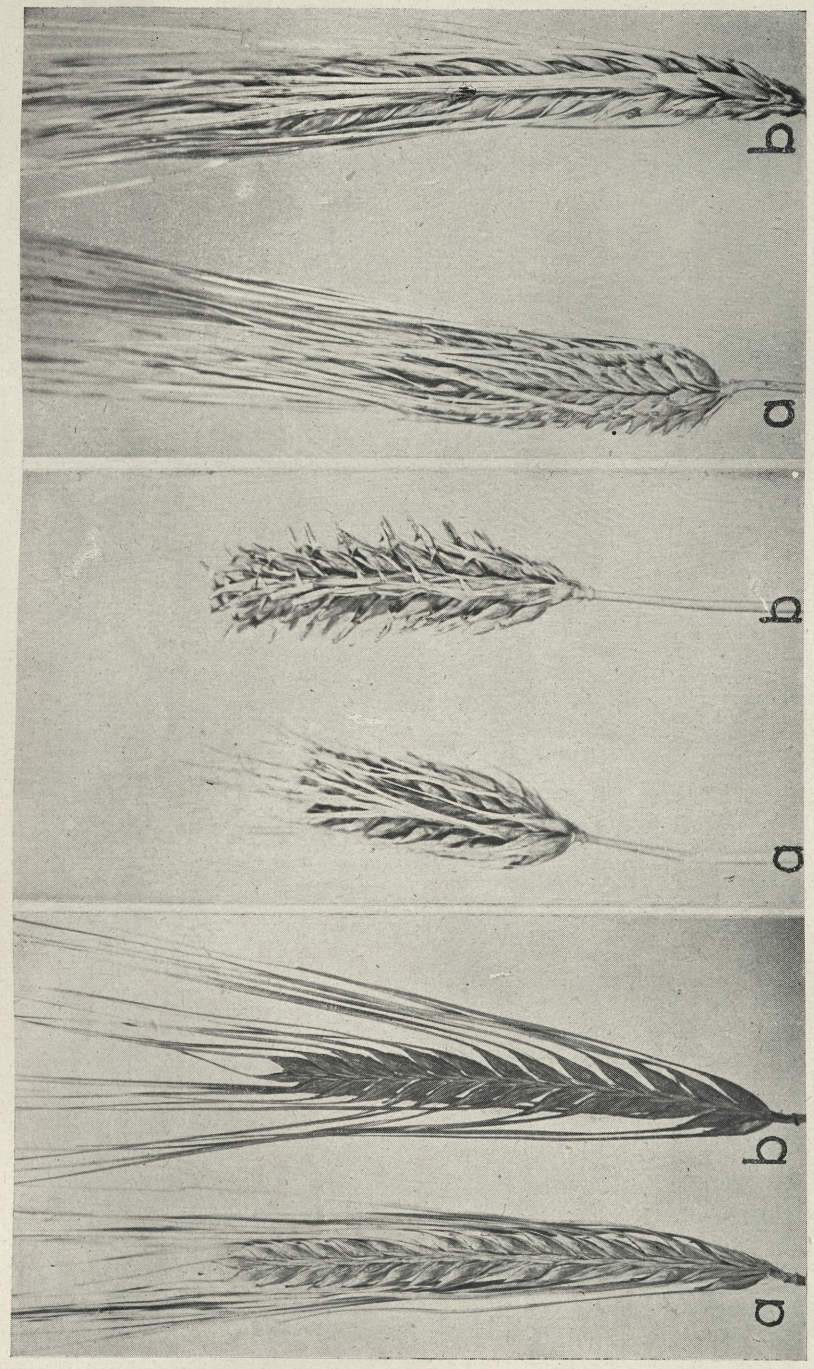

Fig. 7.

J. Sawicki

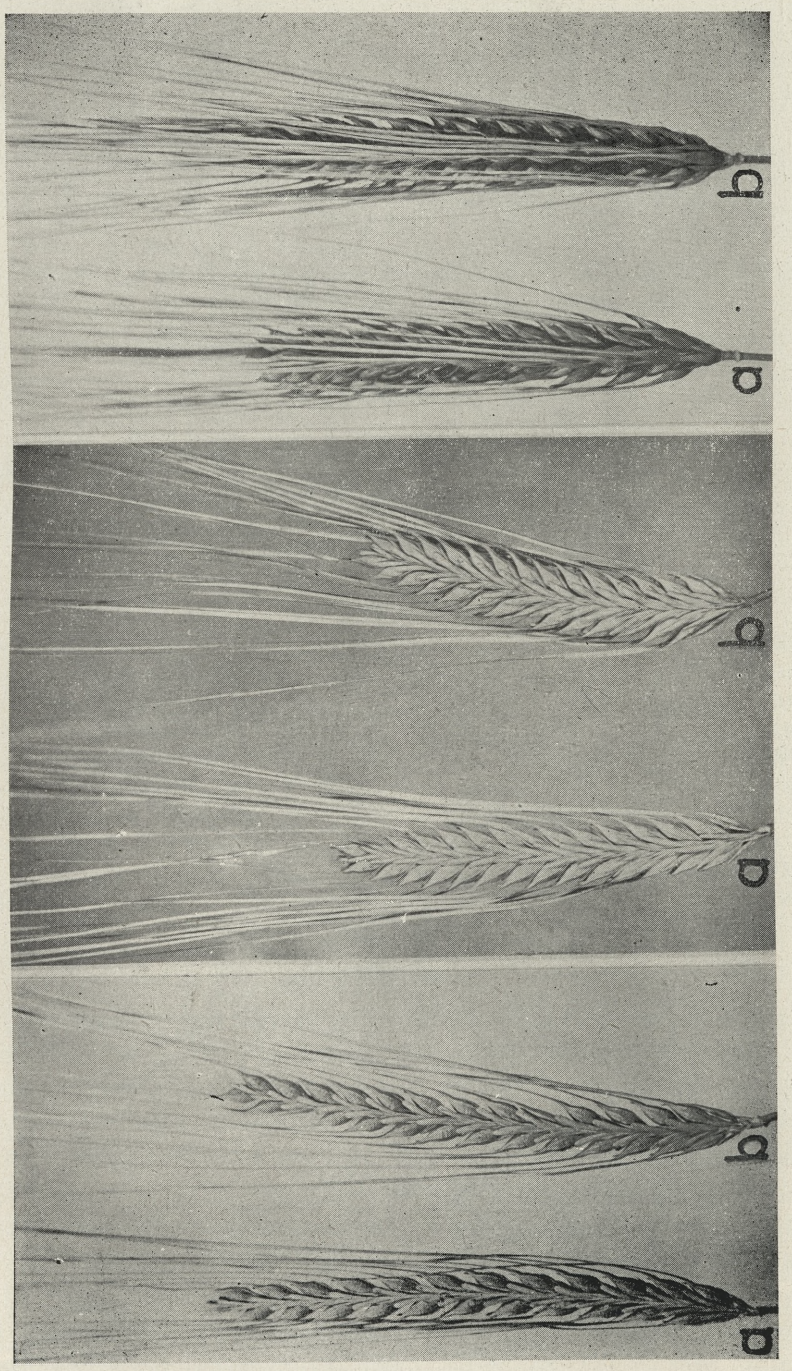

Fig. 8.

J. Sawicki

Badania cyto-ekologiczne, rozmieszczenie geograficzne i ewolucja rodzaju Valeriana L. — Studies in cyto-ecology, geographic distribution and evolution of Valeriana L.

Mémoire

de M^me^ ***M. SKALIŃSKA*** m. c.

présenté dans la séance le 9 Octobre 1950

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Introduction

The present paper deals with chromosome numbers of Polish species of the genus *Valeriana* L. belonging to the section *Officinalis*. Previous studies carried out on plants of various origin (Meurman 1925, Senjaninova 1927, Runquist 1937, Skalińska 1945, 1947, and recently Walther 1949) have shown the existence of a polyploid differentiation within this group. The results however obtained by the above mentioned authors required further studies. The existing cytological differentiation seems to be closely connected with the geographic distribution and ecology, cytological data however available for Valerians from central

and western parts of the European continent are extremely scarce. The present research work has been started in spring 1947 on Polish material from natural habitats as a continuation of the authors studies of Valerians from the British Isles. Cytological investigations were supplemented by field studies on plants from a variety of natural habitats as well as by morphological observations on plants transplanted into approximately uniform conditions.

The results obtained throw some light upon the possible course of evolution within this group.

Material and methods

In the flora of Poland the section *Officinalis* is represented by three species which are distinctly delimited both morphologically and cytologically: (1) *V. officinalis* L. s. str. (*V. exaltata* Mikan, *V. latifolia* Vahl.), (2) *V. tenuifolia* Vahl. (*V. angustifolia* Tausch), and (3) *V. sambucifolia* Mikan (*V. excelsa* Poir.). Specimens of these three species have been collected during four summer seasons (1947—1950); the material originated from 41 natural habitats ranging from the Baltic coast in the north (about 54° 40′ N. Lat.) to the Carpathians and Tatra Mts in the south (about 49° 20′ N. Lat.). In most cases living plants collected in nature were investigated; only in a few instances the material was obtained in the form of seeds which were germinated in the Institute of Plant Anatomy and Cytology of the University of Kraków.

The determination of the numbers of chromosomes was based on root-tip mitoses. The fixatives of Navashin and of Levitsky (5:5) gave in general satisfactory results. The sections, 10 μ thick, were stained with Newton's gentian violet. After fixation the living plants were transplanted into the experimental field of the Institute where they were grown in approximately uniform conditions for comparative morphological studies. In addition, the examination of a number of herbarium specimens supplemente the studies on living plants.

Nomenclature

It has been repeatedly pointed out by various authors that *Valeriana officinalis* L. s. *lato* represents a collective species. On the European continent, chiefly in east-central Europe it can

be easily subdivided into a number of well separated forms; on the other hand in Great Britain there exists a great morphological diversity within this collective species, the various forms representing a more or less continuous series; therefore any attempt to subdivide this polymorphic species into smaller units on morphological basis must result in a failure. The distinct delimination of forms occurring on the European continent should be regarded as representing more primitive conditions, whereas in Great Britain presumably a kind of fusion has taken place between the formerly separated groups which now are linked by a great number of intermediate forms of hybrid origin (Skalińska 1947).

The morphological and taxonomical studies of Walther published in 1949 give a revision of the nomenclature within the collective species. In view of the existing distinct differentiation observable in continental forms, she gave the collective species the rank of a section *Officinalis*. This section is subdivided into two series: (1) *Sambucifoliae* and (2) *Collinae*. The first roughly corresponds to the species *V. sambucifolia* Mikan, as described by Hayek and Hegi, and the second represents Hegi's *V. officinalis* L. with its three forms (var. *latifolia* Vahl. = *V. exaltata* Mikan, var. *media* Koch and var. *tenuifolia* Vahl. = *angustifolia* Tausch.). The forms within *V. officinalis* are regarded by Walther as separate species.

Generally speaking, Walter's conclusions seem to give a workable classification of the continental forms. It may be applied to the Polish representatives of the group owing to their sharp morphological, ecological and cytological delimitation. It seems therefore reasonable to adapt some of the specific names used by this author.

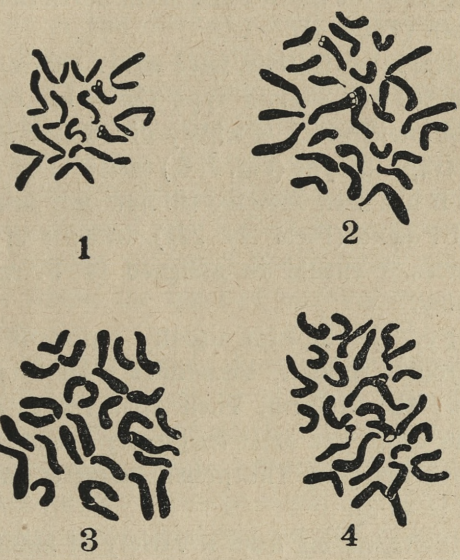
Thus, the name *V. exaltata* Mik. (already considered as a specific name in the paper of Senjaninova 1927) is used by me in the same sense as in Walther's paper to name forms from eastern and central Europe. It corresponds to Hayek's and Hegi's *V. officinalis* L. var. *latifolia* Vahl. together with var. *media* Koch. The latter is somewhat different from the former as far as morphology is concerned; ecologically and cytologically the two forms are alike. In Poland *V. exaltata* is usually diploid ($2n = 14$). It should be added that the name «Var. *latifolia* Vahl» is misleading as it has been used by Drabble (1933) to name some forms

of *V. officinalis* from Great Britain which are not identical with *V. exaltata* and have the octoploid number of chromosomes ($2n = 56$).

The third form of Hegi's *V. officinalis*, viz. var. *tenuifolia* Vahl deserves in my opinion a separate specific name in view of its distinct morphological features as well as its ecology and cytology. It represents a tetraploid type ($2n = 28$). *V. tenuifolia* Vahl has been described for the flora of Poland by Szafer, Kulczyński and Pawłowski (1924) as a form of *V. officinalis* L. occurring in hilly regions chiefly on limestone in southern Poland. *V. tenuifolia* seems to be identical with *V. angustifolia* Tausch described by Kreyer (1930) as a Central-European steppe plant. On the other hand, in Walther's paper the Central-European forms are assigned, together with plants from France and Great Britain, to *V. collina* Wallroth. The forms however described under this name represent a mixture of types. The Polish *V. tenuifolia* which has a high degree of uniformity, seems to correspond exactly to *V. angustifolia* from Upper Austria (Flora Exsicc. Austr.-Hung. N. 3445, coll. by Zimmeter; Kew Herbarium), but is not quite identical with any of the related forms from Great Britain assigned by Walther to *V. collina*, and by Drabble (1932) to *V. angustifolia* Host. It should be added that among the British herbarium specimens listed in Walther's paper under the specific name of *V. collina* not only tetraploids but also octoploids are included (e. g. plants from the Manifold Valley, Staffordshire). Some of the latter are remarkably similar to the continental tetraploids and may be regarded as their parallel forms. In general however the British forms have in their compound leaves a lower number of leaflets than the forms from Poland and Austria. In view of the difficulty of identification, it seems preferable to use for the very uniform species from southern Poland the name *V. tenuifolia* than to assign it to the mixed group described by Walther under the name *V. collina*.

The series *Sambucifoliae* of Walther which corresponds to a single species of Hayek and Hegi's classification, *V. sambucifolia* Mikan, has been subdivided into two separate species: *V. sambucifolia* Mikan and *V. procurrens* Wallroth. Most authors did not make any distinction between the two forms, the first native

in Scandinavia and Central Europe and the second replacing it in Western Europe. According to Sprague (1943) the British form of *V. sambucifolia* is not identical with Central-European forms described under this name. On the basis of morphological and anatomical studies Walther separated the two forms under the above mentioned specific names. This separation seems justified, since the Central-European forms show a high degree of uniformity, sharply contrasting in this respect with the British forms of *V. sambucifolia*. In addition, some well marked morphological differences as well as differences in the time of flowering are observable between specimens of the two forms cultivated in approximately uniform conditions. In accordance with the revised nomenclature of Walther, the Polish plants belong to *V. sambucifolia* in its narrower sense. This species is octoploid ($2n = 56$). The specific value of *V. procurrens* will be discussed later.



Figs. 1—4. Somatic metaphases in root-tips. 1) *Valeriana exaltata* pl. 239 ($2n = 14$), the same as Fig. 7 (Levitsky 5:5 — Gentian violet); 2) *V. exaltata*, pl. 225 ($2n = 28$), the same as Fig. 8 (Navashin-Gentian violet); 3) *V. tenuifolia* pl. 206 ($2n = 28$), the same as Fig. 9 (Navashin-Gentian violet); 4) Polyhaploid twin plant ($2n = 28$) of *V. sambucifolia* ($2n = 56$), the same as Fig. 11 (Navashin-Gentian violet, × 2700).

Valeriana exaltata Mikan

In Poland, *V. exaltata* represents the commonest species within the section *Officinalis*. This species has the widest area of distribution and the greatest ecological range, for it occurs in a variety of habitats. The determination of the chromosome number of this species was carried on on root tips of plants from 27 different habitats ranging from the Baltic coast in the north to the Carpathians Mts in the south (Table I).

The cytological investigations have shown that almost all plants studied represented the diploid type ($2n = 14$, Fig. 1, 5, 6, 7). Thus, it is the diploid which is widely distributed over all Poland, whereas the tetraploid type (Fig. 2, 8,) seems to be extremely rare in this species: it has been detected till now only in a single habitat in west-central Poland (Field No. 225). In spite of some morphological differences it should be assigned to *V. exaltata* together with the diploids.

Morphology. The diploids usually represent vigorous plants without overground runners, sometimes with short underground runners. Height 120—180 cm. Their stem leaves bear (6—) 7—8 pairs of leaflets which are most frequently broadly lanceolate and toothed on both margins. The pollen diameter ranges from 30 μ to 52 μ (Mean = 42·3).

The diploids originating from a variety of habitats represented a group with a high degree of diversity. The cultivation of the plants in approximately uniform conditions during three summer seasons (1948—1950) has shown the existence of genic differences within the diploids studied. They concern chiefly the shape of the leaflets (Figs. 12, 13, 15,) the height of the flowering stems and the time of flowering. Although the tetraploids are a little stouter in general they are morphologically very similar to some types among the various diploids. Their flowers and fruits are somewhat larger, and the size of the pollen grains exceeds that of the diploids. It ranges from 45·4 μ to 52·0 μ (Mean = 48·6).

The shape of the leaflets is determined in general by the ratio width/length. In diploids the most frequent type is represented by broadly lanceolate leaflets in which this ratio ranges from 0·20 to 0·30 (e. g. length 75 mm, width 20 mm; ratio width length = 0·266) (Fig. 13, 2nd row). In shorter and relatively broader

ovate leaflets it exceeds slightly this value (Fig. 13, 2nd row, third leaflet). Narrowly lanceolate leaflets in which the ratio width/length is about 0·14 are relatively rare; they are represented on the same photo, upper row. The leaflets of the tetraploids are larger than those of the diploids. Their shape is broadly lanceolate (ratio width/length = 0·26) (Fig. 13, 3-rd row, second leaflet, Fig. 16).

Toothing of the margins of the leaflets. In most diploids the two margins of the leaflets, or alternatively, only the basiscopic margin, are more or less distinctly toothed. Subentire margins are relatively rare (Fig. 12 left, Fig. 13 top left leaflets). The different shapes and sizes of the teeth, their varying number and more or less asymmetrical and irregular distribution along the margins contribute to increase the diversity in the morphology of the leaflets (Figs. 12, 13, 15). The tetraploids also show some diversity in respect of the toothing of their leaflets: in some plants the toothing is coarse and irregular, in others the margins are only slightly toothed; on the whole, the types of toothing correspond to those found in diploids.

Height of the flowering stem. In uniform garden conditions distinct differences could be observed among the diploids: plants from some habitats had relatively short, stout stems (e. g. from the Hel peninsula) 110 to 130 cm high, while plants from other habitats attained in the same conditions 160 to 180 cm (e. g. plants from Sikornik, nr. Kraków). In the group of tetraploids the height of the individual plants ranged from 145 to 170 cm.

Time of flowering. *V. exaltata* is regarded in general as a late-flowering species: Walther gives July to August as its time of flowering (p. 78). The present studies however have revealed the existence of early-, intermediate- and late-flowering strains among the diploids: in natural habitats the earliest strains begin their flowering already in the first days of June. It is evident that in nature the time of flowering is greatly influenced by the soil, situation, altitude, geographical latitude etc. However the differences with regard to the beginning of flowering were retained by the various strains in conditions of cultivation. It is interesting to note the existence of a kind of latitudinal differentiation in respect of earliness: strains from habitats in the northern parts of Poland (between 54° 50′ and 52° 40′ N. Lat.) are either early (Hel peninsula) or moderately intermediate (Baltic coast, Rozewie).

TABLE I

V. exaltata: localities and habitats of the specimens studied

Field No.	2n	Locality and habitat	North Latitude	Collector
257	14	Rozewie on Baltic coast; at the shore, overflow area	54°50′	M. Piotrowicz
230	14	Hel peninsula (Baltic coast); between Kuźnica and Jastarnia; in a wood on a fixed dune, under *Alnus, Populus tremula* and *Sorbus*	54°40′	M. Piotrowicz
231	14	Lake Kasajno, nr. Giżycko; border of the lake	54°	J. Dyakowska and J. Dobrzańska
232	14	As above	54°	,,
221	14	Primeval forest of Białowieża; wood clearing	52°40′	W. Szafer
227	14	Lake Gopło; border of the lake, in swampy soil	52°40′	J. Dyakowska
244	14	Puszczyków, near Poznań; on a meadow.	52°25′	J. Szulczewski and K. Moldenhawer
225	28	Klęka, nr. Nowe Miasto on river Warta; road side in partial shade	52°05′	D. Marynowska
259	14	Wilanów, nr. Warsaw; roadside, under willow trees	52°12′	K. Wróbel
249	14	Jeziorna, nr. Warsaw; moist ground in a mixed wood *(Alnus, Quercus, Betula, Corylus)*	52°05′	M. Piotrowicz
236	14	Parchacki hill, nr. Puławy	51°25′	Specimens No 236—242 received from the Rural Scientific Institute in Puławy
237	14	Wólka Profecka nr. Puławy; sandy soil	,,	
238	14	Góra Puławska, on sandy soil	,,	
239	14	Kępa, nr. Puławy; on fields	,,	
240	14	Kępa, nr. Puławy; in a copse, rich black soil	,,	
241	14	Włostowice, nr. Puławy	,,	
242	14	Kępa, nr. Puławy; on a field in shade, soil rich in humus	,,	
264	14	Holy Cross Mts; roadside in lower situation	50°50′	M. Piotrowicz
260	14	Nr. Olkusz; on limestone	50°20′	J. Dobrzańska
223	14	Valley of river Prądnik near Ojców; bank of the river	50°10′	O. Laska

Field No	2*n*	Locality and habitat	North Latitude	Collector
224	14	Czyżyny, nr. Kraków; on damp meadows	50°	A. Bajer
228	14	Sikornik, nr. Kraków; on a steep slope along the road in partial shade	50°	A. Bajer and M. Skalińska
246	14	Niepołomicka forest, south-east of Kraków; on a peat moor	50°	M. Piotrowicz
233	14	Valley of river Skawa, nr. Maków (foreland of the Carpathian range) bank of the river	49°45′	E. Banach
265	14	South-west of Jordanów (foreland of the Carpathian range); on a northern slope in stony soil, among *Corylus* shrubs	49°40′	St. Tempka
255	14	Czorsztyn, Pieniny Mts, near the ruins of the castle on a very dry sunny slope	49°30′	A. Bajer
258	14	Czorsztyn, Pieniny Mts; on swampy ground among young willow trees	49°30′	A. Bajer

In the experimental field the first strain begins its flowering already in the last days of May or first days of June; the second, situated in a much more exposed habitat expands its first flowers about the 15-th June. With the advance from the north southwards early strains become rarer; they are replaced by intermediate and late strains which are absent from more northern latitudes; in habitats situated southwards of the 51 parallel late-intermediate and late strains represent the most frequent types. The late strains begin their flowering in the experimental field about one month later than the early strains; the tall and vigorous plants from Sikornik nr. Kraków begin to flower in the first days of July, those from the valley of river Prądnik were slightly earlier (end of June in 1949 and 1950). It has been already proved by the extensive experiments of Turesson (1930) that within a species biotypes native in different latitudes may show a pronounced differentiation regarding their earliness and that the differences are retained when the plants are grown under controlled conditions. The results of the present observations have shown that the diploid forms of *V. exaltata* represent a mixture of various biotypes differentiated with regard

to earliness of flowering; their distribution is correlated to some extent with the geographic latitude.

The tetraploid strain whose natural habitat is situated about 52°, begins in cultivation its flowering simultaneously with the earliest diploid strain from the north (Hel peninsula). This fact deserves mentioning since frequently the tetraploid types are flowering later than the corresponding diploids.

Ecology and geographic distribution. It has been mentioned above that in Poland *V. exaltata* is chiefly represented by diploid strains which have a far wider distribution than the very rare tetraploids found hitherto only in a single habitat. Its large area of distribution is combined with a great ecological range. It is able to grow in a variety of habitats (Table I): in partial shade on moderately damp ground, in open places in swampy soil, on very dry and sunny slopes; one of the most extreme habitats has been found on the Baltic coast where the plants grew on moist ground inundated by the sea. As far as the edaphic conditions are concerned, it should be noted that the diploids occur on a variety of soils; apparently they do not show any preference for soils with either lower or higher acidity; they may grow on limestone in a soil with a relatively high pH value, as well as on a peat moor in acid soil. According to the observations in the experimental field, only in the first stages of development the seedlings require a relatively humid air, but the seeds dispersed by the wind are able to germinate and to develop in the shade of other herbaceous species by which they are protected against drought. Thus, *V. exaltata* is able to develop in a variety of ecological conditions owing to a high degree of adaptability.

Details of the general distribution of this species are given by Hegi and Hayek, by Kreyer (1930), and also by Runquist (1937) for Fenno-Scandia and recently by Walther (1949) for Central Europe. According to Hayek and Hegi, *V. officinalis* is an Eurasiatic species. The data given by Kreyer for his *V. palustris* Kr. (which is a synonyme of *V. exaltata*), by Walther for *V. exaltata*, and complemented by the present studies, show that this species occupies an extensive territory on the European continent: it occurs in the European part of the Soviet Union and also in Poland (from the Baltic coast to the Carpathian foreland), in Austria and Germany. According to Runquist, in Sweden

it extends approximately to the 60° of N. Lat. chiefly occurring along the eastern coast. In Norway it is found on the coast as far as the fjord of Trondhjem. In Finland it occurs in the region of the Finnish gulf. Although Runquist's data are given for *V. officinalis* L. his morphological description as well as the results of his cytological studies show that the plants investigated strictly correspond to *V. exaltata*: they are diploid with 14 somatic chromosomes. On the other hand, a single herbarium specimen from England (Woodstock district), considered by Walther as *V. exaltata* on the basis of a high degree of morphological similarity, represents in fact an octoploid type, by no means identical with the continental *V. exaltata*. The occurrence of diploids in Great Britain is doubtful.

The very wide area of distribution of *V. exaltata* suggests that it represents an old species. The discontinuity within this species seems to be due chiefly to a genic differentiation which could have taken place in various points of its area. It contributed to split the species into different biotypes. The wide area of distribution combined with a great ecological range shows that the observable genic differentiation may play an important part in the evolution of the species leading to the formation of biotypes specialized for various ecological conditions.

Both Walther and Kreyer regard *V. exaltata* (*V. palustris* Kr.) as a purely diploid species. The material from Poland however shows some amount of polyploid differentiation: the incidental occurrence of tetraploids in west-central Poland, in a part of the area which is remote from the putative centre of origin of this species, may represent the first step of a cytological differantiation. It should be added that the very fragmentary records available permit to assume the existence of tetraploid representatives of *V. officinalis* s. str. in Central Europe. Meurman (1925) was the first who determined correctly the basic number of chromosomes in this species; in a specimen derived from the Copenhagen Botanical Garden he found the tetraploid number ($2n = 28$); although the native habitat of the plants examined is unknown, it seems reasonable to assume that they originated from Central Europe. Walther in her morphologic-taxonomical study mentiones some tetraploid species from Central Europe. Besides *V. collina* which is morphologically and ecologically distinctly different from *V. exaltata*, she quotes *V. pratensis* Dierbach, a putative tetraploid

occurring in the plain of the Rhine; the description of this form distinctly disagrees with the features of the tetraploids from western Poland, although the plants also represent early-flowering types. The tetraploid *V. nitida* Kr. described by Kreyer as a species occurring in central Europe is not as tall as *V. exaltata* and represents a late-flowering type. Besides the above mentioned forms no further exact data are known concerning the occurrence in Central Europe of tetraploids similar to *V. exaltata*. It would be extremely interesting to find more westwards some continental forms which would bridge the gap between the diploid and tetraploid forms of *V. exaltata* and the British Valerians represented only by tetraploids and octoploids (Skalińska 1947). In connexion with this problem it should be emphasized that some forms of *V. exaltata* occurring in Poland show a pronounced morphological similarity in stature, shape and number of leaflets as well as other features to some British forms assigned to *V. officinalis* (Figs 15—17; cf. Skalińska 1947, Pls 7, 8). This existence of corresponding forms suggests that the diploids from the European continent may be regarded as putative ancestors of these polyploid types. On the basis of studies of a number of herbarium specimens from Great Britain, Walther arrived to the erroneous conclusion that the diploid *V. exaltata* has also an isolated centre in Great Britain. This conclusion is based on the examination of a specimen from Oxfordshire (Woodstock district, marsh near slade's bottom. C. E. Hubbard and W. B. Turrill. Kew Herbarium). Cytological studies of living plants from the same district carried on in the course of my previous work have proved that the respective plants are octoploids. It is possible however that they have evolved from continental forms with lower numbers of chromosomes. The change from diploid to octoploid conditions would be attained in two steps.

Valeriana tenuifolia Vahl

The area of distribution of *V. tenuifolia* is limited to the hilly region in the south of Poland. Plants from four separate habitats in this region were investigated cytologically. These studies have shown that this species has the tetraploid number of chromosomes ($2n = 28$). The list of habitats is given in Table II. A somatic plate of *V. tenuifolia* is represented on Figs. 3, 9.

Morphology. *V. tenuifolia* seems to be an extremely uniform species. It is represented by slender plants with a rather thin and short flowering stem, in nature usually only 30 to 70 cm high; in uniform garden conditions the flowering stem attains the height of 80 to 100 cm. The stem leaves (Fig. 14) are distinct by the relatively numerous lateral leaflets (10 to 14 pairs). The leaflets are narrowly linear-lanceolate, slightly decurrent, without any toothing; frequently the top pair and the top leaflet are confluent. The rhachis of the leaves is relatively short. The flowers are rather

TABLE II

V. tenuifolia: localities and habitats of the specimens studied

Field No.	2*n*	Locality and habitat	North Latitude	Collector
200	28	Kazimierz on Vistula (Lublin Plateau). Slopes of limestone hills	51°25′	Received from the Rural Scientific Institute in Puławy
248	28	Zelejowa Mt, near Chęciny (Plateau of Little Poland); on limestone	50°50′	W. Szafer
245	28	Westwards of Sandomierz, upper part of a steep slope, in shade	50°35′	M. Piotrowicz
206	28	Bentkowska Valley, north-west of Kraków: shady slope on the bottom of Jurassic limestone rocks	50°5′	M. Skalińska

small, almost white. The diameter of the pollen grains ranges from 36 to 54 μ (Mean = 45·75).

Time of flowering. *V. tenuifolia* is an early-flowering species. In nature it begins its flowering in the last days of May. On the experimental field the specimens brought from natural habitats proved to be earlier in 1949 and 1950 than the earliest strains of *V. exaltata* in the same external conditions: they begun their flowering between the 12-th and the 19-th May, whereas plants of the earliest strain of *V. exaltata* (originating from the Hel Peninsula) expanded their first flowers in the end of May or in the first days of June.

Ecology and geographic distribution. The area of distribution of *V. tenuifolia* in Poland is limited to the hilly regions extending from the Plateau of Little Poland to the Plateau of

Lublin. The plants occur there very locally on limestone slopes, showing a preference for soils with a relatively high pH value; they are found in habitats with a good drainage of the soil but usually on damp and shady places. Concerning ecology, *V. tenuifolia* differs distinctly from *V. exaltata*, being more exclusive than that species in the selection of its habitat. In view of its ecological demands this species is rather rare in Poland. Although the southern part of the area of *V. exaltata* overlaps with that of *V. tenuifolia*, the two species are well separated ecologically since they occur in different habitats.

As far as the general area of distribution of *V. tenuifolia* is concerned this species occurs, according to Kreyer (p. 178), in Bohemia, Württemberg, Austria, Silesia and southern Poland. Although the western forms assigned by Walther to *V. collina* seem to be closely related with *V. tenuifolia*, they are not identical; the main differences concern the number of lateral leaflets which on the average is lower in British forms (6 to 10 pairs), as well as the frequently occurring slight toothing of their margins which in *V. tenuifolia* is wanting.

As pointed out in a previous paper (Skalińska 1947) the distribution of tetraploid Valerians in Great Britain roughly corresponds to that of species which according to Matthews (1937) and Salisbury (1932) represent in the British flora the continental southern geographic element. A common origin of the continental and British forms seems probable; the existing diversity may have resulted from a subsequent regional differentiation. In addition to the purely morphological differences, the occurrence of a polyploid differentiation has been established among the British forms with narrowly linear-lanceolate leaflets (Skalińska 1947). Although Walther regards *V. collina* as a purely tetraploid species, numerous octoploids have been detected among the above forms as a result of cytological studies on British Valerians. It is interesting to note that these forms occur outside the area occupied by tetraploids, extending more northwards and westward. Photos of leaflets of such forms are represented on Plate 7, Fig. 6 of my previous paper. A leaf of a British octoploid plant from Cheddar Gorge is reproduced in the present paper on Fig. 14 together with a leaf of the Polish *V. tenuifolia*. The two leaves show a high degree of similarity; on the whole, the plants may be regarded as corresponding types

although the number of leaflets is somewhat lower in the octoploid than in the tetraploid. The morphological similarity suggests a common origin of the British forms and of those from Central Europe; it also favours the assumption that in Great Britain the change from tetraploid to octoploid conditions enabled the higher polyploids to advance from relatively dry habitats of East Anglia towards the north and west, to conditions with a more oceanic climate which are inaccessible for the tetraploids.

Valeriana sambucifolia Mikan

V. sambucifolia Mikan. (*V. excelsa* Poir.) represents the only octoploid species occurring in Poland. Its main centre of distribution is found in the mountain regions of southern Poland (Tatra Mts, Western Carpathians, Pieniny Mts, Sudeten and Silesia); it occurs however also northwards of this centre in a number of isolated habitats in the plain.

Cytological studies were carried out on root tips of plants originating from ten separate habitats in southern and central Poland, both from the mountains and from the plain (Table III). All plants investigated had 56 somatic chromosomes. A somatic plate of *V. sambucifolia* is represented on Fig. 10.

Morphology. *V. sambucifolia* differs from the two species described above by the presence of overground runners and a strong tendency to vegetative reproduction. The flowering stem is rather short, in natural habitats 60 to 130 cm high; in garden culture some plants attained the height of 165 cm; in the majority of specimens however the height had a range from 75 do 135 cm. The stem leaves bear only 3—4 (—5) pairs of leaflets which are loosely distributed along the relatively long rhachis (Fig. 18). The remarkably low number of lateral leaflets is one of the features of *V. sambucifolia* by which this species may be easily identified and distinguished from *V. exaltata*. The leaflets are usually broadly lanceolate or ovate; the ratio width/length being usually about 0·30. The shape of the leaflets however shows some degree of diversity. In the majority of plants studied the leaflets have a coarse toothing on both margins; in some plants however the teeth are relatively smaller. The flowers are somewhat larger than those

of *V. exaltata*. The pollen diameter ranges from 45·4 to 61·6 μ (Mean = 55·0).

Time of flowering. In natural habitats *V. sambucifolia*

TABLE III

V. sambucifolia: localities and habitats of the specimens studied

Field No.	2n	Locality and habitat	North Latitude	Collector
235	56	Jedlnia nr. Radom. Bank of a rivulet on a moist meadow	51°30′	I. Wichert
203	56	Mników valley, near Kraków. Swampy ground under *Alnus* trees	50°10′	M. Skalińska
266	56	Pieniny Mts. Second peak of Trzy Korony (950 m over sea level). On a shady slope under deciduous trees	49°40′	M. Skalińska and E. Banach
250	56	Western Carpathians. Slope of Babia Góra, roadside (750 m over sea level)	49°30′	E. Banach
217	56	Tatra Mts (limestone part): Mała Łąka valley, on mossy rocks in a stream	49°10′	M. Skalińska
218	56	Tatra Mts (limestone part): Wantule forest in full shade in a ditch. (1500 m o. s. l.)	,,	M. Skalińska
220	56	Tatra Mts (limestone part): Kościeliska Valley, in a ditch.	,,	A. Kłaput
261	56	Tatra Mts (limestone part): Kościeliska Valley, border of a stream	,,	M. Skalińska E. Banach and H. Wcisło
262	56	Tatra Mts: Kościeliska Valley, in a ditch in the higher part of the valley	,,	,,
229	56	Tatra Mts (limestone part); on the northern slope of Giewont (about 1400 m o. s. l.)	,,	A. Kłaput

shows some degree of variability in respect of the beginning of flowering, according to altitude, humidity of the soil and other external conditions; in general however it represents an early-flowering species. In approximately uniform external conditions of the experimental field it begins its flowering very early, simulta-

neously with *V. tenuifolia*. In 1950 almost all specimens of *V. sambucifolia* from various habitats developed already their first flowers on the 12-th May; thus, with regard to the time of flowering this species shows a high degree of uniformity.

Ecology and geographic distribution. It has been mentioned above that in Poland the main centre of distribution of. *V. sambucifolia* is in the mountain regions in the south of the country, and that from this main centre it extends northwards being found in isolated habitats in the plain of central Poland. According to the data based on the study of herbarium specimens it occurs also in Pomerania (Szafer, Kulczyński and Pawłowski 1924 and Walther 1949). Ecologically, this high polyploid seems to be confined to damp and shady habitats. It is rather common in the western (limestone) part of the Tatra Mts where it grows in mountain wood clearings, in valleys on borders of streams and on wet rocks in mountain streams or, less frequently, on steep slopes; its vertical distribution in the Tatra Mts extends to the layer of *Pinus montana*. In Western Carpathians and in Pieniny Mts it is less frequent. In the isolated habitats in the plain its occurrence is confined to swamps, ditches and moist meadows, where it grows under *Alnus* trees.

The general area of distribution of this species is irregular and discontinuous, with one centre in the north, in Scandinavia and Finland, and other centres in mountain regions of Central Europe (Tatra Mts, Carpathians, Sudeten, eastern Alps); isolated habitats in the plain link to some extent the northern and the Central-European centres. Walther presumes that *V. sambucifolia* might be regarded as a northern-mountain species (p. 56). The results of cyto-morphological studies of Runquist (1937) give a strong support to the assumption that it represents a species of northern origin which in the Diluvial period migrated from the arctic southwards to the mountains of Central-Europe. In his paper, Runquist described a form of *V. excelsa (V. sambucifolia)* native at the northern shore of the island Svano (Angermanland); this form is less robust than the typical plants and represents a diploid with 14 somatic chromosomes; it may be considered as a more primitive ancestral type from which the phylogenetically younger octoploids had evolved through chromosome doubling achieved in two steps.

The problem of inter-sterility

The ecological isolation of the three species in Poland does not favour their free intercrossing in nature. Accordingly, spontaneous hybrids have not been found among specimens investigated in the course of the present study.

It should be added that besides the ecological separation another factor may prevent the interspecific hybridization, viz. the different numbers of genomes of the three species. This factor may play an important part in the conditions of experimental field where the plants with different chromosome numbers are grown in close neighbourhood. It is also responsible presumably for the missuccess of artificial crosses. Cross-experiments between *V. tenuifolia* and *V. sambucifolia*, undertaken on a small scale in 1947, have given almost entirely negative results (abortion of embryos, non-germinating seeds, unviable seedlings). The cross-experiments were carried out on potted plants in the laboratory, thus in external conditions which were not very favourable. In view of this and of notable technical difficulties in dealing with the small flower buds, the negative results obtained can not be regarded as wholly convincing. Therefore the problem of the cross-ability of the three karyological types has been attacked on my suggestion in a different way by Miss W. Bieńkowska, at that time a research worker in the Institute of Plant Anatomy and Cytology. In 1949 and 1950 she studied the chromosome numbers of 50 young seedlings of Valerians which germinated in the experimental field. They developed from seeds formed after open pollination of plants belonging to the three species and transplanted from natural habitats. In view of a partial overlapping of their flowering time it could be anticipated that some spontaneous hybrids might be found in the material studied. The close neighbourhood in the experimental field of the three different chromosomic types also seemed to favour the possibility of a spontaneous cross-pollination by insects. In spite of this however not a single hybrid has been found among the 50 seedlings studied. In the material investigated by myself in 1950, I succeeded in detecting only once a hybrid. It has been found among the progeny of *V. sambucifolia* (Field N. 229) from seeds developed after open pollination in the experimental field in 1949 and germinated in the laboratory on Petri-dishes

in 1950. One of these seeds has given a pair of twins: one of the sister-plants represented a smaller polyhaploid seedling ($2n = 28$, Figs. 4 and 11) with only one cotyledon; presumably it developed from a synergid. The second sister-plant was somewhat larger and seemed to be normally developed; this plant however was a hexaploid ($2n = 42$) representing evidently a cross-product of *V. sambucifolia* with a tetraploid plant. Studies on this pair of twins will be published separately in due course. The above observation shows that in rare instances crosses between different chromosomic types are possible, in spite of the existing barrier of incompatibility.

Discussion

The above studies in morphology, cytology, ecology and geographic distribution of the Polish Valerians have revealed considerable differences existing between this group and the British Valerians previously studied by the author.

In the flora of Poland there occur three distinct species well defined morphologically and separated ecologically. On the other hand, in Great Britain it seems impossible to subdivide the collective species *V. officinalis* «sensu lato» into smaller distinct and well separated units owing to the great morphological diversity within the species and the occurrence of a range of intergrading forms which connect the extremes; the various characters of these forms are not regularly correlated.

The distinct morphological separation of the three species occurring in Poland is connected with a cytological differentiation. *V. exaltata* is usually diploid ($2n = 14$); tetraploids with 28 somatic chromosomes seem to represent there only rare exceptions. *V. tenuifolia* is tetraploid ($2n = 28$) and *V. sambucifolia* is octoploid ($2n = 56$). In Great Britain the diploid types are missing; the occurrence of tetraploids and octoploids has been established in the course of the author's previous studies. The existing cytological differentiation however is not strictly connected with the morphology of the plants: the great diversity within each karyological type and the strong overlapping of characters create serious difficulties in a correct identification of the chromosomic types on the basis of purely morphological criteria. The conditions observable in Poland represent probably a more primitive stage in the evolution

of the group than that occurring in Great Britain. This is evident from a careful comparison of the Polish and British forms as well as from the analysis of the differentiation existing within each group.

It has been mentioned already that some of the Polish types have corresponding forms among the British Valerians; the latter represent however types with higher numbers of chromosomes. It is striking that the various biotypes of the Polish diploid *V. exaltata* have much more frequently their parallel forms among the British octoploids than among the tetraploids. On the other hand, corresponding forms of the Polish tetraploid species *V. tenuifolia* have been found both among tetraploid and octoploid specimens of the British *V. angustifolia* Host.

In the British Valerians, the intergrading forms connecting the extremes are presumably of hybrid origin (Skalińska 1947). It may be assumed that a kind of fusion of the formerly separated types has been achieved by their repeated intercrossing, leading to the production of so-called «hybrid swarms» (Allen 1940). In this way presumably the course of evolution of the British Valerians resulted in the creation of a single complex group which is distinctly different from the well separated species from Central Europe.

In connexion with the above considerations, the problem of the origin of *V. procurrens* deserves special attention. It has been already mentioned that Walther subdivided *V. sambucifolia* into two species; the specific name *V. procurrens* has been given by her to forms which are not quite identical with the Central-European *V. sambucifolia* and which replace this species in Western Europe and on the British Isles. According to Walther, the eastern border of its area slightly overlaps the western parts of the areas of *V. exaltata* and *V. sambucifolia*. The former is, according to Hayek and Hegi, an Eurasiatic species and the latter has presumably a northern origin (see above). On the other hand, the origin of *V. procurrens* remains unknown. Its distribution suggests that it could have arisen by hybridization. Morphologically, *V. procurrens* is in some respects an intermediate form between *V. exaltata* and *V. sambucifolia*, or speaking more strictly it seems to be a group of intergrading forms, showing some degree of diversity. To take only one example: the number of lateral leaflets which, according to Sprague (1943,

p. 102) affords the best clue to the subdivision of *V. officinalis*, represents in the forms of *V. procurrens* a rather variable character, namely 4—7 (—8) pairs, their number oscillating between those of *V. sambucifolia* and *V. exaltata*. It may be assumed that the above two species are the ancestors at least of some representatives of this group. Intercrossing could have taken place with the advance of the putative parent species to the west into territories where their ecological separation becomes less marked in a more oceanic climate. It seems plausible that no diploids, but tetraploids of *V. exaltata*, or other tetraploid related forms, could have taken part in the process of hybridization.

In spite of the existing incompatibility barrier between octoploids and tetraploids which usually prevents their intercrossingt, in rare instances hybrids may arise. This is due to a rather frequen, occurrence of giant (unreduced) grains in the pollen of tetraploids as studies on British plants have shown (Skalińska 1947). Such giant pollen grains are able to germinate on the stigma of octoploid plants, giving rise to octoploid hybrids (Skalińska, unpublished data). Another possibility of hybridization may be created by a somatic doubling similar to that observed once in a British tetraploid which developed an octoploid sector in one of its soboles (Skalińska 1947). The doubling of chromosome numbers gives new possibilities with regard to hybridization between related species which have attained the same degree of polyploidy. Subsequently the products of hybridization may continue to cross leading to the formation of large groups with a considerable degree of diversity.

According to Walther *V. procurrens* represents an octoploid species; the British herbarium specimens however analysed by her show that the group is not uniform cytologically: among the plants assigned to this species (p. 59) besides octoploids also tetraploids are found (e. g. plants from Mercombe Wood, coll. T. A. Sprague, Kew Herbarium; living specimens from this locality have been investigated by me in the course of my previous work). The difficulty of a correct determination of representatives of this critical species is increased by the occurrence in Germany of hybrids with *V. sambucifolia* (Walther 1949) and in Great Britain of the wide range of intergrading forms linking it with *V. officinalis* s. str. All the above facts suggest that *V. procurrens* is not a separate

species well defined morphologically but rather a large polyploid complex of hybrid origin.

Recent studies have shown that in many instances polyploid species or races have a wider geographic and ecological range than the corresponding diploids (Hagerup 1932, 1939, Manton 1934, 1937, Anderson and Sax 1936, and many others). The same interrelation has been observed between tetraploid and octoploid Valerians in Great Britain (Skalińska 1947). The area of the tetraploids is limited to the south of England and parts of the Midlands, whereas the octoploids proved able to advance beyond this area to the north of Scotland and to extend notably the geographic and ecological range of this species. In Polish Valerians however the situation is entirely different: the diploid species *(V. exaltata)* has a wider geographic and ecological range than the two polyploid species *V. tenuifolia* and *V. sambucifolia*. The very wide area of *V. exaltata* suggests that it represents an old species which was capable to an expansion over vast territories. The present studies have shown that this species is split into numerous biotypes specialized for various ecological conditions. Presumably the occurring intraspecific differantiation has been attained gradually in the way of a large number of gene-mutations in different points of its area. It should be emphasized that particularly on the diploid level these processes seem to have an evolutionary value, since they lead to the production of small but significant morphological differences which represent the first steps of the subsequent divergence. In consequence of such a purely genic differentiation, an old diploid species, taken as a whole, attains the capacity to persist in a variety of habitats and it may be of a rather common occurrence within its large area.

The above observations on diploid Valerians give further support to the opinion of Gustafsson (1947) that «the diploids are not inferior to polyploids with regard to their abundance and distribution within a definite flora» (p. 280).

Another example of a wide area of distribution of an old diploid species if found in *Aquilegia vulgaris* L. Its area extends over large territories of Asia and Europe. In the northern part of its range there are no other species of this genus; by contrast, a large number of phylogenetically younger species (e. g. *A. alpina* L., *A. Reuteri* Boiss., *A. Einseleana* Schultz, *A. Heankeana* Koch, *A. transsylvanica* Schur) are found near the southern limit of its area.

They attain the greatest diversity in the regions of the Alps and of Transsylvania, representing ecospecies confined to small areas. All these forms are diploid. A morphological analysis permits to assume that they have gradually evolved from *A. vulgaris* by gene mutations followed by an ecological isolation (Skalińska 1940, and unpublished). In the case of *Aquilegia* the differentiation achieved on the diploid level has attained a still higher degree than in *V. exaltata*; it resulted in the production of types which have reached the rank of new species.

In the eastern part of its area, *V. exaltata* is represented only by diploid biotypes. The occurrence of tetraploids in western Poland in one isolated habitat may be regarded as a step of a polyploid differentiation of this species. Difficulties of tracing in Western Europe the distribution of types of *V. exaltata* with doubled chromosome numbers have been pointed out above. According to the details of distribution of *Valeriana* species in Western Europe found in Walther's paper, *V. exaltata* roughly attains the 8° E. Long. more northwards and the 12° E. Long. more southwards, slightly exceeding towards the west the range of *V. sambucifolia*. It is possible that a further advance of these two species has been prevented by a climatic barrier. Their rather abrupt diminution coincides with the appearance of the polyploid complex named by Walther *V. procurrens* and regarded by her as an atlantic species (p. 94). The map of distribution found in Walther's paper (p. 52) suggests that in that region the two formerly separate species have merged into one another to produce a polyploid complex of hybrid origin. The new forms which presumably have arisen by convergence (Huxley 1942, p. 339) represent a discontinuous group of vigorous plants with increased capacity of vegetative reproduction. They seem also to show a higher degree of tolerance for oceanic climatic conditions in Western Europe and the British Isles than their putative ancestors and therefore they proved able of a greater expansion over these territories than the continental species.

Summary

In the flora of Poland the section *Officinalis* of the genus *Valeriana* L. is represented by three species which are sharply delimited both morphologically and cytologically.

V. exaltata Mikan is almost exclusively represented by diploid types ($2n = 14$); this type has been found in 26 different habitats ranging from the Baltic coast in the north to the Carpathians in the south; only in a single habitat in west-central Poland tetraploids ($2n = 28$) have been detected. This diploid species has a very large area of distribution and a great ecological range. It is split into numerous biotypes specialyzed for various ecological conditions. Presumably the occurring intraspecific differentiation on the diploid level has been attained gradually through gene-mutations in different points of its area.

V. tenuifolia Vahl. is a tetraploid species ($2n = 28$) confined to hilly regions in southern Poland where it occurs very locally on limestone, usually in shady places; accordingly, this species seems to be more exclusive in the selection of its habitats than the widely distributed diploids.

V. sambucifolia Mikan. is an octoploid species ($2n = 56$); its main centre of distribution in Poland is found in the mountain regions in the south (Tatra Mts., Western Carpathians, Pieniny Mts., Sudeten and Silesia); from this main centre it extends northwards being found in isolated habitats in the plain of Central Poland. Ecologically this high polyploid seems to be confined to shady and damp habitats. *V. sambucifolia* has an irregular and discontinuous general area of distribution with one centre in the north (Scandinavia and Finland) and other centres in mountain regions of Central Europe.

The distinct separation of the above three species is in sharp contrast with conditions observed on the British Isles where a range of intergrading forms connects the extreme types of Valerians. It may be assumed that a kind of fusion of the formerly separated types has been achieved by hybridization. The probable course of evolution in the group *Officinalis* may be outlined as follows: two Central-European species, *V. exaltata* and *V. sambucifolia* have contributed to the creation of the wide range of forms in Great Britain and Western Europe. *V. exaltata* is an Eurasiatic diploid species. The occasional occurrence of tetraploids in Poland in the western part of its area shows the existence of some polyploid differentiation within this species. A further western expansion of this species seems to be prevented by a climatic barrier in Central Europe. *V. sambucifolia* is an octoploid species presumably of nor-

thern origin. It might have evolved from a more primitive diploid type still occurring in a single habitat in Scandinavia (Runquist 1937). Subsequently the octoploids migrated southwards and westwards in Central Europe. The area of *V. sambucifolia* overlaps in its western part with that of *V. exaltata*, a distinct ecological separation however prevents intercrossing. The rather abrupt diminution of *V. exaltata* and *V. sambucifolia* in Central Europe coincides with the appearance of *V. procurrens* Wallroth — a polyploid complex of hybrid origin. This suggests that in that region the two formerly separate species have merged into one another. Possibilities of their intercrossing in Western Europe were presumably favoured by a less marked ecological separation in a more oceanic climate. In spite of a high degree of incompatibility between different chromosomic types, hybrids may arise in rare instances; subsequently the products of initial hybridization might have continued to cross leading to the production of a large polyploid complex. This phylogenetically younger group formed by convergence shows a higher degree of tolerance for oceanic climatic condition than its putative ancestors and proved able of an expansion over Western Europe and the British Isles. The third species, *V. angustifolia* Host is involved in the process of hybridization in a considerably lower degree.

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Explanation of plates

Figs. 5—7. *Valeriana exaltata* (diploids). Microphotos of somatic metaphases in root-tips: 1) plant 230 from the Hel peninsula; 2) plant 221 from the primeval forest of Białowieża; 3) plant 239 from the surrounding of Puławy (Figs 5 and 6 × 1750, Fig. 7 × 2000).

Fig. 8. *V. exaltata* (tetraploid), plant 225 (× 1750).

Fig. 9. *V. tenuifolia*, plant 206 from Bentkowska Valley (× 1750).

Fig. 10. *V. sambucifolia*, plant 235 from Jedlnia nr. Radom (× 1750).

Fig. 11. A polyhaploid plant with 28 chromosomes from a twin pair of *V. sambucifolia* field No. 229 from the Tatra Mts. (× 1750).

Fig. 12. *Valeriana exaltata* (diploids); Photos of stem leaves less frequent types with relatively narrow leaflets; from left to right: pl. 231, 232, 223 (see Table I) (× 0·33).

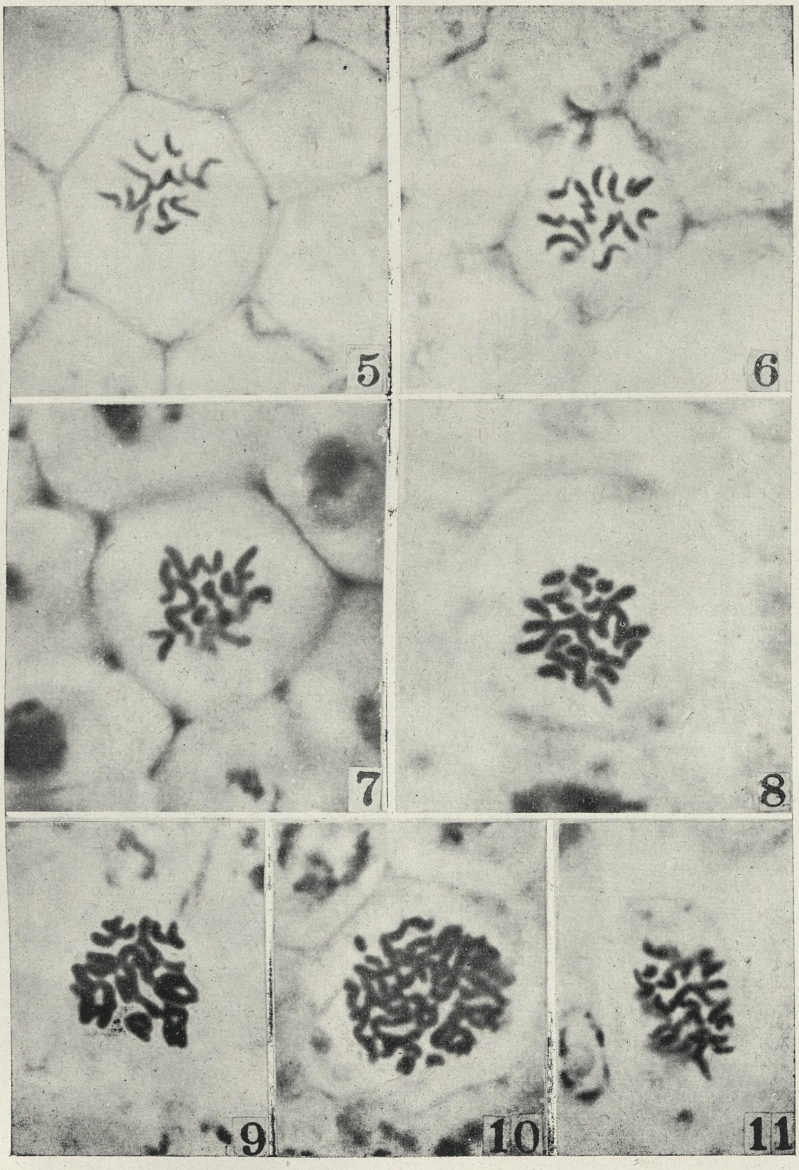

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Fig. 13. Lateral leaflets of diploid and tetraploid plants of *V. exaltata* compared with a leaflet of a British octoploid. The numbers of lateral leaflets in the resp. compound leaves are given in brackets. First row: diploids: pl. 231 (8 pairs); pl. 232 (6 pairs). Middle row: diploids: pl. 221 (8 pairs) pl. 230 (7 pairs) pl. 233 (8 pairs), pl. 223 (8 pairs). Third row: a diploid: pl. 228 (7 pairs), a tetraploid: pl. 225 (7 pairs), a British octoploid from Sapperton Valley, Cotswold Hills representing a corresponding type (6 pairs) (× 0·25).

Fig. 14. Photos of leaves of the tetraploid *V. tenuifolia*, pl. 206, from Bentkowska Valley (left) and of a British octoploid *V. angustifolia* Host from Cheddar Gorge (right) (× 0·33).

Fig. 15. Stem leaf of *V. exaltata* (diploid), pl. 228, (× 0·33).

Fig. 16. Stem leaf of *V. exaltata* (tetraploid), pl. 225 (× 0·33).

Fig. 17. Stem leaf of a British octoploid representing a corresponding type (Sapperton Valley) (× 0·33).

Fig. 18. Stem leaf of *V. sambucifolia*, pl. 203 from Mników Valley, nr. Krakow (× 0·33).

Figs. 12—17, plants from the experimental field, Krakow; fig. 18, plant from its natural habitat.

Wpływ pochodnych pyrazolonu na podziały komórkowe i przemiany kwasu tymonukleinowego w merystemach korzeni Allium cepa L. i Phaseolus multiflorus Willd — L'action des dérivés du pyrazolone sur les divisions mitotiques et sur le métabolisme de l'acide thymonucléique dans les meristèmes des racines de Allium cepa L. et de Phaseolus multiflorus Willd

Mémoire

de M. **A. VORBRODT**

présenté le 9 Octobre 1950 par Mme M. Skalińska m. c. et M. B. Pawłowski m. c.

(Planches 6—7)

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Le problème

Le mécanisme et le dynamisme de la division mitotique ont depuis longtemps attiré l'attention d'un grand nombre de biologues. De nombreuses expériences ont démontré que divers agents

physiques et chimiques sont capables de produire des perturbations particulières au cours de la mitose. Ces recherches, exécutées à l'aide de méthodes biochimiques et histochimiques ont permis d'élucider la part jouée par les procès physico-chimiques au moment de la division cellulaire.

Levis et Bauer (1923, d'après Fell et Hughes) ont décrit les perturbations mitotiques produites par l'action de divers acides. Mottram (1928, d'après Fell et Hughes) a étudié l'influence du dioxyde de carbone. Lewis (1934, d'après Fell et Hughes) et Möllendorf (1938) se sont occupés de l'influence exercée par le milieu hypotonique sur la croissance et la division de cellules cultivées in vitro. On a découvert une série de substances capables de provoquer de perturbations caractéristiques au cours de la division mitotique. Lavan les a désigné sous le terme de substances *c*-mitotiques. Les traits les plus saillants de la *c*-mitose sont, entre autres, les suivants: inactivité des fuseaux, contraction des chromosomes et augmentation de leur viscosité, formation de cellules polyploidales et arrêt de l'activité de certains ferments. Parmis les substances *c*-mitotiqus il faut en premier lieu mentionner la colchicine dont l'action a fait l'objet de recherches de nombreux investigateurs (Levan 1943, 1944, Oestergren 1943, 1944, Łączyńska 1947, Steinegger 1947, 1948, Filutowicz 1950 et d'autres). On a observé l'apparition de phénomènes semblables sous l'influence du acénaphtène (Shumck 1940, d'après Oestergren et Levan) et du camphre. On a démontré expérimentalement que de pareilles perturbations sont également provoquées par les hydrocarbones dits cancerogènes (v. par. ex. Möllendorf 1939, Faller) et par le gaz de moutarde (Fell et Hughes 1949). Parmentier (1949) a décrit l'action antimitotique exercée sur les cellules animales par quelques phénols et amines. Deysson (1949) par contre, a noté un arrêt de la croissance et une perturbation de la mitose dans les racines de l'oignon sous l'influence de l'antipyrine.

L'objet de nos recherches consistait à étudier l'action exercée par les dérivés du phenylpyrazolone sur la croissance et la division des cellules du meristème végétal. La hypersensibilité manifestée à l'égard des dérivés en question par certains individus en voie de traitement par ces dérivés ajoutait un intérêt particulier à notre étude. Il est probable que ces substances agissent antimitotique-

ment sur les cellules de la moelle osseuse. La similitude des processus de division observée chez les cellules animales et végétales nous autorisait à prendre comme matérial d'observation des tissus d'origine végétale, tissus plus commodes à étudier du point de vue méthodique que les tissus des animaux.

Le présent mémoire résume les résultats de nos recherches qui avaient pour but d'étudier: 1° l'action exercée par les solutions aqueuses du phénylpyrazolone sur la croissance et la division des cellules du tissu méristémal de la racine, 2° les perturbations causées par les solutions en question au moment de la division mitotique.

Nous avons également englobé dans nos recherches l'acide thymonucléique auquel Caspersson (1939, 1947) attribue un rôle important dans les procès cellulaires vitaux, surtout au moment de la division. Nous avons essayé de trouver une réponse à la question suivante: quelle est l'influence des dérivés du pyrazolone sur la répartition et le métabolisme de l'acide thymonucléique? Un examen des perturbations dans le métabolisme des acides nucléiques et de leur influence sur le cours de la mitose peut contribuer à éclaircir d'une part la nature des processus vitaux cellulaires et d'autre part à faire mieux comprendre l'importance des acides en question au moment de la division.

Le matériel et les méthodes

Nous avons fait deux séries d'expériences au cours du mois d'avril de l'année 1950. Dans la première série nous avons utilisé comme matériel expérimental 30 bulbes d'oignon *(Allium cepa)*. L'objet de cette série d'expérience consistait à étudier l'influence de deux dérivés du pyrazolone sur la croissance des racines, à savoir: de l'antipyrine *Pyrazolonum phenylodimethylicum* et du pyramidon *Pyrazolonum dimethyloaminophenylodimethylicum* en solution aqueuse à 1/100. Les bulbes étaient placées dans de l'eau ordinaire (eau de robinet) pour 48 heures; les racines ayant atteint pendant ce temps des dimensions convenables, on procédait à la mesure exacte de leur longueurs au moyen d'un vernier, après quoi les bulbes étaient transportées dans des solutions d'antipyrine et de pyramidon. Ensuite on répétait les mesures de longueurs des racines 4 fois à des intervalles de 12 heures. Les résultats des mesures, c'est à dire les moyennes des accroissements en longueur des racines ont été représentés graphiquement.

Dans la deuxième série comme matériel nous avons employé les racines du haricot *Phaseolus multiflorus* provenant de 130 graines dont le poids moyen s'élevait à 1·7 g. Ces expérience avaient pour objet une étude de l'influence exercée par les dérivés du pyrazolone sur la division mitotique et sur le métabolisme de l'acide thymonucléique contenu dans les cellules du meristème des racines. Parmi les graines qui ont germè nous en avons choisi 60 à l'aspect sain et dont les racines avaient atteint 2 cm de longueur. Les plantules séparées en quatre lot de 15 pièces ont été placées dans quatre vases de capacité d'un litre chacun remplis de solutions que voici:

vase No. 1: eau ordinaire, exp. de contrôle
„ „ 2: solution d'antipyrine à 1/1000
„ „ 3: „ de pyramidon à 1/1000
„ „ 4: „ „ „ à 1/500

L'expérience a eu lieu à l'obscurité et à la température de $+18^0$ à $+20^0$ C. Ensuite, après 10, 22, 46, 70 et 144 (= 6 jours) heures nous avons coupés les extrémités des racines sur une longueur de 0·75 cm. Pour les fixer nous les avons placées pour un temps d'une demiheure à une heure dans un mélange d'une solution de sublimé à 6% et d'acide acétique glacial (100 ml de la solution du sublimé pour 2 ml de l'acide acétique). L'inclusion des racines, traitées au préalable par l'alcool (iodé) et par le benzène se faisait dans de la paraffine. A l'aide d'un microtome les bouts des racines ont été découpés en tranches d'une épaisseur de 10 μ et les tranches collées sur des porte-objets albuminés. Chaque racine donnait en moyenne environ 100 sections. Pour colorer les chromosomes et l'acide thymonucléique nous avons employé la méthode de Feulgen et suivi les prescriptions de Romeis (1943). Le cytoplasme était légèrement coloré d'un vert clair. Pour la coloration nous avons également eu recours à l'hématoxyline ferrique de Heidenhain, ainsi qu'à l'hématoxyline acide d'Ehrlich, après avoir préalablement hydrolysé les coupes avec du 1 N HCl, comme dans le procédé de Feulgen.

Dans les calculs des phases mitotiques nous avons suivi en partie la méthode indiquée par Faller (1942). Toutefois dans nos recherches nous avons laissé de coté la phase de reconstruction à cause de perturbations très spéciales provoquées par l'action des substances employées et à cause du manque de traits discriminatifs

nets. En accord avec la majorité des cytologues nous avons distingué les 4 phases suivantes de la mitose: a) prophase, b) métaphase, c) anaphase, et d) télophase.

a) Voici comment Faller décrit la première de ces phases: «Au début de la mitose les cellules du meristème de la racine du haricot ne peuvent pas s'arrondir d'une manière naturelle. On reconnait alors le commencement de la prophase qui se manifeste par un grossissement du noyau et par la concentration de substances qui se colorerent positivement par la méthode de Feulgen». D'après cet auteur le nucléole du noyau demeure visible jusqu'au moment de l'apparition distincte des filaments de chromatine.

b) «On peut ici appliquer une division de la métaphase en b_1 et b_2».

b_1) «La membrane du moyau devient invisible... le déplacement des chromosomes, c'est à dire la métakinese commence (Wassermann 1926, d'apres Faller). Möllendorf appelle ce stade «ungeordnete Bewegung» et le regarde comme le premier degré de la métaphase». Certains botanistes (Strassburger et d'autres) placent ce stade non pas dans la métaphase mais dans la prophase et c'est la raison pour laquelle ils unissent quelquefois ces deux phases en une seule $a+b_1$.

b_2) Ce stade est caractérisé par l'apparition du fuseau et par le groupement ordonné des chromosomes dans le plan équatorial. Si l'on tient compte de l'intensité de coloration on constate qu'à ce moment l'accumulation de l'acide thymonucléique dans le chromosomes atteint son maximum.

c) A l'anaphase on observe la séparation des chromosomes secondaires et leur migration vers les pôles.

d) Comme commencement de la télophase, nous avons pris, en suivant l'exemple de Faller, «le tassement polaire», comme fin la formation de la membrane cellulaire, procès dans lequel le phragmoplaste joue un rôle très actif.

L'influence des dérivés du pyrazolone sur la croissance de jeunes racines

Dans la première série d'expériences nous avons étudié l'influence des dérivés du pyrazolone sur la croissance des racines d'oignons en employant des solutions aqueuses de l'antipyrine et du pyramidon à 1/100. Le tableau ci-dessous résume les résultats des mesures.

Déja au cours de la seconde mesure nous avons pu observer un ralentissement de la croissance accompagné d'un grossissement du diamètre des racines. Pareillement, la croissance de jeunes racines était presque completement arrêtée. On a constaté une sorte de contraction chez les racines exposées à l'action de l'antipyrine pendant 24 heures, toutefois après 36 heures leur longueur

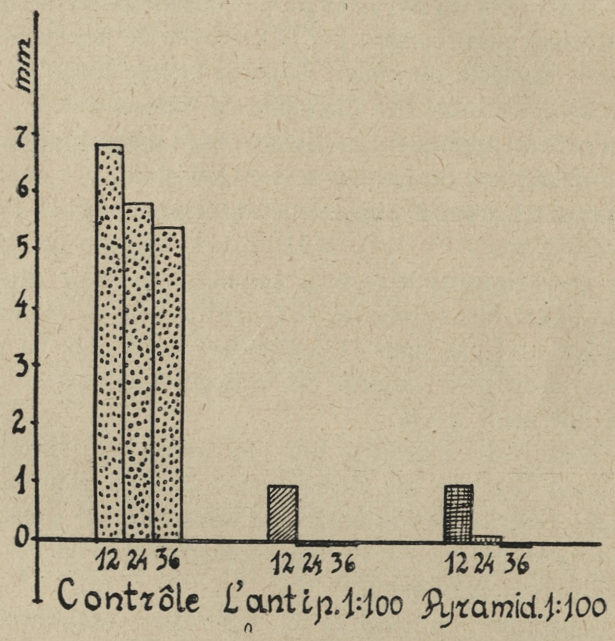

Fig. 1. *Allium cepa.* Elongation des racines de contrôle et expérimentales après 12, 24, 36 h.

n'avait pas subit de changement. L'influence du pyramidon était semblable à celle de l'antipyrine: après 12 heures on a observé également un arrêt partiel de la croissance ainsi qu'un grossisisement du diamètre des racines. L'arrêt de la croissance se prolongeait pendant les 24 heures suivantes et après 36 heures était suivi d'un raccourcissement de la longueur. Les phénomènes de gonflement et de la contraction des racines étaient probablement provoqués par des perturbations dans le régime aqueux des tissus et par leur répercussion sur l'état colloidale du protoplasme. Pour éviter l'apparition de ces phénomènes, attribuables sans aucun doute

à l'emploi de trop fortes doses des dérivés du pyrazolone, nous avons abaissé leurs concentrations dans la deuxième série d'expériences.

Dans cette série, exécutée sur des haricots avec des solutions de l'antipyrine à 1/1000 et du pyramidon à 1/1000 et à 1/500, la longueur initiale des racines était la même dans toutes les séries. Après 3 jours nous avons remarqué que les racines des séries expérimentales étaient plus courtes que les racines de la série de contrôle et que le développement des racines latérales a été fortement enrayé. Après six jours les différences étaient très marquées ainsi que cela résulte des photographies (Fig. 3, 4, 5). L'action de l'antipyrine à 1/1000 était plus faible que celle du pyramidon à 1/1000, et celle-ci à son tour plus faible que l'action du même dérivé employé en solution à 1/500.

Pourcents des phases mitotiques et observations des coupes de contrôle

Avant d'aborder les problèmes qui font l'objet de nos recherches nous avons jugé nécessaire de faire une étude préliminaire des cellules des racines normales. La distinction entre les noyaux en repos et les noyaux au stade de prophase et de télophase finale — noyaux colorés par le procédé de Feulgen — nous intéressait tout particulièrement. Les noyaux en repos ne se colorent presque pas, on aperçoit seulement une faible trace rosâtre de la substance nucléaire dans laquelle se trouvent en petit nombre de fines granulations, positives par raport au réactif de Feulgen, et presque toujours situées près de la membrane nucléaire. Le nucléole est relativment grand, rond, fortement réfractant les rayons lumineux et complétement incolore. Comme dans les cas décrits par Makarov (1948) il est impossible d'observer la structure chromatique.

Au début de la prophase, définie par Jaburek (1929) sous le terme de «sol-gel» (c'est à dire du passage du sol carioplasmatique en gel), a lieu une augmentation du volume du noyau causé par des processus physico-chimiques consistant dans une augmentation de la quantité d'eau. Simultanément a lieu l'accumulation de substances F.+ produite probablement par la synthèse plus active de l'acide thymonucléique et par la décomposition des proteines unissant les chromomères (Caspersson, Brachet 1947). L'appa-

rition de granulations nombreuses est probablement le résultat de l'accumulation de l'acide thymonucléique en assez grandes agglomérations dont l'union conduit à la formation de filaments chromatiques. A la fin de la télophase a lieu la constitution des noyau-fils, au début ils sont petits et renferment de nombreuses granulations F.+ Au cours de cette phase le phragmoplaste et la nouvelle membrane cellulaire sont nettement visibles. Une fois le processus de la télophase achevé, les noyaux reprennent leur aspect de repos et les nucléoles apparaissent. Les noyaux toutefois, sans passer par un repos prolongé, recommencent à se diviser. Ce fait a été également observé et décrit par Faller (1942). Les phases et les détails morphologiques cités nous ont servi de base pour les calculs des pourcents des fréquences des différentes phases mitotiques. Les résultats, basés sur 1500 cellules en état de division sont réunis dans le tableau I.

TABLEAU I

Le pourcentage de phases mitotiques observées dans les cellules normales

Temps d'observation	Prophase	Métaphase		Anaphase	Telophase
	a	b_1	b_2	*c*	*d*
Après 10 h.	47	6	22	7	18
,, 22 ,,	48	10	17	6	19
,, 46 ,,	46	9·6	20·5	7·6	16·3
,, 70 ,,	61	5·7	12·8	6·8	13·7
,, 6 j.	56·6	13·3	15·6	4·4	10·1
,, 8 ,,	45·4	11·1	21·6	5·5	16·4
Moyenne	50·6	9·3	18·2	6·2	15·7

Faller (1942) indique pour les radicules du haricot les nombres suivants: *a*: 33·5—47%, b_1: 4·5—12%, b_2: 10—19%, *c*: 4—8%, *d*: 9—12%. En se basant sur ces données il a également estimé la durée relative des différentes phases: $(a+b_1):b_2:c:d=12:4:1:3$. D'après les observations de Strassburger (1880, selon Faller 1942) et de Jaburek (1929) les durées des différentes phases mitotiques s'élèvent à:

	selon Strassburger	selon Jaburek
a) prophase	180—240 min	156 min
b) métaphase	60 ,,	46 ,,
c) anaphase	20 ,,	27 ,,
d) télophase	40 ,,	33 ,,

Avec les données de Strassburger, pour les durées relatives des phases mitotiques, exprimées au moyen de la méthode de Faller, on trouve les nombres suivants: $a:b:c:d = 9$ ou 12:3:1:2. Dans nos recherches les durées relatives s'expriment approximativement par les relations suivantes: $(a+b_1):b_2:c:d=10:3:1:2$. Il est évident qu'à cause des fluctuations dans le nombre des mitoses au cours de 24 heures ces données ne sont qu'approximatives, néanmoins elles permettent de se rendre compte des durées relatives des différentes phases mitotiques des cellules faisant partie du meristème des racines du haricot.

L'influence des dérivés du pyrazolone sur le pourcentage des phases mitotiques

Une fois connues la morphologie et la durée des phases mitotiques des cellules normales il a été possible d'étudier le matériel provenant des expériences proprement dites. En premier lieu nous avons calculé les fréquences, exprimées en pourcent, des différentes phases mitotiques et nous avons représenté graphiquement les résultats de cette opération (Fig. 2). Remarquons qu'après 24 heures le nombre des cellules au stade de la prophase augmenta, ce qui semble indiquer que les dérivés du pyrazolone agissent au début d'une manière stimulatrice sur les processus cellulaires qui déterminnet le passage des cellules en repos au stade préparatoire de la division. Cependant à mesure que l'action de la substance en question se prolonge, le nombre de télophases augmente en même temps. Ce fait devient particulièrement saillant chez les cellules des racines exposées pendant 46 heures à l'action du pyramidon en solutions à 1/1000. Si nous calculons les durées relatives des phases mitotiques nous obtenons des chiffres qui présentent des écarts considérables par rapport aux nombres normaux. Après 46 heures avec les pyramidon nous avons les résultats suivants $(a+b_1):b_2:c:d = 8:1:1:7$. L'arrêt très marqué de la division cellulaire au stade de la télophase est l'effet de perturbations mitotiques dont il sera question plus loin.

Nous avons constaté une absence complète de divisions dans les radicules qui avaient été exposées à l'action de l'antipyrine à 1:1000 pendant 6 jours, à l'action du pyramidon à 1:1000 pendant 70 heures ou à 1:500 pendant 46 heures. Il résulte de ces nombres

que l'activité du pyramidon est supérieur à celle de l'antipyrine. Si l'on interrompt l'action des dérivés du pyrazolone en transportant les racines des solutions dans de l'eau ordinaire on observe une reprise de l'activité mitotiques des cellules qui, à juger par les pourcents des fréquences des phases mitotiques ne dévie pas de la norme.

Fig. 2. Le pourcentage des phases mitotiques dans les racines du haricot. Les chiffres indiquent le moment de la prise du matériel expérimental: 1-après 10, 2-après 22, 3-après 46, 4-après 70 heures; 5-après 6, 6-après 8 journées (dont 2 jours dans l'eau courante). Dans chaque coupe on a compté 250 cellules au cours de la division; dans les racines de contrôle on a compté au total 1500 mitoses, dans les racines sous l'influence de la solution d'antipyrine 1:1000 —1250 mitoses, dans les racines sous l'influence de la solution du pyramidon 1:1000—1000 mitoses.

Les perturbations de la mitose causées par l'action des dérivés du pyrazolone

Voici la description des perturbations de la mitose observées dans les racines du haricot et causées par l'action des dérivés du pyrazolone:

a) Prophase: Le premier indice de l'activité des agents utilisés consiste dans une augmentation du nombre et des dimensions des granules F.+ (chromocentres) dans les noyaux prophasiques.

Ces chromocentres au fur et à mesure de la durée de l'expérience deviennent de plus en plus gros et finissent par rappeler, au moment de l'arrêt complet des divisions, les chromosomes par leur aspect et par leurs dimensions.

b) Métaphase: Dans les conditions normales tous les chromosomes viennent se placer les uns près des autres pour former le plan équatorial. Dans les coupes toutefois provenant des racines expérimentales certains chromosomes n'arrivent pas à atteindre ce plan. Ces chromosomes en retard se trouvaient tantôt d'un coté, tantôt de l'autre du plan équatorial. Dans certaines cellules la substance F.+ se présentait sous l'aspect des filaments très fins qui aboutissaient aux chromosomes regulièrement disposés (Fig. 9, 11). Il se peut que ces filaments soient des chromosomes démesurement étendus qui ont partiellement réussi à atteindre le plan équatorial et se sont étendus sous l'influence de forces agissant sur leurs télomères. Quelquefois les aspects présentés par ces perturbations étaient tellement anomales qu'en les comparant avec les figures décrites par divers auteurs (Möllendorf 1943, Fell et Hughes 1949), nous avons cru que nous étions en présence d'une sorte de métaphase tripolaire. On a observé l'apparition de phénomènes semblables à la suite de l'action des rayons X (dans les tissus de tumeurs), de l'action de certains hormones sexuels, de hydrocarbones cancerogènes, de gaz de moutarde (ypérite) et d'autres agents. Certaines irrégularités mitotiques se rencontrant également dans les tissus normaux, nous avons exécuté des calculs dont le but était de permettre une comparaison des pourcents des figures mitotiques anomales observées à cette phase dans les coupes expérimentales et de contrôle. Nous avons constaté que dans le tissu normal le nombre maximum de perturbations se rencontre dans le stade b_2 de la métaphase. Les pourcents de perturbations (basés sur l'observation de 300 cellules) s'élevaient

après 10 heures à 13·4% de toutes les cellules à la phase b_2
„ 46 „ „ 14·9% „ „ „ „ „ „ „ „

Les nombres correspondants pour les tissus expérimentaux sont:

antipyrine à 1:1000 après 70 h. — 33·7%
pyramidon „ 1:1000 „ 46 „ — 38·4%

c) Anaphase: Dans ce stade de la division le nombre de perturbations est beaucoup plus petit par rapport au précédent. Dans certaines cellules on pouvait observer la présence de minces anastomoses présentant la réaction positive de Feulgen (F.+) et reliant les groupes de chromosomes en voie de dispersion. On peut interpréter ce phénomène soit en atribuant une viscosité un peu plus grande aux chromosomes, soit en supposant que leur division est incomplète. Certains chromosomes étaient restés en retard au cours de leur acheminement vers les poles cellulaires.

d) Télophase: Dans cette phase le nombre de figures mitotiques anomales atteint son maximum. Les noyaux secondaires en train de formation étaient souvent de grandeur inégale et au lieu de se séparer ils conservaient leur position tout près de la membrane cellulaire. Dans les coupes provenant des racines du haricot exposées pendant 46 heures à l'action du pyramidon à 1/1000, le nombre des cellules avait considérablement augmenté. Leur noyaux étaient petits avec de nombreux chromocentres rapprochés les uns des autres. Dans de nombreuses cellules le phragmoplaste, la membrane cellulaire et le nucléole étaient formés d'une manière anomale (Fig. 7). L'influence de l'antipyrine était semblable à celle du pyramidon, tout en restant un peu plus faible. A cause de perturbations dans le fonctionnement du phragmoplaste la formation des membranes cellulaires était incomplète ou défectueuse, circonstance qui conduisait à l'apparition de cellules binucléaires.

L'influence des dérivés du pyrazolone sur l'acide thymonucléique

En examinant les coupes expérimentales on constate une accumulation considérable d'acide thymonucléique dans les noyaux en repos et prophasiques. Pendant la métaphase et l'anaphase cet acide ne présentait aucun indice d'anomalie. Seuls les chromosomes des racines exposées pendant 70 heures à l'action de l'antipyrine étaient plus courts et plus gros que les chromosomes normaux. Les plus grandes anomalies se manifestèrent au cours de la télophase et de la période de reconstruction. Les noyaux secondaire ne grossissaient pas; situés irrégulièrement ils étaient souvent de grandeur et de forme inégales. Dans de nombreux cas les nucléoles n'apparaissaient pas et la membrane qui sépare les cellules-filles ne se formait pas. Pendant la télophase et la période de recon-

struction la quantité d'acide thymonucléique ne diminuait pas, tout au contraire cet acide s'accumulait sous forme d'agglomérations qui par leur aspect rappelaient les chromosomes et qui souvent se groupaient dans le plan équatorial en deux pôles opposés, voir même dans trois saillies du noyau (Fig. 7, 8). Ce genre d'irrégularités suggére les endomitoses décrites par Dangeard (1947). Le plus souvent toutefois ces agglomérations d'acide thymonucléiques étaient irrégulièrement dispersées à l'intérieur du noyau et parfois même apparaissaient à l'extérieur de la membrane nucléaire.

Discussion

Deysson (1949) a attiré l'attention sur l'influence exercée par des solutions aqueuses de l'antipyrine (à 1/50—1/200) sur les racines de l'oignon. A son avis l'action mitostatique exercée par cette substance se rapproche de l'activité bactériostatique des antibiotiques. Les perturbations causées par les lésions du fuseau et du phragmoplaste conduisent à l'apparition de figures anomales des phases mitotiques et à la formation de cellules bi- et multinucléaires.

A la suite de nos observations préliminaires nous avons dirigé notre attention plus spécialement sur l'influence du degré de dilution des dérivés du pyrazolone sur les phénomènes mitotiques. Il semble que l'emploi de concentrations trop fortes n'est pas indiqué, en effet elles modifient à un haut degré les propriétés physiques du milieu ambiant et il se peut que les effets observés soient attribuables précisément à ces modifications (par ex. hypotonie du milieu extérieur). A notre avis l'arrêt dans le fonctionnement normal du phragmoplaste et du fuseau est le résultat de perturbations profondes qui s'accomplissent dans les processus enzymatiques du cytoplasme et du noyau. En effet les perturbations dans le fonctionnement du fuseau mitotique et dans la structure colloidale du cytoplasme peuvent avoir pour causes uniquement des modifications des propriétés physiques du milieu (milieux iso- et hypotoniques, Lewis 1934, Möllendorff 1938).

De nombreux auteurs ont longuement discuté la fonction et le mécanisme de la formation du fuseau, sans toutefois arriver à élucider définitivement ce problème. Oestergren (1949) suppose qu'il se forme au cours de la division des filaments de nature tacto-

idale qui se raccourcissent ou augmentent en longueur selon que la quantité de substance dont il sont formés diminue ou augmente. Le milieu ambiant du tactoide doit être en état d'équilibre dynamique avec ce dernier par rapport à l'échange continu des particules. Il émet l'opinion que le travail exécuté par la fuseau au cours de la mitose est très rapproché du travail accompli par les forces superficielles. Ehrenberg (1945) a constaté que l'action exercée par de faibles concentrations de corps *c*-mitotiques sur les racines conduit à une contraction et à un grossissement de leur éléments, ce qui a pour effet une augmentation de la courbure du fuseau. Les déplacements accomplis par les chromosomes au cours de l'anaphase semblent être d'autant plus lent que la courbure du fuseau est plus grande.

Les perturbations observées au cours du métabolisme de l'acide thymonucléique se répercutent sans doute aussi sur les fonctions remplies par les acides nucléiques, dont, selon Caspersson, dépendent la synthèse et le métabolisme des protéines. Il semble donc que l'on peut ramener les lésions du phragmoplaste et du fuseau à l'action des facteurs en question, facteurs, qui probablement à travers les perturbations dans la synthèse conduisent à des modifications des propriétés physiques et chimiques des protéines protoplasmatiques. Les perturbations dans le fonctionnement du fuseau et du phragmoplaste seraient donc des phénomenes secondaires.

Disons quelques mots du phénomène de gonflement des racines d'oignons sous l'influence des dérivés du pyrazolone. Nous tenons à sousligner que nous n'avons pas remarqué ce phénomène chez les racines du haricot, ce qu'on peut expliquer soit par l'emploi de concentrations plus faibles des dérivés appliqués, soit par des propriétés différentes des tissus du haricot. C'est un fait connu que l'application des toxines antimitotiques dans de grandes dilutions entraine une augmentation des dimensions des cellules et une diminution de la viscosité de leur cytoplasme (Fell et Hughes 1949). Au contraire, les mêmes substances (par ex. la colchicine, le gaz du moutarde (ypérite) causent une augmentation très marquée de la viscosité des chromosomes. On a également constaté que l'un des principaux phénomènes qui accompagnent l'activité des auxines consiste dans l'abaissement de la viscosité du protoplasme (Northen 1940, 1946 selon Audus). Strügger (selon Audus)

attribue aux auxines une influence positive sur la capacité à gonfler des colloides protoplasmatiques, qui à son tour conduit à un abaissement de la viscosité. Audus écrit (1949): «Les auxines augmentent la capacité à gonfler qui constitue le facteur le plus important de l'accroissement de la cellule. Cela va de pair avec une diminution du poids moléculaire des protéines. Les liaisons entre les bisulfides sont rompues et le nombre de groupes SH s'accroit. Les ferments tels que les déhydrogénases et les carbohydrases sont activés par ces groupes SH. Ainsi s'expliquerait l'influence exercée par les auxines sur les métabolisme». Les faits qu'on vient de mentionner indiquent donc que la croissance normale du tissu dépend de nombreux facteurs. En ce qui concerne le problème en question, on peut donc resumer en deux points suivants l'action exercée par les dérivés du pyrazolone:

1. Augmentation dans les cellules du contenu en eau, qui se manifeste par le gonflement des racines de l'oignon placées dans de solutions un peu plus concentrées de ces dérivés. Probablement les mêmes substances augmentent également la capacité à gonfler de certains colloides du cytoplasme.

2. Les lésions des fuseaux et du phragmoplaste ainsi que l'augmentation de la viscosité des chromosomes sont probablement une conséquence de nombreuses perturbations qui désorganisent la division cellulaire normale. Nous sommes donc en présence d'une influence qui d'une part ressemble à celle des auxines et de l'autre à celle des substances *c*-mitotiques. Il faut également mentionner que l'action exercée sur la division des cellules par les toxines antimitotiques augmente à mesure que leur solubilité dans l'eau décroit. Oestergren (1944) est d'avis que l'activité des substances en question ne dépend que dans une faible mesure de leur structure chimique, mais avant tout de leurs propriétés physiques. Au contraire, d'autres auteurs attribuent l'activité de ces substances à la présence de groupes aminés (Parmentier 1949). Chacune de ces théories permet d'expliquer l'activité plus grande du pyramidon qui se dissout beaucoup plus difficilement dans l'eau que l'antipyrine et contient un groupement aminé.

L'influence des dérivés du pyrazolone sur le métabolisme des acides nucléiques est également intéressante. Suivant Caspersson dans les stades avancés de la mitose a lieu l'hydrolyse des proténes qui unissent les chromomères entre eux. Simultanément a lieu

la synthèse de l'acide désoxyribonucléique conditionnée par les transformations de l'acide ribonucléique présent dans les nucléoles et dans le cytoplasme. En conséquence de quoi apparaissent dans le noyau des granulations distinctement perceptibles et qui se colorent par la méthode de Feulgen. Ces granulations sont toujours rangées près de la membrane nucléaire, ce qui confirme l'opinion de Caspersson suivant laquelle les transformations de cet acide se font surtout dans la région du noyau. Ensuite la quantité d'acide thymonucléique augmente, les filaments de chromatine deviennent perceptibles et finalement on peut apercevoir les chromosomes. L'accumulation la plus grande de cet acide a lieu pendant la métaphase après quoi sa quantité diminue. Enfin, après la formation de noyaux secondaires ont lieu la synthèse des protéines plus complexes et des histones du noyau et la transformation de l'acide thymonucléique en acide ribonucléique du cytoplasme et du nucléole. La synthèse des proteines qui conditionnent la croissance des noyaux et des nucléoles est étroitement liée à la régularité du fonctionnement des acides nucléiques.

En résumé on peut affirmer qu'au début les dérivés étudiés accelèrent la transformation de l'acide ribonucléique en acide desoxyribonucléique; plus tard, au contraire ils paralysent nettement la décomposition de ce dernier. Leur activité rappelle donc celle de la colchicine et des rayons X (arrêt de l'activité de la desoxyribonucléothidase, Lang, Siebert-Oswald 1949, Manoiłow et Semenow 1950). Si donc, après la séparation des chromosomes et après la formation de noyaux secondaires, les cellules et les noyaux cessent d'augmenter leur dimensions, cela tient probablement au manque de matériel de construction consistant principalement en protéines, dans la production desquelles prennent part les acides nucléiques.

La cause de ces phénomènes consiste peut-être dans un arrêt de l'activité de ces acides, arrêt lié aux perturbations qui se présentent au cours de leurs transformations, et qui est dû au fonctionnement anomal des ferments cellulaires. On a constaté notamment que parmis les facteurs indispensables pour assurer le développement normal des tissus et plus spécialement la division des cellules, figurent des protéines qui comprennent le groupement SH. On a démontré que la cysteine ajoutée au milieu dans lequel se développent des cellules agit en stimulant la vitesse de leur division

(Nickerson et Van Rij 1949). On sait également que le fonctionnement de certains ferments (déhydrogénase et carbohydrase) est activé par les groupes —SH (Audus 1949, Brachet 1947 et al.). Du fait que les transformations des acides nucléiques se font simultanément avec des processus oxydo-réducteurs, dans lesquels prennent part de nombreux ferments, il faut conclure que les dérivés du pyrazolone paralysent complétement ou du moins partiellement l'action des ces ferments. La dépendance réciproque observée entre d'une part l'arrêt du metabolisme des acides nucléiques et d'autre part les perturbations de la mitose, plus particulièrement de la télophase, décrites plus haut, témoigne d'un rôle important joué par l'acide thymonucléique dans les processus de division cellulaire et corrobore l'hypothèse de Caspersson.

Conclusion

Une action de courte durée de solutions aqueuses de l'antipyrine (à 1/100, 1/1000) et du pyramidon (à 1/100, 1/500, 1/1000) sur les racines et les tiges de l'oignon et du haricot retarde leur croissance, tandis qu'une action plus longue conduit à son arrêt complet.

L'action du pyramidon est plus intense que celle de l'antipyrine, cette différence peut être expliquée par une solubilité plus faible dans l'eau du pyramidon et par sa structure chimique (présence de groupes aminés).

L'action des dérivés du pyrazolone se manifeste a) par une augmentation du pourcent des telophases, b) par l'apparition de figures anomales dans les phases mitotiques, en particulier dans la méta- et la télophase, c) par un ralentissement très marqué du processus mitotique au cours de la télophase, d) par l'apparition de cellules binucléaires par suite de perturbations survenant au cours de la formation de la membrane cellulaire.

En appliquant la réaction histochimique de Feulgen on a observé des perturbations dans le métabolisme de l'acide thymonucléique qui se manifestaient par une augmentation de sa quantité dans les noyaux en repos et au début de la prophase et aussi par un arrêt de sa décomposition pendant la télophase. Après une cessation complète des divisions on a observé l'apparition dans les noyaux de granules de l'acide thymonucléique (chromocentres) qui par leur aspect rappelaient les chromosomes. On a également constaté

un arrêt dans l'augmentation des dimensions des noyaux-fils et une absence de nucléoles, faits attribuables à des perturbations dans le fonctionnement des acides nucléiques.

La dépendance réciproque observé d'une part entre l'arrêt du métabolisme des acides nucléiques et d'autre part les perturbations de la mitose, plus spécialement de la télophase, décrites plus haut, témoigne d'un rôle important joué par l'acide desoxyribonucléique dans les processus de division cellulaire et corrobore l'hypothèse de Caspersson.

On suppose que les perturbations qui apparaissent au cours des transformations et dans le fonctionnement de l'acide thymonucléique sont causées par un arrêt complet ou du moins partiel du fonctionnement des ferments qui dirigent ces procès.

L'interruption de l'action des dérivés du pyrazolone sur les tissus et le rétablissement de conditions normales font disparaitre les perturbations mitotiques.

J'exprime mes chaleureux remerciements à M. le professeur dr Stanislas Grzycki pour les encouragements et les précieux conseils qu'il m'a prodigués au cours de mon travail.

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Explication des figures

Fig. 3[1]. *Phaseolus multiflorus*. 6-me jour d'expérience. Dans le rang supérieur se trouvent les plantes de contrôle (eau courante), dans le rang inférieur les plantes expérimentales (solution d'antipyrine à 1:1000).

Fig. 4. *Phaseolus multiflorus*. 6-me jour d'expérience. En haut les plantes de contrôle, en bas les plantes expérimentales (solution du pyramidon à 1:1000)

[1] Les photographies (Fig. 3, 4, 5) ont été faites par l'ing. S. Makowiecki, auquel j'exprime mes remerciements.

Fig. 5. *Phaseolus multiflorus*. 6-me jour d'expérience. En haut les plantes de contrôle, en bas les plantes expérimentales (solution du pyramidon à 1:500)

Les figures 6 à 12 représentent le tissue du meristème des racines du haricot *(Phaseolus multiflorus)*.

Fig. 6. Coupe d'une racine de contrôle. Dans les noyaux en repos on aperçoit une petite quantité de granulations F.+ (chromocentres) placées prés de la membrane nucléaire. Les nucléoles sont ronds, brillants, nettement visibles. La disposition des cellules et des noyaux est régulière. Coloration: méthode de Feulgen et vert-clair. Aggrandissement considérable.

Fig. 7. 46 h. dans une solution du pyramidon à 1:1000, le nombre de granulations F.+ dans les noyaux interphasiques est devenu plus grand. Les noyaux secondaires sont placés l'un auprès d'autres; à remarquer l'absence de la membrane bien formée entre les cellules filles. Coloration et aggrandissement comme dans la fig. 6.

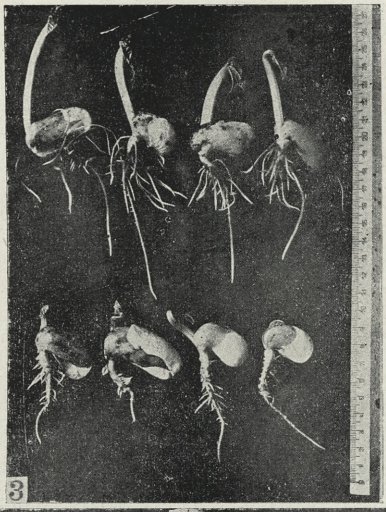
Fig. 8. Coupe expérimentale — 70 h. dans une solution du pyramidon à 1:1000. Absence de divisions, mitotiques. Les noyaux secondaires ont des formes diverses, sont irrégulierèment dispersés et de grandeurs inégales. Les noyaux possédent de nombreux chromocentres qui souvent forment des agglomérations. Absence de nucléoles. Coloration et aggrandissement, comme dans la fig. 6.

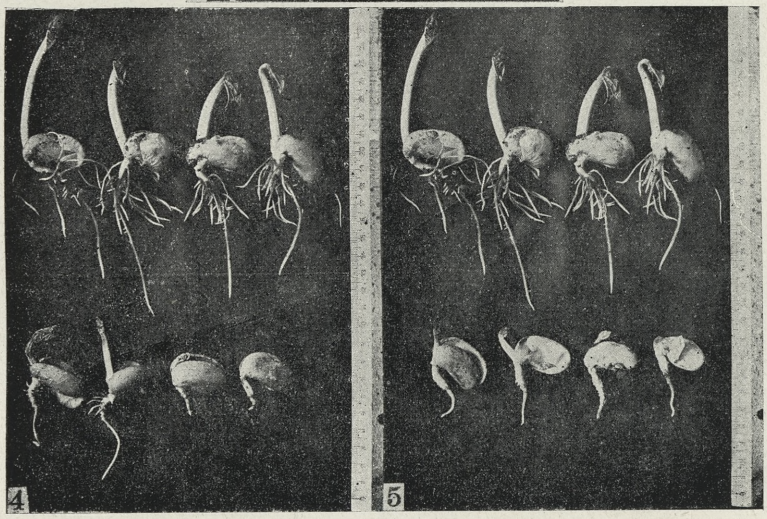
Fig. 9. 70 h. dans une solution d'antipyrine à 1:1000. On voit les filements F.+, qui s'unissent aux chromosomes situés dans le plan équatorial. Coloration comme dans la fig. 6, aggrandissement encore plus considérable.

Fig. 10. Métaphase anomale, qui rappelle par son aspect la métaphase dite tripolaire. Coloration et aggrandissement comme dans la fig. 6.

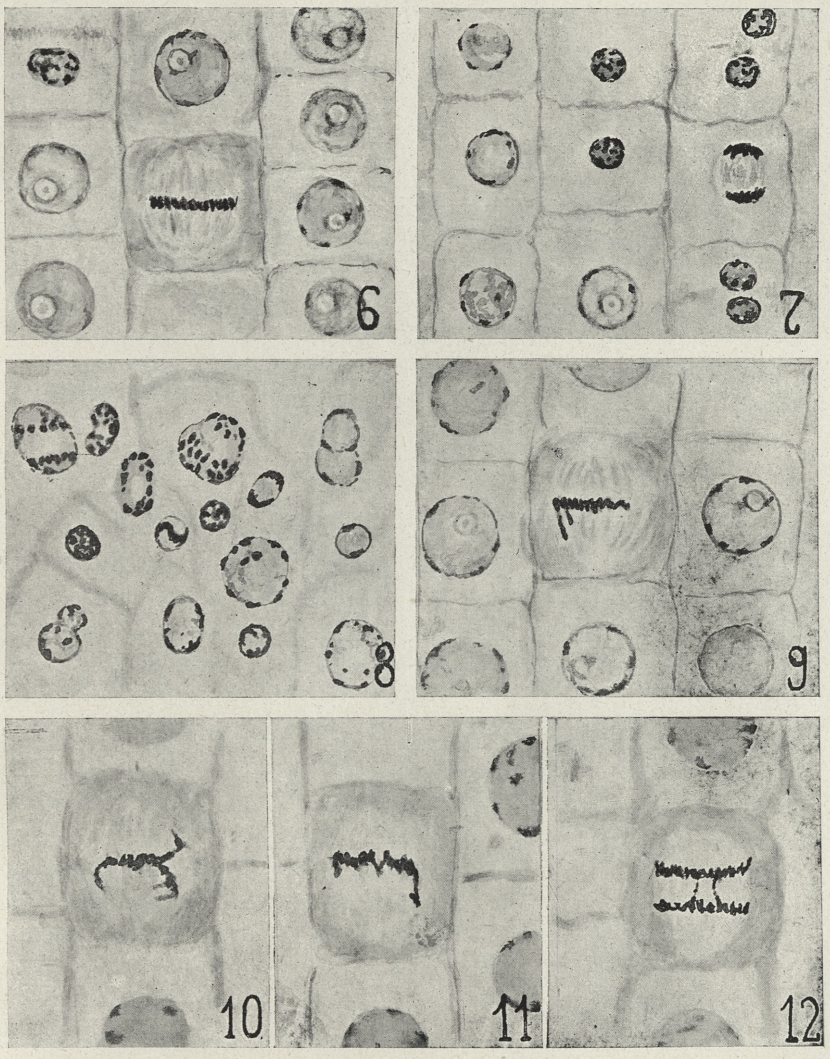
Fig. 11. 46 h. dans une solution du pyramidon à 1:1000. Métaphase anomale (b_2); on voit une deplacement des chromosomes vers le bas. Coloration et aggrandissement comme dans les figures précédentes.

Fig. 12. 70 h. dans une solution d'antipyrine à 1:1000. Anaphase anomale On aperçoit de petites anastomoses de chromatine. Coloration et aggrandissement comme dans les fig. précédentes.





A. Vorbrodt



A. Vorbrodt

Studia nad zróżnicowaniem kariologicznym Cardamine pratensis L. w związku z ekologią. — Studies in karyological differentiation of Cardamine pratensis L. in connection with ecology

Mémoire

de M^lle^ **E. BANACH**

présenté dans la séance du 11 Décembre 1950 par M^me^ M. Skalińska m. c. et M. B. Pawłowski m. c.

(Plate 8)

Cytological studies done hitherto on *Cardamine* have established for this genus the basic chromosome number $x=8$. Most species of the genus *Cardamine* have euploid numbers. The investigations of Manton (1932), which refer to diploid species ($2n=16$) *C. trifolia* L., *C. amara* L., *C. hirsuta* L., *C. impatiens* L., and to the tetraploid species ($2n=32$) *C. flexuosa* With. have been confirmed in the course of the present studies on Polish material from a variety of natural habitats. *C. pratensis* L., differs distinctly from the above mentioned species: within this species there exists a high degree of karyologic differentiation. Previous investigations concerning the chromosome numbers of this species may be briefly summarized as follows: Senjaninova (cit. acc. to Iljinskij 1926) has shown that two species studied by her, *C. pratensis* and *C. dentata*, differ in their chromosome numbers (*C. pratensis*: $2n=24$, *C. dentata*: $2n=72$). Manton (1932) in her study on the phylogeny of *Cruciferae* gives for *C. pratensis* the tetraploid number $2n=32$ and the octoploid number $2n=64$. Flovik (1940) found also for *C. pratensis* $2n=64$. The first aneuploid number ($2n=30$) in this species was established by Lawrence (1931, cit. acc. to Manton 1932). A preliminary note of Lövkvist (1947) bringing the

results of his cytological investigations on *C. pratensis* and *C. dentata* gives interesting data concerning the karyologic differentiation of these two species. On an ample material originating from a variety of natural habitats in southern Sweden Lövkvist has established for these species the following somatic numbers: $2n=30$, 56, 58, 60, 64, 68, 72, 76, 84. He found that the occurrence of the different chromosomic types remains in connection with the water content of the soil. Plants with the lowest chromosome number ($2n=30$) have been found in higher parts and plants with higher numbers ($2n=56$, 60, 64, 68, in lower parts, while near the water or in water plants with numbers $2n=72$, 76. All these types were found in the same meadows.

The aim of this work was to establish somatic chromosome numbers within the species *C. pratensis* in Poland and to study the ecological demands of the particular chromosomic types.

Material and methods

The species *C. pratensis* L. which Schulz (1903) includes in the *Eucardamine* section represents in the Polish flora the holarctic element (Szafer 1949). *C. pratensis* is common in the Polish plain; in mountains it reaches the limit of the higher mountain layer and may be found only sporadically on higher levels (for inst. in the Tatra Mts at the altitude of 1400 m in the neighbourhood of the lake Morskie Oko).

The results obtained are based on a material originating from 41 natural habitats in Southern Poland (Table I). These habitats are situated on the plain as well as in the mountains (Tatra Mts, Pieniny Mts, Babia Góra). The investigated material of *C. pratensis* includes also the variety *C. pratensis* var. *dentata* (Schultes) Neilreich, which in Sweden together with the variety *C. pratensis* var. *palustris* Wimmer et Grabowski has been recognized as a separate species *C. dentata* Schultes (cit. acc. to Hegi).

The somatic chromosome numbers have been established on metaphase plates in root tips. In some cases these numbers have been checked by studies of meiotic divisions in pollen mother cells. A part of the material for cytological studies was fixed in nature and the respective plants were preserved in the form of herbarium specimens; other plants were grown in the Institute and transferred

after fixation of some root tips into the experimental field. The materials were fixed with the use of Navashin and Lewitsky fixatives. Sections 10 μ thick were stained with Newton gentian violet by replacing in some cases clove oil by a solution of phenol in xylene (in a proportion 1:3). Well constrated slides were obtained after prolonging the action of JKJ to 1·5—2 min.

The analysis of metaphase plates presents some difficulty owing to the small dimensions of the chromosomes of *C. pratensis* and their tendency to crowding in the metaphase plates.

The drawings have been done with Reichert oil immersion lens 100×, N. A. 1·30 in combination with Zeiss compensating eyepiece 20× and the Zeiss-Abbe camera lucida. The magnification of the drawings is ±3000×. The microphotographs have been done with Zeiss apochromatic oil immersion lens 90×, N. A. 1·30, Zeiss compensating eyepiece 15×, with the help of Leitz Makam as well as the Practiflex photographic camera. The microphotographs are magnified ±2000×.

Cytological investigations

Cytological results have revealed a wide range of karyological differentiation within the species *C. pratensis* (incl. var. *dentata*). The studied Polish material permit to establish the occurrence of 11 karyological types with the following chromosome numbers: $2n=30$, 32, 38, 44, 50, 58, 64, 68, 72, 76, 78 (Fig. 1—8). With regard to the basic number $x=8$ this range contains 3 euploid types: $2n=32$, 64, 72. The numbers $2n=32$, 64 which represent paired multiples of the basic chromosome number belong to karyologically balanced types in contrast with the unbalanced one $2n=72$. The 8 remaining types have aneuploid numbers $2n=30$, 38, 44, 50, 58, 68, 76, 78. The presumable origin of these chromosomic types will be discussed below.

In spite of a general similarity the Polish material represents a different kind of karyological differentiation than that discribed by Lövkvist for the Swedish material. This author established the following numbers: $2n=30$, 56, 58, 60, 64, 68, 72, 76, 84. Thus in this series there exists a marked gap between the types $2n=30$ and 56. This gap is filled up by new types found in the Polish material ($2n=38$, 44, 50) and by the euploid number $2n=32$ pre-

viously repoıted by Manton (1932) and occurring also in the Polish material. A further chromosomic type $2n=78$ found in the Polish material is not represented in Swedish material. By contrast the types from Sweden $2n=56$, 60, 84 have not been found in the course of the present study.

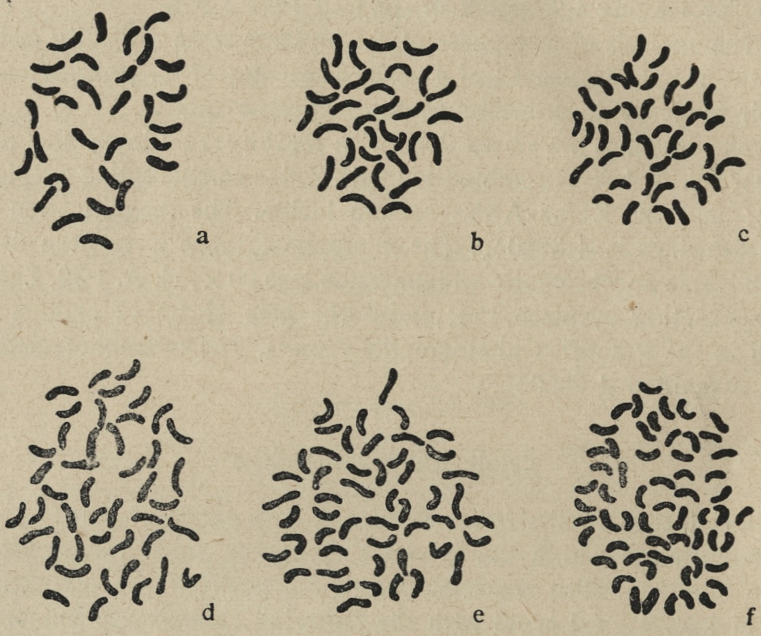


Fig. 1. *C. pratensis* L.: somatic metaphases in root tips a) — $2n=30$, b) — $2n=32$, c) — $2n=38$, d) — $2n=44$, e) — $2n=50$, f) — $2n=58$.

Remarks on the morphology and the habitats of the karyological types

A morphological analysis of the particular karyological types could be carried out only on the basis of a materials grown in approximately uniform conditions. However it is evident from observations done in natural habitats that karyological types with chromosome numbers $2n=30$—64 represent distinctly the morphological type of *C. pratensis*. Nevertheless these types are not morphologically uniform and manifest quantitative differences: biotypes with chromosome numbers ranging from 30 to 38 are less vigorous in nature than the types ranging from 44 to 58. The

type $2n = 64$ seems to be more closely related to lower types in spite of its relatively high chromosome number. In the higher types 68, 72, 76, 78 the features of *C. pratensis* var. *dentata* are

Fig. 2. *C. pratensis* L.: somatic metaphases in root tips a) — $2n = 64$, b) — $2n = 68$, c) — $2n = 72$, d) — $2n = 76$, e) — $2n = 78$.

marked in a higher or lower degree. In general these types show some degree of diversity in their natural habitats. It concerns the vigour of the plants, the size, colour and shape of their petals and the shape and size of the leaflets of the steam leaves. It is in-

teresting to note that among the specimens representing the types $2n = 72$, 76, 78 plants corresponding exactly to *C. pratensis* var. *dentata* (Schultes) Neilreich have been found besides others which were less extreme in their features. These forms have not been found among the $2n = 64$ plants.

In respect of the vertical distribution of the various karyological types it is interesting to note that their greater part is connected with the plain. The following types have been found till now only in these habitats: $2n = 30$, 32, 38, 64, 68, 72, 76, 78. On the other hand the type $2n = 44$ frequent in the plain occurs also in the Carpathian foreland, Pieniny Mts, Babia Góra, as well as in lower situations in the Tatra Mts; it is rarely found there also in higher situations (the neighbourhood of the lake Morskie Oko, about 1400 m). The types 50, 58 have been found till now exclusively in the Tatra Mts. In view of the fact that each of these types has been found only in a single habitat in small colonies comprising only a few plants it is difficult to estimate their ecological requirements.

The distribution of the particular karyological types shows a marked dependence of the moisture of the ground. In the evaluation of the degree of moisture of the habitats, their plant communities were taken into consideration. In case of similar plant communities in different habitats of the same karyological type the floristic composition of a selected habitat has been given (Table I).

On the basis of the results obtained the whole investigated material may be classed into 5 groups which are not uniform karyologically; their representatives within each group however show a high degree of similarity in respect of their ecological demands.

The first group is composed of the types $2n = 72$, 76, 78 originating from 12 habitats. These types proved to be the most exclusive in the selection of their habitats. They are able to a vigorous development only on extremely wet ground near the water or in the water. Observations of the places where they were found lead to the conclusion that the drying out of the soil results in the retreat of these plants to the wetter parts of their habitats and finally in their total disappearing from the previously occupied stands.

The second group is represented by the karyological types $2n = 64$ and 68. The first of these types has been found in two habitats, while the second only in one habitat. They occurred in

TABLE I

List of habitats of *Cardamine pratensis* L.

N°	Chromosome number ($2n$)	Habitats and their floristic composition
36	30	Surrounding of Olkusz; wet parts of meadows by river Biała near the Błędowska desert.
49	30	Gliwice; higher parts of a moist meadow.
54	30	Cegielnia near Olkusz; entirely overgrown ditch across meadows.
117	30	Bieżanów near Kraków; wet parts of meadows; *Caltha palustris* L., *Cirsium rivulare* (Jacq.) Lk., *Equisetum palustre* L., *Lychnis flos cuculi* L., *Polygonum amphibium* L. var. *terrestre* Leysser., *Ranunculus acer* L., *Rumex acetosa* L.
119	30	Bieżanów near Kraków; dry parts of meadows; *Alopecurus pratensis* L., *Anthoxantum odoratum* L., *Arrhenatherum elatius* (L.) Mert et Koch., *Campanula patula* L., *Centaurea jacea* L., *Chrysanthemum leucanthemum* L. *Crepis biennis* L., *Dactylis glomerata* L., *Geranium pratense* L., *Heracleum sphondylium* L., *Holcus lanatus* L., *Knautia arvensis* (L.) Coult., *Plantago lanceolata* L., *Rumex acetosa* L., *Trifolium minus* Sm., *Trifolium pratense* L.
125	30	Kraków-Borek Fałęcki; dry parts of meadows.
133	30	Surrounding of Olkusz; higher parts of banks of river Biała.
24b	32	Bieżanów near Kraków; a moist meadow; *Ajuga reptans* L., *Alectorolophus major* (Ehrh.) Rchb., *Alopecurus pratensis* L., *Campanula patula* L., *Carum Carvi* L., *Chrysanthemum leucanthemum* L., *Knautia arvensis* (L.) Coult., *Luzula multiflora* (Ehrh.) Lej., *Lychnis flos cuculi* L., *Orchis latifolius* L., *Polygala comosa* Schkhr., *Polygonum bistorta* L., *Sanguisorba officinalis* L., *Trifolium arvense* L., *Trollius europaeus* L., *Veronica arvensis* L.
124	38	Kraków-Borek Fałęcki; wet meadow; *Angelica silvestris* L., *Anthoxantum odoratum* L., *Calliergon cuspidatum* Kindb., *Caltha palustris* L., *Carex* sp., *Cirsium oleraceum* (L.) Scop., *Epilobium palustre* L., *Filipendula ulmaria* Max. *Juncus effusus* L., *Lythrum salicaria* L., *Ranunculus acer* L., *Ranunculus flammula* L., *Rumex acetosa* L., *Sanguisorba officinalis* L., *Succisa pratensis* Mnch.
136	38	Czyżyny near Kraków; a wet meadow.
152	38	Mydlniki near Kraków; a wet meadow.
153	38	Kraków-Pasternik, overgrown parts of a ditch.
24a	44	Bieżanów near Kraków; a moist meadow.

Nº	Chromosome number (2n)	Habitats and their floristic composition
26	44	Kraków-Zakrzówek; bank of a ditch between two ponds on a moist meadow.
28	44	Maków Podhalański; grassy bank of river Skawa, about 1·5 m over water level.
29	44	Maków Podhalański; clearings in an *Alnus* scrub on the bank of a tributary of river Skawa on the way to the hamlet Żarnówka.
32	44	Maków Podhalański; scrub at river Skawa on the way to the hamlet Grzechinia; *Alnus incana* Moench., *Anemone nemorosa* L., *Anemone ranunculoides* L., *Cardamine impatiens* L., *Chrysosplenium alternifolium* L., *Galeobdolon luteum* Huds., *Galium vernum* Scop., *Geranium Robertianum* L., *Geum urbanum* L., *Malachium aquaticum* Fr., *Oxalis acetosella* L., *Ranunculus lanuginosus* L., *Stachys silvaticus* L., *Stellaria nemorum* L., *Symphytum tuberosum* L.
60	44	Tatra Mts., Kościeliska valley; scrub at the bank of the stream.
83	44	Babia Góra (Western Carpathians); border of a beach wood on the way to Czarna Hala.
87	44	Pieniny, way to Sokolica; border of a beach wood, among grass.
109	44	Kraków-Zakrzówek; a moist meadow; *Alopecurus pratensis* L., *Cerastium caespitosum* Gillib., *Cirsium rivulare* (Jacq.) Lk., *Heracleum sphondylium* L., *Holcus lanatus* L., *Lychnis flos cuculi* L., *Ranunculus acer* L., *Ranunculus repens* L., *Rumex acetosa* L., *Sanguisorba officinalis* L., *Trifolium pratense* L., *Veronica chamaedrys* L.
121	44	Kraków-Płaszów; a moist meadow.
143	44	Maków Podhalański; moist parts of a wood clearing.
155	44	Tatra Mts., Valley of the lake Morskie Oko; a depression at the border of a wood.
59	50	Tatra Mts, Jaszczurówka; a moist meadow at the border of a wood among grass.
158	58	Tatra Mts, Hruby Regiel; border of a wood, among grass.
118	64	Kraków-Bieżanów; a swampy part of a meadow; *Caltha palustris* L., *Galium palustre* L., *Mentha aquatica* L. *Myosotis palustris* (L.) Lam., *Polygonum amphibium* L. var. *terrestre* Leysser., *Ranunculus repens* L., *Scirpus silvaticus* L.
131	64	Gliwice; a wet meadow.

N°	Chromosome number (2*n*)	Habitats and their floristic composition
129	68	Bieżanów near Kraków; in a swampy ditch across meadows; *Alopecurus geniculatus* L., *Juncus effusus* L., *Lycopus europaeus* L., *Myosotis palustris* (L.) Lam., *Polygonum hydropiper* L., *Ranunculus repens* L., *Rumex cripsus* L.
25	72	Kraków-Zakrzówek; in a swampy ditch between two ponds; *Caltha palustris* L., *Juncus effusus* L., *Carex riparia* Curt., *Galium palustre* L., *Glyceria aquatica* Whlb., *Lysimachia nummularia* L., *Lythrum salicaria* L., *Lycopus europaeus* L., *Myosotis palustris* (L.) Lam., *Oenanthe aquatica* Lk., *Ranunculus repens* L., *Nasturtium amphibium* R. Br.
110	72	Kraków-Zakrzówek; on marshy parts of meadows.
132	72	Gliwice; in a swampy ditch across meadows.
41	76	Mydlniki near Kraków; in an overgrown part of the pond.
55	76	Cegielnia near Olkusz; a swampy ditch across meadows.
130	76	Bieżanów near Kraków; in a swampy ditch across meadows.
151	76	Mydlniki near Kraków; in an overgrown pond; *Alnus glutinosa* Gaertn., *Alisma plantago* L., *Callitriche verna* L., *Equisetum limosum* L., *Juncus effusus* L., *Ranunculus repens* L., *Myosotis palustris* (L.) Lam.
39	78	Kraków-Pasternik; in an overgrown pond; *Alisma plantago* L., *Carex canescens* L., *Glyceria plicata* Fr., *Juncus effusus* L., *Lysimachia nummularia* L., *Ranunculus Flammula* L., *Ranunculus repens* L., *Scirpus silvaticus* L., *Veronica beccabunga* L.
111	78	Kraków-Czyżyny; in a swampy ditch across meadows.
138	78	Kraków-Wieczysta; in a swampy ditch across meadows.
139	78	Kraków-Czarna Wieś; in a swampy ditch across meadows.
147	78	Swoszowice near Kraków; Swampy ground near the border of a pond; *Calliergon cuspidatum* Kindb., *Carex gracilis* Curt., *Comarum palustre* L., *Galium palustre* L., *Lycopus europaeus* L., *Lysimachia vulgaris* L., *Peucedanum palustre* Mich., *Phragmites communis* Trin; *Salix aurita* L.

wetter parts of meadows and in swampy depressions of the ground drying out in the summer.

The third group is represented by the types with the numbers $2n = 32$, 44. In general they grow on moist meadows. The type $2n = 44$ which appears most frequently on moist meadows was found also sometimes on the border of deciduous forests and in scrubs. This is the most widely distributed type in Southern Polanp

(the $2n=44$ plants have been found in 12 habitats). Plants with 50 and 58 chromosome numbers may be probably assigned to the same group.

The type $2n=38$ forms a separate group. Plants of this type studied till now from 4 habitats were found on wet meadows.

The last group consists of plants with $2n=30$ chromosome number (studied from 7 habitats). This type may still find possibilities of development even on dry meadows although this kind of habitat is by no means typical for *C. pratensis*. On moist or wet meadows however it finds favourable conditions and develops more vigorously. The occurrence of this type in habitats which are distinctly different ecologically may be caused by its increased ecological plasticity. It is possible however that this type represents a mixture of genotypes with different ecological requirements. This problem could be solved only in the way of transplant experiments.

The distribution of the various karyological types shows a well marked dependence from the degree of humidity of the habitat: biotypes with lower chromosome numbers show a distinct preference for relatively drier soils whereas types with higher numbers are being found in moister habitats; plants with the highest numbers occur in marshes, ponds and ditches.

Discussion

Aneuploidy in plants is a rather rare phenomenon. The researches of Winge (1940) have shown within the collective species *Erophila verna* a high degree of aneuploid karyological differentiation. *E. verna* occurs in nature in a series of types with chromosome numbers $n=7$—32. The true breeding of the particular types of this species is the result of a regular meiosis and autogamy of the various types. Some grass species of the genus *Poa* also possess a high degree of aneuploid differentiation. Within the strains *Poa alpina* originating from a number of natural habitats Müntzing (1932, 1940) found biotypes with chromosome numbers ranging from $2n=21$ to 33 (besides sporadically appearing biotypes with higher chromosome numbers which have arisen from unreduced gametes). In the *Poa alpina* material Müntzing found besides numerous sexual biotypes, also four apomictic strains with $2n=33$, 35, 37,

38. Three of these types ($2n=33$, 35, 38) represented stabilised clones whereas the fourth ($2n=37$) which was not strictly apomictic manifested in its progeny some degree of oscillation of the chromosome numbers. In the Danish material of *Viola canina* Clausen (1931) found hypertetraploids with a varying number of extra-chromosomes or fragments.

Besides species with a permanent aneuploid differentiation others have also been studied in which the appearance of polysomatic aberrants has been observed only sporadically i. e. among polyploid races (Blackburn 1934, Müntzing 1933, 1937); or in the progeny of hybrids between plants with different chromosome numbers (Skalińska 1938). Aneuploid aberrants represent in most cases unbalanced types with a lowered viability. In general such sporadically occurring polysomatic types have no evolutionary value. In their offspring usually a return to strictly euploid chromosome numbers could be observed

Therefore species which possess constantly an aneuploid differentiation combined with a normal viability are particularly interesting. Lövkvist (1947) was the first who observed within the species *C. pratensis* and *C. dentata* numerous aneuploid types. The present studies on Polish material have enabled us to establish a wide scale of karyological differentiation, which embraces 11 biotypes with euploid and aneuploid chromosome numbers. These biotypes show a normal viability in a variety of natural habitats.

Concerning the origin of aneuploidy it should be mentioned that according to Meurman (1929) and Müntzing (1933, 1936, 1937) this phenomenon is causaly linked with autopolyploidy. In polyploids with frequently occurring polyvalents disturbances in the course of meiosis due to an irregular distribution of chromosomes may lead to the production of aneuploid types.

It may be assumed that besides polyploidy also another factor has presumably played an important part in the karyological differentiation of *C. pratensis*, namely spontaneous crosses. The occurrence of euploid types 32 and 64 suggests that in the first steps of the evolution of this species the doubling of chromosome sets by polyploid mutations could have taken place. On the other hand spontaneous crosses between the older and the newly formed types and, in subsequent stages, a repeated intercrossing of the cross-products could have given rise to a range of aneuploid types. Thus

the aneuploid differentiation was presumably achieved as a second step of the evolution within this species.

Skovsted (1934) explained in a similar manner the karyological differentiation of *Saxifraga granulata* studied by him. Although it is regarded as a good species it has in Denmark a considerable karyological differentiation. The chromosome numbers of this species range between $2n=46$—60. Skovsted assumes that its aneuploid differentiation resulted from crosses of the northern tetraploid race of this species ($n=32$) with the southern diploid one ($n=16$). The fact that the type $2n=48$ was found most frequently in the Danish material favours this assumption.

The study of the Polish material of *C. pratensis* has revealed that the distribution of the various karyological types shows a well marked dependence from the degree of humidity of the habitat: biotypes with lower chromosomes numbers show a distinct preference for relatively drier soils whereas types with higher numbers are being found in moister habitats; plants with the highest numbers occur in marshes, in overgrown parts of ponds and in swampy ditches. The comparison of the ecological demands of the karyological types of *C. pratensis* suggests that the increase of the chromosome numbers enabled the new karyological types to expand over new territories.

The problem of the dependence between the degree of polyploidy and moisture of the habitats of different karyological types has been discussed by Hagerup (1940); according to Melin the arctic diploid species *Oxycoccus microcarpus* occurs in the driest *Sphagnum* bogs. The tetraploid species *O. quadripetalus* may be found in Denmark both in moderately moist as well as in very wet habitats. On the other hand *O. quadripetalus* var. *microphyllus* occurring also in Denmark approaches the arctic species in its ecological requirements. A similar dependence of the moisture of the habitat has been established by Hagerup (1939) for two related species *Deschampsia setacea* ($n=7$) and *D. flexuosa* ($n=14$). In the species *Vaccinium uliginosum* (Hagerup 1933) the arctic diploid form *V. uliginosum* f. *microphylla* occurs in drier habitats as well as in depressions of the terrain, while the tetraploid *V. uliginosum* f. *genuina* in Northern Europe is found exclusively in very wet habitats.

We find an interesting example of ecologic differentiation in the collective species *Valeriana officinalis* in Great Britain (Ska-

lińska 1947). Its tetraploid forms which occupy a relatively small area, occur in rather dry places in hilly regions. On the other hand the octoploids growing within the area of the tetraploids are found exclusively at lower altitudes in habitats with a higher degree of humidity.

The collective species *Erophila verna* gives an example of the ecologic demands of a species with an aneuploid karyological differentiation. Winge observed that the lower types (*Erophila simplex*, $n = 7$) occurred only in sunny dry habitats with low vegetation; *E. duplex* ($n = 15$—20) occurred in pastures, gravel pits, on road borders, while *E. quadruplex* ($a = 26$—32) was found on meadows and swamps.

The above considerations throw some light upon the putative origin of the aneuploid differentiation as well as on the ecology of the particular types within the collective species *Cardamine pratensis* L.

Summary

The intraspecific karyological differentiation of *Cardamine V. pratensis* L. (incl. *C. pratensis* var. *dentata* Schultes/Neilreich) has been studied on plants collected in 41 habitats in Southern Poland: in the plain, in the foreland of the Carpathian range, in the Tatra Mts, in the Western Carpathians (slopes of Babia Góra) and in the Pieniny Mts.

The cytological investigations permit to establish the occurrence of 11 different karyological types with euploid and aneuploid chromosome numbers ($2n = 30$, 32, 38, 44, 50, 58, 64, 68, 72, 76, 78). It may be assumed that this karyological differentiation within the species has been achieved in two main steps: (1) the doubling of chromosomes resulted in the appearance of autopolyploids; (2) spontaneous crosses between the older and the newly formed types and, in subsequent stage, a repeated intercrossing of the cross-products have given rise to a range of aneuploid biotypes which proved able to establish themselves in a variety of habitats.

The distribution of the various karyological types shows a well marked dependence from the degree of humidity of the habitat: biotypes with lower chromosome numbers show a distinct preference for relatively drier soils whereas types with higher numbers are being found in moister habitats; plants with the highest numbers

occur in marshes, in overgrown parts of ponds and in swampy ditches.

This study has been carried out in the Institute of Plant anatomy and cytology of the Jagiellonian University of Kraków. I wish to express my sincere gratitude to Professor M. Skalińska, Head of the Institute for encouragement and valuable criticism and advice during the course of my work. My thanks are due also to all persons who helped me in collecting the plant specimens for investigations and to Dr A. Bajer for the microphotos.

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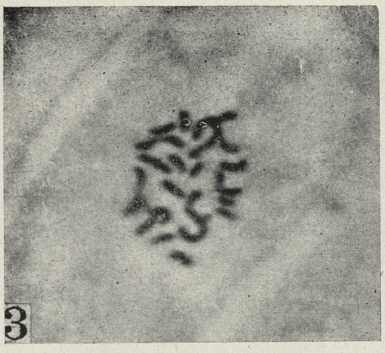
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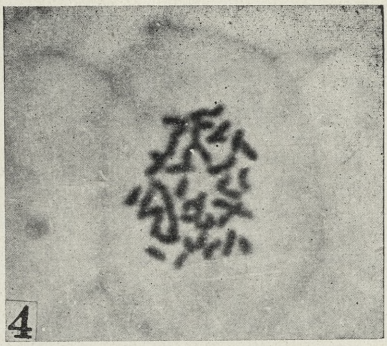
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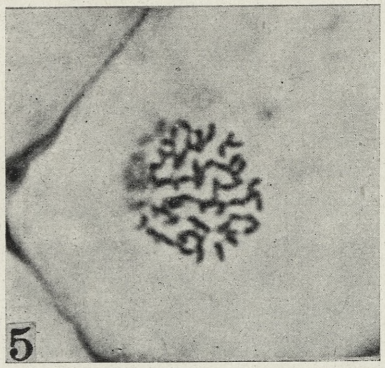
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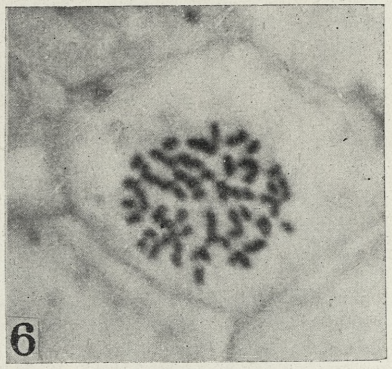
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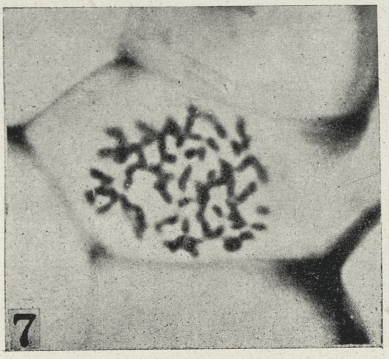
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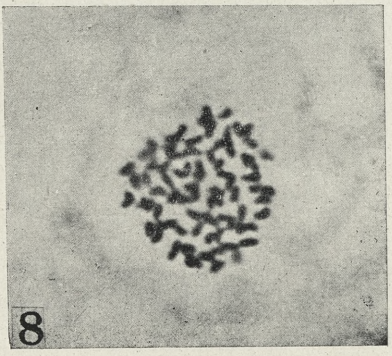
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E. Banach

Explanation of Plate

Cardamine pratensis L.: microphotos of somatic metaphases in root tips.

Fig. 3. — $2n = 30$.
Fig. 4. — $2n = 44$.
Fig. 5. — $2n = 64$.
Fig. 6. — $2n = 68$.
Fig. 7. — $2n = 76$.
Fig. 8. — $2n = 78$.

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