Cytological studies on Cochlearia polonica

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INTRODUCTION

The genus *Cochlearia* is represented in Poland by two species which occur endemically in two distinctly different habitats: *C. polonica* Fröhl. has its natural stands near the town Olkusz, whereas, *C. Tatrae* Borb. grows in Tatra mountains.

The present paper deals only with *C. polonica;* studies on *C. Tatrae* will be published separately.

The stand of *C. polonica* has been discovered by K. P i e c h in 1913 (P i e c h 1924); the plant was then determined as *C. officinalis* L. Later on F r ö h l i c h (1937) recognised it as a new species, on the basis of detailed taxonomical studies.

C. polonica occurs between the town Olkusz and the Błędowska Desert (Pustynia Błędowska), a vast area covered with sand. It is found there sometimes on wet maedows but it grows chiefly in shallow cold water (12—15°C) around the springs of river Biała (Plate I, fig. 1—2). It should be added that it does not occur in drier places exceeding 40 cm above water level and in the occupied area it grows abundantly.

The purpose of this study was:

1. to establish the somatic chromosome number of this species,
2. to investigate the course of meiosis in P. M. C.'s.

MATERIAL AND METHODS.

Living plants were collected in their natural habitats in spring, summer and autumn 1948. They are very difficult to grow in labo-

ratory conditions: after transplanting into flower-pots they perished within a few weeks. Good results however were obtained from cultures in large shallow bassins with running tap water. In these conditions the plants could be kept alive and grew well even during the winter; they flowered in the next spring and developed fruits and seeds in summer 1949. *C. polonica* is a biennial: in the first year it forms rosettes of leaves, in the second year it develops flowers and seeds and perishes soon afterwards.

For root-tips the N a v a s h i n and L e v i t s k y's 5:5 fixatives were used, for buds only N a v a s h i n. Microtome section 10 and 12 μ thick were stained with N e w t o n's gentian violet. It should be noted that the staining with gentian violet after the L e v i t s k y fixative gave less satisfactory results, although the chromosomes were better spaced. In order to find the suitable stages of meiosis in P. M. C.'s, the acetocarmine method of B e l l i n g was applied.

All drawings were made with a Reichert oil imersion lens 100x N. A. 1,30, and a compensating eyepiece Zeiss 20x with the aid of an Abbe camera lucida (magnification 2100x). The photomicrographs were made with the aid of a Zeiss apochromate oil imersion lens 90x and compensating eyepieces 10x or 11x, fig. 3—12 (Plate I), fig. 13—22 (Plate II) and with the aid of Reichert objective 41x and 10x eyepiece fig. 23—24 (Plate II).

MITOSIS.

The somatic chromosome number was established from mitoses in root-tips of 12 plants. In all plants the chromosome number (2n) was 36. In view of the smallness of the chromosomes, it is difficult to say anything about their morphology. It was possible however to distinguish two types of chromosomes according to their size differences: the first were about 1,5 μ long, the second 2,5 μ (text fig. 1 and Plate I, fig. 3).

Textfig. 1, Metaphase from root-tips, (3000 ×). The same as in Plate I fig. 3).
Textfig. 2, Diakinesis. Typical polyvalents from different cells; a. nucleus with only a part of its chromosomes, b. from left to right: 7 hexavalents, 4 pentavalents, 2 quadrivalents, c. from left to right: 11 qudrivalents, 5 trivalents (2800 ×) (compare Plate I figs. 4—12).
Textfigs. 3—5, Top, three heterotypic metaphases in side view. Bottom partial diagrams of the corresponding chromosome configurations. (compare Plate II figs. 15—16). Tiny fragments in the cytoplasm. (2800 ×).
Textfigs. 6—8, Polar view of three heterotypic metaphases showing secondary associations. (compare Plate II figs. 13—14), (2800 ×).

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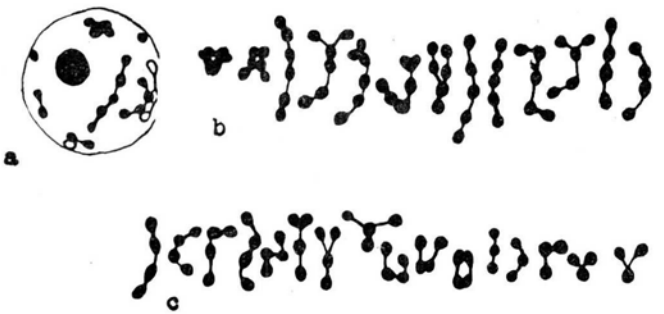

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6 7 8

Previous investigations of other species of *Cochlearia* have shown that this genus has two basic numbers of chromosomes: x=6 and 7 (Löve and Löve 1948, Böcher 1938, Crane and Gairdner 1923, Flovik 1940). The basic number 6 occurs in *C. officinalis L. var. alpina* 2n = 24 (Böcher 1938), *C. officinalis* I. 2n = 24, 24 + 1—4 ff (unpubl. data of Sörensen and Westergaard from Löve and Löve 1948). The basic number 7 has been found in *C. arctica* Schlechtend. 2n = 14 (unpubl. data of Sörensen and Westergaard from Löve and Löve 1948), *C. officinalis* 2n = 28 (Crane and Gairdner 1923), C. danica 2n = 42 (Crane and Gairdner 1923). Thus the number 36 of *C. polonica* suggests that it represents a hexaploid with the basic number 6. Results of studies of meiosis in P. M. C's give further support to this assumption.

MEIOSIS.

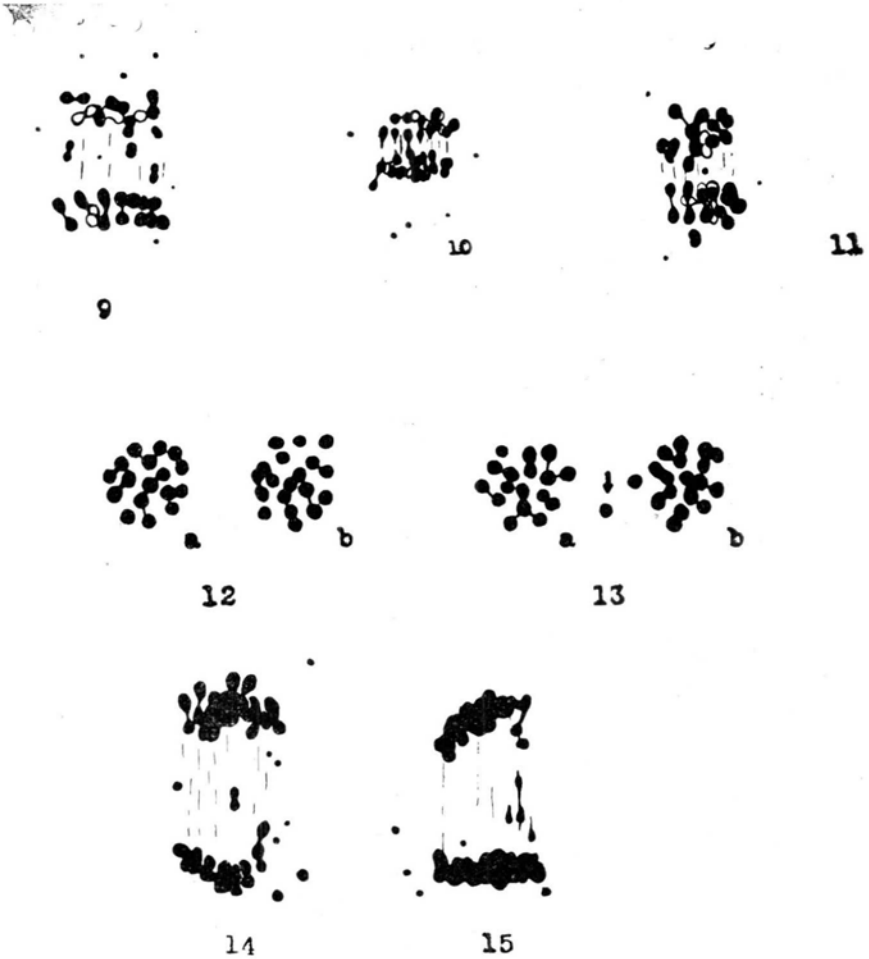
As well known, the Navashin fixative is not suitable for the study of early stages of prophase (Bělař 1928, p. 661). The fixation however was satisfactory for the investigation of diakinesis. Owing to the position of the chromosmes under the surface of the nucleus the analysis of their configurations is easier. In this stage it is possible to observe in addition to bivalents also univalents and polyvalents: tri-, quadri-, penta-, and hexavalents (textfig. 2 and Plate I, figs. 4—12).

The analysis of 50 P. M. C's gave the following results concerning the ocurrence of different polyvalents in diakinesis nuclei:

type of polyvalents	VI	V	IV	III	II	I
min. and max. number in 1 cell	0—1	0—2	0—5	0—4	0—5	0—3

The occurrence of hexavalents in this stage is in accordance with the assumption that *C. polonica* represents a hexaploid with the basic number 6. The various polyvalents occur in the form of rods, rings and Y's (e. g. textfig. 2 polyvalents 12—24 shows different forms of quadrivalents). Among polyvalents the rods represent the most frequent type whereas the rings are relatively rare.

The analysis of the subsequent stages (pro-metaphase and metaphase) is very difficult owing to the crowding of the chromosomes in the equatorial plate. In side view the heterotypic metaphase spindle shows a rather irregular arrangement of the chromosomes in the equatorial plate. This is chiefly due to the occurrence of various



Texfigs. 9—11, Heterotypic anaphase, side view; detailed explanation in text. (compare Plate II fig. 17) (2800 ×).

Textfigs. 12—13, Polar view of two heterotypic anaphases; a. and b. daughter plates; arrow indicates an univalent in cytoplasm (2800 ×).

Textfigs. 14—15, Two heterotypic telophases with lagging chromosomes and dividing chromosomes in cytoplasm; tiny fragments in cytoplasm. (compare Plate II figs. 18—19), (2800 ×).

polyvalents. A partial analysis of a number of metaphase spindles permit to discern the following polyvalents in this stage:

type of polyvalents	VI	V	IV	III	II	I
min. and max. number in 1 cell	0	0—1	0—3	0—4	4—11	1—6

In comparison with the diakinesis the number of hexa-, penta-, and quadrivalents decreases at metaphase and a parallel increase of

the number of bi- and univalents is observable. As well known, in plants with small chromosomes the terminal chiasmata are not always maintained until metaphase, thus in later stages bivalents may be found instead of polyvalents. The polyvalents are visible best if they lie at the periphery of the spindle (for instance rods, textfigs. 3 and 5). In view of the crowding of the chromosomes only rod polyvalents are plainly discernible. In some P. M. C's 1—3 univalents are scattered along the spindle or in the cytoplasm. They are more frequent in the late than in the early metaphase and they result presumably from a precocious breaking of the chiasmata (for instance two univalents on textfig. 4). In some P. M. C's bivalents were found on one pole or sometimes on both poles or they were scattered in the cytoplasm. This may be due in some instances to a precocious seperation of the polyvalents. It is possible hovewer that sometimes the congression of the chromosomes in the equatorial plate is irregular. In this stage in the cytoplasm of some P. M. C's 1—8 tiny fragments are observable. They are similar to the fragments previously observed by some authors in other plants e. g. in P. M. C's of *Prunus laurocerasus* (Meurman 1933).

The polar view of the metaphase spindles helps to some extent to elucidate the difficulties of analysis mentioned above. The various polyvalents and bivalents are not evenly spaced in the plate; on the contrary in many instances they show close associations (textfig. 6—8 and Plate II, figs. 13—14), known as a „secondary pairing" (Lawrance 1931, Darlington and Moffet 1930, Meurman 1933).

The course of anaphase is irregular. This results from the presence at metaphase of polyvalents with odd numbers of chromosomes (tri-. and pentavalents), as well as from the occurrence of univalents both in the spindle and in the cytoplasm. Other irregularities are due to the fact that the chromosomes do not move simultaneously to the poles. Frequently a part of the chromosomes have already reached the poles, whereas the remaining ones are still in the equatorial plate. The separation of the chromosomes which are associated in polyvalents is especially irregular; some are retarded in their movement towards the poles, others may remain as laggards in the equatorial plate (textfigs. 9—11 and Plate II, fig. 17). Thus in the late anaphase between the two groups of chromosomes on the poles frequently there remain uni- and bivalents. In this stage also small chromosomes presumably representing divided univalents have been found in some P. M. C's (textfig. 9 and Plate II, fig. 17).

Here again tiny fragments are observable both in the spindle and scattered in the cytoplasm, their division hovewer could not be observed.

In some cases a transversal orientation of bivalents in relation to the axis of the spindle may lead to further disturbances in the distribution of chromosomes (textfig. 9 and Plate II fig. 17 and textfig. 10).

The polar view of the late anaphase spindles shows that in this stage also the chromosomes are arranged in similar groups as in the heterotypic metaphase (textfigs. 12—13). Judging from the position of such groups in two corresponding anaphase plates in one cell, it is sometimes possible to establish which chromosomes were joined in one group (textfig. 12). In the preceding stage. It should be added however that it is not always easy to imagine the presumable configuration of chromosomes at metaphase (textfig. 13) on the basis of the comparison of two sister anaphase plates. All the above irregularities may contribute to an unequal distribution of the chromosomes in the late anaphase.

The analysis of chromosome distribution at late anaphase in 26 P. M. C's gave the following results:

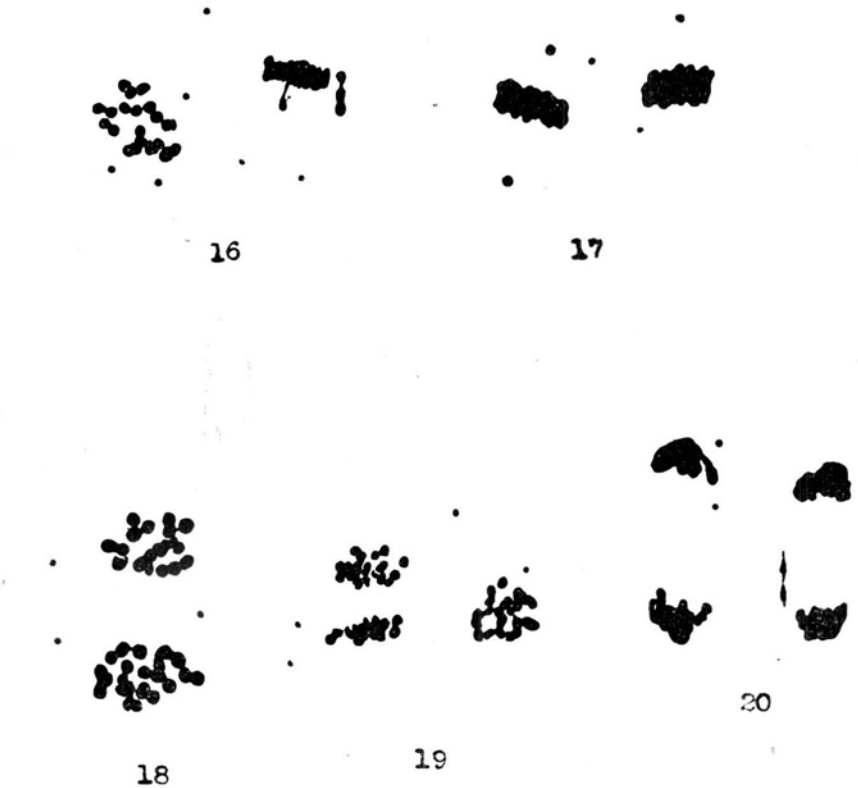
distribution of chromosomes	20+16	19+17	18+18	18+17+1	17+17+2	17+16+3
number of cells	3	4	5	5	4	4

In the early heterotypic telophase the chromosomes which reached the poles are grouped more or less regularly; the univalents and small chromosomes which probably have arisen from their division may be found between the poles. Some univalents are distinctly stretched in the direction of the axis of the spindle (textfig. 14—15 and Plate II, fig. 18—19). Tiny fragments may occur both in the spindle and in the cytoplasm. Some univalents which lie far from the poles probably will be lost in this stage.

In the interkinesis the sharp outlines of the chromosomes included in the daughter nuclei disappear gradually. On the other hand the univalents in the cytoplasm as well as the fragments are still observable. Additional spindles formed by univalents have not been observed. Probably the univalents scattered in the cytoplasm become resorbed in a later stage.

In the homeotypic metaphase the spindles are orientated either parallely or vertically to each other (textfigs. 16—17). The chromosomes are crowded and sometimes they may be arranged irregularly in the equatorial plates. It is remarkable that in this stage in some

plates rod-quadrialents and trivalents are observable (textfig. 16 and Plate II, fig. 21) similarly to polyvalents in the heterotypic division. It seems probable that they derived from the division of hexa-, and pentavalents of the heterotypic metaphase, although usually the association between the chromosomes which form polyvalents do not persist until this stage.



Textfigs. 16—18, Homeotypic metaphases, (compare Plate II figs. 20—22), (2800 ×).
Textfig. 19, Homeotypic anaphase (2800 ×).
Textfig. 20, Homeotypic telophase (2800 ×).

The second anaphase may be more or less regular; sometimes the division and the anaphase movement of the chromosomes does not occur simultaneously in the two spindles within a single cell. In the anaphase (textfig. 19) some chromosomes are lagging; judging from the telophase (textfig. 20) these chromosomes tend to divide frequently. In some P. M. C's fragments are seen again in the cyto-

plasm and the spindles. The tetrads are apparently normaly only occasionally dyads were observed. Presumably they originated from the fusion of the two spindles in the homeotypic metaphase or anaphase, since restitution nuclei never were observed.

The primary pollen grains degenerate frequently and in the mature anthers a variable percentage of sterile pollen ranging from 15%—100%, (Plate II, figs. 23—24), in the average 53%, may be observed. It is possible that the development of the tapetum also plays a part in the abortion of the pollen. In some anthers the tapetum degenerates rather early and this may affect the normal development of the pollen grains. In spite of the observed disturbances, chromosomes counts in the first pollen grain mitosis permit to establish only the normal number 18. Thus probably the pollen grains with deviating numbers are unviable. Normal pollen grains are 3 nucleate; empty pollen grains with a well developed exine could be found in almost all pollen samples.

DISCUSSION

Species within the genus *Cochlearia* show a differentiation concerning both their basic chromosome number and the degree of polyploidy. The series with the basic number 7 is represented by diploid, tetraploid and hexaploid species (*C. officinalis L. var. groenlondica* Gelert. 2n=24 (F l o v i k 1840), *C. arctica* Schlechtend, 2n = 14 (unpubl. data of S ö r e n s e n and W e s t e r g a a r d in L ö v e 1948), *C. officinalis* 2n = 28), C r a n e and G a i r d n e r 1923), *C. Tatrae* Borb. 2n = 42 (Bajer in the press). On the other hand diploid species within the series with the basic number 6 were not found till now. According to previous investigations supplemented by the presented study this series is represented only by tetra- and hexaploides (*C. officinalis L. var. alpina* 2n = 24 B ö c h e r 1938, *C. polonica* Fröhl. 2n = 36).

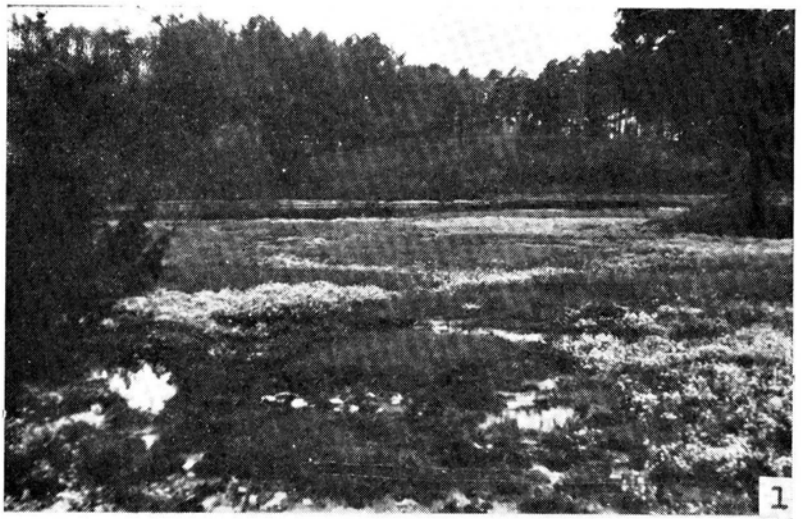
In her paper published in 1932 M a n t o n expressed the opinion that in the evolution of the family *Cruciferae* new genera originated usually as the result of the change of the basic number, whereas new species within a genus have arisen chiefly by polyploidy. Within the genus *Cochlearia* however the two processes mentioned above had presumably contributed to the differentiation of its species.

In *C. polonica* the number of chromosomes as well as the occurrence of polyvalents (hexavalents) at meiosis suggests that this species represents an autohexaploid. This species could have evolved

from a diploid species presumably already extinct. It could have arisen from the union of the normal gamete with an occasionally formed diploid germ cell, giving rise to an autotriploid. A subsequent chromosome doubling of this triploid could lead to the formation of an autohexaploid. Thus its actual chromosome number has been attained in two steps. Numerous examples of chromosome doubling have been described within the last 25 years: e. g. *Nicotiana glutinosa* × *tabacum* (Goodspeed and Clausen 1925), *Raphanus sativus* × *Brasica oleracea* (Karpechenko 1927), *Aquilegia chrysantha* × *flabellata* (Skalińska 1935). The doubling of chromosomes could take place in the growing point of the shoot as in *Primula kewensis* (Newton and Pellew 1929) leading to the formation of an autotetraploid; similarly an octoploid sector has been observed in the runners of a tetraploid *Valeriana officinalis* L. by Skalińska (1947 p. 136).

C. polonica is an endemic autopolyploid species. Contrary to most allopolyploids, the autopolyploids in general do not show any tendency to expand over new territories (Clausen, Keck and Hiesey 1945 pp. 147—148). As well known the increase of the chromosome numbers frequently induces physiological changes (Müntzing 1936, pp. 293—298) which are important for the geographic and ecologic distribution of such forms. In some instances polyploids are able to invade new areas and to colonize quite different habitats; in other instances however their distribution may be restricted only to small areas. This is probably the case in *C. polonica*. Another frequent consequence of polyploidy concerns the life-form. Müntzing (1936), Gustafsson (1947, 1948) and others have shown that in damp habitats polyploid species, prevail; their percentage there is higher than in other plant associations e. g. in the mountain flora. The life-form tends to change with polyploidy: *annuals may change into perennials*. Polyploidy also seems to favour apomixis and vegetative propagation. In the case of *C. polonica* however it should be emphasized that this polyploid species, contrasting with most polyploids from damp habitats, is a biennial not a perennial and its reproduction occurs normally by seeds. Only in rare instances the formation of runners was observed. It ought to be added also that abundant vegetative reproduction of hygrophilous species is by no means limited to polyploids; it occurs as well in a number of diploid perennials from damp habitats (e. g. *Valeriana simplicifolia* 2n = 16, Skalińska, in the press; *Cardamine amara* 2n = 16 E. Banach — unpublished).

PLATE I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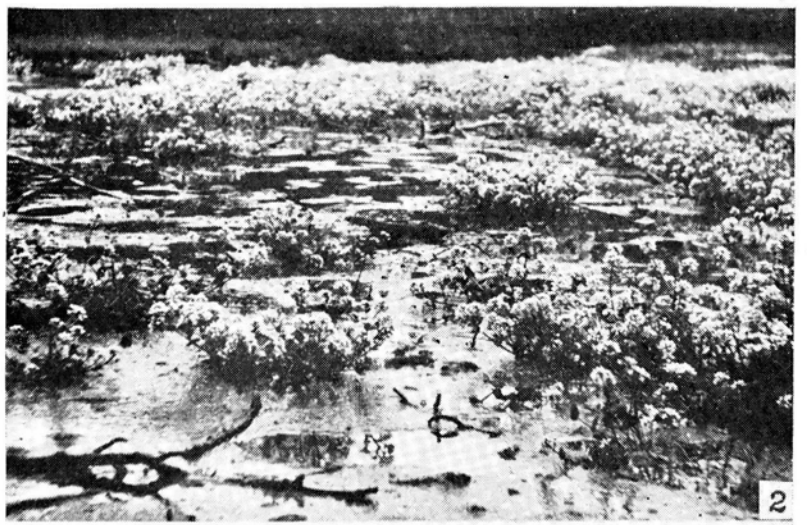
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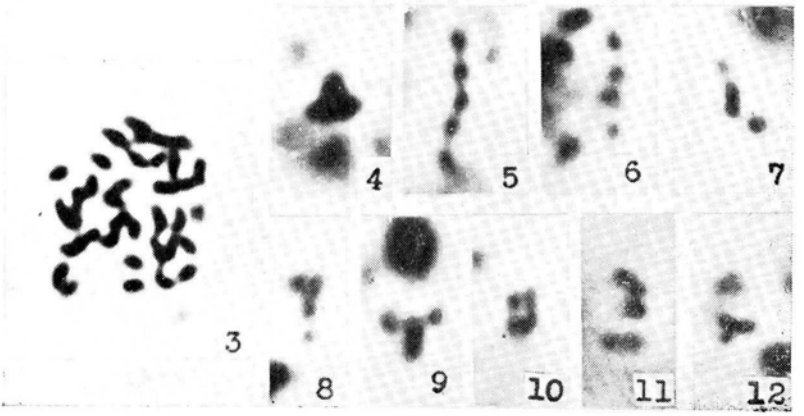
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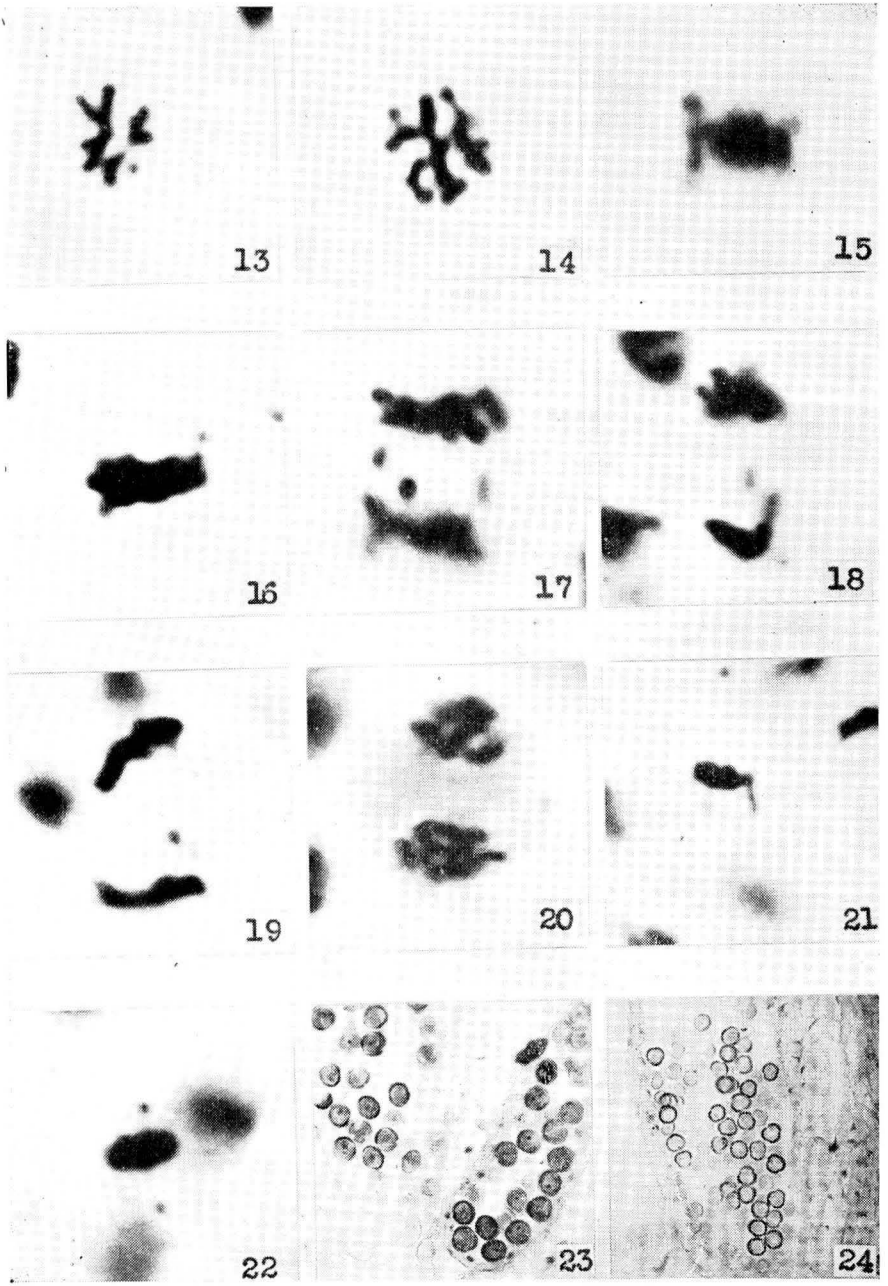
PLATE II



Summary.

1. *Cochlearia polonica* Fröhl. is an autohexaploid with 36 somatic chromosomes; within the genus it belongs to the series of species with the basic chromosome number 6.
2. It represents a biennial endemic Polish species the ecological habitats of which is typical for high polyploids.
3. The study of meiosis in P.M.C's revealed the occurrence of high polyvalents (hexa-, penta-, quadrivalents) in diakinesis and metaphase. The distribution of polyvalent chromosomes to the poles in I anaphase shows some irregularities.
4. The pollen contains a variable percentage of abortive pollen grains (15—100%).

The present work was carried out in the Institute of Plant Anatomy and Cytology of the University of Krakow. In conclusion, the author wishes to express his indebtedness to Professor Dr. M. Skalińska, Head of the Institute for her guidance and valuable criticism throughout the course of the work.

Krakow, 15. V. 1950.

EXPLANATION OF PLATES

PLATE I

Figs. 1—2. Springs of the river Biała: natural stands of *Cochlearia polonica;* plants growing in water and around the springs.

Fig. 3. Metaphase in root-tips (1800 ×).

Figs. 4—12. Polyvalents in diakinesis; 4-ring hexavalent, 5-rod pentavalent 6—7-rod quadrivalents, 8—9-Y quadrivalents, 10-ring quadrivalent, 11-rod trivalent, 12-Y trivalent (3000 ×).

PLATE II

Figs. 13—14. „Secondary pairing" in heterotypic metaphase (polar view) (2850×).

Figs. 15—16. Heterotypic metaphase (side view); polyvalents at the periphery of the plates (2850 ×).

Fig. 17. Heterotypic anaphase with lagging chromosomes (2850 ×).

Figs. 18—19. Heterotypic telophase with lagging chromosomes (2850 ×).

Fig. 20. Homeotypic metaphase (2850 ×).

Fig. 21. A polyvalent at second metaphase (2850 ×).

Fig. 22. Second metaphase. In one spindle two precocious chromosomes near the poles while others are still in the plate (2850 ×).

Figs. 23—24. Anthers with fertile and sterile pollen (250 ×).

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