

DET KONGELIGE DANSKE VIDENSKABERNES SELSKAB

BIOLOGISKE SKRIFTER, BIND VI, NR. 7

CYTOLOGICAL AND EMBRYOLOGICAL
STUDIES IN THE AMPHI-APOMICTIC
ARABIS HOLBOELLII COMPLEX

BY

TYGE W. BÖCHER



KØBENHAVN

I KOMMISSION HOS EJNAR MUNKSGAARD

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1. Introduction.

Arabis Holboellii Hornem. in several respects is a remarkable species. This has particularly been substantiated through investigations during recent years. The following important facts are already available:

(1) The species is very polymorphous, consisting of a number of varieties or microspecies with the chromosome numbers $2n = 14, 21, 28,$ and 42 (ROLLINS 1941, BÖCHER & LARSEN 1950). Among the triploids there is a type with $2n = 21 + 1ff$, which bears plenty of mature seeds. Meiosis on the male side is supplanted by an assyndetic type of division. The result becomes pollen dyads or monads with the unreduced chromosome number. Some preliminary introductory observations of the chromosome numbers of the embryo-sac nuclei indicate apomixis (agamospermy); cf. further BÖCHER 1947.

(2) *Arabis Holboellii* has a disrupted range. It is an American-Greenlandic species with its main distribution in West America. It is absent in the arctic Northeast America, but is found on the Gulf of St. Lawrence. Its distribution would seem to indicate that it belongs to the perglacial survivors, as its often scattered stations can hardly be explained from ecological points of view only, or be due to accidents during its dispersal. It is attached to continental sub- or low-arctic climates and neutral-basic soils and mostly occurs in steppe-like communities. Triploid races have been found in Greenland only, diploid races both in America and Greenland, while tetra- and hexaploids are known from America only (ROLLINS 1941, BÖCHER & LARSEN 1950).

2. Material and Methods, Development of Plants in Culture.

The material of *Arabis Holboellii* mentioned previously (1947) originated from the Botanical Garden of Copenhagen. The Garden had obtained its material from Greenland many years ago, but the place of collection is now unknown. This material has also been used later for the cultivation of new sets of plants and for new fixations. In what follows the whole material from the Botanical Garden of Copenhagen has been termed HBH.

On the Botanical Expedition to West Greenland 1946 I collected seeds in three places in the Søndre Strømfjord, viz. at Itivdlinguaq, Nakajanga (Vandfaldskløften), and the southern slope of Mt. Hassell. The material from these stations in what follows is called S. Str. (Søndre Strømfjord) 10, 9, and 3, the figures referring to the nos. of the localities investigated by the expedition (see BÖCHER 1949).

The material was also supplemented by two seed samples collected by Professor C. A. JÖRGENSEN in 1947 on Østerlien at Godhavn on Disko and one seed sample collected by KNUD JAKOBSEN, M. Sc., at Eqaluit in the eastern area of Archean rock on the Nugssuaq peninsula, also in 1947. The material from the three places in what follows is denoted as Disko 1 and 2 and Eqaluit.

Finally I obtained through the Botanical Garden of Copenhagen seeds of var. *retrofracta* (Graham) Rydb. from Alaska. This material is denoted as Alaska.

The somatic chromosome numbers are distributed as follows in the material:

2 n = 14: Alaska, Disko 1, S. Str. 10, HBH (a few plants).

2 n = 21: Eqaluit, Disko 2, S. Str. 3, S. Str. 9.

2 n = 21 + 1 ff: HBH (many plants).

After sowing of the seeds in April all samples developed rosettes formed during the first season. In pot cultures the plants during the first summer remained completely without flowers; but in the experimental field a number of triploids from S. Str. 3 and 9 succeeded in flowering late in summer. However, they formed only very short flowering shoots close above the rosette (see fig. 16). The formation of long shoots thus in all cases began in the second spring. The appearance of the plants

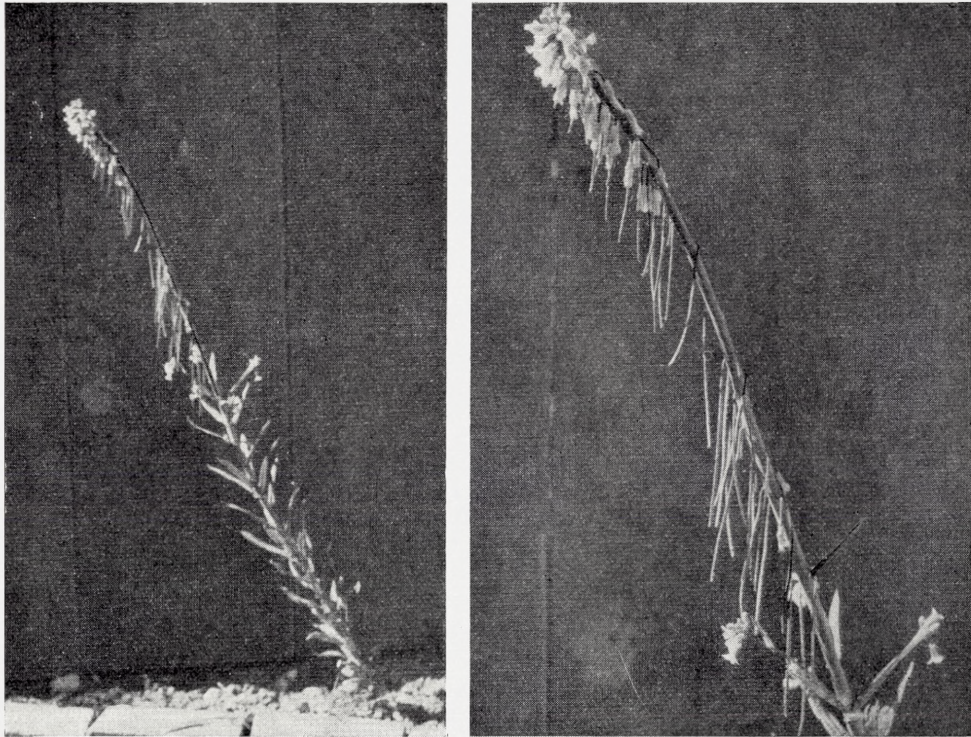


Fig. 1. Typical individual of *Arabis Holboellii* developed from a rosette which did not succeed in bearing flowering shoots in the first season. The plant is triploid and bears plenty of seeds. Material from S. Str. 3 cultivated in Denmark.

is greatly influenced by their flowering late in the first season or not. If only they have formed rosettes they will in the following year as a rule be little branched, with a single vigorous main shoot (fig. 1), whereas after flowering late in the first season (fig. 16 left) they become greatly branched, often with arcuately rising shoots. In the latter case the tip of the main shoot, from which the later flowering in the first season starts, is no doubt dead, while lateral shoots corresponding to the short lateral shoots seen on the plant in fig. 1, have become very long and vigorous (see fig. 2).

The plants die at the close of the second season. Only if the flowering fails or is inhibited (in nature e. g. as a consequence of attacks by *Puccinia Holboellii* Rostr.), or if the fructification fails, the plants may survive, the rosette being retained, or new small rosettes are developed in connexion with the old one. In general the fructification is very considerable. The large number of ripe siliques weigh down the plant so that it comes to slant somewhat (fig. 1); the siliques hang down vertically. The slanting causes the seeds generally to fall at some distance from the base of the mother individual. In nature (Eqaluit, S. Str. 3, 9, and 10) some individuals were always found which did not bear seeds; in rare cases branches on normally fructifying individuals were completely sterile. The same phenomenon may be ob-



Fig. 2. Flowering greatly branched individual of *Arabis Holboellii* developed from rosette, which in the first season succeeded in producing short flowering shoots, the main axis of the plant being unable to stretch. The same triploid race as in fig. 1, cultivated at Vridsløselille, Denmark.

served in cultures (cf. p. 43). As appears from fig. 1 the long inflorescences have nearly ripe siliques below, while at the same time new flowers develop at the top. Thus it is possible to ascertain that a shoot is sterile and fix buds from such a shoot.

At the fixation NAWASHIN's fluid or NAWASHIN-KARPETCHENKO (MÜNTZING's modification) was used, after a short preliminary fixation in CARNOY. Staining was made with FEULGEN's nuclear reaction or NEWTON's iodine gentian violet after hydrolysis in HCl as in the FEULGEN technique.

3. Pollen Development.

Normal Meiosis in Diploids.

(a) Alaska (var. *retrofracta*). Fig. 3a—e shows stages of meiosis in the loculi and fig. 10c a prophase of the first pollen division. There were seven bivalents with complete pairing in all PMCs. In the second metaphase there was a tendency towards secondary association between two chromosomes (fig. 3d), very rarely there were

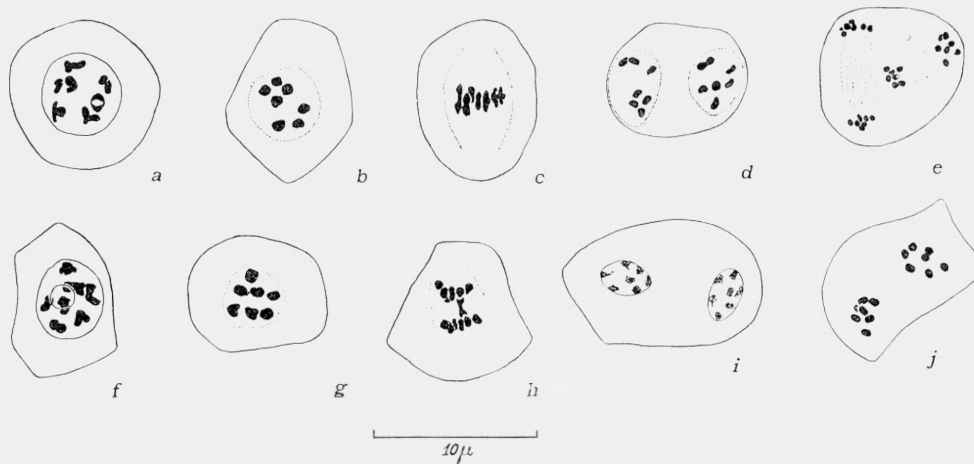


Fig. 3. Meiosis in PMCs in sexual, diploid *Arabis Holboellii*. a—e material from Alaska of var. *retrofracta*; f—j material from West Greenland of var. *typica* (S. Str. 10, Itivdlinguaq). — a, f, diakinesis; b, c, g, metaphase I; h, anaphase I; i, interkinesis; d, early metaphase II; j, metaphase II; e, anaphase II. $\times 2150$.

two groups with two secondarily associated chromosomes. The second anaphase was completely normal and the pollen quite homogeneous (fig. 9 a). A great material of buds was examined, and in no case there appeared to be deviations from a fully normal meiosis. The same applied in the case of the female organs, for which reason this race must be considered normally sexual or amphimictic.

(b) S. Str. 10. In the case of this material there are two widely different types of development. In one part of the material the pollen development was normal (meiotic type), in another part, which will be mentioned below, there was an apomeiotic pollen development¹. In the flower buds in which there was normal pairing the picture was completely in accordance with the Alaska material (see fig. 3 f—j). There were no meiotic disturbances. In the second metaphase there might be a slight secondary association (fig. 3j), and in the first anaphase (fig. 3h) the separation of one of the pairs might be somewhat delayed as compared with the rest. Normal tetrads and homogeneous pollen were formed (fig. 10 b).

Meiotic Pollen Development in Triploids.

Triploids mostly have apomeiotic pollen development. This will be mentioned in detail below. Meiotic pollen formation with pronounced pairing during the first division generally occurs in definite parts of the inflorescence in plants which otherwise proved apomeiotic. However, loculi with meiosis mostly might also have PMCs

¹ *Apomeiosis* means 'any formation of a gametophyte without preceding reduction of the chromosome number' (cf. RENNER 1916, STEBBINS & JENKINS 1939 p. 192, STEBBINS 1941). In the present paper apomeiosis will be used both of the pollen development (including the formation of the male gametophyte) and of the embryo-sac formation (cf. DARLINGTON 1932, 1937).

with total asyndesis, which clearly did not carry through any reduction division. The behaviour of these PMCs will be mentioned in detail in the next section.

(a) HBH. In this material the meiotic type of division was particularly frequent. Probably conditions are these: plants of the HBH material in a comparatively long section of the inflorescence carry through a fairly normal meiosis alongside of other kinds of pollen formation. This view is supported by the fact that in the material both small and larger pollen mother-cells with meiotic division have been found, and it is evident that larger PMCs are preferably formed in the lower and middle part of the inflorescence, while small PMCs are formed at the top (cf. p. 26). For that matter all the triploid material from HBH is characterized by the presence of a supernumerary fragment chromosome, as seen in the root-tip mitosis in fig. 10 d at the bottom of the plate.

In the material where the PMCs were fairly large (fig. 4) a metaphase I with an amazingly regular appearance was observed, a much smaller number of univalents occurring than might be expected beforehand. Whereas the theoretically most probable combination at full syndesis would be 7 bivalents, 7 ordinary univalents and a univalent fragment chromosome or 7 trivalents + the fragment chromosome, there were mostly 10 bivalents, 1 univalent normal chromosome, and the fragment chromosome. There was obviously a difference in the degree of syndesis in the different loculi. In some there were always up to 10 bivalents during metaphase I and corresponding loculi with anaphases gave two groups with 10 or nearly 10 and few univalents, which were seen lagging between the separated groups. Often a large and a small lagging univalent were clearly seen (fig. 4g). In metaphase II there were correspondingly two groups of 10 chromosomes and sometimes a small group with a large chromosome and the fragment (fig. 4j) somewhat removed from one of the groups of 10 chromosomes. In case there were 10 in the groups the second anaphase gave pollen tetrads with 10(—11) chromosomes in the nuclei. Such tetrads have nearly always grains of equal size. In other loculi there was a fairly great number of univalents varying between 3 and 5, besides the fragment (see figs. 4a, e, h). 7 univalents (+ the fragment) were nowhere observed during meta- and anaphase I; but cells of this type must be supposed to occur. Metaphase II pictured in fig. 4i shows two plates, one with 7, the other with 14 (+ 1 ff). Such a distribution may be supposed to have arisen after a metaphase I with 7 bivalents and an anaphase in which all univalents go to one pole. A cell of this type is interesting by giving two pollen grains with 14 + 1 chromosome and two with 7 chromosomes. As both pollen types have one or two chromosome sets they may very well be supposed to be functioning.

In the material in which the PMCs were smaller (fig. 5) there was also a high degree of syndesis. Here there were also PMCs in diakinesis. The cell in fig. 5a contains 9 bivalents, 3 large univalents and the fragment. In the metaphase in fig. 5d there are 8 bivalents and 5 + 1 univalents. In fig. 5b there are probably 1 trivalent, 8 bivalents, and 2 univalents, while the fragment cannot be seen here. In fig. 5c,



Fig. 4. Material from HBH of triploid *Arabis Holboellii*. a—l, meiosis; m—s, apomeiosis resulting in the formation of monad pollen (s). — a, b, c, d, f, metaphase I; e, g, h, anaphase I; i, j, metaphase II; k, anaphase II; l, young tetrad. — m, n, o, semiheterotypical division; p, restitution nucleus; q, the same, the nucleus being much stretched; r, possibly a meta-anaphase II resulting from a cell like fig. 4 p or q; s, young monad pollen. $\times 2150$.

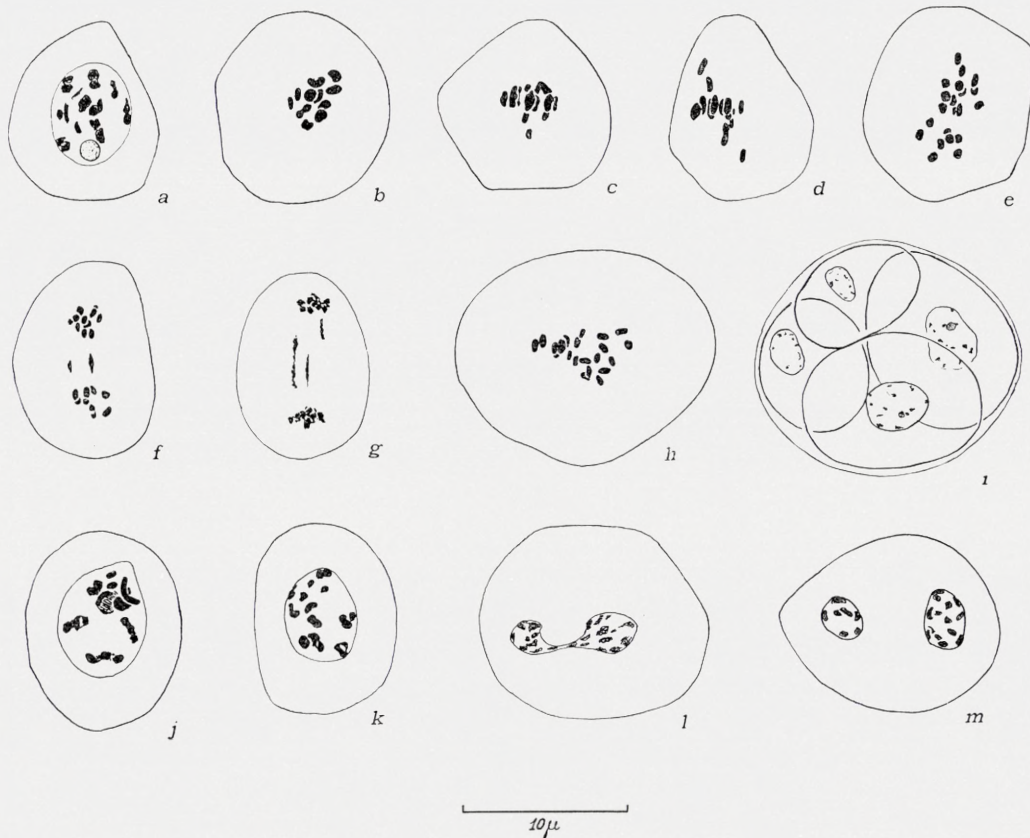


Fig. 5. Material from HBH (a—g). S. Str. 3 (h—i), and S. Str. 9 (j—m) of triploid *Arabis Holboellii*. In the material from HBH and S. Str. 9 the PMCs are rather small as compared with those pictured in fig. 4 and fig. 5 h—i. — a, j, k, diakinesis; b, c, d, metaphase I; f, g, anaphase I (see text); e, semiheterotypical meta-anaphase; h, metaphase II resulting from a restitution nucleus like that pictured in fig. 1; i, tetrad with two large and two small grains; m, interkinesis; in the two nuclei there are 7 and 14 chromosomes. $\times 2150$.

where the chromosomes are seen in side view, there is no doubt about the presence of the trivalent association and here, too, there are 8 bivalents and 2 + 1 univalents. The anaphase seen in fig. 5 f has 9 in the lower and 11 in the upper plate and further a large lagging univalent as well as the fragment, which is also lagging. The cell may be supposed to follow after a metaphase with 1 trivalent and 8 bivalents. Nowhere more than one trivalent configuration were seen.

(b) S. Str. 3. In this material it was sometimes possible to find a few stamens with meiotic division (see p. 20). Homotypical prophases and metaphases showed that often there was an incomplete separation of the nuclei after the first division. In the cell in fig. 5 h the chromosomes formed a connected group, but were distributed so that 11 chromosomes at one end as regards orientation were turned 90° in relation to 10 chromosomes at the other end. Such a cell undoubtedly follows after restitution nuclei greatly constricted in the middle; see S. Str. 9 and FÄGERLIND 1937 (pp. 337—339). The anthers or loculi with meiosis in question were found in flowers which also

had anthers with fully developed dyads. The development in the loculi with meiosis obviously had been slower than in the others (cf. p. 20). A tetrad with two larger and two smaller pollen grains is seen in fig. 5 i. It was found jointly with dyads like the one seen in fig. 6 y, which shows that meiosis and apomeiosis could also take place side by side in the same loculus.

(c) S. Str. 9. Among some potted plants of this type there was one with several large shoots issuing nearly from the base. One of these proved to give only empty, fast withering siliques, whereas there was clearly nothing wrong about the fructification on the other shoots. The top flowers on one of the fertile shoots and on the sterile one were fixed. It proved that the fertile shoot had regular dyad formation, while in the sterile shoot there was a meiosis which was very irregular, but in which the degree of syndesis as in the HBH material was astonishingly high. There was a typical diakinesis with a varying, but mostly low number of univalents. The nuclei might be very difficult to interpret, but the occurrence of trivalents was beyond doubt. In a cell as the one shown in fig. 5 j there are three, perhaps four trivalents. In the metaphases it might be judged that there were often more than 7 bivalents. Anaphase I was very irregular and mostly resulted in an extremely uneven distribution of the chromosomes or in a peculiar form of constricted restitution nuclei, which developed into dumbbell-shaped interkinesis nuclei (fig. 5 l). In the same loculus more than half of the PMCs might have this appearance. They no doubt arise after such semi-heterotypical anaphases in which two separate chromosome groups have been on the point of developing. An interkinesis with the distribution 7—14 chromosomes is seen in the cell in fig. 5 m. Such cells were seen here and there and perhaps they may sometimes give functioning pollen grains.

In the material there were many signs of degeneration of PMCs, and accordingly ripe anthers contained comparatively few pollen grains. The pollen was irregular, often shrivelled, and of nearly the same order of magnitude as in diploid plants with a normal tetrad formation.

The tapetal cells in the sterile shoot behaved differently. They worked loose and were rounded off like PMCs and at this stage were always binuclear. PMCs in meta-anaphase I were always surrounded by a fringe of such loose, binuclear tapetal cells. There were no signs, however, that these should ever come to form pollen.

The strange behaviour of the tapetal cells and the degeneration of PMCs suggest a disturbed physiological equilibrium, and this perhaps is the most important thing in connexion with the fact that the shoot was completely sterile. Most of the pollen is bad and it will only extremely rarely be able to germinate or be of any importance for the pseudogamy (cf. p. 40). One EMC in metaphase I with 10 bivalents was found (fig. 13 k), which shows that meiosis in the ovules may follow the same main lines as at the pollen formation. If EMCs are subject to the same physiological factor as PMCs, there may also be degeneration of them, which of course results in sterility. But it is furthermore very conceivable that possibly formed megaspores with reduced chromosome numbers (about 10) will be unable to develop embryo-sacs. Below (p. 43) we shall return to the problem of sterility in *Arabis Holboellii*.

Apomeiotic Pollen Formation in Diploids and Triploids.

Formation of pollen with the unreduced chromosome number was very frequent in *Arabis Holboellii*. In many cases there seemed in the same type to be either meiotic or apomeiotic pollen formation, and the two types of formation were not connected by intermediate types. In the HBH material there was, however, proof that meiotic and apomeiotic divisions may take place side by side, occasionally even in the same loculus, and in such cases it is possible—apart from the result of the division, reduction or no reduction—to find intermediate forms between the two types of division.

Semiheterotypical Type of Division.

(a) HBH. In some loculi in flowers with a meiotic type of division and large PMCs there was a number of cells with greatly reduced syndesis or total asyndesis. During the meta- and anaphase the univalents were scattered on the spindle as first described by ROSENBERG (1917, 1926–27) for *Hieracium* and by him termed semiheterotypical meta- and anaphase. As in the case of *Hieracium* the result may be two nuclei, often with greatly different chromosome numbers (cf. ROSENBERG 1917, fig. 26 B); but mostly the result is a restitution nucleus (cf. fig. 4 m–p) with the unreduced number. The restitution nuclei may develop further and pass into homotypical division. The result then will be pollen dyads, or the whole process for some reason stagnates so that also the second division fails to appear. The uninuclear interkinesis in question then gives rise to a monad pollen grain. The cells shown in fig. 4 p–q were found together with PMCs in the second prophase, metaphase, and telophase. Thus their division had been delayed, but otherwise there was hardly any delay in their development. As in neighbouring cells at the end of the second division a demarkation was taking place in the plasma which is included in the resulting pollen grains. In several cases the nuclei were in a state corresponding to that found in recently developed dyads in other types of *Arabis Holboellii* (cf. fig. 6 t, y), where the chromosomes, apart from the chromocentres, had become nearly invisible. The cells in fig. 4 p, q therefore should no doubt be interpreted as sources of monad pollen. The cell in fig. 4 r is interesting because it makes it probable that a kind of restitution nuclei may also develop during the second division. The cell was found together with PMCs in the second division, while the cells in figs. 4 m–o were found with cells in the first division. Its chromosomes formed a connected rather long figure; but outside this the small extra chromosome furthermore was seen isolated from the others. This isolation may most naturally be supposed to have arisen during the first division. The cells fig. 4 l and s were found together in the same loculus with nearly ripe tetrads. In fig. 4 s there can be no doubt that we have a monad. In this and other cases there was often a depression on one side of the young monad pollen grain. A corresponding depression is seen in the process of formation in figs. 4 p–q. Presumably this is connected with the stretching and bending of the nucleus taking place, an extreme case of which is seen in fig. 4 q.

In some respects this material resembles arctic *Poa* (NYGREN 1950). In *Poa flexuosa* tetrads, dyads and monads occur in the same loculus and a kind of restitution nuclei are formed during the second division. Here, however, the chromatides separate, thus giving rise to tetraploid monads.

In the material with small PMCs also asyndetic cells were found (fig. 5e), which probably also would develop into dyads or monads. Furthermore there were in some loculi distinct signs of the first division having become abnormal. Late prophase cells or diakinesis-like cells obtained oblong nuclei, which in some cases might seem to undergo a contraction, in other cases obtain an interkinesis-like character. Conditions in these loculi clearly to a considerable degree approached those found in flowers with complete or nearly complete suppression of the first division.

The *Pseudoillyricum* Type and the Pseudohomotypical Type of Division.

The first of these terms refers to ROSENBERG's *Hieracium pseudoillyricum* type, which in all essentials corresponds to one of the completely asyndetic types found in *Arabis Holboellii*. The *Hieracium pseudoillyricum* type is mentioned on pp. 326—330 in ROSENBERG 1926—27.

The material to illustrate the asyndetic types is richest among the triploids, for which reason the account is introduced with a mention of them. As compared with the types of pollen formation mentioned above, it is in the first place remarkable that the diakinesis in most cases is missing. The late interkinesis nuclei and uninuclear cells with homotypical prophases described below may indeed look like asyndetic diakineses, but are distinct from these by being without the large nucleolus and by the smaller diameter of the nucleus. The other characteristic feature is that there is only one cell-division, perhaps at times none at all, and that during the metaphase no paired chromosomes are ever seen, but only secondarily associated chromosomes. These metaphases clearly correspond to homotypical or pseudo-homotypical metaphases. The result of the cell-division which can be observed is a regular formation of dyads. The succession of the various stages during the whole process is often difficult to make out. According to FAGERLIND's investigations (1947 a and c) of the macrogametophyte formation in *Taraxacum* and *Erigeron annuum* we should be very attentive to the placing of cells with one contraction nucleus. For the sake of the discussion of this feature we shall begin by mentioning the Eequaluit- and Disko 2-material, which contains all stages.

(a) Eequaluit. Fig. 6a—t. Corresponding to the diakinesis there is a mitotic-looking prophase, only essentially different from such a phase by the size of the nucleus and the nucleolus (fig. 6a). The chromosomes are long and large parts of them are euchromatic and colourless. The stage follows after early prophases, in which only very small chromocentres are coloured and the chromosomes are still more filiform. In some cases there is at these stages a synzesis contraction, but this is not pronounced in the present material.

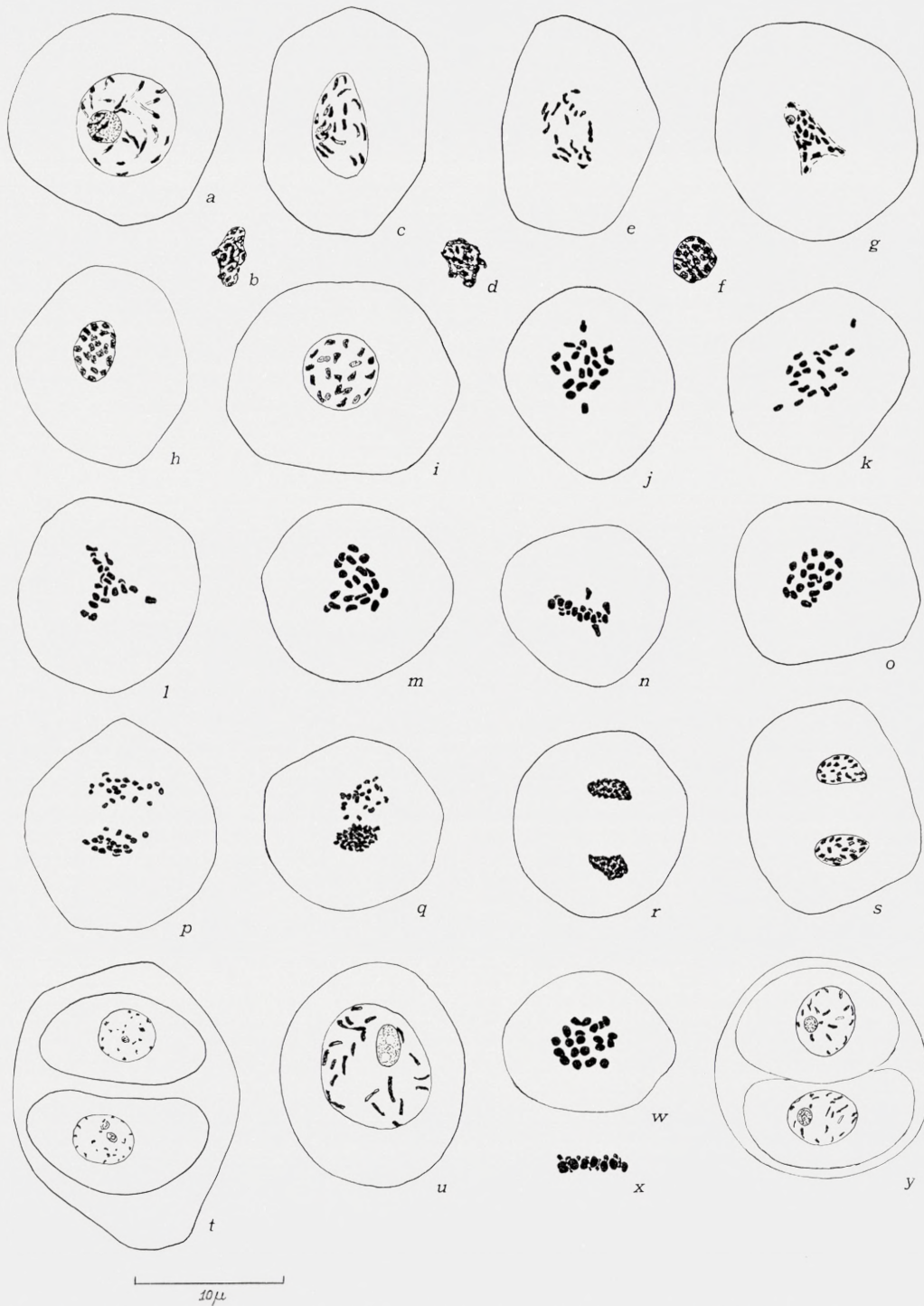


Fig. 6. Apomeiotic and asyndetic division in PMCs of triploid *Arabis Holboellii* from Egeluit (a—t) and S. Str. 3 (u—y) — a and u, mitotic prophase; c, e, g, PMCs from the same anther showing transitional stages between prophase and the contraction stage. — b, d, f, three nuclei in the contraction stage. — h, i, interkinesis. — j, k, prometaphase I. — l, m, prometaphase II (cp. text). — n, o, and w, x, metaphase. — p, q, anaphase. — r, telophase. — s, very young dyad. — t, y, young dyads. $\times 2150$.

In some flowers the anthers of which otherwise only contained cells in the prophase, there were a few loculi the development of which was ahead of that of the others. In these there were transitions between the prophases corresponding to diakinesis (fig. 6a) and contraction nuclei (fig. 6g). FAGERLIND (1947b), who has studied contraction stages in great detail, is of opinion that the contraction in question corresponds to the one normally taking place during ana- and telophases. If therefore the above-mentioned stages in *Arabis Holboellii* should also correspond to telophases, we should previously have a chance of finding signs of a division. I also succeeded in doing so in the case of the Ekaluit material. The large prophase nucleus stretches and becomes oblong or somewhat flattened while at the same time the chromosomes increase in colouring capacity and condensation. The nucleolus decreases considerably in size (fig. 6c). Then a stage follows in which the nuclear membrane often can no more be distinguished and possibly also in certain cases is dissolved, and in which it seems that the nuclear spindle is active or is developing. Already the stretching of the nucleus is indicative of this, but in a cell as the one in fig. 6e there is an arrangement of the still rather long chromosomes which reminds of the arrangement of univalents during the semiheterotypical division mentioned above. No doubt the disappearance of the nucleolus and the degeneration of the nuclear membrane together with the stretching of the nucleus are sufficient evidence that the stages in question correspond to a division. Probably the spindle has not succeeded in developing normally and the ana-telophatic contraction sets in at an advanced stage. The first division of the meiosis only manages to be faintly indicated.

The sequence of the next stages is not immediately obvious and might best be judged after analysis of the distribution of the stages in whole loculi. Unfortunately all the PMCs in the same loculus mostly were at the same stage. Hence the material became rather sparse, but it might be supplemented somewhat by studies of the stages occurring in the same anther. Furthermore it was in several places evident that the parts of the anthers which were averted from the central axis of the flower developed fastest. The loculi situated farthest out showed a more advanced development than those in the interior. The following example (Table 1) shows the distribution in all the 24 loculi of a flower. Stamens 2 and 5 contain comparatively young stages and probably are the two outer, shorter stamens in the flower. In stamens 1—4 the outer loculi clearly showed a more advanced development.

Both contraction nucleus and interkinesis cover several stages and may be difficult to distinguish. No doubt the chromosomes in the pronounced contraction nuclei are in the same state as in typical interkinesis. Interkinesis therefore is used to denote cells with rounded-off, not greatly contracted nuclei, in which the chromosomes have the structure of interkinesis. As appears from figs. 3i and 5l—m it is always possible during the interkinesis to count the chromosomes with fairly great certainty. They are comparatively little changed. They are more diffuse at the edge and less stainable. Hence it is difficult or impossible to draw a sharp distinction between the interkinesis proper and the prophase of the division following. The

Table 1.

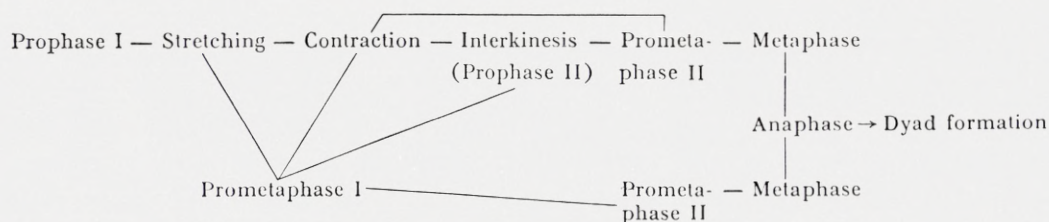
Stamen no.	Inner loculi	Outer loculi
1. Left half [as seen from the central axis of the flower]	Interkinesis [uninuclear]	Interkinesis [uninuclear] Prometaphase II
1. Right half [as seen from the central axis of the flower]	Interkinesis [uninuclear]	Interkinesis [uninuclear] Prometaphase } II Metaphase }
2. Left half	Prophase I Stages of stretching and contraction [cf. fig. 6c—e]	Prometaphase I
2. Right half	Prophase I Stages of stretching and contraction [cf. fig. 6c—e]	Contraction nucleus Prometaphase I and metaphase
3. Left half	Interkinesis [uninuclear] Contraction nucleus	Prometaphase II Anaphase Telophase Anaphase Prometaphase II — metaphase Contraction nucleus
3. Right half	Prometaphase I	Contraction nucleus Metaphase [a single cell]
4. Left half	Contraction nucleus	Prometaphase II Metaphase [a few cells]
4. Right half	Prometaphase I	Contraction nucleus
5. Left half	Prophase I	Prophase I
5. Right half	Prophase I Contraction nucleus	Prophase I
6. Left half	Contraction nucleus Metaphase and Prometaphase I	Interkinesis [uninuclear]
6. Right half	Prometaphase I	Contraction nucleus

chromosomes in the latter are fairly scattered and are more distinctly delimited, and furthermore the nucleolus is often visible. In the table such prophases and interkineses proper are merged and termed interkinesis.

The most difficult problem is that of the so-called prometaphases (fig. 6j—m). As in *Taraxacum* and other apomicts (cf. FAGERLIND 1947 c p. 388) these are remarkably common, so common that they must be of quite a different signification and duration than in plants with a normal meiosis. In *Arabis Holboellii* it was nearly impossible to find them in the diploid or triploid material of a meiotic type of division. In the apomeiotic material there was partly a clear transition from the interkinesis to the prometaphase and further to the metaphase and the anaphase, partly a very great probability of transition from the stretching stage to the prometaphase and from that further to the contraction phase. While comparatively compact prometaphases as e. g. in fig. 6 l—m have no doubt developed from contraction nuclei or interkineses, it is not possible in such cells as those in fig. 6j—k, because of the spreading or repulsion of the chromosomes, which exceeds the diameter of the interkinesis nuclei, to imagine such a development. It is natural to imagine the stretching stage (fig. 6c—e) continued by further condensation of the chromosomes, and a stamen like no. 2 in Table 1 greatly suggests that such a development takes place. Very diffuse prometaphases were particularly frequent in some loculi. Hence they might be assumed to belong to the first division, whereas the denser ones continuing in meta- and anaphase, would belong to the second division. If the degree of condensation in the chromosomes had been greatly different in the case of the two divisions as in *Taraxacum*, the two types would have been easy to distinguish. However, there is practically no difference in the condensation between the first and the second division in *Arabis Holboellii*; if anything, it is a little greater in the second division, and hence the interpretation offered in Table 1 by terming the prometaphases I and II is not without elements of uncertainty.

If the two types of prometaphase are interpreted correctly, the question arises whether prometaphase I always develops into a contraction nucleus, or whether it may also become a metaphase. I have seen much in the material to suggest that both possibilities are realized. Thus the question arises whether the contraction stage with subsequent interkinesis may also follow after metaphases, which thus are asyndetic first metaphases. I have repeatedly seen contraction nuclei in the same loculus as regular plates of metaphase and hence have pondered on the possibility that conditions should correspond to *Taraxacum* and *Erigeron*, where the metaphases are clearly superseded by the contraction phase, the latter to begin with having the plate form of the metaphase (see figs. in FAGERLIND *loc. cit.*). But in *Arabis Holboellii* the contraction nuclei were always oblong to irregularly spherical (fig. 6 b, d, f), sometimes 3—4-lobed. Therefore I believe that possible first metaphases only exceptionally develop further into contraction nuclei and interkineses. If so, these stages are rather left out, and, in so far as the metaphases are continued in anaphases, they must therefore be characterized as pseudohomotypical (cf. GUSTAFSSON 1935 b). My view

of the pollen formation therefore is that partly we have a nearly total suppression of the first division and a development corresponding to that of the *Hieracium pseudoillyricum* type, partly a pseudohomotypical division, which, however, in the Egluit material does not pass through any asyndetic diakinesis. This may be illustrated diagrammatically as follows:



Contraction nuclei may be found together with meta- and anaphases. Thus it seems that the interkinesis may be left out, which does not seem strange, since the chromosomes just have an interkinetic structure during the contraction stage.

The *Pseudoillyricum* type no doubt is the one requiring the longest time. Here there are two repulsions and two telophatic contractions, whereas at the pseudohomotypical division there is only one repulsion, which reaches its maximum during prometaphase I. After that there is an increasingly great attraction until the chromosomes form a dense lump during the telophase (cf. fig. 6p—r).

All the peculiarities mentioned probably can be referred to a definite one, viz. the fact that the process of division as a whole takes place very fast, so that the stages found under normal meiosis are often only indicated or left out. The greater speed also appears from the fact that flowers containing stages between prophase I and fully developed dyads are much rarer than flowers in diploid amphimicts containing all stages of meiosis. If a section is made right through the upper part of the inflorescence in these, several flowers will contain meiosis, while in apomeiotic forms following the *Pseudoillyricum* type only one or two flowers show the stages corresponding to meiosis.

(b) Disko 2 (fig. 7i—t). On the whole the same stages were found here as in the preceding material, also transitions between prophase I and the contraction nucleus, but no stages in which a dissolution of the nuclear membrane seemed to be indicated. In return the synzesis stage was frequent during the early prophase. In the material anaphases were very frequent. One cell in the anaphase (fig. 7s) contained two chromosomes in each plate with a completely mitotic appearance, while the rest had retained the degree of condensation which is common both in normal homotypical anaphases and in the corresponding anaphases in apomeiotic flowers. A clear deviation was the occurrence of secondary associations, which are already indicated during prophase II (fig. 7l), but later become greatly pronounced in the metaphase. Many examples might be found of approaches between two and two chromosomes (particularly fig. 7n and p), and the secondarily associated chromosomes might

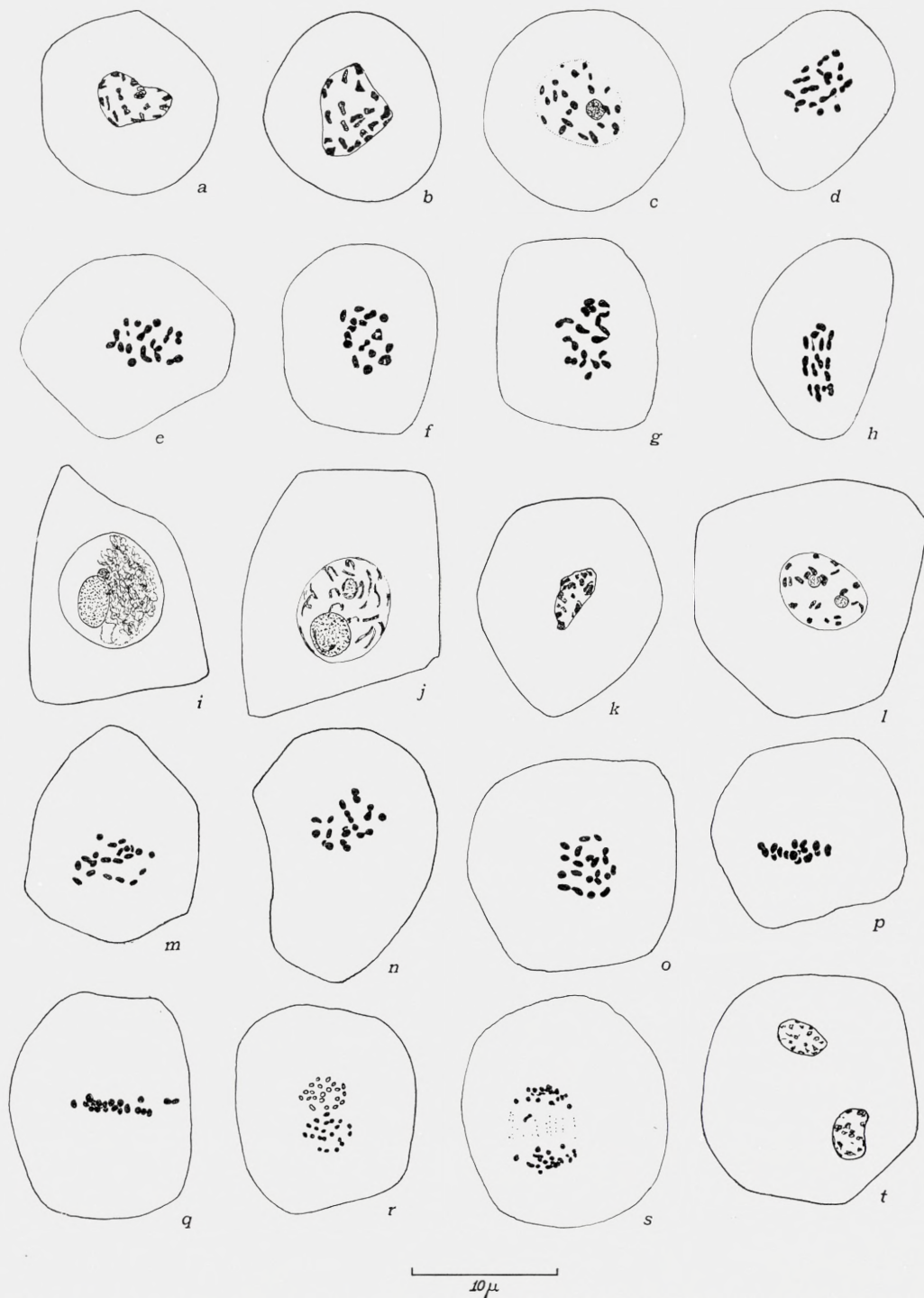


Fig. 7. Apomeiotic and asyndetic development in PMCs of triploid *Arabis Holboellii* from HBH (a—h) and Disko 2 (i—t). — a, b, interkinesis (a somewhat contracted nucleus in fig. a). — c, prophase II (cp. text). — d—g, metaphases showing secondary associations between the chromosomes. — h, the same stage but the chromosomes arranged as in a semiheterotypical anaphase. — i, very early prophase. — j, asyndetic, mitotic prophase. — k, contraction stage. — l, prophase II. — m, prometaphase. — n, o, p, q, metaphase. — r, s, anaphase. — t, dyad nuclei formed. $\times 2150$.

sometimes (fig. 7p) be placed with nearly polarly oriented centromeres (cf. the pseudogemini formation in *Taraxacum* studied by GUSTAFSSON 1935a); but the general impression was that this was an accident or the result of narrow room in the equatorial plane of the spindle. As a rule the centromeres of both associated chromosomes were in the equatorial plane (fig. 7n, o, and q farthest to the right).

(c) S. Str. 3 (fig. 6u—y). In this material some of the same stages were found, which is evidence that the pollen formation follows the same lines as described in the cases (a)—(b). However, as mentioned, there is not always a sharp distinction between flowers with meiosis and flowers with apomeiosis. Reduction and non-reduction may take place in the same flower and even sometimes in the same loculus. In one case the three loculi preferably contained dyads, while the fourth had meta- and anaphase II, which had developed after a meiotic first division. Flowers which in this way had no homogeneous pollen formation seemed to occur in a place in the raceme where there is a transition from asyndesis to syndesis (cf. pp. 24—28).

(d) HBH (fig. 7a—h). With the exception of stages with extreme or great nuclear contraction and with anaphases nearly all the stages described under (a) and (b) were found here.

The secondary association between the chromosomes during the metaphase was of special interest here. This association was more pronounced than in the other cases, the connexion between the chromosomes apparently being closer. Furthermore there were several cases of pseudotrivalents. In figs. 7d, e, f there are one pseudotrivalent and numerous groups with two associated chromosomes. In fig. 7g there are two groups with three, and four groups with two secondarily associated chromosomes. The number of completely isolated chromosomes varied between five and ten. Among these the small extra chromosome always occurred. In the cells pictured in fig. 7d—g all the centromeres were in the equatorial plane.

A possibly important deviation from the other material was the fairly frequent occurrence of cells with an appearance like fig. 7h. They were found together with cells with regular or somewhat diffuse metaphase plates. As in these the chromosomes were secondarily associated, but were placed together within a spindle-shaped area. The arrangement greatly reminded of that of univalent chromosomes during semi-heterotypical meta-anaphase or of that seen in the cell in fig. 4r, which probably belongs to a homotypical division. It was mentioned above that in some of the triploid HBH material some PMCs were seen in which the nucleus, which was in the late prophase, was extended and assumed an oblong, often somewhat curved form. Such cells can easily be imagined to have given rise to the deviating metaphases mentioned. These did not seem likely to continue as anaphases, but looked like restitution nuclei. If this view is correct, they will finally form monad pollen.

(e) S. Str. 10. Diploid type, cf. p. 7. Besides quite normal meiosis (fig. 3) regular dyad formation and no doubt monad formation, too, were found here. Whereas there were numerous preparations showing finished dyads or pollen of dimensions which would presuppose monad formation, there was only a single preparation

showing stages of dyad formation (fig. 8). The same contraction stages with interkinesis-like chromosomes were found (fig. 8a—b), and also young dyads in which the delimitation of the resulting pollen grains had not yet taken place (fig. 8d). The dyad formation took place with great regularity as in the triploids. The agreement with these obviously was very great, even though there seemed to be asyndetic diakineses here (cp. fig. 3a, f with fig. 8c). Unfortunately the meta- and anaphase were not seen, but in principle it can be said that the division was completely in accordance

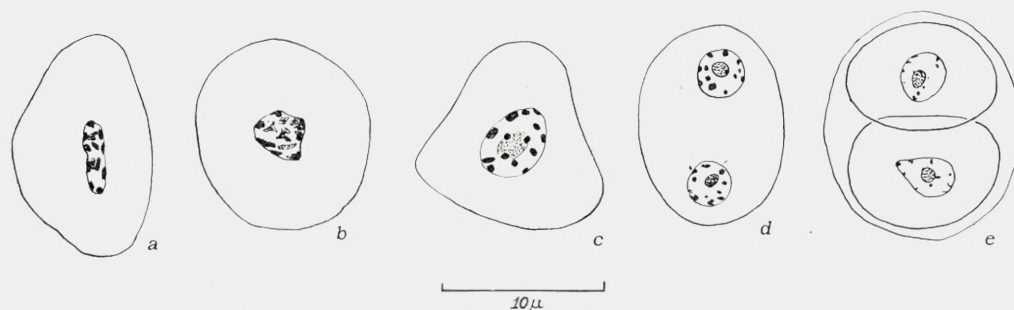


Fig. 8. Apomeiotic and asyndetic division in PMCs of diploid *Arabis Holboellii* from S. Str. 10 (Itivdlinguaq). — a—b, contraction stage and interkinesis — c, prophase II or perhaps asyndetic diakinesis. — d, dyad nuclei with unreduced chromosome number. — e, young dyad. $\times 2150$.

with the triploid material, the resulting dyad nuclei containing 14 chromosomes. The dyad cells were smaller than in the triploids just as the pollen mother-cells were.

The apomeiotic process in this material took place on other plants than the meiotic ones. There were no signs of asyndesis where the meiotic type occurred, and no signs of syndesis where the apomeiotic pollen formation took place.

(f) HBH (diploid type). In the embedded material from HBH there was, besides that originating from triploid plants, some which originated from one or a few diploid plants. The pollen formation could not be studied here, but the pollen completely corresponded to that found in the S. Str. 10 material with apomeiotic pollen formation. The embryo-sacs in the diploid HBH material as well had generally unreduced chromosome numbers (see p. 33).

Survey of Pollen Formation.

I. Prophase with diakinesis.

a. Meiotic division with reduction of the chromosome number.

1. Normal meiosis and tetrad formation in diploids.

2. Approximately normal meiosis with varying number of univalents.

Tetrad formation, pollen with varying chromosome numbers, mostly ab.

9—11. Triploids.

b. Apomeiotic development.

1. Asyndesis. Semiheterotypical meta- and anaphase followed by the formation of restitution nuclei. The restitution nucleus develops further. After homotypical division dyads with unreduced chromosome numbers were formed. Triploids.
2. As the preceding type, but the restitution nucleus does not develop further or is followed by a kind of homotypical restitution nucleus. The result is monad pollen with unreduced chromosome numbers. Triploids.

II. Prophase without diakinesis, asyndesis prevalent. Apomeiotic development.

1. First division indicated by the formation of contraction nucleus. The second division normal; regular formation of dyads; pollen with unreduced chromosome numbers. Diploids and triploids.
2. The first and second division not separated by a contraction nucleus stage; hence pseudohomotypical metaphases. Dyad formation. In case the prometaphases are succeeded by restitution nuclei there is a possibility of monad formation. Diploids(?) and triploids.
3. The first division indicated by stretching of the nucleus and contraction. The second division is not carried through, but is supplanted by a formation of a kind of restitution nucleus. Monad formation. Pollen with unreduced chromosome numbers. Triploids(?).

With the exception of type II 3 numerous observations underlie the types. The fact that it has not been possible to provide sufficient material to illustrate monad formation is no doubt due to the fixation of the lowermost buds in the racemes having taken place too late; cf. pp. 24–28 and table 5.

4. Pollen Cytology.

Both in the microscopical preparations made by microtome technique and in preparations of pollen from living plants or herbarium material there proved to be most striking differences in size between the fully developed pollen grains. Flowers from the same individual might contain widely different size classes. It was soon evident that the differences found could not alone be due to the method of formation of the pollen or to the chromosome number. The size of the pollen grains also seemed to depend on a physiological factor which decreased or increased in the longitudinal direction of the inflorescence.

**Size of the Pollen as Compared with the Chromosome Number
and the Method of Pollen Formation.**

(a) S. Str. 10 (diploid). A longitudinal section through the upper part of an inflorescence showed that the lowermost flowers (no doubt in the middle of the raceme) contained very large pollen grains of ab. 17.6μ in diameter. The next size of bud contained fully developed, not yet separated dyads the greatest breadths of which were ab. 10μ . The smallest buds contained stages of the dyad formation. The very large round pollen grains in the lower flowers could not be dyad pollen, for in other preparations this was found to have a diameter of ab. 14.3μ . Typical tetrad pollen in material from S. Str. 10 with normal meiosis, finally, had a pollen diameter of ab. 11μ . Measurements of 50 pollen grains of respectively tetrad and dyad pollen are recorded in Table 2. Furthermore a measurement of 50 pollen grains from one of the loculi with the above-mentioned large pollen grains has been included. It appears that these just have the dimension to be expected from monad pollen and therefore no doubt are so. If the radius of a pollen grain is known, a pollen grain with half the volume will have a radius which is ab. 0.8 times smaller ($\sqrt[3]{0.5} = 0.794$). The graduation from ab. 17.6 to 14.3μ and further to 11.0μ is in perfect agreement with the series monad—dyad—tetrad.

Table 2.

	Pollen diameter in μ							n
	11.0	13.2	14.3	15.4	17.6	19.8	22.0	
Meiotic tetrad pollen	72	28	50
Mainly apomeiotic dyad pollen ...	2	14	64	18	2	50
Mainly apomeiotic monad pollen	2	2	20	56	16	4	50

Figs. 9b and c show the dimensions of fully developed pollen in S. Str. 10 with apomeiotic pollen development. Fig. 9b no doubt is dyad pollen, whereas fig. 9c shows monad pollen and double pollen grains with several nuclei the nature of which will be discussed below. For comparison reduced tetrad pollen from the Alaska material is shown in fig. 9a and in fig. 9d very large unreduced pollen grains from the diploid material from HBH. These pollen grains must be supposed to be monad pollen.

(b) HBH (triploid). Because of the other factors which interfere and influence the size of the pollen grains it is very difficult to give definite measures of tetrad, dyad, and monad pollen here. Tetrad pollen often has pollen diameters of ab. 11 – 13μ and dyad pollen 15 – 18μ , while corresponding monad pollen presumably reaches a size of ab. 22μ . A measurement of 50 pollen grains in a loculus from the upper region of the inflorescence, where the adjoining smaller flowers showed dyad for-

mation, gave the following picture: 11μ 4 per cent.; 13.2μ 8 per cent.; 15.4μ 32 per cent.; 17.6μ 54 per cent., and 19.8μ 2 per cent. The maximum is at 17.6μ . In some older, larger flowers partly small pollen grains of $11-13 \mu$, partly large rounded-off grains of $20-22 \mu$ were seen. It must be assumed that partly tetrads, partly monads were formed here, a view which is supported by the above-mentioned fact that meiosis and the formation of restitution nuclei may take place side by side in the HBH material (cf. fig. 4). Fig. 9g shows examples of pollen from the older flower and fig. 9f pollen from the flower which because of the homogeneous unreduced pollen and its situation near a flower with dyad formation must be assumed to be dyad pollen. Fig. 9e shows an example of pollen from a material in the neighbourhood of which meiosis with a high degree of syndesis and with regular tetrad formation was seen.

Both the diploid and the triploid material mentioned above originated from the upper part of the inflorescence and therefore no doubt are comparable. If we include the reduced pollen from the Alaska material in our considerations (fig. 9a), we get the following picture: tetrad pollen $9-11 \mu$ (somatic diploid), $11-13 \mu$ (somatic triploid); dyad pollen $13-15 \mu$ (diploid), $15-18 \mu$ (triploid); monad pollen $15-20 \mu$ (diploid), $20-22 \mu$ (triploid). This gives a difference in size between diploid and triploid unreduced pollen of ab. $2.5-3.5 \mu$, a difference which is in good agreement with the difference between the pollen mother-cells (cp. fig. 3f-j with fig. 5a-g or fig. 8a-e with fig. 7a-h).

The Size of the Pollen Grains as Compared with Physiological Differences in the Longitudinal Direction of the Raceme.

Anthers from the same flower were squashed on the slide and placed in a drop of orcein-acetic acid-glycerine. The flowers were chosen from different heights in the raceme. In two plants of S. Str. 3 and three of HBH, one of Disko 2 and one of S. Str. 9 measurements were made of 50 pollen grains from the same flower at different heights of the raceme. Furthermore five other plants of S. Str. 3 and HBH were examined, in which a smaller number of pollen grains were measured. In all cases it proved that the size of the pollen grains decreased the higher the flowers were found in the raceme. Likewise it was evident that there was no even decrease, but a definite graduation. The pollen from the same flower belonged to definite size classes. In some flowers there was one maximum, in others two or three. From Table 3, which gives a comparison of the material, it appears that 10 flowers have one maximum, whereas 8 flowers have bimodal and 1 flower has trimodal distribution.

While such bi- or trimodal distributions might fairly easily be understood from the investigations of the size of tetrad, dyad, and monad pollen described above, it was much more difficult to understand why the maxima found within the same individual did not correspond. Particularly difficult, perhaps, were the individuals

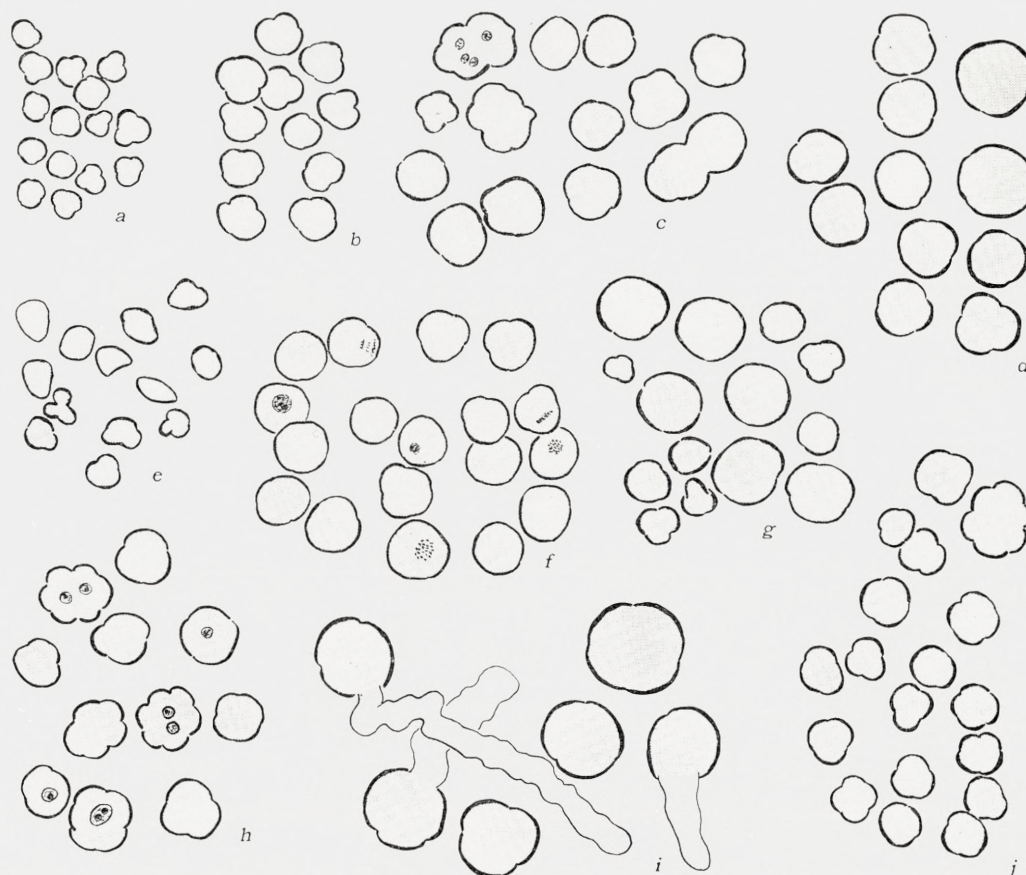


Fig. 9. Pollen samples of *Arabis Holboellii*. a—d, diploids; e—j, triploids. — a, tetrad pollen, Alaska material. — b, dyad pollen; c, monad pollen (with double pollen grains), material from S. Str. 10; d, monad pollen, diploid material from HBH. — e, tetrad pollen in triploid material from HBH. — f, dyad pollen; g, monad and tetrad pollen in triploid material from HBH. — h, dyad pollen with double pollen grains, material from Equaluit. — i, monad pollen, j, dyad pollen in material from S. Str. 3; in j one double pollen grain, in i three pollen grains have germinated. $\times 400$.

from S. Str. 3 (1) and HBH (1), in which flowers from four heights were examined and four maxima appeared.

In the plant S. Str. 3 (1) there were maxima at 13.2, 19.8, 24.2, and 30.8 μ . If a monad pollen measures 30 μ , a corresponding dyad pollen will be ab. 24 μ and the tetrad pollen 19.2 μ . While the two highest maxima in this plant are easily explained as monad and dyad pollen, the next maximum at 19.8 μ cannot be explained as tetrad pollen; for in the first place there is a maximum at 13.2 μ which will not be explained, secondly it was evident from the greatly rounded form of the pollen grains that tetrad pollen was out of the question. Supposing that the smallest pollen grains of ab. 13 μ are tetrad pollen, corresponding monads will just have a diameter

Table 3.
Pollen measurements in triploid *Arabis Holboellii*.

Material	Position of flower	Pollen diameter in μ												n	
		6.6	8.8	11.0	13.2	15.4	17.6	19.8	22.0	24.2	26.4	28.6	30.8		33.0
S. Str. 3 [1]	top	8	42	30	12	6	2	50
	upper	2	22	12	16	30	18	50
	middle	12	54	6	4	22	2	50
	lowest	10	58	14	4	10	2	50
S. Str. 3 [2]	top	14	20	40	26	50
	upper	2	6	0	2	14	74	2	50
	below	4	0	0	10	66	12	2	2	4	50
S. Str. 9	upper	6	8	40	40	6	50
	lowest	4	56	36	4	50
Disko 2	upper	4	4	60	32	50
	lowest	2	4	4	0	2	20	58	10	50
HBH [1]	top	2	68	28	2	50
	upper	..	4	32	42	12	10	50
	middle	..	2	2	20	12	0	12	44	8	50
	lowest	..	4	6	12	6	6	2	2	20	34	8	50
HBH [2]	upper	6	28	58	2	6	50
	middle	2	0	0	4	16	2	16	22	10	8	18	2	..	50
HBH [3]	upper	4	64	12	8	12	2	50
	middle	2	2	24	40	16	16	50

of ab. 20 μ . If this holds true, there are two types of monads, small and large ones, and the small ones have a diameter which is ab. two thirds of that of the large ones.

In several cases (S. Str. 3 (2), S. Str. 9, and Disko 2 in Table 3 and Upernivik Isl. in Table 4) it appears that the pollen maximum of the upper flowers occurs in a place which is two thirds or nearly two thirds of the maximum of the lower flowers. This would seem to indicate that PMCs of two or more sizes are produced. And indeed, this is exactly the case. In the HBH material there were very clearly two sizes of PMCs, as appears from a comparison between fig. 4 and fig. 5. The same applied to the S. Str. 3 material (the small cell in fig. 6w originated from another flower than the two large cells in fig. 6u and y) and the Egluit material (cp. fig. 6h—s with fig. 6t). The difference between the two types of PMCs very nearly corresponds to the two thirds ratio mentioned above.

From this knowledge we shall try to understand the four maxima in the plant HBH (1). The lowermost flower no doubt particularly produces large rounded monad

Table 4.
Pollen measurements in *Arabis Holboellii* (herbarium material).

	Material and collector	Pollen diameter in μ													n	
		10.8	12.6	14.4	16.2	18.0	19.8	21.6	23.4	25.2	27.0	28.8	30.6	32.4		34.2
Greenland	Upernivik Isl. [M. P. PORSILD] ¹	12	22	30	14	10	8	4	50	
	Upernivik Isl. [M. P. PORSILD] ²	4	14	24	32	22	4	..	50	
	Umanaq Storø [M. P. PORSILD]	6	10	18	38	20	8	50	
	Sarfanguaq [M. P. PORSILD]	10	44	18	12	6	4	4	2	50	
	Ikertoq fjord [O. HAGERUP]	2	6	20	36	16	14	6	..	50	
	Scoresby Sound [N. HARTZ].....	2	8	18	36	22	6	4	2	0	2	50	
N. America	Dahlem [Bot. Gardens] [A. LANGE]..	2	6	12	18	38	16	4	4	50
	Ritzville [Adams Co.]	2	0	2	6	4	2	2	6	12	16	34	10	2	2	50
	Washington [VASEY]	6	14	42	26	10	2	50	
	Big Horn [ROLLINS].	10	26	36	20	6	2	50	

¹ Small bud in upper part of the raceme.

² Vigorous flower in lower part of the raceme.

pollen grains and some small, often shrivelled and degenerated grains which presumably are failing tetrad pollen grains. The flower examined in the middle of the raceme no doubt mainly has dyad pollen and failing tetrad pollen. Then in the upper part of the raceme follows a flower in which the greater part of the pollen is tetrad pollen which has not failed. These grains have a diameter of about 13.2μ . Fully developed, not shrunken tetrad pollen grains corresponding to monad and dyad pollen of 26.4 and 22μ should have a diameter of about 17μ . In the uppermost flower there is, indeed, normal tetrad pollen, but here of a grade still smaller, viz. about 10μ . 13 is nearly four fifths of 17 , and 10 nearly four fifths of about 13 . Here there seem to be two grades of four fifths, but added up this corresponds to about two thirds. It might be supposed that the difference of two thirds often found was based on two preceding cell divisions of the archesporial cells which each involved

a reduction in the size of the cell of about four fifths (0.8). Of course this is only a hypothesis, and even though this explanation may seem somewhat complicated, it has some probability as it is based on the fact that PMCs of two sizes are produced one of which is about two thirds of the other.

In respect of the sizes of PMCs conditions in *Arabis Holboellii* remind not a little of those in *Euhieracium*, in which ROSENBERG (*loc. cit.*) and later GENTCHEFF & GUSTAFSSON (1940a) have shown that peripheral florets often have large, but centrally placed florets have small PMCs.

The development of the inflorescence in *Arabis Holboellii* in this connexion is remarkable. It is conspicuous that the lower flowers of a raceme bear long maturing siliques while development of flowers and divisions in PMCs and EMCs still take place at the top. Everything seems to indicate that the development near the top takes place at a slower rate than at the base of the raceme. This would go very well with the above-mentioned theory of two extra divisions of the archesporial cells in the upper part of the raceme.

Nuclear Divisions in the Fully Developed Pollen Grain and the Occurrence of Double Pollen Grains.

Fig. 10 gives some material to illustrate the pollen mitoses. The first pollen mitosis takes place nearly at the time when the preparation or introduction to meiosis or corresponding processes take place in the ovules. The degree of condensation of the chromosomes during the metaphase varies somewhat. In general it is somewhat higher than in mitoses in somatic cells and closely approaches to that found in the homotypical metaphases. Fig. 10d shows a root-tip mitosis and figs. 10e—h the first pollen mitosis in the HBH material (dyad pollen with unreduced chromosome number). Figs. 10l—m show metaphases in similar pollen from the Disko 2 material.

Fig. 10c shows a reduced pollen grain from the Alaska material with $n = 7$. Fig. 10b shows a corresponding pollen grain from the diploid, meiotic S. Str. 10 material. Below on the right in fig. 10n the opposite is seen, an unreduced monad pollen grain from the apomeiotic S. Str. 10 material. Here there is a prophase in the generative nucleus with 14 chromosomes.

After the first pollen mitosis the generally known polar contrast between the two nuclei as a rule makes its appearance. Besides morphologically they differed by their properties of colour. After a combination of FEULGEN and gentian violet staining the vegetative nucleus after sufficient differentiation turned blue, while the generative nucleus was red.

Division of the generative nucleus takes place both in diploids and triploids with unreduced chromosome numbers before the pollen germinates (fig. 10i). It was a curious fact that in some of the diploid or triploid monad grains finally 6 nuclei were found, 2 vegetative and 4 male nuclei (fig. 10j). Obviously the first mitosis in the pollen grain does not always result in a polar distinction between a generative

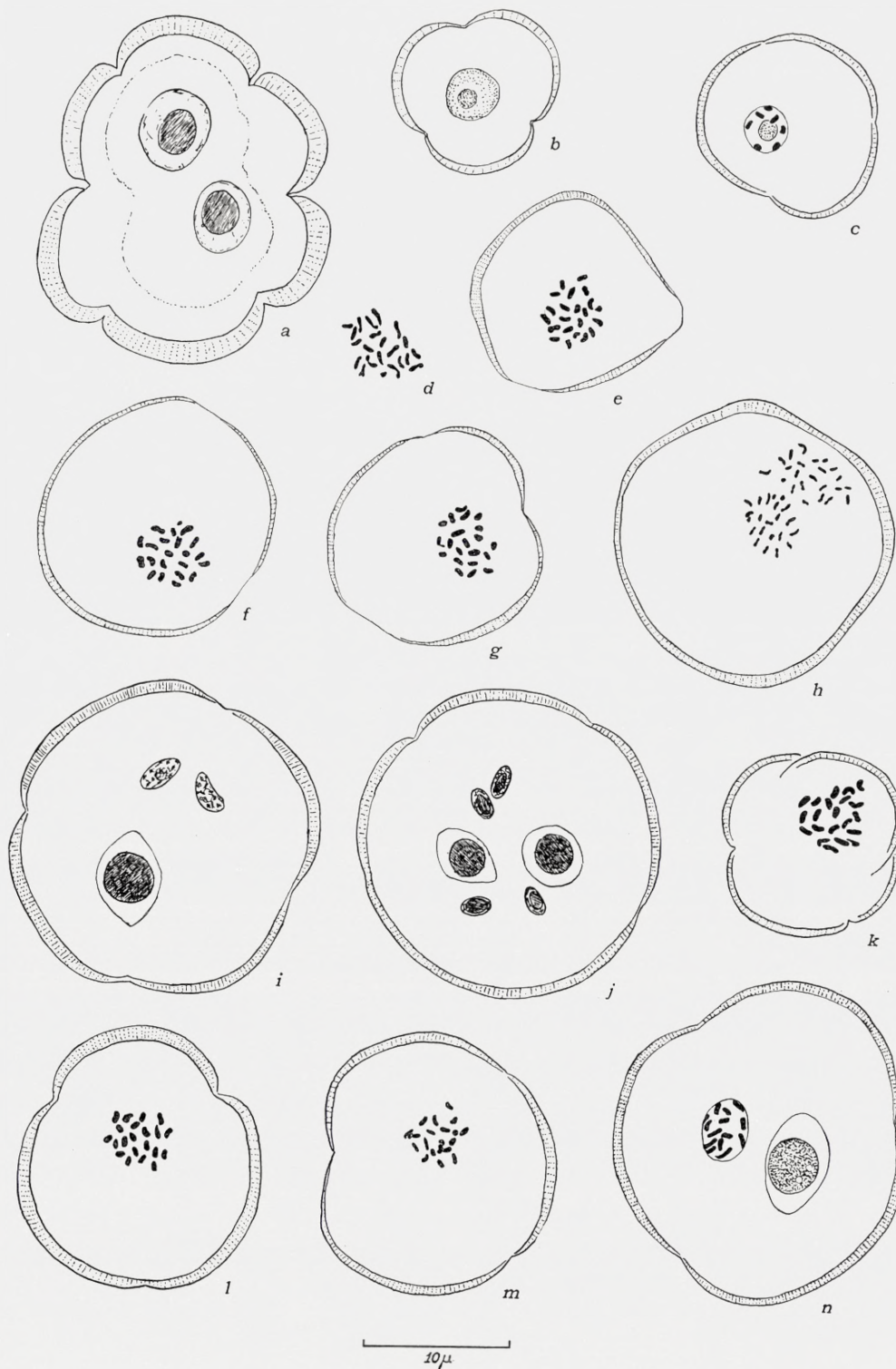


Fig. 10. Pollen of *Arabis Holboellii*; and for comparison a metaphase plate from a root tip of a triploid HBH plant (d). — b—c, reduced pollen grains from diploids. — a, e—n unreduced pollen grains from triploids (a, e—m) and diploids (n). — a, binuclear double pollen grain from the Egoaluit material. — e—h, mitosis in dyad pollen from the HBH material. — i, j, small monad pollen grains from triploid HBH material; in j two vegetative nuclei and two pairs of male nuclei (cp. text). — k, pollen mitosis in material from S. Str. 3. — l—m, mitoses in unreduced dyad pollen of Disko 2 material. — n, prophase in generative nucleus in unreduced monad pollen of diploid material from HBH. $\times 2150$.

nucleus and a vegetative one. The following explanation seems natural: at the monad formation the nuclear division seems to have been retarded, while the transformation or development of PMCs into pollen grains, which is highly due to the activity of the tapetum, has been forced. The first mitosis in the pollen grain in a way comes to correspond to one of the meiotic divisions and takes place at a time when the substances which must be supposed to be determinative of the polar contrast between generative and vegetative nucleus, are not yet present. Therefore only the next division corresponds to the first pollen mitosis, and then follows a third division in which both generative nuclei divide.

The occurrence of double pollen grains is of fairly great interest. In the *Equaluit* material these were frequently found, often several in each cross section of loculi with mature pollen grains (fig. 9h). In the *S. Str. 3* material they were rare (fig. 9j at the top). In both cases they were found jointly with pollen which must be supposed to be dyad pollen, and they always contained two large nuclei (fig. 10a), while the surrounding single grains had one large nucleus. In a double grain the two nuclei were not completely separated and in another it seemed as if they had fused or that the original nucleus had started a division without finishing it and instead had formed a hexaploid restitution nucleus (fig. 9h below). The formation of double pollen grains presumably is connected with the fact that the pollen development has taken place at a time when the nuclear division which normally results in the separation of two dyad cells has not yet been completely finished. The cells from which the double pollen grains originate must for some reason be delayed as regards the nuclear division itself.

In some flowers in the *HBH* material some large round pollen grains were found which contained two identical, closely joined, hemispherical cells. In this case a separation of the dyad cells had taken place, although it was greatly delayed in relation to the pollen development, and hence the two cells had been surrounded by a common pollen wall.

In the diploid apomeiotic material from *S. Str. 10* double pollen grains were found, but here in connexion with pollen grains of a size which made it fairly probable that it was a question of monad pollen. Whereas the surrounding single grains here often had two equally large nuclei, there were four in the double grains (fig. 9c at the top). The formation of two equally large nuclei in monad pollen before the differentiation of any generative nucleus was mentioned above. No doubt the four nuclei in the double pollen grains represent two sets of such nuclei of equal size. Finally such double pollen grains therefore must be able to obtain 12 nuclei. Their formation, for that matter, has not been explained, but there is reason to suppose that the division of PMCs was delayed by the formation of restitution nuclei. In most PMCs the nucleus does not reach the stage of division before the development of the pollen grain wall; but in some cases the division takes place during the wall-formation, and so there is often time for yet another nuclear division before the division differentiating the sex cells sets in. In fig. 9d there is a sample of pollen from diploid

apomeiotic material from HBH. This, too, is no doubt monad pollen. Two of the grains were larger than the others and also had larger nuclei. Therefore they may be tetraploid and have possibly arisen in a similar way as the presumed hexaploid nucleus in the pollen grain in fig. 9h below.

5. Embryo-sac Development.

Diploids with Normal Meiosis and Formation of Embryo-sacs with Reduced Chromosome Number.

(a) Alaska. Here a number of EMCs in the prophase were seen. A few of these had got as far as the diakinesis (fig. 11 a). As might be expected from the fully normal meiosis in the male organs, the diakinesis on the female side was completely normal with full syndesis and seven bivalents.

(b) S. Str. 10. Besides prophases of the same type as in the Alaska material a metaphase seen obliquely from the side, with typical formation of bivalents, was found here. Furthermore a late second anaphase (fig. 11 b) which will result in a linear tetrad with reduced chromosome number. This completely normal development was ascertained in the same part of the material, where also a normal reduction took place in PMCs.

(c) HBH. The greatest part by far of the diploid material from HBH proved to be apomeiotic; but in a single embryo-sac two of the four nuclei might be counted and proved to have 7 chromosomes. Surrounding somatic cells had about 14.

Diploids and Triploids with Apomeiotic Development and Formation of Embryo-sacs with Unreduced Chromosome Number.

(a) S. Str. 10 (diploid). After the prophase in EMCs follows a formation of more or less regular metaphase plates in which total asyndesis (fig. 11 d) or the occurrence of single bivalents (fig. 11 e) was ascertained.

The embryo-sac mother-cells are either subepidermal or a parietal cell¹ is cut off which may divide so that a one-layered tapetum consisting of few cells arises over the sporogenous cell. In both cases the embryo-sac mother-cell becomes the source of a dyad the nuclei of which have unreduced chromosome numbers. In the case pictured in fig. 11f the upper dyad cell has degenerated while the lower one under vacuolization has divided into a binucleate embryo-sac. This development was frequent in the present material. Several instances of mitosis in quadrinucleate embryo-sacs were found and in every case the chromosome number proved to be unreduced (fig. 11 e). The mature embryo-sac could not be studied in this material.

¹ In most species of *Cruciferae* examined, no parietal cell is formed. But e. g. in *Alyssum macrocarpum* parietal cells were observed by RIDDLE (1898).

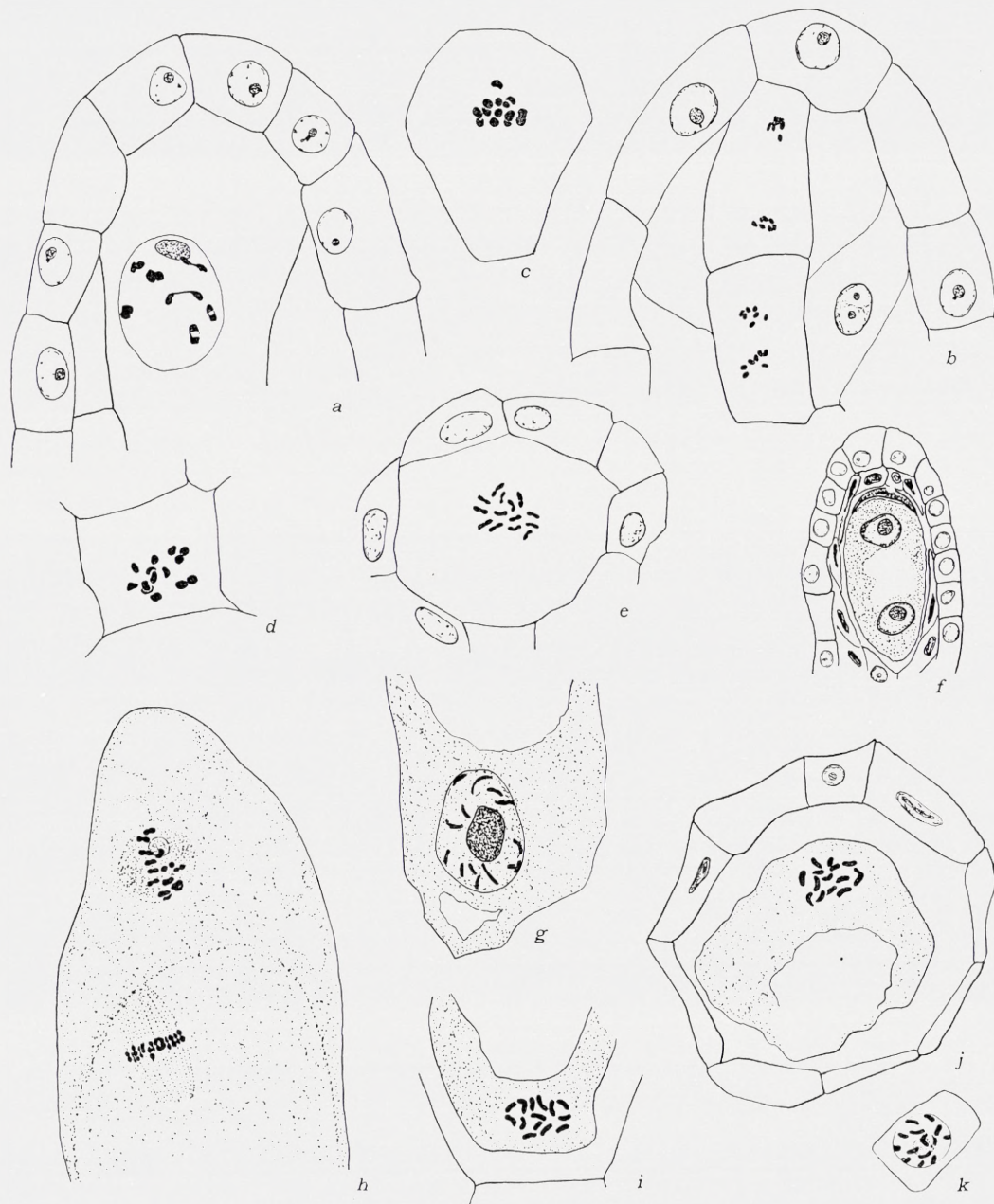


Fig. 11. Embryo-sac formation in diploid *Arabis Holboellii*. a, diakinesis in EMC from the Alaska material. — b, formation of megaspore tetrad in diploid, sexual plant of the S. Str. 10 material. — c—d, metaphases in EMCs in plants with apomeiotic embryo-sac formation in other plants of the S. Str. 10 material, in d complete asyndesis. — e, mitosis in quadrinucleate embryo-sac showing the unreduced chromosome number (S. Str. 10). — f, degeneration of micropylar dyad cell and development of the lower one to a binucleate embryo-sac (*Taraxacum* type) in material from S. Str. 10. — g, prophase in the basal (chalazal) nucleus in a binucleate, unreduced embryo-sac from the HBH material. — h, two meiosis-like divisions in quadrinucleate embryo-sac; i, a mitosis from the chalazal part of the same embryo-sac, cp. text. — j, mitosis in quadrinucleate embryo-sac from the HBH material. — k, mitosis (prophase) from the integument tissue. All except f $\times 2150$; f $\times 850$.

(b) HBH (diploid). Besides a single instance of the formation of an embryo-sac with reduced chromosome number (see above) numerous instances of the formation of embryo-sacs with unreduced numbers were found here. The first metaphase in EMCs could not be found in the material, but there were numerous mitoses in bi- or quadrinucleate embryo-sacs and the chromosome number was 14 (fig. 11 i—j). A prophase from a somatic division in integument tissue, also with 14 chromosomes, has been included for the sake of comparison (fig. 11 k). Fig. 11 g shows a prophase in the chalazal end of a binucleate embryo-sac. The nuclei at this stage (cf. fig. 11 f) are always very large, probably because the embryo-sac has spread at the expense of the surrounding cells, which degenerate.

Fig. 11 h—i deserves particular mention. These cells originate from the same quadrinucleate embryo-sac. All the four nuclei were dividing at the same time. At the chalazal end there were two mitoses of a completely normal appearance (fig. 11 i), while the divisions at the micropylar end were meiosis-like with greatly condensed chromosomes. The centromeres had split and the anaphase had just started (fig. 11 h). It is of interest that no degenerating tapetal layer nor any degenerating micropylar dyad cell could be observed here. The quadrinucleate embryo-sac thus has arisen by a division of the embryo-sac mother-cell. Assuming that the first of the two divisions resulting in the formation of the quadrinucleate embryo-sac was purely mitotic, corresponding to the division by which the parietal cell in many cases is formed, the next two divisions will correspond to meioses. Thus the one in fig. 11 h will be a kind of homotypical division, and, indeed, it cannot be denied that the similarity to a homotypical division is striking. It is remarkable that the divisions of the chalazal end are purely mitotic. In the embryo-sac in question, alongside of the vacuolization a separation of the nuclei of the egg-apparatus takes place which develops meiosis-like with condensed chromosomes, and the nuclei of the antipodal system, which develop purely mitotically. In the adjacent ovules this was repeated, although here with the essential limitation that only one of the micropylar divisions had condensed chromosomes.

A number of fully developed embryo-sacs could be studied. A normal-looking egg-apparatus and a central nucleus had developed. In some of the nuclei about 14 chromocentres could be counted. A case of fusion of two polar nuclei into a central nucleus was also observed. The nuclei of the antipodal system seem to perish soon, as generally in *Cruciferae* (see further SCHNARF 1929 p. 146).

(c) Disko 2 (triploid), fig. 12. In this material particularly beautiful instances of the first division of the EMC were found. The prophases in every case were completely asyndetic and the same applied to the metaphases. This complete asyndesis is in good agreement with the investigations of the pollen formation in this type, which did not show any signs of syndesis and in which meiotic divisions were not found at all.

Both completely mitotic prophases (fig. 12 a) and more diakinesis-like pro-phases with rather short thick chromosomes (fig. 12 c) were observed, but also an

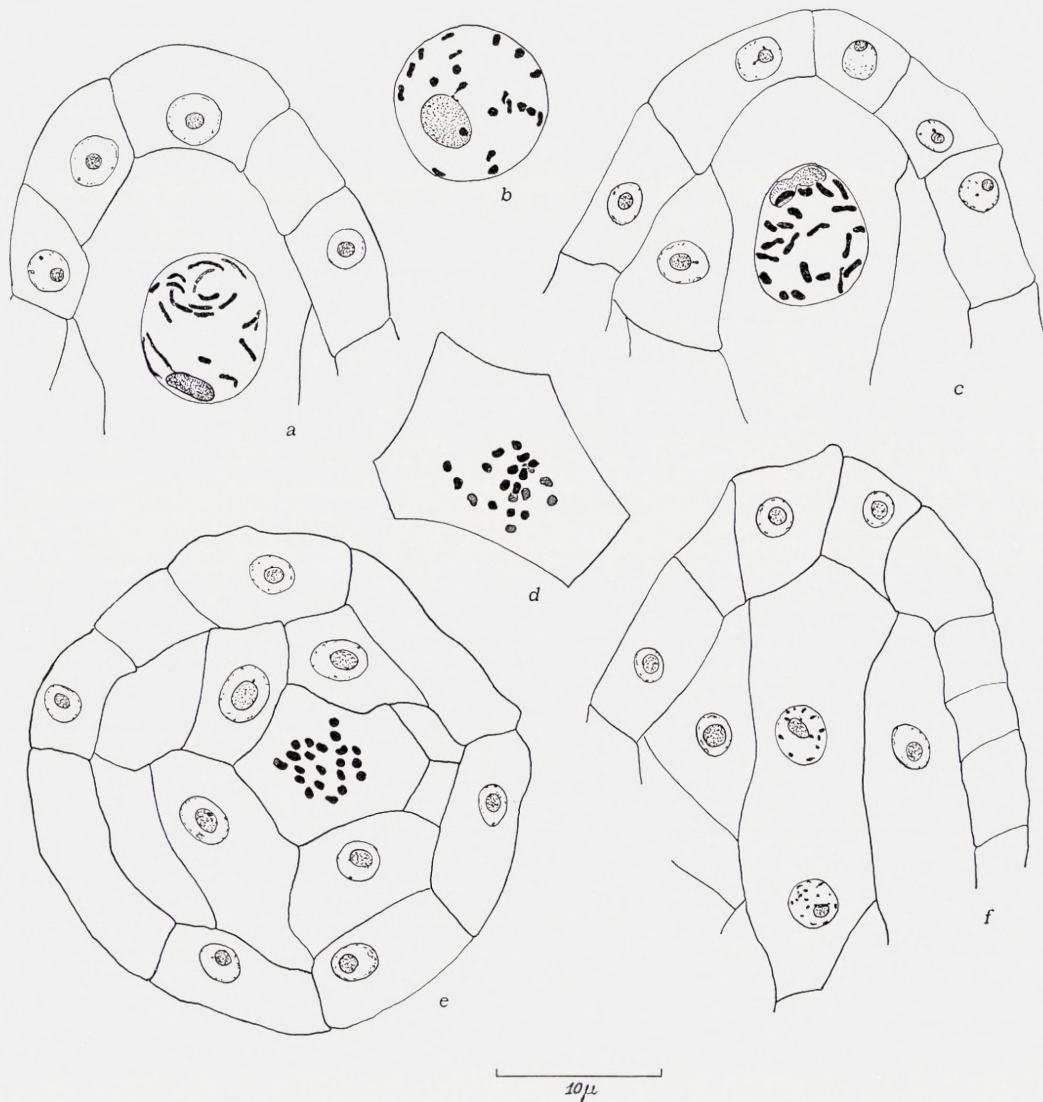


Fig. 12. Formation of unreduced embryo-sacs in triploid material of *Arabis Holboellii* from Disko 2.—a, mitotic prophase in EMC; b, asyndetic diakinesis; c, transitional type between mitotic prophase and asyndetic diakinesis.—d, prometaphase in EMC.—e, asyndetic metaphase (pseudohomotypical metaphase).—f, dyad nuclei; the lower one is untouched by the knife and has about 21 chromosomes. $\times 2150$.

EMC, the nucleus of which completely corresponded to the asyndetic diakineses described in *Taraxacum* by GUSTAFSSON 1935 figs. 24—34. This cell (fig. 12b) and the cell in fig. 12c were found near ovules with EMCs in the metaphase. The metaphases were completely asyndetic, with the same chromosome condensation as in the diakinesis. The completely mitotic prophase was seen in an ovary in which the rule otherwise was that a parietal cell developed at the top. The physiological state in the ovary in question thus conditioned that the first division was mitotic regardless

of the question whether this results in the formation of a parietal cell. In other ovaries there is a state in which the first division resembles a meiosis and here no parietal cells are formed. In numerous cases a larger sporogenous cell with a nucleus at a synzesis-like stage was seen below the parietal cell. In a single case there was here a somewhat lengthy nucleus, in which in contrast to the usual single nucleolus there were two nucleoli of unequal size. The form of the nucleus and the number of nucleoli suggest that a restitution nucleus had developed here in the same way as in the Egoaluit material (see below).

In one of the siliques in which a parietal cell only rarely develops a lengthy binucleate EMC was also seen (fig. 12f), probably a young dyad in which no wall formation had taken place. The chromocentres in the chalazal nucleus untouched by the knife amounted to about 21. In another part of the material an instance of degeneration of the upper dyad cell was observed.

(d) Egoaluit (triploid). This material on the whole is in agreement with the preceding material. Asyndetic metaphase in an EMC and dyad formation were observed.

The development of a parietal cell was very frequent and the further division of this cell transversely to the longitudinal direction of the ovules was seen in several places. Below the parietal cell there was either a large cell the nucleus of which was at a synzesis-like stage, or there were dyads in which the upper cell as a rule was the smallest and had the smallest nucleus. In many places there were partly ovules with a parietal cell, partly some without such a cell. In a silique cut longitudinally an interesting transition from one to the other was observed; it was possible here to study six ovules in a row:

- (1) Parietal cell present; below it a dyad, the chalazal cell of which is the largest (fig. 13h).
- (2) As in no. 1.
- (3) No parietal cell, two dyad cells of equal size.
- (4) No parietal cell, the micropylar dyad cell is the smallest (fig. 13f).
- (5) As no. 4.
- (6) As nos. 4—5.

The transition from cases with to cases without parietal cells takes place in no. 3. Besides there was mostly no appreciable morphological difference between the micropylar dyad cell and the parietal cell, and it seems natural to suppose that the upper dyad cells might frequently behave as parietal cells and hence did not degenerate.

On an analogy of the Disko material a restitution nucleus was found in an EMC developed below a parietal cell (fig. 13b and g). In this nucleus 21 chromosomes were counted. Its strange, rather long form would seem to indicate that the univalents during a semiheterotypical metaphase and anaphase had been placed on a nuclear

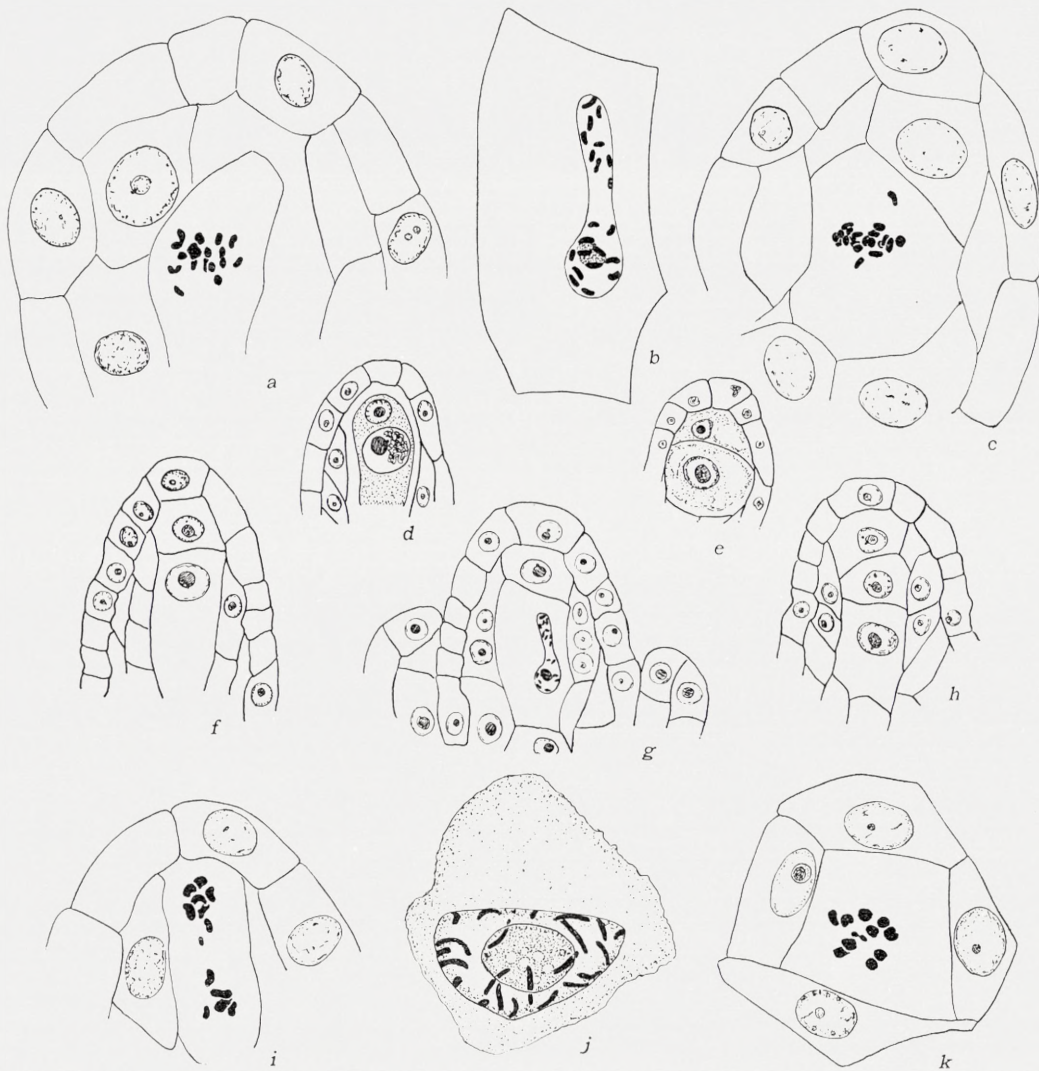


Fig. 13. Embryo-sac formation in triploid material of *Arabis Holboellii*. a, and c—d, material from S. Str. 3; b and f—h, material from Eequaluit; e, i—j, HBH material; k, material from S. Str. 9 (sterile branch on otherwise fertile plant). — a, c, metaphase in EMCs, in a the asyndesis is not complete, some bivalents being formed. — d, a case of failing cell-wall formation between a parietal and a sporogene nucleus. — e, dyad; micropylar cell small; f, dyad, micropylar cell, may develop into a parietal cell. — h, dyad developed below a parietal cell. — g, restitution nucleus in the same position as the dyad in fig. h; b, the restitution nucleus at higher magnification. — i, semiheterotypical anaphase in EMC with high degree of pairing. — j, prophase in nucleus in binucleate embryo-sac; about 21 chromosomes. Metaphase plate with 10 bivalents and one dividing univalent in EMC from sterile branch. a—c, i—k $\times 2150$; d—h $\times 850$.

spindle stretching in the longitudinal direction of the cell. The nucleus now was in the prophase.

(e) S. Str. 3 (triploid). A completely asyndetic diakinetik stage and a completely asyndetic metaphase (fig. 13c) were seen in several EMCs, but further some corresponding stages in other EMCs, in which a pairing between some of the chromo-

somes was evident. In a diakinesis e. g. 6II and 9I were seen, and a corresponding metaphase is pictured in fig. 13a. In other EMCs 3II and 15I, or 1II and 19I were found. Completely asyndetic stages were particularly found where no parietal cell had been formed. Examples were seen of dyads the upper cells of which were degenerating. Fig. 13d shows a case of no wall being formed between the nucleus of the parietal cell and the sporogenous cell, which here is in a synizesis-like state.

(f) HBH (triploid). The EMCs are mostly developed without preceding formation of parietal cells. The upper dyad cell is small (fig. 13e) and has been seen in a degenerating and compressed state. In a number of binucleate embryo-sacs with very large nuclei about 21 chromosomes in the prophase might be counted (fig. 13j). The mature embryo-sacs have a normal-looking egg-apparatus, in which the synergid nucleus mostly is found nearly in the middle of the cells with a large vacuole turning down towards the rest of the embryo-sac (fig. 14a and c); but they may also be placed lowermost in the cells close to the egg-cell nucleus (see fig. 14b). The central nucleus arises by fusion of two nuclei of equal size (fig. 14a) and is always easy to distinguish from the other nuclei in the embryo-sac because of its size. The nuclei of the antipodal system perish at an early stage.

Examples of Triploids with a High Degree of Syndesis during Meiosis in the Embryo-sac Mother-cell.

(a) S. Str. 9. As mentioned above, p. 11, fixations were here made of flowers at the tip of a shoot the siliques of which did not develop. The meiosis in PMCs showed a high degree of syndesis, but at the same time many signs of degeneration of PMCs at an early stage. In this material also a metaphase in polar view was found in an EMC. There were here 10 bivalents and one univalent which seemed to be dividing (fig. 13k).

(b) HBH. In this material, as will be remembered, meiotic divisions are frequent and the degree of syndesis is high. In part of a raceme in which meiosis with a high degree of syndesis was predominant in the PMCs (fig. 5), also a metaphase was found in an EMC. The chromosomes were scattered down through the cell (fig. 13i) as during a semiheterotypical anaphase. There were 9 bivalents and 3 univalents besides the small extra fragment chromosome, which is seen in the middle of the cell. The bivalent chromosomes showed no signs of tending to part, and there were indications that the result would be a restitution nucleus or perhaps two nuclei with respectively $12 + 1$ and 9 chromosomes.

Survey of Embryo-sac Development.

- I. Meiotic division with full pairing; formation of linear tetrad and embryo-sacs with reduced chromosome number. Diploids.
- II. Meiotic division with varying, often high degree of syndesis. Constant occur-

rence of univalents. Probability of the formation of megaspore cells with deviating, unbalanced chromosome number and consequent sterility, or of formation of restitution nuclei with subsequent development of dyads the upper cell of which degenerates (the *Taraxacum* type). The embryo-sac gets an unreduced number. Triploids.

- III. After a mitotic asyndetic prophase or an asyndetic diakinesis an asyndetic metaphase appears. The division results in dyads the upper cell of which generally dies, and the embryo-sacs get unreduced chromosome numbers. Sometimes, however, both nuclei formed by division of the EMCs take part in the embryo-sac formation (the *Antennaria* type). Diploids or triploids.

In spite of eager search no contraction nuclei were found during the apomeiotic and asyndetic embryo-sac formation (type III); but there were asyndetic diakineses with large nucleoli. These facts both indicate that conditions on the female side do not completely correspond to those on the male side, where contraction nuclei were of frequent occurrence and where no typical diakineses were seen. Considering, furthermore, the fact that in the case of S. Str. 3 EMCs with total asyndesis and EMCs with partial syndesis and bivalent formation were found in the same ovary, there can hardly be any doubt that there is a difference between conditions in stamens and ovules. All things considered, Type III comes under what GUSTAFSSON (1935b) denoted as pseudohomotypical division; cp. further the discussion pp. 53—55.

6. Pseudogamy and Embryo Development.

The above-mentioned cases of embryo-sac formation with unreduced chromosome numbers like the frequently occurring apomeiotic pollen formation testify to *Arabis Holboellii* propagating apomictically. In order to investigate the form of a possible apomixis in more detail, a number of castrations, some germination experiments with pollen, and some investigations of the endosperm- and embryo-development in diploid and triploid plants of the HBH material which had apomeiotic pollen and embryo-sac development were made.

Castration Experiments.

In 1944 some castrations of flowers belonging to the HBH material were made, and in 1950 of flowers from the S. Str. 3 and S. Str. 9 material. After the emasculation all the castrated flowers were examined under a strong magnifying glass or a preparation microscope in order to make sure that no pollen should have escaped from the anthers and settled on the stigma during the emasculation. After the castration the plants were placed in a hothouse or in a window in the laboratory and were constantly kept under observation.

Castration without after-treatment. On three plants of the HBH material respectively 8, 8, and 4 flowers were emasculated. The siliques began to stretch, but their growth soon stopped, after which they withered. From the S. Str. 3 material two plants were used, on which respectively 4 and 6 flowers were emasculated. The result was as in the preceding case: prompt stopping of the growth and at last, when the top flowers of the raceme had faded, a general withering.

The S. Str. 9 material behaved somewhat differently. Here 8, 2, and 2 flowers on three different plants were castrated. In all the three plants the siliques grew out at nearly the same rate as in non-castrated flowers, but at a length of about 1 cm. the growth ceased. With the exception of one silique no mature seeds were formed, but the siliques withered. This one silique only reached a length of 2 cm., and it only contained 2 seeds, which ripened. As the air in the laboratory room may have contained pollen which after the castration accidentally may have settled on the stigma of the silique in question, there is hardly any reason to assume that the two seeds should have developed without stimulation by pollen.

Control flowers of the S. Str. 3 and 9 material were emasculated and then pollinated with pollen from HBH plants. This gave quite a normal development of siliques. Two flowers on a S. Str. 9 plant were castrated and then smeared with pollen on the outside of the ovary while no pollen was put on the stigma. In both cases the siliques soon withered.

Castration with succeeding hormone treatment. In a number of emasculated flowers of the S. Str. 3 and 9 material siliques and stigmas were smeared with 0.1 ‰ β indolyl acetic acid or "carpon" solution. The active constituent in "carpon" is β naphthoxy-acetic acid. 1 tabloid was dissolved in 5 l. water. 4 castrated flowers on 3 different plants from the S. Str. 3 material were treated with "carpon". None of the 12 siliques showed any signs of development. The same thing happened after the treatment with β indolyl acetic acid of 8 flowers of the S. Str. 9 material. The only effect appearing was that the peduncles 24 hours after the treatment straightened themselves horizontally from the stem, while otherwise they were directed downwards. This effect prevailed for a very short time only.

After this experiment there can be no doubt that *Arabis Holboellii* requires pollination in order to develop seeds in the siliques. An attempt at replacing a possible stimulation by pollen by adding hormones proved abortive.

Germination Experiments with Pollen Grains.

Pollen from the S. Str. 3, S. Str. 9 and the HBH material was sown in 0.5 and 1 ‰ cane-sugar solution placed as a hanging drop in a moist chamber. After three quarters of an hour the pollen grains germinated. The germination continued for 6–8 hours after the beginning of the experiments. The longest pollen tubes became about 20–23 times the diameter of the pollen grain.

From the S. Str. 3 material both the largest and the next largest pollen grains

germinated (presumably monad and dyad grains; cf. fig. 9i). In the S. Str. 9 and HBH material plentiful germination of large and medium-sized pollen grains was seen, whereas small pollen grains, which must be supposed to be tetrad pollen, rarely germinated. Pollen with an unreduced chromosome number thus are capable of pollination, while the tetrad pollen of the triploids, which generally has a deviating chromosome number, more exceptionally is able to pollinate. Perhaps only the tetrad pollen grains which happen to contain a whole or two whole sets of chromosomes ($n = 7$ and 14) can be of importance for the pollination.

Embryo- and Endosperm-Development.

Material of diploid and triploid HBH plants with an apomeiotic embryo-sac development were examined. No fundamental differences between the diploid and the triploid plants were observed. In both cases it proved to be possible to count the same chromosome number (or number of chromocentres) in embryo-sacs and embryos. Also the endosperm nuclei were counted; they were tetraploid in diploid material and hexaploid in triploid material (fig. 14d—e); these observations show that there is no fertilization of either the egg-cell nucleus or the central nucleus. Both in diploids and triploids the latter has been seen to be formed by fusion of nuclei of equal size (cf. fig. 14a).

The apomictic embryo formation is further corroborated by studies of the behaviour of male cell nuclei in the embryo-sacs. The male nuclei are easy to recognize because of their dimension and often lengthy form. They are seen in the ripe pollen grains in fig. 14f and here are drawn to the same scale as fig. 14g—j, where they occur in embryo-sacs.

Fig. 14g shows a case in which the nuclei of the egg-apparatus are situated in a slightly different way, the egg nucleus here apparently being situated closer to the micropyle than the synergid nuclei. These were situated at the same height, while the egg nucleus and, fairly close to it, two male nuclei were seen at deeper focussing. There were no distinct boundaries between synergids and egg cells, nor any sign of degeneration of any kind at the micropylar end, which would seem to indicate that the male nuclei in this case had forced their way into a young embryo-sac in which the egg-apparatus for some reason had not yet finished its development.

In fig. 14h the synergids are degenerating, while a male nucleus is seen close to the egg nucleus as well as the central nucleus. In fig. 14i nearly the same situation is seen, only that here there is a greater distance between egg nucleus and central nucleus. Here there are two male nuclei and their contours were indistinct, which would seem to indicate incipient degeneration.

The case pictured in fig. 14j perhaps is the most interesting one. Here the embryo-sac was not found in quite a lengthwise position, but is seen somewhat foreshortened. With the highest focussing of the microscope a distinctly delimited, but degenerating synergid cell was seen here. Its nucleus had a completely blurred contour. A dark-

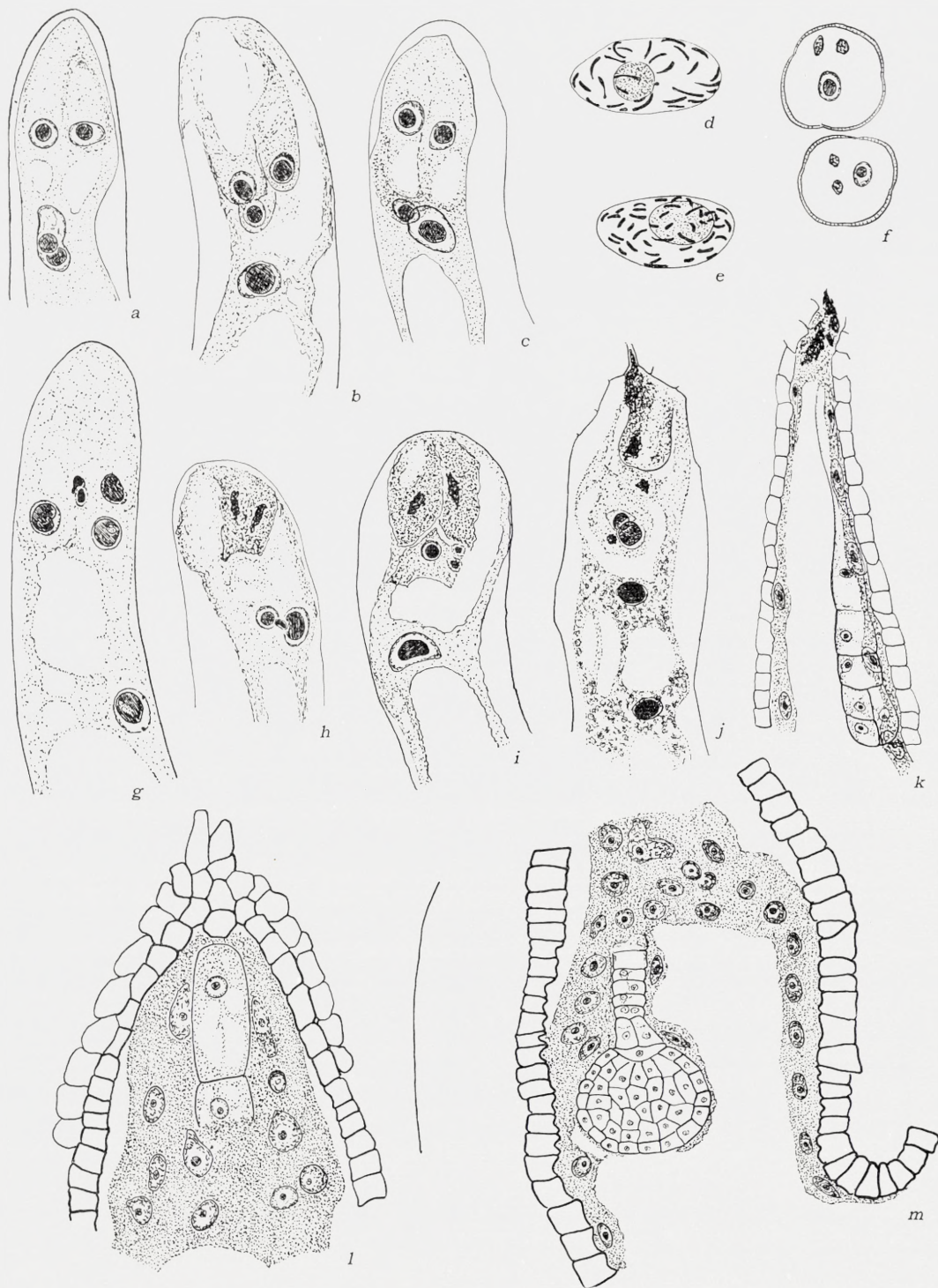


Fig. 14. a, b, c, mature embryo-sacs, in a, the fusion of the two polar nuclei is seen. — d, prophase in endosperm nucleus in diploid material; about 28 chromosomes. — e, prophase in endosperm nucleus in triploid material; about 42 chromosomes. — f, two pollen grains showing the two male nuclei at the same enlargement as figs. g—j. — g—h, the behaviour of the male nuclei in the embryo-sac, cp. text; in fig. h—j, degeneration of two or one synergid cell. — k, young embryo; at the micropylar end the last remains of the synergid may be seen. — l, micropylar end showing large haustorial cell terminating the suspensor. — m, embryo with adherent part of the suspensor. a—c, f—j $\times 850$; d—e $\times 2150$; k—m $\times 400$.

coloured part of the plasma was wedged in a little between two of the cells surrounding the embryo-sac. This is best interpreted as a rest of the pollen tube. Closely outside this synergid cell a small nucleus with an indistinct contour was seen, presumably the one male nucleus in degeneration. The other synergid nucleus was sharp at about the same high focussing. It showed no signs of degeneration. Below this the other male nucleus appeared, again with signs of degeneration, and close to it the egg nucleus. There was no central nucleus, but instead two nuclei of equal size separated by vacuoles. Round these two nuclei the cytoplasm was vesicular-granulate. The two nuclei no doubt have arisen by division of the central nucleus, and the drawing thus showing the first stage of the endosperm formation. On the whole the embryo-sac in fig. 14j, like the other embryo-sacs depicted, testify to a pseudogamous development of both embryo and endosperm.

It is remarkable that the central nucleus does not seem to be fertilized as it happens e. g. in pseudogamous *Potentilla* and *Poa* species (GENTCHEFF & GUSTAFSSON 1940b, HÅKANSSON 1943). *Arabis Holboellii* obviously belongs to the same pseudogamous group as the *Ranunculus auricomus* complex (HÄFLIGER 1943) and *Rudbeckia laciniata* (FAGERLIND 1946); but I am not quite sure that *Arabis Holboellii* has never any fertilization of the central nucleus. I have seen a case in which the central nucleus had divided the first time, the nuclei containing three nucleoli and looking larger than e. g. in the embryo-sac in fig. 14j. For that matter FAGERLIND in the case of, amongst others, *Ranunculus auricomus* and *Rudbeckia laciniata* emphasizes, that the parthenogenesis in fact probably is autonomous; but the autonomous process cannot in pseudogamous forms be started because the absence of pollination leads to a milieu unfavourable to the vitality of both the egg cell and the central cell. The carpel tissue requires pollination in order that the physiological conditions necessary for a continued development can arise.

The synergid degeneration is a clear sign that the pollen tube has forced its way into and exhausted the two male nuclei. Rests of the synergids are also seen during the first stages of the embryo development, when the embryos are 2- to 6-celled, while at that time it was not possible to see the sure rests of the male nuclei (cf. fig. 14k above).

Already from the earliest embryo development the embryo is closely surrounded by endosperm. The endosperm is found along the edge of the embryo-sac in a thin layer, but fills up a greater area at the micropylar end, where the embryo develops, and at the chalazal end, where there are generally some nuclei of particularly large dimensions, but hardly with a chromosome number deviating from the others. The endosperm gets a considerable start of the embryo.

The embryo development offers no appreciable new features. It corresponds to the development in the other *Cruciferae*, which has been so thoroughly studied, amongst others, by SUÈGES (1919). A large haustorial cell develops at the micropylar end of the suspensor, and the suspensor itself consists in older embryos of at least 12, and up to 14 cells (cf. fig. 14l—m).

The above-mentioned investigations decidedly indicate that in diploid and triploid forms of *Arabis Holboellii* with apomeiotic embryo-sac development, there is an apomictic endosperm- and embryo-development. The species has diplospory, pseudogamy, and parthenogenesis. On the other hand it is very probable that diploids with a normal meiosis have a normal or amphimictic development.

7. The Occurrence of Seed-sterility in *Arabis Holboellii*.

Both in nature and in culture experiments *Arabis Holboellii* proves to be strange by its alternation between great fertility and total sterility. In nature conditions in the localities examined by me mostly are these: some plants in a population are totally sterile while the rest teem with long siliques full of seeds. Involuntarily one comes to think of a segregation. However, there are both in nature and frequently in experiments plants which have a branch or a definite part of a shoot that is completely sterile and this indicates a phenotypical determination of the sterility.

It has been mentioned above (p. 11) that top flowers in a completely sterile shoot showed a deviating behaviour of the tapetal cells and had a partial degeneration of PMCs, a fact which suggests a disturbed physiological equilibrium. Likewise the physiological differences found in the longitudinal direction of the inflorescence which are reflected in the size of the PMCs and in the method of pollen formation have been mentioned above. These and other experiences are of importance in a discussion of the causes of the sterility. According to occurrence a distinction can be made between the following cases:

(1) Sterility in the upper part of the raceme. A considerable number of plants of different origin have at the top a small collection of flowers which wither while the flowers found immediately below develop large fertile siliques (figs. 15 and 16 on the right). In many flowers the transition is very well-defined, in others we find between the large fertile siliques and the fading flowers and buds one or more siliques which begin to develop normally, but which soon check their growth and which do not succeed in bearing ripe seeds. They behave completely as siliques in castrated flowers.

Such cases of sterility may be connected with the occurrence of meiotic divisions in the upper part of the raceme, which gives worthless pollen for the pseudogamy and perhaps abortive embryo-sacs. On the other hand the sterile siliques are found immediately below the fading flowers and buds, and hence it is also possible to imagine that the physiological condition at the top of the raceme is unfavourable and prevents seed development in the siliques.

(2) Sterility in the lower part of the raceme. This form was seen partly on individual plants in culture in the open (fig. 15 on the right), but never much pronounced here, partly on plants which had been brought into the laboratory and



Fig. 15. On the left experimental plants from the S. Str. 9 and S. Str. 3 material, on the right from the HBH material. — On the S. Str. 9 plant there is a shoot which is sterile at the top but otherwise completely fertile, and a completely sterile shoot. The plant of the S. Str. 3 material is sterile at the very top and below at rather a long stretch. It was placed in the laboratory with less access to light. Among the HBH plants there are four with withering buds at the top and at the transition to the fertile siliques some other siliques whose development has stopped. The plant no. 2 from the right has at the top a small bunch of buds which will hardly develop. Next follows fully developed flowers, and finally, again with a well-defined transition, nearly mature siliques. The plant on the extreme right is sterile in the lowermost and parts of the upper part of the inflorescence, and it continues flowering while the flowering has ceased in the case of most individuals.

were used for pollen investigations. These plants suddenly had been subject to altered conditions and became more lanky than the corresponding open-air plants, presumably because of the smaller amount of light in the laboratory. The rate of growth thus here was increased artificially and hence there is a possibility that the optimum condition (rate of development) for seed development, which is normally present in the lower and middle part of the raceme has been shifted upwards here. A raceme from the S. Str. 3 material with sterility in the lower part is seen in fig. 15 left.

(3) Sterility comprising a whole main shoot on a plant the other shoots of which are fertile. This form of sterility is more enigmatic, but it may be connected with competition between the shoots for access to certain substances, or perhaps differences in time of development between the shoots. To each main



Fig. 16. On the left three experimental plants of *Arabis Holboellii* cultivated at Vrdsloselille in Denmark. 1—2 belong to the S. Str. 3 material, while no. 3 belongs to the S. Str. 9 material. They were all started as germlings in spring and formed rosettes, but in the autumn two of them (1 and 3) began flowering without the stem stretching. This kind of plants mainly flowered at the end of the main shoot, but besides consisted of a dense cluster of leaf rosettes, which the following summer each developed into a shoot; cf. fig. 2. — On the right an individual from the S. Str. 3 material collected 28th of July in Greenland. 4 branches are fertile, or at the very top have withering flowers, while 2 branches are mainly sterile.

shoot corresponds a leaf rosette, which develops in the first season (see fig. 16 left). These rosettes are placed in such a way that they will inevitably dome to compete. Fig. 15 shows on the left an instance of sterility of this type in a plant of the S. Str. 9 material, in which the phenomenon seems particularly frequent and in which the above-mentioned cytological difference between sterile and fertile shoots on the same individual has been ascertained.

Out of 10 potted individuals of the type S. Str. 9, 7 had complete fertility, while 3 had a shoot that was completely sterile. Fig. 16 on the right shows an individual collected in Greenland and belonging to the S. Str. 3 material on which the majority of the silicles on two of the main shoots are sterile.

(4) Sterility comprising the whole plant. A completely sterile and a

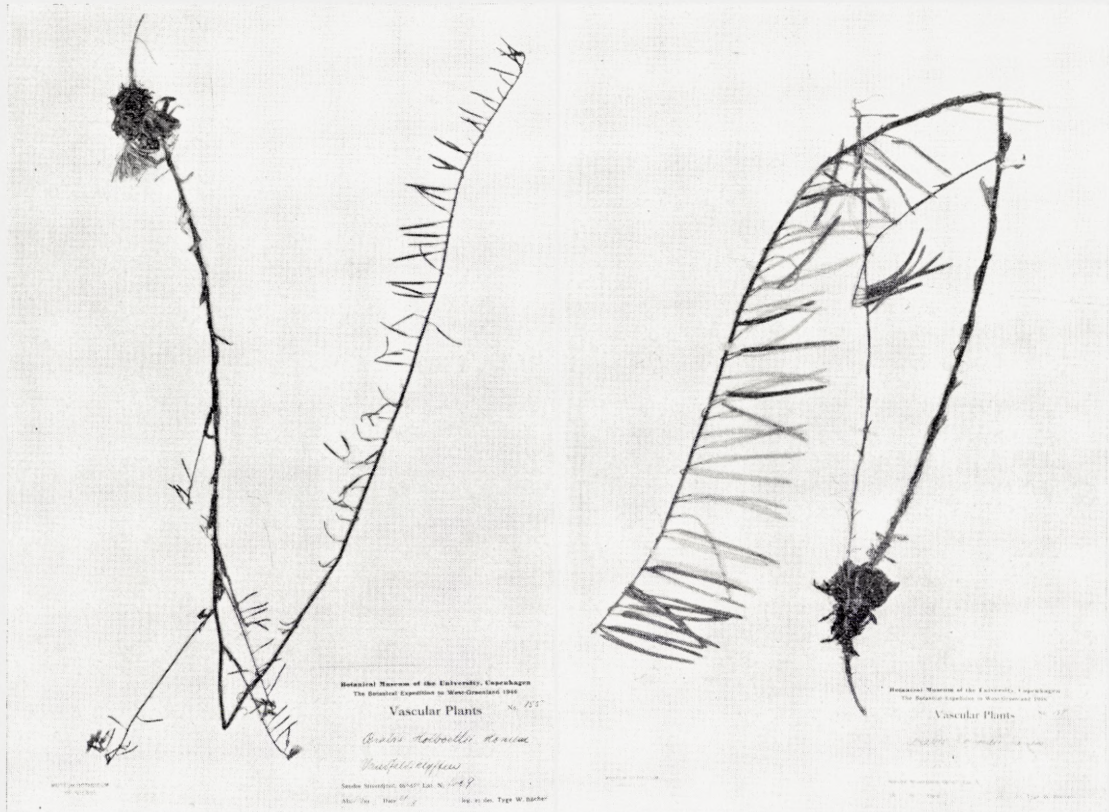


Fig. 17. Two individuals of *Arabis Holboellii* collected in Greenland 21st of August at Vandfaldskloften (S. Str. 9). The individual on the left is completely sterile; it is still flowering on the lateral shoots and at the base a new rosette is developing. Hence the individual will no doubt be at least triennial. The individual on the right is completely fertile; its rosette is withering and no new rosette is being formed. The flowering is long over.

completely fertile plant collected on the same day in the place from which the S. Str. 9 material originates is pictured in fig. 17. It is characteristic that the flowering continues on the sterile individual while all the fertile individuals at the time of the collection (21st of August) had long ceased flowering. It might be thought that the sterile individual had a slow growth because of some external factor (e. g. soil factor, root competition, or the like). In other localities, too, I have noticed that the sterile individuals were retarded. It is of great importance for the understanding of this form of sterility that in culture experiments under the same conditions, in the experimental field and in pots, with S. Str. 9 or other types, not a single completely sterile individual appeared. This greatly supports the view that sterility comprising whole plants is also phenotypically determined.

All the four types of sterility thus can be phenotypically determined, and at any rate the most probable cause seems to be disturbances (changes) in the rate of

growth and development. The plants, shoots, or parts of the raceme in question are either inhibited by an external factor or, in case (2), forced at too high a rate. A hormone production corresponding to a definite rate of development is no doubt a condition of the release of the apomixis mechanism found in *Arabis Holboellii*.

8. The Polymorphy of *Arabis Holboellii* in Relation to its Cytological and Embryological Behaviour.

ROLLINS (1936) characterizes *Arabis Holboellii* as a "huge polymorphous species" and writes "that one of the most complex problems presented in the genus *Arabis* centers around this species and its allies". In his paper of 1941 he takes a survey of the varieties and their distribution in West America and also discusses the systematic rank of the various types. In spite of the fact that ROLLINS himself has pointed out the existence of polyploidy in *Arabis Holboellii*, he sees no reason to divide it into microspecies. He thinks that "the graduation of characters throughout the varietal series is too complete to allow the admission of even the leading varieties to specific rank". He says himself about his varieties that they may not be entirely natural in every case and points out the importance of further studies, not least cytogenetic studies.

The investigations described above highly contribute to explaining the complex nature of *Arabis Holboellii* and throws new light on the systematic problems. The species includes both amphimictic and apomictic races, and there is a great probability that there are both total apomicts and races which sometimes produce seed sexually. The physiological difference found in the longitudinal direction of the raceme perhaps in certain cases may condition apomictic embryo formation in the lower and middle part of the raceme and amphimictic embryo development at the top. Under such circumstances the systematic conditions become much more difficult to interpret than in the case of constantly agamospermous plants, which may be divided into a number of comparatively constant agamospecies.

To the student of *Arabis Holboellii* in Greenland, both in nature and in the herbarium, it seems undeniably strange to read about it as a "huge polymorphous complex"; for in Greenland it shows a fairly moderate variation in spite of the fact that the Greenland population includes both sexual diploids and apomictic diploids and triploids.

It is not possible on the basis of pollen investigations in herbarium material to form a completely clear picture of the distribution of the diploids and the triploids in Greenland. For this purpose the material of flowers in the herbarium is too small and the distinction between diploids and triploids by means of the size of the pollen too uncertain. From the material in Table 4 as compared with the material in Tables



Fig. 18. Three typical plants, belonging to the Alaska material (var. *retrofracta*), on the left, diploid var. *typica* from S. Str. 10, in the middle, and triploid var. *typica* from S. Str. 3, on the right. Phot. May 1949 soon after the flowering had started in var. *typica*.

2—3 it can be concluded that the plants in Ikertoqfjord, Umanaqfjord, and Upernivik Island must be triploid. The material from Sarfanguaq may very well have been diploid, whereas the Scoresbysund plants in all probability were triploid. On the other hand it can be stated with rather great certainty that all the samples mentioned because of the great pollen variation must be apo- or amphiapomicts. As the material from S. Str. 10 (Itivdlinguaq) included both amphimicts and apomicts, it can be established that all the Greenland populations known so far contain apomicts. In all triploids and some diploids (the HBH material) apomixis is prevalent. The Greenlandic *Arabis Holboellii* is what BABCOCK & STEBBINS (1938) would call a mainly agamic complex.

The various types of *Arabis Holboellii* investigated by me in culture show different vigour and are not of equal height, and they deviate from each other as regards earliness. The Alaska material of var. *retrofracta* was very late as compared with all the Greenland material of var. *typica* (fig. 18). The difference between diploid and triploid Greenland plants is mainly quantitative, diploids from S. Str. 10 proving to be somewhat lower than the triploids from S. Strøm fjord (fig. 18). However, the S. Str. 3 plants were taller than the other triploids. There is no doubt that nearly

every Greenland population has its own peculiarities, not only morphological ones, but also as regards cytological-embryological behaviour. The populations seem to behave like a series of formae apomictae or formae amphi-apomictae or perhaps agamotypes (cf. TURESSON 1926, 1943). These have presumably arisen each in its place and are both geographically isolated from each other and genetically distinct, as the exchange of genes by hybridization between such forms will generally be excluded.

It is of great interest that in two cases diploids are amphi-apomicts. Thus the diploid population in Greenland is highly predisposed to asyndesis and agamospermy. Amphi-apomictic diploids will easily produce triploid offspring by fusion of a reduced and an unreduced gamete, and among the triploids it is evident that preferably such individuals as possess a hereditary tendency towards apomixis will be preserved. If thus there has once developed a predisposition conditioning a regular asyndesis-apomixis mechanism in a diploid plant, the result will easily be an increasing frequency of triploid apomicts with mainly apomeiotic pollen and embryo formation.

There is reason to compare the genus *Crepis*, which has been so thoroughly investigated by BABCOCK and co-workers. From BABCOCK & STEBBINS (1938) the following data may be adduced. With the exception of *Crepis acuminata* the diploid *Crepis* species in North America have small areas of distribution, while the polyploids occupy wider areas. Within the section *Psilochaena* (cf. BABCOCK & JENKINS (1943), STEBBINS & JENKINS (1939)) there is an agamic complex of nine species, seven of which include partly sexual diploids, partly polyploid apomicts. The species may, e. g., have the chromosome numbers 22, 33, 44, and 77. The authors think that the sexual diploids form the starting-point of the polymorphy in the group; but the diploids, for that matter, are mutually well separated genetically. It is the polyploid apomicts, both allo- and autopolyploids, which "exhibit the enormous amount of intergradation between the various species as represented by their original, diploid forms. The agamic *Crepis* complex, therefore, shows very marked concentrations of variability at or near the centers of distribution of the diploid sexual forms, and a progressive "thinning out" of the biotypes at greater and greater distances from these centers".

In *Arabis Holboellii* we have a complex of forms which highly remind of the *Crepis* complex. In the first place there are both in var. *typica*, var. *retrofracta*, and var. *pinetorum* races with different chromosome numbers which form polyploid series (ROLLINS *loc. cit.*, BÖCHER & LARSEN) and both var. *retrofracta* and var. *typica* have sexual diploids and polyploids, of which at any rate the triploids within var. *typica* are apomicts. The centre of origin of the species is no doubt West America, where it exhibits an enormous multitude of forms and where it caused similar difficulties to the systematists as groups of the genera *Poa* and *Potentilla*. According to ROLLINS there are five "varieties" in West America. Among these var. *retrofracta* has a wide distribution. It has mostly the chromosome number $2n = 14$ (two stations in Wyoming, two in Colorado, and one in California). It is rarely tetraploid ($2n = 28$; material

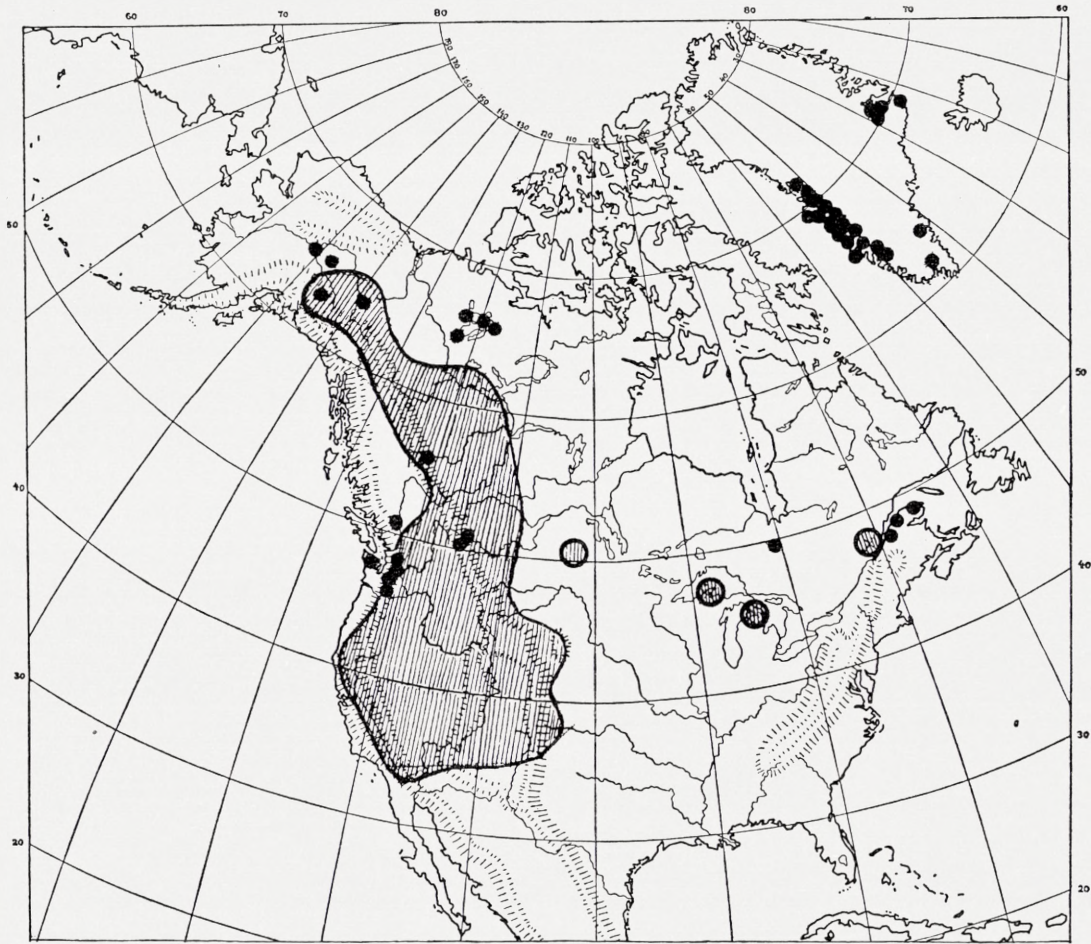


Fig. 19. Total range of the collective species *Arabis Holboellii* (West American range according to ROLLINS, Greenland range according to BÖCHER). Solid dots: var. *typica*, hatched areas: other varieties.

from Utah). My material from Alaska, as stated above, was also diploid and furthermore normally sexual. Probably the most original, quite normal sexual types of the species are found within the *retrofracta* complex. Among the other American varieties var. *pinetorum* (with $2n = 28$ or 42) has a fairly small, southern distribution (ROLLINS *loc. cit.*, maps on p. 442). Var. *typica*, however, is the variety with the widest distribution by far. It ranges from West America to Scoresbysund in East Greenland (fig. 19). Between Greenland and West America it is found in Quebec. The Greenland populations thus are peripheral in the total area of the species and as sexual diploids in Greenland seem to be rare, the species here is comparatively stable with moderate variation. However, it has hardly stagnated in its evolution in Greenland. A population as the one in Itivdlinguaq (S. Str. 10) or perhaps other diploid populations will be able to produce new types, if occasionally fertilization takes place

between reduced sexual cells. And triploids with restitution nucleus formation as introduction to the embryo-sac formation may also be supposed to give variation in the offspring, provided that there is pairing and chiasma formation. Indeed, in triploids of *Arabis Holboellii* cases of bivalent formation and the formation of restitution nuclei as well have been seen during the macrosporogenesis, a fact which may be considered as being of importance for the understanding of the rise of new races (cf. DARLINGTON 1932, BERGMAN 1935, 1941).

Presumably it will be the task of American workers to fathom the American races of *Arabis Holboellii*. At present I am myself collecting material for a more detailed judgment of the problem of microspecies as regards the Greenland material, but out of regard for the whole would like also to include American material to a limited extent in the investigations.

9. Discussion.

According to GUSTAFSSON's survey of Apomixis in Higher Plants (1946—47) *Arabis Holboellii* is the first and so far the only example of a plant with agamospermy belonging to the *Cruciferae*. Therefore there is reason to make certain comparisons with other plants with similar conditions, particularly, of course, species with pseudogamy and diplospory. Summing up, GUSTAFSSON writes about pseudogamous and autonomous apomicts that meiosis rarely degenerates or is mitotized and that chromosome pairing is not inhibited, but that in many syndesis even is generally good. In this respect *Arabis Holboellii* is peculiar and heterogeneous. In diploids an either-or has been found: regular meiosis or apomeiotic pollen and embryo-sac formation. In triploids in some material only apomeiotic development has been found, while in other material either meiosis or apomeiosis has been observed. Finally, flowers with both types have been found in some of the plants examined and during the macrosporogenesis a development which must be characterized as something between a meiotic and an apomeiotic process. The fairly great complexity in *Arabis Holboellii* reminds of that found in other similar groups, e. g. *Euhieracium* and certain *Calamagrostis* species. The surveys on pp. 21—22 and 37—38 concerning the pollen and embryo-sac formation in *Arabis Holboellii* do not, it is true, show quite so great a many-sidedness as e. g. within a complex like *Calamagrostis purpurea* (cf. NYGREN 1946); but it should be kept in mind that at present we know *Arabis Holboellii* fairly thoroughly only from an area where its variation is comparatively small. A corresponding investigation of material from West America, where the species shows great morphological plasticity, probably will give a number of data which added to the results communicated by me can easily come to give a picture just as complex as that known of e. g. *Taraxacum* and *Euhieracium*, of apomeiotic *Poa*- and *Calamagrostis*-species (MÜNTZING 1940, NYGREN 1946, 1950), or certain groups of species of the genus *Potentilla* (cf. e. g. A. & G. MÜNTZING 1941, RÜTISHAUSER 1948).

A remarkable feature is the very regular dyad formation taking place in certain diploids and all triploids. The process resulting in the formation of dyads occurs as regularly as a normal meiosis. Obviously a physiological equilibrium has been reached which conditions that in the greater part, sometimes the whole of the inflorescence, there is an asyndetic pollen and embryo-sac formation. The syndetic type, the genuine meiosis, which in triploids involves a high degree of pollen lethality and no doubt also frequently seed sterility, in such a milieu comes to look like an abnormal process. LEVAN (1940) has described an equally regular asyndetic pollen formation in the triploid, probably apomictic *Allium amplexans*. In *Hieracium* species with complete univalence, too, dyad formation may occur with a very great regularity.

Asyndesis can be modificative or genotypical. In the latter case it may be due to lack of chromosome homology or the activity of special asynaptic genes. In *Arabis Holboellii* the asyndesis is both modificative and genotypical. It is clearly modificative in all the cases in which the same individual has syndetic and asyndetic development in different parts of the inflorescence; but it is also evident that there is a genetic basis of the asyndesis. The triploids would hardly be able to exist without this basis. It can no doubt be expressed in the way that *Arabis Holboellii* in many cases in diploids and in all triploids has genes for asyndesis or apomeiotic development, but that these do not always succeed in manifesting themselves in the whole of the inflorescence. The genes presumably condition a definite hormone concentration and this, again, is a condition of the asyndesis and the production of unreduced embryo-sac nuclei and pollen. The most important factor to the triploid plants is only the presence of the state conditioning the asyndesis-apomixis mechanism in so great a part of the inflorescence that a sufficient fructification is secured. Besides, it is well-known that asyndesis and dyad formation can be stimulated by changes in temperature (KAGAWA 1939) just as meiosis in e. g. *Hieracium robustum* at a low temperature is changed into mitosis (GUSTAFSSON & NYGREN 1946), and no doubt it should be taken for granted that comparatively small changes in temperature or the like will be sufficient to make *Arabis Holboellii* change from apomeiotic to meiotic development, and such a change, like those which are due to the presumed induced differences in rate of growth, will easily result in a change from fertility into sterility, as is not rarely observed in *Arabis Holboellii*.

A strange feature is the high degree of syndesis found in triploids when these for some reason change from asyndesis into syndesis. The high percentage of bivalents condition that the tetrads become rather regular and that genuine dwarf pollen is rare. The syndesis must be supposed to be connected with structural changes in the form of interchange. The small extra chromosome also clearly testifies to the occurrence of such structural changes in the species. This was very constantly present in the triploid material from HBH and in this material has persisted since the first investigation of the plants in 1936 (see BÖCHER 1938 figs. 2—3), that is, as the species is biennial, through seven generations.

As mentioned above, the pollen formation offers several problems. As it takes its course in most cases in the materials from Eqalet and Disko 2, the similarity to ROSENBERG's *Hieracium pseudoillyricum* type seems very great. According to ROSENBERG (1926—27) the process of division begins with a mitotic-looking prophase which is succeeded by a contraction phase and later by an interkinesis with one nucleus. Then a "fast somatische Teilung" follows, which must be paralleled to the homotypical division. The result is a dyad formation. Apart from the interesting stage seen in the Eqalet material of *Arabis Holboellii*, which probably corresponds to the first division, the resemblance between *Hieracium pseudoillyricum* and *Arabis Holboellii* is nearly complete; for the division in the latter can very well be called nearly somatic, the degree of contraction of the chromosomes being the only factor that distinguishes it from a mitosis, and it must be admitted that the contraction is not much greater than in pollen mitoses. It is of interest that ROSENBERG also in *Hieracium pseudoillyricum* could establish the occurrence of semiheterotypical metaphases. But the semiheterotypical division generally was checked at an early stage by the onset of the contraction phase. In *Arabis Holboellii*, too, it appears that the contraction phase, as it were, takes the first division by surprise or catches up with it, and this at a time when the chromosomes have not yet reached the degree of contraction characteristic of meiosis.

From normal meiosis to the *Pseudoillyricum* type there has no doubt been a shortening of the whole process of pollen formation, and this shortening is still greater when the first and the second division are merged and the metaphases become pseudohomotypical. Finally a culmination in respect of shortening is probably reached if both divisions are suppressed and the PMCs after the formation of restitution nuclei become monad pollen. I am thinking of restitution nuclei following after quite asyndetic stages such as the prometaphase. The great frequency of this stage is highly indicative of its being of comparatively long duration. During the prometaphase the spindle is no doubt active, and in cases of a relatively low degree of precocity the chromosomes will be arranged in an equatorial plane and later split up, while conditions in the case of a high degree of precocity will render such an arrangement impossible. The chromosomes then will remain scattered on the spindle and the prometaphase as in the case of the semiheterotypical meta-anaphase will become a restitution nucleus (cp. the resemblance between the prometaphases in fig. 6j—k and the stages in figs. 4m—n and 5e).

It is remarkable that as regards the apomeiotic process there does not in *Arabis Holboellii* seem to be a complete correspondence between male and female organs. Probably the same flower or part of the inflorescence as a rule has both apomeiotic pollen formation and apomeiotic embryo-sac formation, but these do not occur in the same way, as the contraction stage seems to be absent on the female side and the diakinesis mostly on the male side. The divisions in the EMCs in some cases can be characterized as pseudohomotypical; but as in a good number of cases bivalents have been seen among univalents during the diakinesis and the metaphase, it is impossible to refer

all apomeiotic divisions in EMCs to a definite type. Probably there are both semi-heterotypical and pseudohomotypical divisions, and small differences in external conditions may cause now one, now the other type to predominate. It was characteristic that all totally asyndetic divisions were found in subepidermal EMCs, while EMCs with the occurrence of bivalents and restitution nuclei were found when a parietal cell had been given off above the EMC. The division which leads on to embryo-sac formation has here been retarded by the insertion of the parietal cell, and temporal retardation leads the division in a meiotic direction. The difference between the male and the female side, too, can be connected with the time factor; for, as mentioned above, the development of EMCs only begins at a time when the pollen formation is finished and when, as a rule, the first pollen division takes place, i. e. that the division of the female organs starts long after that of the male ones. And indeed the occurrence of diakinesis in EMCs and now and then bivalents is decidedly a step in a meiotic direction.

These results are in good agreement with those of GENTCHEFF & GUSTAFSSON (1940 a), who in *Hieracium* on the male side found "a tendency to increase the degree of precocity", while on the female side there was "a lower degree of precocity owing to different time-action of the prophase starting forces in relation to growth of the loculi and the nucellus".

GUSTAFSSON (*loc. cit.* p. 94) summarizes his own, GENTCHEFF'S and NYGREN'S investigations of the time-relationship of meiosis and corresponding types of division as follows: "The division takes a mitotic course when it occurs too late (complete mitotization) or when it appears prematurely (partial mitotization, asyndesis)." It is evident that in *Arabis Holboellii* the tendency towards premature development is slighter and slighter upwards in the raceme. For a triploid plant we may show this development diagrammatically (Table 5), all the time keeping in mind that the female organs are later than the male ones.

It should be noted that the diagram is an attempt at bringing the majority of observations of *Arabis Holboellii* under a uniform point of view: the dependence of the cytological-embryological process on the rate of development, which, again, must be supposed to be connected with differences in concentration or a limitation in time of the production of certain growth substances. On the male side there is a foundation of most of that listed in the diagram. As for the female organs no comparisons have been made between embryo-sacs from the lower and the upper part of the raceme. Therefore the diagram on the female side is chiefly hypothetical, though it should be noted that the asyndetic diakineses and pseudohomotypical metaphases in the Disko 2 material were just found in flowers fairly far down in the inflorescence and that the partly syndetic stages of division in the S. Str. 3 material and the syndetic semiheterotypical metaphase in the HBH material originated from flowers at the top of the racemes. Of course several modifications of the series set up in the diagram are possible. Thus the transition between small and large PMCs can no doubt take place both within the syndetic area (HBH) and within the asyndetic area.

Table 5.

Development as a consequence of physiological differences in the longitudinal direction of the raceme	<i>Male side</i> (mainly based on observations)			<i>Female side</i> (mainly hypothetical)			Position in the raceme
Delayed	<i>Syndesis</i> 2 divisions	<i>Tetrads</i> with reduced number	<i>Small PMCs</i> perhaps after extra division of archespore cells	?	?	Early stopping of floral development. Sterility	At the top
"Normal"	<i>Syndesis-asyndesis</i> 2(1-0) divisions	<i>Tetrads</i> (dyad-monads)	The same	<i>Syndesis-asyndesis</i>	Tetrad or dyad (restitution nucleus)	Sterility or apomixis and fertility	
Premature	<i>Asyndesis</i> ; 1(-0) division	<i>Dyads</i> (monads)	<i>Small PMCs</i> <i>Large PMCs</i>	<i>Syndesis-asyndesis</i>	Restitution nucleus; dyads; degeneration of upper dyad cell	Apomixis and fertility	
Greatly premature	<i>Asyndesis</i> ; (1-)0 division	(Dyads) <i>Monads</i>	The same	(Syndesis) <i>Asyndesis</i>	The same or pseudohomotypical division; development of both dyad cells	Apomixis and fertility	Below

The chief tasks at future continued investigations of the *Arabis Holboellii* complex will include (1) supplementary investigations of the embryo-sac formation, particularly at different heights in the raceme, and of the monad formation in the anthers in the lower part of the raceme, (2) more investigations of pseudogamy, (3) experimental investigations of modificative seed sterility, (4) experiments of hybridization between sexual diploids and apomictic diploids and triploids, (5) culture experiments with different races (agamotypes) of the species in order to study the variation in more detail. To these tasks should finally be added a number of special taxonomic and cytogenetic investigations of West American forms.

10. Summary.

1. In a material of diploid and triploid plants of *Arabis Holboellii* var. *typica* from Greenland and diploid plants of var. *retrofracta* from Alaska the pollen and embryo-sac formation have been investigated. In some of the diploid material the meiosis was quite normal, for which reason the plants in question must be considered sexual or amphimictic. In the rest of the material there proved to be a behaviour deviating greatly from a normal meiosis and a number of indications of apomixis.

2. The following signs of apomixis are present: (a) the pollen formation to a wide extent is asyndetic and apomeiotic, mostly corresponding to that in the *Hieracium pseudoillyricum* type. Mostly dyad pollen and, by suppression of both divisions, monad pollen develop. The pollen grains have the unreduced chromosome number; (b) the macrosporogenesis follows the same main lines as also here there may be total asyndesis. Semiheterotypical division and formation of restitution nuclei may occur, too. The embryo-sac nuclei get the unreduced chromosome number. (c) The unreduced pollen is capable of germinating; castration causes sterility. Male nuclei have been observed in the embryo-sac near the egg nucleus and the central nucleus, but no fertilization of these. The endosperm nuclei are hexaploid in triploid material and tetraploid in diploid material. This goes very well with the fact that fusion of the two polar nuclei into the central nucleus has been observed. The one synergid cell or both synergids degenerate after the male nuclei have penetrated into the embryo-sac. The embryo develops from the micropylar end of the embryo-sac and starts its development after the central nucleus has begun the formation of endosperm. All these facts taken together show that we have to do with pseudogamy and agamospermy (diplospory and parthenogenesis).

3. Besides the apomeiotic pollen formation meiosis with tetrad formation has been found in some triploids. During this meiosis there is a pronounced syndesis with formation of up to 10 bivalents. The high number of bivalents must be connected with structural anomalies in the form of interchange. Also the presence of a fragment chromosome in a triploid is indicative of structural changes. The tetrad pollen frequently gets chromosome numbers between 9 and 11. It seems to a wide extent to be unable to germinate.

4. Sterility, which appears as an early cessation of the development of the siliques, may include parts of racemes, main shoots on plants, and whole plants. It is modificative and possibly is connected with retardation of growth and connected change from apomeiotic to meiotic development.

5. The pollen grains decrease in size upwards in the racemes. The size of the pollen grains depends on the chromosome number, the way of formation (tetrads, dyads, or monads), and of the size of PMCs. It is assumed that those at the top of the raceme start with a diameter about one third shorter than those farther down.

A frequent distribution in the raceme is this: at the top, tetrad pollen, in the middle of the raceme, mainly dyad pollen, and below, dyad and monad pollen. All observations indicate that the development in the lower part of the raceme is faster than farther up. It is premature below and at the very top possibly often delayed in relation to a normal development, which involves meiosis. Asyndesis—syndesis, the size of PMCs, and perhaps the way of embryo-sac formation as well thus depend on physiological differences in the longitudinal direction of the raceme.

6. *Arabis Holboellii* is an amphi-apomictic complex, but the Greenland population investigated in the present paper represent a comparatively rigid part of the species, while in West America the species is very plastic. Here diploids are comparatively widely distributed, thus also tetra- and hexaploids, whereas triploids so far have been found in Greenland, only. The difference between the variabilities in Greenland and West America is presumably due to the fact that apomixis is prevalent in the Greenland populations, while the American populations include numerous amphimictic diploids, perhaps amphimictic polyploids, too, which by intercrossing can carry the variability to so critical heights that taxonomists have difficulty in unravelling the various types and units.

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