MEDDELELSER OM GRØNLAND
UDGIVNE AF
KOMMISSIONEN FOR VIDENSKABELIGE UNDERSØGELSER I GRØNLAND
Bd. 125 · Nr. 4

I.

STUDIES ON THE EMBRYOLOGY AND CYTOLOGY OF SAXIFRAGA

BY

L. HARMSEN

WITH 17 FIGURES IN THE TEXT

KØBENHAVN
C. A. REITZELS FORLAG
BIANCO LUNOS BOGTRYKKERI A/S
1939

While at The Danish Arctic Station in Godhavn, Greenland, my attention was drawn by Mr. M. PORSILD to *Saxifraga stellaris var. comosa*, and he suggested that I should go into the question of the fructification of this plant, of which no ripe seeds had been observed in Greenland. The plant is nevertheless common in this region, as it has a very abundant vegetative reproduction by bulbils.

For this purpose a very copious material was gathered and preserved in order to include as many phases of development as the time of the year permitted; unfortunately, the vegetation period was already well advanced (end of August), so that the youngest stages of the formation of embryo-sacs are lacking; there is also an absence of fully developed embryos. Whether or not the latter can succeed in developing fully will presumably depend on the mildness of the autumn season.

Material and Methods.

The material was collected in a wet depression near The Danish Arctic Station and fixed on the spot for five or ten minutes in Carnoy's solution after which it was fixed for 24 hours in a solution of formalin-chromic-acetic acid (Karpechenko, modified by MABEL L. RUTTLE, 1927). After being paraffined in the usual manner it was cut in a thickness of 0.015 to 0.025 mm. Staining was for the most part done in Heidenhain's iron-haematoxylin: mordanted for 2 minutes in 2.5 per cent. iron-alum and after that stained for one minute in 0.5 per cent. haematoxylin, both at 62° C.; then differentiated, first in 5 per cent. iron-alum and finally in 2.5 per cent., which gave good, clear preparations; in a few cases I also made use of iodine-gentiana-violet, magdala red-aniline blue and Feulgen's nucleal stain.

Saxifraga stellaris L. **var. comosa** RETZ.,

belongs to the section *Boraphila* ENGL. (fig. 1); it has spatulate leaves close to the ground, racemous, very branchy inflorescence, in which only the flowers of the main axis and sometimes the terminal flower of some few strong shoots develop, whereas the others turn into bulbils

which, in rare cases, have small and weak stamens. Thus each plant has only one or very few blooms; their structure is normal, with five

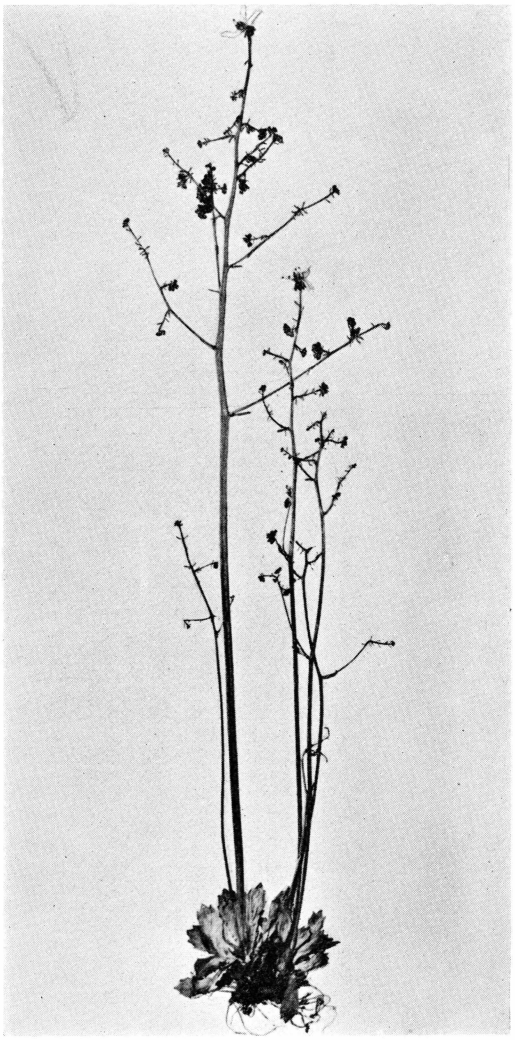

Fig. 1. Habitus picture of *Saxifraga stellaris* var. *comosa*. This specimen has only two blooms, otherwise solely bulbils, many of which fell off during preparation. Half nat. size.

sepals, five petals of equal size, ten stamens and two carpels; very often, however, there are three, and sometimes four carpels.

The ovules are anatropous and have only one integument with three or four layers of cells (fig. 2); as the youngest stages are lacking in the material, it cannot be seen whether it was formed by fusion of

the two as otherwise is the normal in *Saxifraga*. Among Saxifragaceae MAURITZON (1933) gives one integument in *Peltiphyllum peltatum*, *Tanakaea radicans* and *Choristylis rhamnoides;* all other species examined have two integuments, which as a rule have two layers of cells, probably

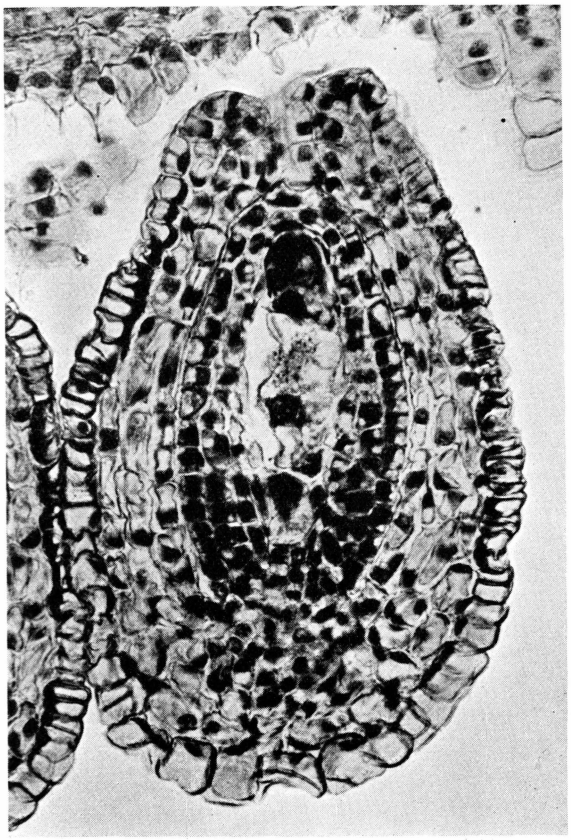

Fig. 2. Embryo-sac. ca. × 400.

indicating that one integument of three to four layers is a product of the fusion of the two-layer integuments.

The earliest phase in my material is shown in fig. 3, viz. nucellus with the embryo-sac mother-cell formed of the innermost cell of the tetrad; the other tetrad cells have already degenerated. The nucleus divides in the usual manner into eight, and the two polar nuclei fuse (fig. 4) and form the central nucleus, which lies a little below the middle of the embryo-sac. The mature embryo-sac is shown on fig. 5 and is of the usual appearance, with two synergidae (no hook-shaped projections observed), one egg-cell, one central nucleus and three small antipodal cells.

On the stigma of somewhat older ovaries there is a quantity of pollen; but despite its abundance, and though continued examinations were made with various staining methods, it was impossible to find a single one that had germinated; nor could I find any pollen-tube in style, on placenta or in micropyle, which means that no fertilization was observed direct. A large number of the embryo-sacs perish, but

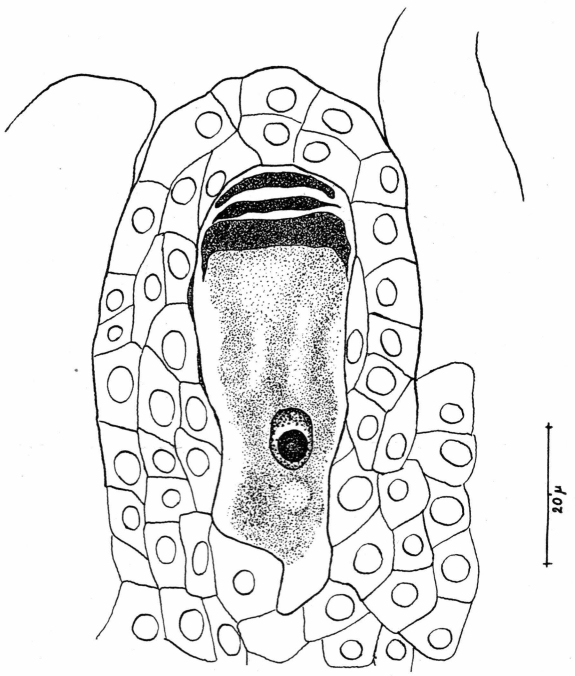

Fig. 3. Nucellus with embryo-sac mother-cell. The three degenerated tetrad cells can also be seen. × 900.

embryo and endosperm were formed in some of them. The earliest phases in the material have triple-cell embryo and trinuclear endosperm (fig. 6). Endosperm development seems to proceed in the manner described (1907) by H. O. JUEL (figs. 8—11); a small chalazal part is formed with cells rich in plasma, and a larger, central or micropylar part with large vacuoles in the cells, which at a rather late stage centre round about the free nuclei. The development of the embryo (figs. 6—11) is also the same as that with which we are already familiar in *Saxifraga*, there being a five-celled suspensor with the outermost cell developed as a haustorium, and the embryo proper, which in the present material does not get beyond the spherical form. Many embryos degenerate like the embryo-sacs before reaching full development; possibly

this applies to them all, as ripe, germinative seeds of this species have never been found in Greenland.

The first stages of embryo and endosperm development seem to proceed normally and in the usual manner for *Saxifraga* species. Re-

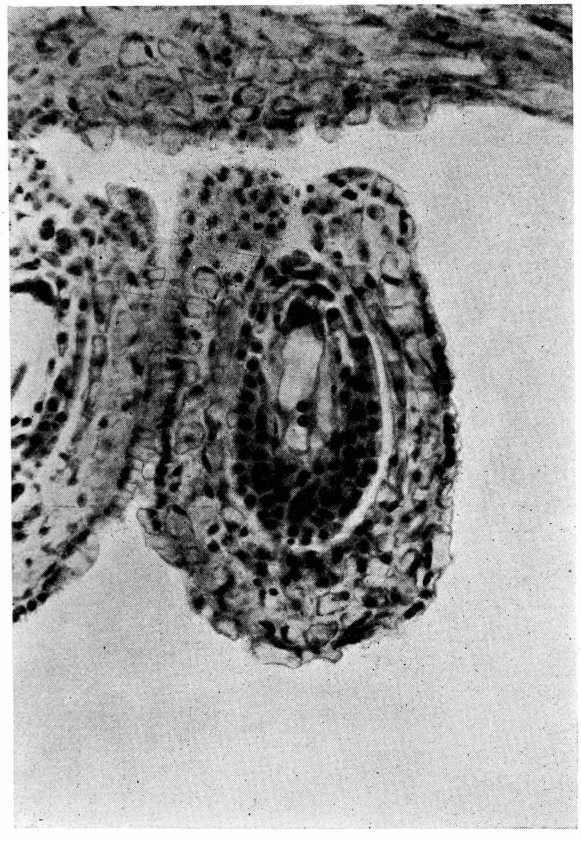

Fig. 4. Young embryo-sac. × 430.

peated chromosome counts in the metaphase stage of mitoses in embryo cells (fig. 12) give 64; endosperm counts, from both the chalazal and the micropylar parts, give 96 (fig. 13), which would seem to indicate dual fertilization, though this was not observed direct; in that case the haploid number should be 32. SKOVSTED (1934) and BØCHER (1938) give n = 14 for *Saxifraga stellaris* L. BØCHER (1938) gives n = 28 for *Saxifraga stellaris var. comosa* (determined on material from Lapland).

The chalazal part of the endosperm often contains nuclei which must be presumed to have been formed by the fusion of several. In

that case the chromosome number would be correspondingly increased, and this has in fact been found, as about 1100 chromosomes were counted in an astonishingly regular metaphase (fig. 14). Up to 14 nucleoles were observed in the nucleus. In the chalazal endosperm there was also a division stage which must be explained as amitosis (fig. 15).

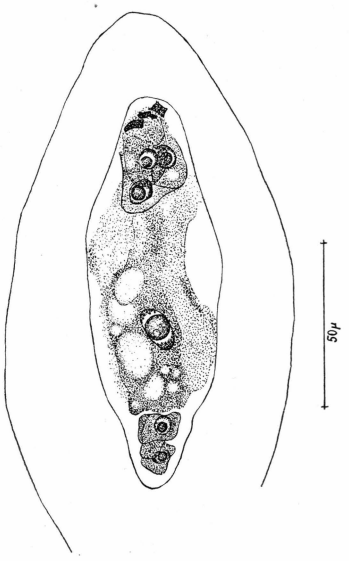

Fig. 5. Ripe embryo-sac. × 430.

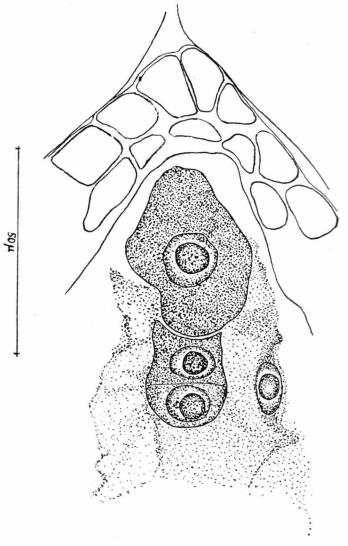

Fig. 6. Triple-celled embryo. × 540.

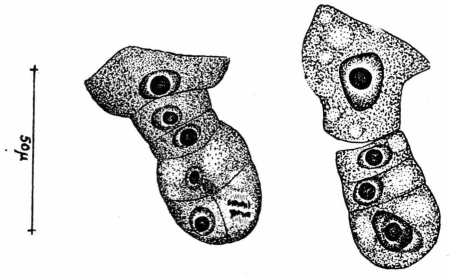

Fig. 7. Young embryos. × 408.

Otherwise, cell division in embryo and endosperm seem to proceed quite regularly; unfortunately, nothing has been found to show whether or not the same is the case with pollen development; but as the pollen grains vary greatly in size (fig. 16), there are grounds for assuming irregularities in the meiotic division with the consequent unequal distribution of the chromosomes. In all probability the irregularities are due to the exterior conditions, no doubt extreme temperatures most of all, as the somatic divisions did not manifest similar characteristics.

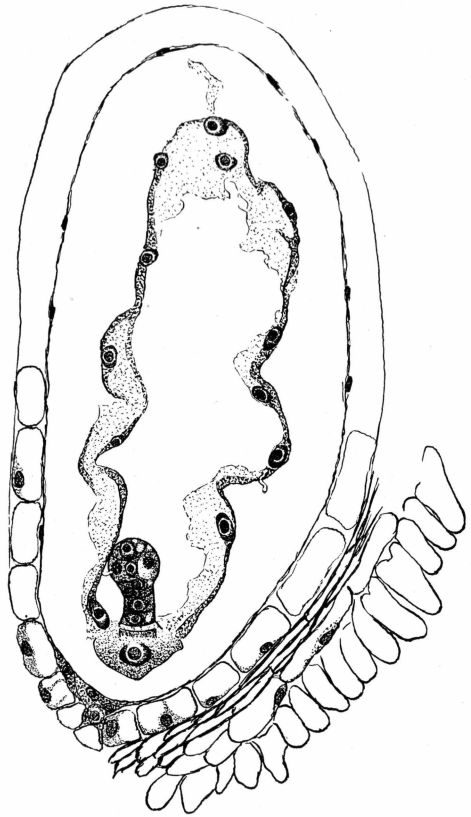

Fig. 8. Longitudinal section of immature seed, endosperm nuclei still free. × 210.

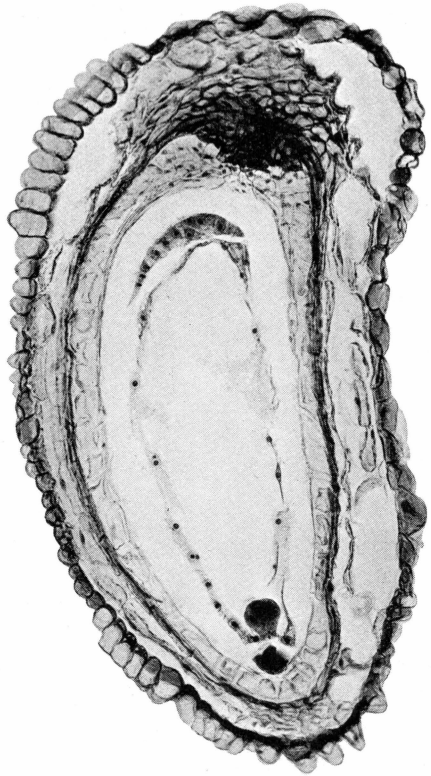

Fig. 9. Longitudinal section of immature seed, the central endosperm with free nuclei, the basal one divided into cells. ca. × 100.

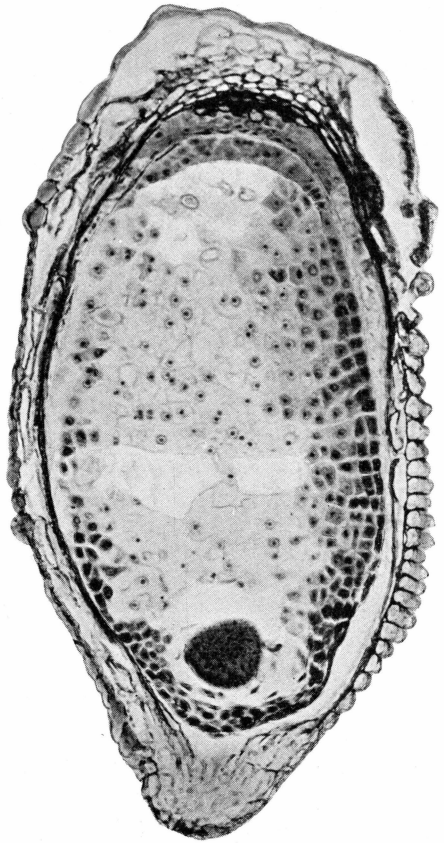

Fig. 10. Longitudinal section of immature seed, the endosperm cells delimited, the embryo globular. ca. × 100.

The smallest two pollen grains on the figure are from an anther on a bulbil.

As was already shown by SCHOLANDER (1934), the structure of the inflorescence of *Saxifraga stellaris* is quite different to that of *S. stellaris*

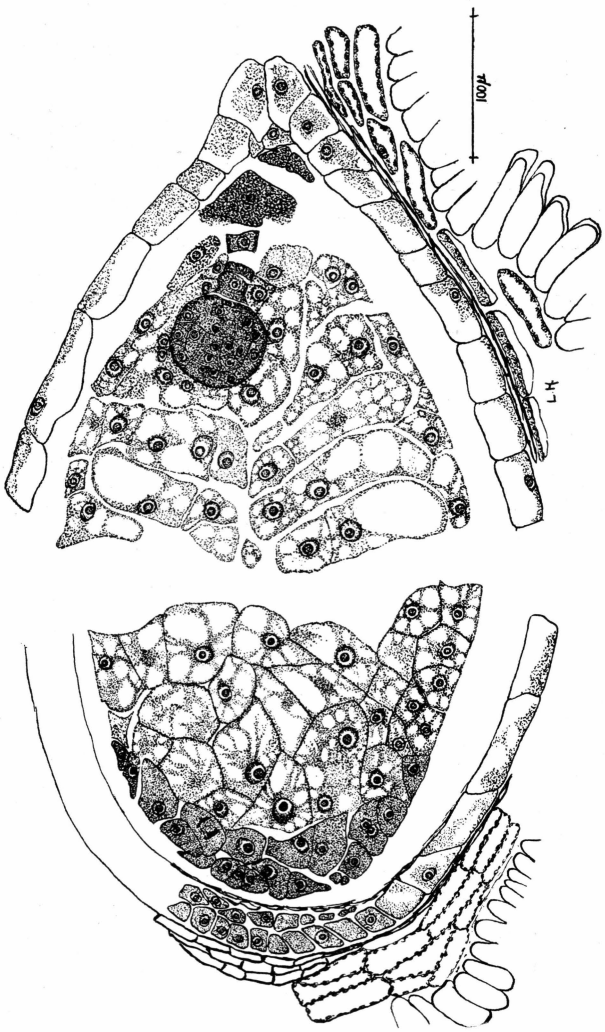

Fig. 11. As fig. 10. × 210.

var. comosa; in the latter there is a pronounced, vigorous central axis with weaker shoots, whereas on *S. stellaris* the main axis is not much pronounced and the shoots are almost just as strong.

The origin of the many bulbils is uncertain. As already stated, they are sometimes furnished with stamens, and this indicates the

possibility that they are formed by the transformation of blooms, similar to what happens with viviparous grasses.

As the geographic distribution and ecology of the two forms differ, and as *S. stellaris var. comosa* also deviates from the other known species of *Saxifraga* in having only one integument, it would seem justifiable to regard it as a separate species, which in that case must be called *Saxifraga foliolosa* R. Brown (1821).

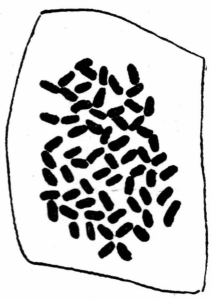

Fig. 12. Mitosis in embryo cell, 2n = 64. × 2450.

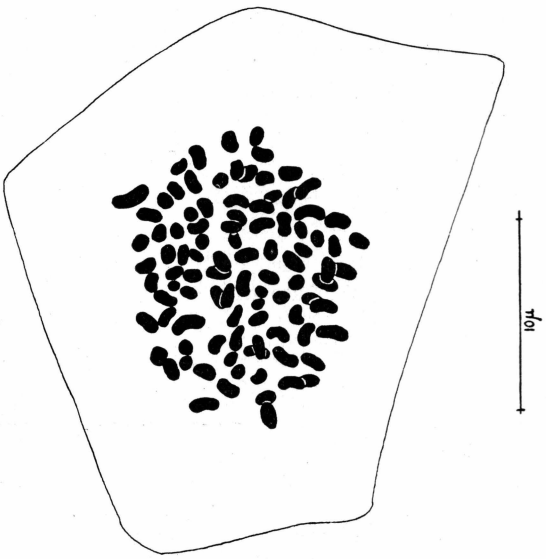

Fig. 13. Mitosis in endosperm cell, 3n = 96. × 2450.

To judge from the chromosome count, *Saxifraga foliolosa* must be considered as a polyploid form, possibly of *S. stellaris* or a related species. It is the general rule that the polyploid forms are more robust in their development than the corresponding diploid forms (Müntzing), but this has not been observed with certainty in this case. On the other hand, as already stated there is a considerable morphological difference between *S. foliolosa* and *stellaris*, especially as regards the structure of the inflorescence and the integuments.

As in many arctic grasses such as *Poa*, *Deschampsia* and *Festuca*, there is a tendency towards viviparity parallel with the increased chromosome number (Flovik, Müntzing). Corresponding to the numbers 28 and 32, belonging to plants from different localities, we know that for instance *Poa alpina* includes forms with mutually different chromosome numbers but constant numbers within each form.

Both species are associated with wet soil: bogs, snow-patches, along watercourses, wet depressions and the like. In the arctic region

S. foliolosa has a much more northerly range than *stellaris*, a circumstance that is repeated among other plants (for example, see HAGERUP).

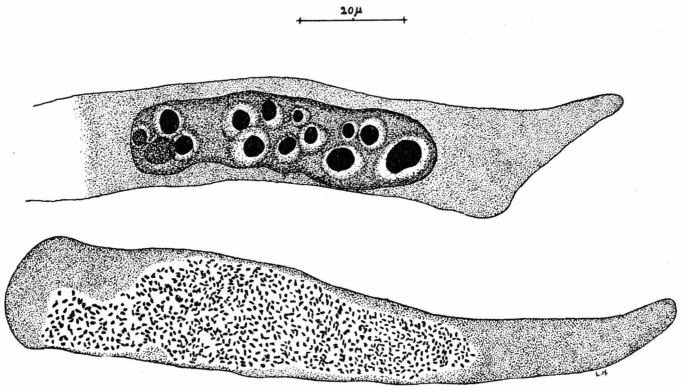

Fig. 14. Cells of the basal endosperm: above, nucleus with 14 nucleoles, below, metaphase with ca. 1100 chromosomes. × 680.

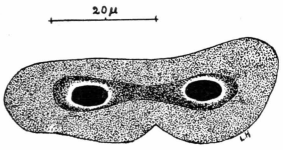

Fig. 15. Amitosis in cell of basal endosperm. × 680.

In the Alps the position seems to be reversed, for there *S. stellaris* ranges higher up (HEGI); consequently, there is a possibility of its being another form. Furthermore, *S. stellaris* seems to be more oceanic and *foliolosa* more continental (BØCHER).

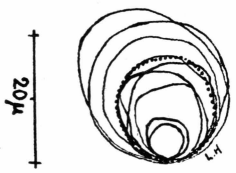

Fig. 16. Variation of pollen grains. The normal size is indicated by the dotted line. The two smallest are from the anther on a bulbil. × 860.

The two species also display differences in their geographical distribution (fig. 17; for distribution in Greenland see BÖCHER, map), *S. foliolosa* being circumpolar, high-arctic (-alpine), whereas *stellaris* is subarctic-temperate-alpine, with its principal distribution in the mountain regions of Europe.

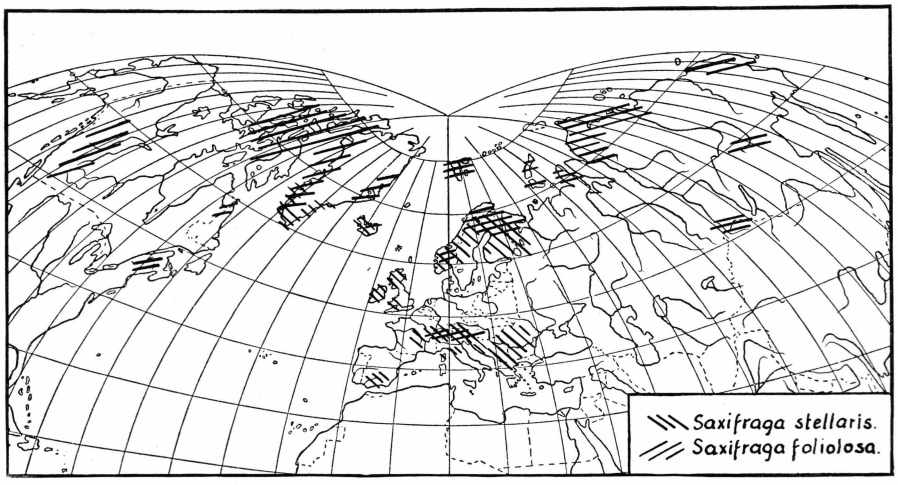

Fig. 17. Map sketch showing distribution of *Saxifraga stellaris* and *S. foliosa*. Compiled on the basis of Engler & Irmscher: Saxifraga, with some additions.

If *S. stellaris* is regarded as the fundamental form of *S. foliolosa*, the increased chromosome number and its viviparity have enabled it to spread to high-arctic regions and to parts with a more continental climate.

Summary.

Saxifraga stellaris var. comosa is examined embryologically and cytologically:

there is only one integument;
embryo-sac development appears to be normal;
no pollen-tubes or fertilization observed;
a number of embryo-sacs degenerate, whereas embryo and endosperm form in others; many embryos degenerate;
no ripe, germinative seeds ever found in Greenland;
chromosome number in embryo 64, in endosperm 96;
pollen irregular;
S. stellaris and *S. stellaris var. comosa* differ as to morphology, ecology and geographical distribution;
the variety should be regarded as a separate, polyploid form: *Saxifraga foliolosa* R. Brown.

BIBLIOGRAPHY

Arwidsson, Th.: Einige neue Gesichtspunkte zu den Chromosomenzahlenbestimmungen. Svensk Bot. Tidskrift, Bd. 32, 1938.

Bøcher, Tyge W.: Biological Distributional Types in the Flora of Greenland. Meddelelser om Grønland, Bd. 106, Nr. 2, 1938.

— Zur Zytologie einiger arktischen und borealen Blütenpflanzen. Svensk Bot. Tidskrift, Bd. 32, 1938.

Dahlgreen, K. V. Ossian: Notes on the ab initio Cellular Endosperm. Botaniska Notiser, Lund 1923.

— Die Befruchtungserscheinungen der Angiospermen. Hereditas X, 1927.

— Die Morphologie der Nuzellus. Jahrb. für wiss. Bot., Bd. LXVII, 1927.

— Hakenförmige Leistenbildungen bei Synergiden. Bericht. d. Deutsch. Bot. Ges., Bd. XLVL, 1928.

— Zur Embryologie der Saxifragoideen. Svensk Bot. Tidskrift, Bd. 24, 1930.

Engler, A. und Irmscher, E.: Das Pflanzenreich, IV. Saxifraga. 1916—19.

Flovik, Karl: Cytological Studies of Arctic Grasses. Hereditas XXIV, 1938.

Gäumann, E.: Über die Entwicklungsgeschichte einiger Saxifragaceen. Vorläufige Mitteilung. Svensk Bot. Tidskrift, Bd. 12, 1918.

Hagerup, O.: Empetrum hermaphroditum (Lge.) Hagerup, a New Tetraploid, Bisexual Species. Dansk Botanisk Arkiv, Bd. 6, 1927.

— Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. Hereditas XVI, 1932.

— Studies on Polyploid Ecotypes in Vaccinium uliginosum L. Hereditas XVIII, 1933.

— Studies on the Significance of Polyploidy. II. Orchis. Hereditas XXIV, 1938.

Juel, H. O.: Studien über die Entwicklungsgeschichte von Saxifraga granulata Nov. Acta Reg. Soc. Sci. Upsaliensis. Ser. IV, Vol. I, Nr. 9, 1907.

Mauritzon, Johan: Studien über die Embryologie der Familien Crassulaceae und Saxifragaceae. Lund 1933.

Müntzing, Arne: The Evolutionary Significance of Autopolyploidy. Hereditas XXI, 1936.

Rocén, Th.: Beitrag zur Embryologie der Crassulaceen. Svensk Bot. Tidskrift, Bd. 22, 1928.

Rosendahl, C. O.: Die Nordamerikanischen Saxifraginae und ihre Verwandschafts-Verhältnisse — — —. Beibl. Nr. 83 zu d. Bot. Jahrb., Bd. 37, 1906.

Samuelsson, G.: Studien über die Entwicklungsgeschichte der Blüten einiger Bicornes-Typen. Svensk Bot. Tidskrift, Bd. 7, 1913.

Schoennagel, E.: Chromosomenzahl und Phylogenie der Saxifragaceen. Bot. Jahrb., Bd. 64, 1931.

Scholander, P. F.: Vascular Plants from Northern Svalbard. Skrifter om Svalbard og Ishavet, Nr. 62, 1934.

Schürhoff, P.: Zur Zytologie von Saxifraga. Jahrb. für wiss. Bot., Bd. 64, 1925.

Skovsted, A.: Cytological Studies in the Tribe Saxifrageae. Dansk Botanisk Arkiv. Bd. 8, 1934.

Warming, Eug.: The Structure and Biology of Arctic Flowering Plants. 4. Saxifragaceae, 1. Morphology and Biology. Meddelelser om Grønland, Bd. XXXVI, 1909.

Færdig fra Trykkeriet den 1. September 1939.