Drawings of Scandinavian Plants 89–90

C iopodium L.

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89. **Chenopodium polyspermum** L. 1753

Annual, 20—100 cm high, erect, ascending or decumbent, branched. Lower branches often long, ascending. Stem angular, striated. Foliage green, sometimes reddish, glabrous. Leaves alternate, except for the opposite lowermost ones. Lower leaves ovate to elliptical or spatulate, up to 8 cm long, length usually twice the breadth, rounded to cuneate at base, more or less tapering to a long petiole. Margins entire or at base with a single, small tooth on each side. Apex obtuse to acute. Upper leaves ovate to lanceolate, usually petiolate, entire. Inflorescences both axillary and terminal, either composed of solitary or few-clustered flowers in dichasia, or of clustered flowers in lax to rather dense, short, spike-like cymes. Flowers perfect, perianth glabrous, mostly 5-merous. Perianth lobes free, only united below, ovate to elliptical, narrowed at base, obtuse to acute, almost entirely rounded on the back, at anthesis green, the outer parts becoming light and membraneous at maturity, only partly covering the seed. Pistil short, with two small, sometimes insignificant stigmas, papillate to the base. Stamens 1—3, rarely 5. Seeds horizontal, at maturity detached from the perianth, orbicular, brownish to black, 0.8—1.1 mm in diameter, mostly rounded in transection. Pericarp rather thick, yellowish to reddish-brown, closely radially rugose. Testa lustrous, obscurely reticulate, with small irregular pits. Radicula rather short and broad, attached to the seed. Embryo annular.

Flowering time: July to September.

Chromosome number: 2n=18.

Variation: Two main form series can be distinguished but may only represent modifications. Plants belonging to var. *polyspermum* are characterized by a dark green colour, ascending to decumbent stem, usually ovate, obtuse leaves and loose inflorescences. Those of var. *acutifolium* (SM.) GAUD. are usually pale green, often somewhat reddish, erect, with ovate to elliptical, acute leaves and rather dense, spike-like inflorescences. However, according to JÖRGENSEN (1973), there seems to be only a slight correlation between leaf shape and type of inflorescence.

Habitat and distribution: *C. polyspermum* occurs as a weed on most waste ground such as road-sides, and in gardens and on other cultivated ground, as well as on shores and river banks. It is distributed throughout most parts of Europe but is rare in the Mediterranean area, in Asia Minor and in the USSR especially in the European parts, in the western parts of Siberia and in the region of Caucasus. It has been introduced into South Africa and North America. In Scandinavia rather common and more or less established in the southern parts. *C. polyspermum* is widely distributed in Denmark except for the northern and western parts of Jylland,

[1] ENGSTRAND is responsible for the drawings and GUSTAFSSON for the text.

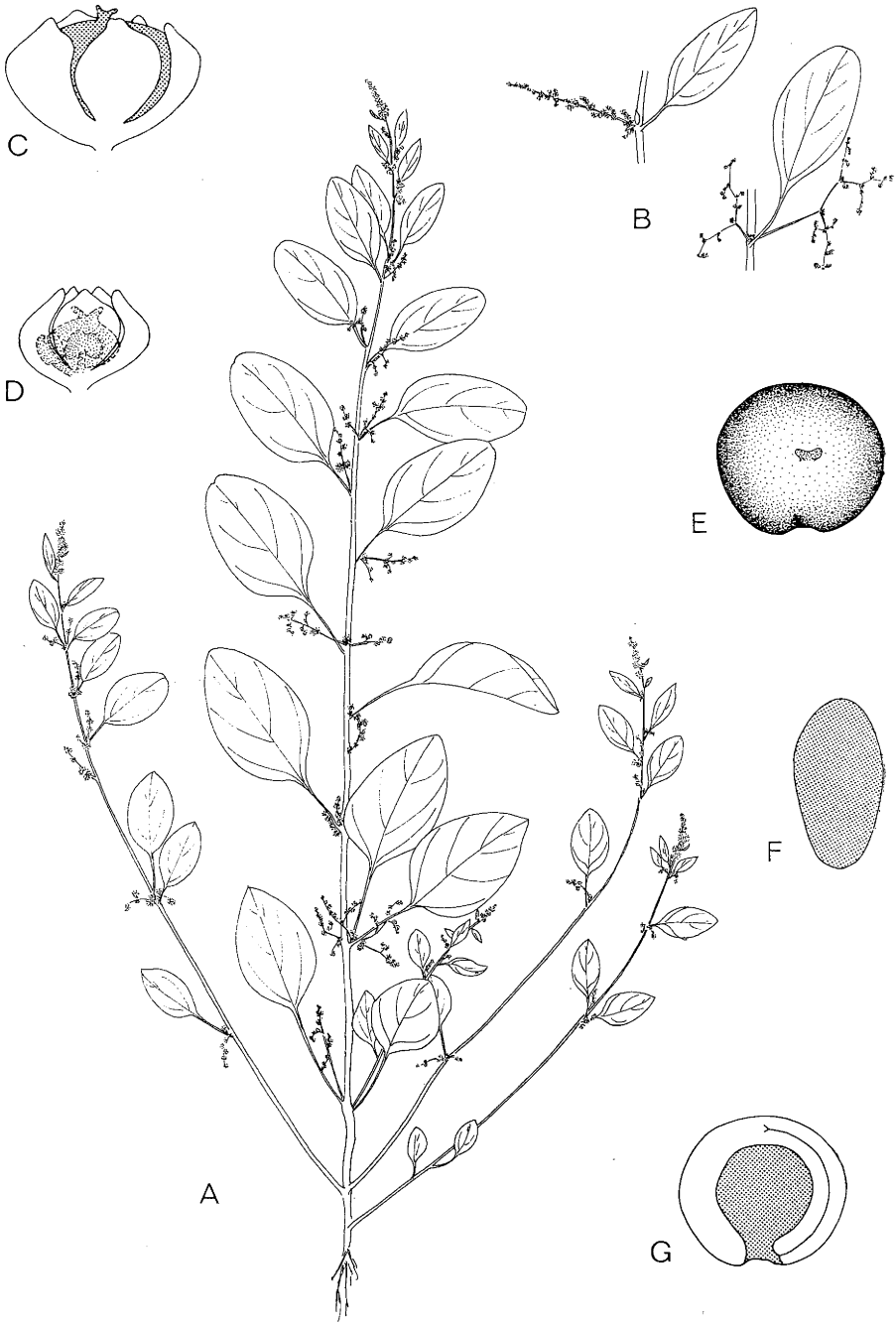

Fig. 89. *Chenopodium polyspermum* L. — A: Habit. — B: Leaves and inflorescences of var. *polyspermum* (to the right) and var. *acutifolium* (to the left). — C: Fruit enclosed in the perianth. — D: Hermaphrodite flower. — E: Fruit with pericarp. — F: Seed in transection. — G: Section through a seed, showing the embryo. — A—B: ×0.5. — C—G: ×20.

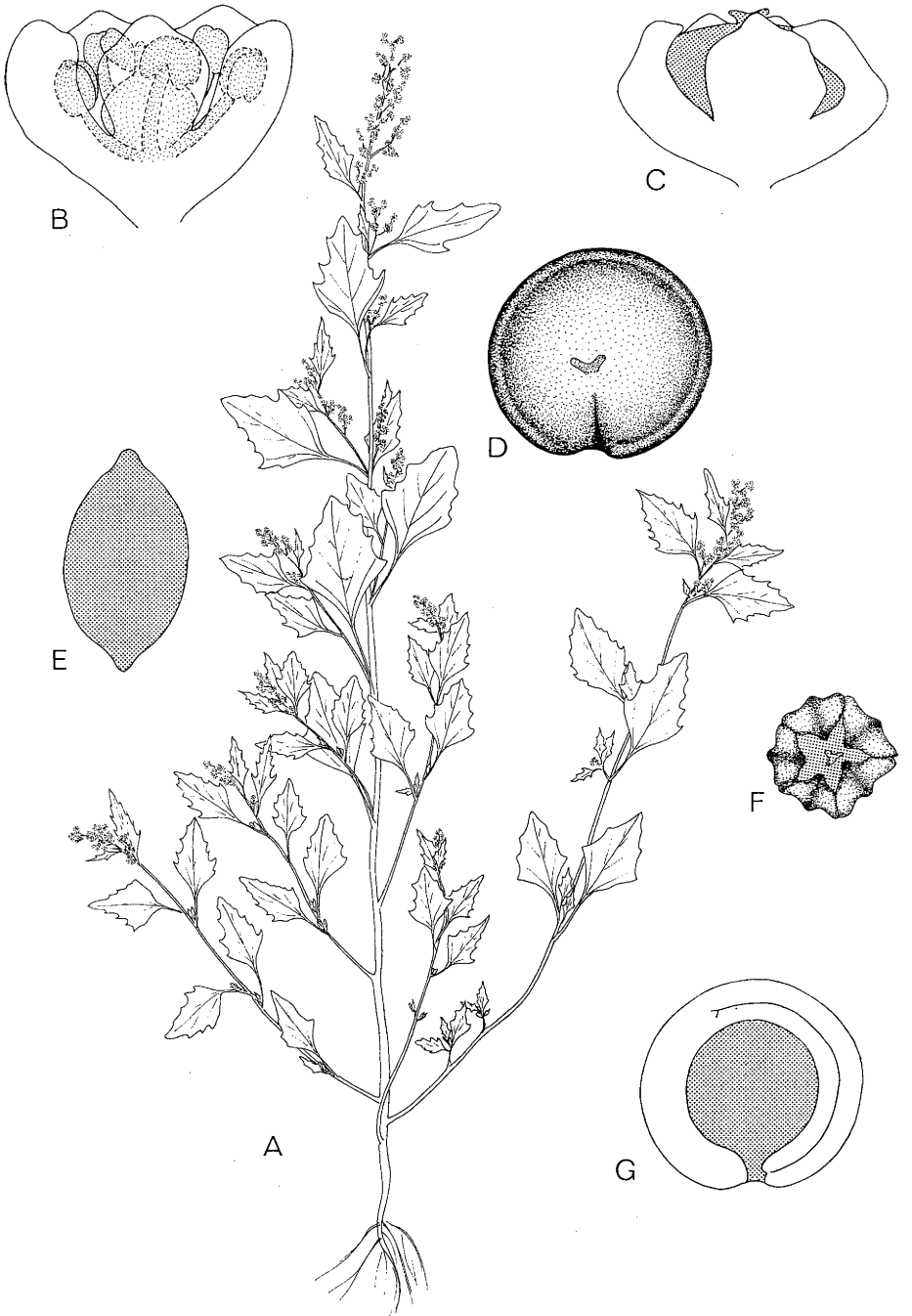

Fig. 90. *Chenopodium murale* L. — A: Habit. — B: Hermaphrodite flower. — C: Fruit enclosed in the perianth. — D: Fruit with pericarp. — E: Seed in transection. — F: Perianth seen from above. — G: Section through a seed, showing the embryo. — A: ×0.5. — B—E, G: ×20. — F: ×10.

and in Sweden northwards to the provinces of Värmland—Gästrikland, rare further north. In Norway mainly distributed around Oslo, and in Finland in the southern to southwestern parts.

90. **Chenopodium murale** L. 1753

Annual, up to 100 cm high, rather stout, erect mainstem, mostly branched. Lower branches ascending to decumbent. Stem ± striated, angular. Foliage mostly dark green, farinose at least in young stages of development, with an unpleasant smell. Leaves alternate, the lowermost ones sometimes opposite. Size and shape of leaves very variable, the lower ones rhomboid to ovoid, usually 2—8 cm long, 1—1 1/2 times as long as broad, the cuneate base gradually tapering to the long petiole. Margins of upper parts irregularly dentate, with coarse, acute forward-pointing teeth, almost completely entire basically. Apex acute. Upper leaves ovate, elliptical or lanceolate, petiolate, sharply dentate, acute. Inflorescences mostly composed of clustered flowers in rather loose, cymose panicles, situated both terminally and in axils. Flowers perfect, 5-merous, mostly farinose. Perianth lobes united to a length of one third, ovate, obtuse to acute, more or less fringed at the summit, conspicuously keeled on the back, mostly light green except for the whitish, membraneous summit. The seed more or less covered by the perianth lobes, but upper parts sometimes detached from the seed. Pistil with 2—3 rather long, papillated stigmas. Seed horizontal, orbicular, large, 1.0—1.5 mm in diameter, black, compressed in the outer parts, thus being keeled in transection. Pericarp usually yellowish to brownish, papillate, closely adherent to the testa. Testa lustrous, closely and minutely pitted. Radicula short and broad, closely adpressed to the seed. Embryo annular.

Flowering time: July to September.

Chromosome number: 2n=18.

Variation: *C. murale* is variable in most vegetative characters such as height, shape and size of leaves, dentation and degree of vestiture, and different forms have been distinguished (for further information see AELLEN 1960).

Habitat and distribution: *C. murale* occurs as a weed especially on ground rich in nitrogen, usually close to human settlement, as in farmyards, along village streets, on cultivated ground and, rarely, on sea shores. In recent times introduced into most parts of the world, probably indigenous in the Mediterranean area and in the southern and southwestern parts of Asia. In Scandinavia *C. murale* is a rare, casual weed, which seems to have been more common previously and then often in connection with shipping. In Denmark most records originate from the islands and in Sweden from localities along the south and west coasts, but scattered localities occur northwards to the province of Norrbotten. In Norway reported from single localities northwards to Trondheim. Only a few records are known from Finland.

Comments: *C. murale* has been confused with other species, particularly with *C. rubrum* and *C. urbicum*. But, in fact *C. murale* is rather easily recognized by its conspicuously keeled perianth segments, which are only united below and are often fringed at the summits, and by the black, rather large, horizontal, sharply keeled seeds, with a minutely pitted testa.

LITERATURE CITED

AELLEN, P. 1960. Chenopodium. — In HEGI, Illustrierte Flora von Mitteleuropa, 2. Aufl., III (2). — München.

JÖRGENSEN, P. M. 1973. The genus Chenopodium in Norway. — (In press).

Pollen Ontogeny in Some Species of Campanulaceae. A Study by Electron Microscopy

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ABSTRACT

DUNBAR, A. 1973. Pollen ontogeny in some species of Campanulaceae. A study by electron microscopy. — Bot. Notiser 126: 277—315.

The organelle population of some *Campanula* and of *Jasione montana* pollen has been investigated and their distribution and possible functions have been linked to the ontogeny of the pollen wall and its pores. The early pollen mother cells are connected by cytoplasmic bridges, disappearing at prophase of meiosis. Callose is synthesized adjacent to the inside of the mother cell wall. After meiosis an accumulation of the rough endoplasmic reticulum portends cytokinesis between the nuclei. The tetrad cells synthesize a callose layer of their own; an electron transparent space separates the callose layers of the individual cells. The partition is not completed towards the former pollen mother cell wall. A primexine template is initiated adjacent to the inside of the callose. Mitochondria are largely in association with the inside of the plasma membrane of the tetrad cells. The primexine template curves locally into the cells; a fibrous material fills the space thereby formed, indicating the future pore. Polysomes are observed close beneath the plasma membrane probably in correlation to the forming probacula. A protectum is initiated simultaneously with the first indication of lamellae basically to, and in contact with the probacula and locally with the proaperture. Coincidently finger-like inclusions appear in the fibrous material of the pores; it is suggested that they give rise to the outer part of the operculum, the inner part being formed on units of granular material. Released from the tetrad, the microspores rapidly gain in size. In *Campanula* the tectum becomes spongy; in *Jasione* a common tectum with perforations is formed. The rest of the wall shows but small difference between the genera. The parallel development and difference in pattern between the operculum and the pollen wall are discussed.

ABBREVIATIONS AND TERMINOLOGY

GA=glutaraldehyde
PTA=phosphotungstic acid
RNA=ribonucleic acid

The terms used to describe the different layers of the pollen wall are mainly the ones introduced by FAEGRI (1956) and FAEGRI and IVERSEN (1950). The terms used in the introduction of *Campanula* and *Jasione* pollen grains are listed by ERDTMAN (1952) and ERDTMAN et al. (1961).

INTRODUCTION

The pollen grains in *Campanula* and *Jasione* are radially symmetrical, isopolar, zonotreme 3—5-porate (*Jasione* 3-porate), spheroidal and tectate. In *C. rotundifolia* the polar axis is about 29 μ, the equatorial diameter about 33 μ, whereas in *J. montana* they are 22×25 μ (cf., e.g. ERDTMAN et al. 1961). Spinules are evenly distributed over the non-apertural surface of the pollen grains.

A spheroid shape of Campanulees and a surface provided with small asperities may have been first described by CANDOLLE (1830) and thereupon by FRITSCHE (1832). The latter also reported *Campanula* pollen to be provided with 4—5 pores. That the pollen of *Campanula americana* possess two nuclei at the time of anthesis was first described by BARNES (1885) who further stated that the smaller nucleus persisted, and either with or without division copulated with the female pronucleus. FISCHER (1890) described the exine of Campanulaceae pollen as consisting of two layers and as having short rods. In distinction from FRITSCHE he stated the number of pores to be no more than 4. He described the pores as small and round and reported the intine to be strikingly thick beneath the pores. DRAHOWZAL (1936) in a detailed study on the number of pores in *Campanula rapunculoides* reported them mostly to be 4, although 3- and 5-porate pollen grains also occurred. A further review of the literature regarding light microscopical studies on Campanulaceae pollen can be found in ERDTMAN (1952) where also a description of the pollen morphology is given.

BADRÉ et al. (1972) studied the fine structure of *Heterochaenia* pollen. A short report on the fine structure and shape of some Campanulaceae pollen (DUNBAR in press) revealed a striking variation between some genera in this family. Beyond these reports very little is known about the fine structure of the Campanulaceae pollen.

In the present study an attempt is made to investigate the general changes in the cell organelles during the pollen ontogeny and to relate the findings to the developmental processes which lead to the formation of the pollen wall and its pores. Further the environment of the pollen mass, i. e. associations with contiguous cells and tissues, is briefly dealt with.

MATERIAL AND METHODS

METHODS FOR THE TRANSMISSION ELECTRON MICROSCOPY. Anthers of *Campanula rapunculoides* L., *Campanula persicifolia* L., *Campanula rotundifolia* L. and *Jasione montana* L. were cut into segments if they were near maturity, immediately after immersion in the fixative, whereas young anthers were fixed intact. Half of the anthers from each flower bud were fixed in a stock solution of 0.1 M GA in 0 1 M cacodylate-HCl buffer at pH 7—7.2, the others were fixed in 0.1 M GA in 0.1 M phosphate buffer (MILLONIG's in PEASE 1964) at pH 7.2. In some of the young material the stock solution was diluted with distilled water. The material was transferred without rinsing to osmium tetroxide in the same buffer (cacodylate-HCl buffer or phosphate buffer) followed by dehydration in an acetone series, starting at 30 % acetone and proceeding to 95 % within 50 minutes; 5 changes followed of 100 % acetone within 1 hour. The anthers were infiltrated for several days with MOLLENHAUER's epon-araldite mixture no. 1 (MOLLENHAUER 1964). Some material was rinsed briefly in distilled water and put into 1 % uranyl acetate (aqueous) for 4 hours at +20°C before dehydration in the acetone series. Sections of all material studied were cut for examination with the light microscope. Sections for examination by the electron microscope were cut with a DuPont diamond knife using an LKB Ultrotome. Some of all sectioned material was examined unstained. Some sections were PTA stained according to PEASE (1966). Where no staining information is included in the figure legend, the sections were stained with 1 % aqueous uranyl acetate for 5 minutes at 42°C and for an additional 5 minutes at room temperature followed by lead citrate (REYNOLDS 1963) for 10 minutes. Micrographs were taken with a Zeiss EM-9S microscope on Gevaert 23 56 film if no other information is included in the figure legend.

METHODS FOR SCANNING ELECTRON MICROSCOPY. The surface of a metal holder was covered with a thin layer of glue which was then allowed to partly dry. The air-dried (chemically untreated) pollen grains were dusted over the holder and adhered to the surface of the glue. The pollen grains were then coated with a film of about 20 nm thickness, of evaporated gold/palladium. During evaporation the holders were rotated on a helical path giving maximum variation of angle relative to the evaporating source. A Stereoscan Mk IIa (Cambridge Scientific Instrument Co.) at the Swedish Geological Survey was used for examination and for taking the micrographs.

Table 1. The species represented in the 16 stages of ontogeny.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
C. rapunculoides			+		+	+	+	+	+	+	+	+	+	+	+	+
C. rotundifolia		+											+	+	+	+
C. persicifolia																+
J. montana	+			+										+		+

STAGES AND EVENTS DURING THE ONTOGENY OF CAMPANULA AND JASIONE POLLEN

RESULTS

To aid in the presentation of the results the period of development has been divided arbitrarily into 16 stages (see above). At each stage only such features will be described which differ from those of the preceding stages or are in some way of special significance.

Stage 1. Pollen Mother Cells with Cytoplasmic Connections

Between the wall of the tapetal cells and the one of the pollen mother cells, a mass of medium density is evident (Fig. 3 A). Cytoplasmic bridges are obvious between the neighbouring pollen mother cells (Fig. 1 A). Ribosomes occur together with other organelles in the connecting cytoplasm (Fig. 1 C). The nucleus is mostly located near one of the connecting bridges, *i.e.* the one belonging to a pollen mother cell having its nucleus also located near the same connection (Fig. 1 A). Blebs occur adjacent to the cytoplasmic side of the nucleus envelope (Figs. 1 B, 2 B). The envelope is provided with many pores (Figs. 1 B, 2 A). A spheriod configuration of the endoplasmic reticulum (Fig. 1 A) is found in most of the pollen mother cells. It includes tightly packed ribosomes, sometimes along with unidentified inclusions (Fig. 2 D). An accumulation of elements (rods) appear from combined data of cross and longitudinal sections to be rod-shaped. While lacking a limiting membrane they are surrounded by the rough endoplasmic reticulum (Figs. 2 C, E, 3 A). Dilated cisternae of the endoplasmic reticulum include tubuli. They are observed adjacent to the nucleus envelope (Fig. 2 A). Lipid droplets are evenly dispersed in the cytoplasm. Further components of the cytoplasm are a great number of mitochondria, ribosomes, Golgi bodies and vacuoles, some of the vacuoles having dense inclusions.

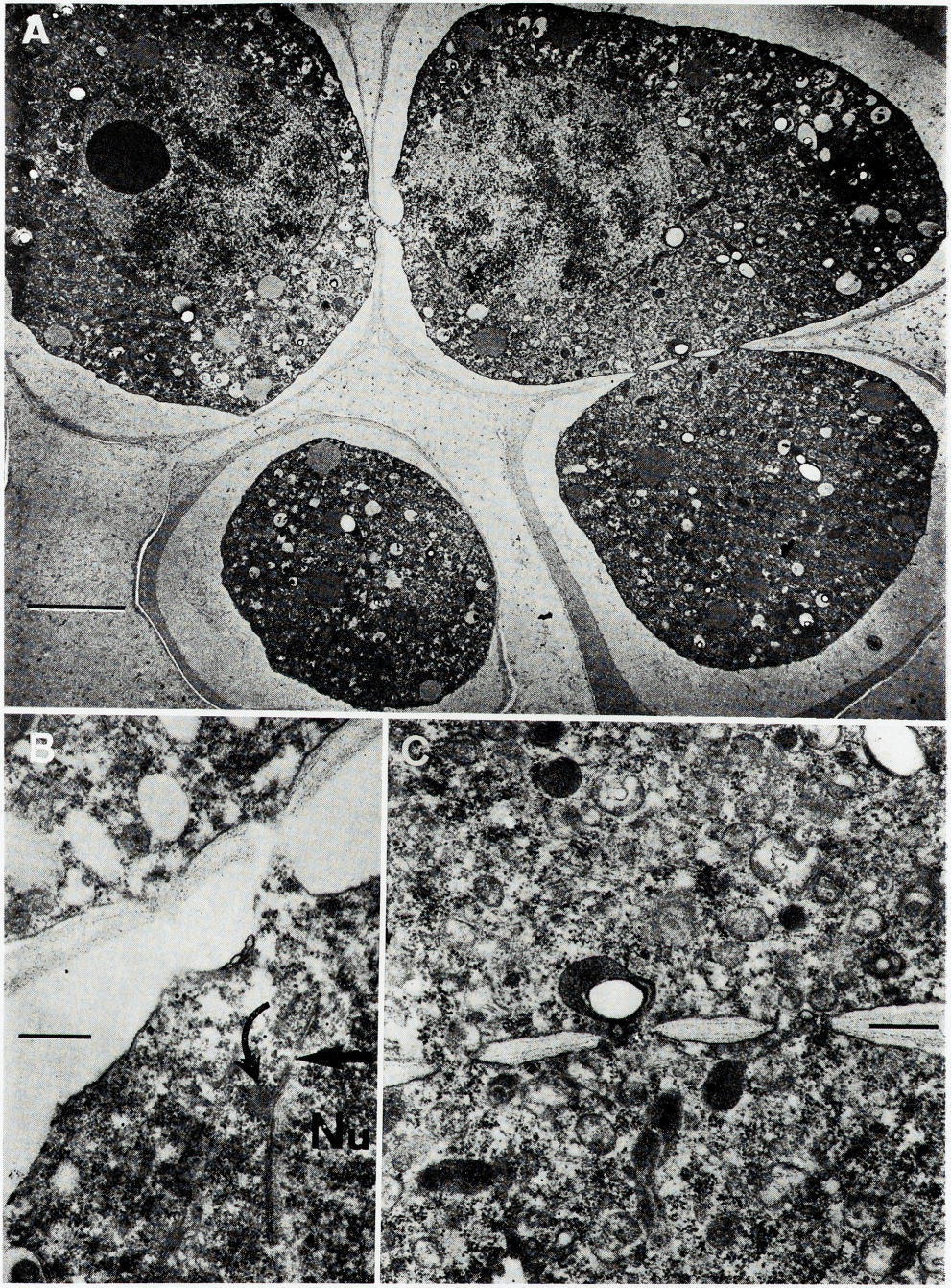


Fig. 1. Legend see page 282.

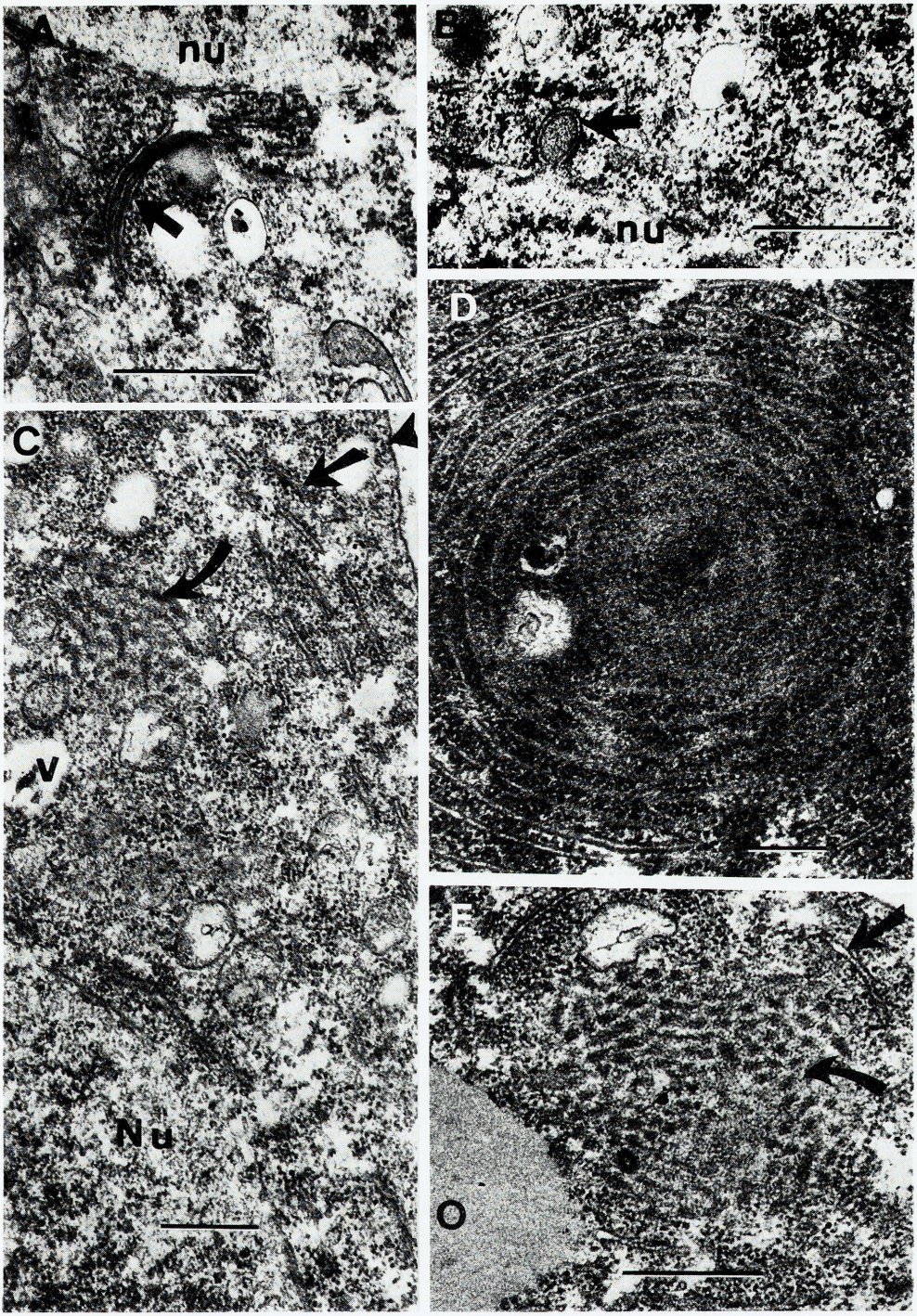


Fig. 2. Legend see page 282.

Stage 2. Pollen Mother Cells during Prophase of Meiosis

A callose layer is forming in contact with the inside of the pollen mother cell wall (Fig. 3 B). The rough endoplasmic reticulum is a conspicuous feature of the cytoplasm, while Golgi bodies are small and only consist of a few straight cisternae. The nucleus occupies a considerable portion of the cell. The chromosomes have formed bivalents with synaptinemal complexes (Fig. 3 C).

Stage 3. Young Tetrad Immediately after Meiosis

The tapetal cell wall is apparently in a phase of dissolution only remaining as loosely composed fibres (Fig. 4 A). Between this remainder and the tetrad, ribosome-like elements are found. The former pollen mother cell wall is locally interrupted (Fig. 4) and at such zones the ribosome-like material comes into contact with the callose (Fig. 4 C). Inclusions of fibrous material are evident in the outermost part of the callose layer (Fig. 4 A). Dense droplets are attached to the plasma membrane, to the nuclear envelope and are membrane-bounded elsewhere in the cytoplasm (Fig. 5 A, B). The four nuclei are in a tetrahedral position with the result that each of the nuclei is surrounded by an about equal volume of cytoplasm. Cytokinesis is portended between the nuclei by an accumulation of segments of the rough endoplasmic reticulum (Fig. 6 A). The accumulation of rods observed at stage 1 is evident in the cytoplasm, although from now on without any surrounding cisternae of the endoplasmic reticulum. Occasionally a spheroid of ribosome-like elements is found close outside one of the nuclei (Fig. 5 B). The number of lipid droplets and of ribosomes appears to have decreased, which may be due to an increased volume of cytoplasm. Apart from the organelles portending cytokinesis, the organelle population is evenly dispersed in the cytoplasm.

Fig. 1. *J. montana* stage 1. — A: A partly connected quartet of pollen mother cells. Material of medium density is dispersed in the theca loculus between the mother cells. Approx. ×6,500. The marker is 2 μ. — B: Detail of Fig. 1 A. Nucleus (Nu), blebs of the perinuclear cisterna (bent arrow), pores of the cisterna (long arrow). Approx. ×20,000. The marker is 0.5 μ. — C: Detail of Fig. 1 A. Ribosomes occur frequently in the cytoplasmic bridges. Approx. ×20,000. The marker is 0.5 μ.

Fig. 2. *J. montana* stage 1. A: A pair of intracisternal tubules (arrow) close to the perinuclear cisterna. Nucleus (Nu). Approx. ×38,000. The marker is 0.5 μ. — B: Blebs of the perinuclear cisterna (arrow). Nucleus (Nu). Approx. ×40,000. The marker is 0 5 μ. — C: Segments of the rough endoplasmic reticulum (arrow) outside an accumulation of granular units (bent arrow). Nucleus (Nu), vacuole (V), plasma membrane (arrow-head). Approx. ×28,000. The marker is 0.5 μ. — D: A configuration of the endoplasmic reticulum is illustrated surrounding closely packed ribosomes, which appear to be more closely arranged towards the centre. EMG taken with a Philips 201 electron microscope. Approx. ×55,000. The marker is 0.2 μ. — E: Accumulation of granular units (bent arrow), segment of the surrounding endoplasmic reticulum (arrow), lipid droplet (0). Approx. ×40,000. The marker is 0.5 μ.

Fig. 3. A: *J. montana* stage 1. Tapetal cell (T) withdrawn from its wall (arrow). Pollen mother cell including a granular accumulation; part of the units is longitudinally sectioned (bent arrow). Nucleus (nu) with nucleolus. Mass of dense material (short arrow) between the pollen mother cell wall and that of the tapetum. Approx. ×11,000. The black marker is 10 μ. — B, C: *C. rotundifolia* stage 2. B: Pollen mother cell withdrawn from its wall (arrow). Callose (C) begins to form. Rough endoplasmic reticulum (R), chromosomes on the left-hand side. Approx. ×25,000. The marker is 0.5 μ. — C: Nucleus envelope (bent arrow). Three more or less longitudinal sections of chromosomes paired into bivalents, with synaptinemal complexes (arrow). Approx. ×52,000. The marker is 0.2 μ.

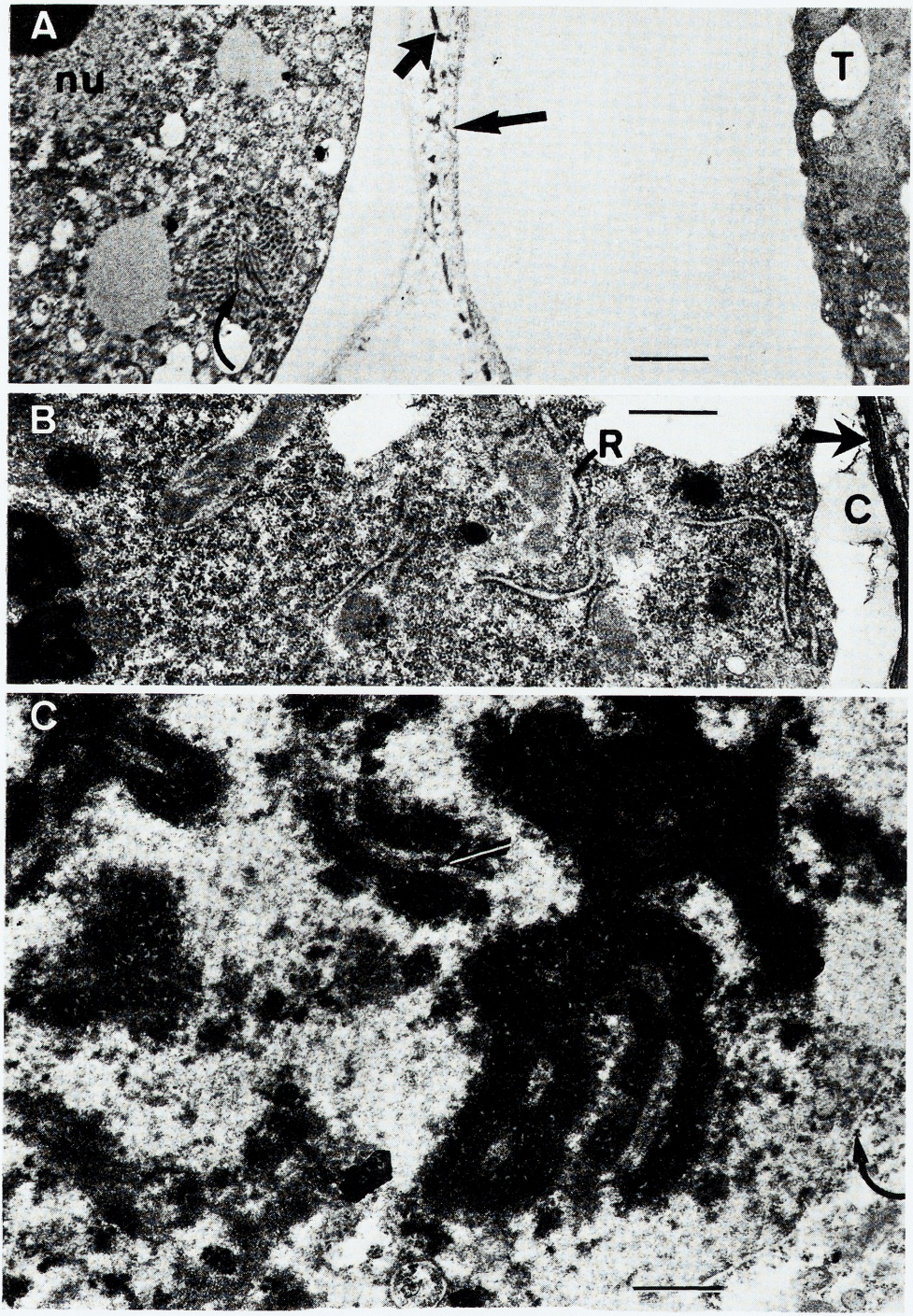


Fig. 3. Legend see page 282.

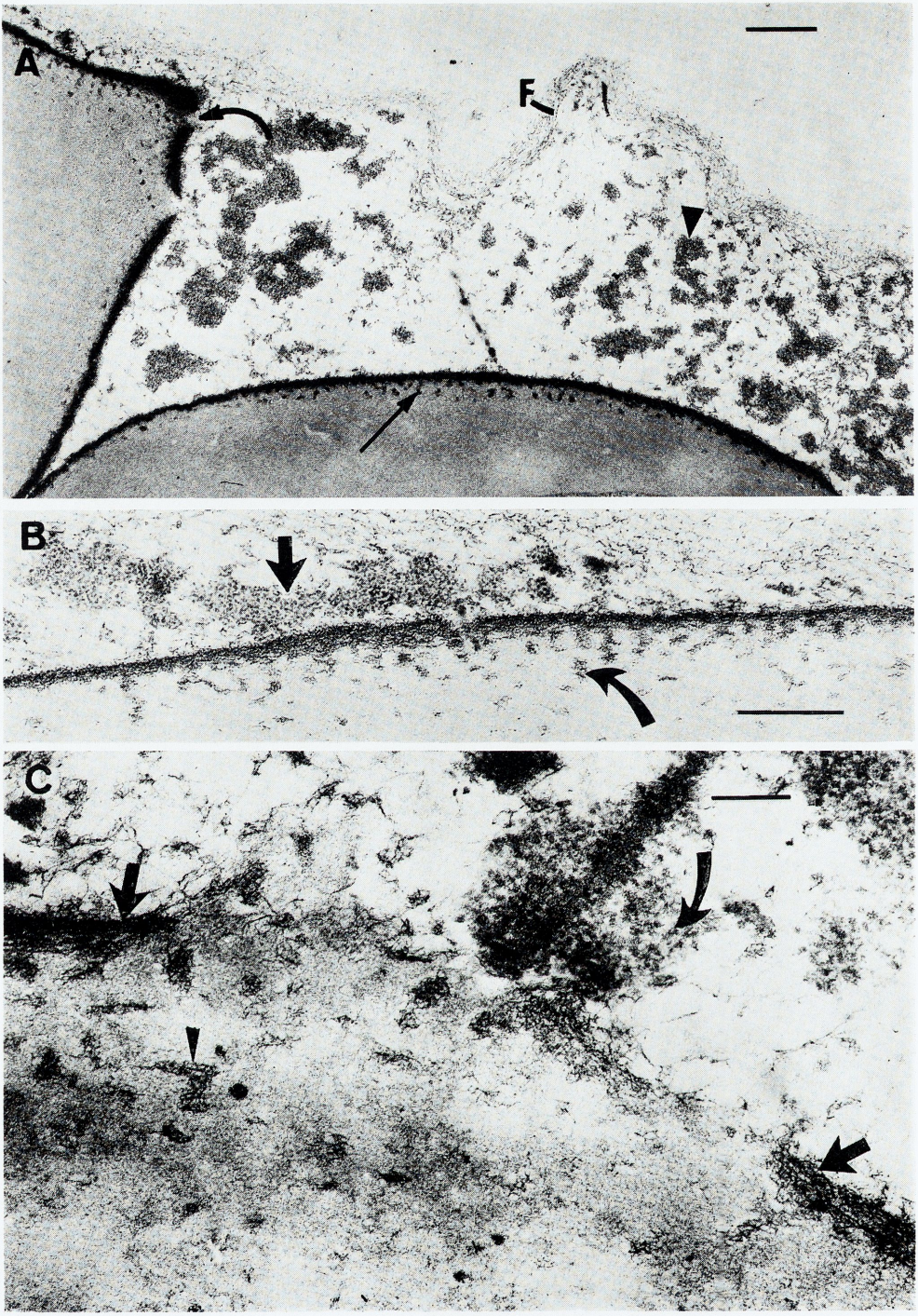


Fig. 4. Legend see page 286.

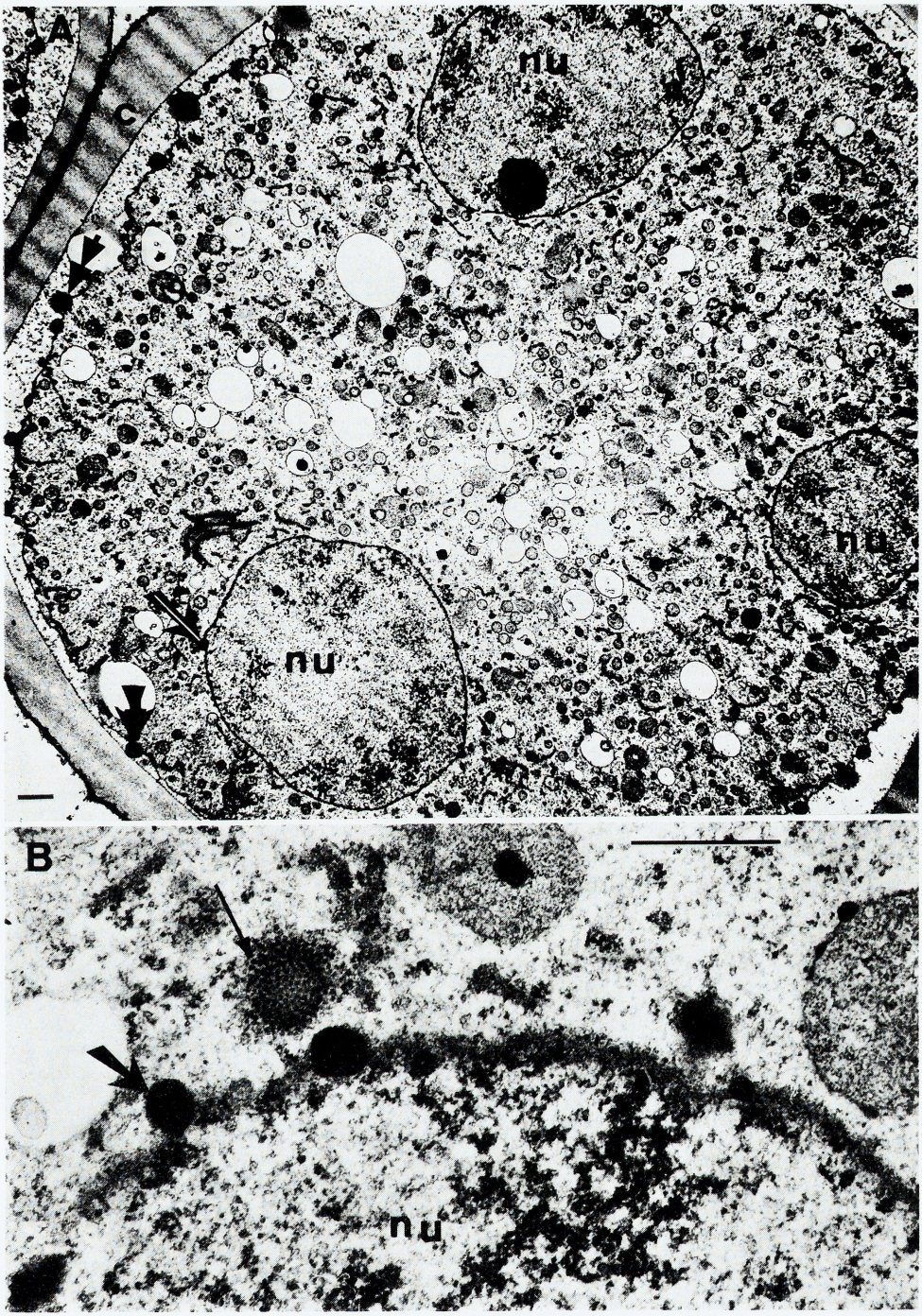


Fig. 5. Legend see page 286.

Stage 4. Early Tetrad

Structures of different shape are located outside the tapetal cells along with a layer of granular-fibrous material (Fig. 7). The cytokinesis between the nuclei has given rise to a tetrad of cells, which are further separated by a callose layer of their own. Dense droplets are associated with the plasma membrane of the tetrad cells.

Stage 5. Initiation of the Primexine Template

A thin layer of the primexine template has formed adjacent to the inside of the callose layer (Fig. 6 B). Dense droplets occur less frequently at the plasma membrane, while such droplets are no longer evident associated with the nucleus envelope. Plastids, which apparently display a secretory activity are found in the cytoplasm. Golgi bodies with adjacent vesicles are numerous, while almost no lipid droplets are present. The accumulation of rods reveal connecting fibrils in cross-sections (Fig. 6 C).

Stage 6. Further Development of the Primexine Template

The primexine template first observed at stage 5 has developed into an intensely dense and compact layer, although it has formed irregularly with local interruptions (Figs. 8 A, 9 A). These "open" zones, however show a thin fibrous layer which is comparable to the early primexine template of stage 5.

Stage 7. Mitochondria in Contact with the Plasma Membrane

The plasma membrane is slightly convoluted. Vesicles are found adjacent to its outside. Mitochondria are located in contact with the plasma membrane, close together or at some distance from each other (Fig. 8 B). The mitochondria are obviously much larger than the rest of the mitochondria population. The nucleus envelope is undulated and has prominent pores (Fig. 8 D). Microtubules occur beneath the plasma membrane and Golgi bodies with adjacent vesicles are found in the peripheral cytoplasm (Fig. 8 D).

Fig. 4. *C. rapunculoides* stage 3. — A: The callose envelope (C) of two former pollen mother cells prior to cytokinesis. Dense inclusions (arrow) line the periphery of the callose layer. A granular mass occurs outside the callose (arrow-head) bordered by a fibrous layer (F) probably the former wall of the tapetal cells. Note interruptions of the callose border (bent arrow). Approx. ×10,000. The marker is 1 μ. — B: Ribosome-like elements (arrow) outside the callose layer. Inclusions of the callose (bent arrow). Approx. ×30,000. The marker is 0.5 μ. — C: Former pollen mother cell wall (arrows) with interruption. Ribosome-like elements (bent arrow), fibrous material similar to the material of the border is embedded in the callose (arrow-head). Approx. ×55,000. The marker is 0.2 μ.

Fig. 5. *C. rapunculoides* stage 3. — A: Three of the four nuclei are seen (nu). Callose layer (C), dense droplets (double-headed arrows) associated with the plasma membrane and perinuclear cisterna (arrow). Approx. ×5,500. The marker is 1 μ. — B: Droplets of the perinuclear cisterna (arrow). Accumulation of units appearing like ribosomes, a low dense centre occurs in each unit (thin arrow). Nucleus (Nu). Approx. ×42,000. The marker is 0.5 μ.

Fig. 6. *C. rapunculoides*. — A: Stage 3. Detail of Fig. 5 A. Segments of the endoplasmic reticulum seem to accumulate (between arrows) between the nuclei illustrating the first evidence of the future cytokinesis. Mitochondria appear to divide (short arrow), Golgi body (G), vacuole with inclusion (V), dense droplets on the nuclear envelope (bent arrow). Approx. ×11,000. The marker is 1 μ. — B, C: Stage 5. B: Early tetrad cell. Callose (C), a thin layer of primexine template (bent arrow), nucleus (nu). Granular mass of material outside the callose (arrow). Approx. ×5,000. The marker is 1 μ. — C: Granular accumulations (arrow) connected by fibres (bent arrow). Approx. ×54,000. The marker is 0.2 μ.

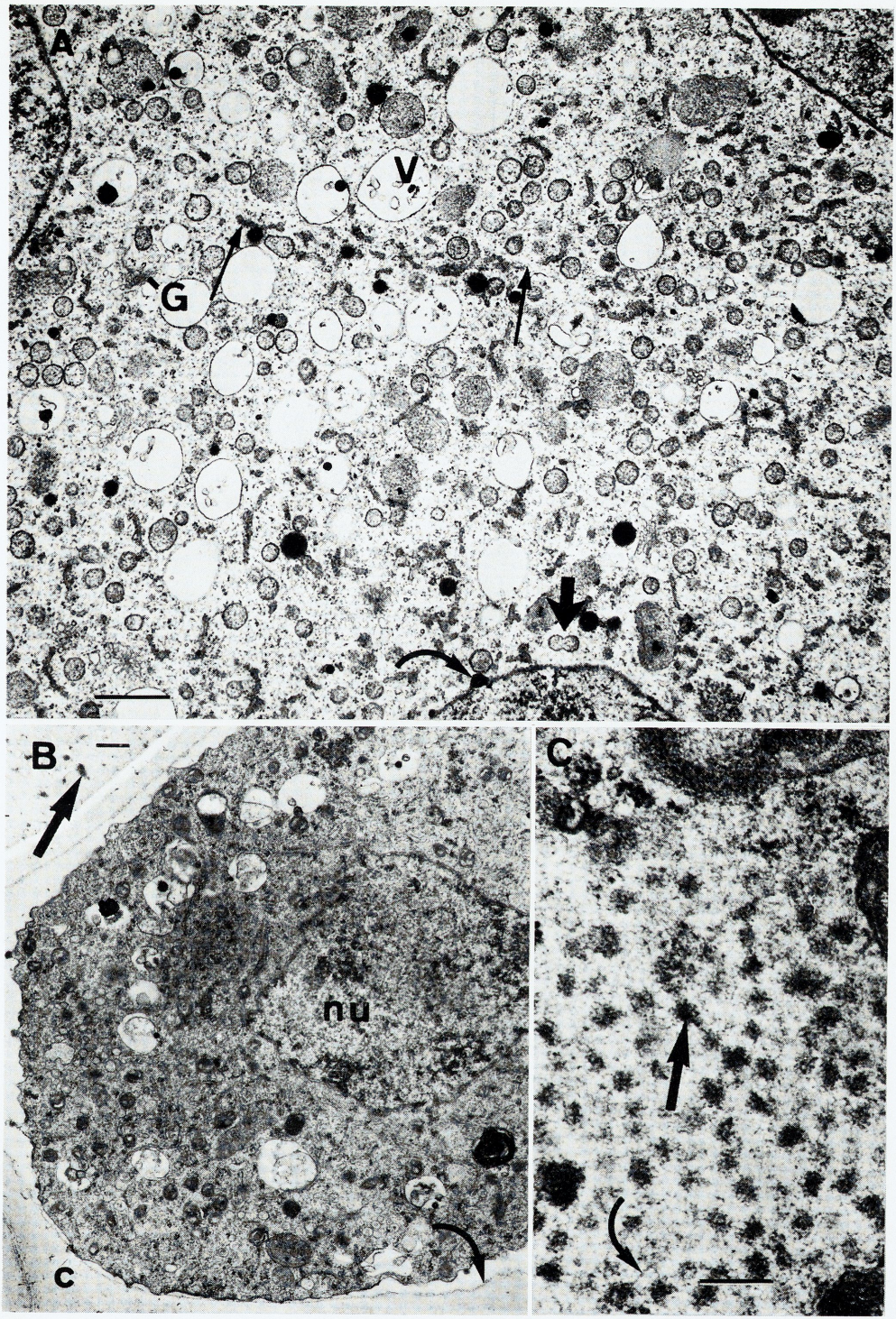


Fig. 6. Legend see page 286.

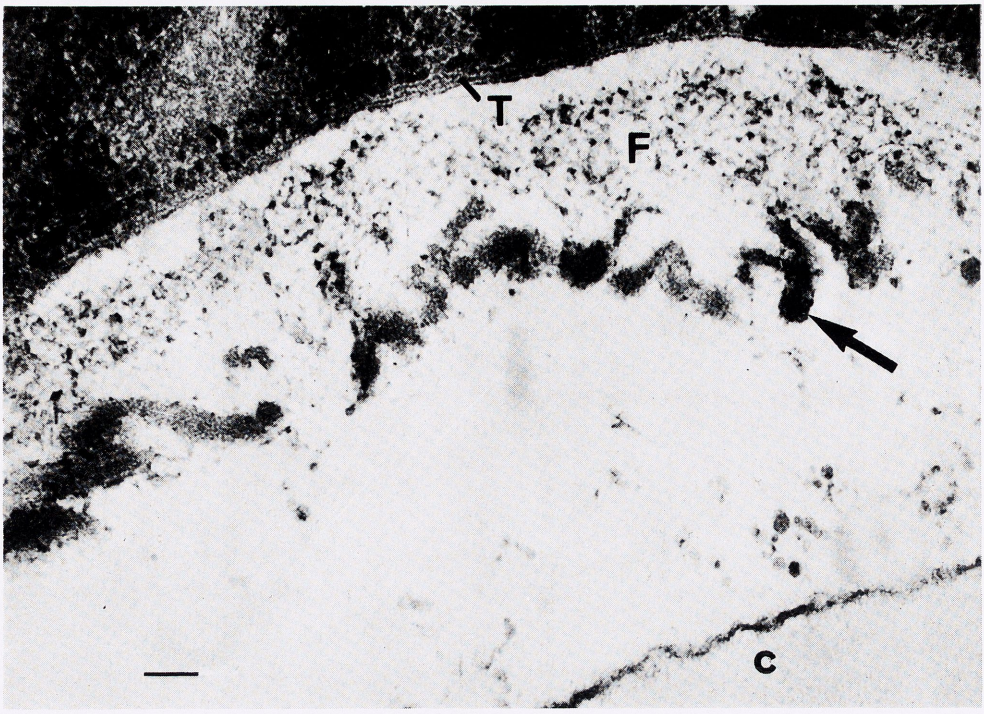


Fig. 7. *J. montana* stage 4. Multi-shaped structures (arrow) associated with granular-fibrous material (F) outside a tapetal cell (T). The structures may represent modified Ubisch bodies. Callose (C) of a tetrad. Approx. ×70,000. The marker is 0.1 μ. EMG A. v. HOFSTEN. Jeol 100 B.

Stage 8. Initiation of the Apertures

The primexine template becomes curved locally into the tetrad cell, and a fibrous material is evident in the space thereby formed (Fig. 8 C). These zones will give rise to the future pores of the pollen wall. A close contact between Golgi body with vesicles and the rod-accumulation is evident in the peripheral cytoplasm (Fig. 8 E).

Stage 9. Formation of Probacula

The primexine template has altered into a loosely composed layer, which in cross-sections appears like a net-work. Into this net-work probacula are laid down (Fig. 10 B). Polysomes are obvious in the cytoplasm close beneath the plasma membrane. There seems to be a correlation between their position and the probacula (Fig. 10 B). Further segments of the rough endoplasmic reticulum are found in the peripheral cytoplasm, although in no particular zone (Fig. 10 B). Microtubule-complexes occur in contact with the nucleus envelope (Fig. 9 C). Multi-vacuolar bodies are present in the peripheral cytoplasm (Figs. 9 B, 10 A) and are further observed at the cell surface adjacent to the inside of the plasma membrane. Ribosomes are arranged in a spheroid construction with an electron transparent matrix. Unidentified structures are included in this matrix (Fig. 9 B).

Stage 10. Formation of Protectum, Endexine and Operculum

As in stage 3 a substance is located outside and in contact with the former pollen mother cell wall and fibrous material is included in the outer part of the callose layer (Fig. 11 B). A protectum has formed adjacent to the top of the probacula (Figs. 11 B, D, 12 B). This layer consists of globular units slightly denser than the probacula. The units are connected laterally by less dense bridges. The protectum is distally adjacent to the callose layer (Figs. 11 B, 12 B). In the proaperture the fibrous material includes units which are surrounded by a border. The fine structure of the included material is less coarsely fibrous than the general filling of the aperture (Fig. 11 C, E). In material treated with GA and osmium tetroxide but lacking section staining, the filling of the proaperture is electron transparent, while its inclusions show about the same density as the young wall (Fig. 11 A).

The first indication of the footlayer and endexine is represented by somewhat undulating lamellae, in contact proximally with the plasma membrane and distally with the base of the probacula. The layer consists of several lamellae on top of one another. Each lamella is made up of an electron transparent layer sandwiched between dense leaflets, the whole lamella being slightly thicker than the plasma membrane (Fig. 12 B). Occasionally the lamellae branch (Fig. 13 A). These lamellae are also obvious at the apertural margins (Fig. 11 E). Segments of the rough endoplasmic reticulum, ribosomes and polysomes are numerous in the cytoplasm beneath the plasma membrane.

Stage 11. The Callose Is Dissolved

There are rods and differently shaped bodies outside the tapetal cells (Fig. 12 A). They seem to consist of an outer layer and a matrix; the latter has sometimes disappeared (Fig. 12 A). The outer layer stains more heavily than does the matrix. No Ubisch bodies are found outside the tapetal cells.

The microspores are released from the tetrad by the dissolution of the callose. At non-apertural parts of the wall the footlayer and endexine has become thicker by accretion of additional lamellae. It is possible to distinguish the footlayer from the endexine since the former now consists of a continuous layer while the endexine is lamellated (Fig. 14 A—C). Between the endexine and the plasma membrane lamellae are found which evidently approach the endexine to contribute to this layer (Fig. 14 C). Configurations, which may be termed "plasmalemmasomes" are evident immediately outside the plasma membrane of the microspore (Fig. 14 B).

In the ektexine some of the probacula branch (Fig. 14 A, C). The first indication of spinules is observed between the general structures of the protectum (Fig. 14 A, C). As in the earlier stages plastids displaying secretory activity are present; their product has become more prominent (Fig. 14 A).

Stage 12. Further Development of the Young Wall

Slightly later in the development the filling of the aperture decreases in stainability. Some of its inclusions lie distally in the aperture. These inclusions have achieved a density and fine structure similar to that of the ektexine (Fig. 13 B). At the apertural margin the endexine has become broad and its outermost part towards the aperture consists of lamellae with a low dense core which continues as white lines in the non-lamellated endexine (Fig. 13 B). At non-apertural parts of the wall the endexine is filled by closely packed low dense lines (Fig. 14 D). Only in some zones of the wall lamellae are found between the endexine and the plasma membrane. The protectum has grown by accretion of sporopollenin,

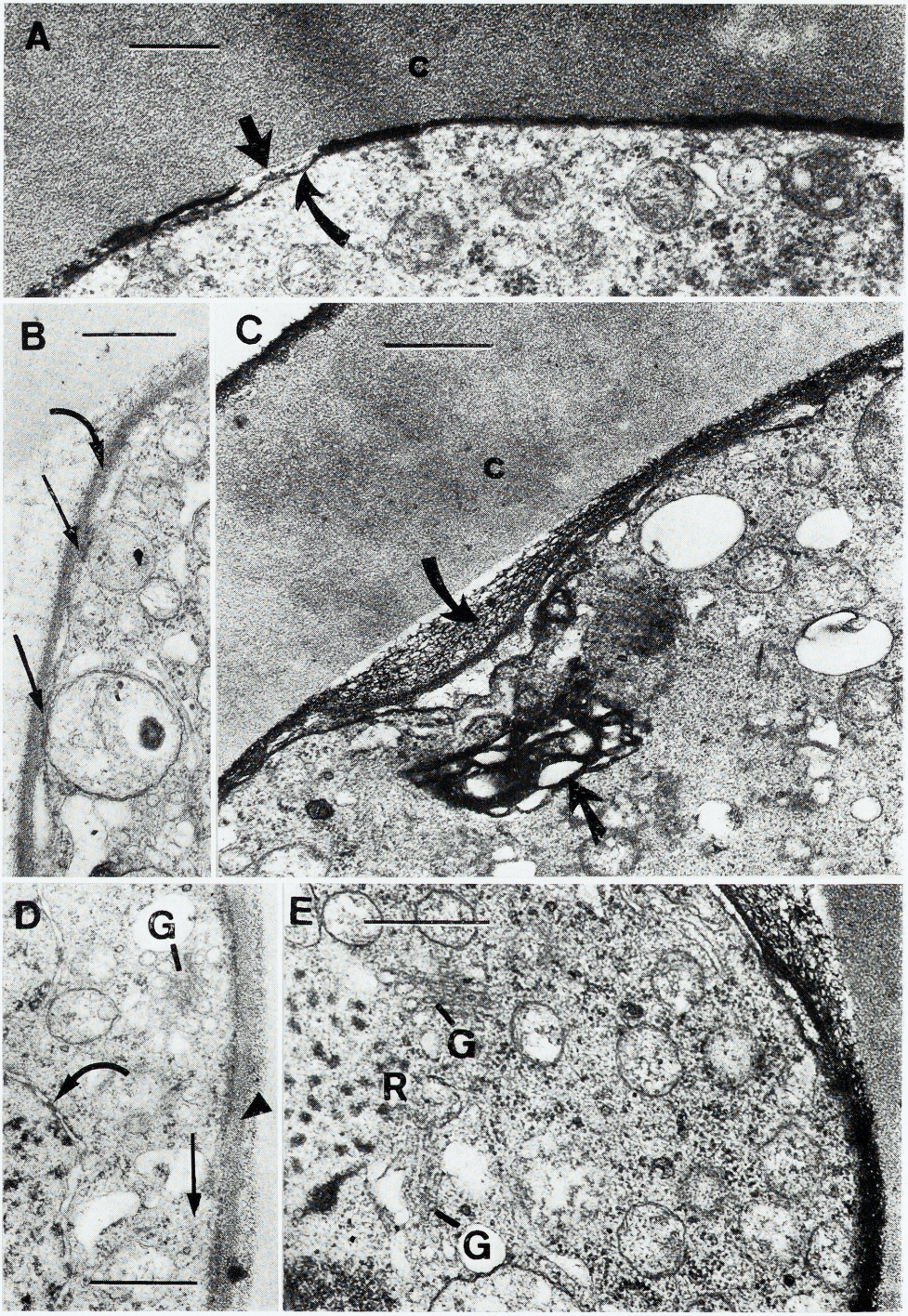


Fig. 8. Legend see page 292.

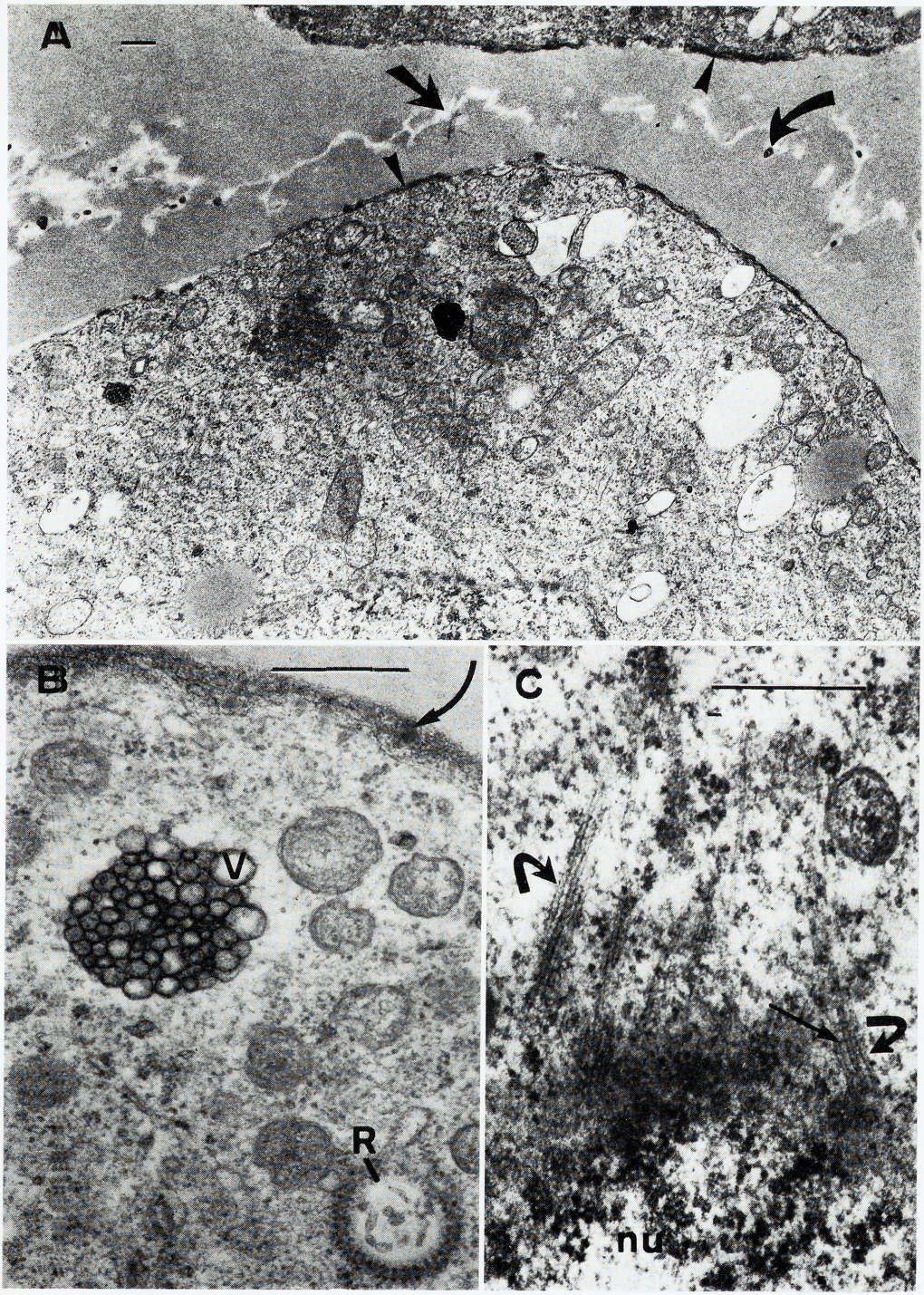


Fig. 9. Legend see page 292.

which apparently has been contributed to its outside, since the earlier observed globular units of the layer now constitute an inner part of the protectum (Fig. 14 D). Where spinules are forming these units show continuity with the probaculum located inside the spinule.

Stage 13. Prevacuolar Stage

As the microspore enters the later stages of ontogeny prior to mitosis it rapidly increases in size. Its wall grows by further accretion of sporopollenin. The protectum has in the *Campanula* pollen developed into a spongy layer (Fig. 15 B). Spinules are formed at some distance from each other. They are divided basically and their "roots" are connected to the adjacent ektexine (Figs. 15 B, 18 D, E, 21 A). The footlayer is thick and continuous. It is in direct continuity with the short bacula (Fig. 15 B). A surface membrane covers the ektexine partly (Fig. 15 B). The outer part of the endexine is continuous with the base of the footlayer. Beneath this part the endexine consists of lamellae with a low dense core. There is a great difference in the stainability of the ekt- and endexine, the latter being much more heavily stained (Fig. 15 B).

The filling of the aperture has increased and consists of granular units. In material treated with uranyl acetate before dehydration its granular fine structure is enhanced (Figs. 12 C, 15 C). The filling adheres to the margin of the aperture (Fig. 12 C).

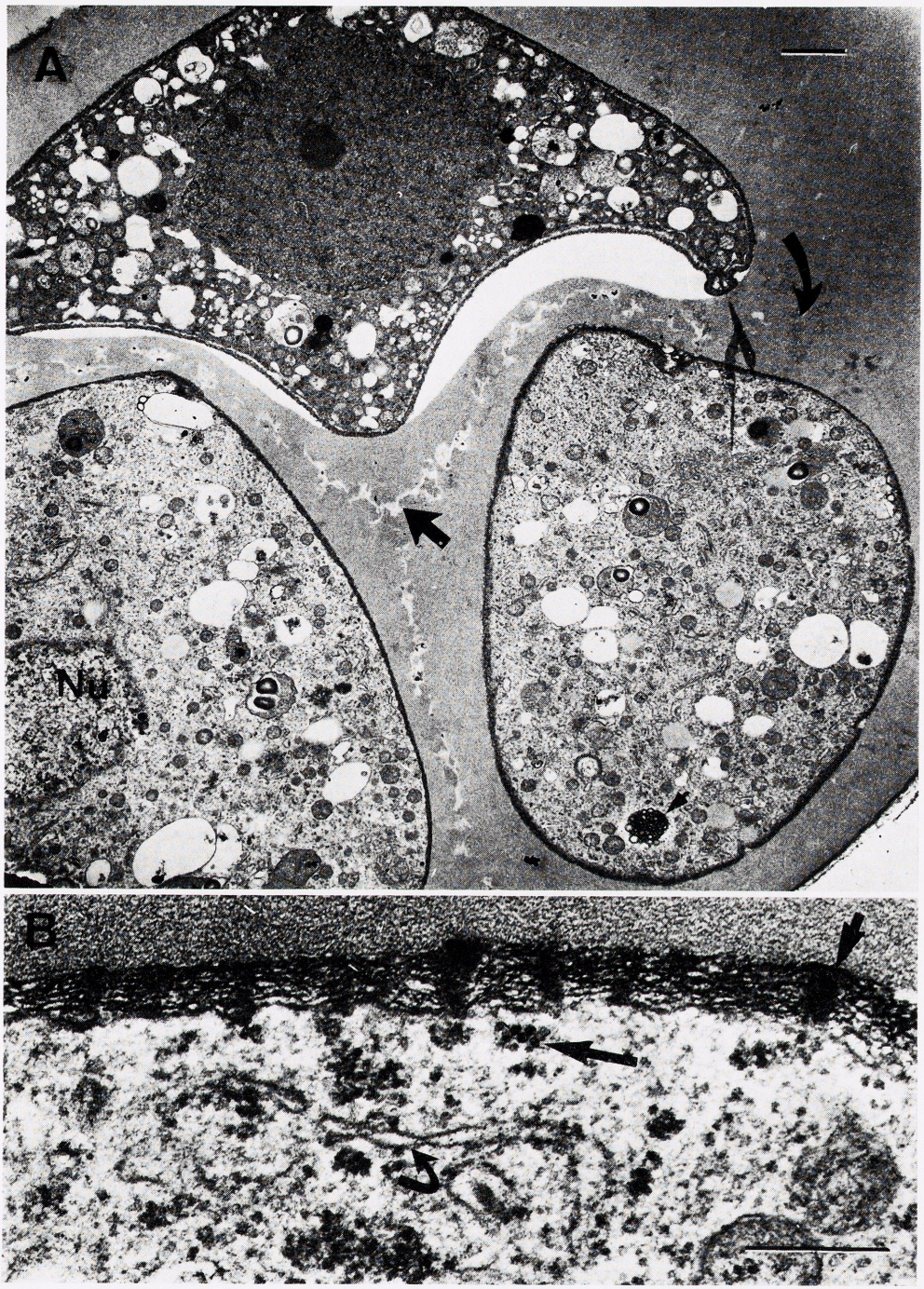
A conspicuous component of the cytoplasm is the dilated cisterna of the rough endoplasmic reticulum including tubules (Fig. 15 A). Cross-sections reveal that several tubules occur in one cisterna (Fig. 15 D) which is also evident in longitudinal sections (Fig. 15 E). The tubule is 330 Å thick in diameter, and shows a medium density, slightly enhanced in sections stained by uranyl acetate followed by PTA (Fig. 15 A).

The product of plastids observed in ear-

Fig. 8. *C. rapunculoides*. — A: Stage 6. Part of a young microspore of a tetrad. The primexine template is in some regions interrupted (arrow), plasma membrane (bent arrow). Callose (C) (see also Fig. 9 A). Approx. ×26,000. The marker is 0.5 μ. — B, D: Stage 7. B: Mitochondria (M) are located adjacent to the plasma membrane (arrows) primexine template (bent arrow). Approx. ×13,000. The marker is 1 μ. — C, E: Stage 8. C: The primexine template makes infoldings filled with a fibrous material (bent arrow). This will become the aperture. Callose (C), multi-vacuolar structure (arrow). Approx. ×30,000. The marker is 0.5 μ. — D: The primexine template (arrow-head). Microtubules (arrow) can be traced beneath the plasma membrane. Golgi body (G). The nucleus envelope shows prominent pores (bent arrow). Approx. ×15,000. The marker is 1 μ. — E: A cross-section of the granular rods (R). Golgi bodies (G). At the upper right corner is a part of a proaperture. Approx. ×35,000. The marker is 0.5 μ.

Fig. 9. *C. rapunculoides*. — A: Stage 6. Two tetrad cells with a partition between their callose layers (arrow). Dense inclusions in the separating space (bent arrow). The early primexine template has formed locally (arrow-heads). Approx. ×5,000. The marker is 1 μ. — B, C: Stage 9. B: Detail of Fig. 10 A. Cross-section of probacula (bent arrow) in the primexine template, accumulation of vacuoles of different size and content (V), ribosome configuration with inclusion (R). Approx. ×40,000. The marker is 0.5 μ. — C: Several microtubule-complexes (bent arrows) adjacent to a nucleus (Nu). Connection between the microtubules (arrow). Approx. ×45,000. The marker is 0.5 μ.

Fig. 10. *C. rapunculoides* stage 9. — A: Three of the microspores of a tetrad enveloped in callose. The callose of each microspore is limited (arrow); the border is incomplete towards the surface of the entire callose (bent arrow). The microspore at the top of the Figure has a more electron dense nucleus and cytoplasm than the others and may abort. Nucleus (Nu), group of vacuoles (arrow-head). Approx. ×9,500. The marker is 1 μ. — B: Probacula (short arrow) in the primexine template. Polysomes (arrow), the rough endoplasmic reticulum (bent arrow). Approx. ×40,000. The marker is 0.5 μ.



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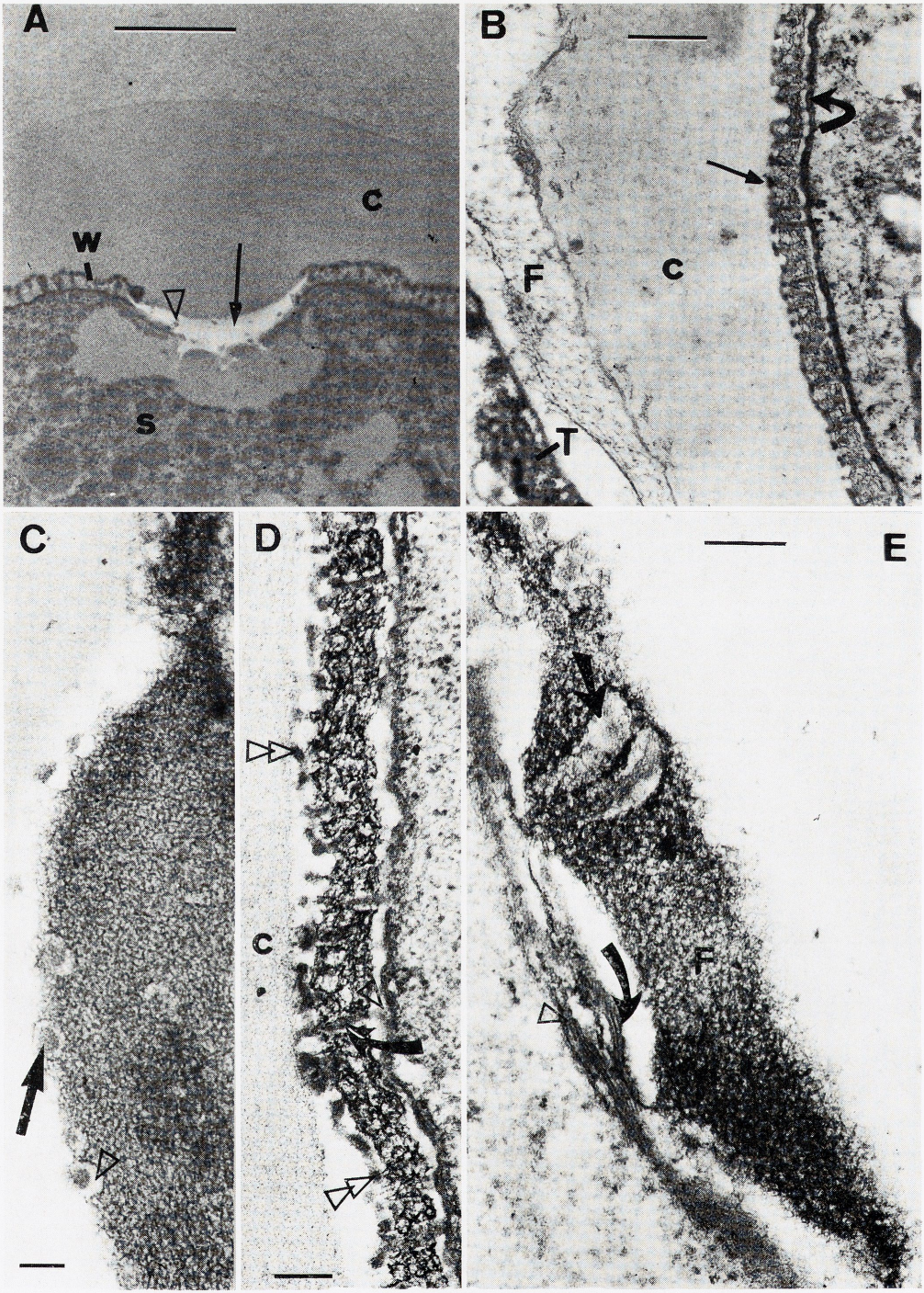

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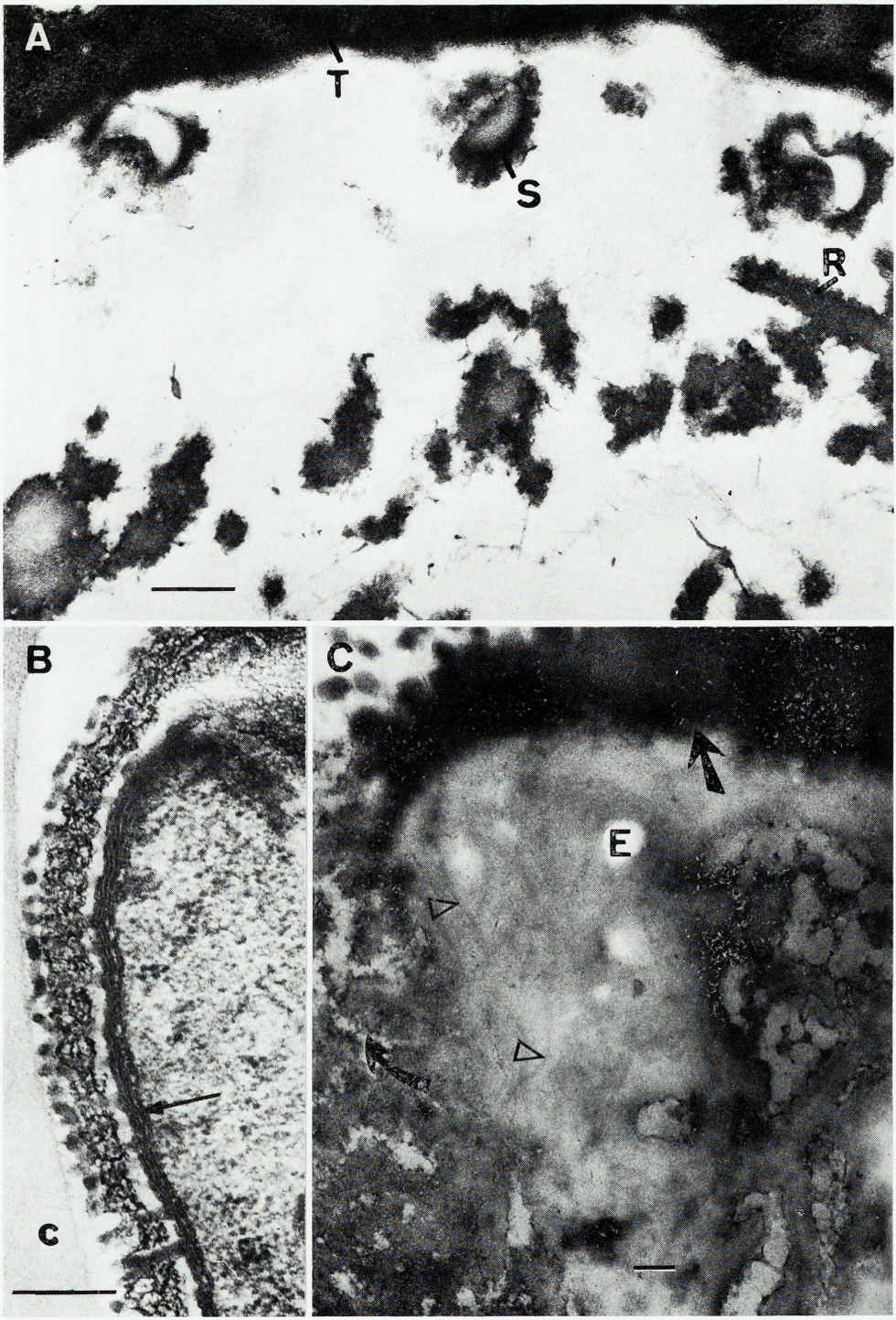


Fig. 12. Legend see page 296.

lier stages appears altered. A contraction of the material seems to have occurred.

Stage 14. Vacuolar Stage

A major component of the cell is a big vacuole. The plasma membrane is slightly withdrawn from the microspore wall (Fig. 16). In some species an intine has begun to form in the space created by the withdrawing plasma membrane (Fig. 17 A). A common tectum has developed in *J. montana* in clear distinction from the spongy layer of the *Campanula* pollen. The tectum is perforated. In the perforations and on the surface a fibrous material is evident. A surface membrane covers the spinules and general tectum. The endexine is lamellated at the apertural margin and non-lamellar elsewhere, although open spaces remain in the layer. A core of low density is obvious in the lamellae of the apertural margin (Fig. 17 B) and white lines are closely packed in the endexine of non-apertural zones. The endexine stains more heavily than does the ektexine. The break-down of the tapetal cells has begun and organelles of these cells surround the microspore mass (Fig. 17 B). Dense bodies occur along with the organelles of the tapetum. Their periphery is more dense than their centre (Fig. 17 B). They lack lamellae (cf. DUNBAR 1973 a).

Stage 15. Post Microspore Mitosis

A granular material is dispersed in the theca loculus (Fig. 18 B). The microspores have undergone mitosis. There is an electron transparent irregularly outlined space between the generative and the vegetative cells (Figs. 18 A, C, 19 A). This space is continuous with the extracellular space beneath the exine (Figs. 18 A, 19 A). The plasma membrane of the pollen is strongly convoluted and numerous vesicles are evident in the extracellular space beneath the exine (Fig. 18 B).

Fig. 11. *C. rapunculoides* stage 10. — A: GA fixed — osmium treated material, no section stain. The material of the young aperture appears osmiophobe (arrow) in contrast to its osmiophile inclusions (arrow-head). Callose (C), young wall (W), cytoplasm (S). Approx. ×17,000. The marker is 1 μ. — B: Non-apertural part of the microspore. Tapetum (T), fibrous material (F) outside the callose wall (C). Protectum (arrow), endexine (bent arrow) is forming. Approx. ×23,000. The marker is 0.5 μ. — C: Fibrous filling of a young aperture. Cross-section of inclusions (arrow). An electron transparent space (arrow-head) is evident around the inclusion. Approx. ×65,000. The marker is 0.1 μ. — D: Higher magnification of non-apertural part of the young wall similar to the one in Fig. 11 B. Callose (C), protectum (double-headed arrows), probacula (bent arrow) in contact with the young endexine (arrow head). Approx. ×42,000. The marker is 0.2 μ. — E: Fibrous material (F) of aperture. Longitudinal section of inclusions (arrow). Lamellae (bent arrow) beneath the aperture partly fused with the plasma membrane (arrow-head). Approx. ×64,000. The marker is 0.2 μ.

Fig. 12. *C. rapunculoides.* — A: Stage 12. Tapetum (T), rod-shaped (R) and multi-shaped bodies (S) outside the tapetum. Approx. ×56,000. The marker is 0.2 μ. — B: Stage 10. Early stage of endexine (arrow). At the upper right side is an apertural margin. Callose (C). Approx. ×30,000. The marker is 0.5 μ. — C: Stage 13. Material treated with uranyl acetate before dehydration. Apertural margin with the dark stained ektexine (arrow), low dense endexine (E) in which low dense lines can be traced in spite of the lack of contrast (arrow-heads). Note the granular units (bent arrow) filling the aperture and adhering to the apertural margin. Approx. ×60,000. The marker is 0.1 μ.

Fig. 13. *C. rapunculoides* stage 10. — A: Lamellae at the margin of an aperture. Some of the lamellae appear to branch off (arrow) from the ones underlying the non-apertural part of the wall. Inclusion of the aperture filling (bent arrow). Approx. ×80,000. The marker is 0.1 μ. — B: Stage 12. The filling of the aperture in Fig. 13 A is almost dissolved, while the inclusion at the top of the aperture is non-dissolved (arrow-head). At the apertural margin lamellae are shown with an electron transparent core (arrow) which continues in the layer of endexine as white lines (double-headed arrow). Approx. ×55,000. The marker is 0.1 μ.

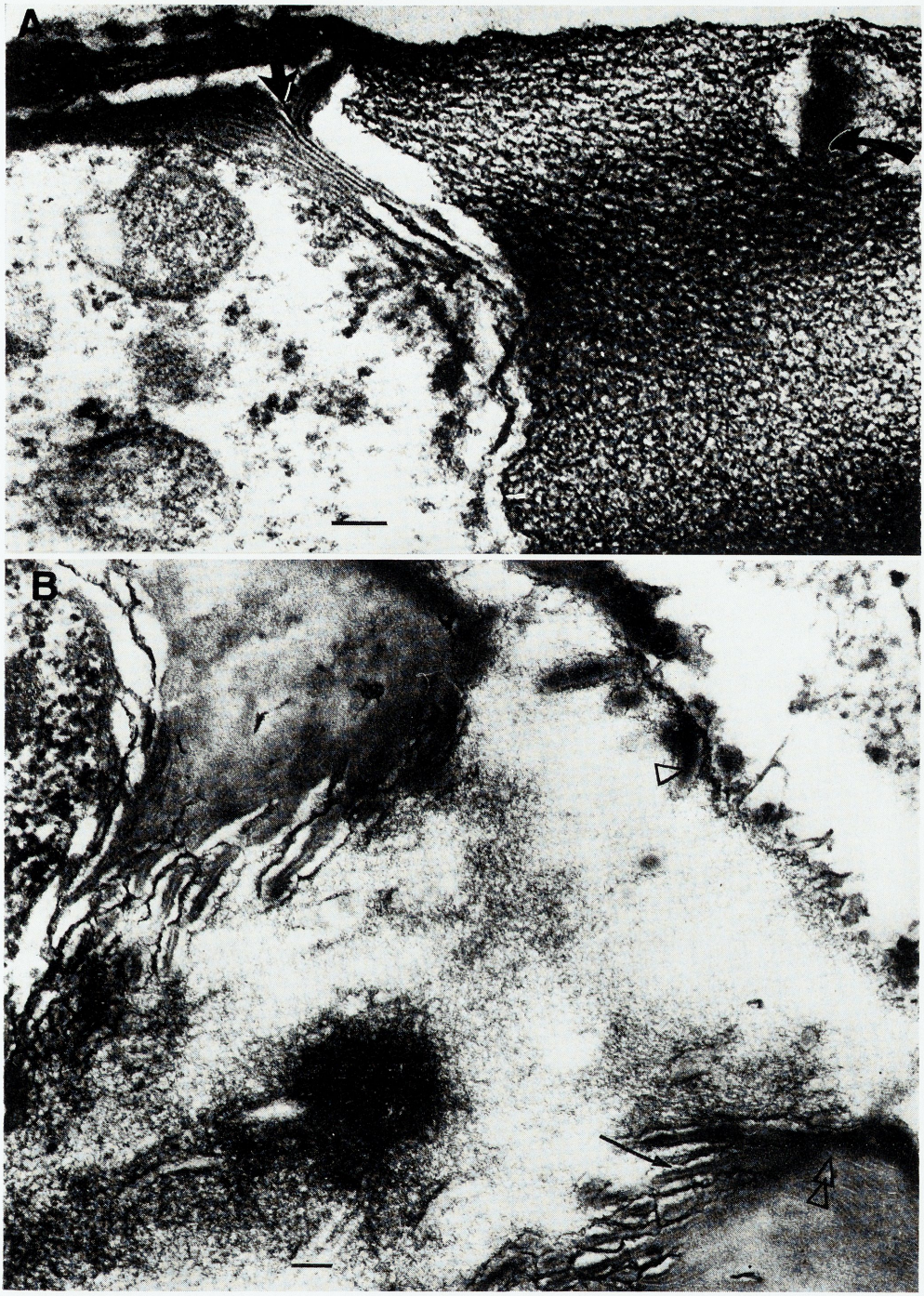


Fig. 13. Legend see page 296.

In *C. rotundifolia* a thick footlayer has formed beneath the spongy units of the tectum (Fig. 19 A, C). Spinules are connected to the adjacent ektexine by their "roots" (Fig. 19 C). The endexine is lamellated and becomes broad at the apertural margin (Fig. 19 A, D). White lines can be traced in the non-apertural endexine and in the lamellae at the apertural margin (Fig. 19 D).

Stage 16. The Intine Has Formed — Wall Thickenings in the Endothecial Cells

Near anthesis wall thickenings are formed in the endothecial cells (Fig. 20 A). An intine has formed, which becomes rather broad beneath the pores where it is transversed by trabeculae. The trabeculae appear tubular and are limited by a border (Figs. 19 D, 21 B). The generative cell appears to lie free in the vegetative cytoplasm (Figs. 19 D, 20 B, 21 A). Several layers of the rough endoplasmic reticulum surround the generative cell (Fig. 21 A). Many starch grains have developed along with "storage bodies". In the cytoplasm of the generative cell the nucleus occupies most of the space. Mitochondria, Golgi bodies and the rough endoplasmic reticulum are frequently present in the cell.

The fibrous material observed at stage 14 on the surface and in the perforations of the tectum has disappeared. A surface membrane covers the ektexine outwardly. Tangential sections of the pollen grain reveal the continuity of the membrane between the units of the ektexine. Treatment with uranyl acetate before dehydration emphasizes this feature (Fig. 19 B).

DISCUSSION

Connection Between Pollen Mother Cells

Bridges of cytoplasm connect adjacent pollen mother cells during stage 1. Whether they represent former plasmodesmata which have become altered into broad zones during ontogeny or whether they have formed *de novo* is not clear. The possibility that they have existed previously along with plasmodesmata may not be excluded although in unpublished material of this period (DUNBAR) no cytoplasmic bridges are observed. Regardless of their origin they provide a possibility for the pollen mother cells to exchange cytoplasmic organelles. Cytoplasmic connections between pollen mother cells were observed by HESLOP-HARRISON (1964, 1966, 1968) during meiotic prophase; they were considered to be eliminated by the rapid growth of the callose. WEILING (1965) studied plasmatic connections between pollen mother cells of *Lycopersicum esculentum* and *Cucurbita maxima;* in the former the plasmatic connections were present before the plasmodesmata disappeared; mitochondria and other organelles were observed in the connections. In *Helleborus foetidus* similar connections were described by ECHLIN and GODWIN (1968); the connections persisted during the formation of callose but were sealed off by the deposition of thick cal-

Fig. 14. *C. rapunculoides.* — A—C: Stage 11. A: A microspore released from the callose envelope. Plastids displaying secretory activity (arrow-heads), nucleus (nu), filling of aperture (thin arrow), prospinule (double-headed arrow), lamellae beneath the endexine (bent arrow). Approx. ×10,000. The marker is 1 μ. — B: Detail of Fig. 14 A. Plasmalemmasomes are evident adjacent to the outside of the plasma membrane (arrow). Approx. ×25,000. The marker is 0.5 μ. — C: Part of non-apertural wall with lamellae (arrow) beneath the endexine. Note unit of ektexine portending a spinule (arrow-head); branching probacula (double-headed arrow). Approx. ×26,000. The marker is 0.5 μ. — D: Stage 12. The primexine template appears as a reticulum. Note the circular units of the protectum (arrow). White lines are obvious in the inner endexine while the outer part differentiates into a footlayer. Approx. ×70,000. The marker is 0.1 μ.

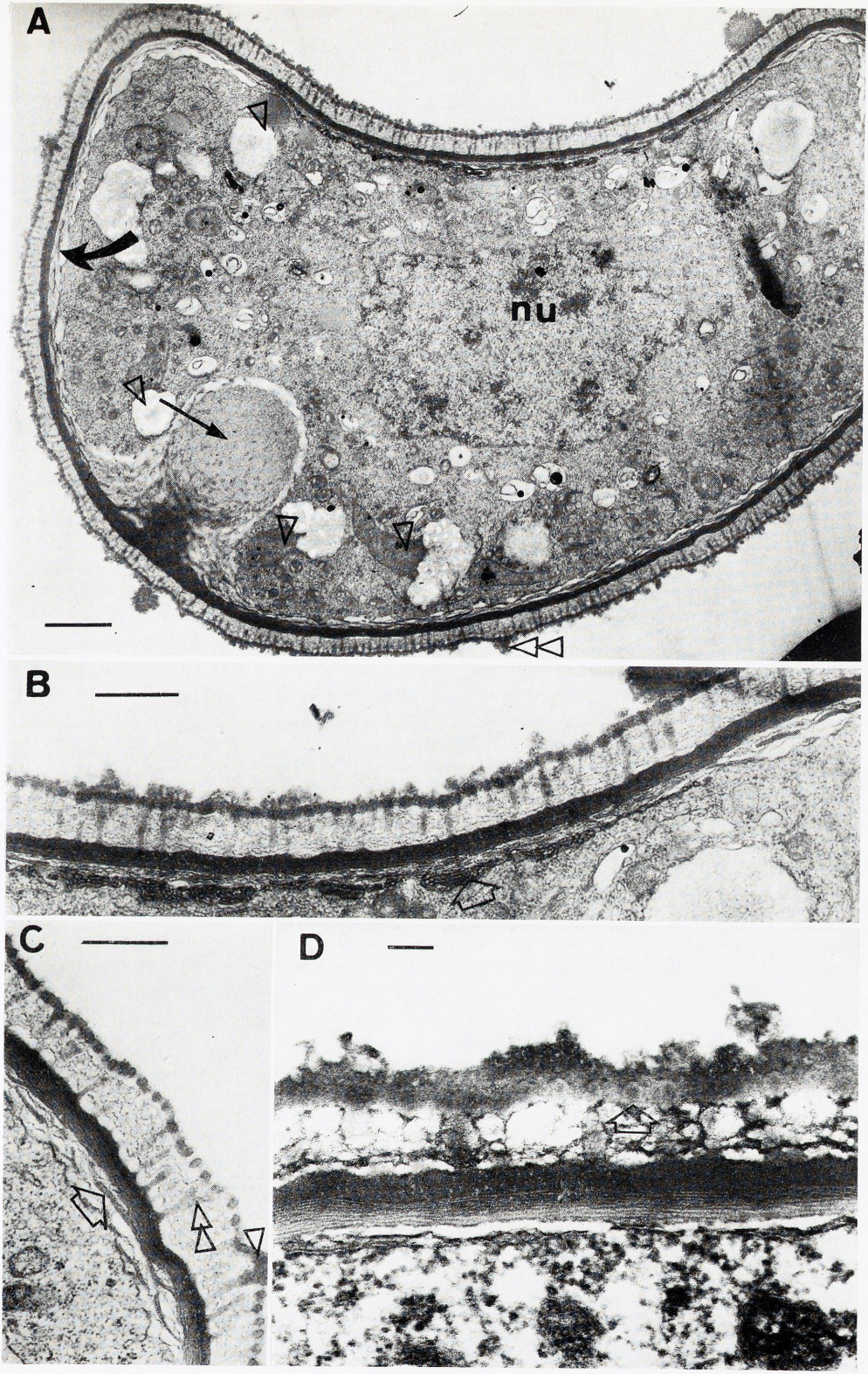


Fig. 14.

lose walls at the first meiotic division. Cytoplasmic bridges extend through the wall and callose layer at scattered points in pollen mother cells of *Nicotiana tabacum* (LEDBETTER & PORTER 1970) and they were interpreted as reminiscent of connections found between germ cells in many forms of animal and plant cells. In the few gymnosperms investigated so far, no plasma channels are formed, and the cells within a microsporangium are asynchronous in development (VASIL & ALDRICH 1970).

Callose

The first indication of callose synthesis is observed at prophase of meiosis and occurs in contact with the pollen mother cell wall from which the protoplast is withdrawn. The callose synthesis extends to fill the entire space between the pollen mother cell and its former wall. During this period Golgi bodies are scarce and insignificant, while the rough endoplasmic reticulum is a major component of the cytoplasm. It seems likely that the endoplasmic reticulum is involved in the synthesis of callose. Similar observations are reported by ANGOLD (1967) in *Endymion non-scriptus* where a "beaded" endoplasmic reticulum is evident at metaphase of the first meiotic division, and by DUNBAR (1973 b) in *Eleocharis* where blebs pinched off from the perinuclear cisterna contribute to the vesicular endoplasmic reticulum of the cytoplasm at prophase of meiosis. On the other hand ECHLIN and GODWIN (1968) considered Golgi bodies to be closely associated with the formation of callose in *Helleborus foetidus* at prophase of meiosis.

In the present study at stage 4, the tetrad cells have synthesized a callose layer of their own which is delimited by an electron transparent space. However the demarcation does not reach the former pollen mother cell wall; a broad part of the outer callose layer is left intact. This feature would indicate that the cytokinesis, portended at stage 3, results in a complete division of the pollen mother cell into four microspores only after the dissolution of the callose at stage 11.

Experimental work has shown the callose layer to be a barrier, at least to large molecules (HESLOP-HARRISON 1966). SOUTHWORTH (1969) showed in experimental work with tracers that acetate and glucose can pass into callose-encased microspore walls. Further it was demonstrated by ROWLEY and DUNBAR (1970) that small molecules, such as iron, easily penetrate the callose layer. In the present study a mass of material is evident outside the tetrad from stage 3 and until stage 11, when the callose dissolves. During this period first the primexine template and thereupon the exine are initiated and develop (the latter only to a certain degree). The material outside the tetrad may well constitute a source for substrate required, since it seems rather unlikely that the tetrad cells are able to produce all the precursors needed for the synthesis of the different extracellular layers

Fig. 15. A, B, D, E. *C. rapunculoides* stage 13. — A: Sections stained with uranyl acetate followed by PTA. Intracisternal microtubules (bent arrow). Approx. ×35,000. The marker is 0.5 μ. — B: Non-apertural part of the exine. Spinule divided basically (arrow-head). Bacula (E), sculptured units of the ektexine (S) covered by a surface membrane (arrow), footlayer (F), white lines in the part of the endexine which is continuous with the ektexine (bent arrow), lamellated endexine with white lines (arrow-head). Approx. ×35,000. The marker is 0.5 μ. — C: *C. rotundifolia* stage 13. Material treated with uranyl acetate before dehydration. Units of granular fine structure fill the aperture (Gr), endexine (H). The white lines can be traced in the endexine in spite of the lack of contrast (arrow-head); see also Fig. 12 C. Approx. ×60,000. The marker is 0.1 μ. — D, E: Different parts of same EMG. D: A pair of intracisternal tubules (arrow) in cross-section. — E: Longitudinal section of tubules included in cisternae of the rough endoplasmic reticulum (arrow-head). Approx. ×50,000. The marker is 0.1 μ.

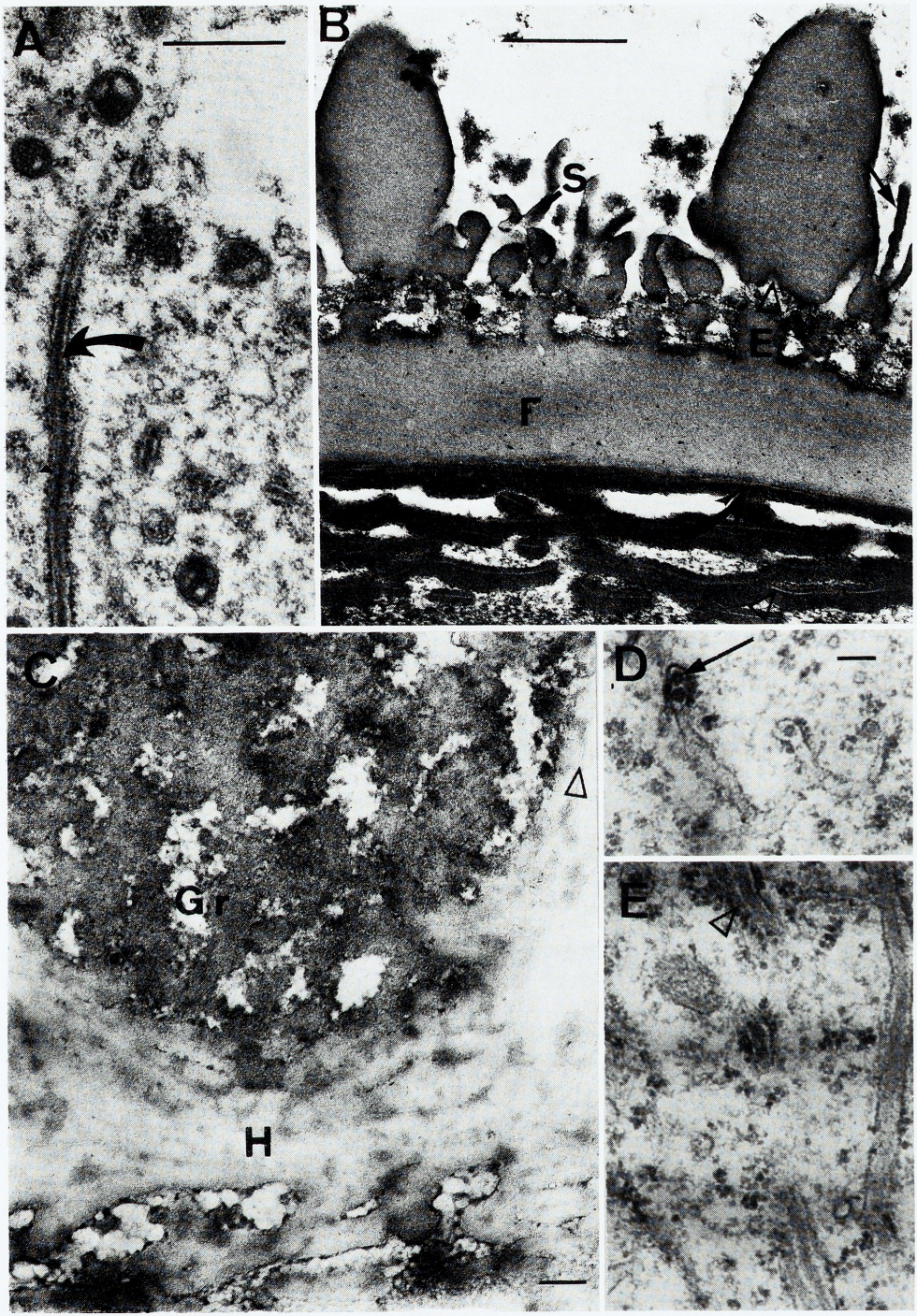


Fig. 15.

without the existing of a route for substrate from outside the tetrad.

Intracisternal Tubules

Dilated cisternae of the rough endoplasmic reticulum which include tubules are obvious during the ontogeny of *Campanula* and *Jasione* pollen. Their size, 330 Å in diameter, and their intracisternal location distinguish them from the 270 Å thick microtubules of widespread occurrence in the cytoplasm. Further the interior of the intracisternal tubules shows a higher electron density than that of the cytoplasmic microtubules. The tubules are well stained by PTA which may indicate the presence of both proteins and polysaccharides (DUNBAR 1973 b). Similar structures are occasionally found in cells of higher plants undergoing secondary wall formation (HEPLER & NEWCOMB 1964). Of importance is the fact that the tubules are not affected by low temperature or by treatment with colchicine nor by pepsin digestion; hence the tubules may not be closely related chemically to either cytoplasmic or ciliary microtubules (STEER & NEWCOMB 1969). On the other hand colchicine-resistant tubules are reported in *Saprolegnia* (HEATH & GREENWOOD 1970). Intracisternal microtubules with a diameter of 170 Å are reported in *Vacuolaria virescens* (HEYWOOD 1969) to occur during the entire development including those cells undergoing mitosis and cytokinesis.

The tubules are probably a general phenomenon since they are reported to occur in lower as well as in higher plants. Hitherto they have not been reported in pollen ontogenesis, which may be due to the fact that they are sensitive to fixation. On the other hand the presence of this highly specialized system in the *Campanula* and *Jasione* pollen may reflect the requirement of a specific and as yet unknown process in the pollen.

Accumulation of Rod-shaped Units

The rods which consist of a granular material and have fibrous connections are strikingly similar to nematosomes in neurons of rats (GRILLO 1970) and to the extranucleolar body reported in the oocyte of the prepubertal mouse (CHOUINARD 1973), where their occurrence could be related to the stages of differentiation and development of the oocyte. It was postulated that they represent accumulation products of specific gene activities. In the present study they are observed during stages 1—8. At stage 8 there seems to be an interaction between the rods and Golgi bodies. This would suggest that they represent storage material utilized by the pollen with the aid of the Golgi bodies.

Concentric Configuration of Endoplasmic Reticulum

At stage 1 a concentric parallel configuration of endoplasmic reticulum is evident in most of the pollen mother cells. A similar configuration occurred in the tetrad cells of *Eleocharis* (DUNBAR 1973 b) and in the young microspore of *Betula verrucosa* immediately after release from the tetrad (DUNBAR, unpublished). A similar configuration is furthermore observed in pollen tubes of *Lycopersicum peruvianum* (DE NETTANCOURT et al. 1973). While its function is not clear, the fact that this phenomenon is observed in genera so widely unrelated, suggests that it reflects an activity common among angiosperms.

Fig. 16. *C. rapunculoides* stage 14. — A: An aperture is shown on the left. Spinule (W), tectum (E), footlayer (F), endexine (N), nucleus (nu), a big vacuole (V). Approx. ×17,000. The marker is 1 μ. — B: Aperture. Note difference in stainability between ekt- and endexine. Low dense cores of the lamellae continue in the endexine as white lines. The filling of the aperture consists of multi-shaped units. Approx. ×30,000. The marker is 0.5 μ.

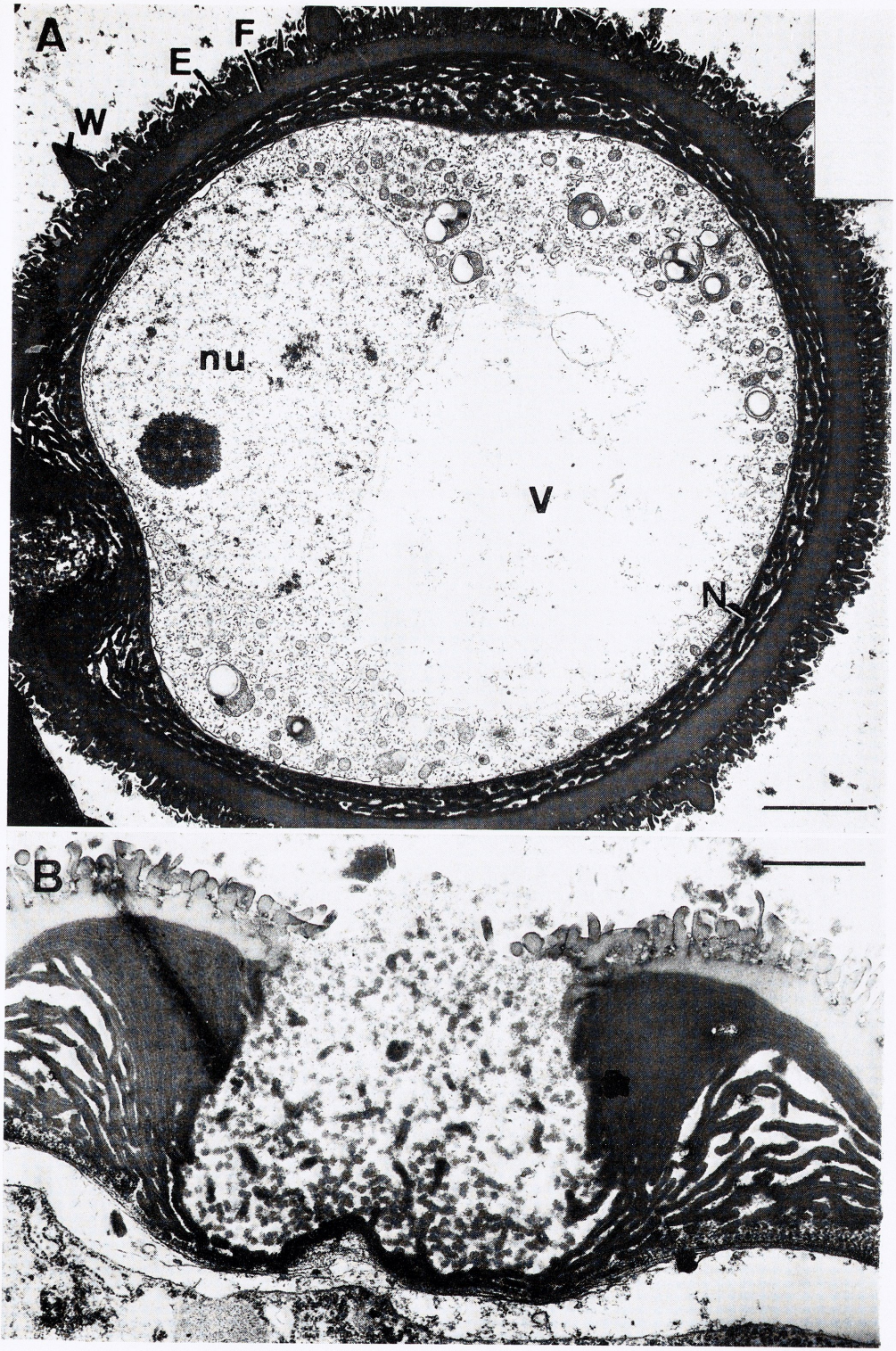


Fig. 16.

Chromosomes

At stage 2 at prophase of meiosis, homologous chromosomes are evidently paired into bivalents and synaptinemal complexes are formed between them. MOENS (1968) demonstrated that the two axial cores of a set of homologous chromosomes participated in the formation of a synaptinemal complex in the sporocytes of *Lilium longiflorum*.

Plastids

While starch grains are a general feature in the cytoplasm of mature pollen grains very little is known about their ontogeny. In the present study, however, plastids displaying secretory activity are observed during stages 3—13. The product alters gradually, finally to become a material of a certain contraction and a stainability not too unlike that of starch grains. Hence it is suggested that this activity may reflect a synthesis of starch, since at maturity the vegetative cytoplasm contains a large number of starch grains.

Secretory Activity

The dense droplets observed associated with the plasma membrane and nucleus envelope at stage 3, and with only the plasma membrane at stages 4 and 5, are morphologically similar to the ones reported in *Eleocharis* during successive stages of development, the first of which was related in time to the initiation of the exine; the process was interpreted as a possible synthesis of some of the monomers required for exine formation (DUNBAR 1973 b). DICKINSON (1970) observed spherical inclusions in the protoplast of the young grain of *Lilium longiflorum* which by reason of their staining characteristics were believed to be droplets of saturated lipid and it was expected that they were a source of osmiophilic material of the wall although the frequency of the inclusions remained unchanged until after the release of the microspores from the tetrad. If they were related to the metabolism of the wall at all, it would appear to be in connection with the later stages of the exine, or of the intine. In *Campanula* the droplets are obvious during stages 3 and 4, while the first exine is observed at stage 9. In *Epilobium* lipid droplets are numerous in a similar position, associated with the plasma membrane, although at a later stage of ontogeny, when a pollen wall with a thick footlayer has developed; they are interpreted as lipids transported through the wall from the outside slightly earlier in time (ROWLEY, personal communication).

Mitochondria

VAZART (1970) demonstrated that the mitochondria are evenly dispersed beneath the plasma membrane and closely arranged during probacula formation in *Linum*. Mitochondria are found in a similar position in *Campanula* shortly after the formation of the primexine template. It is not known whether this feature could at all be related to wall formation in any other sense than that of a response to the energy requirement of wall metabolism.

Polysomes

At the period of probacula formation polysomes are evident close beneath the plasma membrane probably situated in

Fig. 17. *J. montana* stage 14. — A: An aperture is shown in the left upper corner. Tapetum residue with dense bodies (arrow-head). Perforated tectum (T) laid upon bacula, footlayer (F), dense stained endexine beneath the footlayer. The intine is broad beneath the pore (I), nucleus (Nu), a big vacuole (V), rough endoplasmic reticulum (thin arrow), Golgi bodies (G), mitochondria (M). Approx. ×17,000. The marker is 1 μ. — B: Detail of Fig. A. Perforation of the tectum (arrow). Approx. ×38,000. The marker is 0.2 μ.

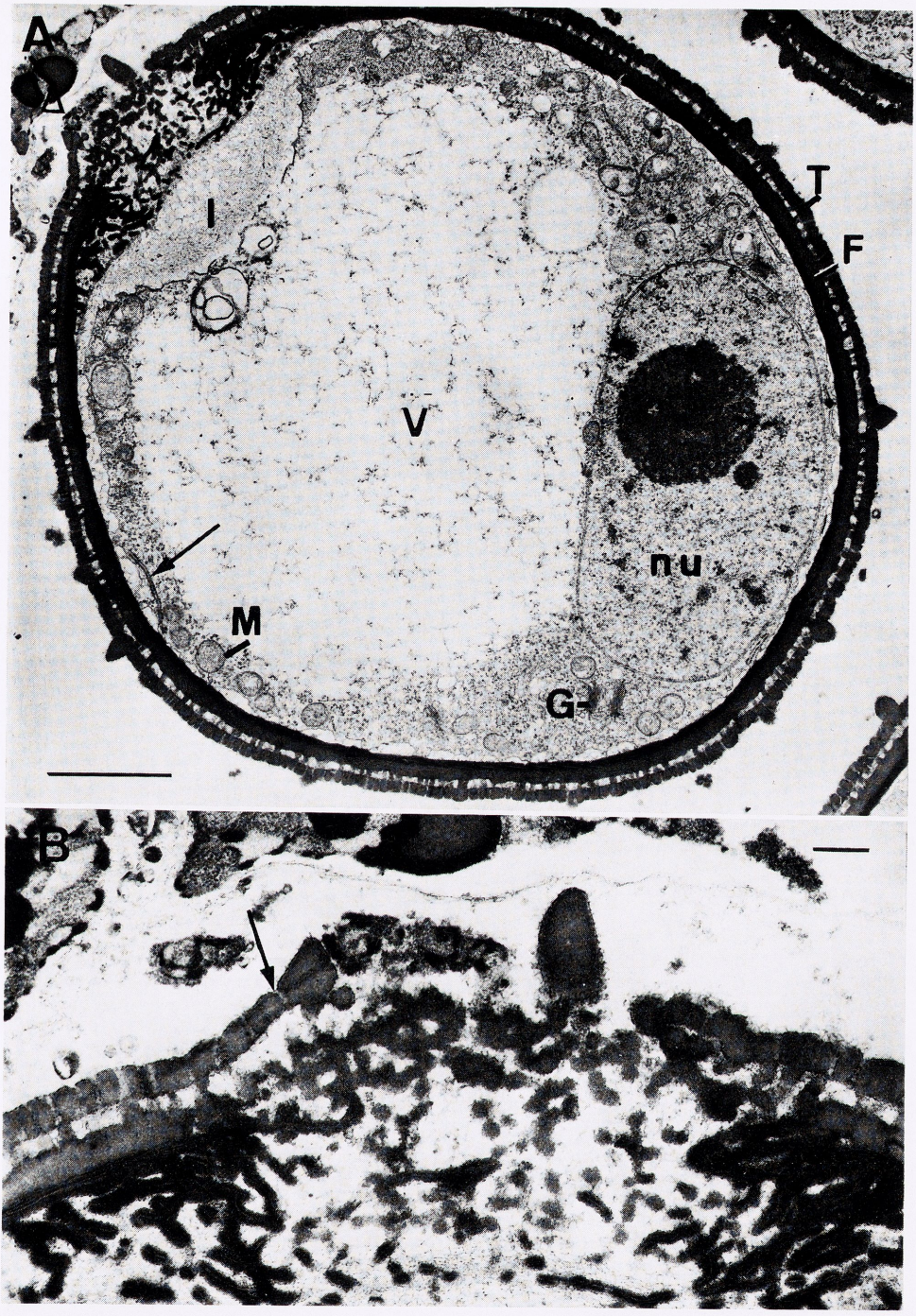


Fig. 17.

correlation to these units of the early wall. However their position cannot be interpreted as exactly beneath each probacula. HESLOP-HARRISON (1968) observed ribosomes accumulated into polysomes beneath probacula in *Lilium longiflorum*. It is suggested that in the present material they are involved in the synthesis of specific proteins required for the formation of the young pollen wall.

Helical polysomes have been found in a number of animal and plant cells, always associated with some change in cell development. In correlation with the fusion of the egg and sperm nuclei in cotton embryogenesis ribosomes became arranged into long helical polysomes, surrounding the plastids and mitochondria, and it was suggested that the stimulus for the formation of the helical polysomes was synthesized or released by the fusing nuclei (JENSEN 1968). An inactivation and storage of the ribosomes has also been suggested (WOODING 1968) as a response to environmental influence forming compact helices of the considerable ribosome population in companion cells in mature secondary phloem of *Acer pseudoplatanus*. In the present study helical polysomes are found during a metabolically active stage of development when the first exine is deposited into the primexine template. The polysome configuration surrounds unidentified units which appear to have been morphologically altered. It is not clear if this phenomenon reflects an inactivation of cytoplasmic components or leads to a transformation of the cytoplasm of the microspore in preparation for wall formation and requiring new messenger RNA from the nucleus residing in its cytoplasm.

Plasmalemmasomes

Zones of membrane configurations become evident at stage 11 associated to the plasma membrane at concave non-apertural parts of the microspore. Foldings of the plasma membrane have been called plasmalemmasomes (EDWARDS 1962, MARCHANT & ROBARDS 1968). They are in *Campanula* of great similarity to the plasmalemmasomes reported by MARCHANT and MOORE (1973) in fungi. They were demonstrated both with conventional fixation methods and with freeze-etching to have a variable morphology. Further they were stated not to be fixation artefacts, but to be a product of the plasma membrane. The plasmalemmasomes in the present study may constitute a form of membrane storage set up to provide for the rapid growth of the microspore and its expanding cytoplasm at the very time when the microspore is released from the tetrad. Other functions for plasmalemmasomes may, however, be considered. At a

Fig. 18. A—D: *C. rapunculoides* stage 15. A: Part of the generative cell wall (arrow) continuous (at the point of the star) with the extracellular space beneath the endexine (E). Approx. ×25,000. The marker is 0.5 μ. — B: Semi-tangential section of non-apertural part of the pollen wall. Spinule in this section slightly divided basically (arrow-head), sculptured part of the ektexine (S), footlayer (F), endexine (arrow). Note the gradual change in stainability of the sporopollenin of the ekt- and endexine. A granular-fibrous material (L) is evident in the theca loculus. Approx. ×13,000. The marker is 1 μ. — C: Part of the generative wall. Cross-section of what appears to be an extension of the cytoplasm (arrow-head). Approx. ×70,000. The marker is 0.1 μ. — D: Tangential section of the pollen wall. Spinules are sectioned at different levels and show the connection of their roots with the adjacent ektexine (arrow-heads). (cf. Fig. 18 E). Approx. ×11,000. The marker is 1 μ. — E, F: scanning electron micrographs DUNBAR (1973, Grana). E: *C. persicifolia*. Part of aperture with operculum (thin arrow), spinule with "roots" (arrow-head), lira (bent arrow) separated by striae (grooves). Note the coarse pattern of the operculum. Approx. ×54,000. — F: *J. montana*. Spinules with less distinct roots (arrow-head) than in *Campanula* (cf. Fig. 18 E). An irregular pattern of protrusions covers the surface between the spinules. Approx. ×30,000.

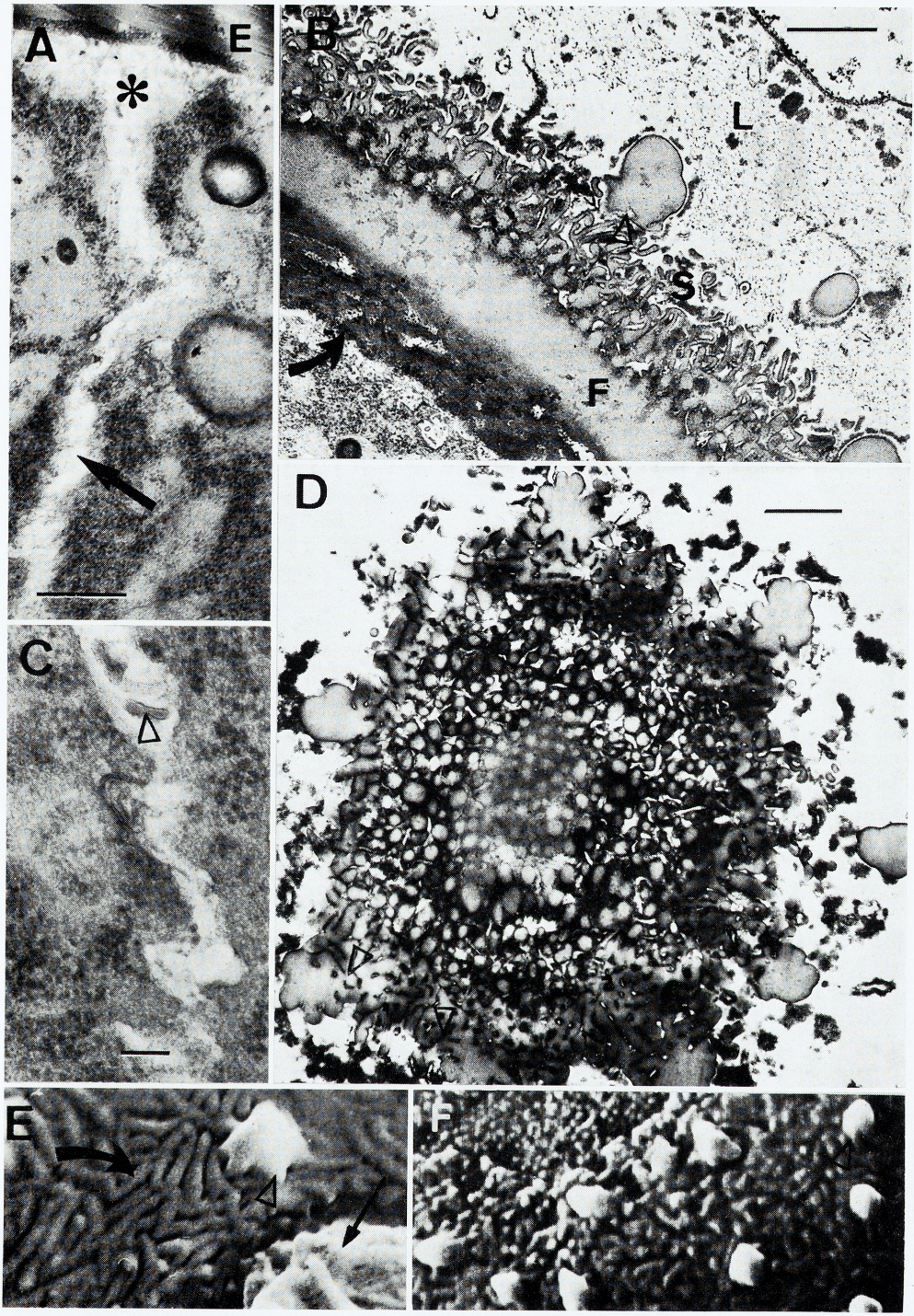


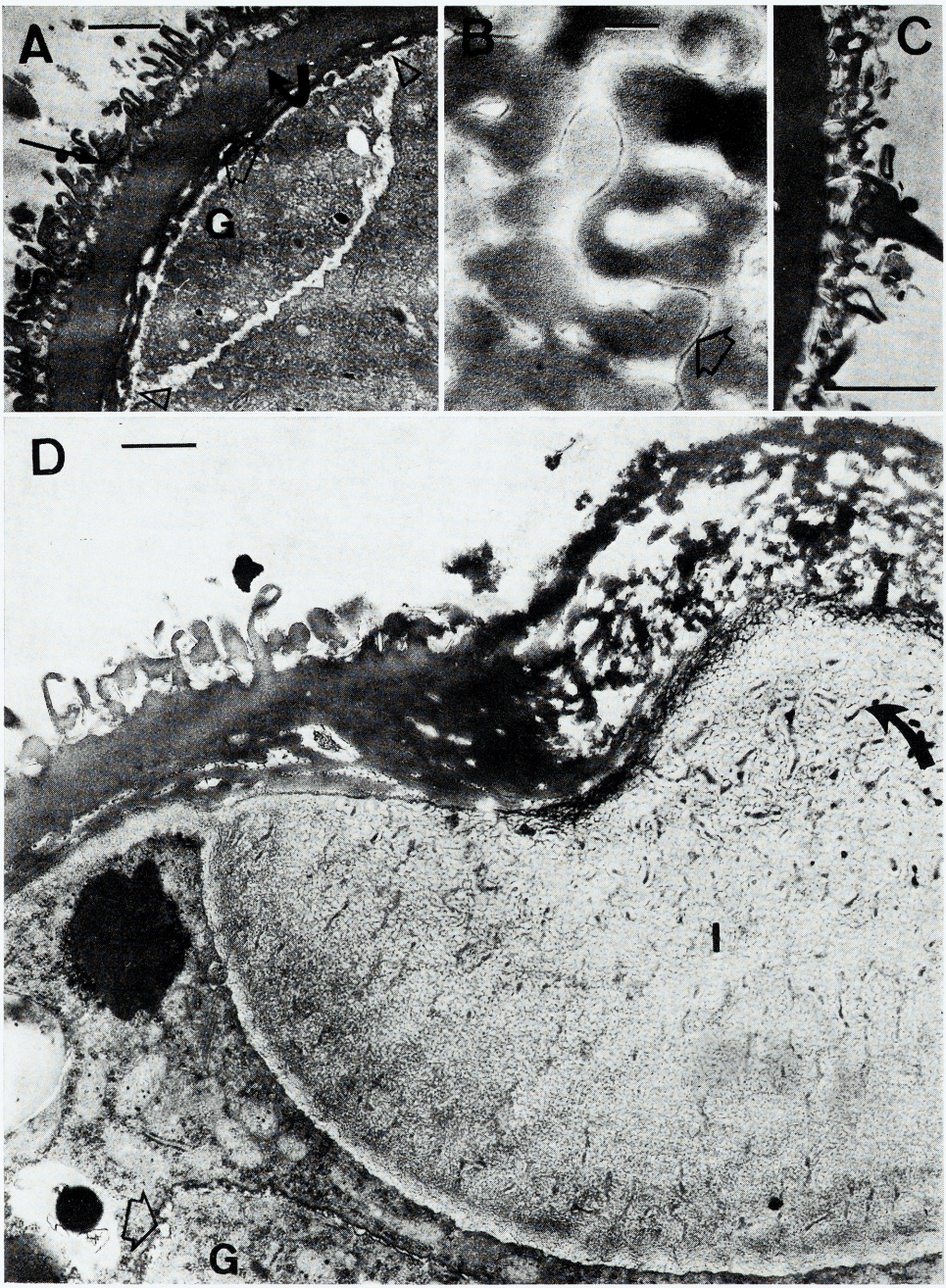
Fig. 18.

later stage of ontogeny than in the present study, an ATPase reaction was demonstrated at the plasmalemmasome region in nearly mature pollen of *Menyanthes trifoliata* (ROWLEY et al. 1973); the results were interpreted to indicate a release of ATPase into the cell walls. This result supports the generally accepted fact, that pollen grains during different stages of ontogeny secrete enzymes, as for instance, wall-softening enzymes prior to the pollen tube formation in *Artemisia* (SOUTHWORTH & BRANTON 1971). Therefore it may not be excluded that also in the present material plasmalemmasomes are involved in the translocation of proteins.

The Generative Cell

A temporary occurrence of callose between the generative and vegetative cells is suggested by several authors. ANGOLD (1968) suggested that callose was involved in the structure of the generative cell wall in *Endymion non-scriptus*. GÓRSKA-BRYLASS (1970) reported callose in, among others, the pollen of *Campanula trachelium* and it was stated that the "callose stage" is a clearly defined stage of gametic differentiation structurally but also physiologically, the significance being increased by the fact that this stage is not an isolated phenomenon in the male gametogenesis of higher plants but has also been observed in spermatids of homosporous and heterosporous pteridophytes and liverworts (GÓRSKA-BRYLASS 1968, 1969). The first-formed layer of the generative cell wall in the present study has in all the species investigated, a rather electron transparent structure during the time of connection with the extracellular space, and may well be composed of callose. Whatever the composition of this wall it changes considerably, undergoing reduction, to become thin and undulating at stage 16. STRANDHEDE (1973) points out that the content in the space between the generative and the vegetative cells of *Eleocharis* seems to change continually and is sometimes a complex of vesicles which renders the term "wall" unsuitable at least in the vesiculated stage. Vesicles present in excess in the generative cell wall are further reported by LEDBETTER and PORTER (1970) in pollen of *Saintpaulia ionantha*. In *Saintpaulia* a sheath of the rough endoplasmic reticulum was observed by those authors to surround the generative nucleus, while in all the species investigated in the present study several layers of the rough endoplasmic reticulum surround not the generative nucleus but the generative cell. Furthermore, in *Eleocharis* the sperm cells were surrounded by rough endoplasmic reticulum (DUNBAR 1973 b). The extensive endoplasmic reticulum is likely to contribute to the synthesis of the plasma membrane of the generative as well as sperm cell walls, since it is known to be a highly differentiated membrane system in association with which phospholipids and protein can be synthesized, stored and modified (WHALEY et al. 1971). In the cytoplasm of the generative cell numerous mitochondria, Golgi bodies and segments of the rough endoplasmic reticulum are located, while no starch grains are found. This partly supports the observations by BURGESS (1970) in the generative cell of *Endymion non-scriptus* where the cytoplasm contains many mitochondria and dictyosomes and no plastids, although in

Fig. 19. *C. rotundifolia*. — A—C: Stage 15. A: Non-apertural part of a pollen grain. Generative cell (G), generative cell wall continuous with the extracellular space (arrowheads). The tectum (arrow), on top of the short bacula, footlayer (bent arrow), lamellated endexine (short arrow). Approx. ×9,500. The marker is 1 μ. — B: Tangential section showing the surface of the ektexine. Material treated with uranyl acetate before dehydration. The continuity of the surface membrane between the units of the wall (arrow) is emphasised by the treatment, and so is the fine structure of the ektexine. Approx. ×70,000. The marker is 0.1 μ. — C: Non-apertural part of the pollen wall. Spinule with



dividing base. Approx. ×15,000. The marker is 1 μ. — D: Stage 16. Aperture and apertural margin. Part of the generative cell (G). The generative cell wall (arrow) seems no longer to be continuous with the intine (cf. Fig. 19 A). The thick intine (I) beneath the aperture has inclusions (bent arrow). There are white lines in the broad part of the endexine. Approx. ×20,000. The marker is 0.5 μ.

contrast to the situation in the present material the generative cell in *Endymion* does not contain any membrane system corresponding to the endoplasmic reticulum.

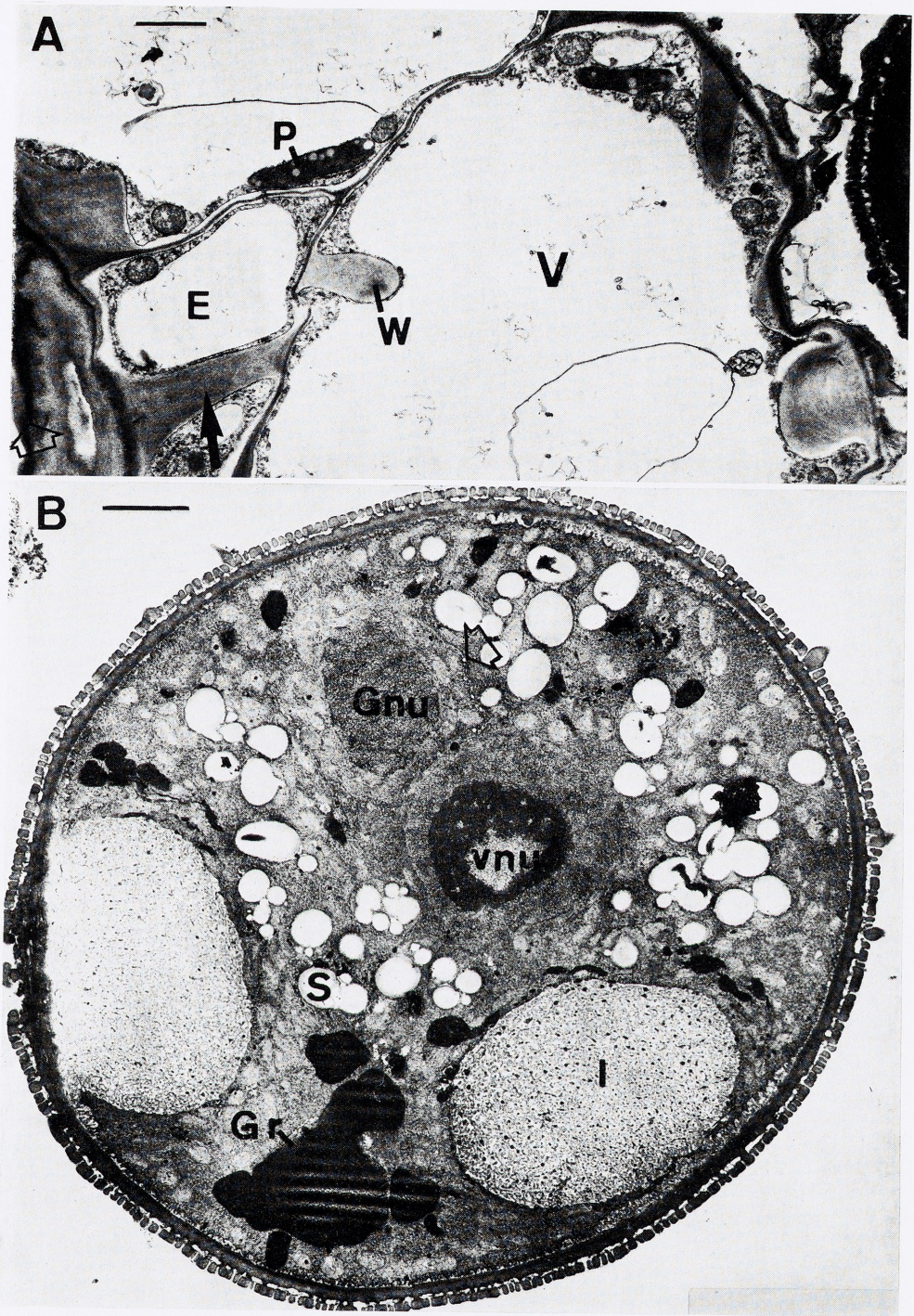
The Pollen Wall

The young wall consists at first of straight probacula. The developmental process leading to an ektexine with short bacula evenly distributed on top of a footlayer and covered by a spongy tectum with large spinules provokes a number of questions. In *Campanula* it has been possible to follow the ontogenesis during the critical period of early wall formation, while this has not been the case for *Jasione*. It would be of interest to study this period more closely, although it is likely that the developmental process of its wall is a general one, since it leads to the formation of a more common ektexine, where less short bacula are evenly distributed on a footlayer and where these bacula are covered by a tectum more usual among angiosperm pollen. Both genera have in common, however, the characteristic basically divided spinules.

The probacula in *Campanula* have a constant height during the unbranched period. At stage 9 they are enclosed in the primexine template. At stage 10 the primexine template has apparently shrunk, since the base of the probacula reaches outside this layer. The primexine template, however, surrounds the probaculum to its very top but does not enclose the protectum. This feature may illustrate the shrinkage of the primexine template rather than a formation of the protectum outside the primexine template. On the other hand the protectum may as well be an outgrowth of the probacula. It has become generally accepted that at least the early stages of exine development take place while the tetrad cells are still embedded in callose (GODWIN 1968, ECHLIN & GODWIN 1968, HORNER & LERSTEN 1971). The callose has been suggested to impose a pattern via the primexine template on the developing exine (WATERKEYN & BIENFAIT 1970) and to do so combined with the influence from the plasma membrane (DUNBAR 1973 b). Contrarily it is suggested to be questionable that a "callose template" could directly control the exine pattern, since in *Sorghum* the entire microspore wall, except for the primexine (primexine template) forms after callose dissolution (CHRISTENSEN et al. 1972); it is recognized, however, that the callose could indirectly affect exine structure via a primexine-mediated influence. In the present study the protectum at stage 10 is in direct contact with the callose layer and an influence from this layer cannot be entirely excluded.

At stage 11 the probacula branch. The process of cleavage leads to a differentiation of the bacula which in a later period gives rise to the spongy layer on top of the short bacula. The short bacula which are continuous with the footlayer are supposed to be homologous to the unbranched base of the young probacula. The question of how the developmental sequence of the roots of spinules occurs may possibly be explained by the branching of the probacula from which a further outgrowth could give rise to the roots. The roots again may distally fuse

Fig. 20. *J. montana* stage 16. — A: Tissue of the anther wall. Part of a pollen grain right-hand side of the Figure. Endothecial cell with wall thickenings (W), chloroplast (P), a central vacuole (V), epidermis cell (E) with thick outer wall; a cuticle (short arrow) covers the surface of the anther. Thick anticlinal wall between epidermal cells (arrow). Approx. ×5,000. The marker is 2 μ. — B: Two apertural regions are shown, the right-hand one demonstrated only by the thick intine (I). Generative cell wall (arrow), nucleus (GNu), vegetative nucleus (VNu), starch grains (S), "storage body" (Gr). The perforations of the tectum are broader than at stage 14 (Fig. 17 A). Approx. ×6,000. The marker is 2 μ.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and in such a way constitute the solid part of the outer spinule. Contrarily the spinules may as well form outwardly at first, secondarily to become fused with the probacula, since it is observed that an outer layer adheres, at stage 12, to the protectum. How, in fact, the complicated structure of the spinules does form, can not be explained by the data gained in the present study.

The data obtained in the present study point to the fact that the footlayer originates by the gradual alteration of the lamellae of the endexine which fuse to give rise to a continuous layer. In a strict sense this would mean that the footlayer is a product of differentiation of the endexine. At stage 12 the footlayer has become broader and is similar in fine structure to the rest of the ektexine. Near maturity the entire ektexine decreases in stainability and a clear distinction between the end- and ektexine not only as to their staining properties but also to their fine structure is evident. No white lines are visible in the ektexine but they do remain in the endexine until maturity.

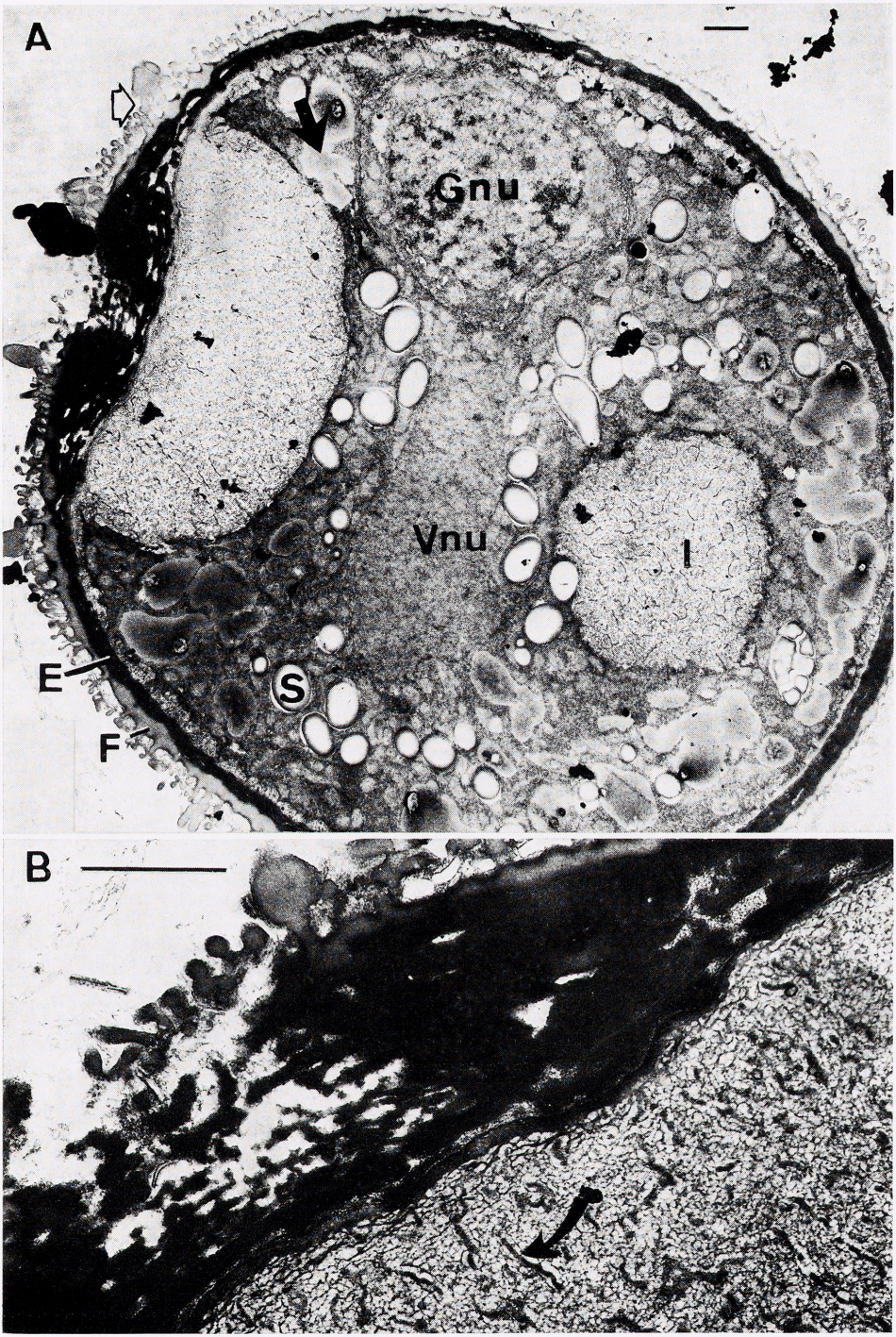
Operculum

The pores are provided with a cover at maturity, an operculum. In contrast to the sculptured ektexine of the pollen wall, the surface of the operculum has a coarser pattern, however, slightly similar to the common pattern of the ektexine. The operculum of the pore in *Poa annua* (ROWLEY 1964) was formed by the ektexine and suspended on a continuous sheet, probably the endexine, originating between the exine and intine. The pattern of operculum formation in maize (SKVARLA & LARSON 1966) follows a somewhat different course from that in the present study, paralleling that of the exine so that the mature operculum has a spinulate tectum supported by bacula and a footlayer. In the present study finger-like inclusions of the proaperture become gradually altered to achieve at stage 12 a density and fine structure similar to that of the ektexine. Since these inclusions become located distally in the aperture, associated with its surface, it is likely that at maturity they will constitute the outer sculptured part of the operculum. A granular material forms units which gradually fill the pore and furthermore adhere to its margins. The material differs in fine structure and stainability from both ekt- and endexine. Near maturity the filling of the pore is still rather loosely arranged. Probably this is the material which constitutes the inner part of the operculum. Conditions for an easy rupture may be expected for an operculum. That the operculum in fact consists of an outer and an inner layer becomes evident in scanning electron micrographs. The difference in pattern between the sculptured part of the operculum and the general ektexine may be influenced genetically, but there is also reason to believe that the pollen wall expands (BANERJEE et al. 1965) resulting in increased dimensions of all exine parts, while no stretching of the operculum may occur. This process of the general surface would surely bring about an alteration of its sculpture and result in a pattern differing from that of the operculum.

ACKNOWLEDGMENTS

This work was partly supported by a grant from the Royal Foundation for Swedish Cultural Activities. I wish to thank Professor F. FAGERLIND for guidance and

Fig. 21. *C. persicifolia* stage 16. Two apertural regions are shown, the right-hand one only by the underlying intine (I). Ektexine with spinules. Note the "roots" of the spinule base (short arrow). Continous footlayer of the ektexine (F), dense stained endexine (E) broad at apertural margin, generative cell with nucleus (GNu), vegetative nucleus (VNu), starch grains (S), "storage body" (arrow). Approx. ×6,000. The marker is 1 μ. — B: Apertural region. White lines are discernible in the endexine, inclusions in the intine (bent arrow). Approx. ×40,000. The marker is 0.5 μ.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Cytological Studies in Homalothecium geheebii (Mild.) Wigh comb. nov. (Bryophyta) and Its Distribution in Scandinavia

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ABSTRACT

WIGH, K. 1973. Cytological studies in Homalothecium geheebii (Mild.) Wigh comb. nov. (Bryophyta) and its distribution in Scandinavia. — Bot. Notiser 126: 316—324.

Homalothecium geheebii was described as a species of *Brachythecium,* later it was transferred to the genus *Camptothecium.* It is now transferred to the genus *Homalothecium* because of morphological and cytological affinities with that genus.

Nine gatherings of *Homalothecium geheebii* have been studied cytologically and the chromosome complement has been found to consist of 10 chromosomes, 4 long and 6 small. This chromosome formula is compared with that of species in *Homalothecium, Camptothecium,* and dioecious species in *Brachythecium* with n=10. The morphological and cytological relations between these three genera are discussed.

The distribution and the habitat of *Homalothecium geheebii* in Scandinavia are discussed.

INTRODUCTION

Homalothecium geheebii was collected for the first time in 1864 by MILDE (GEHEEB 1870). These specimens were discussed by MILDE (1869 a) in Bryologia silesiaca under the name of *Brachythecium laetum* (BRID.) B. S. G., a species with smooth seta. These misidentified specimens proved to be identical with a new species with rough seta collected by GEHEEB. This new species was described as *Brachythecium* (*Hypnum*) *geheebii* by MILDE (1869 b, c) in two papers which appeared in the Botanische Zeitung and the Hedwigia. In the first journal the paper was published on the 3rd December 1869, but it seems impossible to find out when the other paper was published as the documents with date of publication were destroyed in 1945. JURATZKA (1870) discussed this new species and reported the Hedwigia as the journal in which it was described. On the other hand BOULAY (1884) referred to the Botanische Zeitung for the description. It thus seems to be impossible to decide where *Homalothecium geheebii* was described for the first time.

Several pleurocarpous mosses were earlier described as belonging to the genus *Hypnum.* This large genus was split into a number of genera, e.g. *Brachythecium* which was described by BRUCH et al. (1853). This explains why the new species was described by MILDE as *Brachythecium* (*Hypnum*) *geheebii.* The species is thus to be regarded as belonging to the genus *Brachythecium,* and *Hypnum* only indicates the earlier generic name of the species in *Brachythecium.*

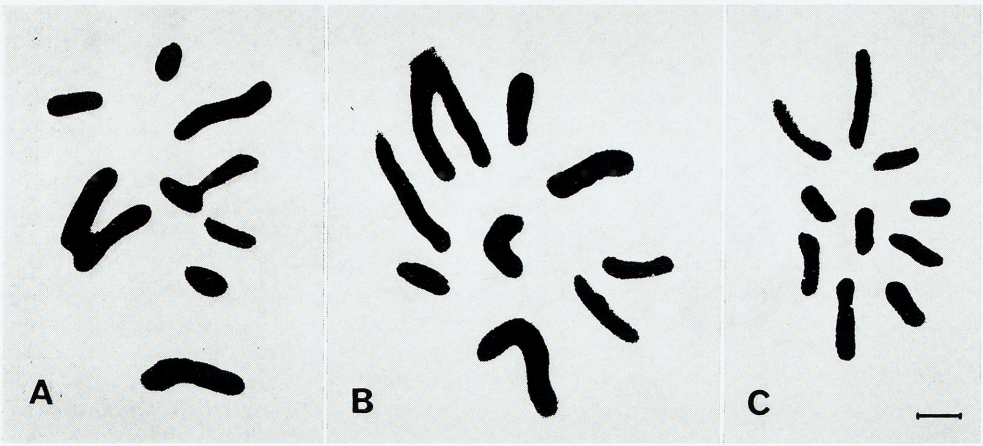

Fig. 1. Photomicrographs of gametophytic mitoses of *Homalothecium geheebii*, n=10. Scale=1 μ.

LINDBERG (1882), however, transferred the species to *Hypnum* (*Brachythecium*) *gehebii* (MILD.) LINDB. (sic!) and it must thus be regarded as a species of *Hypnum*.

Homalothecium geheebii has also been treated as a species of *Camptothecium* by KINDBERG (1894). The generic name was wrongly spelt *Campothecium*, but later it was corrected by KINDBERG (1896).

This species has thus earlier been treated as belonging to three different genera, viz. *Brachythecium*, *Hypnum*, and *Camptothecium*.

MATERIAL AND METHODS

Mitotic chromosomes were counted in the meristematic tissues of the gametophytes. The cytological techniques and cultivation methods are the same as those used by WIGH & STRANDHEDE (1971) and by WIGH (1972 a). The photographic techniques are described by WIGH (1973, in press).

The specimens studied cytologically were collected in Asker, Akershus, Norway, five gatherings from Groset (reference numbers: 72—209, 72—210, 72—211, 72—212, and 72—214) and four from Skaugumsåsen (reference numbers: 72—216, 72—217, 72—218, and 72—219).

RESULTS AND DISCUSSION

The Chromosome Complement in Homalothecium geheebii

This is the first report on the chromosome number in *Homalothecium geheebii*. Nine gatherings from two adjacent localities have been studied. All the gatherings have the same chromosome complement, viz. 4 long and 6 small chromosomes (Fig. 1). This chromosome formula is similar to that of several other species of the genus *Homalothecium* (p. 320). It is possible to distinguish one minute chromosome from the other 5 small ones in some species of *Homalothecium*, but as the 6 small chromosomes in *H. geheebii* are of gradually decreasing length it is not possible to make this distinction in that species.

Some Remarks On the Morphology of Homalothecium geheebii

Homalothecium geheebii was previously placed in the genera *Brachythecium* or *Camptothecium* by most authors. It was placed in *Hypnum* by LINDBERG (1882), but as it is not related to *Hypnum* (sensu stricto) according to current taxonomic

delimitation, this combination will not be further discussed here.

The earlier treatment of *Homalothecium geheebii* in two genera is due to the fact that it combines characters of both *Brachythecium* and *Camptothecium* (*Homalothecium*). The species is characterized by the strongly plicated leaves with rather short cells. Plication is typical of *Camptothecium* and the areolation is more in agreement with that of species of *Brachythecium*. The broadly decurrent leaves have also been regarded as a character typical for *Brachythecium*. Authors stressing the importance of the plicated leaves have placed the species in the genus *Camptothecium* and authors regarding the areolation as being more important have placed it in the genus *Brachythecium*.

The present author has placed the species in *Homalothecium* mainly on cytological evidence, but also because of the plication of the leaves. In its habit it sometimes resembles small forms of *Homalothecium sericeum*. Among the species of *Brachythecium* it agrees most closely with *B. populeum* in habit. Sometimes these two species have been confused, but *Homalothecium geheebii* is readily distinguished from *B. populeum* by the strongly plicated leaves. The two species have, however, the long nerve in common which has probably caused the confusion.

The Morphological Relations Between Homalothecium and Camptothecium

The genus *Homalothecium* is characterized by the erect or almost erect capsule lacking cilia and by the more or less reduced segments, *Camptothecium* by the more or less curved capsule, well-developed cilia and segments. On the other hand the two genera have the strongly plicated leaves and areolation in common.

The form of the capsules and the development of the peristomes are however not always constant within a species. The character of erect versus curved capsules is of little taxonomical value since the form is variable in *Homalothecium lutescens* (HEDW.) ROBINS., for example. The most common type of this species has a curved capsule, but the variety *fallax* (PHILIB.) BREIDL. has an erect capsule. The development of the peristome is variable in *Homalothecium aureum* (SPRUC.) ROBINS., according to ROBINSON (1962).

This variation makes it difficult to separate the two genera and some authors are of the opinion that the differences do not afford sufficient basis for generic separation. MÖNKEMEYER (1927 p. 791) wrote: "Die Gattung *Homalothecium* ist von *Camptothecium* durch kein durchgreifendes Merkmal zu trennen." The same opinion has been expressed by DIXON (1924), ROBINSON (1962), and LAWTON (1965) for example. The fact that several species have been transferred from one genus to the other illustrates that it is difficult to maintain a clear-cut distinction between the two genera. On the other hand modern studies in Brachytheciaceae treat *Homalothecium* and *Camptothecium* as two genera, e.g. PODPĚRA (1954) and TAKAKI (1955 a).

The present author considers, both from morphological and cytological points of view, that some species of *Camptothecium*, e.g. *C. lutescens* and *C. aureum*, must be regarded as belonging to *Homalothecium*. There are no differences in the gametophytes, and the differences in the sporophytes of the two genera are but small. The chromosome complement in these two species is discussed on p. 320. These two species have already been transferred to *Homalothecium* by ROBINSON (1962).

The Cytological Relations Between Homalothecium and Camptothecium

Chromosome numbers published for the two genera are given in Table 1. In Table 2 the chromosome numbers published are shown in relation to the numbers of species and populations studied.

The chromosome number n=10+? published by SMITH & NEWTON (1968) for

Table 1. Published chromosome numbers in *Camptothecium* and *Homalothecium*. n: chromosome numbers in the gametophyte, P: numbers of populations studied, acc.: accessory chromosomes, *: chromosome numbers reported for the first time in the present paper. — All species of *Homalothecium* except *H. aristatum* and *H. laevisetum* have also been treated as belonging to the genus *Camptothecium*. *H. nitens* has also been treated in the genus *Tomenthypnum*.

Taxon	n	P	Author
Camptothecium auriculatum (LINDB.) BROTH., as *Brachythecium subauriculatum* CARD.	11	1	YANO (1957 a, b)
Homalothecium aristatum LAZ.	8	3	LAZARENKO et al. (1970, 1971)
H. aureum (SPRUC.) ROBINS.,	10	6	WIGH (1972 a and unpublished)
as *H. nevadense* (LESQ.) REN. et CARD.	12	1	STEERE (1954), STEERE et al. (1954)
H. fulgescens (C. MÜLL.) JAEG.	8	1	LAWTON (1971)
H. geheebii (MILD.) WIGH	10	9	WIGH*
H. laevisetum LAC., as *H. tokiadense* (MITT.) BESCH.,	11	1	YANO (1957 a, b)
as *H. laevisetum*	11	2	INOUE (1964, 1967.
H. lutescens (HEDW.) ROBINS.	8	1	HOLMEN (1958)
	10	2	WIGH & STRANDHEDE (1971)
	10	26	WIGH (1972 b, 1973)
	10+1 acc.	2	WIGH (1972 b, 1973)
	11	1	SMITH & NEWTON (1968)
	12	1	LAZARENKO & LESNYAK (1966), LAZARENKO et al. (1971)
	12	1	VISOTSKA (1967), LAZARENKO et al. (1971)
	14	1	HO (1956)
H. nitens (HEDW.) ROBINS.	10	1	WIGH (1972 b)
	12	1	HOLMEN (1958)
	12	1	LAWTON (1971)
H. phillipeanum (SPRUC.) B. S. G.	10	1	WIGH (unpublished)
	16	1	VISOTSKA (1967), LAZARENKO et al. (1971)
H. sericeum (HEDW.) ROBINS.	8	1	HOLMEN (1958)
	8	9	SMITH & NEWTON (1968)
	8	1	VYSOTSKAYA & FETISOVA (1969), LAZARENKO et al. (1971)
	9	1	SMITH & NEWTON (1968)
	10	1	RAMSAY (1969)
	10	5	WIGH (1972 a, b, 1973)
	10+1 acc.	5	WIGH (1972 b, 1973)
	10+3 acc.	1	WIGH (1973)
	10+?	1	SMITH & NEWTON (1968)
	10+1m	1	RAMSAY (1969)
	11	2	SMITH & NEWTON (1968)
	11	2	WIGH & STRANDHEDE (1971)
	11+1m	1	SMITH & NEWTON (1968)
	11+2m	1	RAMSAY (1969)

Homalothecium sericeum is not given in Table 2 as the exact chromosome number is uncertain. The chromosome numbers n=10, n=10+1 accessory chromosome, and n=10+3 accessory chromosomes are grouped together as the basic number for these populations is n=10. The chromosome numbers n=11 and n=10+1m are grouped together as the m-chromosomes are regular members of the chromosome set. The same is the case with n=12 and n=11+1m.

Table 2. The frequencies of species and populations of *Camptothecium* and *Homalothecium* in relation to the chromosome numbers published.

Chromosome numbers	Species	Populations
8	4	16
9	1	1
10, 10+1 acc., 10+3 acc.	6	58
11, 10+1m	4	10
12, 11+1m	4	6
11+2m	1	1
14	1	1
16	1	1

Some chromosome numbers published for *Homalothecium lutescens* and *H. sericeum* have been discussed by WIGH (1972 b, 1973 in press). It seems likely that certain populations in both species with more than 10 chromosomes must be regarded as n=10 with a varying number of accessory chromosomes, so that the number of populations with n=10 or n=10 plus accessory chromosomes in Table 2 will be greater than shown.

In *Homalothecium aureum* the basic chromosome number is n=10 (Table 1). In the population studied by STEERE et al. (1954) there were another two, very small, chromosomes which perhaps are accessory chromosomes. Further studies are required to confirm this.

YANO (1957 a, b) reported n=11 in *Camptothecium auriculatum* (Table 1), and gave the chromosome formula n=11 =V(H)+2V+6(4v+2j)+2m(h). This formula is noteworthy as it shows two small and one large heterochromatic chromosomes, 2m(h) and V(H) respectively. As a rule the same numbers of large and small heterochromatic chromosomes occur in a species. One of the small chromosomes is perhaps an accessory chromosome. The relation between accessory chromosomes and m-chromosomes was discussed by WIGH (1973 in press).

The information available in Tables 1 and 2 indicates the occurrence of at least two basic chromosome numbers in the genera *Homalothecium* and *Camptothecium,* viz. x=8 and 10. Other possible basic numbers are x=11 and 12, more doubtful basic numbers are x=9, 13, and 14. It is evident that the dominating basic number is x=10.

Except for *Camptothecium auriculatum,* all the species in Table 1 from which mitotic chromosomes have been studied and classified have 4 long chromosomes, viz. *Homalothecium aureum* (WIGH 1972 a and unpublished), *H. geheebii* (p. 317), *H. laevisetum* (YANO 1957 a, b and INOUE 1967), *H. lutescens* (WIGH 1972 b), *H. nitens* (WIGH 1972 b), *H. phillipeanum* (WIGH unpublished) and *H. sericeum* (WIGH 1972 a, b). The other chromosomes have been grouped in different ways but as a rule one minute chromosome has been recognized together with 5 or 6 small chromosomes.

Homalothecium geheebii thus shows great cytological resemblance to several species of *Homalothecium,* p. 317.

There are no cytological differences between the genera *Homalothecium* and *Camptothecium.* The type species for the two genera, *H. sericeum* and *C. lutescens,* have very similar chromosome complements (WIGH 1972 b). Thus there is no reason to distinguish between the two genera from a cytological point of view.

The Morphological Relations Between Brachythecium and Homalothecium

There is no fundamental morphological difference between the genera *Brachythecium* and *Homalothecium.* As a rule the latter genus is characterized as having straight and strongly plicated leaves with linear cells, but there are species of *Brachythecium* which also have strongly plicated leaves with similar areolation, e.g. *B. glareosum* (SPRUC.) B. S. G. and *B. turgidum* (HARTM.) KINDB. The straight leaves are characteristic for *B. mildeanum* (SCHIMP.) SCHIMP. for example. Despite these resemblances *Homalothecium* is re-

Table 3. Dioecious species of *Brachythecium* with at least one population with 10 chromosomes. L, S, and M denote long, short, and minute chromosomes respectively.

Taxon	Chromosome formula	Author
Brachythecium buchananii (HOOK.) JAEG.	n=10 (no formula)	SANNOMIYA (1955)
	n=10=4L+5S+1M	INOUE (1967)
	n=11=2L+8S+1M	CHOPRA & KUMAR (1967)
	n=22=4L+16S+2M	CHOPRA & KUMAR (1967)
as *B. buchananii* var. *japonicum* CARD.	n=10=3L+6S+1M	YANO (1954, 1955, 1957 b)
B. kuroishicum BESCH., as *B. decurrentifolium* BROTH.	n=10=3L+6S+1M	YANO (1954, 1955, 1957 b)
B. procumbens (MITT.) JAEG.	n=10=2L+7S+1M	CHOPRA & KUMAR (1967)
B. wichurae (BROTH.) PAR.	n=10=4L+5S+1M	INOUE (1967)

garded as a well-defined and natural genus by most authors. The most useful character for distinguishing the two genera is the strongly plicated leaves in *Homalothecium*.

The Morphological and Cytological Relations between Homalothecium geheebii and Brachythecium

The genus *Brachythecium* has been divided into a number of sections and subgenera. These units are mostly neither morphologically nor cytologically homogeneous but the splitting of the genus into smaller units must be regarded as an attempt to make this large genus easier to handle. The main lines in this division of *Brachythecium* are rather clear even if some species are controversial and the taxonomical status of the subdivisions varies, e.g. section versus subgenus.

Among the species in *Brachythecium*, *Homalothecium geheebii* appears to be most closely related to species in the section *Salebrosa*. This section is treated in the broad sense by some authors, but some, as for example NYHOLM (1965), have divided the section into two groups, section *Albicans* and section *Salebrosa* in a more restricted sense. The former section is distinguished from the latter in having shorter leaf cells and more broadly decurrent leaves. *Homalothecium geheebii*, however, does not fit very well into any section of *Brachythecium*. It is distinguishable from most species in the section *Salebrosa* by the very rough seta and by the leaves which are more deeply plicated than in most species in this section. The presence of short cells in the leaves of *Homalothecium geheebii* has been the main reason for placing the species in the section *Albicans* by NYHOLM, another being the rough seta. It differs from the species in this section in having strongly plicated leaves. As the species cannot conveniently be placed in any section of *Brachythecium* a new section to accommodate it, *Pseudocamptothecium*, has been described by SZAFRAN (1961).

As *Homalothecium geheebii* is a dioecious species only dioecious species of *Brachythecium* can be discussed when comparing the chromosome complements. Some dioecious species of *Brachythecium* have the same chromosome number as *Homalothecium geheebii*, n=10 (Table 3). All these species belong to the section *Salebrosa* as defined by TAKAKI (1955 b).

It must be noted that *Brachythecium decurrentifolium* is dioecious according to YANO (1957 a, b), but it is treated by TAKAKI (1955 b) as a synonym of the autoecious species *B. kuroishicum*.

Among the species in Table 3 *Brachythecium wichurae* and one population of *B. buchananii* have a chromosome comple-

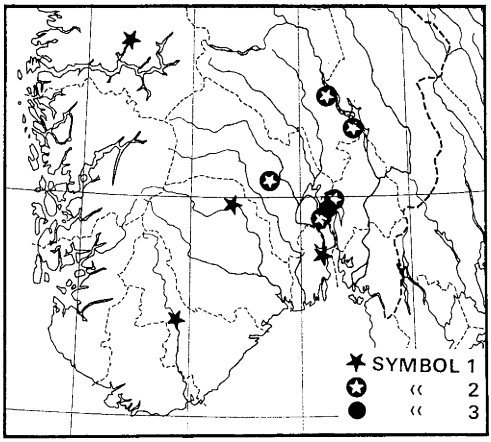

Fig. 2. The distribution of *Homalothecium geheebii* in Scandinavia. Symbol 1: Localities from which it has been collected during the 19th century only. Symbol 2: Localities from which it has been collected during the 20th century. Symbol 3: Localities from which gatherings have been studied cytologically.

ment similar to that of *Homalothecium geheebii,* p. 317.

If the different chromosome numbers reported for *Brachythecium buchananii* refer to different species or to intraspecific chromosome races is not known, but as the species is very variable according to TAKAKI (1955 b) it seems likely that the chromosome numbers refer to different taxa.

Brachythecium wichurae is not morphologically related to *Homalothecium geheebii.* The leaves are not so strongly plicated as in the latter species, and the nerve is shorter. On the other hand *B. buchananii* has strongly plicated leaves in common with *Homalothecium geheebii,* but the nerve is shorter than in *Homalothecium geheebii.*

The Distribution of Homalothecium geheebii in Scandinavia

Homalothecium geheebii was first collected in Scandinavia in 1868 by F. KIAER, p. 324. In Norway, in the vicinity of Oslo, several adjacent localities are known, but outside this area the species is restricted to 7 localities according to available herbarium material. It is remarkable that the species has not been collected from 4 of these localities since the 19th century (Fig. 2). During the last few decades the species has been studied by Professor PER STÖRMER (pers. comm.), who has reported several new localities.

The species is confined to Europe. Apart from Norway it is known from some Continental countries, but is, however, not known from Denmark, Finland and Sweden. This is remarkable since the habitat in which it grows in Norway is not so specialized that a similar habitat could not be found in the other Scandinavian countries. The species is not western in distribution so the presence of *Homalothecium geheebii* in Norway cannot be explained in that way. As the species is so easily recognized, and as it has been known in Norway for such a long time it cannot be assumed that it has been overlooked in the other Scandinavian countries.

The Habitat of Homalothecium geheebii

Homalothecium geheebii is reported to grow on porphyry in Norway. It has been collected by the present author on this substratum in a more or less shady forest together with *Brachythecium populeum* (HEDW.) B. S. G. and *B. reflexum* (STARK.) B. S. G. According to herbarium material, in Norway it is also known to grow together with *Anomodon rugelii* (C. MÜLL.) KEISSL., *A. longifolius* (BRID.) HARTM., *Mnium stellare* HEDW., and *Tortula ruralis* (HEDW.) CROME. The species grows in similar habitats in other European countries. In Germany it is reported to grow on basalt and phonolite together with *Anomodon rugelii* (GEHEEB 1870), and together with *Brachythecium reflexum* and *Grimmia hartmanii* SCHIMP. on a similar substratum (JURATZKA 1870). *Homalothecium geheebii* grows on andesite in Hungary, often together with *Anomodon rugelii* (VAJDA 1961, BOROS 1968).

CONCLUSIONS

Morphologically *Homalothecium geheebii* resembles *Brachythecium* in areolation and *Homalothecium* in having strongly plicated leaves.

Chromosome numbers are known in some 35 species of *Brachythecium*. More than 10 of these species are dioecious, but only one species and one population of a polymorphous species have a chromosome complement similar to that of *Homalothecium geheebii*. In *Homalothecium* 8 species have been cytologically studied (*Homalothecium geheebii* is not included) and 6 of these species have a chromosome set agreeing with that of *Homalothecium geheebii*.

The two species of *Brachythecium, B. wichurae* and *B. buchananii* with chromosome complements similar to that of *Homalothecium geheebii* do not seem to be morphologically related to the latter species. *Brachythecium buchananii* shows closest morphological affinity with *Homalothecium geheebii,* but this species of *Brachythecium* is also reported to have the chromosome number n=22, which is unknown in the genus *Homalothecium* (Tables 1 and 3).

Homalothecium geheebii (MILD.) WIGH comb. nov.

Brachythecium (*Hypnum*) *geheebii* MILDE, Bot. Zeitung 27: 823 (1869) and Hedwigia 8: 161 (1869), see p. 316. — *Hypnum* (*Brachythecium*) *gehebii* (MILD.) LINDBERG (sic!), Bot. Notiser 1882: 195 (1882). — *Camptothecium geheebii* (MILD.) KINDBERG, Can. Rec. Sci. 6: 73 (1894).

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APPENDIX

Herbarium specimens studied

The following 91 herbarium specimens from Norway have been studied. The abbreviations for the Herbaria follow LANJOUW & STAFLEU (1964). The numbers in parentheses refer to the authors' determinations.

A k e r s h u s: Asker, Bergsfjell: B. KAALAAS 27. 9. 1891 BG(48), 11. 9. 1897 BG(36), 13. 9. 1897 S(62), Sept. 1897 L(22), S(56). Groset: F. CONRAD 7. 5. 1891 BG(37), C(7), GB(1), O(270, 271). Groset-Stokkerdalen: F. KIÆR 17. 7. 1880 O(276). Skaugumsåsen: R. FRIDTZ 10. 10. 1886 BG(42), E. JÖRGENSEN 1. 5. 1892 BG(49), S(59), UPS(24), B. KAALAAS 31. 5. 1885 BG(39, 40, 44, 45), C(4), 11. 10. 1886 BG(43), 31. 5. 1887 UPS(28), 7. 5. 1891 BG(50), 3. 5. 1901 BG(38), UPS(31), B. KAALAAS and R. FRIDZ 10. 10. 1886 O(281), F. KIÆR 19. 8. 1868 O(269), 9. 9. 1868 O(266, 272, 282), TRH(10), 17. 7. 1880 H(12), 16. 6. 1882 O(275, 283), 10. 10. 1886 O(267), F. KIÆR and S. LINDBERG 16. 6. 1882 S(63), UPS(29), S. LINDBERG 16. 6. 1882 H(13, 14, 15, 16, 17, 18), LD(21), O(278), S(57). Stokkerdalen between Hvalstad and Groset: E. JÖRGENSEN 1. 5. 1892 BG(41), O(268, 272, 280). — Bærum, Kolsås, between Dalbo and Knabberud: F. KIÆR 28. 6. 1884 O(284). Kolsås, Knabberud: F. KIÆR 26. 6. 1884 O(274). Kolsås, Stensås: B. KAALAAS 20. 5. 1907 BG(47). Överland: P. STÖRMER 7. 6. 1957 O(273). Tanum-uren: B. KAALAAS 2. 5. 1890 BG(46), S(61), UPS(30).

A u s t - A g d e r: Bygland, Fröysnes: N. BRYHN 23. 7. 1894 BG(33), O(255), 24. 7. 1894 BG(32), July 1894 C(3, 5), O(254), S(64), TRH(9), July 1895 UPS(26), July 1896 S(58).

B u s k e r u d: Eggedal, Öydegården: P. STÖRMER 28. 7. 1965 O(259). — Lier, Asdöl: P. STÖRMER 24. 5. 1959 O(258).

O s l o: Vettakollen: B. KAALAAS 10. 5. 1891 BG(51).

O p p l a n d: Östre Toten, W. Leirsjöen, S. the chapel of Totenvik: P. STÖRMER 7. 8. 1946 O(264), UPS(23).

S o g n a n d F j o r d a n e: Balestrand, Fjaerland, Mundal: N. BRYHN Aug. 1893 LD(20), 8. 8. 1899 BG(35), TRH(11), Aug. 1899 C(2, 6), H(19), O(253), S(52, 54, 55, 60), UPS(25).

T e l e m a r k: Tinn, Vestfjorddalen, near Dale or Kvitå: F. KIÆR 7. 8. 1890 O(279).

V e s t f o l d: Borre, Falkensten: E. JÖRGENSEN 20. 9. 1890 BG(34), O(256, 257, 260, 261), TRH(8).

Studies in African Cyperaceae VIII

The Taxonomic Position of Abildgaardia Vahl and Nemum Hamilton

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ABSTRACT

LYE, K. A. 1973. Studies in African Cyperaceae VIII. The taxonomic position of Abildgaardia Vahl and Nemum Hamilton. — Bot. Notiser 126: 325—329.

Abildgaardia VAHL and *Nemum* HAMILTON are regarded as related genera distinct from *Fimbristylis* VAHL and *Scirpus* L. (in its modern sense). A new tribe Abildgaardieae K. LYE, trib. nov. mainly based on an unusual embryo type, is established for these two genera. The concept of *Abildgaardia* is extended to include *Bulbostylis* KUNTH ex C. B. CL.

Three new combinations are coined, viz. *Nemum bulbostyloides* (HOOPER) K. LYE, *Nemum equitans* (KÜKENTH.) K. LYE and *Abildgaardia boeckeleriana* (SCHWEINF.) K. LYE.

Abildgaardia VAHL

in Enum. Plant. 2: 296 (1806) emend. K. LYE

Herbae annuae vel perennes, hermaphroditae. *Spiculae* undique imbricatae. *Squamae* plurifariae vel distichae. *Perigynium* nullum. *Stylus* bifidus vel trifidus; basi deciduus vel persistens. *Caryopsis* trigona vel compressa. *Embryo* turbinatus.

TYPUS GENERIS: *Abildgaardia ovata* (N. L. BURM.) KRAL in Sida 4: 71 (1971), syn. *Abildgaardia monostachya* VAHL in Enum. Plant. 2: 296 (1806).

The genus *Abildgaardia* has only been accepted by a relatively small number of cyperologists, and in most revisions it has been included in *Fimbristylis* VAHL. During the years 1806—1970 a total of 37 species combinations were coined in *Abildgaardia* (cf. Index Kewensis). At that time the generic concept was not, however, well founded, so most of the names have to be disregarded as belonging to other genera. It is only since 1965 (cf. VAN DER VEKEN 1965) a better understanding of this genus has been established (cf. GORDON-GRAY 1971, KRAL 1971, LYE 1971). It is most unfortunate that HOOPER & NAPPER (1972) in an important reference work followed the old treatment of CLARKE (1902) sinking *Abildgaardia* in *Fimbristylis* while retaining *Bulbostylis* as a separate genus.

VAHL (1806) based his genus *Abildgaardia* on two species, viz. *A. monostachya* VAHL, now known as *A. ovata* (N. L. BURM.) KRAL (Fig. 1), and *A. tristachya* VAHL, now known as *A. triflora* (L.) ABEYWICKR. (Fig. 2). *A. ovata* is the lectotype of this genus. The genus *Abildgaardia* was described to include species of *Fimbristylis*-like habit but with (at least partly) opposite glumes and flattened spikelets. My concept of the genus includes VAHL's species, but in addition includes *Bulbostylis* KUNTH ex C. B. CL. as well as a few species of *Fimbristylis* with filiform leaves and *Abildgaardia*-type embryos.

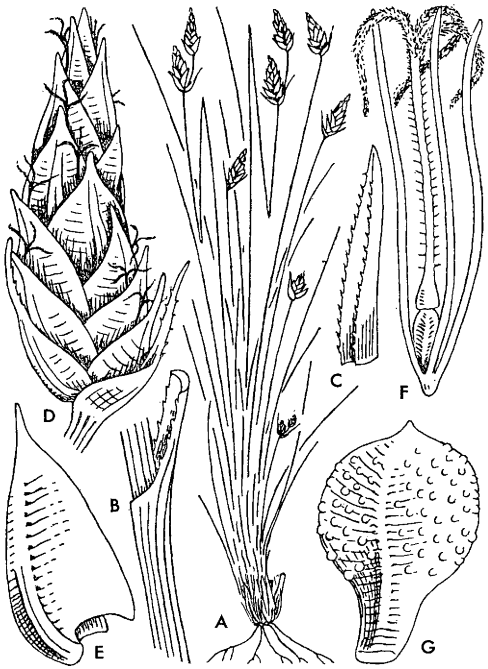

Fig. 1. *Abildgaardia ovata* (N. L. BURM.) KRAL, syn. *A. monostachya* VAHL, the type-species of *Abildgaardia* VAHL. — A: Habit, c. ×0.4. — B: Throat of leaf-sheath. — C: Leaf-apex. — D: Spikelet showing opposite lower glumes. — E: Glume. — F: Flower with ovary and three filaments. — G: Achene. — Drawn from HAINES 4026 (Makerere Hill, Kampala, Uganda). Original by RICHARD WHEELER HAINES.

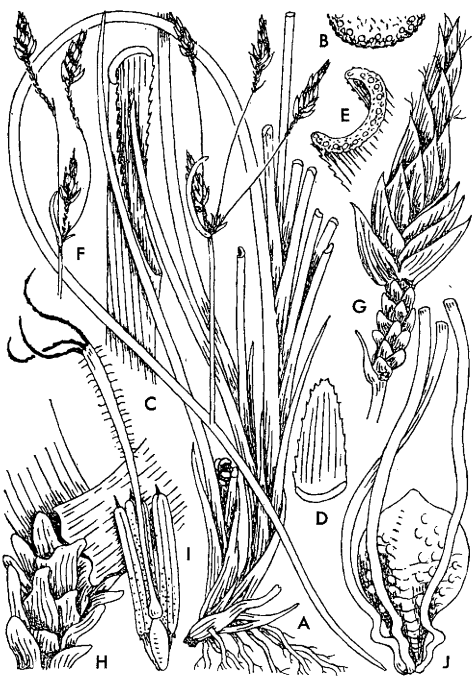

Fig. 2. *Abildgaardia triflora* (L.) ABEYWICKR., syn. *A. tristachya* VAHL. — A: Habit, c. ×0.4. — B: Transect of culm. — C: Throat of leaf-sheath. — D: Leaf-apex. — E: Transect of leaf. — F: Inflorescence. — G: Spikelet. — H: Rachis of spikelet showing two glumes and many small scale-like projections. — I: Flower. — J: Achene with persistent filaments. — Drawn from HAINES 4198 (Dar es Salaam, Tanzania). Original by RICHARD WHEELER HAINES.

Recent embryological studies have shown that *Abildgaardia* VAHL is not related closely to *Fimbristylis* VAHL but to *Bulbostylis* KUNTH ex C. B. CL. (cf. SHAH 1965, VAN DER VEKEN 1965). The only character separating the two genera *Abildgaardia* and *Bulbostylis* appears to be that at least the lower glumes are distichously arranged in *Abildgaardia* while they are all spirally arranged in *Bulbostylis*. In other genera of Cyperaceae the character "distichously or spirally arranged glumes" is only regarded as a separating character at varietal or subspecific level, cf. *Fuirena stricta* STEUD. var. *stricta* and *F. stricta* var. *chlorocarpa* (RIDLEY) KÜKENTH. and *Cyperus michelianus* (L.) LINK subsp. *michelianus* and *C. michelianus* subsp. *pygmaeus* (ROTTB.) ASCHERS. & GRAEBN. (cf. HOOPER & NAPPER 1972).

Other characters such as deciduous or persistent style-base were found to be of no taxonomic value separating the two genera since both characters are represented in each genus (cf. LYE 1971, KRAL 1971, GORDON-GRAY 1971). GUAGLIANONE (1970) has shown that intraprophyllar buds are lacking in *Fimbristylis* but pres-

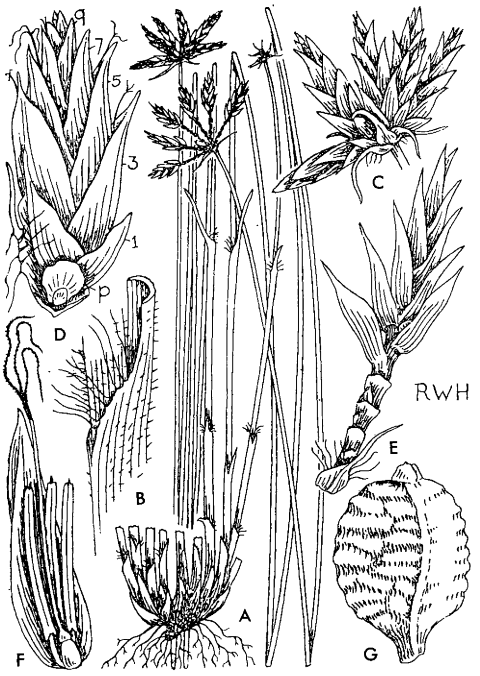

Fig. 3. *Abildgaardia pilosa* (WILLD.) NEES, syn. *Bulbostylis pilosa* (WILLD.) CHERM. — A: Habit, c. ×0.4. — B: Throat of leaf-sheath. — C: Inflorescence. — D: Flowering spikelet; P=prophyll; glumes numbered. — E: Mature spikelet with lower glumes and achenes fallen. — F: Glume with flower. — G: Achene. — Drawn from HAINES 3019 (Lagos, Nigeria). Original by RICHARD WHEELER HAINES.

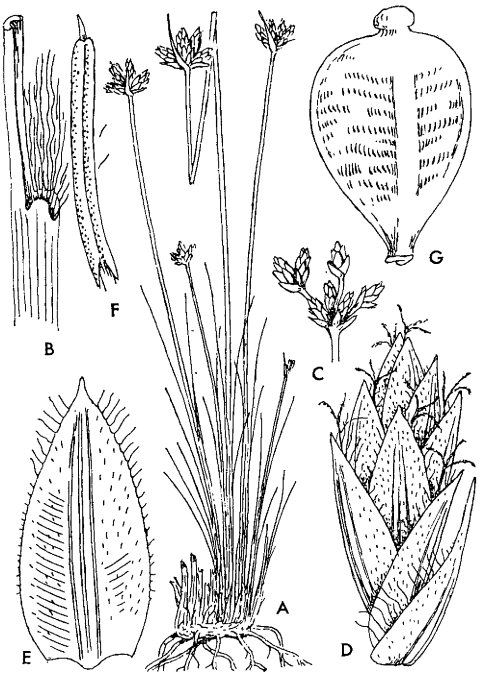

Fig. 4. *Abildgaardia boeckeleriana* (SCHWEINF.) K. LYE, syn. *Bulbostylis boeckeleriana* (SCHWEINF.) A. A. BEETLE. — A: Habit, c. ×0.4. — B: Throat of leaf-sheath. — C: Inflorescence. — D: Spikelet. — E: Glume. — F: Anther. — G: Achene. — Drawn from HAINES 4081 (Mukoko, Masaka). Original by RICHARD WHEELER HAINES.

ent in *Bulbostylis,* and although this may be a useful character separating these two genera, it cannot be used for separating *Bulbostylis* from *Abildgaardia* since some species of *Abildgaardia* also have a congested inflorescence and intraprophyllar buds, e.g. *Abildgaardia pilosa* (WILLD.) NEES (Fig. 3). In general morphology also the similarity between say *Abildgaardia hygrophila* (GORDON-GRAY) K. LYE and *Bulbostylis contexta* (NEES) BODARD is striking. An East African species also, *Abildgaardia boeckeleriana* (SCHWEINF.) K. LYE, comb. nov., syn. *Scirpus boeckelerianus* SCHWEINF. in Bull. Herb. Boiss. 2, Append. 2: 50 (1894) and *Bulbostylis boeckeleriana* (SCHWEINF.) BEETLE, is sometimes confused with *Abildgaardia pilosa* (cf. Fig. 4). Another character which may be useful in the classification of these genera is the presence or absence of minute scales supporting the glumes on the rachilla, but the nature of these scales is not fully understood. It is possible that it is only a scale-like elongation of the very irregular, nodular rachilla. These scales are best developed in *Abildgaardia ovata* (N. L. BURM.) KRAL and *A. triflora* (L.) ABEYWICK. (cf. Fig. 2), but remnants of such scales are also found in *Bulbostylis.*

Since the two genera *Abildgaardia* and

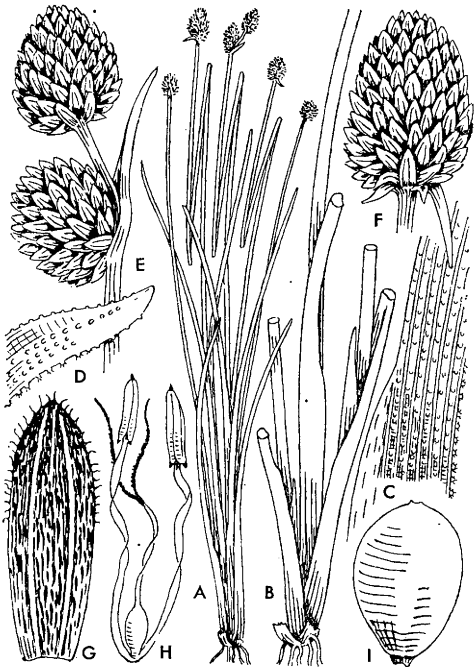

Fig. 5. *Nemum spadiceum* (LAM.) DESV. ex HAM., syn. *Scirpus angolensis* C. B. CL. — A: Habit, c. ×0.4. — B: Plant-base. — C: Throat of leaf-sheath. — D: Leaf-apex. — E: Inflorescence of two spikelets. — F: Inflorescence of one spikelet. — G: Glume. — H: Flower. — I: Achene. — Drawn from ROBINSON s.n. (near Kasama, Zambia). Original by RICHARD WHEELER HAINES.

Bulbostylis can be interpreted in so many different ways, and since at least four possibly equally important characters can be used as the main separating criterion, and since none of these characters can be combined to give a better generic circumscription, the present author has found it impossible to retain the genus *Bulbostylis*.

It is of course unfortunate that so many well-known names in *Bulbostylis* will have to be rejected. Although *Bulbostylis* has been conserved over many older genera, it has not been conserved over *Abildgaardia*, and since it also was conserved erroneously as *Bulbostylis* KUNTH, although KUNTH has not described this genus (cf. HOOPER 1968), it is better for the name to be forgotten or preserved for a section of *Abildgaardia* only.

Nemum DESV. ex HAMILTON
in HAM. Prodr.: 13 (1825)

The genus *Nemum* is not accepted by most cyperologists, and it is usually included in *Scirpus* L. s. lat. (cf. HOOPER & NAPPER 1972), although the remote relationship to *Scirpus sylvaticus* L., the type-species of *Scirpus* L., is striking. *Nemum spadiceum* (LAM.) DESV. ex HAM., syn. *Scirpus angolensis* C. B. CL., the type-species of *Nemum* (cf. Fig. 5) has been found to have the same embryo-type as *Abildgaardia* (cf. VAN DER VEKEN 1965), and the present author therefore believes that *Nemum* is more closely related to *Abildgaardia* than to any other genus.

Nemum differs from *Abildgaardia* in having glumes persistent on the rachilla after the nutlets have fallen, lack of distinct midrib, and more numerous and more densely set flowers.

It is a small genus of only three species. The type-species occurs scattered throughout tropical Africa with an isolated occurrence in the West Indies. *Nemum bulbostyloides* (HOOPER) K. LYE, comb. nov., syn. *Scirpus bulbostyloides* S. HOOPER in Kew Bull. 26: 581 (1972), is restricted to a few mountains in West Africa, while *Nemum equitans* (KÜKENTH.) K. LYE, comb. nov., syn. *Scirpus equitans* KÜKENTH. in Wiss. Ergebn. Schwed. Rhod.-Kongo-Exped. 1911—12, i: 7 (1921), only occurs in Zambia and the Congo.

Abildgaardieae K. LYE, trib. nov.

Herbae annuae vel perennes, hermaphroditae. *Spiculae* undique imbricatae. *Squamae* plurifariae. *Perigynium* nullum. *Stylus* bifidus vel trifidus; basi deciduus vel persistens. *Caryopsis* trigona vel compressa. *Embryo* turbinatus.

TYPUS TRIBERIS: *Abildgaardia* VAHL in Enum. Plant. 2: 296 (1806).

This tribe is designated to include genera with many-flowered bisexual spikelets as in Scirpeae, but Abildgaardieae differs in its very distinctive embryo type, open or congested anthela, and the constant absence of perianth segments. The embryo is turbinate with a germinative groove perpendicular to the plane of the first leaf; the radicle and coleoptile are basal, separated by a groove (cf. VAN DER VEKEN 1965).

This tribe comprises the genera *Abildgaardia* (including *Bulbostylis*) with about 120 species, *Nemum* with 3 species, and possibly also the monotypic genus *Nelmesia* (cf. VAN DER VEKEN 1955), but more research is needed to establish the correct taxonomic position of this genus.

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Studies in African Cyperaceae IX

The Morphology of Coleochloa Gilly and Afrotrilepis J. Rayn.

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ABSTRACT

HAINES, R. W. & LYE, K. A. 1973. Studies in African Cyperaceae IX. The morphology of Coleochloa Gilly and Afrotrilepis J. Rayn. — Bot. Notiser 126: 330—339.

The morphology of three species of *Coleochloa* (*C. microcephala, C. abyssinica, C. setifera*) and one of *Afrotrilepis* (*A. pilosa*) is studied in some detail. The hairy ring below the ovary is not a perianth or utricle, but probably a cupular outgrowth of the receptacle equivalent to that of *Scleria,* and the spikelets are of racemose structure, not cymose as stated by some modern authors.

The genera *Coleochloa* and *Afrotrilepis* show relationship with the West African *Microdrachoides* and the South American *Trilepis.* All these genera are related to *Scleria* and can be classified inside Sclerieae. No close relationship with Mapanieae was found.

INTRODUCTION

The Cryptangieae of BENTHAM (1883), often considered a part of Sclerieae, have been well treated systematically by GILLY (1943 "Lagenocarpieae"), NELMES (1953) and others. Their morphology is, however, but poorly understood, particularly as regards the membranous utricle-like covering of the fruit which has been variously interpreted by different workers. Recently KOYAMA and MAGUIRE (1965) and KOYAMA (1971) have proposed a new major group, their "Mapanioideae", to include Cryptangieae and Mapanieae, distinguished primarily by fruit structure, but without consideration of the very relevant work of RAYNAL (1963).

The present studies on *Coleochloa* are

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Fig. 1. *Coleochloa microcephala* NELMES. — A: Habit drawing, c. ×0.9. — B: Detail from inflorescence. — C: Base of sheath (1/2 removed) showing prophylls and peduncles. — D: Part of lower culm covered by leaf-sheaths and leaves; the scar from a fallen leaf below. — E: Female spikelet; glumes numbered. — F: Bisexual spikelet; glumes numbered. — G: Female flower with outer wall ("utricle") of fruit split to expose the central mass which has been broken across. — H: Leaf-base showing the hairy ligule. — I: Tip

of pistil with 3 rows of spicules and 3 style-branches. — J: Diagram of bisexual spikelet; glumes numbered. — K: Female spikelet; glumes numbered. — L: Section of leaf. — M: Male spikelet; glumes numbered. — N: Plant-base. — Drawn from PÓCS 6058/A (Uluguru mountains, Tanzania, 1600 m). — Original by RICHARD WHEELER HAINES.

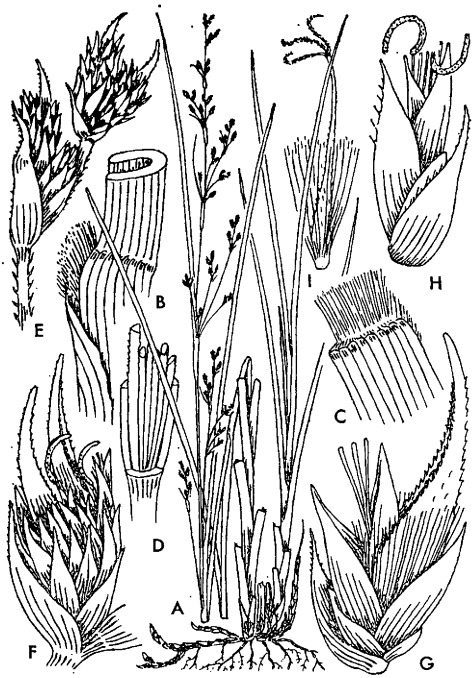

Fig. 2. *Coleochloa abyssinica* (HOCHST.) GILLY. — A: Habit drawing, c. ×0.4. — B: Leaf-base with ligule. — C: Ligule. — D: Base of sheathing bract (1/2 removed) showing peduncles. — E: Detail from panicle. — F: Spikelet-cluster. — G: Bisexual spikelet. — H: Male spikelet. — I: Female flower with surrounding hairs. — Drawn from HAINES 4232 (foothills of Mt. Kadam, Karamoja, Uganda, 1500 m). — Original by RICHARD WHEELER HAINES.

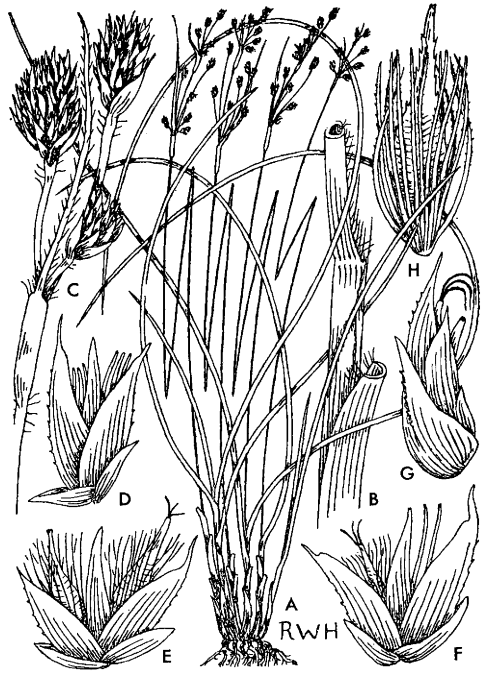

Fig. 3. *Coleochloa setifera* (RIDL.) GILLY. — A: Habit drawing, c. ×0.4. — B: Culm with leaf-bases. — C: Detail from panicle. — D: Male spikelet. — E: Female spikelet. — F: Bisexual spikelet. — G: Female spikelet. — H: Female flower with surrounding hairs. — Drawn from MILNE-REDHEAD & TAYLOR 8594 (Matagoro Hills, Tanzania, 1330 m). — Original by RICHARD WHEELER HAINES.

based on three collections drawn for our proposed "Sedges and Rushes of Uganda and Kenya", viz. *Coleochloa microcephala* NELMES, a fine sheet from the Uluguru Mountains of Tanzania at 1600 m, rich in flowers and fruits, PÓCS 6058/A, kindly lent by the East African Herbarium, *C. abyssinica* (HOCHST.) GILLY from Karamoja, Uganda at 1500 m, HAINES 4232, and *C. setifera* (RIDL.) GILLY from the Matagoro Hills of Tanzania, MILNE-REDHEAD and TAYLOR 8594. The studies also include the West African *Afrotrilepis pilosa* (BOECK.) J. RAYN.=*Eriospora pilosa* (BOECK.) BENTH. RAYNAL (1963) found the inflorescences of *Coleochloa* and *Afrotrilepis* "absolument identique", the separation, at subgeneric level by CLARKE (1902) and at generic by GILLY (1943), being based on vegetative characters.

MORPHOLOGY

Habit and Stems

Coleochloa microcephala (Fig. 1) is a tufted perennial growing on "rocky pave-

ments, commonly forming clumps on wet rocks; 6,000—8,500 ft." (NAPPER 1964).

Other species of *Coleochloa* (Figs. 2 and 3) have similar tufted habits and may, as stated by RAYNAL (1963) in his generic description and as seen in our *C. abyssinica,* be stoloniferous. *Afrotrilepis pilosa* has thick trunks, horizontal at the base, branching and upright above (BENTHAM 1881 b). A section of the trunk (Fig. 4 F) shows a central triangular stem surrounded by old leaf bases intermingled with adventitious roots to form a hard mass. Similar formations are found in related genera, *Trilepis, Cephalocarpus, Everardia,* and *Afrotrilepis,* and outside the Cyperaceae in *Vellozia* (NEES 1842, GILLY 1941 a, 1941 b and 1943, TROLL 1943). *Afrotrilepis jaegeri,* figured by RAYNAL (1963), has branching leafy stems but no thick trunks, and young, though already flowering, plants of species that later develop trunks may be thin-stemmed (CHEVALIER 1933 *Afrotrilepis pilosa,* GILLY 1942 *Cephalocarpus rigidus*).

Leaves

In all *Coleochloa* species (cf. Figs. 1—3, CLARKE 1909 Tab. 140, SCHÖNLAND 1922 Pl. 75) there are a few stiff scales at the base of each culm. In *C. microcephala* they are split below but not near the tip, so that they do not form separate setae as in many other sedges. The foliage leaves are distichous and separated into a distinct sheath and blade by a slight groove on the outer surface and a hairy ligule within. The sheath is stiff, compressed from side to side and open with overlapping margins for most of its length, resembling the sheaths of grasses, not joined to a tube as in most sedges. Succeeding sheaths overlap, closely embracing the flattened stem. The blade is stiff with inrolled margins and a hairy midrib and is eventually deciduous immediately above the ligule. Deciduous blades are rare in sedges but known outside Lagenocarpeae in *Pycreus mundtii.*

In *Afrotrilepis* the leaves are arranged spirally, the sheaths are closed and the blades are long persistent, at length turning downward to clothe the trunks (BENTHAM 1881 b and our Fig. 4). RAYNAL (1963) gave detailed drawings of the throat of the sheath, with a hairy ligule on the margin at the base of the blade and a lingula (antiligule) opposite. Below the lingula the sheath is folded and RAYNAL suggested that this indicated a closure of a formerly open sheath along the line of the fold. But, since closed sheaths are the rule in Cyperaceae and the open sheath of *Coleochloa* an exception, it seems improbable that the closed should have been derived from the open.

Panicle

Coleochloa microcephala, C. abyssinica and *Afrotrilepis pilosa* have well-developed lax panicles spread over about half the length of the culms, while *Coleochloa setifera* and *Afrotrilepis jaegeri* have relatively compact panicles of few spikelets, confined to the ends of the culms. In both *Coleochloa* and *Afrotrilepis* many branches may arise in the axil of an inflorescence bract, their bases enclosed by a series of delicate membranous prophylls, all facing the same way and entirely hidden in the bract sheath. Such tandem branching is found in several groups of sedges and has been discussed by HAINES (1966) but its nature is not understood.

The spikelets are set in dense clusters at the ends of the branches, each subtended by a bract but without the 2-keeled adaxial prophyll commonly found in Cyperaceae. The loss is probably associated with the close crowding as in the subgenus *Vignea* of *Carex* (KÜKENTHAL 1909) and *Schoenus* (HAINES 1966).

Spikelet

In *Coleochloa microcephala* most of the spikelets are 2-flowered, either with both the flowers male or with the lower flower

Fig. 4. *Afrotrilepis pilosa* (BOECK.) J. RAYN. — A: Habit drawing, c. ×0.9. — B: Detail from panicle. — C: Spikelet-cluster with peduncle. — D: Leaf-section. — E: Base of sheathing bract (1/2 removed) with numerous peduncles and delicate prophylls. — F: Cross-section of trunk. — G: Spikelet with two male flowers; glumes numbered. — H: Spikelet with one male flower; glumes numbered. — I: Bisexual spikelet with one male and one female flower; glumes numbered. — J: Female flower with surrounding hairs. — K: Section through female flower showing the wide air-space between the outer wall and the endosperm. — Drawn from HAINES 3223 (Igboora, Nigeria). — Original by RICHARD WHEELER HAINES.

female and the upper male. In these spikelets there are 4 glumes in more or less distichous arrangement, the lowest short, obtuse and empty, the next 2 longer, awned and each subtending a flower, and the uppermost again empty. NAPPER (1964) described spikelets with 2 female flowers as occurring in the genus, and we have seen them in *C. setifera.* Some female spikelets may be 1-flowered and 3-glumed, with the flower subtended by the middle glume (Fig. 1 K). Each flower is enwrapped by the glume above, thus in the 4-glumed bisexual spikelet the female by the 3rd and the male by the 4th glume. The flowers are, however, set as usual in Cyperaceae, with a face of the ovary and a pair of the stamens adaxial, the unpaired border of the ovary and the unpaired stamen facing away from the spikelet axis (Fig. 1 J). Where there are 5 glumes the lower 2 are empty (Fig. 3). The female and bisexual spikelets are found towards the apex of the spikelet-cluster, the purely male below.

Coleochloa and *Afrotrilepis* are exceptional among Lagenocarpeae in having bisexual as well as unisexual spikelets. The other African genus *Microdrachoides* and all the American genera have only unisexual spikelets. As in *Scleria,* some species of which have very similar bisexual spikelets (RAYNAL 1963, HAINES & LYE 1972), the unisexual types appear to be reduced from the bisexual.

This account of the spikelet as having a series of glumes arranged on a simple rachilla, some subtending flowers, though agreeing with the classical concepts of BENTHAM (1881, 1883), CLARKE (1902) and NELMES (1953) is still controversial.

RICHARD (1851) described *Coleochloa* (*Eriospora*) *abyssinica* as having 1-flowered unisexual spikelets set in pairs, male and female, the pair forming what is here described as a single bisexual spikelet. PAX (1886), KERN (1961) and KUKKONEN (1967) have described the male flowers of various Sclerieae and Lagenocarpeae as set on a branch of the axis bearing the female flower.

KOYAMA (1971 Fig. 11) gives a plan showing a much reduced prophyll present at the base of the staminate branch of *Afrotrilepis* confirming its true lateral nature, but his drawing of the spikelet does not show the prophyll and we have not been able to find it in our material. RAYNAL's (1963) opinion that "la meilleure explication de l'épillet androgyne soit la plus simple: toutes les fleurs sont latérales sur un axe simple" seems justified.

Flower

The male flower consists of stamens only but the female flower is more complex, the pistil being set in a ring of hairs joined together below. In *Microdrachoides* and many other Lagenocarpeae the hairs spring from a 3-lobed structure described by GILLY (1943) as a perianth and used by him to distinguish the tribe from Sclerieae which had the pistil set in a cupule but no perianth. KOYAMA & MAGUIRE (1965) believed the perianth in Cryptangieae to be represented by the "utricle" of the fruit, the hairy "squamellae" being "metamorphosed glumes rather than floral segments". They supported this interpretation by finding the abscission of the fruit above the squamellae, whereas in most Cyperaceae the perianth falls with the fruit. But in fact the hairy ring or hairy squamellae fall with the fruit. So the site of abscission cannot be used to exclude their perianthal nature. Indeed the abscission type is of little value, for in *Pycreus* the fruit falls leaving the stamens behind, whereas in most Cypereae the stamens fall with the fruit.

Hairy perianths are indeed found in some sedges, as *Eriophorum* and *Carpha,* developed for dispersal of the fruits by wind. But undoubted perianths are unknown outside Rhynchosporeae, Scirpeae and, if considered a separate tribe, Schoenoplecteae. Again perianths are nearly always 6 or more membered in Cyperaceae,

only reduced to 3 in a few species or perhaps individuals of *Fuirena*. It seems more likely that, following a recent suggestion of KOYAMA (1971), the hypogynous squamellae of Lagenocarpeae are equivalent to the "discoid organ", the cupule of *Scleria*. This cupule is, as GILLY (1943) said, "formed by hypanthial growth of the pedicel" as a special organ, not a perianth. BLASER (1941) found the cupule of *Scleria reticularis* vascularised by bundles which turned back to enter the ovary, not ending freely as would be expected for perianth bundles. In the section *Ophyroscleria* of *Scleria* the margin of the cupule is hairy, though the hairs are short (NEES 1842, PIÉRART 1951), and with a lightening of the fruit for wind or bird transport could have developed into a hairy ring. BLASER's observation also controverts the suggestion made by KERN (1962) and KUKKONEN (1967) that the cupule represents a ring of glumes.

Fruit

The pistil, which matures while the associated staminate flower is still undeveloped and hidden in its glume, is not remarkable apart from the 3 rows of spicules running from its base to the point of division of the style branches and the lack of any sharp demarcation between the ovary and style (Fig. 1 I). The ripe fruit, however, which projects above the rest of the spikelet, closely resembles in superficial appearance that of a *Carex*. It has an outer "utricle", triangular in section and set with spines along its margins. The body narrows to a beak from which 3 style branches, eventually dry and fragile, spread. This "utricle" is separated by a wide air space from the mass of hard endosperm within, but joined to the pistil just below the attachment of the style branches.

Fig. 2 shows a young pistil of *Coleochloa abyssinica* comparable to CLARKE's (1909) figure of *Afrotrilepis pilosa*, also a mature fruit set in its spikelet. Figs. 3 and 4 show fruits of *Coleochloa setifera* and *Afrotrilepis pilosa* comparable to RAYNAL's fine drawings of *A. pilosa* and *A. jaegeri*. Fig. 1 G shows the "utricle" split to expose the central mass which has been broken across, comparable to NEES' drawing of *Trilepis lhotzkiana* (NEES 1842 Tab. 29, 11 m 19).

NEES described the "utricle" as a "Perigynium femineum utriculiforme rostratum, ore ad stylo cohaerente". BENTHAM (1883 p. 1068), however, recognized NEES' perigynium as a part of the ovary, a "nucis pericarpium" and GILLY (1943) described the beak as made "of pericarp tissue". Yet NELMES (1953) again described the female flower as "surrounded by a trigonous, sac-like membranous utricle from the apex of which the 3 stigmas are only just exserted, the style being situated in the upper beak-like portion of the utricle".

RAYNAL (1963) would appear to have settled the matter. He found the vessels in the spine-bearing ribs at the corners of NELMES' supposed utricle continued directly into the 3 style branches, proving the "utricle" to be in fact the outer coat of the ovary and the air space a split in the substance of the ovary wall.

KOYAMA and MAGUIRE (1965), however, again found the fructification of Cryptangieae "not a fruit of true achene type" but "a compound structure of an achene and a utricle". Their excellent figures of transverse sections of the ovaries of *Didymiandrum, Trilepis* and *Everardia* show the three "utricular vascular bundles" clearly and their longitudinal sections show the bundles running toward the style branches, but they did not follow the bundles into the branches themselves. Our Fig. 1 G, showing the ribs that contain the bundles continued directly into the style branches, confirms RAYNAL's account and controverts the utricular theory of NEES, NELMES and KOYAMA & MAGUIRE. For the bundles of a true utricle should be confined to the

utricle itself, not continue into the style branches.

Utricles in the sense of urceolate structures surrounding but separate from pistils are indeed found in *Bisboekelera* and *Calyptrocarya* of Sclerieae, springing from the receptacle and enclosing the ovary. But KOYAMA's (1965) longitudinal sections from these genera show three things. They are covered by an epithelium on both inner and outer surfaces, not on the outer surface only. They are open above, not attached to the style. Vascular bundles could not pass from them into the style branches. So they are not equivalent to the membranes round the pistils of *Coleochloa* and *Afrotrilepis*. KOYAMA compares them to the thickened ring at the base of the pistil in *Bequerelia*. This is clearly a cupular structure similar to the cupule of *Scleria,* not a part of the ovary wall, nor a perianth, nor an aggregation of glumes. PIÉRART (1951) believed *Scleria* to show an evolutionary trend from a simple to a more elaborate type of cupula and *Bisboekelera* and *Calyptrocarya* show a continuation of this trend.

In the floating fruits of *Oxycaryum* (*Scirpus*) *cubense* and *Cyperus nudicaulis* the maturing ovary wall becomes spongy and filled with air to give buoyancy. *Cladium mariscus* again has a bulky, spongy outer coat covering a hard inner coat which contains the seed (KOWAL 1958). In all these the wall has become divided into 3 layers, an outer pericarp, a spongy mesocarp and a dense endocarp, woody in *Cladium*. In *Coleochloa* and *Afrotrilepis* a similar process has led to the formation of a continuous air space which separates the pericarp to form a spurious "utricle". In *Didymiandrum* and *Everardia* the mesocarp remains spongy (KOYAMA & MAGUIRE 1965), never breaking down to a continuous space, and the endocarp becomes woody as in *Cladium* (MAREK 1958). In *Trilepis* (GILLY 1943) it remains thin though hardened, while in *Coleochloa* and *Afrotrilepis* it is membranous and closely adherent to the endosperm.

LAGENOCARPEAE AND SCLERIEAE

BENTHAM (1881 a English version, 1883 Latin) defined his new tribe Cryptangieae from Sclerieae as having the "spikelets unisexual", so including *Trilepis* (*Fintelmannia*) but excluding *Afrotrilepis* and *Coleochloa* (both included in *Eriospora*), a most unnatural arrangement. PAX (1886) combined the genera and later (1887) put them with *Scleria* in Sclerieae but separated them from *Cryptangium* and *Lagenocarpus*. CLARKE (1895) recombined the tribes under Sclerieae.

GILLY (1943) again separated his Lagenocarpeae from Sclerieae, changing the name as PFEIFFER (1921) had sunk the genus *Cryptangium* in *Lagenocarpus*. Lagenocarpeae had a perianth, not a cupule, Sclerieae the reverse. But his so-called perianth is, in fact, a cupule and the distinction fails. Other criteria such as shrubby habit, beaked achenes, inflated ovary walls, and separately deciduous style branches may also fail, in some species of *Lagenocarpus* for example. So we agree with CLARKE (1895) and RAYNAL (1963) in keeping the tribes united for the present.

The proposal of a group "Mapanoideae" to include Sclerieae, Lagenocarpeae and Mapanieae, made by KOYAMA and MAGUIRE (1965) and KOYAMA (1971) is not acceptable. They are said to share a "peculiar compound fruiting structure" and "a conspicuously cymose partial inflorescence". The fruits of *Afrotrilepis* and *Coleochloa* are in fact of purely ovarian origin apart from the hairy ring, and the spikelets are racemose in structure.

DEVELOPMENT AND DISPERSAL

GILLY (1943) believed that his Lagenocarpeae were derived independently from other Cyperaceae through *Prionium* (Juncaceae). So he suggested that an "arborescent or subarborescent" habit preceeded the herbaceous type. But if Cyperaceae are monophyletic, shrubbiness confined

to a few genera without other features that might appear primitive, would be a specialized feature. It could be secondarily lost, as seems probable in *Afrotrilepis jaegeri* and possibly in *Coleochloa.*

GILLY (1943) associated the African *Coleochloa, Afrotrilepis,* which he separated as a section of *Trilepis,* and *Microdrachoides* with the South American *Trilepis* as a natural group. He gave an interesting map supporting a South American origin, perhaps at a time when the two continents were nearer together than they are now. The thin but hardened endocarp of *Trilepis* appears more primitive than the membranous endocarp of the African genera. RAYNAL (1964) described *Scleria guineensis* with a thin fragile pericarp in contrast to the thick type usually found in the genus and suggested this might show a transition to the membranous pericarp of the *Afrotrilepis* type.

The species all grow on exposed rocks, often separated by great expanses of lowland forest, and the specialization of the fruit appears related to this distribution, the lightness and hairiness probably allowing for wind dispersal, epizooic carriage by birds and attachment to damp rocky surfaces.

If these surmises, and those of NELMES (1951) and HAINES & LYE (1972) are correct, the lowland Sclerieae have given rise to two upland groups, Cariceae in Malaysia, now world wide but still mainly montane in the tropics, and the *Trilepis* group in South America, exclusively montane and only spread as far as Africa.

JAEGER (1971) describes *Afrotrilepis jaegeri* J. RAYNAL as growing in a "zone marginale des hauts sommets de Loma", "en équilibre avec le milieu et à l'abri de toute compétition", an extreme example of a refuge situation.

SUMMARY

Three species of *Coleochloa,* viz. *C. abyssinica* (HOCHST.) GILLY, *C. microcephala* NELMES and *C. setifera* (RIDL.) GILLY and one of *Afrotrilepis* viz. *A. pilosa* (BOECK.) J. RAYN., are drawn with dissections and their morphology discussed. The spikelets are racemose with all flowers set laterally on a rachilla in the axils of glumes. They may be purely male, purely female or bisexual, often with one male above a single female flower. Below the ovary is a hairy ring, often lobed in related genera, probably a cupular outgrowth of the receptacle equivalent to the cupule of *Scleria,* not a perianth or utricle. The outer part of the ovary wall becomes inflated. This part of the pericarp is separated by an air space from the rest of the ovary. The vascular bundles that end in the style branches are attached to the interior surface of this outer part of the pericarp, which has been mistaken for a utricle partially fused with the ovary wall.

The association of these African genera with *Microdrachoides* and with the South American *Trilepis,* all plants of rocky outcrops, within the tribe Sclerieae is confirmed, but not any close association with Mapanieae.

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Helichrysum monogynum, a New Species from Lanzarote, Canary Islands

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ABSTRACT

BURTT, B. L. & SUNDING, P. 1973. Helichrysum monogynum, a new species from Lanzarote, Canary Islands. — Bot. Notiser 126: 340—344.

Helichrysum monogynum is described from the Famara mountain massif, Lanzarote, eastern Canary Islands. The nearest relative of the new species appears to be the South African *H. ericifolium*. *H. monogynum* is a tetraploid species, with chromosome number 2n=28. Data on local distribution and ecology of the new species are provided.

During botanical field work in Lanzarote, eastern Canary Islands, in February and March 1971, one of us (P. S.) came across what appeared to be an undescribed species of *Helichrysum*. The plant was found in three localities situated near each other in the western part of the island (see below).

One-and-a-half years later a herbarium sheet of the same plant, collected March 1972 in approximately the same area of Lanzarote by Mr. J. ØVERGAARD, Oslo, was handed over to the Botanical Museum, Oslo for identification.

Helichrysum monogynum BURTT & SUNDING, sp. nov.

Planta canariensis nulli arcte affinis sed cum *H. ericifolio* LESS. austro-africano comparanda. Ab hac species foliis spatulatis nec linearibus, capitulis haud homogamis sed florum singulum femineum praeditis ad apices ramorum congestis, facile distinguitur.

Suffrutex nanus caespitosus irregularis ramis primo albolanatis deinde glabris exterioribus prostratis. *Folia* alterna, spatulato-oblanceolatis, apicibus acutis recurvis, ad basin auriculatum attenuata, 1—1.7 cm longa, 1.75—3 mm lata, omnino griseolanata. *Capitula* plura in apicibus ramulorum brevium aggregata, ramulis ad apices ramorum etiam aggregatis; involucrum cylindricum, c. 5 mm longum, basi lanatum; bracteae c. 16, c. 6-seriatae, oblongae, exteriores breviores (c. 1.75 mm) et rubescentes, interiores gradatim longiores et pallescentes basi gradatim angustatae. Flores in capitulo 10—12; flos femineus singulus marginalis ceteris hermaphroditis. *Corolla* 2.5 mm longa; lobi 5 anguste triangulares, apice extra stipitato-glandulosi. *Antherae* parte fertili fere 1 mm longa, basi cauda vix 0.5 mm, apice appendice acuto vix 0.5 mm. *Stylus* 2 mm longus, apice in ramos duos stigmatiferos 0.5 mm longos truncatos divisus. *Ovarium* 0.5 mm longum, glabrum, et floris feminei et hermaphroditorum ovulatum.

Fig. 1. *Helichrysum monogynum* BURTT & SUNDING, specimen from the locality "eastern Famara Valley, plateau ab. 1 km S of Iglesia", drawn by Mrs. DAGNY TANDE LID (×0.8). To the right, a flowering branch somewhat enlarged (×2.4).

Canary Islands. Lanzarote, 1 km NW of Los Valles, 460 m, 4 March 1971, P. SUNDING 2515 (holotypus O, isotypus E); ibidem, Vistas de las Nieves, 0.5 km SW of Ermita de las Nieves, 540 m, 4 March 1971, P. SUNDING 2514 (O); ibidem, eastern Famara Valley, plateau ab. 1 km S of Iglesia [i.e. Ermita de las Nieves], 500 m, March 1972, J. ØVERGAARD (O, E).

TAXONOMY

The weak representation of *Helichrysum* in the Canary Islands is remarkable: it is doubled by the discovery of this new species (LEMS 1960, ERIKSSON 1971). The only other native *Helichrysum, H. gossypinum* WEBB (syn. *H. webbii* (SCH. BIP.) CHRIST), is also endemic to Lanzarote, the remaining islands having only introduced species, if any. Whereas the affinity of *H. gossypinum* with the endemic species of Madeira (LOWE 1868) is fairly definite, *H. monogynum* is a much more difficult species to place.

It has no close affinity with any northern species of *Helichrysum*. In consequence one turns to South Africa, where the genus reaches its peak of development. Here the relationship seems to be with the widespread and polymorphic *H. ericifolium* LESS. This species may be distinguished in all its forms by the narrow linear leaves and, on dissection, by its papillose achenes. Nevertheless there seems to be no fundamental structural difference and there can be little doubt that this is where the affinity of *H. monogynum* lies. *H. ericifolium* has heads of about the same size containing 10—12 flowers which are, however, all hermaphrodite; the habit is sometimes rather more erect than in *H. monogynum*, but both species are distinctly woody at the base and both grow on bare stony ground. In addition to the papillose achene *H. ericifolium* differs in having finer pappus setae and less markedly hairy tips to the corolla lobes. In *H. monogynum* these hairs on the corolla

lobes are well developed and each ends in a conspicuous orbicular cell.

The state of infrageneric classification in *Helichrysum* is indicated by the fact that the group in which *H. ericifolium* falls is called § *Oxybelia* by DE CANDOLLE (1838 p. 171), § *Ericifolia* by HARVEY (in HARVEY & SONDER 1865 p. 208) and § *Praecincta* by MOESER (1910 p. 298). These changes are nomenclatural only, the circumscription of the group varies little: in no case is the taxonomic rank clearly indicated. It does not seem possible, formally, to do more than place *H. monogynum* in subgenus *Helichrysum.*

If one judges on the external appearance of the capitula, then size and colouring recall two species in other genera: *Gnaphalium genevoisii* EMBERGER from the high mountains of Morocco and *Anaphalis aurora* RECH. FIL. from Iran. The idea of an affinity outside *Helichrysum* may seem ridiculous, but it is all too well known that generic limits in this part of Compositae are highly unsatisfactory. However *Gnaphalium genevoisii* differs from *H. monogynum* in its numerous female flowers, more compact habit and subrotund leaf-blade. It may not be a typical *Gnaphalium,* at least it has no close affinity amongst Mediterranean species, but it is too distinct from *H. monogynum* to permit a useful discussion of their affinities alone. *Anaphalis aurora* is a good species of *Anaphalis* with female sterile disc flowers: those of *H. monogynum* are fully fertile. *Anaphalis* is as yet unknown from North Africa or the Mediterranean.

CYTOLOGY

Fixations of *H. monogynum* inflorescences in various stages of development were made in the field by P. S. [voucher specimens: SUNDING No. 2514 (O)]. The material has been studied by Garden Curator LIV BORGEN, Botanical Garden, Oslo, who counted the chromosome number $2n=28$ in pollen meiosis. The basic number in the genus *Helichrysum,* as in *Gnaphalium* and other related genera, is $x=7$ (FEDOROV 1969); *H. monogynum* thus appears to be a tetraploid species. The second endemic Canarian *Helichrysum* species, *H. gossypinum,* has not yet been studied cytologically.

LOCAL DISTRIBUTION AND ECOLOGY

The top plateau and the western precipices of the southern part of the Famara mountain massif were explored on 4th and 6th March 1971. *Helichrysum monogynum* was found in three places, viz.:

(1) 1 km northwest of Los Valles, 460 m s. m. Type locality (SUNDING No. 2515).

The plant was growing here on a slightly sloping surface near the precipice toward the west side of the plateau. The bedrock was a soft, light basaltic rock. According to data presented by HAUSEN (1959) and FUSTER et al. (1968), the actual area has basaltic rocks of petrographically rather uniform composition, mainly picritic basalt and olivine basalt. Although the underlying bedrock was easily weathered, there was only a thin, loose soil layer (finely grained soil mixed with gravel and stones) above it, the reason probably being strong wind erosion combined with grazing.

Whereas the western precipices of the Famara mountain are known to possess a rich and interesting flora (with, among others, the second endemic *Helichrysum* species, *H. gossypinum*), the top plateau is in its general character rather poor. The plant community of the *H. monogynum* locality was open and poor in species, with *Launaea arborescens* and *Helianthemum canariense* as the most conspicuous plants. The species composition of a typical part of this stand will be apparent from Table 1, Column 1.

The place and the adjacent areas had been subject to pronounced grazing, as also shown by the plant cover, which was mostly dominated by grazing indicators such as *Cynara cardunculus* var. *ferocis-*

Table 1. Species composition of three stands, one from each of the three *Helichrysum monogynum* localities mentioned in the text. Cover/abundance according to the BRAUN-BLANQUET scale (BRAUN-BLANQUET 1964).

Stand No.	1	2	3
Altitude, m	460	500	540
Area analyzed, m^2	10	10	50
Slope, degrees	2	20	4
Exposure	SW	S	SW
pH	—	—	7.3
Total cover of vegetation, %	<5	10	<5
Helichrysum monogynum n. sp.	1	1	1
Anagallis arvensis L. f. *azurea* HYL.	—	1	—
Bromus madritensis L.	—	1	—
Euphorbia obtusifolia POIR. ssp. *regis-jubae* (WEBB) MAIRE	—	1	—
Hedypnois rhagadioloides (L.) SCHM.	—	—	1
Helianthemum canariense (JACQ.) PERS.	2	2	2
Launaea arborescens (BATT.) MURB.	1	1	1
Launaea nudicaulis (L.) HOOK. FIL.	+	—	1
Linum strictum L.	—	1	—
Lotus lancerottensis WEBB	1	1	1
Micromeria varia BENTH.	—	1	+
Phagnalon rupestre (L.) DC.	—	1	—
Reichardia tingitana (L.) ROTH	—	—	1
Romulea columnae SEB. var. *grandiscapa* (WEBB) PIT.	—	1	—
Stipa capensis THUNB.	—	1	—
Teloschistes sp.	1	—	1
Number of species, vascular plants	5	12	8
Number of species, lichens	1	0	1

sima, Odontospermum intermedium, and *Euphorbia obtusifolia* ssp. *regis-jubae* (cf. SUNDING 1972 p. 156).

(2) Vistas de las Nieves, 1 km southwest of Ermita de las Nieves, 500 m s.m. (only a few specimens were seen and no collection was made).

Steeper slope (inclination approx. 20°), resulting in less loose soil and more solid rock on the surface. Better protected and less wind-eroded than in the first-mentioned locality. Higher number of associated plant species (see Table 1, Column 2) and probably less influenced by grazing.

(3) Vistas de las Nieves, 0.5 km southwest of Ermita de las Nieves, 540 m s.m. (SUNDING No. 2514).

Near the edge of the mountain plateau where it drops abruptly down to the sea shore. Ecological conditions very much the same as in the first stand, thus much grazed and wind-eroded. A soil sample from the rhizosphere of *H. monogynum* gave pH 7.3 (measurement by glass electrode pH-meter). For associated plant species, see Table 1, Column 3.

Helichrysum monogynum was not seen anywhere in the areas between the three mentioned localities or in adjacent areas, although it might have been overlooked (when not in flower) among the equally greyish-green *Phagnalon rupestre* and *Helianthemum canariense.*

With the specimens of *H. monogynum* collected by Mr. ØVERGAARD somewhat more towards the east, very few data on the ecology of the locality were given; the only information being that the plant was rare and grew "together with *Odontospermum*".

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Evolutionary Trends in the Atriplex triangularis Group of Scandinavia

I. Hybrid Sterility and Chromosomal Differentiation

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ABSTRACT

GUSTAFSSON, M. 1973. Evolutionary trends in the Atriplex triangularis group of Scandinavia. I. Hybrid sterility and chromosomal differentiation. — Bot. Notiser 126: 345—392.

The *Atriplex triangularis* group is composed of diploid (2n=18) annual species restricted to littoral biotopes. The main aim has been to elucidate the type and degree of heterozygous chromosomal alterations within populations, as well as chromosomal differentiation and hybrid sterility of intra- and interspecific combinations of populations. The breeding system, dispersal and germination are discussed. Crossing experiments within *A. longipes* ssp. *praecox* give rise to F_1 hybrids with reduced male fertility, while crosses within *A. triangularis* and *A. longipes* ssp. *longipes* have high fertility values. Little or no correlation exists between geographical distance separating parent populations and fertility of hybrid offspring. Within taxa chromosomal and morphological differentiation have probably evolved separately. Fertility of interspecific crosses is relatively high, except for crosses entailing *A. longipes* ssp. *praecox*. Hybrid seeds show reduced germination. Baltic populations of *A. longipes* ssp. *praecox* have been crossed with populations of *A. longipes* ssp. *longipes* from the Baltic and other regions. Hybrid sterility is most pronounced in the Baltic region, where the taxa occur sympatrically. In hybrid plants a strong correlation exists between reduced fertility and the frequency of bridges at anaphase I. Bridge formation is probably due to crossing-over within paracentric inversions in a heterozygous state rather than to breakage and reunion phenomena. Formation of bivalents is normal, even in hybrids with reduced fertility.

CONTENTS

INTRODUCTION

The present paper presents the results of experimental work on the *Atriplex triangularis* complex in Scandinavia. *A. triangularis* in its broadest sense is distributed throughout most parts of the world and is more or less restricted to littoral biotopes. In the *A. triangularis* group, which is composed of a polymorphic aggregate of annual species, the most conspicuous differentiation appears to have taken place in the northeastern part of North America and in the central, western and northern parts of Europe. This polymorphism is perhaps most marked in Scandinavia where a great number of taxa have been distinguished on an exclusively morphological base. The taxonomy and morphological variation within the *A. triangularis* complex will be treated in a separate paper, but the following taxa are recognized in this paper: *A. calotheca* (RAFN) RAFN & FRIES,[1] *A. glabriuscula* EDMONDST., *A. longipes* DREJ. ssp. *longipes*, ssp. *praecox* (HÜLPH.) TURESS. and *A. triangularis* WILLD. *A. glabriuscula* and *A. triangularis* have large areas of distribution, while the others are more or less restricted to the coasts of Scandinavia.

The wide morphological variation is due to a high degree of ecological differentiation and to spontaneous hybridization. The importance and effects of ecological differentiation in the genus *Atriplex* has already been outlined by TURESSON (1922 a, 1922 b and 1925). However, very little attention has been paid to the cytological variability and differentiation both within and between the different species of *Atriplex*. Thus a great part of the present investigation has been performed to elucidate the occurrence and degree of chromosomal rearrangement within populations, as well as between intra- and interspecific combinations of populations. In addition, studies of the breeding system and dispersal, supplemented by field studies of population structure and ecology seemed desirable.

[1] According to the typification made by TASCHEREAU 1972 *A. hastata* L. is identical with *A. calotheca* (RAFN) RAFN & FRIES. However, the name *A. hastata* L. has been rejected here as being a long-persistent source of error (cf. HANSEN & PEDERSEN 1968 and TASCHEREAU 1972).

Table 1. Morphological appearance of the taxa in the *A. triangularis* complex.

Taxon	*A. calotheca*	*A. glabriuscula*	*A. longipes* ssp.		*A. triangularis*
			longipes	*praecox*	
Height (cm)	20—100	10—80	15—60	5—20	10—100
Lower leaves					
Form	Triangular-hastate	Triangular-hastate	Rhomboid	Rhomboid-ovate	Triangular-hastate
Base	Truncate	Truncate	Cuneate	Cuneate	Truncate
Length/width ..	0.9—1.3	0.9—1.4	1.6—2.7	1.5—2.5	0.9—2.0
Margins	Laciniate	Dentate-sinuate	Entire-dentate	Entire-dentate	Dentate
Bracteoles	Herbaceous All sessile	Fleshy All sessile	Herbaceous Axillary stalked	Herbaceous All ± sessile	Herbaceous All sessile
Form	Triangular	Rhomboid	Rhomboid	Rhomboid-ovate	Triangular-rhomboid
Margins	Laciniate	Entire-dentate	Entire-dentate	Entire-dentate	Entire-dentate
Surface	Veined	Smooth	± reticulate	Veined	Veined
United	At base	To one half	At base	At base	At base

MATERIAL

The present investigation has been carried out at the Institute of Plant Taxonomy, Lund, during the years 1968 to 1972 and is almost entirely based upon material collected by the author. Most of the cultivated material, including artificially produced hybrid progenies, is preserved at LD.

Seeds of spontaneous material were collected at random throughout the entire populations and sown in pots at the Lund Botanical Gardens. In the spring about 30 representatives of each population were transplanted into individual pots. Those kept in greenhouses were mainly used for crossing experiments, while those grown outdoors in the Gardens were used for comparative cultivation experiments.

The origins of these populations are listed at the end of this paper. The methods used are presented in the respective sections.

DELIMITATION OF TAXA

The morphological differences between the species are few and rather slight. The most relevant characters are the shape and size of leaves and bracteoles. Most other characters, such as the appearance of the perianth and the size and shape of seeds overlap in different species. The appearance of the species is summarized in Table 1 and Fig. 1.

A. calotheca (RAFN) RAFN & FRIES: Lower leaves triangular to hastate, laciniate, upper ones irregular laciniate, often with a somewhat cuneate base. Inflorescence branched, dense. Bracteoles green, triangular, herbaceous, sessile, united at base only.

A. glabriuscula EDMONDST.: Lower leaves triangular to hastate, dentate to sinuate, upper ones triangular to rhomboid, dentate to entire. Inflorescence loose, branched or unbranched. Bracteoles in the upper part green to brown, at base brown to black, rhomboid, thick and fleshy, sessile, dentate to entire, united to the middle.

A. longipes DREJ. ssp. *longipes*: Height usually more than 20 cm. Lower leaves rhomboid, dentate to entire, often prominent basal lobes, upper ones rhomboid to lanceolate, often entire. Inflorescence loose, branched or unbranched. Bracteoles green, rhomboid to triangular, herbaceous, dentate to entire, the axillary ones at least with long stalks, united at base.

A. longipes DREJ. ssp. *praecox* (HÜLPH.) TURESS.: Height usually less than 20 cm. Lower leaves rhomboid to ovate, dentate to entire, often prominent basal lobes, upper ones ovate to lanceolate, often

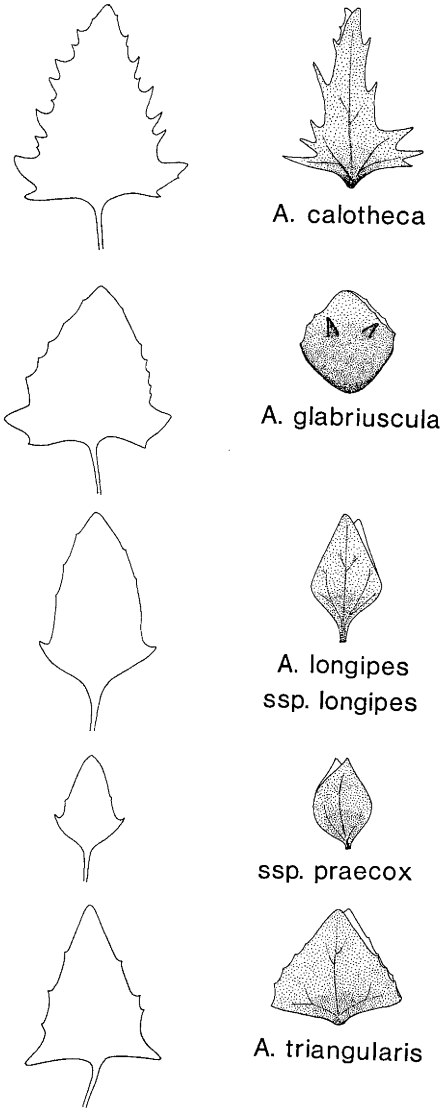


Fig. 1. Morphological appearance of lower leaves and bracteoles of the taxa within the *A. triangularis* complex.

entire. Inflorescence often unbranched, loose. Bracteoles green, rhomboid to ovate, herbaceous, usually entire, tapering to a short petiole, united at base.

A. triangularis WILLD.: Lower leaves triangular to hastate, dentate, upper ones triangular to rhomboid, rarely lanceolate, dentate. Inflorescence loose, branched or simple. Bracteoles green to brown, triangular to rhomboid, herbaceous, sessile, united at base only.

HABITAT

All the species within the *A. triangularis* group are true halophytes and almost exclusively restricted to littoral biotopes. The only exception is *A. triangularis*, which may occur more or less temporarily as a weed along roadsides, in farm-yards, etc. The littoral biotopes inhabited by the *Atriplex* species may be divided into two categories according to degree of exposure to the sea. The most exposed biotopes, such as sandy or rocky shores, the lower parts of salt-marshes and some communities of seashore meadows are greatly influenced by violent storms, tides or washing by the sea, particularly during spring and autumn. It is not uncommon that whole plants are covered by water for short periods of time, or that part of a population is washed away before setting seed. The vegetation is dominated by prostrate ecotypes of common halophytes and other species. In these habitats most of the species in the *A. triangularis* group are represented by forms having a prostrate habit, long basal branches and a short main stem.

The other kind of biotopes need more sheltered conditions for their development, such as those found in calm bays. In these biotopes the littoral zone is dominated by belts of *Phragmites* or marshes. In the marshes the *Atriplex* species often inhabit communities dominated by *Scirpus maritimus*. Other widely distributed species in this habitat are *Phragmites communis, Scirpus tabernaemontani, Aster tripolium* and *Triglochin maritimum*. Where the water is more brackish *Scirpus maritimus* is more or less replaced by *Phragmites communis* and/or *Scirpus tabernaemontani*. The substrate is usually a soft, muddy or sandy soil, and in fact

Table 2. Habitats of the taxa within the *A. triangularis* complex. + indicates that the taxa is common in the habitat, (+) that it is rare.

Kind of biotope	EXPOSED BIOTOPES						MORE SHELTERED BIOTOPES	
	Shores with rubble	Sandy beach	Salt-marshes occurring in			Marsh-land	The *Scirpus maritimus* community	The *Phragmites* community
			The Baltic	W. Scandinavia	N. Norway	SW. Denmark		
Vegetation type	Low	Low—tall	Low	Low	Low	Low	Tall	Tall
Substrate	Stones, gravel	Sand	Stones, gravel mud	Stones, gravel, sand, mud	Stones, gravel, mud	Sand, mud	Sand, mud	Sand, mud
A. calotheca		(+)					+	
A. glabriuscula	+	(+)		+			+	
A. longipes ssp. *longipes*				(+)			+	(+)
ssp. *praecox*			+		+			
A. triangularis	+	+	+	+	+	+	+	+

the *Scirpus maritimus* community is often a pioneer in muddy bays. These marshes are probably not wholly natural, as they have been influenced by man for a long time. They are often heavily grazed. In these biotopes the *Atriplex* species are represented by forms having an erect habit, ascending branches and a long, stout main stem.

The different taxa have somewhat different ecological amplitudes and preferences (Table 2). *A. triangularis* is most unspecific in this respect, it inhabits most communities in all kinds of maritime biotopes, from sandy or rocky shores to different seashore meadows and salt-marshes. *A. glabriuscula* is also rather tolerant. Along the Scandinavian west coast it is fairly common in exposed stony marshes or on shores covered with rubble, but on both sides of Öresund (The Sound) it is found predominantly growing in marshes dominated by *Scirpus maritimus*. The other taxa are to a greater extent restricted to a certain kind of biotope.

A. calotheca and *A. longipes* ssp. *longipes* are mainly restricted to marshes, preferably to *Scirpus maritimus* communities, *A. longipes* in the lower parts and *A. calotheca* also in drier areas higher up in this zone. In addition *A. longipes* ssp. *longipes* may inhabit exposed marshes along the Swedish west coast, and *A. calotheca* may rarely occur on sandy shores or on banks of seaweed.

A. longipes ssp. *praecox* is confined to seashore meadows and marshes with a low vegetation type. It is usually found growing in the lower, rather wet parts, mainly in more or less ephemeral areas with very little vegetation, sometimes in pure gravel. In the Baltic region *A. longipes* ssp. *praecox* may grow together with *Agrostis stolonifera, Festuca rubra, Juncus gerardi, Eleocharis uniglumis, Glaux maritima, Plantago maritima, Triglochin maritimum* and low biotypes of *Aster tripolium*. In the northern parts of Norway *A. longipes* ssp. *praecox* inhabits salt-marshes, which are rather different from the southern Scandinavian ones. According to GILLNER (1955) they are transitional between the Arctic forms and those typical for the boreal parts of the Atlantic region. The primary communities are dominated by *Puccinellia* species, either

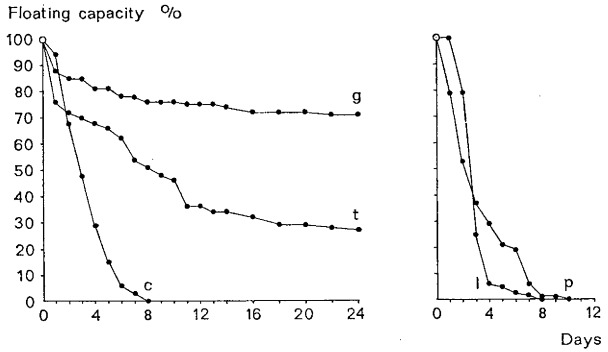


Fig. 2. Floating capacity of bracteoles (including seeds) of the taxa in the *A. triangularis* complex. Floating capacity (vertical axis) in relation to time (horizontal axis). — c: *A. calotheca.* — g: *A. glabriuscula.* — l: *A. longipes* ssp. *longipes.* — p: *A. longipes* ssp. *praecox.* — t: *A. triangularis.*

P. maritima or *P. phryganodes,* followed by a zone of *Carex* species, *C. subspathacea, C. salina* and *C. mackenziei,* further a zone of *Mertensia maritima, Juncus gerardi, Gentiana detonsa, Plantago maritima* and *Festuca rubra. A. longipes* ssp. *praecox,* together with *A. triangularis,* is found growing in the lower parts of these marshes.

DISTRIBUTION

A. calotheca: Rather common along the coasts of western Scandinavia, from the eastern part of Jylland to Halland, rare northwards to Oslo. Rare in the Baltic region, but more common on the island of Öland.

A. glabriuscula: Mainly distributed in the western parts of Scandinavia, rare in the northern parts of Norway and in the Baltic region.

A. longipes ssp. *longipes:* Rather common in the southern parts of Scandinavia northwards to Oslo, and along the Swedish east coast northwards to Stockholm.

A. longipes ssp. *praecox:* In the northern part of Norway common from Bodö to the Varanger Peninsula, in Sweden exclusively along the east coast, from Blekinge to Gästrikland and on the islands of Öland and Gotland, in Finland north to Vaasa and on the Åland archipelago.

A. triangularis: Frequently occurring along all the coasts of Scandinavia.

DISPERSAL

Local dispersal: The local dispersal is conditioned by a combination of dispersal by the agency of wind and sea-water. The range of dispersal by wind is probably very limited, as the *Atriplex* seeds enclosed in their bracteoles are relatively heavy. Probably the greatest part of even the local dispersal occurs by the agency of water. In nature, floating fruits have often been observed. The fruits are mainly dispersed one at a time.

Dispersal to new localities: Dispersal of the *Atriplex* fruits from one locality to another probably occurs exclusively by the agency of the sea. The dispersal range is probably correlated with the floating capacity of the bracteoles enclosing the seeds. Many factors may of course influence the effectiveness of the dispersal, for example the salinity of the sea-water and currents. But on the whole the greater the floating capacity of the bracteoles, the greater is the chance of successful dispersal. In order to test the length of the floating period 100 fruits of each taxon were placed in containers filled with sea-water with a salinity of 1.5 %. Circulation of the water was maintained all the time by means of an electric pump and the water was sprinkled all over the surface. The numbers of floating fruits were controlled day by day. The results are summarized in Fig. 2.

The floating capacity of fruits belonging

to *A. calotheca* and *A. longipes* decreased very rapidly. After 2—3 days only 50 % of the fruits were still afloat and after 8 and 10 days respectively all had sunk. The floating capacity of the fruits of *A. glabriuscula* and *A. triangularis* is rather high. After 24 days 71 % and 27 % respectively were still afloat. The bracteoles of *A. glabriuscula* are particularly well adapted for dispersal by sea. The two bracteoles are united above the middle, are thickest in the basal parts and become hard when mature. The tissue of the bracteoles is characterized by large, air-containing cells at least in the basal parts. The other species have herbaceous bracteoles, united only at the base and built up of a rather compact cell structure, except for those of *A. triangularis* which are somewhat loose in the basal parts. Most likely the shape and size are of subordinate importance, as the relatively large and laciniated bracteoles of *A. calotheca* are not advantageous in this respect. Fruits of *A. calotheca* and *A. longipes* keep floating long enough for dispersal at least for short distances and those of *A. glabriuscula* and *A. triangularis* even for long distances.

However, the relation between dispersal range and the morphology of the bracteoles must be interpreted carefully. The fruits of the perennial species *A. recurva* D'URV. have a limited floating capacity, although it has hard and fleshy bracteoles, united above middle, like those of *A. glabriuscula* (GUSTAFSSON 1970). But, in the *A. triangularis* group there may exist a correlation between the area of distribution and the dispersal range, as the species with the greatest floating capacity also have the largest distributional areas. *A. triangularis* is distributed throughout almost the whole world, and *A. glabriuscula* in the western to the northwestern parts of Europe and in the northeastern part of North America. But, the wide area of distribution at least of *A. triangularis* may to some extent be due also to human agency. On the other hand *A. calotheca* and *A. longipes* have restricted areas of distribution, being chiefly limited to Fenno-Scandia.

FLOWERS, FLOWERING TIME AND BREEDING SYSTEM

The species of the *A. triangularis* group are monoecious with both male and female flowers. Bisexual flowers have not been observed in this group. The female flowers are highly reduced, both perianth and stamens being completely lacking. The ovules are protected by two leaf-like bracteoles, which are rather small before anthesis, but grow very rapidly after pollination. The two stigmas are papillated to the base and exceed the bracteoles in length at anthesis. The male flowers have five-lobed perianths and five stamens placed opposite the lobes. The gynoecium is reduced to a small remnant in the centre. Before anthesis the filaments are relatively short and the whole stamen bent inwards and protected by the perianth. Some hours before anther dehiscence the filaments increase in length very rapidly and finally become bent outwards as are the thecae. Dehiscence is extrorse and the most terminal male flowers are the first to dehisce. The pollen grains are rather small, 20—30 μ, spheroidal and polyporate.

The flowering period of *A. longipes* differs somewhat from that of the other species, although overlapping occurs in some regions. In the Baltic region, *A. longipes* ssp. *praecox* starts flowering in the middle of May, ssp. *longipes* at the end of May, the flowering period lasting till the end of June in both taxa. *A. longipes* has already reached maturity when the other species start flowering at the end of July or beginning of August. But occasionally, particularly in populations from west Sweden, a few individuals can still be found flowering at the beginning of August when the other species begin to flower. The degree of overlapping is about one week in culture experiments

Table 3. Degree of protogynous development in the *A. triangularis* complex. + indicates well-developed female flowers with visible papillae and successful pollination, (+) that only a few flowers were successfully pollinated, — unsuccessful pollination. Further explanation in the text.

Taxon	Days before anther dehiscence										
	0	1	2	3	4	5	6	7	8	9	10
A. calotheca	+	(+)	(+)	—	—	—	—	—	—	—	—
A. glabriuscula	+	+	(+)	—	—	—	—	—	—	—	—
A. longipes											
ssp. *longipes* ...	+	+	+	+	+	+	+	(+)	—	—	—
ssp. *praecox*	+	+	+	+	+	+	+	+	(+)	—	—
A. triangularis	+	+	(+)	—	—	—	—	—	—	—	—

and probably about the same in nature. In northern Norway, *A. triangularis* and *A. longipes* ssp. *praecox* flower at the same time, from the middle of July to the beginning of August. However, in cultivation experiments populations of *A. longipes* ssp. *praecox* originating from Norway flower at the same time as those from the Baltic region, indicating that genes for early flowering are present. In the Norwegian populations, their action is apparently inhibited by environmental conditions. The long, severe winter in that region does not admit of germination until April or May, and despite a rapid rate of development the *praecox* populations are not able to flower until the middle of July.

The length of the flowering period varies greatly for each plant depending on natural conditions but usually lasts for five to ten days. In *A. longipes* the flowers situated in the axils are female, while the terminal inflorescences are composed of both male and female flowers arranged in spikes. In the other species almost all the pistillate flowers are situated terminally.

In order to investigate the length of the protogynous stage, i.e. the time lapse between stigma receptivity and anther dehiscence, plants from three populations of each taxon were emasculated before anthesis. Only the most terminal male flowers were left as these dehisce first and were carefully isolated in pergamine bags. The development of the stigmas and the shape of the papillae in the female flowers were observed. As far as possible plants at varying stages of development were chosen, and before pollination the stigmas were checked to see that they were free of pollen grains. Each individual was then pollinated with pollen from another individual from the same population. Anther dehiscence of the terminal flowers was observed. The number of days that elapsed between pollination and anther dehiscence was determined. After one to two weeks it was possible to see if the female flowers had been successfully pollinated or not, as the swelling of the ovules and the growth of the bracteoles take place rather quickly. The results are shown in Table 3.

In populations of *A. longipes* the stigmas of the female flowers are receptive up to eight days before anther dehiscence and this stage of protogyny seems to be of equal length in both terminal and axillary female flowers. In populations of the other species the male and female flowers seem to be well developed simultaneously, or the female flowers one day earlier than the male flowers. The figures in this experiment are of course not definite, other populations may have a more extended protogynous stage under other conditions. But the tendency is quite clear. *A. longipes*

has a relatively long period of protogyny, *A. calotheca, A. glabriuscula* and *A. triangularis* at least, a very short one.

The relation between self- and cross-fertilization is rather difficult to estimate. The relatively long protogynous period of *A. longipes* will favour the amount of seeds obtained by cross-pollination and at least a great number of the pistillate flowers will be pollinated by foreign pollen. The protogynous stage is less evident within the other species and as no system of self-incompatibility has been developed the degree of self-fertilization may be rather high or even dominant. However, the number of spontaneous hybrids found and the maintenance of chromosomal heterozygosity in some populations of *A. longipes* and *A. triangularis* at least, indicate that cross-fertilization is not particularly uncommon. The most effective breeding system from an evolutionary point of view should be that of *A. longipes*. Development of protogyny increases the proportion of cross-fertilization, which may give rise to new, positive gene combinations and the maintenance of heterozygosity in the populations. But, at the same time, the formation of an adequate number of seeds every year is guaranteed by the possibility of inbreeding. The species may be designed as more or less facultatively autogamous.

The small, smooth pollen grains, highly reduced female flowers with prolonged, papillated stigmas reaching beyond the bracteoles and the lack of nectar indicates wind pollination. On the other hand, small beetles have sometimes been observed crawling in a fortuitous manner over the terminal inflorescences.

GERMINATION

The species belonging to the *A. triangularis* group are all annuals, and their reproductive capacity is entirely dependent on adequate seed formation and a relatively high germinating capacity. Most other halophytes are perennials with a greater or lesser degree of vegetative propagation. Further, it is important, at least for annuals, to keep the seeds in a viable condition. MILTON (1939) has shown that seeds of different halophytes were found buried in the mud of a salt-marsh, but in very few species are the seeds buried in a viable condition or in large numbers, except for *Glaux, Agrostis* and *Puccinellia*. A prominent feature was the small number of seeds of the dominant species, but instead these halophytes had a high degree of vegetative propagation to compensate the low reproduction ability by seeds.

One of the greatest inhibiting factors as regards the germination of halophytes is the negative effects of a too high concentration of sodium chloride in sea-water. The great majority of halophytes germinate best under fresh-water conditions, but some germination may be expected in water with up to 2 % NaCl. The most tolerant in this respect is *Salicornia stricta,* in fact it is the only species which can withstand a salinity of 5—10 %. In *Aster tripolium* an increase in the salt concentration also delays the start of germination (MONTFORT & BRANDRUP 1927). A similar reaction has been recorded by BEADLE (1952) for five halophytic *Atriplex* species native to Australia. Germination is inhibited by a high Cl^- concentration in the fruit sheath, and germination does not occur until this concentration has been reduced by rainfall. It is evident that a reduction in the salinity of the surface layers of the soil is necessary for successful germination. In marshes of the Northern Hemisphere such a reduction occurs in the spring time when the seeds of many halophytic species germinate.

Germination within the A. triangularis Group

In natural habitats the seeds germinate exclusively in the spring, germination in the autumn has not been observed. Under

natural conditions the seeds have a period of dormancy lasting for about six to eight months, from August to the end of March. The length of the period during which the seeds are viable has not been investigated in detail, but only a very low percentage of seeds that have been kept in a refrigerator or at room temperature for two years or more will germinate. The seed reserve is probably very small. The germination experiments indicate that all the viable seeds germinate at the first occurrence of suitable conditions, i.e. in the springtime.

There is no reason to believe that *A. triangularis* and related species react in any different way from other halophytes, as regards the negative effects of high salt concentrations on germination. On the other hand, very little is known about the effects of solutions with different degrees of salinity on seeds before germination. It is possible that seeds exposed to sea-water with a high salinity for a long period of time, except during the germinating period, will be permanently damaged, which decreases the capacity to germinate. In this case the damage is irreparable even if germination takes place under fresh-water conditions.

The aims of these experiments have been to investigate the germination of dimorphic seeds, of spontaneous seeds, the role of seed dormancy and cold treatment, and the effects of different salt-water solutions on germination. All the germination experiments are based on spontaneous seeds collected in 1969 and 1970 and the analyses were carried out in 1970 and 1971. Well-developed seeds of different populations were selected at random, sown in pots containing two parts of sterilized soil to one part sand. The pots were then placed out of doors in the Botanical Gardens, Lund. The number of germinated seeds was investigated in the spring.

Germination of dimorphic seeds: Heteromorphic seeds are found in the *A. triangularis* group as well as in other *Atriplex* species, for example in *A. hortensis* and *A. nitens*. In these species both vertical and horizontal seeds occur, some of them are small, black and have a thick testa, others rather large, light brown and have a thin testa. BECKER (1913) has shown that these seed types show different germination responses, the brown seeds germinating more rapidly than the black ones and probably also in greater numbers. In *A. triangularis* sensu lato the seeds are dimorphic too, i.e. brown seeds and somewhat smaller black ones. However, the differences in size are small and both types have a thick testa.

Table 4. Germination of large brown, and small black seeds in *A. longipes* and *A. triangularis*. 50 seeds of each category were used each time.

Date	*A. longipes*		*A. triangularis*	
	Large Brown %	Small Black %	Large Brown %	Small Black %
1.3	—	—	—	—
15.3	2	4	2	—
21.3	2	6	10	4
30.3	12	14	24	16
5.4	40	38	42	34
12.4	68	82	80	72
19.4	86	90	94	98
1.5	86	90	94	98

Fifty seeds of each type were sown in 1971 and the germination was controlled eight times, from March to May. The results are shown in Table 4. At the beginning of the germinating period a greater percentage of the brown seeds of *A. triangularis* germinated but this difference disappeared at the end of the period. In *A. longipes* the situation was quite the opposite, the number of germinated black seeds being greater each time. On the whole, the differences between the two types of seeds of the *A. triangularis* group seem to be small, and in fact the maximum values were reached simultaneously.

Germination of spontaneous seeds: The germination of 48 populations in all,

Table 5. Germination of seed samples originating from different populations in nature. n indicates the number of seed samples (=populations) investigated.

Taxon	Germination %								n
	0	40	50	60	70	80	90	100	
A. calotheca	—	—	1	—	1	1	1		4
A. glabriuscula	—	—	—	—	2	1	—		3
A. longipes									
ssp. *longipes*	—	2	1	1	4	3	1		12
ssp. *praecox*	—	—	—	3	3	5	1		12
A. triangularis	—	—	—	5	5	6	1		17
Total	—	2	2	9	15	16	4		48
%	—	4.2	4.2	18.8	31.3	33.3	8.3		

originating from seeds collected in nature, was checked in the spring. The results are summarized in Table 5. About 8 % of the populations germinate to between 90 and 100 %, 83 % to between 60 and 90 % and the rest to between 40 and 60 %. There are only small differences between the taxa, some populations showing reduced germination, others not.

Seed dormancy and the effects of cold treatment: Well developed seeds from six populations were treated according to three different methods over a period of five months. Six germination tests were carried out, one at the beginning of the testing period and then one each month. Method 1: For each test 30 seeds from each of the 6 populations (5×6×30) were sown in pots and placed out of doors in the Botanical Gardens. Method 2: 5×6×30 seeds were kept in paper bags at room temperature (18°C). Method 3: In addition, for each test 30 seeds from 3 populations (5×3×30) were kept in paper bags in a refrigerator (−5°C).

The frequency of germinated seeds is shown in Fig. 3. Only a small number of the seeds kept at room temperature or in the refrigerator germinated throughout the entire period. The highest values were 6 and 8 % respectively. The germination of the seeds sown out of doors was much higher. After one month only 3 % of the seeds had germinated, but then germination increased successively. After two months 29 % had germinated and 69 % after five months. Although the number of seeds used is limited, the tendency is quite obvious. Seed dormancy or cold treatment alone is not sufficient to give a high percentage of germinated seeds. Apparently they need at least a short period of dormancy combined with continuous fluctuations of temperature

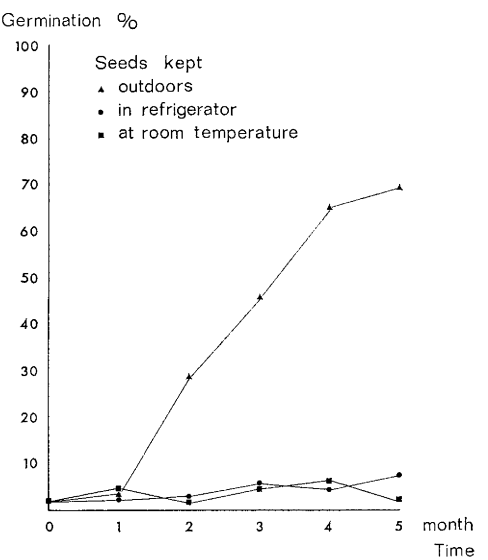


Fig. 3. Germination of seeds treated according to three different methods over a period of five months. The seed samples have been kept outdoors in the Botanical Gardens, in a refrigerator (—5°C) and at room temperature (18°C) respectively.

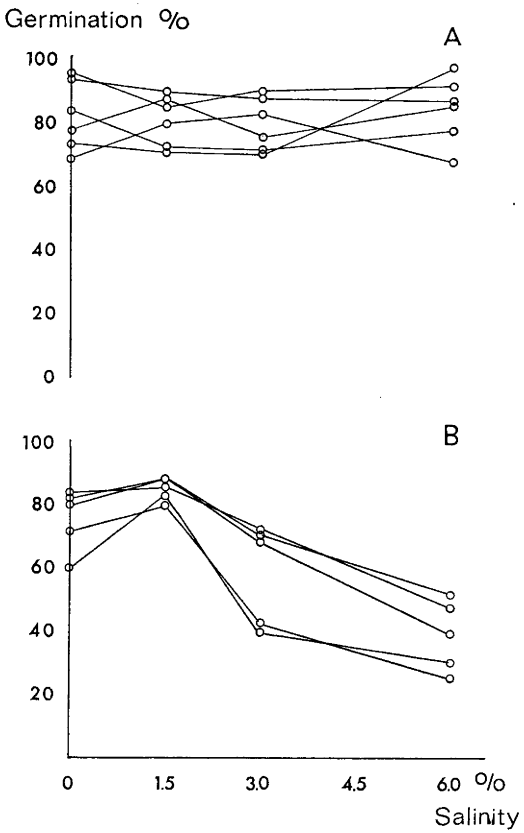


Fig. 4. The effects of different salt-water solutions on germination. — A: Seed samples of populations from the western parts of Scandinavia. — B: Seed samples of populations from the Baltic region. — Further explanation in the text.

above and below zero, as was the situation during the winter in the Botanical Gardens. The same situation is probably found in natural habitats.

Effects of different salt-water solutions on germination: Fifty seeds from eleven populations, five originating from the Baltic region and six from the western part of Scandinavia, were treated in different solutions for about two months: a) pure tap water, b) sea-water with a salinity of 1.5 %, c and d) sea-water with salt added raising the salinity to 3 and 6 % respectively. After treatment the seeds were sown in pots which were placed out of doors in the Botanical Gardens. In order to avoid counteracting the effects of a possible uptake of salt-water by the seeds the pots were not watered until the spring.

Germination is shown in Fig. 4. The seeds from the western part of Scandinavia are not affected by the solutions used, not even by 6.0 %, which has about twice or three times the salinity of the sea-water in this area. The minimum values for tap water and for the solution with a salinity of 6.0 % are almost the same, 69 and 68 % germinated seeds respectively. The germination of the seeds from the Baltic region is, however, lower. Germination in tap water varies between 60 and 84 %. Obviously there is an increase in germination in the 1.5 % solution, a fact which is hard to explain. In solutions with a salinity of 3.0 and 6.0 %, germination decreases successively and varies in the latter from between 26 and 52 %. Apparently seeds originating from the Baltic region where the sea has a low salinity, are more sensitive to solutions with a high salinity than those from the western part of Scandinavia where the sea has a relatively high salinity. The same physiological reaction pattern has been observed in *Aster tripolium* (MONTFORT & BRANDRUP 1927). Seedlings originating from parents from saline habitats have a better root growth in salt-water solutions than those from fresh-water habitats.

CYTOLOGY

Aims and Methods

The purposes of the cytological investigation have been to determine the chromosome number and to study the chromosome morphology in detail if possible. Further, investigations of meiosis and male fertility were carried out in order to estimate the degree of cytological variation and structural rearrangements within populations of different taxa.

A. calotheca	2n=18	WULFF 1936
A. glabriuscula	2n=18	WULFF 1936, LÖVE & LÖVE 1956, TASCHEREAU 1972
A. longipes		
ssp. *longipes*	2n=18	GUSTAFSSON 1972
ssp. *praecox*	2n=18	GUSTAFSSON 1972
	2n=36	LAANE 1966
A. triangularis	2n=18	WINGE 1917, WULFF 1937, HEISER & WHITAKER 1948, TARNAVSCHI 1948, HULME 1958, GADELLA & KLIPHUIS 1966, NOBS et al. 1971
	2n=36	HEISER & WHITAKER 1948

The somatic chromosomes have been studied in root tips and two different methods used:

(1) After cooling over a night at 5°C, the root tips were fixed in the Svalöv modification of Navashin-Karpeschenko. The root tips were then embedded in paraffin wax and sectioned at 10 µ. The sections were stained in 1 % crystal violet.

(2) The root tips were pretreated in 0.2 mM solution of oxyquinoline at 5°C over a night and then fixed in Carnoy (3:1). After hydrolysis for 8—10 minutes in 1-N HCl at 60°C they were stained in Feulgen for 2—3 hours. The root tips were then squashed in 45 % acetic acid.

Meiosis was studied in squash preparations. Young buds were fixed in Carnoy (3:1) and stained in Feulgen as described above. Several other pretreatments and fixatives were tested, but without satisfactory results.

Chromosome Number and Morphology

All the species are diploid and have 2n=18. No plant with a deviating number has been found, although specimens of 132 populations have been investigated. The different populations determined from each taxon are listed at the end of this paper.

A detailed analysis of the chromosomes is rather difficult, as they are small and very similar. All are metacentric to submetacentric and with a length of between 2 and 3 µ. The different pairs of homologues are difficult to identify. Now and then one pair with small satellites has been noticed in good preparations. This pair is submetacentric, with the satellite localized to the shortest arm. It is also the largest pair and at least sometimes possible to identify. Once or twice another pair with satellites has been observed too, but the satellites of this second pair are too small to be identified with certainty. The other pairs seem to have the same morphology, they are more or less metacentric and non-satellited. The differences in length are small and gradual. It has not been possible with the techniques used in this investigation to observe any differences between the karyotypes of the taxa. Further, no chromosomal rearrangement has been observed in mitotic chromosomes. The karyotype is in accordance with those of other European *Atriplex* species studied by the author.

Previous Reports

The basic number of the genus *Atriplex* is x=9. Most species are diploid, but tetraploids also occur. In the *A. triangularis* group the chromosome numbers given above have previously been reported.

HEISER and WHITAKER counted the chromosome number 2n=36 in root tips of *A. triangularis* and it may be a determination of endomitotic cells, which are common in this material. LAANE determined n=18 in young buds of *A. longipes* ssp. *praecox* originating from the Varanger Peninsula, Norway. However, all populations (13) from northern Norway counted by the author have 2n=18.

Endomitosis

Endomitosis, i.e. formation of large cells containing multiples of the normal

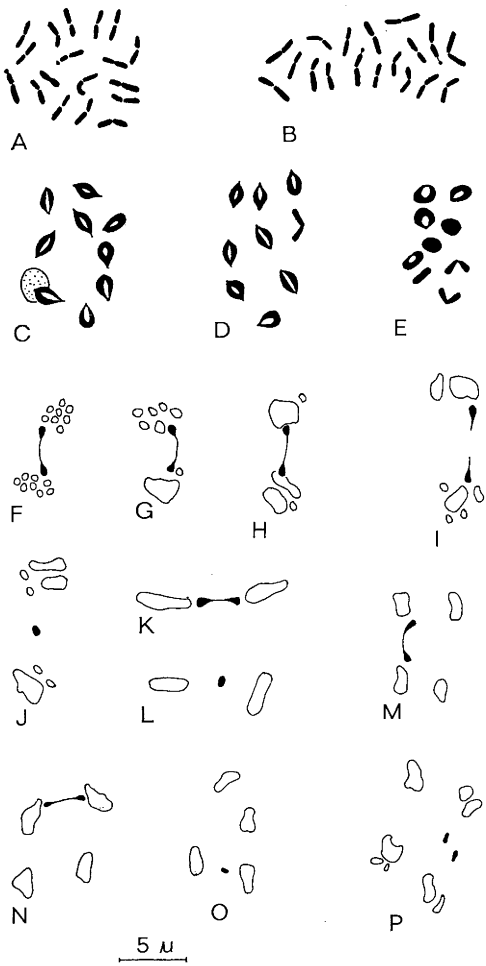


Fig. 5. Mitosis and meiosis in natural populations of the taxa in the *A. triangularis* complex. — A—B: Somatic metaphase plates (2n=18). — C—P: Meiosis. — C—E: Bivalent formation at diakinesis—metaphase I. — C: 9 ring bivalents. — D: 8 ring and 1 rod bivalent. — E: 7 ring and 2 rod bivalents. — F—J: Disturbances at anaphase I—telophase I. — F—H: Formation of one bridge. — I: Broken bridge. — J: Lagging chromosome. — K—L: Disturbances at metaphase II. — K: Remaining bridge. — L: Lagging chromosome. — M—P: Disturbances at anaphase—telophase II. — M—N: Remaining bridge since A I. — O: One laggard. — P: Two laggards.
A: *A. glabriuscula*, population GMK. — B, D: *A. calotheca*. — B: GHA. — D: GHB. — C: *A. longipes* ssp. *praecox*, G 116. — E, G—M, O, P: *A. triangularis*. — E, P: G 219. — G, I, J: G 233. — H: GAA. — K, L, M: G 170. — O: G 240. — F, N: *A. longipes* ssp. *longipes*. — F: G 113. — N: G 106.

chromosome number, is not uncommon in plant and animal tissues. The multiples of the somatic number arise through a doubling of the chromosomes without normal cell division. BERGER (1941) found in *Spinacia* that the frequency and polyploid level increase along the root tips. In the most distal parts exclusively diploid cells are observed, while both tetra- and octoploid cells occur in more differentiated regions. Such endomitotic cells have been noticed in many plant species of different families. In the *A. triangularis* group as well as in the entire family Chenopodiaceae, endomitosis is common and occurs in almost every root tip. Both tetraploid and octoploid cells have been observed, but cells belonging to the same cell layer often have a similar level of polyploidy. The size of the cells are quite different, the tetraploid cells being larger than the diploid ones, but smaller than the octoploid cells.

The function of these giant cells is somewhat problematic, but STEBBINS (1971) suggests that these polyploid cells with large nucleoli may be capable of synthesizing a great amount of proteins during a short period of time.

Table 6. A survey of meiosis in some populations of *A. longipes* and *A. triangularis*. Only populations with meiotic disturbances are noted. The origins of the populations are listed at the end of the paper.

Taxon	Population code	Diakinesis—Metaphase I	Anaphase
A. longipes ssp. *longipes*	G 106	9 bivalents	A II: Bridges and laggards
	G 113	9 bivalents	A I: Bridges
	G 142	9 bivalents	A I: Laggards
	G 145	9 bivalents	A II: Laggards
ssp. *praecox*	G 109	9 bivalents	A I: Bridges and laggards
	G 112	9 bivalents	A I: Bridges and laggards
	G 121	9 bivalents	A I: Bridges
			A II: Laggards
	G 154	9 bivalents	A I: Bridges and laggards
A. triangularis	G 170	9 bivalents	A I: Bridges
	G 219	9 bivalents	A II: Bridges and laggards
	G 233	9 bivalents	A I: Bridges and laggards
	G 240	9 bivalents	A II: Bridges and laggards
	GAA	9 bivalents	A I: Bridges and laggards
			A II: Bridges and laggards
	GBD	9 bivalents	A I: Bridges
	GBH	9 bivalents	A II: Bridges and laggards
	GBI	9 bivalents	A I: Bridges and laggards
	GBK	9 bivalents	A I: Bridges

Meiosis

The main purpose of the investigation of meiosis has been to examine the causes of reduced fertility in populations of *A. triangularis* and *A. longipes*. The number of plants examined in each population is rather small, because almost only plants with reduced fertility have been investigated.

The results, summarized in Table 6, are intended only to point out the meiotic disturbances observed in these plants. At diakinesis and metaphase I nine ring bivalents are usually formed, but occasionally one or two rod bivalents are present. The size of the bivalents is about the same, but at diakinesis one bivalent seems to be somewhat larger than the others. This bivalent probably consists of the largest chromosome pair with satellites, and at diakinesis it has been observed attached to the nucleolus. No disturbances at all have been observed at diakinesis and metaphase I, nine bivalents were found in all the 17 populations investigated. At anaphase I many plants have a normal separation of the chromosomes, nine to each pole. However, in all the seventeen populations there are plants with formation of bridges and laggards. Usually only one bridge is noticed but now and then two bridges are present. The fragments are usually small, sometimes too small to be easily identified, and the corresponding dichromatid is usually rather large. Variation in size of the fragments occurring in the same plant has not been observed. At metaphase II two cases of bridges remaining from anaphase I were observed (populations G 170 and GBH). Bridges have surprisingly often been observed at anaphase II, mostly remaining from anaphase I, but bridges apparently formed at anaphase II are occasionally present. Laggards are rather common both at anaphase I and II. Usually only one laggard is present at A I and one to four at A II. At meta-

phase II one such lagging chromosome has been observed lying outside the centre of the division. Regular tetrads are generally developed. Micronuclei and extra microspores are very rare even in plants with acentric fragments and bridges.

The disturbances are of the same kind in the whole of the material, bridges and laggards at anaphase. The formation of bridges and fragments may be the result of heterozygosity for paracentric inversions and/or meiotic breakage and reunion processes at prophase. An examination of pairing at pachytene is desirable in order to be certain of this. Unfortunately this is very difficult in material with such small chromosomes, but other information is available. These two hypotheses have been discussed in an earlier paper (GUSTAFSSON 1972). The disturbances found are probably due to heterozygosity for paracentric inversions on the following indications:

(1) Side-arm bridges and other asymmetrical configurations formed by chromatic breakages and reunions have not been observed, neither in the spontaneous material nor in artificial hybrids.

(2) Differences in the size of the fragments within an individual have not been observed.

(3) The variation of male fertility in various hybrid combinations can hardly be explained by chromatid and/or subchromatid crossovers, but rather by heterozygosity for paracentric inversions.

(4) In some crosses, e.g. GK 22, 28, 39 and 63 where one bridge was observed at anaphase I, all the hybrid plants have highly reduced fertility. If breakages and reunions have caused the situation observed there may possibly have been a conspicuously wider variation in male fertility.

(5) It is difficult to believe that breakage phenomena may cause great differences in the male fertility of adjacent populations of the same taxon such as those observed in *A. longipes* ssp. *praecox* from the Åland Islands. In two populations 50 % of the plants have reduced fertility, while almost all plants in two other populations are highly fertile.

Male Fertility

It has not yet been possible to investigate the meiotic behaviour of many individual plants and populations. However, variation in male fertility may indicate the frequency of heterozygous chromosomal rearrangements within populations. Male fertility has been determined as the percentage of well-developed pollen grains stainable in cotton blue. Usually three tests have been performed within each plant, as the percentage of morphologically good pollen may be influenced by environmental conditions. The results obtained are based on material raised from spontaneous seeds and cultivated in the Botanical Gardens, Lund.

In this paper, male fertility, morphologically good pollen and pollen fertility are terms used to express the same phenomenon, i.e. the frequency of stainable pollen. Naturally the stainability is not equal to the maximum number of germinating pollen grains, but it is the most adequate and the easiest way to estimate the number of well-developed pollen grains.

In Table 7 the fertility values found in different taxa are summarized. They constitute the average values from three tests grouped in intervals of 10 % as there is a certain degree of variation within plants. The variation is slight in plants with high fertility values, but somewhat greater in those with reduced fertility. The variation within populations of *A. longipes* and *A. triangularis* is shown in Figs. 6 and 7.

A. calotheca: In all the material examined, 83 plants from 6 populations, the fertility value is high. Six specimens have between 80 and 90 % and the remainder more than 90 % stainable pollen. Thus

Table 7. Male fertility in populations within the *A. triangularis* group showing regional variation. n indicates the number of plants investigated and N the number of populations represented in each region.

Taxon	Origin	Male fertility %							
		50	60	70	80	90	100	n	N
A. calotheca		—	—	—	6	77	83		6
A. glabriuscula		—	1	1	4	97	103		6
A. longipes ssp. *longipes* ..	Baltic	—	—	1	3	84	88		6
	W. Sweden	—	1	2	14	79	96		5
	Denmark	—	1	—	1	63	65		5
ssp. *praecox*	Åland	—	5	5	1	26	37		4
	E. Sweden	1	3	3	7	54	68		6
	Norway	2	—	1	3	51	57		4
A. triangularis	Baltic	—	—	—	4	86	90		5
	W. Sweden	2	—	3	14	137	156		8
	Denmark	1	—	—	5	96	102		6

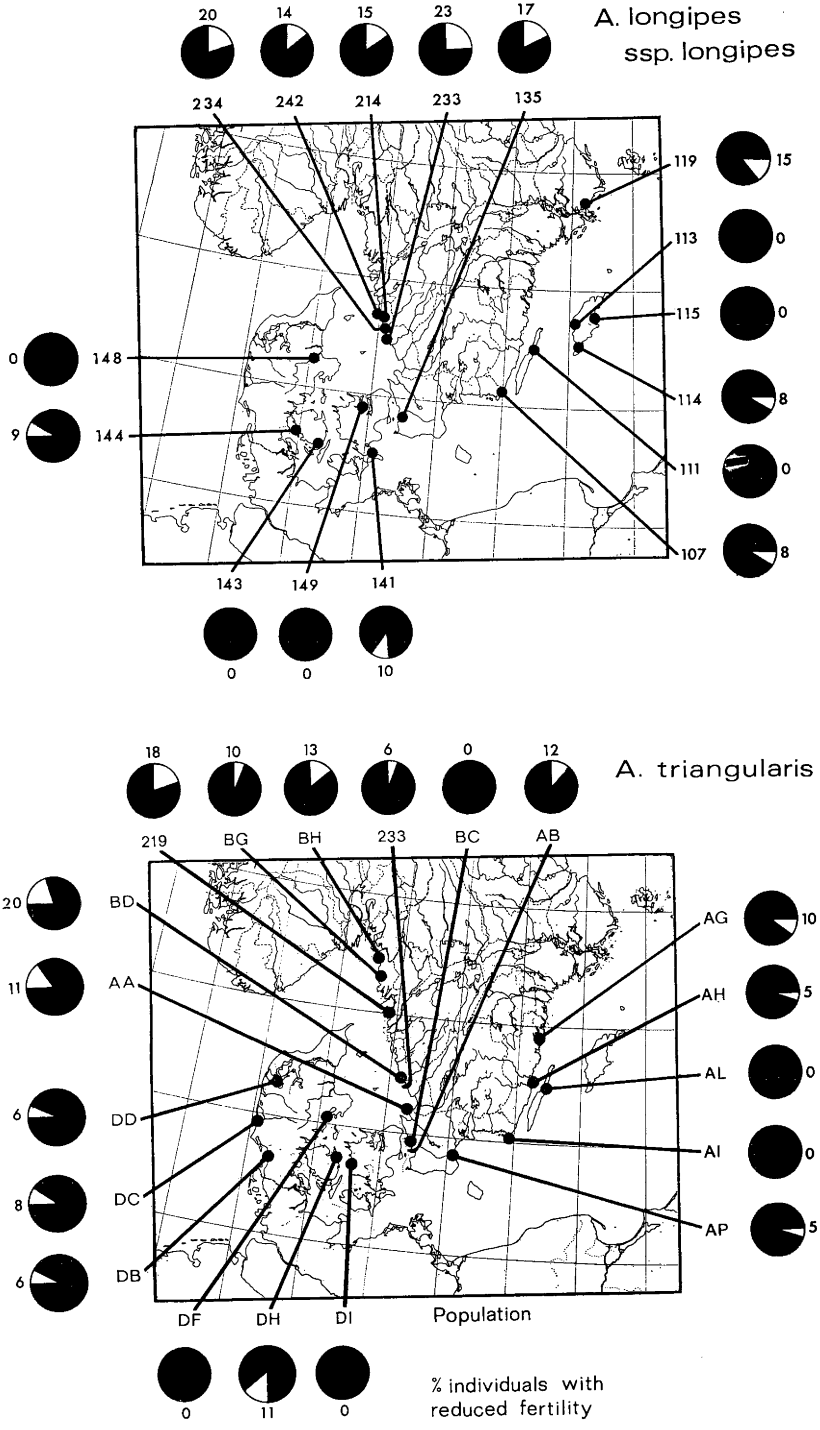
populations of *A. calotheca* seem to consist of plants with high fertility.

A. glabriuscula: In the six populations investigated about 94 % of the plants are almost completely fertile, i.e. > 90 %. The two individuals with the lowest values together with two of those having a fertility value between 80 and 90 % belong to the same population (GMK). The other populations consist solely of highly fertile plants.

A. longipes ssp. *longipes:* 249 plants from 16 populations have been investigated and most of them have high fertility values. The variation within populations from the Baltic region and from Denmark is small, about 95 % being almost completely fertile. In six populations there are no plants at all with reduced fertility and in the other five only one or two plants with 80 to 90 % good pollen. Only two plants (from populations G 119 and G 144) fall below 80 %. The variation is greater in populations from the western part of Sweden, all five populations having plants with reduced fertility. In all the mean degree of reduction is about 18 %. The increase in plants with fertility values between 80 and 90 % is obvious compared with other populations and further three plants have between 60 and 80 % stainable pollen. The reduction is distributed about equally in the different populations.

A. longipes ssp. *praecox:* Only two of fourteen populations are composed of exclusively highly fertile plants (populations G 88 and G 160). All the others have at least one individual with reduced fertility. A total of 162 plants has been investigated. The reduction in fertility is especially pronounced in populations G 87 and G 89, both originating from the Åland archipelago (Fig. 7). In these populations about half of the plants have less than 80 % stainable pollen. But on the other hand populations G 88 and G 90 from the same area have almost exclusively highly fertile plants. The amount of variation is about equal in all the other populations, a few specimens having reduced fertility. Taken as a whole 19 % of the material shows reduced fertility, 7 % having between 80 and 90 % and 12 % less than 80 % good pollen. The frequency of plants with reduced fertility is fairly high, at least compared with the other taxa.

A. triangularis: In all 19 populations have been investigated, 5 originating from the Swedish part of the Baltic, 8 from the Swedish west coast and 6 populations


A. longipes
ssp. longipes
20
14
15
23
17
234
242
214
233
135
119
15
113
0
115
0
0
148
114
8
9
144
111
0
107
8
143
149
141
0
0
10
A. triangularis
18
10
13
6
0
12
219
BG
BH
233
BC
AB
20
BD
AG
10
11
AA
AH
5
AL
0
6
DD
AI
0
8
DC
AP
5
6
DB
DF
DH
DI
Population
0
11
0
% individuals with
reduced fertility

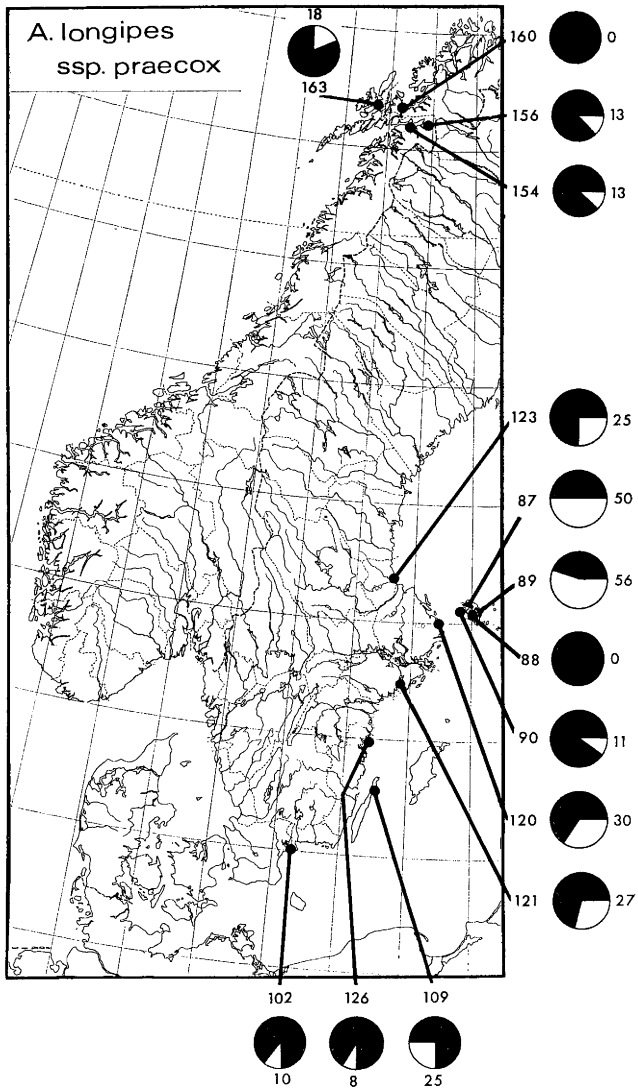


Fig. 6 (left). The frequency (%) of plants with reduced fertility (less than 90 % stainable pollen) in different populations of *A. longipes* ssp. *longipes* and *A. triangularis*.

Fig. 7 (right). The frequency (%) of plants with reduced fertility (less than 90 % stainable pollen) in populations of *A. longipes* ssp. *praecox*.

from Denmark. Most populations consist of plants with high fertility values. However, in some populations from the Swedish west coast a certain amount of variation has been observed (Fig. 6). Seven of eight populations include individuals with reduced fertility and four of these populations have plants with a fertility value below 80 %. Variation is greatest in population GBD where about 20 % of the plants have below 90 % and in two individuals the fertility value is as low as 50 to 60 %. Taken as a whole, 12 % of the plants investigated from this region show reduced fertility. The corresponding figures for the other regions are much lower, about 5 %. Three populations originating from the Swedish east

coast and four from Denmark have one or two plants with reduced fertility, but all have fertility values above 80 %, except for one plant belonging to population GDC which has 57 %.

CROSSING EXPERIMENTS

The occurrence of numerous hybrids in nature indicate that barriers to gene exchange are poorly developed in the *A. triangularis* group. But as most of the natural hybrids investigated probably represent both primary hybrids and derivates of different backcrosses towards the parents, it seemed desirable to test the vigour and male fertility of artificially produced hybrids. Hitherto only limited information on the existing crossing barriers in the whole genus *Atriplex* has been available, and no experimental work has been carried out in the *A. triangularis* group. In 1925 TURESSON reported two crossing combinations, artificially produced, one between *A. littoralis* L. and *A. patula* L. and one between *A. triangularis* and *A. littoralis*. But, as he failed in the emasculation of the female parents, the results obtained must be interpreted with discretion. He found that the hybrid plants of both combinations were almost completely sterile. The same results have been obtained in controlled crossing experiments (HULME 1958). In addition, meiosis in a spontaneous hybrid plant between *A. littoralis* and *A. patula* has been investigated by the author (GUSTAFSSON unpublished). Although these species belong to the same section the bivalent formation is highly irregular and the pollen fertility very low. Seed-setting was very low, and none of the seeds germinated.

NOBS et al. (1971) report artificially produced viable hybrids between *A. triangularis* and *A. rosea* L. Although these two species belong to different sections, they produce viable hybrid progenies both in F_1 and F_2. All the hybrid plants examined were highly sterile. These results are confirmed by other crossing experiments between species of different sections. Seeds are easily obtained from crosses between *A. longipes* and the lignified, perennial *A. recurva* D'URV., from the Aegean islands of Greece. However, no hybrid seed of this cross has hitherto germinated. These facts indicate that crossing barriers are built up rather slowly within the genus and that most crosses within sections will be easily obtained and even species hybrids might be partially fertile.

The aim of the crossing experiments has been to throw light upon three main problems: (1) The degree of chromosomal heterozygosity within certain populations. (2) Crossing ability between isolated populations within taxa, with special reference to geographical distance and morphological differentiation. (3) The mode and the strength of internal barriers in crosses between taxa.

The code to the parent populations used in the crossing experiments is preserved at and available from the Botanical Library, Lund, Sweden.

Methods

One difficulty has been the emasculation process, as the flowers are small and the terminal inflorescences consist of both male and female flowers. However, in addition to the terminal inflorescences *A. longipes* has axillary flowers which are exclusively female. This species is also protogynous. Thus, *A. longipes* can be easily used as female parent, if the terminal inflorescences are removed. But it was sometimes even possible to use the other species as well if repeated emasculations were performed. Another great problem has been the different times of flowering of the different species. Crosses between *A. longipes* ssp. *praecox* and in particular *A. calotheca* and *A. glabriuscula* are difficult to perform as *A. longipes* ssp. *praecox* flowers early and the others late. But crossing was possible to some extent when representatives of *A. calotheca* and *A. glabriuscula* were kept in a greenhouse during the vegetative period, and those of ssp. *praecox* placed outdoors. At the onset of anthesis in *A. calotheca* and *A. glabriuscula* emasculated representatives of *A. longipes* ssp. *praecox* were moved into the greenhouse and pollination performed.

Table 8. Germination of hybrid seeds in crosses within and between taxa. n indicates the number of crosses investigated.

	Germination %												
	0	1	10	20	30	40	50	60	70	80	90	100	n
Crosses within taxa													
A. longipes ssp. *longipes* .	13	2	10	8	4	4	4	1	—	—	2		48
ssp. *praecox*	5	7	10	7	2	—	—	—	—	—	—		31
A. triangularis	15	21	10	5	1	1	—	2	—	1	1		57
Total	33	30	30	20	7	5	4	3	—	1	3		136
%	24.3	22.1	22.1	14.7	5.1	3.7	2.9	2.2	—	0.7	2.2		
Crosses between taxa													
calotheca×*glabriuscula* ..	—	—	—	—	1	—	—	—	—	—	—		1
calotheca×*longipes*	—	1	—	1	2	1	—	—	—	—	—		5
calotheca×*praecox*	2	—	1	—	1	—	—	—	—	—	—		4
calotheca×*triangularis* ..	2	—	4	—	—	1	1	—	—	—	2		10
glabriuscula×*longipes* ...	—	—	3	2	—	—	—	—	—	—	—		5
glabriuscula×*praecox* ...	—	—	—	—	—	1	—	—	—	—	—		1
glabriuscula×*triangularis*	2	1	4	1	1	—	—	—	—	—	—		9
longipes×*praecox*	8	6	10	3	1	—	—	1	—	—	—		29
longipes×*triangularis* ...	5	2	6	7	1	—	2	—	—	—	—		23
praecox×*triangularis* ...	6	1	3	—	—	—	—	—	—	—	—		10
Total	25	11	31	14	7	3	3	1	—	—	2		97
%	25.8	11.3	32.0	14.4	7.2	3.1	3.1	1.0	—	—	2.1		

The effectiveness of emasculation was controlled every day and before pollination the stigmas were checked to see that no pollen was present. Each plant was isolated by applying a pergamin bag to the branches. Pollen was repeatedly brushed onto the surface of the stigma and the plant again isolated. After successful pollination the swelling of the ovules and growth of the bracteoles could be seen within a week or two.

A varying number of F_1 plants were raised, depending mainly on the number of germinated seeds, but in some crosses up to 15 hybrid plants were investigated. The degree of vegetative development and the male fertility of the hybrid plants were noted. In addition to male fertility meiosis was investigated in several crosses with reduced fertility.

Germination of Hybrid Seeds

The germination response of 233 crossing combinations was tested in 1971 and 1972. The hybrid seeds were treated in the same way as spontaneous seeds had been (cf. page 354) and placed outdoors in the Botanical Gardens. The number of seeds available in the crosses varied as the number of female flowers that were successfully pollinated varied. Occasionally a too small number of seeds may have affected the results, but on the whole the results seem to be relevant.

The results are summarized in Table 8. The crosses tested have been divided into two major groups, one representing crosses between populations within taxa and the other crosses between taxa. Germination in both intra- and interspecific crosses is very low. The majority of the crossing combinations, about 83 %, germinate to less than 30 % and only 2—3 % to more than 80 %. In interspecific crosses those between populations of *A. longipes* ssp. *praecox* and other species seem to germinate to a somewhat lesser extent than the others. The two groups seem to have about the same variation amplitude. The low figures for the germination of hybrid seeds are also confirmed by other observations. Seeds produced by F_1 hybrids germinate so

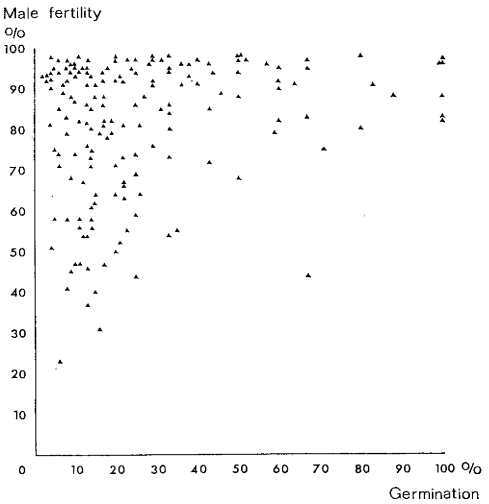


Fig. 8. Correlation between germination of hybrid seeds and male fertility of the F_1 plants.

poorly that it is difficult to get a sufficient number of F_2 individuals, although a large number of seeds were used.

The results diverge greatly from those obtained in normal populations where the majority germinate to more than 60 % (cf. Table 5). The decreased germination of hybrid seeds may be due to physiological disturbances and/or anatomical deficiencies. The shape of most seeds seems quite normal, but some observations indicate abnormal development. The testae of some non-germinated seeds were removed around the radicle and after some days the growth of the radicle was quite normal. Probably it had not been able to break through the testa, which perhaps was somewhat thicker than normal or did not split in the normal way. Defects probably caused by physiological unbalance have been observed as well. In some hybrid seeds the natural period of dormancy was disturbed. Germination occasionally occurred during the winter season (December and January), when the temperature rose above zero. But of course it resulted in unsuccessful development, as sooner or later the seedlings died when the temperature dropped. Seeds obtained from natural populations are completely dormant during the winter season and germination does not take place until March or April.

It is also possible that some of the non-germinated seeds have aneuploid chromosome numbers or other types of abnormal chromosome complements. In fact, no hybrid plant with a deviating chromosome number has been observed despite disturbances at meiosis. Probably some kind of selection of such gametes or seeds occurs.

The correlation between germination of hybrid seeds and male fertility of the F_1 plants is shown in Fig. 8. Only a low correlation exists. Crossing combinations with low fertility values also have a low percentage of germinated seeds while those with high fertility values may have both low and high degrees of germination.

Viability and Seed-setting

Most of the germinating seeds give rise to vegetatively well-developed hybrids, at least as vigorous as the parent species. In fact some of the F_1 plants display a certain heterosis effect. In a few exceptional crosses (e.g. Gk 40), especially those with low fertility values, some of the F_1 plants were weak in early stages of development, while others in the same crosses were quite normal. The fertility values of the weak plants agreed with those of the normal ones.

An abnormal development of the leaves was occasionally observed, both in intra- and in inter-specific crosses. Such leaves were either deformed or consisted of two united leaves. Further, in some crosses parts of the branches were accrete to the stem. However, hybrid plants with such abnormalities seemed to be quite normal in other respects.

As a rule seed-setting was good, even

in hybrids with meiotic disturbances and reduced male fertility. But in some crosses (e.g. Gk 40) with low fertility values a large number of unsuccessfully pollinated female flowers were observed. Sometimes the bracteoles of the non-pollinated flowers enlarged very rapidly and new female flowers developed within them. Such reflowering usually resulted in at least some production of seed.

Chromosome Number

The chromosome number of a large number of hybrid plants has been investigated. All hybrids investigated have $2n=18$. Aneuploid seeds are either never formed or they do not germinate.

Meiosis

In order to investigate the causes of reduced male fertility in hybrids, meiosis was examined in some crossing combinations. As a rule only one or two hybrid plants from each crossing combination has been investigated, preferably those ones with low fertility values. Meiosis was investigated in 42 crosses, 24 representing population crosses within taxa and 18 between taxa. The results are shown in Table 9. The pairing of the chromosomes is mostly quite normal, even in hybrids with low fertility values. Nine ring bivalents are usually formed but occasionally one or a few rod bivalents are visible. Configurations consisting of more than two chromosomes have not been seen. However, in two crosses, Gk 31 and Gk 80, univalents were observed in one cell each, other metaphase groups in the same crosses being quite normal.

At anaphase I the number of cells with visible disturbances is often rather high, but they are exclusively of two kinds, viz. the formation of bridges and laggards. Hybrid plants with both one and two bridges were noticed. It has been impossible to localize the bridges to particular chromosome pairs, as the chromosomes were small and of equal size. But, in hybrids having two bridges, they always occur in different chromosome pairs. The bridges may or may not be torn apart at anaphase I indicated by remaining bridges at later stages of meiosis. Thus, most of the bridges observed at anaphase II are persistent but new bridges are occasionally formed. The fragments are usually rather small and the corresponding dichromatids consequently relatively large. The number of laggards varies between one and six at both anaphase I and II. In the crosses number Gk 20 and Gk 336 chromosomes lying outside the spindle axis have been noticed at metaphase II.

The different types of meiotic behaviour causing formation of bridges and fragments at anaphase I have been discussed previously (cf. p. 360 and GUSTAFSSON 1972). The most relevant interpretation in this case is that bridges and fragments are caused by paracentric inversions. The inversions are probably dissimilar in the different crosses, as the frequencies of crossovers within the inverted segments vary greatly. In cross number Gk 92 about 2 % of the crossovers occur within the inverted segment, but in cross number Gk 40 the corresponding figure is about 16 %. However, this difference in the number of bridges formed does not necessarily imply that the inverted segment in the first cross is small and in the second large. If terminal chiasmata are the most usual type, the number of bridges is influenced not only by the size but also by the position of the inverted segment. Inversions do not seem to be more common in hybrids between taxa than in those representing population crosses within taxa. In fact, the greatest number of meiotic disturbances are found in population crosses within *A. longipes* ssp. *praecox*.

CHIASMA FORMATION

The position of chiasmata may or may not remain constant during the different

Table 9. A survey of meiosis in F_1 hybrids representing crosses within and between taxa. The figures indicate the number of cells counted.

Cross no.	% good pollen	Metaphase I	Anaphase—Telophase I				Anaphase—Telophase II		
			Normal	Bridges 1	Bridges 2	Lag-gards	Normal	Bridges	Lag-gards
Crosses within A. longipes ssp. **longipes**									
3—01	59	—	—	—	—	—	164	2	3
4—08	86	9 bivalents	—	—	—	—	47	1	—
16A—16	97	9 bivalents	106	—	—	—	67	—	—
88—01	83	9 bivalents	—	—	—	—	151	1	1
92—01	88	—	62	1	—	—	—	—	—
92—02	88	9 bivalents	—	—	—	—	63	1	—
94—02	74	9 bivalents	—	—	—	—	124	1	—
95—01	85	9 bivalents	76	—	—	1	—	—	—
98—03	62	9 bivalents	34	2	—	—	83	3	3
107—02	84	—	84	4	—	—	—	—	—
107—03	85	9 bivalents	71	2	—	—	—	—	—
Crosses within A. longipes ssp. **praecox**									
22—10	46	—	—	—	—	—	54	4	2
22—13	47	9 bivalents	30	19	1	—	—	—	—
28—08	63	9 bivalents	—	—	—	—	100	1	—
39—01	40	9 bivalents	90	14	—	4	—	—	—
40—03	62	9 bivalents	71	14	—	—	—	—	—
58—03	70	9 bivalents	104	5	—	3	—	—	—
63—02	62	9 bivalents	96	6	—	4	—	—	—
70—01	81	9 bivalents	98	3	—	6	40	3	6
72—01	80	9 bivalents	102	3	—	—	—	—	—
80—04	84	9 bivalents	—	—	—	—	74	4	2
84B—02	36	9 bivalents	—	—	—	—	90	2	3
Crosses within A. triangularis									
299—04	72	—	—	—	—	—	71	1	—
302—01	96	9 bivalents	103	—	—	—	—	—	—
315—02	81	9 bivalents	56	—	—	—	68	—	4
383—03	90	—	58	—	—	—	—	—	—
398—02	74	—	—	—	—	—	72	2	—
A. longipes ssp. **longipes**×ssp. **praecox**									
9—16	82	9 bivalents	108	3	—	—	126	4	—
13—03	69	9 bivalents	114	10	2	—	116	4	2
13—12	82	—	—	—	—	—	76	1	1
13—14	84	—	49	2	—	—	—	—	—
16B—22	48	9 bivalents	98	11	2	6	48	2	2
17—12	62	—	—	—	—	—	242	13	4
27—02	59	9 bivalents	143	14	—	2	66	2	1
27—06	84	9 bivalents	69	1	—	1	—	—	—
31—10	78	9 bivalents	86	1	—	4	—	—	—
31—17	59	9 bivalents	56	3	—	2	17	—	1
32—02	65	—	—	—	—	—	87	1	1
32—15	45	9 bivalents	34	3	—	—	92	3	—
140—02	85	9 bivalents	71	1	—	1	—	—	—
155—02	97	9 bivalents	94	—	—	—	—	—	—
166—02	80	9 bivalents	75	5	—	1	—	—	—
A. calotheca×**A. glabriuscula**									
356—02	98	9 bivalents	64	—	—	—	—	—	—

Table 9.

Cross no.	% good pollen	Metaphase I	Anaphase—Telophase I				Anaphase—Telophase II		
			Normal	Bridges 1	Bridges 2	Laggards	Normal	Bridges	Laggards
A. calotheca × A. longipes ssp. **longipes**									
216—01	85	—	—	—	—	—	114	1	—
333—02	97	—	—	—	—	—	129	—	—
A. glabriuscula × A. longipes ssp. **longipes**									
336—01	87	—	—	—	—	—	65	1	—
A. glabriuscula × A. triangularis									
353—01	96	9 bivalents	60	—	—	—	76	—	—
355—01	98	9 bivalents	154	—	—	—	—	—	—
A. longipes ssp. **longipes × A. triangularis**									
330—03	98	9 bivalents	46	—	—	—	—	—	—
A. longipes ssp. **praecox × A. triangularis**									
20—02	60	9 bivalents	219	40	4	1	—	—	—
20—12	96	9 bivalents	291	—	—	—	111	—	—

stages of meiosis giving rise to two types of chiasmata, interstitial and terminal. Interstitial chiasmata, found in species with large chromosomes, such as *Allium fistulosum* and *Vicia faba* (SWANSON 1965), can be localized either close to the centromeric region or anywhere along the chromosome. In plants with smaller chromosomes, such as species of *Campanula* (GAIRDNER & DARLINGTON 1931) and *Anemone* (MOFFETT 1932), all the chiasmata are terminal or have become terminalized at metaphase I.

All species in the *A. triangularis* group seem to have terminal chiasmata. Some information, available from plants heterozygous for inversions, supports the theory that the terminal chiasmata have arisen in the most distal parts of the chromosomes, rather than that they have become terminalized (GUSTAFSSON 1972). In ring bivalents two chiasmata are usually formed, one on each side of the bivalent, but bridge formation at anaphase II indicates that three may occur, two within the inverted arm and one in the other arm. Further, the number of chiasmata seems to be similar in normal plants and those that are heterozygous for paracentric inversions (GUSTAFSSON 1972).

CROSSOVERS IN HYBRIDS HETEROZYGOUS FOR PARACENTRIC INVERSIONS

The most typical features of heterozygous paracentric inversions are loop formation at prophase stages of meiosis and the formation of bridges and fragments recognizable at anaphase. However, if the inverted segment is very small it is possible that no loop will be formed, but that instead the corresponding segments will be unpaired. If no chiasma occurs within the loop all the chromatids will give rise to functional gametes, two will contain the inverted segment and the other two will be normal. But if chiasmata occur within the inverted segment different types of configurations can be observed at anaphase I and/or II, depending on the number of chiasmata involved and on which chromatids they occur between. A single crossover within the loop will result in a bridge and fragment visible at A I and cause inviability of at least

two gametes. The acentric fragment is always of the same size in a specific inversion independent of the position of the chiasma within the loop. Further, the more distal the inverted segment is the smaller the fragment will be.

Two chiasmata inside the loop may give rise to two bridges within the same chromosome pair at A I. But two chiasmata, one inside and one outside the inverted segment, may also result in an association of the two chromatids in a chromosome and the formation of a fragment at A I. In this case the bridge is first visible at A II. A triple crossover, one outside and two inside the loop, will give rise to two chromosomes with associated chromatids, two fragments at A I and two bridges at A II.

In most hybrids of the *A. triangularis* group only one bridge is visible at A I, caused by a single crossover within the inverted segment. In these hybrids the inversion differences of the parents are restricted to segments situated in one chromosome. In addition, two bridges have been observed simultaneously in four crosses, which indicate that the inversion differences comprise two different chromosomes. In all these four crosses populations of *A. longipes* ssp. *praecox* are involved.

At anaphase II two kinds of bridges have been observed, the most common being bridges remaining from A I. More rarely new bridges are formed at A II. In many higher plants the bridges will be broken by movement at anaphase I or cut by the cell wall. A cell wall is usually developed at the end of the first meiotic division, at right angles to the dividing axis. But in the *Atriplex* species, as well as in many other plants, no wall is developed until the second division has been completed. At that time both the walls are formed simultaneously. Obviously, the anaphase movement is not always strong enough to cause the bridges to break and this will result in remaining bridges at A II (cf. SHARP 1934). The new bridges formed at A II have probably arisen by two chiasmata, one inside and one outside the inverted segment.

No other anaphase configurations arising from other types of crossovers, have been observed in the *A. triangularis* group. This may be due to difficulties of observation as the chromosomes are small.

CORRELATION BETWEEN THE FREQUENCY OF BRIDGES AND HYBRID FERTILITY

The correlation between the number of bridges present at anaphase I and male fertility in F_1 hybrids is shown in Fig. 9. The correlation is high. In hybrids with high fertility values (80—90 %) the frequency of bridges is usually 0—5 %, but in those with highly reduced fertility (40—60 %) the frequency is at least 5—10 %. This high correlation indicates that reduced male fertility is to a great extent dependent on heterozygosity for paracentric inversions. Two simultaneously formed bridges, caused by two different inversions, have only been observed in hybrids with fertility values below 70 %.

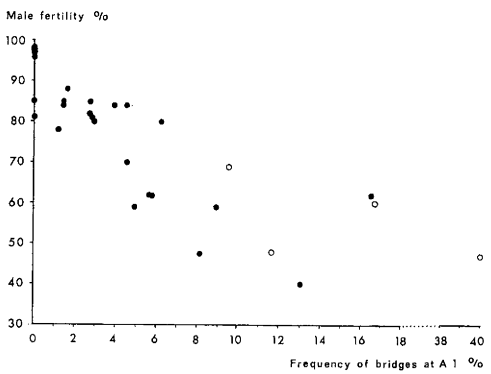


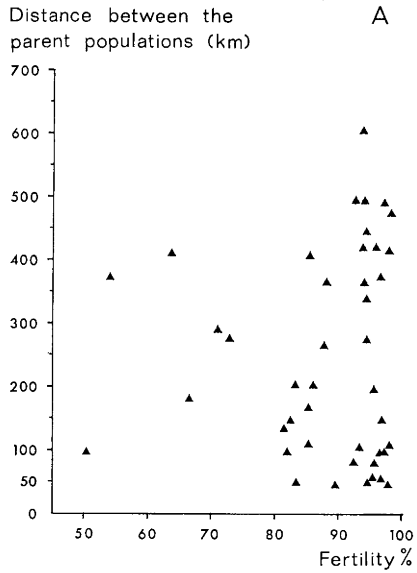
Fig. 9. Correlation between the frequency of bridges at anaphase I and male fertility in different F_1 plants. The diagram is only based on data from the crossing experiments. Dots indicate observation of one bridge and circles formation of two bridges simultaneously.

Table 10. Mean fertility values of F_1 progenies in crosses within *A. longipes* ssp. *longipes* and ssp. *praecox*. The parent populations are combined to represent different regions. n indicates the number of crosses carried out.

Parent × Parent		Male fertility % in F_1								
		30	40	50	60	70	80	90	100	n
A. longipes ssp. **longipes**										
CROSSES WITHIN REGIONS										
Baltic	Baltic	—	—	1	—	1	4	3		9
W. Sweden	W. Sweden	—	—	—	—	—	2	3		5
Denmark	Denmark	—	—	—	—	—	3	8		11
Total		—	—	1	—	1	9	14		25
%		—	—	4.0	—	4.0	36.0	56.0		
CROSSES BETWEEN REGIONS										
Baltic	W. Sweden	—	—	1	—	2	1	3		7
Baltic	Denmark	—	—	—	1	—	2	10		13
W. Sweden	Denmark	—	—	—	1	—	—	2		3
Total		—	—	1	2	2	3	15		23
%		—	—	4.3	8.7	8.7	13.0	65.2		
A. longipes ssp. **praecox**										
CROSSES WITHIN REGIONS										
E. Sweden	E. Sweden	—	4	4	3	2	1	3		17
Norway	Norway	1	—	—	—	—	—	—		1
Total		1	4	4	3	2	1	3		18
%		5.6	22.2	22.2	16.7	11.1	5.6	16.7		
CROSSES BETWEEN REGIONS										
E. Sweden	Åland	—	—	2	1	2	1	1		7
E. Sweden	Norway	—	5	3	1	1	1	—		11
Åland	Norway	—	—	1	—	—	—	—		1
Total		—	5	6	2	3	2	1		19
%		—	26.3	31.6	10.5	15.8	10.5	5.3		

Hybrid Fertility in Relation to Geographical Distance between Parent Populations

One of the most interesting aspects of hybrid sterility within taxa, has been to estimate the degree of cytological differentiation between adjacent populations compared with those that are more widely separated. Adjacent populations usually have greater opportunities for gene exchange than distant ones, particularly when the geographical isolation is pronounced and has been established for a long time. In Scandinavia the geographical isolation is not more than about 8,000 years old, i.e. since the last glaciation. But populations of *A. longipes* at least may be divided into different more or less natural regions. *A. longipes* ssp. *longipes* inhabits marshes along the coasts of Denmark, and the western and the eastern parts of Sweden. The opportunities for gene exchange between populations of *A. longipes* ssp. *longipes* belonging to different regions are rather small at present, as populations in Denmark are separated from the Swedish ones by Öresund and populations belonging to the western and the eastern part of Sweden respectively do not meet. The populations of *A. longipes* ssp. *praecox* used for experimental purposes originate from three regions, the Swedish part of the Baltic, the Åland



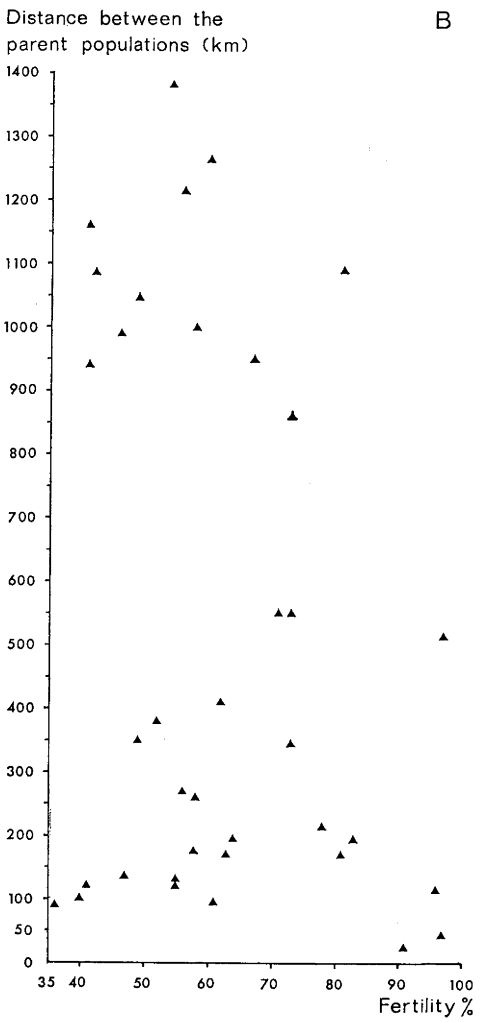


Fig. 10. Hybrid fertility (%) in relation to geographical distance (km) between parent populations. — A: Crosses within *A. longipes* ssp. *longipes*. — B: Crosses within *A. longipes* ssp. *praecox*.

archipelago and the northern part of Norway. The populations from Norway at least are well isolated from the others. The gene exchange between populations of Åland and those from the Swedish mainland is limited, if it occurs at all. The area of distribution of *A. triangularis* is more continuous. It is common along all the coasts of Scandinavia except for the northernmost part of Norway, but for practical reasons the experimental material has been divided into five regions, Norway, Finland, Denmark, east and west Sweden. The crossing experiments include population crosses within *A. longipes* ssp. *longipes,* ssp. *praecox* and *A. triangularis*.

CROSSES WITHIN A. LONGIPES SSP. LONGIPES

The fertility values of 48 crosses are summarized in Table 10 and Fig. 11. In crosses between populations belonging to the same region 23 of a total of 25 have more than 80 % stainable pollen, and the remainder between 50 and 60 %, and 70 and 80 %, respectively. Eighteen of 23 combinations representing population crosses between different regions have more than 80 % stainable pollen. In the other five crosses male fertility varies between 50 and 80 %, i.e. two crosses between 70 and 80 %, two between 60 and 70 %, and finally one cross with

50 to 60 %. However, the hybrid fertility is high both between adjacent populations and between geographically distant ones. Only in crosses where populations G 111 from the province of Öland and G 214 from Halland are involved mean values below 80 % are found (cf. Fig. 11). Those crosses representing the greatest distances, Gotland×Denmark, are almost completely fertile. The geographical distance between the parent populations in relation to their F_1 fertility is shown in Fig. 10. There is evidently no correlation between the distance between the populations crossed and the male fertility of their hybrids. Those hybrid progenies with low fertility values represent crosses both between adjacent and distant populations.

CROSSES WITHIN A. LONGIPES SSP. PRAECOX

In all, 37 crosses performed between populations from Norway, the Åland archipelago and the Swedish part of the Baltic, germinated successfully. Mean fertility values for different F_1 families are shown in Table 10 and Fig. 11. The number of hybrids with reduced male fertility is fairly high in crosses within regions as well as between populations from different regions. Seventy-eight % and 84 % of the crosses respectively have mean values below 80 % stainable pollen. One cross, between two adjacent populations from Norway, has a fertility value as low as 36 %. In fact, it is the lowest value for all crosses where populations from Norway are involved. The two most distant crosses, Norway×Blekinge and Norway×Öland, have 54 % and 60 % morphologically good pollen. This reduction in fertility between adjacent populations is also obvious in crosses between populations from the Swedish east coast. Two of the crosses, with the highest fertility values found, have been carried out between some of the most widely separated populations in this area, Blekinge×Uppland and Blekinge×Södermanland. By contrast, the cross between Uppland and Södermanland is semi-sterile, the male fertility being 55 %.

There is either no correlation between hybrid fertility and the distance between the parent populations or it is very low (cf. Fig. 10), but on the other hand the frequency of reduced fertility may be somewhat higher in crosses between regions than within (cf. Table 10).

CROSSES WITHIN A. TRIANGULARIS

Mean fertility values of 88 F_1 progenies are summarized in Table 11 and Fig. 11. In crosses between adjacent populations from the Swedish west coast 51 % have a fertility above 90 %, 23 % have a fertility between 80 and 90 %, and 26 % of the crosses have less than 80 % stainable pollen. The variation within combinations is rather great. Ten crosses have been carried out between populations G 221 and G 233 and the male fertility values of the crosses vary between 60 and 97 %. Six crosses are almost completely fertile, one cross has a mean value of 90 %, another 75 % and two between 60 and 70 %. The variation amplitude is about the same in other repeated combinations. Only two crossing combinations have total mean values below 80 % good pollen, one between two populations from the province of Bohuslän (G 166×G 221) and one between two populations from the province of Halland (G 167×G 236). All other combinations have higher mean values including the crosses between the most distant populations, Skåne×Bohuslän. There seems to be little or no correlation between geographical distance between the crossed populations and their hybrid fertility in this region.

The number of crosses between populations within other districts is small, but the pattern of variation is similar. Some of the crosses are highly fertile, others have more or less reduced fertility. However, none of the crosses between popula-

Table 11. Mean fertility values of F_1 progenies in crosses within *A. triangularis*. The parent populations are combined to represent different regions. n indicates the number of crosses carried out.

Parent × Parent	Male fertility % in F_1						
	50	60	70	80	90	100	n
A. triangularis							
CROSSES WITHIN REGIONS							
E. Sweden E. Sweden	—	2	—	2	—		4
W. Sweden W. Sweden	1	2	9	11	24		47
Denmark Denmark	—	1	—	2	2		5
Total	1	5	9	15	26		56
%	1.8	8.9	16.1	26.8	46.4		
CROSSES BETWEEN REGIONS							
E. Sweden W. Sweden	—	—	1	4	1		6
E. Sweden Finland	—	—	2	1	1		4
E. Sweden Norway	—	—	—	1	1		2
E. Sweden Denmark	—	1	2	4	—		7
W. Sweden Finland	—	—	1	—	1		2
W. Sweden Denmark	—	1	—	2	3		6
Denmark Finland	—	1	1	2	1		5
Total	—	3	7	14	8		32
%	—	9.4	21.9	43.8	25.0		

tions from the eastern part of Sweden is completely fertile, all four combinations have less than 90 % stainable pollen. But on the other hand entirely fertile combinations may not be represented because of the limited number of crosses performed. In crosses between regions the combination E. Sweden × Denmark seems to have a high frequency of crosses with reduced fertility, while crosses representing W. Sweden × Denmark have relatively high fertility values.

On the whole crosses within regions have a higher frequency of fertility values above 90 % than crosses between regions.

Correlation between Morphological Differentiation and Hybrid Fertility

One of the main purposes of the crossing experiments within taxa has been to examine the degree of correlation between morphological and cytological differentiation. A relatively large number of crosses has been carried out both between populations representing different morphological types and between populations belonging to the same type. Populations of *A. longipes* and *A. triangularis* have been used and the results are summarized in Table 12.

A. longipes ssp. *longipes:* The morphological diversities between populations of *A. longipes* ssp. *longipes* are fairly small, as most of the size characters are overlapping. In fact the most prominent differences are in habit. The Baltic populations are usually composed of individuals with a much-branched, ascending habit. The basal branches are often long and rigid and the whole plant gives a stout impression. On the other hand plants from populations from Denmark and west Sweden are sometimes taller and often have an erect habit. The basal branches are usually few and pointing upwards, the leaves and the bracteoles often larger.

The results for male fertility in F_1 hybrids are summarized in Table 12. The highest frequency of hybrids with reduced fertility is found in crosses between the

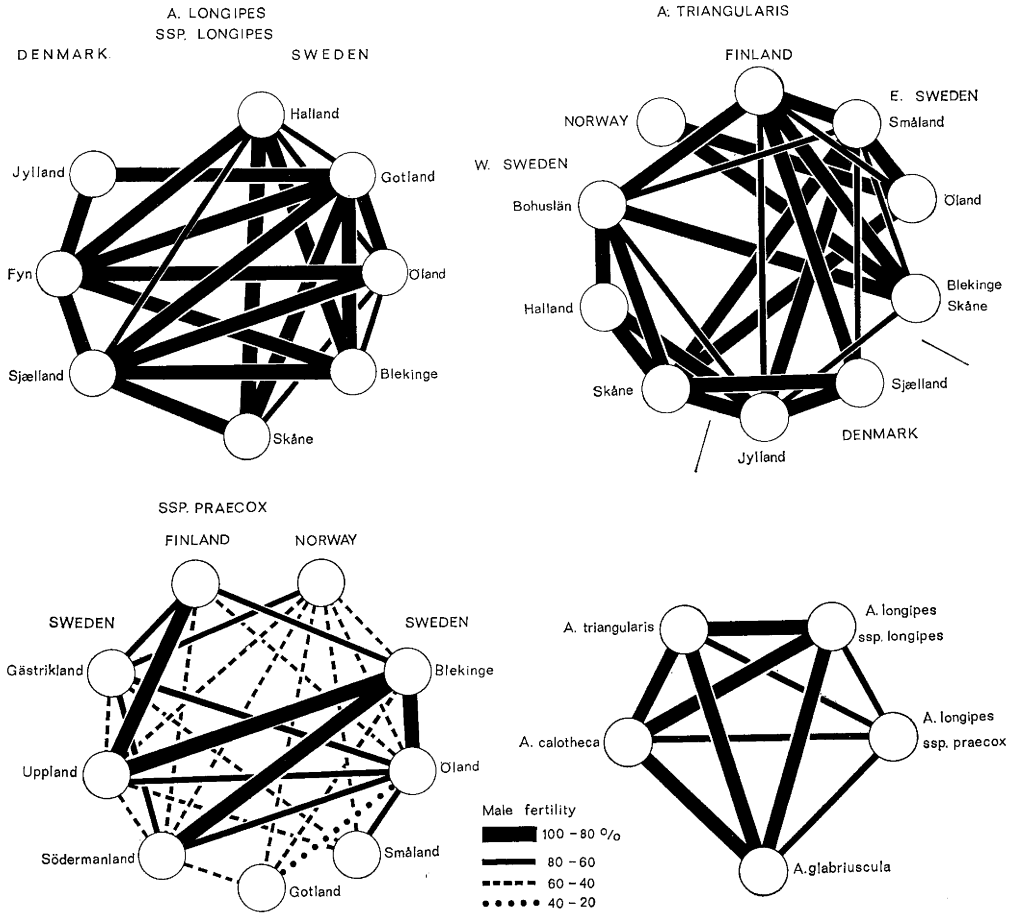


Fig. 11. Crossing polygons indicating mean fertility values (F_1) in crosses within and between taxa in the *A. triangularis* complex. In crosses within *A. longipes* and *A. triangularis* the parent populations are combined to represent different provinces of Sweden and Denmark. The crossing polygon of crosses between taxa is based on average fertility values of all crosses carried out in each combination.

morphological types, 20 % of the hybrids have less than 80 % stainable pollen. The corresponding figure for crosses within types is 11 %. Thus in *A. longipes* ssp. *longipes* correlation between morphological differentiation and hybrid sterility seems to be rather low.

A. longipes ssp. *praecox:* The morphological variation is relatively small both within and between populations. However, two form series may be distinguished, based mainly on vegetative characters. One is characterized by being low, usually 6—12 cm, small and narrow leaves, 1—2 cm long, few branches and a more or less erect habit. The other one is taller, has larger leaves and a much-branched, ascending habit.

The crossing experiments include only population crosses within the Baltic region. Only four populations of the tall

Table 12. Hybrid sterility in relation to morphological differentiation in *A. longipes* and *A. triangularis*. Explanation of the morphological types in the text. n indicates the number of crosses carried out in each combination.

Taxon	Morphological type Parent × Parent		Male fertility % in F_1							
			40	50	60	70	80	90	100	n
A. longipes ssp. *longipes*	Baltic	Baltic	—	1	—	1	4	3	9	
	Danish	Danish	—	—	1	—	5	13	19	
	Baltic	Danish	—	1	1	2	3	13	20	
A. longipes ssp. *praecox*	Small	Small	2	4	1	4	1	3	15	
	Small	Tall	2	2	3	—	1	1	9	
A. triangularis	Prostrate	Prostrate ...	—	1	2	6	4	17	30	
	Erect	Erect	—	—	4	2	9	4	19	
	Prostrate	Erect	—	—	1	4	12	9	26	

types have been used, as this type is rather rare, and unfortunately no cross between these populations is available. Thus, the fertility values of crosses between 12 populations belonging to the small type are compared to the fertility values of crosses between the types. The results are shown in Table 12. The fertility values of both the combinations show a wide variation, from 40 to 100 % stainable pollen. Eleven of 15 population crosses within the small types have fertility values less than 80 %, one between 80 and 90 % and three above 90 %. The fertility values of crosses between the morphological types have about the same distribution. Most crosses have reduced fertility, 7 of 9 have below 80 %. Obviously there is no significant difference between the two categories of crosses and consequently there is little or no correlation between morphological and cytological differentiation.

A. triangularis: The morphological variation within *A. triangularis* is rather wide and a large number of taxa have been described. However, these can be divided into two different form series, one erect and one more or less prostrate. The erect types occur on maritime biotopes with tall vegetation, such as marshes dominated by *Scirpus maritimus* and with a belt of *Phragmites*. They are mostly tall, often with rather few ascending branches, have relatively large, green leaves and large bracteoles. By contrast the prostrate types inhabit exposed biotopes with sparse or low vegetation. These types have long, rigid, prostrate to ascending basal branches and a rather short main stem. The leaves are often small, triangular and often somewhat reddish, and the bracteoles small.

Within *A. triangularis* 19 crosses have been carried out between erect parents, 30 between prostrate parents and 26 between an erect and a prostrate parent. Populations originating from Norway and Finland have been excluded, as seeds of the two morphological types were not kept separate. The fertility is relatively high in crosses between parents of the erect and the prostrate type. In fact, not more than 5 crosses have less than 80 % good pollen. Crosses within the morphological types have a higher frequency of fertility values below 80 %, 9 of 30 in crosses between parents with a prostrate habit, and 6 of 19 in crosses between parents with an erect habit. Thus, in *A. triangularis* it seems likely that a cytological differentiation that gives rise to reduced hybrid fertility is not correlated with morphological differentiation.

Crosses between Taxa

The crossing experiments comprise all taxa, including crosses between *A. longipes* ssp. *longipes* and ssp. *praecox*. Populations of ssp. *longipes* and ssp. *praecox* were crossed in different directions, in order to compare the degree of reduced fertility in crosses representing parents from the same geographical region on the one hand and on the other hand parents from different geographical regions. In crosses between species the most interesting point seemed to be an estimation of the highest possible degree of gene exchange within the *A. triangularis* group, i.e. the existence of or lack of highly fertile F_1 progenies of a given repeated combination. The distribution of the fertility values are summarized in Tables 13, 14 and 15, and shown in Fig. 11.

MORPHOLOGICAL VARIATION WITHIN DIFFERENT F_1 FAMILIES

The morphological variation within F_1 families representing the crosses *A. calotheca*×*A. longipes, A. calotheca*×*A. triangularis* and *A. longipes*×*A. triangularis* is shown in Figs. 12 and 13. These F_1 progenies like those of most other interspecific crosses, are fairly homogeneous, and the morphological variation is small in characters such as habit and general shape, base angle and margins of both leaves and bracteoles. The small amount of variation indicates that the genes determining these special characteristics of the species occur in a homozygous state in the parents. By contrast different size characters show a certain amount of variation, as do colour, the perianth and the dentation on the back of the bracteoles. The variation in these last-mentioned characters indicates gene heterozygosity in the parents.

The morphological differences are sometimes great between different F_1 families belonging to the same crossing combination, and this is particularly true in combinations where different female parents have been used.

MORPHOLOGICAL VARIATION, GERMINATION, FERTILITY AND SEED-SETTING IN CROSSES BETWEEN TAXA

A. longipes ssp. *longipes*×ssp. *praecox*

The appearance of the F_1 families is very variable, and all forms from typical ssp. *longipes* to ssp. *praecox* have been observed. The differences are most prominent in characters such as height, leaf-shape and size of bracteoles. A few cases of reflowering have been observed.

Germination of hybrid seeds: The germination is highly reduced, in 27 of 29 hybrid progenies less than 30 % of the seeds germinated.

Fertility in the F_1 hybrids: The male fertility of 68 crossing combinations is summarized in Table 13. Populations of ssp. *longipes* from different regions, the Baltic, W. Sweden and Denmark, were crossed with populations of ssp. *praecox* originating from Norway, Åland and E. Sweden. Most of the crosses show reduced fertility, only 11 have more than 90 % stainable pollen. This reduction is especially pronounced in crosses with populations of ssp. *praecox* from Norway. Only one of ten crosses has more than 90 % and eight have fertility values less than 50 %. But the degree of reduced fertility is also high in crosses with ssp. *praecox* from the Baltic region (E. Sweden and Åland). Baltic populations of *A. longipes* ssp. *praecox* have been crossed with populations of ssp. *longipes* originating from the Baltic region, the western part of Sweden and from Denmark. The distribution of the fertility values in these crossing combinations appears to vary. Crosses within the Baltic region where the two taxa occur sympatrically, have a higher frequency of combinations with low fertility values than those between populations with an allopatric distribution. The differences are shown in Fig.

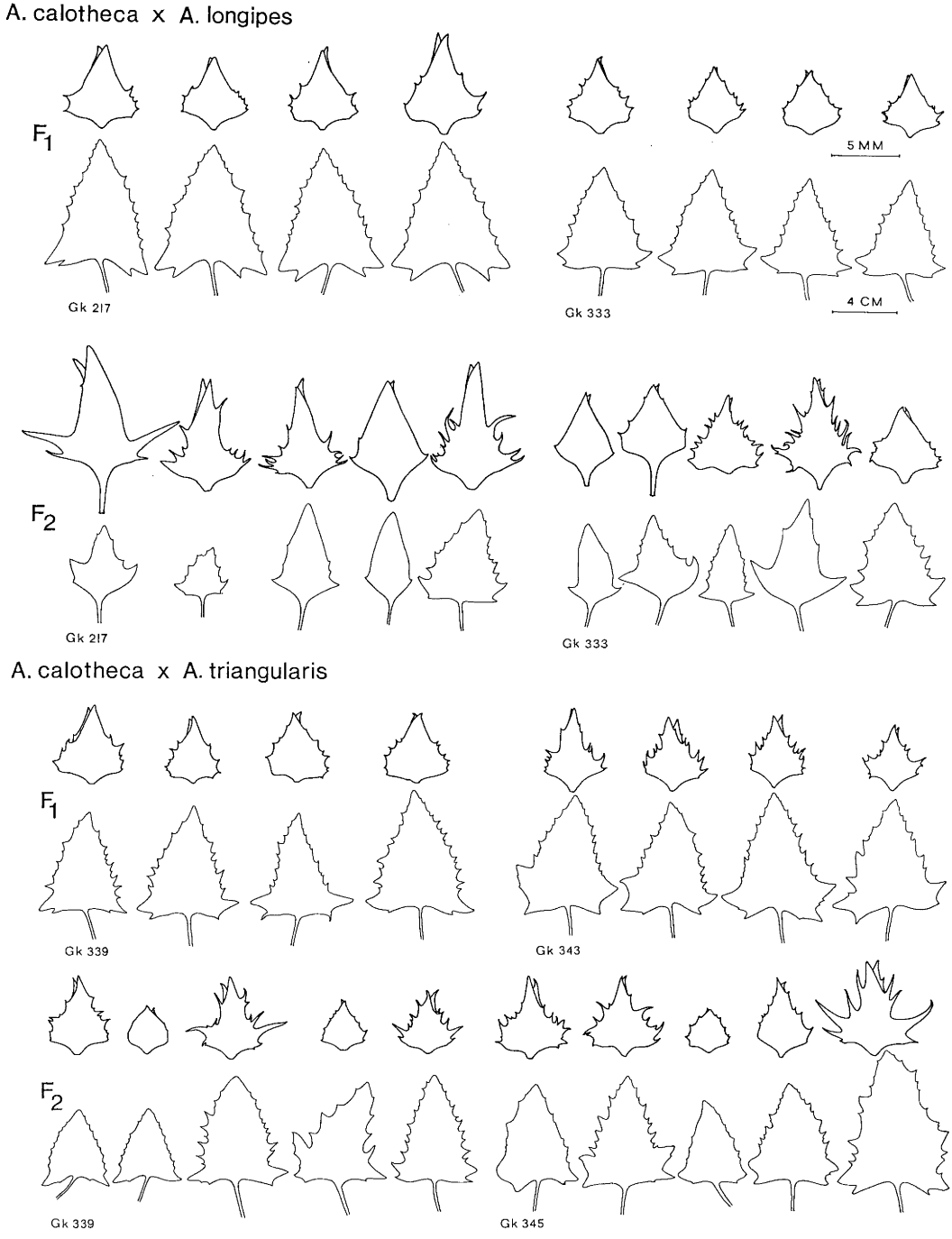


Fig. 12. Variation in lower leaves and bracteoles in F_1 and F_2 progenies of crosses between *A. calotheca* and *A. longipes*, and between *A. calotheca* and *A. triangularis*.

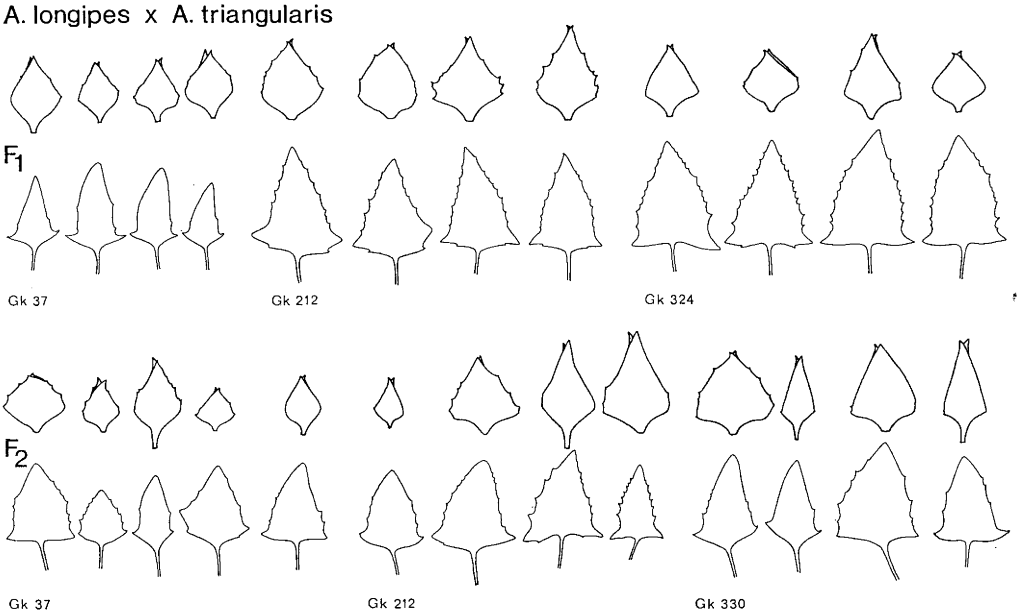


Fig. 13. Variation in lower leaves and bracteoles in F_1 and F_2 progenies of crosses between *A. longipes* and *A. triangularis*. — Scales as in Fig. 12.

14. Sixty-eight % of the crosses between sympatric populations have a mean value of less than 70 % stainable pollen, but only 36 % of these crosses represent allopatric distribution. The number of crosses analysed is high and the difference in distribution obvious. One such case is shown in Fig. 15. Population G 121 of ssp. *praecox* is crossed with 11 populations of ssp. *longipes*, four originating from the Baltic, two from the western part of Sweden and five from

Table 13. Mean fertility values of F_1 progenies in crosses between populations of *A. longipes* ssp. *longipes* and ssp. *praecox*. The parent populations are combined to represent different regions. n indicates the number of crosses carried out.

A. longipes ssp.		Male fertility % in F_1									
longipes origin	× *praecox* origin	20	30	40	50	60	70	80	90	100	n
Baltic	Norway	—	1	2	—	—	—	—	1		4
Baltic	E. Sweden	—	1	1	5	5	3	2	1		18
Baltic	Åland	—	—	1	2	—	—	—	1		4
W. Sweden	Norway	—	1	1	—	—	—	—	—		2
W. Sweden	E. Sweden	—	—	1	—	1	4	3	1		10
W. Sweden	Åland	—	—	—	—	—	1	—	—		1
Denmark	Norway	1	1	1	—	—	1	—	—		4
Denmark	E. Sweden	—	—	2	—	4	3	3	5		17
Denmark	Åland	—	—	—	3	2	1	—	2		8
Total		1	4	9	10	12	13	8	11		68

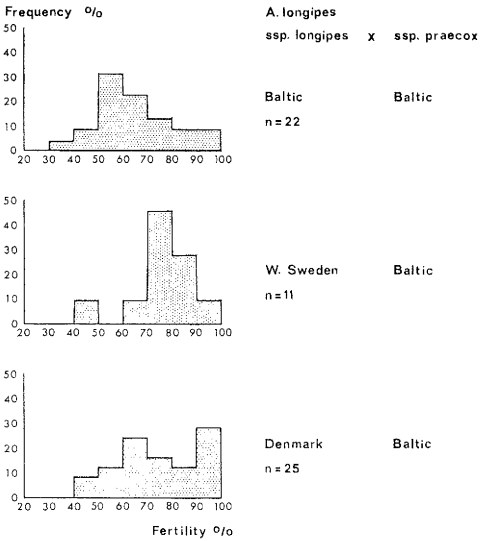


Fig. 14. Distribution of fertility values in F_1 progenies representing crosses between *A. longipes* ssp. *longipes* and ssp. *praecox*. The parent populations of ssp. *longipes* originate from three regions, the Baltic area, the western parts of Sweden and Denmark, those of ssp. *praecox* exclusively from the Baltic region. n indicates the number of crosses carried out.

Denmark. The average values of these combinations are 69 %, 86 % and 81 % stainable pollen respectively. Three crosses have a fertility of more than 90 %, two between G 121 and *longipes* populations from Denmark and one with ssp. *longipes* from W. Sweden. All the others have a fertility between 60 and 80 %. The degree of hybrid sterility is obviously not correlated with the geographical distance separating the parent populations. The strongest crossing barriers seem to be developed in the Baltic region, where the taxa occur sympatrically.

Fertility in the F_2 progenies: Eighty F_2 plants representing 18 progenies have been studied and they show a conspicuous amount of variation, from 30 to 100 % good pollen. Fifty % of the F_2 plants have a fertility greater than 80 %, about 33 % between 60 and 80 %, and 18 % between 30 and 60 %.

Seed-setting: Generally good.

A. calotheca×*A. glabriuscula*

Only two crosses have been made, mainly because emasculation is difficult to perform in these species. The F_1 progenies are more or less intermediate between the species. The hybrids have usually dentate to somewhat laciniate lower leaves and triangular or rarely rhomboid, sessile bracteoles which are thick to herbaceous and dark in colour at the base, herbaceous and green in the upper parts. The margins of the bracteoles are laciniate to dentate, the back with or without appendages. The surface has indistinct veins. The inflorescences are branched and rather loose.

Germination of hybrid seeds: 30 to 40 % of the seeds germinated. Only one progeny was investigated.

Fertility in the F_1 hybrids: Both the crosses are surprisingly fertile, the average values are 97 and 80 % stainable pollen. In one cross all the F_1 plants are almost completely fertile, but in the other the variation amplitude is greater, between 67 and 97 %.

Fertility in the F_2 progenies: Two F_2 progenies have been studied and 16 of a total of 18 plants have a fertility of more than 90 %.

Seed-setting: Good.

A. calotheca×*A. longipes*

Altogether hybrid seeds of seven crosses germinated, five between *A. calotheca* and *A. longipes* ssp. *longipes* and two between *A. calotheca* and *A. longipes* ssp. *praecox*. In crosses with ssp. *praecox* all the hybrids have triangular, laciniate lower leaves and dentate upper ones. The bracteoles are sessile, with or without appendages on the back and the margins intermediate. The F_1 progenies of crosses with ssp. *longipes* have dentate or laciniate lower

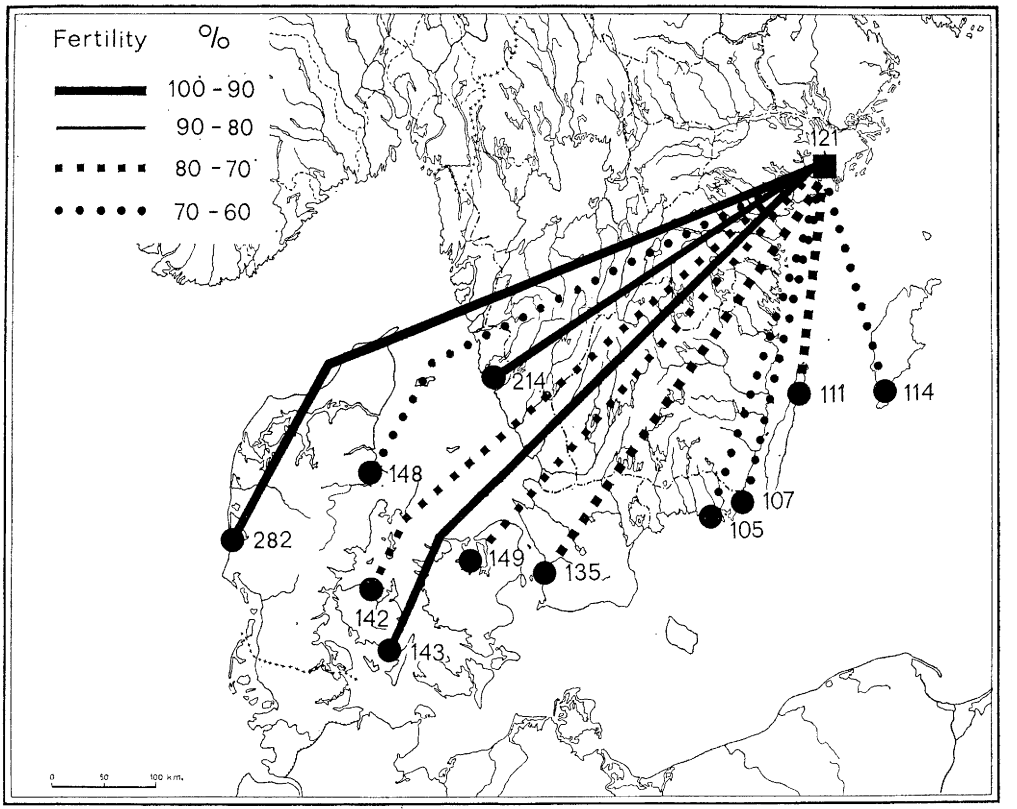


Fig. 15. Fertility values in crosses between eleven populations of *A. longipes* ssp. *longipes* (dots) and population G 121 of ssp. *praecox* (square).

leaves, with a truncate or cuneate base. The upper leaves are usually dentate. The bracteoles are sessile or sometimes shortly stalked, laciniate or intermediate. The inflorescences are rather loose. The variation in leaves and bracteoles are shown in Fig. 12.

Germination of hybrid seeds: In crosses

Table 14. Mean fertility values of F_1 progenies in crosses between taxa. n indicates the number of crosses carried out, and M the mean value of crosses belonging to the same crossing combination.

Crossing combination	Male fertility % in F_1									
	30	40	50	60	70	80	90	100	n	M
calotheca×*glabriuscula*	—	—	—	—	—	1	1	2	88	
calotheca×*longipes*	—	—	—	—	1	1	3	5	90	
calotheca×*praecox*	—	—	1	—	—	1	—	2	72	
calotheca×*triangularis*	—	—	—	1	—	2	5	8	88	
glabriuscula×*longipes*	—	—	—	—	—	2	3	5	91	
glabriuscula×*praecox*	—	—	—	1	1	—	—	2	70	
glabriuscula×*triangularis*	—	—	1	—	1	1	4	7	85	
longipes×*triangularis*	—	1	3	—	2	5	11	22	83	
praecox×*triangularis*	1	2	—	2	3	4	1	13	72	

Table 15. Distribution of male fertility values of F_2 plants representing different crossing combinations. n indicates the number of F_2 plants and N the number of F_2 progenies investigated.

Crossing combination	Male fertility % in F_2									
	30	40	50	60	70	80	90	100	n	N
Within *A. longipes*										
ssp. *longipes*	—	—	—	—	1	1	11	13	2	
ssp. *praecox*	—	4	9	3	11	14	14	55	10	
Within *A. triangularis*	—	—	1	5	7	17	28	58	9	
calotheca×*glabriuscula*	—	—	—	1	—	1	16	18	2	
calotheca×*longipes*	—	—	—	1	1	7	32	41	7	
calotheca×*praecox*	—	—	—	6	2	12	7	27	2	
calotheca×*triangularis*	—	—	—	—	2	12	28	42	7	
glabriuscula×*longipes*	—	—	—	—	—	6	19	25	3	
glabriuscula×*triangularis*	—	—	—	—	1	19	15	35	5	
longipes×*praecox*	1	2	11	17	9	15	25	80	18	
longipes×*triangularis*	—	—	—	3	17	36	91	147	15	
praecox×*triangularis*	—	2	2	8	12	23	44	91	9	

with ssp. *longipes* the germination varies between 1 and 50 %, and in crosses with ssp. *praecox* between 0 and 40 %.

Fertility in the F_1 hybrids: In crosses between *A. calotheca* and *A. longipes* ssp. *longipes,* 3 F_1 families have more than 90 % stainable pollen, 1 F_1 family between 80 and 90 % respectively 70 and 80 %. In crosses with ssp. *praecox,* 1 F_1 progeny has a fertility between 50 and 60 % and the other between 80 and 90 %.

Fertility of the F_2 progenies: In F_2, both the combinations show a certain amount of variation, from 60 to 100 % good pollen. In the combination *A. calotheca*×*A. longipes* ssp. *longipes* most of the F_2 plants have a fertility greater than 90 %. In crosses *A. calotheca*×*A. longipes* ssp. *praecox* about half of the F_2 plants have a fertility of between 80 and 90 %, a quarter greater than 90 % and between 60 and 80 % respectively.

Seed-setting: Good.

A. calotheca×*A. triangularis*

Eight crosses germinated successfully and all the F_1 hybrids were vigorous. The appearance of the different F_1 progenies varies, six progenies have more or less laciniate lower leaves, the others dentate. The upper leaves are mostly triangular, dentate or rarely laciniate, and often have prominent basal lobes. The bracteoles are usually triangular, sessile, dentate or intermediate, with or without appendages on the back. The surface is often veined. The inflorescences are loose, at least not so compact as in *A. calotheca.* The variation of leaves and bracteoles is shown in Fig. 12.

Germination of hybrid seeds: The hybrid seeds of two crosses did not germinate at all. In four crosses less than 20 % germinated, in two crosses between 40 and 60 %, and in further two 90 and 100 % of the seeds germinated.

Fertility in the F_1 hybrids: Seven of eight crosses have a male fertility of between 80 and 100 %. One cross, Gk 340, represented by one F_1 plant only, has 61 % stainable pollen. The variation within crosses is rather small.

Fertility of the F_2 progenies: In 28 of 42 F_2 plants derived from 7 progenies the fertility is greater than 90 %, in 12 the fertility lies between 80 and 90 % and in 2 between 70 and 80 %.

Seed-setting: Good.

A. glabriuscula×*A. longipes*

Five crossing combinations between *A. glabriuscula* and *A. longipes* ssp. *longipes* and two with ssp. *praecox* have been investigated. The shape of the lower leaves varies in the different F_1 families, some of them have leaves similar to those of *A. glabriuscula,* others to those of *A. longipes* or are intermediate. The margins are usually dentate, rarely entire. The bracteoles are mostly rhomboid, united only at the base, shortly stalked or sessile, green in the upper part, sometimes black and fleshy at the base. The margins are entire or dentate, appendages present or lacking on the back. The surface is generally veined.

Germination of hybrid seeds: The five crosses between *A. glabriuscula* and *A. longipes* ssp. *longipes* have a germination of 10 to 30 %, and the only cross investigated between *A. glabriuscula* and *A. longipes* ssp. *praecox* 40 to 50 %.

Fertility in the F_1 hybrids: All the five crosses between *A. glabriuscula* and *A. longipes* ssp. *longipes* have more than 80 % stainable pollen, two between 80 and 90 % and three between 90 and 100 %. Both the crosses with ssp. *praecox* have reduced fertility, one having 73 % and the other 67 %.

Fertility of the F_2 progenies: All the F_2 plants raised from crosses between *A. longipes* ssp. *longipes* and *A. glabriuscula* have fertility values greater than 80 %, 19 between 90 and 100 %, and 6 between 80 and 90 %. No F_2 progeny has been obtained between *A. glabriuscula* and *A. longipes* ssp. *praecox,* due to poor germination.

Seed-setting: Good.

A. glabriuscula×*A. triangularis*

Seven crosses have been investigated. The lower leaves are triangular to hastate, dentate and with a truncate or subcordate base. The upper leaves are triangular to lanceolate and often have prominent basal lobes. The bracteoles are variable, in some F_1 families being similar to those of *A. glabriuscula,* i.e. rhomboid, united to the middle, thick and fleshy at the base, in others more like those of *A. triangularis,* triangular to rhomboid, only united at the base and more herbaceous. The margins are usually somewhat dentate and the back often has appendages.

Germination of hybrid seeds: Seeds of two crosses did not germinate at all. In the other seven crosses germination varied between 1 and 40 %.

Fertility in the F_1 hybrids: Four crosses have fertility values greater than 90 %, one cross has between 50 and 60 %, one between 70 and 80 % and one between 80 and 90 %.

Fertility of the F_2 progenies: Fifteen of 35 F_2 plants have more than 90 % stainable pollen, 19 between 80 and 90 % and 1 F_2 plant between 70 and 80 %.

Seed-setting: Good.

A. longipes×*A. triangularis*

In all 35 crosses germinated, 22 between *A. triangularis* and *A. longipes* ssp. *longipes,* 13 with ssp. *praecox.* The different crosses show great morphological diversity. The appearance of the hybrid plants in many crosses is similar to that of *A. triangularis,* even in some crosses where *A. longipes* is the pistillate parent. In others, intermediates or hybrids similar to *A. longipes* occur, particularly in crosses between ssp. *praecox* and *A. triangularis.* Part of the morphological variation is shown in Fig. 13.

Germination of hybrid seeds: The germination is highly reduced, the crosses between ssp. *longipes* and *A. triangularis* showing a variation from 0 to 60 %, but 20 of 23 crosses have less than 30 %. In ssp. *praecox*×*A. triangularis* all the crosses have less than 20 % germinated seeds.

Fertility in the F_1 hybrids: Crosses with high fertility values as well as those with

Table 16. The relation between male fertility in F_1 hybrids and their corresponding F_2 progenies after self-fertilization. n indicates the number of F_2 plants investigated.

F_1	Male fertility % in F_2								
	30	40	50	60	70	80	90	100	n
Crosses within taxa									
100—90	—	—	—	—	1	1	11		13
90—80	—	—	1	—	2	9	20		32
80—70	—	—	2	1	9	10	15		37
70—60	—	2	2	3	6	8	4		25
60—50	—	—	—	4	—	2	—		6
50—40	—	2	5	—	1	2	3		13
Crosses between taxa									
100—90	—	—	—	2	2	38	140		182
90—80	—	—	—	6	13	42	77		138
80—70	—	—	2	8	13	35	39		97
70—60	—	2	3	8	8	7	9		37
60—50	—	1	5	8	1	6	6		27
50—40	1	1	3	4	7	3	6		25

reduced values occur in both crossing types. The frequency of reduced fertility is highest in crosses between ssp. *praecox* and *A. triangularis*. 8 crosses of 13 have below 80 % good pollen. The corresponding figures in crosses with ssp. *longipes* are 6 out of 22.

Fertility of the F_2 progenies: In crosses between ssp. *longipes* and *A. triangularis* most of the F_2 plants have a fertility of 80 to 100 %, some between 60 and 80 %. The fertility of F_2 plants, representing *A. longipes* ssp. *praecox*×*A. triangularis*, shows considerable variation, from 40 to 100 %. Sixty-seven of 91 F_2 hybrids have more than 80 % stainable pollen, 20 have between 60 and 80 %, and 4 between 40 and 60 %.

Seed-setting: Good.

FERTILITY AND MORPHOLOGICAL VARIATION IN THE F_2 PROGENIES

Eighty-nine F_2 progenies were raised by selfing of the F_1 hybrids, which represented different crossing combinations at different levels of fertility. Due to poor germination it was sometimes difficult to get progenies with enough F_2 plants, although a large number of seeds was sown. Most of the F_2 individuals were vegetatively well developed and seemed to be at least as vigorous as the parent species. The aims were twofold: to investigate the fertility of the F_2 progenies in relation to that of the F_1 hybrids, and further to study morphological variation in different F_2 progenies.

Fertility: The fertility values of F_2 in different crossing combinations are summarized in Table 15. In most crosses between taxa there are almost completely fertile F_2 hybrids as well as some with more or less reduced fertility. The greatest variation is shown in crosses with *A. longipes* ssp. *praecox* as one parent, from 30 to 100 % stainable pollen. In fact population crosses within *A. longipes* ssp. *praecox* and *A. triangularis* have a greater proportion of F_2 individuals with reduced fertility than crosses between taxa have.

The relation between fertility in F_1 and that of the F_2 progenies is shown in Table 16. F_2 progenies of highly fertile F_1 hybrids are generally almost completely fertile, but some individuals have decreased fertility values. Progenies raised from semi-sterile F_1 hybrids show great

Table 17. The number of F_2 plants with a male fertility greater than, equal to and less than that of their corresponding F_1 hybrids.

Crosses	F_2 plants with a fertility			
	greater than that of F_1	equal to that of F_1	less than that of F_1	Total n
within taxa	80	34	12	126
	63.5	27.0	9.5	%
between taxa	219	209	78	506
	43.3	41.3	15.4	%

variation in fertility ranging from below the F_1 value to almost 100 % good pollen. F_2 individuals with restored fertility are even produced when the F_1 hybrids have fertility values as low as 40—50 %. The number of F_2 individuals with a fertility greater than, equal to and less than that of their F_1 hybrid is summarized in Table 17. In crosses within taxa 64 % and between taxa 43 % of the F_2 hybrids have increased fertility, while the corresponding figures for reduced fertility are 10 and 15 %. Obviously restoration of fertility occurs more quickly in intraspecific crosses than in interspecific crosses.

Morphological variation: The morphological variation in F_2 progenies, representing interspecific crosses, is shown in Figs. 12 and 13. In all the interspecific crosses within the *A. triangularis* group the number of F_2 hybrids resembling one of the parent species in all characters is low. Most of the F_2 plants differ from their parents in at least one character and further, the morphological characters seem to be combined independent of one another. For instance, in crosses between *A. calotheca* and *A. longipes* there are F_2 hybrids characterized by rhomboid, laciniate leaves as well as triangular ones with entire margins, combined with entire or laciniate bracteoles. The occurrence of these recombination types indicates that the genes conditioning the shape of the leaves and bracteoles are mainly inherited unlinked, and this is probably also true of genes influencing the shape of the leaves in relation to those determining the leaf margins. Hybrids with entire leaves but with laciniate bracteoles and vice versa have been observed both in different F_2 progenies of *A. calotheca*×*A. longipes* and of *A. calotheca*×*A. triangularis*. Further, the occurrence of transitional forms both between laciniate and entire bracteoles, and leaves with truncate and cuneate bases, indicate interaction between more than one pair of genes with complementary effects, rather than incomplete dominance. This suggestion is supported by the fact that some progenies of spontaneous hybrids with an intermediate leaf form keep this character in later generations.

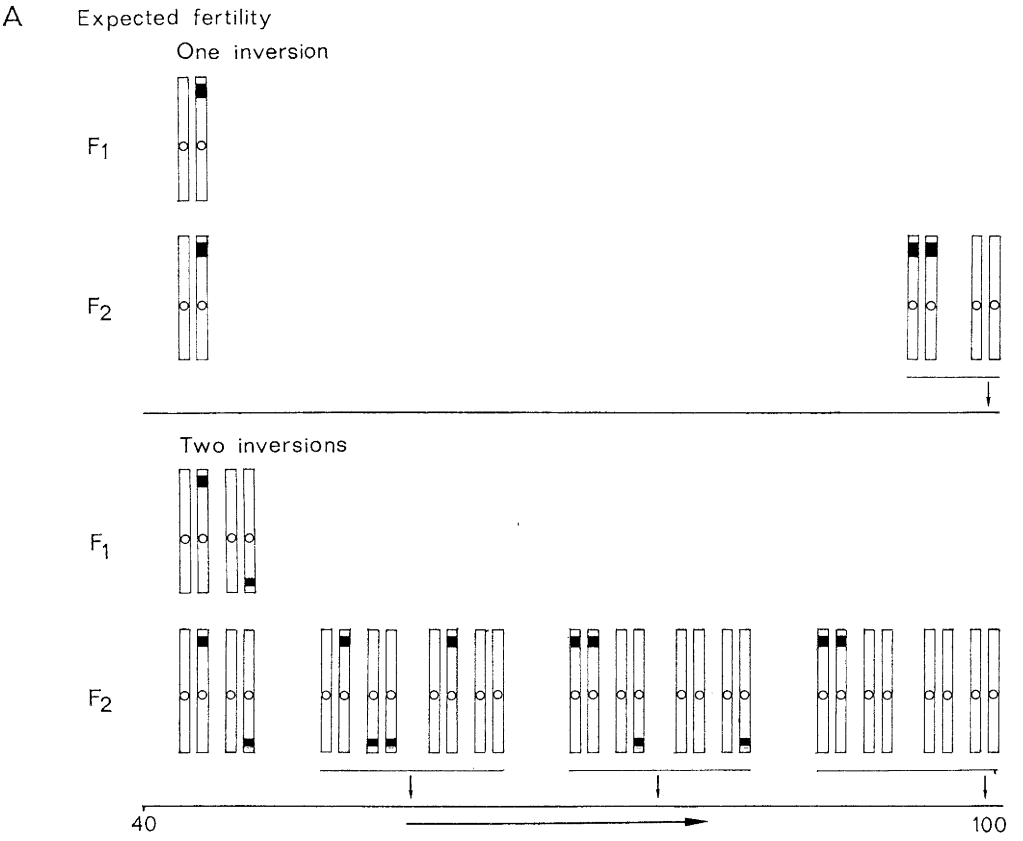
Some spontaneous as well as artificially produced hybrids between *A. calotheca* and *A. longipes* possess long-stalked, axillary bracteoles with laciniate margins. In crosses between *A. glabriuscula* and *A. longipes* F_2 plants with long-stalked, axillary bracteoles with dark, fleshy and thick bases have been observed. Certain F_2 specimens derived from crosses between *A. calotheca* and *A. glabriuscula* have rhomboid thick and fleshy bracteoles with laciniate margins. Thus, genes conditioning the different characters of the bracteoles seem also to be inherited unlinked.

SUMMARY AND DISCUSSION

The most significant internal crossing barrier is the reduced germination of both hybrid seeds and seeds produced by F_1 plants. The differences in germination between spontaneous seeds of different populations and seeds of different crosses are considerable. In the majority of both intra- and interspecific crosses the germination is less than 30 % and as many as 24 to 26 % of the crosses did not germinate at all. By contrast, seeds obtained in natural populations usually ger-

minate to at least 60 %. Seeds with anatomical and physiological disturbances occur in population crosses as well as in species crosses. Hybrid seeds with decreased or delayed germination have been observed in many other species aggregates, e.g. in the *Nigella arvensis* coenospecies (STRID 1970) and in the *Malcolmia maritima* complex (STORK 1972). In the *A. triangularis* group as well as in *Nigella arvensis* and *Malcolmia maritima* the lack of or at least the low degree of correlation between male fertility and germination is marked. STRID concludes that "the germination data are too haphazard to permit dependable conclusions on the taxonomy and evolution of the group". But in fact, the lack of correlation indicates that germination and male fertility reflect different processes going on in hybrid plants and are controlled by different genes or gene blocks, which are independent of one another rather than linked together. However, it is very difficult to say which are most relevant as a measurement of hybrid unbalance. In the *A. triangularis* group at least both are important and express a general degeneration of the hybrid plants.

In both intra- and interspecific crosses germinated seeds give rise to viable hybrids which are usually vegetatively well developed, with different degrees of male fertility. Particularly in crosses with populations of *A. triangularis, A. longipes* ssp. *longipes* and ssp. *praecox* the decrease in male fertility is highly correlated with the frequency of inversion bridges found at anaphase I. As a rule only one bridge has been observed in each cell. Two bridges, formed simultaneously, have only been observed in four crosses, all involving populations of *A. longipes* ssp. *praecox*. The relatively rare occurrence of two bridges gives the impression that reduced fertility is mostly due to different, single inversions in the different crosses. But, this assumption is contradicted by the results obtained in the F_2 generation. The expected variation in fertility of the F_2 progenies, derived from selfed F_1 plants heterozygous for one and two inversions respectively, are shown in Fig. 16. The total size of the inverted segment(s) is presumed to be similar, and thus probably results in an approximately equal number of crossovers within the inverted segments. Selfing of F_1 hybrids heterozygous for one small or large inversion, results in an equal number of F_2 plants which either are almost complete fertile or have a male fertility and chromosomal constitution similar to that of the F_1 hybrid. On the other hand, selfing of F_1 plants heterozygous for two inversions of different size will give rise to F_2 progenies with a various degree of reduced fertility, ranging from that of the F_1 hybrid to complete fertility. The distribution of the fertility values in two crosses, Gk 32 and Gk 40, is shown in Fig. 16. In both these crosses only one bridge has been observed in each cell, indicating the occurrence of one inversion. The two F_2 progenies show conspicuous variation in male fertility and are not segregated into the two expected categories when one inversion is involved. The same variation pattern is observed in most F_2 progenies derived from semifertile F_1 hybrids (cf. Table 16). This variation is, however, very similar to the expected one, derived from hybrids heterozygous for two inversions. Further, the chances of crossovers occurring simultaneously in two small inverted segments must be considered small. This will probably explain the low frequency of crosses where two bridges have been observed at the same time. The fertility of the F_2 plants may also be affected by other factors such as minor chromosomal alterations, hardly visible at meiosis, and fertility-affecting genes. But the strong correlation between bridge formation and fertility in F_1 indicates that at least a great part of the decreased fertility is caused by the effects of inversions even in F_2. Probably two or more small inversions are involved rather than a single,



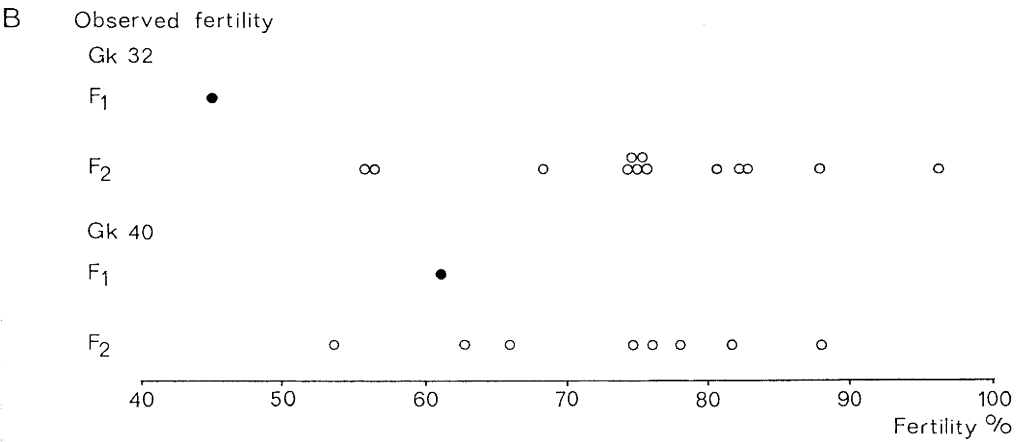


Fig. 16. Variation in male fertility in F_2 progenies derived from selfed F_1 plants heterozygous for one and two inversions respectively. — A: Expected variation in fertility. — B: Observed variation in fertility of crosses Gk 32 and Gk 40. Dots represent fertility values of selfed F_1 plants and circles fertility values of the F_2 plants. Further explanation in the text.

larger one. The size of the inversions is difficult to estimate, as not only the length but also the position of the inverted segments may effect bridge formation, especially if terminal chiasmata are the most frequent. Similar results have been obtained in some other plant genera. Detailed cytological analysis of hybrids between *Lilium martagon* and *L. hansonii* indicate that the parent species differ by small paracentric inversions in about one half of the chromosomes (RICHARDSON 1936). Artificial hybrids between diploid species of *Paeonia* show heterozygosity for small paracentric inversions as well as for translocations (STEBBINS 1938) and populations of *Paris quadrifolia* exhibit a high degree of inversion polymorphism (GEITLER 1938).

However, heterozygosity for paracentric inversions does not explain the reduced fertility of F_2 plants originating from highly fertile F_1 hybrids. This decrease may be due to a number of different causes such as general physiological unbalance, disharmony between the parent genomes, but also to fertility-affecting genes and small chromosomal rearrangements. STEBBINS (1950) has pointed out that gametes with very small duplications or deficiencies due to small rearrangements of the chromosomes may be viable, but an accumulation of two or more deficiencies will be lethal or semi-lethal. Such an accumulation is highly probable after selfing of a F_1 hybrid. Despite the inversions at least very few barriers causing hybrid sterility have been evolved in the *A. triangularis* group. A great number of both the intra- and interspecific crosses are fertile or almost so. The degree of reduced fertility in intraspecific crosses is most pronounced within *A. longipes* ssp. *praecox*, only 5 to 17 % of the crosses have fertility values greater than 90 %. The corresponding figures for crosses within *A. longipes* ssp. *longipes* and *A. triangularis* are higher, 56 to 65 and 25 to 46 % respectively. In these taxa it is quite obvious that the geographical distance between the crossed populations is of subordinate importance. The investigation on floating capacity shows that the dispersal range of the bracteoles is quite sufficient to allow gene exchange at least between adjacent populations. However, crosses between adjacent populations have as high a degree of reduced hybrid fertility as those between more distant populations. Even crosses between populations growing 1,000 kilometers apart, may be quite fertile. The whole *A. triangularis* complex has migrated to Scandinavia during the last 10,000 years, i.e. since the last glaciation. The cytological differentiation within taxa has probably evolved during this period of time, as no old isolated populations have existed in the area. It may also be so that regional differentiation has not arisen because the period of isolation has been too short.

The low correlation between hybrid fertility and geographical distance has also been observed in the *Plantago maritima* complex (GREGOR 1939 and MOORE et al. 1972) and in the *Potentilla anserina* aggregate (ROUSI 1965). The crossing experiments show that crosses performed between populations from different continents are not more sterile than those within continents. The situation in the *Streptanthus glandulosus* complex is quite the opposite. The fertility of hybrids between isolated populations decreases as the distance between crossed populations increases (KRUCKEBERG 1957). Similar results have been obtained in the *Gilia achilleafolia* complex (GRANT 1954). The relation between hybrid fertility and geographical distance between parents is obviously quite different in different species complexes. The presence or absence of such a correlation is probably mainly due to both the duration and the degree of isolation (i.e. opportunity for gene exchange), also to the type of hybrid sterility, the degree and rate of chromosomal differentiation in the species and the effects of evolutionary factors.

On the whole, in crosses between popu-

lations of *A. longipes* ssp. *longipes* and ssp. *praecox* from the Baltic region, where they occur sympatrically, hybrid sterility is more pronounced than in crosses where populations of ssp. *longipes* originate from other regions. It is probable that selection for reproductive isolation plays or has played an important role in the Baltic region. The occurrence and importance of such a process, termed the Wallace Effect, has been tested and verified in mixtures and mutant strains of *Drosophila* species in particular. KNIGHT et al. (1956) report that ethological barriers between mutant strains of *Drosophila melanogaster* have increased in strength during successive generations of artificial selection. Selection for cross-incompatibility has also been observed between sympatric species of the leafy-stemmed *Gilias* (GRANT 1966). Sympatric speciation and chromosomal evolution may be possible if the selection for crossing barriers is fairly strong and the frequency of hybridization and introgression is prevented or at least reduced.

In interspecific crosses all combinations, except those entailing *A. longipes* ssp. *praecox*, have average fertility values greater than 82 %. Each combination has a few crosses with reduced fertility, but the majority have fertility values greater than 90 %. The average fertility values of crosses between *A. longipes* ssp. *praecox* and the other taxa are about 70 %. The number of crosses between *A. calotheca* — *A. glabriuscula* and *A. longipes* ssp. *praecox* is small, but it seems probable that a weak crossing barrier exists between *A. longipes* ssp. *praecox* and the other taxa. The evolution of this barrier may be comparatively old, as the taxa of the *A. triangularis* group have probably migrated to Scandinavia from different directions. The distribution patterns indicate that populations of *A. longipes* ssp. *praecox* have reached Scandinavia from the northeast, and that *A. calotheca, A. glabriuscula, A. longipes* ssp. *longipes* and *A. triangularis* have arrived from the south.

Weak internal crossing barriers or lack of them has been observed in other halophytic species as well. Thus, there seems to be little or no cytogenetic differentiation between diploid populations within the *Plantago maritima* complex. The only existing internal barrier to gene exchange exists in Europe, where crosses between diploid and tetraploid populations give rise to sterile hybrids (MOORE et al. 1972). In most hybrid progenies obtained from crosses within the *Potentilla anserina* group, the pollen seems to be as good as that of the parents and seed fertility as high. Further, the meiotic behaviour within these hybrids is quite normal (ROUSI 1965).

Disagreement between morphological and cytological differentiation has been observed in *A. triangularis* as well as in *A. longipes* ssp. *longipes* and ssp. *praecox*. The lack of correlation indicates that chromosomal and morphological differentiation have probably evolved separately within the taxa and are probably conditioned by different evolutionary factors. Evolution within *A. longipes* ssp. *praecox* has led to cytological differentiation, mainly inversion polymorphism, morphological differentiation is slight. By comparison the other taxa are morphologically rather distinct and in *A. triangularis* especially different morphological types have evolved. Cytologically the variation within these taxa is slight, and the differences between taxa small.

In the genus *Galeopsis* (MÜNTZING 1930) and in the *Mimulus guttatus* complex (VICKERY 1959) evolution of crossing barriers appears also to be independent of morphological and physiological differentiation.

Analysis of hybrid progenies shows that genes determining the characters of the taxa occur in a homozygous state in the parents and that most characters are inherited independent of one another.

Further, at least some characters are conditioned by more than one pair of genes with complementary effects.

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APPENDIX

Code to populations kept in cultivation and used in the experimental studies. The following abbreviations are used. Countries: Sw.=Sweden, No.=Norway, Dk.=Denmark, Fi.=Finland. Provinces of Sweden: Bl.=Blekinge, Boh.=Bohuslän, Gotl.=Gotland, Hld.=Halland, Gästr.=Gästrikland, Öl.=Öland, Österg.=Östergötland, Sk.=Skåne, Sm.=Småland, Söderm.=Södermanland, Uppl.=Uppland.

Population code	Origin
A. calotheca 2n=18	
GHA	Sw. Sk. Vikhög
GHB	Sw. Sk. 3 km N Barsebäckshamn
GHC	Sw. Sk. Skanör
GHD	Sw. Sk. Bunkeflo
GHF	Sw. Hld. Falkenberg, Skreanäs
GHG	Sw. Hld. Tvååker, Björkäng
GHH	Sw. Sk. Gässie
GKA	Dk. Sjælland. Köge
GKB	Dk. Sjælland. Dragör
A. glabriuscula 2n=18	
GMA	Sw. Sk. Vikhög
GMB	Sw. Sk. Barsebäckshamn
GME	Sw. Sk. Torekov
GMF	Sw. Hld. Falkenberg, Morup
GMG	Sw. Hld. Åsa, Stenudden
GMI	Sw. Hld. Kuggaviken
GMK	Sw. Hld. Falkenberg, Skreanäs
GML	Sw. Hld. Tvååker, Björkäng
GMM	Sw. Hld. Varberg, Getterön
GMO	Sw. Boh. Strömstad, Tjärnö
GPA	Sw. Öl. Albrunna
GQA	Dk. Fyn. Kerteminde
A. longipes ssp. **longipes** 2n=18	
G 65	Dk. Falster. Orehoved
G 66	Dk. Sjælland. 3 km NW Præstö
G 67	Dk. Jylland. Ringköbing
G 69	Dk. Sjælland. Köge
G 105	Sw. Bl. Hasslö
G 106	Sw. Bl. Torhamn
G 107	Sw. Bl. Kristianopel
G 111	Sw. Öl. Kårehamn
G 113	Sw. Gotl. Klintehamn
G 114	Sw. Gotl. Fidenäs
G 115	Sw. Gotl. Botvaldevik
G 119	Sw. Uppl. Roslagskulla, Östanå
G 132	Sw. Sk. Landskrona
G 135	Sw. Sk. Lomma
G 136	Sw. Sk. Bunkeflo
G 138	Sw. Sk. Skanör
G 141	Dk. Mön. Ulfshale
G 142	Dk. Fyn. Onsevig
G 143	Dk. Fyn. Taasinge, Vindeby
G 144	Dk. Fyn. Assens, Torö Huse
G 148	Dk. Jylland. Udbyhöj
G 149	Dk. Sjælland. Orö
G 180	Sw. Boh. Hogdal, Landholmen
G 214	Sw. Hld. Ölmevalla
G 233–10	Sw. Hld. Falkenberg, Skreanäs
G 234	Sw. Hld. Tvååker, Björkäng
G 242	Sw. Hld. Onsala, Vässingsö

Population code	Origin
A. longipes ssp. **praecox** 2n=18	
G 101	Sw. Sk. Tosteberga
G 102	Sw. Bl. Listerlandet, V:a Näs
G 108	Sw. Öl. Köpingsvik
G 109	Sw. Öl. Källahamn
G 112	Sw. Öl. 1 km WSW Norra Udden
G 116	Sw. Gotl. N. Själsö
G 117	Sw. Gotl. 3 km SE Fårösund
G 118	Sw. Gotl. Fårö, at the church
G 120	Sw. Uppl. Väddö, Kvarnsand
G 121	Sw. Söderm. Mörkö, Hörningsholm
G 122	Sw. Söderm. Muskö, Guldboda
G 123	Sw. Gästr. Bönan, Utvalsnäs
G 124	Sw. Österg. Gryt. Strömmen
G 126	Sw. Sm. Loftahammar, Flatvarp
G 128	Sw. Sm. Figeholm, Kråkelund
G 87	Fi. Åland. Lemland, Norrby
G 88	Fi. Åland. Lemland, Herrö
G 89	Fi. Åland. Lumparland, Svinö
G 90	Fi. Åland. Eckerö, Storby
G 150	No. Nordland. Mosjöen, Dagsvik
G 151	No. Nordland. Sommerset, Mörsviksbotn
G 152	No. Nordland. Bognes, Innhavet
G 154	No. Nordland. Ballangen, Arnes
G 155	No. Nordland. Narvik, Skjomnes
G 156	No. Nordland. Narvik, Rombaksbotn
G 157	No. Nordland. Ofotfjorden, Bogen
G 158	No. Troms. Skånland, Breistrand
G 159	No. Troms. Hinnöya, Fauskevåg
G 160	No. Troms. Hinnöya, Medkila
G 161	No. Troms. Kvaefjord, Straumen
G 162	No. Troms. Gullesfjord, Gombogen
G 163	No. Troms. The strait of Sortland, Sigerfjord
A. triangularis 2n=18	
G 165	Sw. Boh. Havstenssund
G 166	Sw. Boh. Orust, Varekilsnäs
G 167	Sw. Hld. Kuggaviken
G 170	Sw. Hld. Trönninge, Laxvik
G 206	No. Vestfold. Tönsberg, Mostranda
G 208	No. Östfold. Fredriksstad, Slevik
G 209	No. Östfold. Holmestrand
G 216	Sw. Boh. Hällekind
G 219	Sw. Boh. The bridge at Stenungsund
G 221	Sw. Boh. 3 km SW Tjuvkil
G 223	Sw. Boh. Marstrand
G 232	Sw. Hld. Steninge
G 233	Sw. Hld. Falkenberg, Skreanäs
G 236	Sw. Hld. Varberg, Getterön
G 238	Sw. Hld. 3 km NW Frillesås
G 239	Sw. Hld. Åsa
G 240	Sw. Hld. Åsa, Stenudden
G 241	Sw. Hld. Tjolöholm
G 249	Sw. Sk. Simrislund
G 265	Sw. Sm. Västervik, Segersgärde
G 270	Sw. Öl. Köpingsvik
G 271	Sw. Öl. Mörbylilla
G 272	Sw. Öl. Karlevi
GAA	Sw. Sk. Torekov
GAB	Sw. Sk. Vikhög
GAF	Sw. Sm. Västervik, Segersgärde
GAG	Sw. Sm. Västervik
GAH	Sw. Sm. Mönsterås, Björnö
GAI	Sw. Bl. Torhamns udde
GAK	Sw. Bl. Hasslö
GAL	Sw. Öl. Kårehamn
GAM	Sw. Öl. Albrunna
GAO	Sw. Bl. Norje
GAP	Sw. Sk. Kivik
GBA	Sw. Sk. Skanör
GBC	Sw. Sk. 3 km N Barsebäckshamn
GBD	Sw. Hld. Björkäng
GBG	Sw. Boh. Fjällbacka, Långasjö
GBH	Sw. Boh. Strömstad, Tjärnö
GBI	Sw. Boh. Grebbestad, Svinanäs
GBK	Sw. Hld. Varberg, Apelviken
GDB	Dk. Jylland. V. Vedsted
GDC	Dk. Jylland. 5 km N Nymindegab
GDD	Dk. Jylland. Oddersund
GDE	Dk. Jylland. Skagen
GDF	Dk. Jylland. Aarhus, Ajstrup strand
GDG	Dk. Fyn. Middelfart, Gamborg
GDH	Dk. Fyn. Kerteminde
GDI	Dk. Sjælland. 2 km N Tjæreby
GGA	Fi. Nyland. Tvärminne
GGB	Fi. Åland. Lemland, Lemström
GGC	Fi. Åland. Eckerö, Storby