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Biology of generative reproduction of *Colobanthus quitensis* (Kunth) Bartl. from King George Island, South Shetland Islands

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Abstract: Our macroscopic observations and microscopic studies conducted by means of a light microscope (LM) and transmission electron microscope (TEM) concerning the reproduction biology of *Colobanthus quitensis* (Caryophyllaceae) growing in natural conditions in the Antarctic and in a greenhouse in Olsztyn (northern Poland) showed that this plant develops two types of bisexual flowers: opening, chasmogamous flowers and closed, cleistogamous ones. Cleistogamy was caused by a low temperature, high air humidity and strong wind. A small number of microspores differentiated in the microsporangia of *C. quitensis*, which is typical of cleistogamous species. Microsporocytes, and later microspores, formed very thick callose walls. More than twenty spheroidal, polypantoporate pollen grains differentiated in the microsporangium. They germinated on the surface of receptive cells on the dry stigma of the gynoecium or inside the microsporangium. A monosporic embryo sac of the *Polygonum* type differentiated in the crassinucellar ovule. During this differentiation the nucellus tissue formed and stored reserve materials. In the development of generative cells, a male germ unit (MGU) with differentiated sperm cells was observed. The smaller cell contained mainly mitochondria, and the bigger one plastids. In the process of fertilization in *C. quitensis* only one nucleus of the sperm cell, without cytoplasm fragments, entered the egg cell, and the proembryo developed according to the Caryophyllad type. Almost all *C. quitensis* ovules developed and formed perispermic seeds with a completely differentiated embryo both under natural conditions in the Antarctic and in a greenhouse in Olsztyn.

Key words: Antarctic, *Colobanthus quitensis*, microsporogenesis, sexual reproduction, seed development.

Introduction

Colobanthus quitensis (Kunth) Bartl. is the only indigenous dicotyledonous plant growing in the Antarctic (Scottsberg 1954; Bravo *et al.* 2001). Its main range of occurrence is in South America, where it reaches the central Andes. Occurring in extremely cold regions of the world, this species had to develop a number of adaptation mechanisms which enable it to survive long periods of frost and maintain metabolism in relatively low temperatures during the growth season (Bravo *et al*. 2001; Sierra-Almeida *et al.* 2007).

Harsh environmental conditions in the Antarctic are shaped not only by low temperatures, but also by cyclic freezing and thawing, wetting and drying, high intensity of solar radiation in summer, including UV-B, shortage of light in winter, salinity and strong winds (Ruhland and Day 2001; Alberdi *et al.* 2002). In order to fully adapt to the Antarctic conditions, this species had to develop a strategy of generative reproduction, which would be efficient in extreme weather conditions. *C. quitensis* produces abundant flowers almost every year, but short and cool summers often cause the seeds to lose viability (Edwards 1974; Convey 1996). Warmer summers favour sexual reproduction in Antarctic flowering plants and the seeds formed then are heavier and more viable (Day *et al*. 1999).

So far, mainly macroscopic observations of sexual reproduction in *C. quitensis* have been conducted (Holtom and Greene 1967; Convey 1996; McGraw and Day 1997; Ruhland and Day 2001; Krna *et al.* 2009). Very few articles concerning generative reproduction in this species contain results of anatomical studies performed by means of microscopic techniques, especially an electron microscope (Sadowska 1998; Giełwanowska *et al.* 2006, 2007).

The aim of our study was to analyse microscopically individual stages of generative reproduction in *C. quitensis* growing in natural conditions in the Antarctic and in a greenhouse in Olsztyn.

Material and methods

Plant material. — Developing flower buds and seeds of *Colobanthus quitensis* were collected during the 26th Antarctic Expedition organised by the Department of Antarctic Biology of the Polish Academy of Sciences in Warsaw. Flower buds in various development stages were gathered from December 2001 to March 2002 in the vicinity of the Polish *H. Arctowski* Antarctic Station (62°09.8’ S and 58°28.5’ W). The seeds were collected from March to May 2002. Some of them were placed on moist filter paper and kept at room temperature in a laboratory at the *Arctowski* Station to check seed germinability directly after harvest. Having been transported to Poland and stored at room temperature for about 13 months from the moment of collection, the seeds were sown. Some of them were placed on MS medium in Petri

dishes, and when seedlings appeared, they were planted into pots with garden soil. The seeds sown directly into pots also produced plants.

Almost all the *C. quitensis* plants from the seeds brought from the Antarctic have been growing, flowering and bearing fertile seeds in a greenhouse until the present moment. The new seeds have dispersed and produced new plants. In this way the culture of *C. quitensis* has been continued under greenhouse conditions of about 20°C since 2002.

Entire *C. quitensis* plants were also collected in the vicinity of the Polish *H. Arctowski* Antarctic Station. They were prepared for transportation and brought to Olsztyn, where they were planted in pots with garden soil with a view to continue the culture in a greenhouse.

Light and electron microscopy. — The material for the study under light and electron microscopes was prepared by a standard method of fixing developing *Colobanthus quitensis* flower buds in a 3.5% glutaraldehyde solution in a phosphate buffer with pH 7.0 at room temperature for 10 hours, and next post-fixing them in a 2.5% aqueous solution of osmium tetroxide for 8 hours. Then, after rinsing and dehydration in a graded alcohol and acetone series, the material was embedded in epoxide resin Poly Bed 812. Semi-thin and ultra-thin sections were prepared on a Leica ultramicrotome (Ultracut R) using glass and diamond knives. The semi-thin sections were stained with toluidine blue and toluidine blue with azure B according to Pearse (1962). After that they were observed and photographed under light microscopes Olympus and Nikon Optiphot II. All the specimens intended for photographing in light microscopes were embedded in glycerol. The ultra-thin sections (60–90 nm) were fitted on 300-mesh copper grids and contrasted with a saturated aqueous solution of uranyl acetate and lead citrate according to Reynolds (1973). The electron microscope observations were carried out and photoelectronograms taken in a transmission electron microscope (TEM) JEOL JEM 100S.

Results

Colobanthus quitensis, growing both under natural conditions in Antarctic tundra (Fig. 1a–c) and in a greenhouse (Fig. 1d–i), produced small, inconspicuous flowers with white and green perianths. These single bisexual flowers terminated module shoots or grew in axils of xerophytic leaves. Stamens and carpels most often covered with five elements of the undifferentiated perianth developed in closed flower buds hidden among the newest leaf blades. A different number of perianth elements were also observed, for example 4 or 6. The perianth consisted of green floral leaves arranged in two whorls. The leaves were not fused but their margins were appressed (Fig. 2a). In natural conditions, in the Antarctic, developing flower buds almost always remained closed until the stage of maturing seeds. In greenhouse conditions, on the other hand, they opened much earlier, even before pollen maturation.

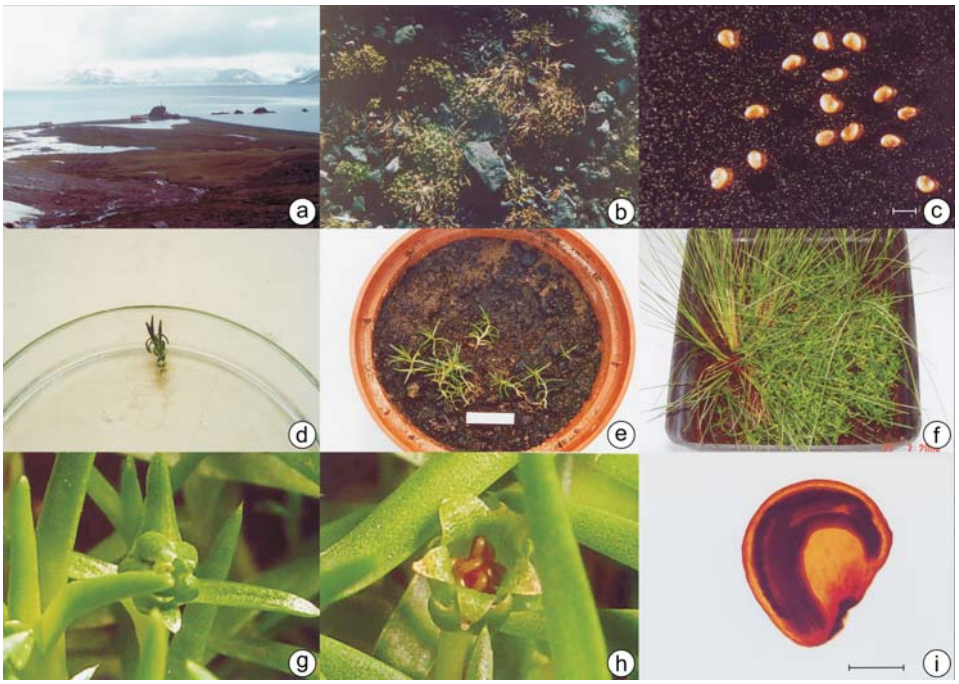

Fig. 1. **a**. Part of the west coast of Admiralty Bay with the buildings of the Polish *Arctowski* Antarctic Station. **b**. *Colobanthus quitensis* and *Deschampsia antarctica* plants growing in Jasnorzewski Gardens, where the plants and seeds used in the study came from. **c**. Seeds collected from plants growing in the vicinity of the station. Scale bar 3 mm. **d–f**. *Colobanthus quitensis* plants growing in the laboratory and greenhouse of the University of Warmia and Mazury in Olsztyn at a temperature of about 20°C. **g–h**. Bags with seeds maturing in greenhouse conditions. **i**. Mature pearlwort seed with a visible embryo. Scale bar 1 mm.

Structure of microsporangium and development of pollen. — Five stamens with short filaments usually differentiated in *Colobanthus quitensis* flowers. Patch-like structures covered with epidermis developed symmetrically on both sides of the slightly widened basal part of the filament. They were built of tightly fused cells. Their ultrastructure resembled the secretory tissue. Cells of these patch-like structures contained thick cytoplasm with organelles and a centrally located nucleus with a large nucleolus. Among the organelles, small plastids with starch, mitochondria, numerous sections of endoplasmic reticulum and tiny vesicles with osmophilic material were interesting to note. As the stamens matured, cells of the patches on their filaments vacuolised.

Two microsporangia (Fig. 2a) that were regularly circular in shape in cross section (Fig. 2e, f) differentiated in the anther. Three layers of cells were visible in the microsporangium wall. Cells with starch, which transformed into endothecium were visible under the epidermis, the outermost layer, almost on its entire length. Only in some places an intermediate parenchyma layer was found. A nutritive tissue, the tapetum, differentiated inside (Fig. 2b–g). The central part of the micro-

sporangium was occupied by the archesporial tissue, which gave rise to microspores as a result of meiosis. Before meiosis began, microspore mother cells built special callose walls. Callose surrounded microsporocytes until the tetrad stage (Fig. 2d). After meiosis, when callose degraded, microspores were released from tetrads, and a complex cell wall, the sporoderm, formed on each of them (Fig. 2g–i).

The developing mother cells of the male gametophyte were surrounded and nourished by the cell tapetum from the premeiotic stage, through meiosis, to the stage of bicellular pollen grains. Cells of the secretory tapetum were mono- or binucleate. They retained their individuality until the stage of male gametophyte. In the thick cytoplasm of tapetal cells, even before the release of microspores from tetrads, osmophilic droplets appeared and moved to the pollen sac (Fig. 2b, c, e–g). Apart from small lipid droplets, vast areas of osmophilic material could be observed in the central part of the protoplast in tapetal cells and on the boundary with the pollen sac. This material in the form of proorbicules moved from various regions of the tapetum towards pollen grains, or in the form of bigger structures, orbicules, fused with the developing sporoderm (Fig. 2i, arrowheads). Numerous apertural sites devoid of electron-dense sporopollenin material differentiated in the forming sporoderm (Fig. 2g, h). In the photograms it could be observed that the orbicules were deposited on the surface of pollen grains until the final stage of tapetum disintegration and disappearance. At this time, endothecium with distinct ridge-like wall thickenings was already fully developed in the microsporangium wall (Fig. 2f, arrow).

In the case of microspore or pollen protoplast death, most likely resulting from temperature or osmotic stress, it was found that the tapetum did not always degrade. Instances of the endothecium with woody ridges, which did not differentiate and microsporangia in which the typical layers were not formed in the sporoderm were also observed (Fig. 2j, arrows).

Development and structure of the embryo sac. — Most often 15–40 ovules differentiated from the centrally located vertical placenta in the five-chambered ovary. Very young ovules were convexities of the meristem. As they developed, a single outer integument differentiated, followed by the second, inner one. When both the integuments were present, an archesporial cell, which divided mitotically, differentiated subepidermally from the nucellus. This division gave rise to two cells: a parietal cell close to the micropyle and a basal one at the chalazal end. The basal cell became the megaspore mother cell (megasporocyte) in a short time (Fig. 3a). The megasporocyte underwent meiosis, which resulted in a linear megaspore tetrad. Three megaspores at the mycropylar end degenerated. The chalazal megaspore turned into a functional one. The analysis of embryological specimens in the consecutive stages suggested that the embryo sac of *C. quitensis* conforms to the most common *Polygonum* type. The functional megaspore increased in volume,

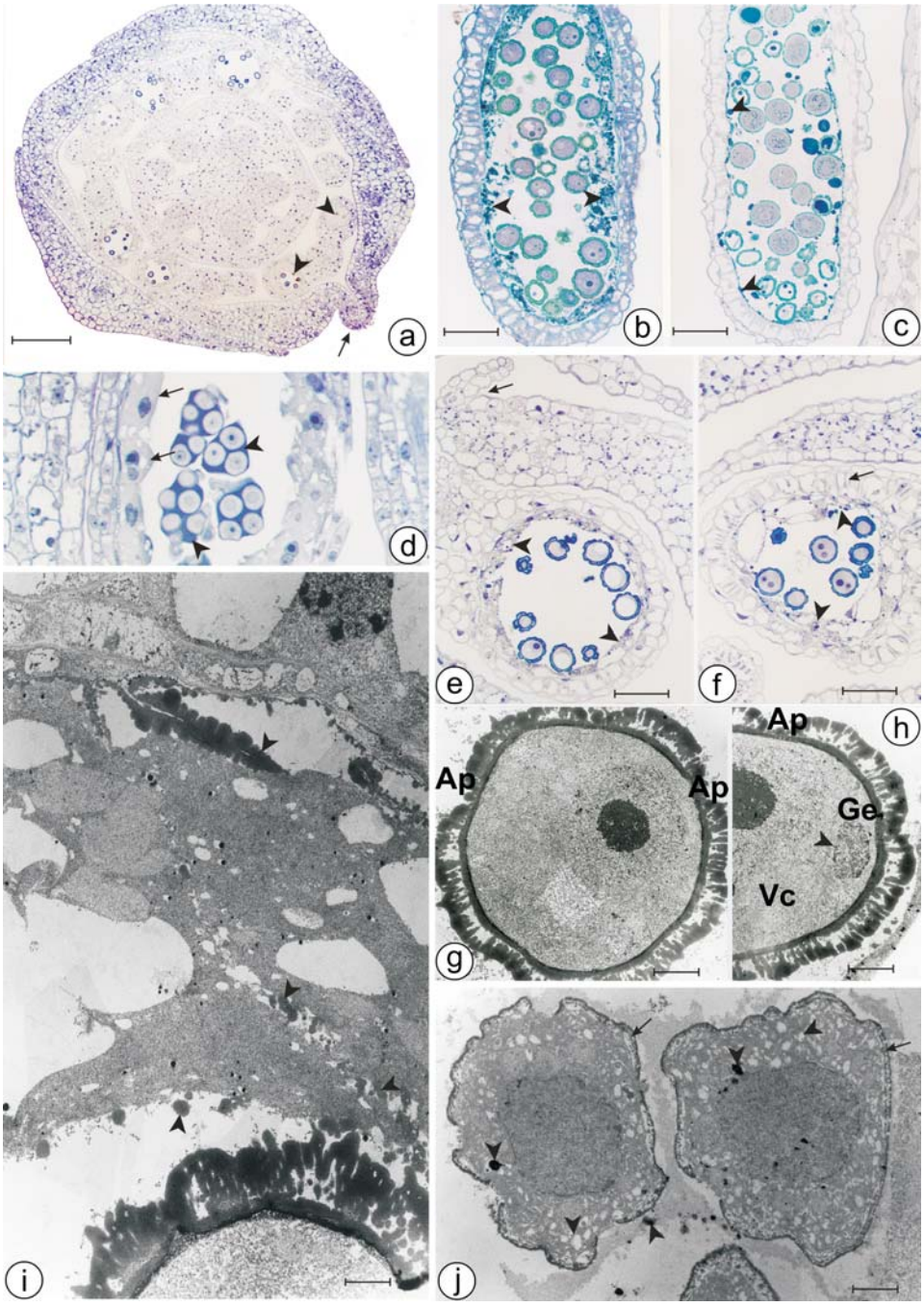

Fig. 2. **a**. Closed bud of *Colobanthus quitensis* at the stage of maturing pollen. Five elements of the perianth in two verticils are visible. The margins of two sepals are touching (arrow), the surfaces of the leaflets fit tight. Parts of six stamens are visible. Two microsporangia (arrowheads) are differentiating in the stamen head. Ovule are visible in a five-chambered ovary, on the surface of the axial placenta. →

elongated along the micropylar chalazal axis and began mitosis going through the free nuclear stage of embryo sac formation, followed by the cellularisation stage.

In *C. quitensis*, ovules with two developed nucelli, where two fully formed embryo sacs were found, were recorded only sporadically. During embryo-sac formation intensive growth of the entire ovule was observed in this plant. Integuments elongated and completely covered the nucellus. Nucellus cells grew and filled with thick cytoplasm and reserve materials. An especially large amount of reserve materials appeared at the chalazal end of the nucellus. The differentiating embryo sac also grew fast. Following karyokineses the differentiation process of the seven celled embryo sac took place. After cytokinesis three micropylar nuclei formed the egg apparatus consisting of an egg cell and two synergids (Fig. 3b). The synergids surrounded the egg cell that was pyriform. The widened end of the egg cell was turned towards the central cell. The filiform apparatus was clearly visible in the synergids. The electronograms showed that the condition of the synergids differed. In one of them the cytoplasm was thick and contained the nucleus and the remaining organelles, while in the other the cytoplasm disorganised.

The middle, biggest part of the embryo sac was filled with the central cell with two nuclei from the two poles of the embryo sac. These were polar nuclei. The nucleus from the chalazal end moved in the direction of the micropyle, towards the nucleus from the micropylar end. Before fertilisation the polar nuclei fused into a secondary nucleus of the embryo sac surrounded by cytoplasm with starch grains. Vacuoles occupied the greatest part of the central cell. The cell wall of the large central cell formed characteristic outgrowths located mainly at the micropylar end.

Pollination, fertilisation and embryo development. — When pollen grains were mature and contained a two-celled male gametophyte, microsporangia of *C. quitensis* ruptured at a point called stomium on the inner, medial side of the theca (Fig. 3d). After dehiscence some pollen were deposited on the surface of receptive

Scale bar 300 µm. **b**. Microsporangium with bicellular pollen grains with a distinct sporoderm, disorganising tapetum (arrowheads) and differentiating endothecium. Scale bar 70 µm. **c**. Microsporangium with disorganising pollen grains and tapetum (arrowheads). Scale bar 70 µm. **d**. Fragment of a microsporangium with microspore tetrads. Very thick callose walls of microspores (arrowheads) are visible. Tapetal cells (arrows) with distinct nuclei and dense cytoplasm are also visible. Scale bar 80 µm. **e**. Mononucleate pollen grain in the microsporangium. Perianth element with a tilted edge is visible (arrows). Scale bar 80 µm. **f**. Structure of the microsporangium with bicellular pollen grains. Disorganising tapetum (arrowheads) and endothecium (arrow) are visible. Scale bar 80 µm. **g**. Ultrastructure of a microspore. Sexine, nexine and intine are visible in the sporoderm with distinct apertures (Ap). Scale bar 5 µm. **h**. Ultrastructure of bicellular pollen grains. Cell wall (arrowheads) separating a generative cell (Ge) and vegetative cell (Vc) is visible. Scale bar 5 µm. **i**. Fragment of the cytoplasm of the tapetum and sporoderm. Deposits of fatty substances (arrowheads), numerous drops, proorbicules and orbicules are visible. Layers of sexine, nexine and intine are visible in the sporoderm. Scale bar 2 µm. **j**. Disturbed pollen development. Pollen grains with an insufficiently developed sporoderm (arrows), optically electron empty and dense droplets are visible (arrowheads). Scale bar 5 µm.

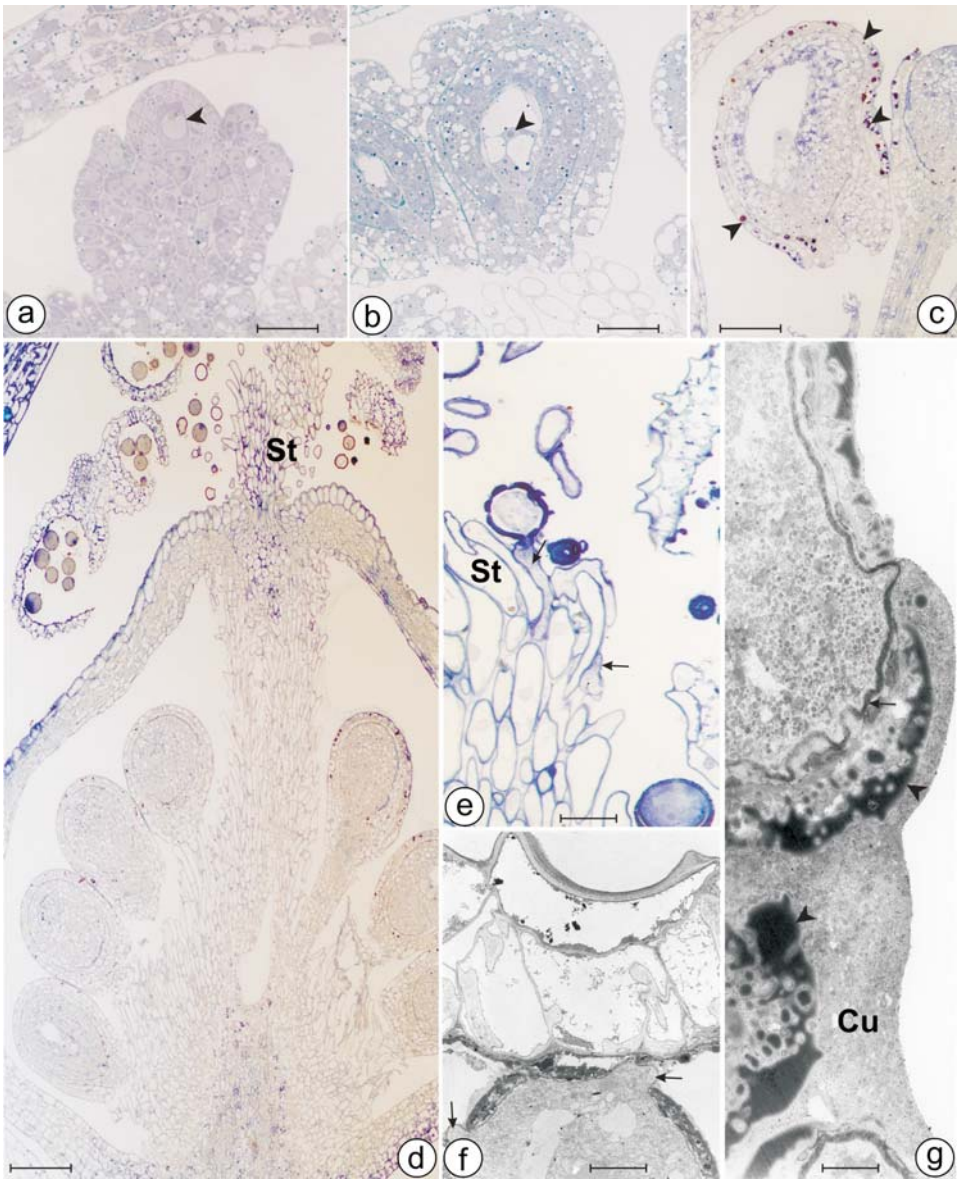

Fig. 3. **a**. Ovule of *Colobanthus quitensis* with differentiated envelopes and an archesporial cell (arrowheads). Scale bar 70 µm. **b**. Embryo sac with an egg apparatus on the micropylar pole. The nucleus of the egg cell (arrowhead) and synergids are visible. Scale bar 70 µm. **c**. Proembryo with prosuspensor are visible. Reserve substances are accumulated in nucellar cells, especially in the internal part. An especially large amount of clearly osmophilic lipid substances is gathered in the cells of the future seed coat (arrowheads). Scale bar 100 µm. **d**. Longitudinal section through a closed flower bud at the stage of theca dehiscence. Pollen reaches the surface of the dry, feathery sigma (st). In the ovary numerous ovules with embryo sacs are visible. Scale bar 200 µm. **e**. Germinating pollen grains (arrows) on the stigma (St) of a closed flower. Scale bar 40 µm. **f**. TEM Germinating pollen grain inside the microsporangium. Two pollen tubes (arrows) growing through two neighboring apertures are →

cells of the five-branched feathery stigma and some stayed in the microsporangium (Fig. 3d, e).

Under Antarctic conditions elements of the perianth remained tightly closed during theca dehiscence in the majority of *C. quitensis* flowers. Flowers with open perianths were observed only on plants growing in special niches, i.e. warm and well sheltered depressions of the ground. In several cases single thecae were observed to stick out of the perianth in the flower buds of plants found in the Antarctic. In these buds stamens were visible in a place of uncharacteristic connection between two perianth elements (Fig. 2a, arrow). However, under laboratory conditions *C. quitensis* flowers always opened after pollen maturation.

In the closed *C. quitensis* flowers thecae were located and opened at the height of the stigma. As the style is short, the stigmata lie almost directly on top of the ovary (Fig. 3d, e). *C. quitensis* has a dry stigma. The pellicle, a distinctly osmophilic protein-lipid layer, was visible on the outside of the epidermal cell cuticle.

After the rupture of the theca most pollen grains reached the stigma surface. They germinated there and their pollen tubes grew towards the ovary (Fig. 3d, e). The remaining pollen grains germinated inside the microsporangium. Very often several tubes grew from one pollen grain (Fig. 3f, arrows). The pollen tubes growing on the surface or between the cells of the stigma not always exhibited specifically directed growth. Sometimes, while growing, they wound around the elongated cells of the stigma and criss-crossed. In Fig. 3g the apical ends of the pollen tubes grow in various directions in the pellicle, where fine granular and fibrous structures with different degrees of osmophily are visible. At the apical end of the tubes cytoplasm with numerous, characteristic vesicles could be observed. Most of the vesicles had an electron-dense content. Droplets of the material secreted by the cytoplasm of the pollen tube into the cell wall close to the tube apex were visible outside the intine (Fig. 3g). Numerous pollen tubes grew into the ovary, continued growing on the placenta surface and reached the ovules. Single *C. quitensis* pollen tubes were observed to grow through the ovule micropyle and approach the embryo sac. The tube grew into it through the degenerated synergid. The cytoplasm of the tube carried male gametes with a different protoplast composition to the vicinity of the egg cell. One sperm cell contained cytoplasm with a large number of plastids, while the second, smaller one, had mainly mitochondria in its cytoplasm. This was visible in the electron microscope images, even though we failed to record them photographically to complete the documentation. The fusion of one sperm nucleus with the egg cell nucleus and the nucleus of the second sperm cell close to the central cell nucleus were observed in the semi-thin specimens. It appeared that only the sperm cells nuclei penetrated the egg cell. This is indicated by

visible. Scale bar 6 µm. **g**. TEM Fragments of two pollen tubes growing on the surface of the stigma. The intine (arrow) and electron-dense components (arrowheads) secreted by the protoplasts of pollen tubes are visible. At the surface of pollen tubes there is a layer of pelicula (Cu). Scale bar 0.6 µm.

the fact that no strongly stained cytoplasm fragments of the sperm cells were found close to the clearly visible fusiform sperm nuclei.

The proembryo development in *C. quitensis* conformed to the Caryophyllad type, in which the zygote divides transversally. Then the apical cell division is transverse and the basal cell neither participates in the formation of the proper embryo nor divides (Fig. 3c).

After the process of double fertilisation the nucellus tissue of *C. quitensis* ovules was observed to increase in volume. The cells grew and their cytoplasm became thicker. They filled with reserve materials in a short time. In this way the perisperm, the nutritive tissue of the developing young sporophyte, was formed.

Seed germination and plant growth. — Directly after harvest *Colobanthus quitensis* seeds did not germinate under any conditions. They might have been in deep primary dormancy. Seed germination results obtained over the next two years after the seeds had been brought to Poland from the Antarctic were low (2–4%). That is why we do not present these data in this work. However, the tetrazolium test showed that both the caryopses collected in 2002 and those after two-year storage had about 80% of viable embryos. At present the seeds produced by the plants growing in a greenhouse at a temperature of 20°C are released from capsules in the immediate vicinity of plants, germinate, and the seedlings give rise to new plants. The majority of them flowered in the second and third year. Sparse flowers appeared on *C. quitensis* plants at different times. One to five flower buds usually developed on one individual from April to August each year at different time intervals.

Discussion

The adaptation of spermatophytes to specific environmental conditions is visible in their full development cycle, which leads to flower and seed formation (Edwards 1974), Brown (1912) and Holdgate (1964) observed flowering and seed-dispersing specimens of the Antarctic vascular plants, *Colobanthus quitensis* and *Deschampsia antarctica* Desv., in the communities of South Georgia, Holtom and Greene (1967) and Greene and Holtom (1971) in the archipelagos of the South Orkney Islands and South Shetland Islands, Komárková *et al*. (1985, 1990), Ruhland and Day (2001) on the islands along the west coast of the Antarctic Peninsula, and recently Krna *et al.* (2009) on the Antarctic Peninsula.

Our detailed study on the generative tissues development, both male and female, and electron microscope observations of mother cell formation and gametophyte development confirm the above-mentioned authors' opinion that *C. quitensis* is adapted to extreme conditions of the Antarctic environment. Despite this, as the growth season in the Antarctic geobotanical zone is extremely short in the context of the full development cycle of a spermatophyte and the

plants are subjected to a number of stress factors, more or less distinct disturbances are observed at different stages of this cycle.

The summer season is assumed to last about 5 months, approximately from November to March, considering above all night and day length. If the snow-cover period in the areas free from ice is taken into account, the growth season might be shortened by 1–2 months for the seed-bearing plants that occur there. Irrespective of the remaining factors affecting the plants, this time is critical to the success of seed formation (Edwards 1974).

In the season 2001–2002, *C. quitensis* flower buds with meiosis in the microsporangia were collected in the second half of December 2001 and in January 2002 on King George Island in the vicinity of the Polish *H. Arctowski* Antarctic Station. At this stage the flower buds were completely hidden among the youngest leaves. Flower development on one plant was rather synchronous. On the neighbouring plants slight differences could be observed in the growth phases of flower buds, but on individuals from different microhabitats the development span of flower buds was up to 6–7 weeks. There were also marked differences in the number of produced flowers, as in *D. antarctica* (Giełwanowska *et al*. 2005).

Perianth elements in *C. quitensis*, though not fused, remained tightly connected even at the stage of mature pollen grains and differentiated embryo sac. When the plants with mature, but closed flowers were transferred to the laboratory at the Polish *H. Arctowski* Station and kept in dry air at room temperature (approx. 20°C), all the flower buds opened after about half an hour. Open *C. quitensis* flowers were also observed on plants growing in pots under greenhouse conditions in Olsztyn. In natural habitats, close to the Polish *H. Arctowski* Antarctic Station, only a few open flowers of *C. quitensis* were found. These were single specimens that formed cushions 4–7 cm in diameter growing in niches well sheltered by rocks. Based on this observation it can be stated that cleistogamy in *C. quitensis* (Giełwanowska *et al.* 2007), the same as in *D. antarctica* (Giełwanowska *et al.* 2005), is most probably conditioned by low temperatures, high air humidity and strong wind. Parodi (1949) called *D. antarctica* flowers cleistogamous, because he had observed the chaffs in the flowers to be fused together for 2/3 of their length from the base.

Levkovsky *et al.* (1981) suggested that cleistogamy in Poaceae occurring on Arctic islands is induced by low temperatures (3.5–5.0°C) and high air humidity. Therefore, this phenomenon should rather be designated as cryocleistogamy.

In the vicinity of the Polish Antarctic Station, in the season 2001–2002, mature beige-brown fruits with seeds appeared on the plants of *C. quitensis* and *D. antarctica* at the same time, that is towards the end of the summer in March 2002. In this time period, plants with developing flower buds were sporadically found, but no further development was observed.

Microsporocyte and microspore development. — Between ten and twenty microspore mother cells differentiated in each of the two microsporangia in the *C. quitensis* theca. The androecia in *C. quitensis* flowers had a similar number of

microsporangia, which produced a comparable number of microspores. A similar number of pollen grains, most often 60–90, was formed in the thecae of flowers developing in both natural (in the Antarctic) and greenhouse (in Olsztyn) conditions. *Colobanthus quitensis* flowers with four or six stamens instead of five, which is typical of this species, were sparse. In this respect *D. antarctica* was similar. Three stamens with microsporangia developed in its chasmogamous flowers, the same as in the cleistogamous ones. Four microsporangia usually differentiated in each anther, but anthers with three microsporangia were also observed, which might be treated as an indication of miniaturisation or oligomerisation (Giełwanowska *et al.* 2005). However, clear differences were visible in the number of pollen grains between the microsporangia of closed and open *D. antarctica* flowers. Ten to thirteen microspore mother cells usually differentiated on a stamen of a chasmogamous *D. antarctica* flower, and 40–52 pollen grains matured after meiosis. In cleistogamous flowers, which formed in the second half of the growth season, only 20–30 pollen grains developed in the microsporangia. Such few microspores have never been described in other grass species.

Further development of microsporocytes and microspores of *C. quitensis* progressed in a similar manner to other dicots. As early as in the first meiotic prophase microsporocytes were surrounded with a thick callose wall and remained in such isolation until the tetrad stage. Callose began to hydrolise there when the formation of the sporoderm began, the same as in other flowering plants (Heslop-Harrison 1968; Heslop-Harrison *et al.* 1986). Further pollen development progressed in a similar way to most flowering plants. The generative cell remained close to the sporoderm after mitosis, but it was separated from the vegetative cell by a very delicate wall with a small amount of fibrous material. Unlike other angiosperms, *C. quitensis* did not form the typical wall with callose and other polysaccharides (Huang and Russel 1992). It is different in the microspores of many angiosperms with binucleate pollen described in literature (Mascarenhas 1989; Raghavan 1997). In these species, shortly after mitosis, the generative cell separates from the sporoderm and completely embeds itself in the cytoplasm of the vegetative cell. It often changes shape from lenticular to fusiform and pointed at the ends. The elongated shape and common transfer of sperm cells and the vegetative nucleus, that is the male germ unit (MGU), have a cytoskeletal basis (Van Lammeren *et al.* 1985, 1989). The male germ unit of *C. quitensis* was observed in a transmission electron microscope. Unfortunately, the attempt to photograph the only object was unsuccessful. However, clear morphological differentiation of the sperm cells with such features as in *Plumbago zeylanica* (Russell and Cass 1981; Russel 1984) or *Brassica* (Dumas *et al.* 1985) was visible.

The mature trinucleate pollen of *C. quitensis* is partly released from the theca, reaches the surface of the feathery stigma and germinates there. The pollen grains that remain in the microsporangium germinate inside the theca, the same as in other cleistogamous flowers. Two to three pollen tubes growing from one pollen

grain were observed in semi- and ultra thin sections. Due to the fact that pollen grains of this species have 20–26 pores (Sadowska 1998), a greater number of pollen tubes can be produced, which helps the MGU to reach the embryo sac.

Changes in tapetal cells. — A single-layered secretory tapetum differentiated in the microsporangia of *Colobanthus quitensis*. Cells of this tissue had thick cytoplasm and were binucleate. They retained their individuality until the stage of mature pollen, and then their cytoplasm gradually degraded and the protoplasts degenerated. The time when the tapetum was present in the *C. quitensis* theca was strongly correlated with the development of the male gametophyte, except for one instance. An intact tapetum survived in the microsporangia of a *C. quitensis* flower where microspore protoplasts degenerated probably as a result of a stress factor (or several factors). The tapetal cells did not degenerate along with microspore mother cells, but remained viable and continued to develop. They gathered a lot of osmophilic material. The cytoplasmatic changes within this tissue are connected with the protection and nourishment of sporogenic cells and the formation of sporoderm parts (Pacini 1990; Rowley 1993).

Female gametophyte development. — In *Colobanthus quitensis* the female gametophyte development conformed to the monosporic *Polygonum* type. Occurring in as many as 95% of flowering plant species, this type of embryo sac development is the most common. In *C. quitensis* the archesporial mother cell cut off a parietal cell, and the megasporocyte differentiated from a deeper-located cell. The megasporocyte of *Stellaria media* (Rodkiewicz and Bednara 1976), which, like *C. quitensis*, belongs to the family Caryophyllaceae, develops in the same way. After meiotic division the chalazal megaspore became the functional one, which developed into the female gametophyte. Finally, as a result of meiosis, four magaspores with linear arrangement were formed. The same as in the *Polygonum* type, three megaspores degenerated, and the chalazal one went through three mitotic cycles, which gave rise to eight nuclei of the embryo sac. The organisation of the embryo sac in this species was similar to *Deschampsia* (Giełwanowska *et al*. 2005).

Following the process of fertilisation, a proembryo develops in the ovule according to the Caryophyllad type. Before the proembryo development in *C. quitensis*, an increase in the volume of the nucellus tissue was observed in the ovule. The cells grew and filled with reserve materials in a short time. The perisperm, the nutritive tissue of the young sporophyte, regarded as an evolutionarily old tissue with reserve materials stored in the seed and additionally one that is easier to form, is developed in this way.

Cleistogamy and possibilities of cross-pollination. — Ensuring gene exchange between genetically different individuals, cross-pollination enables greater species plasticity and adaptation to changeable environmental conditions. Therefore, in the course of evolution organisms, which reproduce sexually by cross-pollinations, obtained an advantage over others (Raghavan 1997).

Under extremely unfavourable conditions of the Antarctic environment a specific type of cleistogamy developed in *C. quitensis*, namely cryocleistogamy. The size of flowers is very similar. All of them have similarly structured androecium and gynoecium. No differences in the cell ultrastructure of these flower parts were observed, and the ultrastructural examination of the pollen did not show any variations in the structure of the pollen sporoderm from different flowers. The flowers opened only under conditions favourable to the transfer of pollen grains directly to the stigma of the gynoecium. In all the flowers pollen germinated both on the surface of stigmata and inside microsporangia, despite the fact that the microsporangia always opened.

It appears that cleistogamy in both the Antarctic species of vascular plants is an adaptation that developed in the course of evolution when *C. quitensis* and *D. antarctica* occupied the unfavourable Antarctic environment, and at present it is their constant feature. However, the species in question have retained their ability to open flowers, and the clearly less cleistogamous pearlwort still tends to release pollen. This adaptation is still developing in this species. The morphology of all *C. quitensis* and *D. antarctica* flowers indicates that both cross- and self-pollination are possible. It appears that features such as close contact of stigma with thecae, a very small amount of pollen produced in a flower and its almost 100% viability cause the studied species to prefer autogamy. Environmental conditions permitting, flowers of both species open and cross-pollination, in which genes are exchanged, is possible. On the one hand, autogamy precludes genetic interchange and limits the evolutionary variation of species. On the other hand, however, ensuring reproductive success of the vascular plants which grow in the Antarctic conditions, it appears to be advantageous to them.

Ecophysiology of dormancy and seed germination. — Seeds of wild plants usually have a programmed strategy of generative regeneration which consists in a cyclical pattern of seasonal dormancy variations repeated every year. Nevertheless, the expression of this pattern depends on certain environmental conditions, such as suitable temperature, light, cyclic drying and wetting and nitrate concentration in the substrate (Bouwmeester and Karssen 1993; Derkx and Karssen 1994; Baskin and Baskin 1998). Preliminary studies conducted do not allow to conclude unambiguously. However, it might be presumed that polar vascular plants living in exceptionally extreme conditions, including *Colobanthus quitensis* and *Deschampsia antarctica*, require certain variable temperature and light to break dormancy (Giełwanowska *et al.* 2005). Moderate nitrate concentrations might also favour germination. The conditions present during flowering, seed formation and maturation are also likely to affect the dormancy pattern of these species, which is indicated by varying germination results recorded for *D. antarctica* seeds collected at different times. According to Edwards (1974), *D. antarctica* produces sufficiently large (at least 1.4 mm long) caryopses, which are able to germinate, only in the years when the mean temper-

ature of the period from October to March is above 0.5°C. Lewis Smith (1994) writes that germination may be inhibited by diurnal freeze-thaw cycles and depends on temperatures remaining continuously above 2–5°C for several days. Seed establishment is sporadic, occurring only in warmer, sunnier summers (Lewis Smith 2003). According to Convey (1996), it takes at least 18 months for *D. antarctica* to progress from flowering to seed production. However, if summer is short and cold, seeds fail to mature and lose viability. Warmer summers improve sexual reproduction, enhancing inflorescence development and increasing the production of heavier and more viable seeds (Day *et al.* 1999).

Especially difficult seed dormancy release and good retention of seed viability appear to be adaptation features that might allow the seeds to survive under extreme conditions of the Maritime Antarctic. In this way germination probability may be minimalised when there is no guarantee for further growth and development. Our observations suggest that *C. quitensis* seeds, like *D. antarctica* caryopses, enter a deep secondary dormancy, the release of which requires certain conditions. Seeds of this species probably need long exposure to white light, several-week low-temperature stratification and at least a few days of incubation at about 20°C in order to germinate.

As yet, the strategy of generative reproduction in *C. quitensis*, the same as in *D. antarctica*, has not been sufficiently well examined. It is essential to continue the studies, which would explain the ecophysiological conditions of flowering as well as development, maturation, dormancy and germination of seeds in the harsh environment of the Maritime Antarctic.

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