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Reproductive biology of *Callitriche cophocarpa* Sendner (Callitrichaceae)

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&

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ABSTRACT

JÄGGI, M. & C. D. K. COOK (1998). Reproductive biology of *Callitriche cophocarpa* Sendner (Callitrichaceae). *Candollea* 53: 101-115. In English, English and German abstracts.

A population of *Callitriche cophocarpa* Sendner in the Werdkanal near Rottenschwil, Kanton Aargau was studied. In spite of having a very efficient means of vegetative spread it does reproduce sexually and forms a seed bank. A seed to seed life history was observed. Isozyme electrophoresis revealed that the population contained more than one genotype but insufficient evidence was found to document outbreeding although it is likely. It is an amphibious species but unusual in the genus because the terrestrial state very rarely developed flowers. Plants growing in water flowered freely and set good seed. The floating rosettes are unisexual: males develop from branches arising near the apex of the main axis and females from branches near the base. Epihydrophily (pollination on the water surface) was documented. The pollen is mostly dispersed in clumps. A unique feature is that wind, rain or waves are needed to transfer the pollen from the anthers over the floating leaves to the surface of the water. Anemophily may be effective over very short distances. In the Werdkanal *C. cophocarpa* showed regular flowering and subsequent fructification on totally submerged parts of the plants. Hypohydrophilous pollination may also take place but we were not able to show it on plants in cultivation.

ZUSAMMENFASSUNG

JÄGGI, M. & C. D. K. COOK (1998). Reproduktionsbiologie von *Callitriche cophocarpa* Sendner (Callitrichaceae). *Candollea* 53: 101-115. Auf Englisch, englische und deutsche Zusammenfassungen.

Eine Population von *Callitriche cophocarpa* Sendner aus dem Werdkanal, nahe bei Rottenschwil im Kanton Aargau, wurde untersucht. Trotz stark ausgeprägter vegetativer Vermehrung pflanzt sie sich auch sexuell fort und bildet eine Samenbank. Die Entwicklung konnte von Samen zu Samen verfolgt und beobachtet werden. Anhand von Enzymelektrophorese konnten wir zeigen, dass die Population aus mehr als einem Genotyp besteht. Fremdbestäubung konnte nicht eindeutig nachgewiesen werden, obschon sie sehr wohl möglich ist. Dies ist für diese Gattung aussergewöhnlich, handelt es sich doch um eine amphibische Art, deren Landpflanzen nur spärlich Blüten bilden. Als Wasserpflanze blühte sie häufig und entwickelte viele Samen. Die Schwimmblattrosetten sind eingeschlechtlich: männliche Blüten entspringen auf Seitentrieben, nahe der Hauptstengelspitze, und weibliche Blüten auf Seitentrieben, nahe der Basis. Epihydrophilie (Bestäubung auf der Wasseroberfläche) wurde nachgewiesen, wobei der Pollen meist in Klumpen verbreitet wird. Wind, Regen oder Wellen werden benötigt, um ihn von den Antheren über die Schwimmblattrosetten auf die Wasseroberfläche zu transportieren. Es handelt sich hier um ein einmaliges Merkmal. Anemophilie (Windbestäubung) kann über sehr kurze Distanzen ebenfalls stattfinden. Im Werdkanal zeigt *C. cophocarpa* häufig Blüten- und darauffolgende Fruchtbildung an submersen Teilen der Pflanze. Hypohydrophilie (Unterwasserbestäubung) könnte ebenfalls vorkommen, konnte aber an den kultivierten Pflanzen nicht demonstriert werden.

KEYWORDS: *Callitriche cophocarpa* – Floral morphology – Hydrophily – Seed bank – Sexual determination.

Introduction

The family Callitrichaceae has one genus, *Callitriche* with 17 species; it is almost cosmopolitan but with most species found in the temperate zones of both hemispheres. They are annual or perennial herbs which grow entirely submerged, amphibious or terrestrial. The flowers are unisexual, highly reduced and lack a perianth. The floral biology has received considerable attention (SCHOTSMAN, 1967, 1982; MARTINSSON, 1991a; PHILBRICK, 1984, PHILBRICK & ANDERSON, 1992). SCHOTSMAN (1982) distinguished two different pollination modes in *Callitriche*: “contactors” in which there is direct contact between anther and stigma, and “non-contactors” where there is no contact between anther and stigma. The “contactors” are autogamous and have small anthers, low pollen:ovule ratios, and are pollinated either above or below the water. The “non-contactors” have larger anthers, high pollen:ovule ratios, and are pollinated in air, at the water surface or below the water. In most species of *Callitriche* both genders develop in close proximity, either both in one leaf axil or in opposite pairs. When this is the case, geitonogamy (second order autogamy) is the usual fertilization system; in these plants there is little opportunity for cross-fertilization. PHILBRICK & ANDERSON (1992) found in *Callitriche heterophylla* Pursh emend. Darby and *C. verna* L. an internal geitonogamy in which pollen grains germinate within closed anthers and the pollen tubes pass internally through vegetative tissues to the female flower; this system virtually precludes cross-pollination. This system is an adaptation analogous to cleistogamy and indicates selection pressure favouring inbreeding. Among the “non-contactors” SCHOTSMAN (1982) pointed out, that even within aerial pollinated species there is a strong tendency towards geitonogamy. She considered cross-pollination between neighbouring individuals to be possible. Except the existence of a hybrid (*C. cophocarpa* × *C. platycarpa*) she presented no additional evidence; MARTINSSON (1991b) confirmed Schotsman’s conclusions. OSBORN & PHILBRICK (1994), using molecular techniques, showed some genetic variability within a population of *C. hermaphroditica* L. (a totally submerged species) which is additional evidence that cross-fertilization does take place in this genus.

The aim of this study was to investigate cross-fertilization in *Callitriche*. As a suitable species *C. cophocarpa* Sendner was chosen. Male and female flowers develop on floating rosettes derived from separate branches. Anthers and the distal parts of the stigmas are exposed above the water surface. *Callitriche cophocarpa* can spread vegetatively so it was necessary to demonstrate that there was a seed to seed turnover within the chosen population. The total life history was examined with particular emphasis on floral development. Experiments to find out pollen longevity and time of stigmatic receptivity were also undertaken. Isozyme electrophoresis was used to investigate allelic segregation within the population.

Materials and methods

Examined sites

The natural drainage system of the River Reuss between Merenschwand and Bremgarten was modified from 1857 to 1860 by digging new canals. Additional ones were constructed between 1972 and 1982. One of these new canals (constructed in 1975 and 1976), the Werdkanal was chosen for this study (1:25.000, Blatt 1110, Coordinates: 670.30/240.26 – 670.30/239.95 der Schweizerischen Landestopographie). The examined part of the Werdkanal is about 375 m long and has a solid concrete base with an open grid of concrete blocks along the banks. It is about 1.5-2 m wide and the water is approximately 0.7 m deep; the substrate is mostly loose mud about 0.3 m deep. The water is slow flowing; it is clear except in May and June when it is over fertilized by run off from the surrounding fields. Very few trees and shrubs grow along the banks; the site gets much sunlight. The canal is cleaned once a year, between August and September with a

drag-plough which is pulled downstream. The banks are mown between July and September. The cut off plant material floats downstream to a pump-station where it is arrested by a grid and removed with a rake. For a full description of the site with the associated plant species see JÄGGI (1996).

Living samples

Cuttings were collected before the flowering season in May 1995 and planted in the University of Zürich Botanic Garden in flat plastic basins half filled with garden soil. Single shoots were fixed with staples and the basins filled with tap water. The basins were placed in two different experimental greenhouses: cold (7-)11-21(-26)°C and warm (16-)18-26(-30)°C. Plants were cultivated in 10 cm and 1 cm deep water, and on wet mud. In June additional material was collected and planted outdoors in the botanic garden. In July *C. cophocarpa* started to flower in the Werdkanal so an additional 100 cuttings were collected and transferred to the botanic garden. To test the viability of seeds two additional fruit collections were made in August; the muddy substrate was sieved (width of sieve-meshes: 0.5 mm and 0.2 mm) and the mericarps removed with a pair of forceps. They were stored either wet in tap water or allowed to dry in petri dishes. In September, after the canal had been cleaned, material was collected for electrophoresis and to estimate the number of mericarps in the seed bank. Nineteen mud and plant samples were taken at distances of 15 m. The 19 plant samples were cultivated in separate containers in the warm house for electrophoretic examination. A final visit in November 1995 was made to record how *C. cophocarpa* had recovered from the cleaning process.

Anatomy and morphology

For scanning electron microscopy flowering shoots were fixed in FAA and then preserved in 70% ethanol. For microtome sections male and female flowers were selected from fixed material (70% ethanol). They were dehydrated in a graduated series of ethanol and then evacuated before being embedded in 2-hydroxyethyl-methacrylate (HEMA) as described by IGERSHHEIM & CICHOCKI (1996). The sections were stained with toluidine blue (GERLACH, 1977) before mounting.

Breeding system

Three plants from submerged culture were chosen for observations. Each was placed in a single, transparent plastic container. The shoots were kept in tap water and placed in front of a window. The plants were observed for three consecutive days using a swivel-arm binocular microscope.

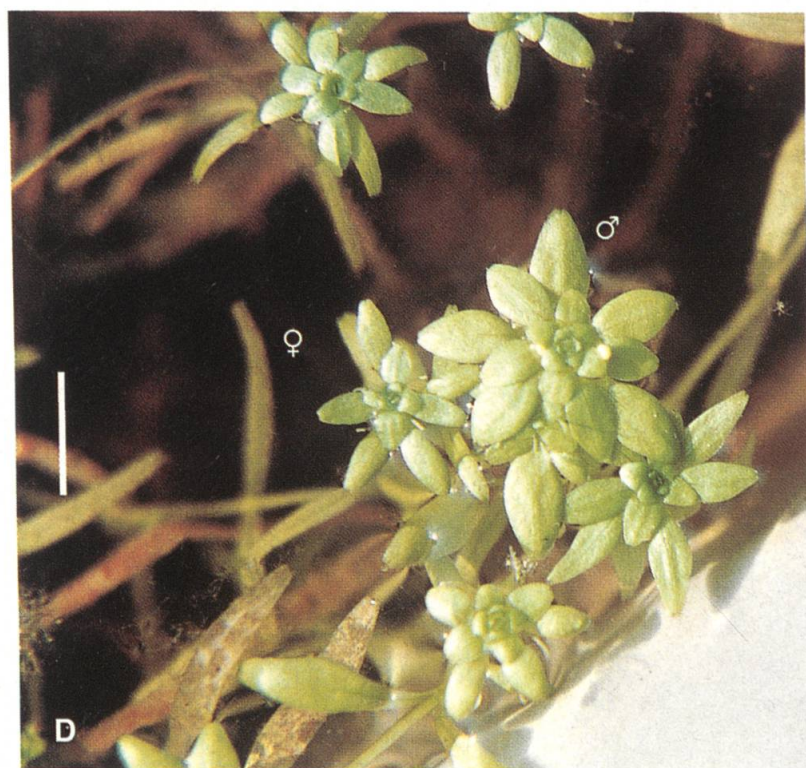
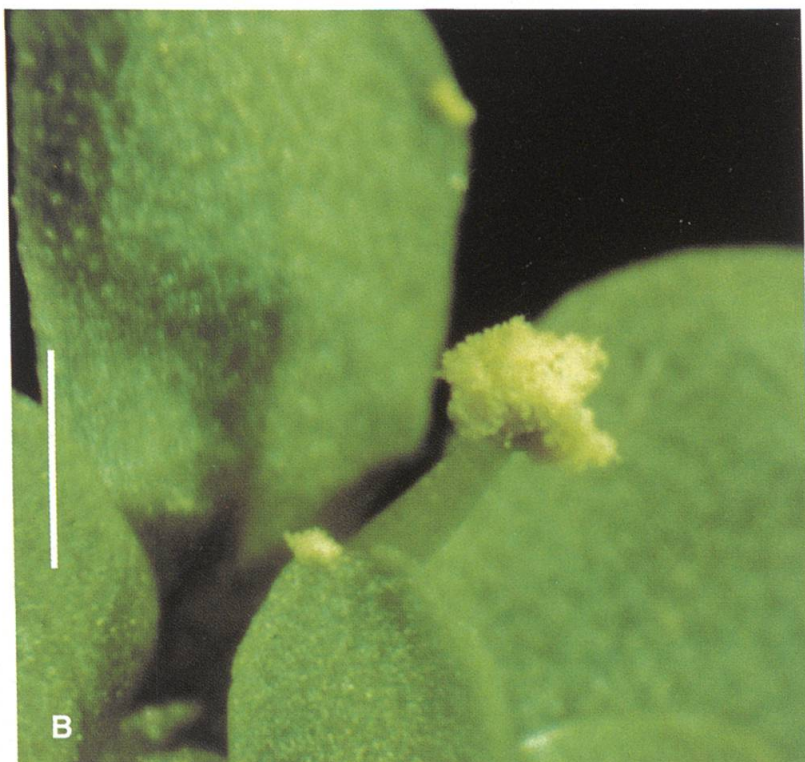
Pollen germination

The following experiments were executed to test the viability of pollen; for full details see JÄGGI (1996):

1. Mature pollen in petri dishes on distilled water, incubated in the dark.
2. Mature pollen in petri dishes on Brewbaker-kwack medium (KEARNS & INOUE, 1993) mixed with different sucrose solutions, incubated in light. Experiment repeated with cavity microscope slides.



Fig. 1. – Flowers of *Callitriche cophocarpa*: **A**, two male flowers emerging from a floating rosette; **B**, male flower after anther dehiscence showing clumped pollen grains exposed on anther flaps. **C**, female rosette with emergent stigmas (scale bars = 1 mm); **D**, female (♀) and male (♂) floating rosettes (scale bar = 1 cm).



3. Mature pollen in small petri dishes on Brewbaker-kwack medium (KEARNS & INOUE, 1993) mixed with 1% agar or 1.5% gelatine (GERLACH, 1977), incubated in light. Experiment repeated only with agar and a smaller range of sucrose solutions.
4. Mature pollen with one stigma was placed on slides on 1% agar medium (GERLACH, 1977) mixed with 10 ml distilled water, 1% boric acid and 10 ml sucrose; half of the slides were incubated in the dark and half in light.
5. Wet pollen after floating for 24h and 48h was tested as above (No.: 4).

Pollen counts

Mature anthers were fixed in a solution of 3:1, 70% ethanol:acetic acid (GERLACH, 1977). Nine anthers were investigated. The pollen grains were suspended in a solution of 0.9 ml 70% ethanol, 2-4 drops of detergent and 2-4 drops of 5% methylene blue. The mixture was poured into a 1 ml calibrated tube (DAFNI, 1992). The number of pollen grains in a known volume of the suspension ($3 \times 3 \times 0.1 \text{ mm} = 0.9 \text{ mm}^3$) at a magnification of $100 \times$ was counted, using a Neubauer counting chamber. These counts were extrapolated (Factor 1111) for the original volume of 1 ml ($\equiv 1000 \text{ mm}^3$). Twenty counts per anther were the basis for estimating the average number of pollen grains.

Mericarps and seedlings

The collected plant material and the mud samples were sieved (width of the sieve-meshes from the top to the bottom sieve: 5.0 mm, 1.0 mm, 0.5 mm and 0.2 mm). The mericarps were stored wet in small glass jars or dried in petri dishes. Mericarp viability was tested with the dry-stored samples. Thirty dried mericarps were kept in distilled water. A total of 190 dried seeds, ten from each of the 19 mud samples, was tested in tap water. The samples from mud yielded many seedlings which were later cultivated in sterilized soil and placed in the cold greenhouse.

Electrophoresis

Non-flowering shoots from 19 samples of *C. cophocarpa* and a shoot of *Asplenium rutamuraria* L. as control marker were chosen for electrophoretic investigations. Seven starch gels were prepared using gel-buffer (dilution from 1:14 to 1:28) and the strongest extraction buffer was used following SOLTIS & SOLTIS (1989). The electrode buffer "tris-citrate" was modified from pH 7.0 to pH 7.2 by decreasing the citric acid molarity and increasing the concentration of tris. Horizontal starch gel electrophoresis was executed for 6 h at 30 mA and 100 V at the start and 45 mA and 170 V at the end. A total of 17 enzyme systems was prepared using the techniques described by SOLTIS & SOLTIS (1989).

Results

Life history

Seeds germinate underwater. The seedlings and submerged shoots have long internodes with linear leaves. When the shoots reach the water surface the internodes become truncated and the leaves become spatulate. These morphogenetic changes result in apical rosettes which float

on the water surface. Terrestrially cultivated plants develop linear to spatulate leaves which are smaller than floating or submerged ones but still form very loose apical rosettes. The control of internode truncation and leaf shape is complex and described by JÄGGI (1996). In *C. cophocarpa*, the male and female flowers are born on different rosettes and are thus spatially separated on the plants (Fig. 1D). Male-flowering rosettes develop from the apical part of the main axis. Female flowers develop on shoots which arise at the basal, submerged part of the main axis. This results in a pattern where, on each shoot system, most of the male rosettes are at the centre of the cushion while the females are at the periphery. However, the arrangement of the flowers was not as regular as that illustrated by MARTINSSON (1991a). The genders were always on separate shoots. The female flowers remain fertile longer than the males so over a long period it is difficult to measure the ratio of males to females. A mature cushion of *C. cophocarpa* is made up of a mosaic of male and female rosettes. Floral initiation takes place under water. Mature female and male flowers were never observed on submerged parts of the cultivated shoots, but *C. cophocarpa* in the Werdkanal showed regular flowering and subsequent fructification on totally submerged parts of the plants.

After the canal is cleared with a drag-plough in late summer *C. cophocarpa* is the first plant to recover. Even one-noded portions can root and develop into new plants.

In nature, many fruits develop. Germination takes place in autumn. The seedlings grow a few centimetres before they stop growing during winter. In November 1995 many seedlings were found in the Werdkanal at the time when the mature plants had died back to their stolons.

The male flower

The male flower of *Callitriche* consists of one naked stamen (Fig. 1A). The filament is slender and elongates while the pollen matures. The anther is reniform with longitudinal dehiscence slits which become continuous over the apex (Fig. 1B).

In *C. cophocarpa* the young anther is 4-sporangiate (Fig. 2A), divided by a connective and by two septas. The septas usually become detached from the endothecium and shrink by the time the pollen ripens (Fig. 2B) or, occasionally it may persist until the anther dehisces.

The angiosperm anther wall usually has four layers; in *Callitriche* HEGELMAIER (1864) found only two layers, the epidermis and a fibrous layer including thickened endothecial cell walls. SCHOTSMAN (1982) mentions the endothecium with its thickened cell walls as important for this specialized kind of anther dehiscence. The young anther of *C. cophocarpa* (Fig. 2A) shows clearly two different cell layers; the epidermis consists of flat hexagonal cells, except a few square ones which form a dehiscence line. The inner layer, the endothecium, consists of large square cells with thickened sidewalls. These thickened walls suggest a dehiscence mechanism which functions by desiccation. Intermediate layers could not be seen in the sections, but fragments of a third cell layer were sometimes visible. These fragments could be part of a secretion tapetum, the innermost layer of the anther wall. It disintegrates by the time the pollen ripens and consequently no additional layer is visible.

At anthesis the filaments elongate lifting anthers about 0.5 cm over the water surface. The anther opens along longitudinal dehiscence slits. The two resulting anther-flaps stretch before they fold back and wither. The pollen grains stick to these anther-flaps (Fig. 1B). The process of dehiscence lasts about half an hour in cultivation. Pollen remains stuck to the anther flaps unless it is mechanically removed by wind or water. Due to wind and water most pollen lands on the surface of the water. The pollen grains float on water for at least three days. The pollen with its reticulate exine is illustrated on Fig. 3.

As subsequent anthers develop and emerge above the water surface, the older ones fold back and are withdrawn under water. This way additional pollen may be placed on the water surface or, perhaps, it may sink and reach stigmas situated below the water surface.

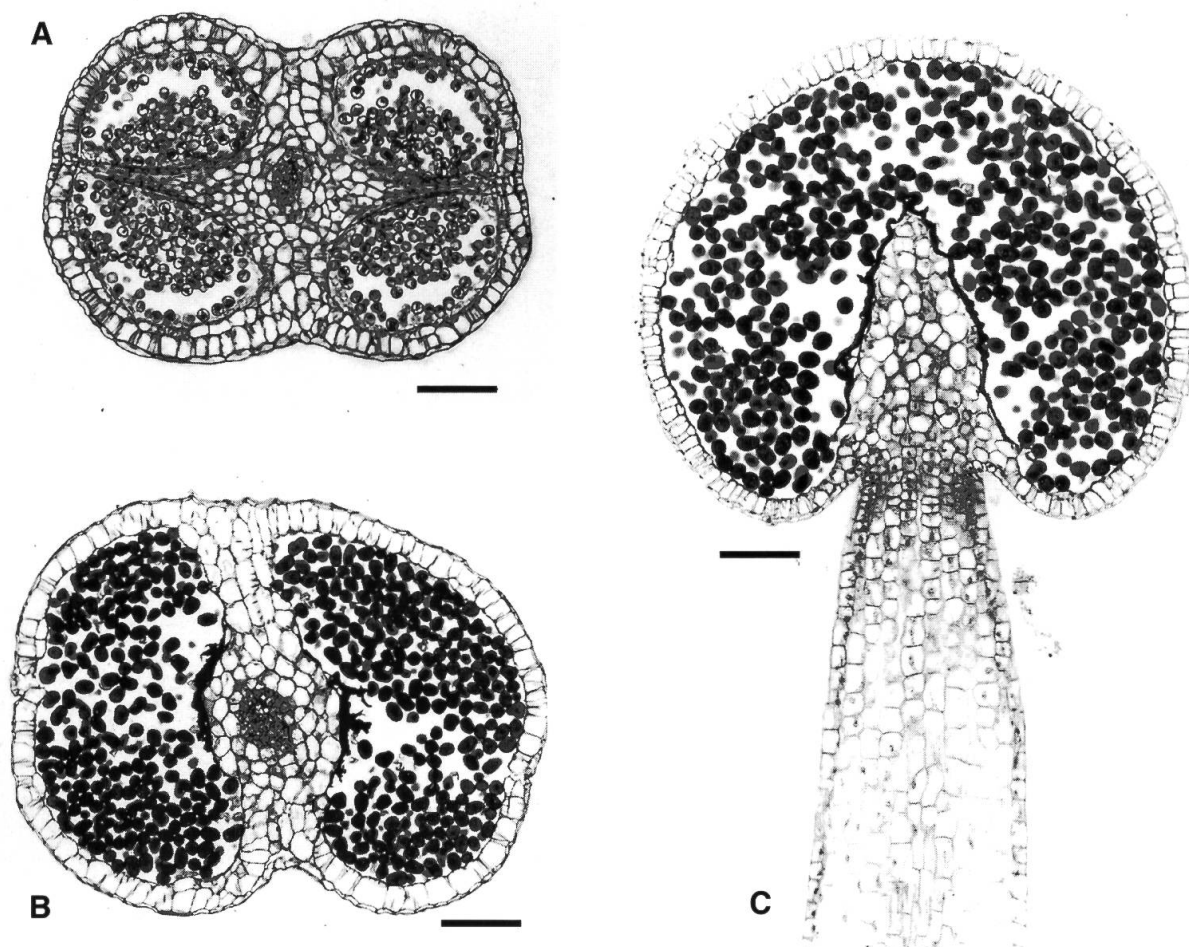


Fig. 2. – Anthers of *Callitriche cophocarpa*: **A**, **B**, transverse sections through anthers; **A**, a young 4-sporangiate anther showing the connective and two septas; **B**, a mature anther where the septas have disintegrated and the outer wall is almost reduced to a single-layered endothecium; **C**, longitudinal section through a mature anther with the connective detached from the the top of the endothecium (scale bars = 100 µm).

Female flower and fruit

The female flower of *Callitriche* consists of one, naked, four-locular ovary. Each loculus contains a single anatropous, unitegmic, tenuinucellate and pendulous ovule. There are two free and elongate styles which are papillate and stigmatic at anthesis. The ovary remains under water while the distal parts of the stigma emerge into air (Fig. 1C, D).

The fruit is four-lobed and each lobe is keeled (fig. 4A). At maturity it splits into four, flattened mericarps. The anatomy of the fruit of *Callitriche* has been described by HEGELMAIER (1864), FASSETT (1951) and SCHOTSMAN & HALDIMANN (1981). The exocarp, the epidermis of the fruit consists of one layer. The mesocarp consists of one layer and the endocarp is composed of two layers of criss-crossed fibres. In *C. cophocarpa*, an additional layer of parenchymous cells occurs locally or is absent.

The fruit-coat of *C. cophocarpa* bears special structures which probably help to reinforce the seed-coat. The seed-coat itself is composed of two layers, each with lignified fibrous cells (Fig. 4) which build a criss-cross pattern. As the mericarp ripens, the parenchymous cell layer

between the endocarp and the endosperm changes into a sticky layer and unites completely with the endocarp (Fig. 4B). It is not visible in the mature mericarp. Using the terminology adopted by SCHOTSMAN & HALDIMANN (1981): the young mericarp shows, starting from the outside (Fig. 4B), the epidermis (exocarp) which consists of one, thick, cell layer of polygonal or square cells; adjacently are one or partially two parenchymous cell layers (mesocarp). The layer connected to the endocarp shows thickened cell bases. These probably strengthen the endocarp. They are made up of two criss-crossed layers consisting of very thin-walled fibrous cells. The exocarp, mesocarp and endocarp build together the pericarp. The next strata is a single cell layer of parenchymous cells; this layer is part of an integument and may represent the testa as suggested by SCHOTSMAN (1967). Finally the secondary endosperm of large oil-filled and loosely packed cells surrounds the young dicotyledonous embryo.

By the time the four mericarps separate the pericarp becomes detached from the mericarp. The thickened cell bases of the innermost parenchymous layer of the mesocarp stick to the endocarp and form a honey-comb like pattern on the surface. The mature embryo is surrounded by endosperm and protected by the strengthened endocarp.

Pollen: quantity and germination

Pollen grain number per anther was found to range from 3722 to 9333; the average was 6746.80.

The pollen of *C. cophocarpa* is presented aerially (Fig. 1B). It is dispersed in the air or on the water surface (in vitro experiments, SCHOTSMAN, 1982). On the surface of the water the pollen is mostly in clumps of grains. The pollen is unwettable and if the water tension is reduced with detergent, the pollen rapidly sinks.

The pollen morphology is described by MARTINSSON (1993), see also Fig. 3. She wrote that the pollen of *C. cophocarpa* is of the aerial type with a well-developed exine, but it also has

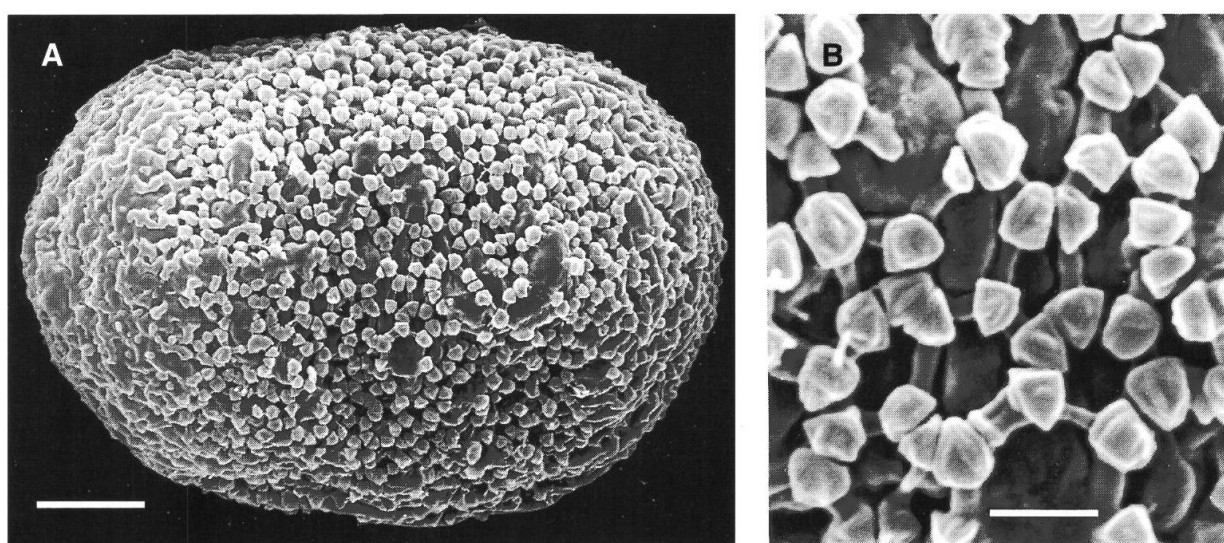


Fig. 3. – SEM pictures of pollen of *Callitriche cophocarpa*: **A**, an entire pollen grain (scale bar = 10 μ m); **B**, detail of the exine ornamentation (scale bar = 0.6 μ m).

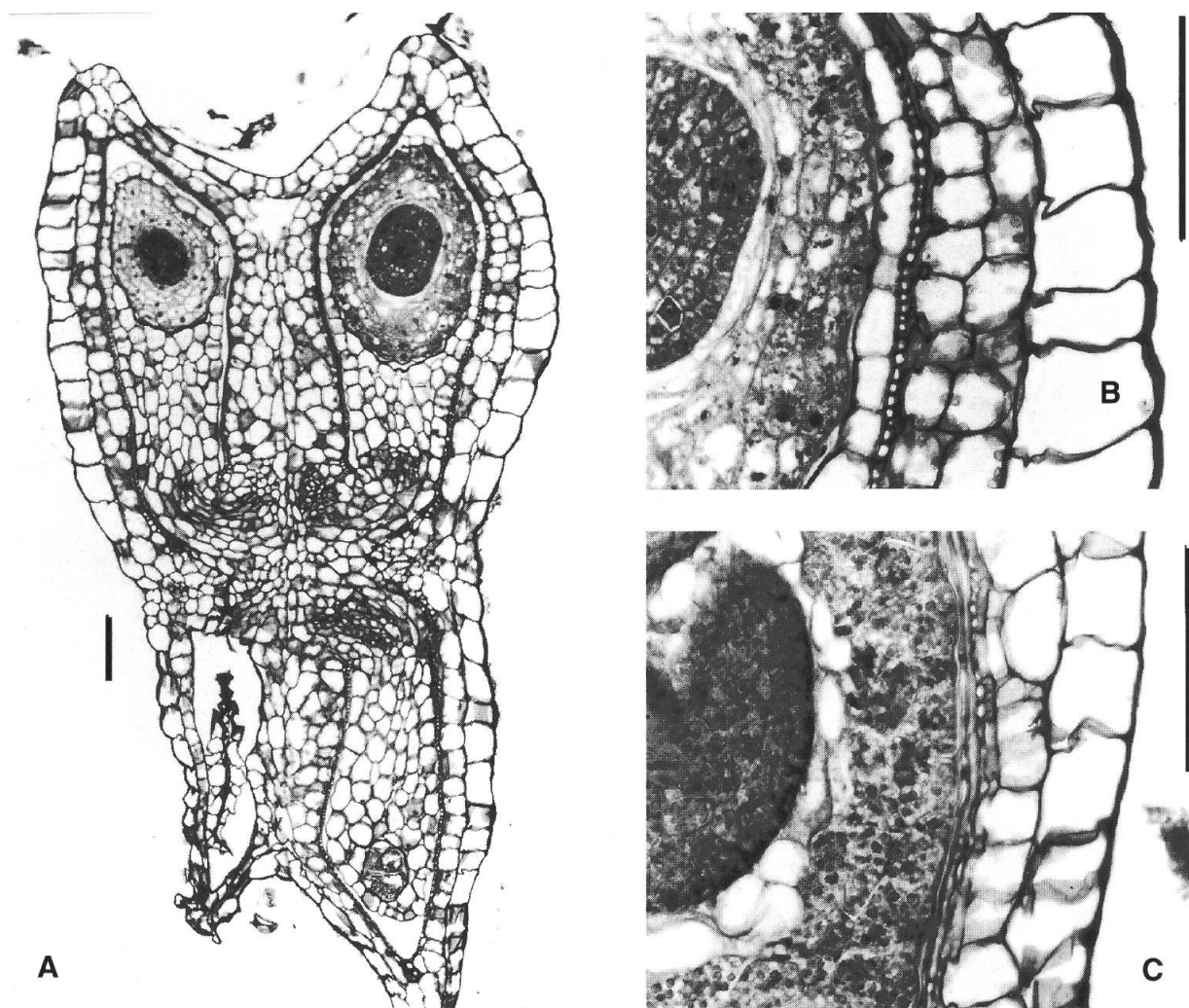


Fig. 4. – Fruits of *Callitriche cophocarpa*: transverse sections of fruits: **A**, a young fruit showing two fertile mericarps; **B**, a magnification of part of A showing the different cell layers starting from the outside: the exocarp, a two-layered, parenchymatous mesocarp, the endocarp, the testa which at this stage consists of a parenchymous cell layer; **C**, transverse sections of a mature mericarp with the hardened testa (scale bars = 100 μ m).

a leptomata. She suggests that the occurrence of leptomata could be a way of coping with size increase of the pollen in contact with water while retaining the well-developed exine as a floating device.

The pollen germination experiments which were executed with distilled water and liquid Brewbaker-Kwack medium (KEARNS & INOUE, 1993) were not successful because the pollen grains came to lie on the periphery of the solution droplets where they could not be observed. On a Brewbaker-Kwack medium stabilized with 1% agar or 1.5% gelatine (GERLACH, 1977) the pollen grains did not germinate. However, when receptive stigmas were added to the medium, some pollen grains germinated. The closer the pollen was placed to the papillate stigma the better it germinated. The maximum length of the pollen tube in culture was 40 μm .

Flowering success

SCULTHORPE (1967) writing about flower production in *Callitriche*, says that the amphibious species bear most flowers in the axils of the uppermost (floating or aerial) leaves. Therefore, many more terrestrial plants were cultivated than submerged ones. Against all expectations, *C. cophocarpa* when cultivated terrestrially, very rarely developed flowers: from 100 samples taken out of the “Werdkanal” one flowered and from 100 additional samples taken from a nearby canal the “Alte Jonen” none flowered. The submerged shoots, cultivated in 60 pots, flowered well. Over a period of 2-3 weeks there were up to ten rosettes of each sex flowering at any one day.

Pollination

In nature, *C. cophocarpa* grows in water and develops cushions made up of many floating-leaved rosettes. These cushions range in size from a few centimetres to approximately half a metre in diameter. The flowers develop over the whole cushion but male and female flowers develop on different shoots so that the individual rosettes within the cushion are unisexual. There is very rarely any direct contact between male and female flowers. In cultivation without wind or water movements the pollen grains remain attached to the anther flaps. In nature the pollen grains are soon transferred from the anther flaps to the surface of the water by wind and/or water movements. The pollen grains are unwettable and float for, at least, three days.

Pollen dispersal in natural habitats is difficult to observe and the cultivated shoots of *C. cophocarpa* could never be arranged in vitro as they are found in nature. The floating ability of the pollen shows that transport on the water surface is possible. If a male rosette is situated at the periphery of a cushion, pollen could easily float downstream to reach another plant or population of *C. cophocarpa*. Theoretically, outbreeding is possible but wind or waves are needed to transfer pollen over the leaves of the mother rosette. Most male rosettes, however, are trapped in the cushion by surrounding female rosettes; inbreeding through geitonogamy (second order autogamy) in this situation is more probable. Around the individual leaves in the floating-leaved rosettes, the surface tension of the water is lowered because the adaxial leaf surface is unwettable. This may ease the passage of pollen to receptive stigmas because some pollen grains are “sucked in” between the leaves. The flowers certainly show epihydrophily. Anemophily, the transfer of pollen to stigmas directly by wind, cannot be totally ruled out but is unlikely to play an important role in cross fertilization. The large amount of mericarps found in the mud samples collected in autumn 1995 indicates that there is a fairly high fruit-set and that pollination is effective.

Mericarps and seedlings

Most mericarps which were collected were subsequently found not fully mature. Mature mericarps stored in tap water immediately after collection germinated within one week. The dry mericarps were stored for about two and a half to three months before their viability was tested in distilled water.

Mericarps collected from mud were stored dry for about four months. A total of 190 mericarps were tested in tap water. After one week, four seedlings grew – each from a different mud sample. This 2.1 % germination is very low.

The seedlings collected in nature from mud samples and cultivated in the cold house grew very quickly. They developed small linear leaves. Unfortunately, a mass development of algae reduced the number of seedlings.

When cultivated, *C. cophocarpa* germinates in autumn. The seedlings grow a few centimetres before they cease growth to over winter. The last visit at the “Werdkanal” in November 1995 showed that at least some mericarps in nature also germinate in autumn. Active vegetative growth also takes place before winter, at the time when the parental plants die down to the perennial stolons.

Electrophoresis

Of the 17 tested enzyme systems SkD, PGM and DIA showed heterozygosity. Full details of the work are reported in JÄGGI (1996).

Discussion

Although essentially an amphibious plant, *C. cophocarpa* was found to flower only when growing as an aquatic. Terrestrially cultivated plants grew well and appeared robust but they very rarely developed flowers. Sexual determination follows a morphogenetic pattern: branches from the base of the main axis develop female flowers while those near the apex of the main axis develop males. All floating rosettes are unisexual. It was difficult to estimate the ratio of male to female flowers because the females remain receptive for a long time, on a day to day basis there is considerable variation. Each female flower develops a maximum of four ovules. The male flowers have but one anther; each anther contains between 3722 and 9333 (mean 6746.8) pollen grains. This is fairly high compared to the average number of 3425 pollen grains per anther that MARTINSSON (1996) found in Swedish material of *C. cophocarpa*. In cultivation unpollinated flowers developed no seed, therefore, agamospermy is unlikely to occur.

The flowers show no features that may be interpreted as supporting biotic pollination and, indeed, no animal visitors were observed. The question is – is the pollination anemophilous or hydrophilous? Epihydrophilous pollination was observed. At anthesis the carpels and basal part of each stigma are held under water; the distal parts of the stigmas emerge into air between the floating leaves (Fig. 1C & D). The adaxial surface of the floating leaves is unwettable; this causes local changes in the water tension and a meniscus is formed along the leaf margins. The pollen is unwettable and floats on the surface of the water. When grains drift near a floating rosette, the pollen is drawn between the leaves towards the stigma. The stigma is wettable. When in contact with the stigmatic surface the pollen germinates and its tube enters the stigmatic tissue. The pollen sticks together in clumps on the water surface which probably helps to insure that all four ovules become fertilized following a single contact. Clumped pollen has been reported from

other hydrophilous plants (PHILBRICK & LES, 1996). The pollen, because of its unwettability, is stuck to the water surface and is transported by water movements rather than by wind.

This kind of epihydrophyllous pollination is found in the genus *Elodea* (COOK & URMI-KÖNIG, 1985). Male flowers of *Elodea* scatter their pollen directly onto the surface of the water. In *C. cophocarpa* the pollen is not simply deposited onto the water. Stamens emerge between the rosette leaves but develop near the middle of the rosette and not at its edge. They are held about 0.5 cm above the rosettes. Each anther dehisces apically and the resulting two flaps slowly stretch horizontally. There is no explosive mechanism. In cultivation, without wind and water movements, the pollen remains on the flaps and, only when they wither does it eventually fall onto the leaves; very few grains land directly onto the water surface. To reach to the water the pollen must be dislodged by wind or water movements. Both are effective in nature. Pollen is not easily dislodged from the flaps by blowing over the flowers. We were not able to observe direct aerial transference of pollen from an anther to a stigma but it could play a role in pollination.

Considering the main features of the wind pollination syndrome as presented by COOK (1988), some features in *C. cophocarpa* indicate pollination by wind: pollen size (30–32 μm), quantity, unisexual flowers with reduced perianths, and reduced stamen and ovule numbers. However, other features are not ideal for anemophily. The pollen grains are not smooth but have a reticulate surface with leptomatas that may reduce the resistance to drying; also they are somewhat sticky and are spread in clumps and not as single grains. Pollen grains floating on the water show no signs of plasmolysis which may be interpreted as an adaptation to hydrophily (DAUMANN, 1963). The anthers are held, at most, 5 mm above the water surface which is very low for efficient long-distance dispersal of pollen by wind. The stigmas are simple; the lower part is in water, the emergent part rarely reaches more than 1.5 mm above the water surface. This is also far from being an ideal situation to trap wind-born pollen grains. In anemophilous aquatics, reviewed by COOK (1988), the stamens and stigmas are usually held high above the water; an exception is *Limnobium* where the stigmas are borne low down but they are branching and spread over the water surface.

Pollen transfer directly from anther to stigma by wind is perhaps possible within a single cushion of *C. cophocarpa* but this would probably result in geitonogamy (second order autogamy). If outcrossing occurs in *C. cophocarpa* it is the result of epihydrophily and not caused by direct anemophily. The cushions in the “Werdkanal” were surrounded and overgrown by other plants and it is unlikely that pollen grains could find their way by air through this dense vegetation to reach a receptive stigma. Another fact is that the anthers are exposed only a few millimetres above the water surface and the pollen grains are mostly dispersed in clumps. This “heavy load” is unlikely to be transported by wind, at least not for long distances. If the clumped pollen grains fall onto the leaf surface of a floating-leafed rosette, it sticks to its surface and can only be removed by water, for example rain, waves or by strong currents that flood the rosettes. The pollen grains have to be transported over the rosette onto the water surface if cross-pollination is to take place. It is for this stage that the vector wind is important. Once beyond the floating rosette further transportation of the pollen grains takes place on the water surface. Floating pollen, even if only moved by a slow water current, is more likely to find its way to a neighbouring cushion and a receptive stigma than wind-born pollen. The remarkable distribution of the sexes with the male rosettes in the centre and the females around the periphery add additional evidence to this hypothesis. We conclude that *C. cophocarpa* is essentially epihydrophilous but that wind plays an important role in moving the pollen beyond the leaves of the floating rosettes. Under experimental conditions female and male flowers were never observed on submerged parts of the cultivated shoots, but *C. cophocarpa* in the Werdkanal showed regular flowering and subsequent fructification on totally submerged parts of the plants. Hypohydrophilous pollination may also take place.

Unfortunately, the electrophoretic tests could not prove outcrossing although it cannot be ruled out. The isozymes SkD (shikimat-dehydrogenase), PGM (phosphogluconate-dehydrogenase) showed heterozygosity but there was no evidence of allelic segregation. Perhaps this heterozygosity is fixed or it is a consequence of polyploidy (PHILBRICK & ANDERSON, 1992),

however, which mechanism it is remains unknown. The isozyme DIA (diaphorase) showed heterozygosity and allelic segregation. This could have arisen when two colonizers with different DIA enzyme systems established two independent populations which later spread by vegetative means, or a spontaneous mutation may have been spread by vegetative fragmentation.

In nature, the pollination is effective and a high seed-set was observed. Also, a seed bank exists. Mericarps were recovered from samples collected at regular distances of over a 270 m long part of the "Werdkanal". Helped by cutting and dragging, the seeds are dispersed along the canal. The mericarps were observed to germinate in cultivation and in nature. Genetic turnover (seed to seed life history) in *C. cophocarpa* certainly coexists with vegetative spread. SABBATINI & MURPHY (1996) observed the response of *Callitriche* and *Potamogeton* to cutting, dredging and shade in English drainage channels. They consider the survival strategy of both plants, *C. stagnalis* and *Potamogeton crispus*, is essentially similar (competitive-disturbance tolerator), but the results suggested that *C. stagnalis* was the more competitive of the two species, while *P. crispus* was better in tolerating disturbance and shade-stress. Their results also demonstrated that phenology may be affected by management disturbance (removal of standing crop produced a strong regrowth response in *P. crispus* in mid to late summer, but in *C. stagnalis* the standing crop showed no such recovery). It was interesting to see in Switzerland that *C. cophocarpa* was among the first species to recover from the cutting process in September. This could be due to different competitors than seen in the above mentioned case, or *C. cophocarpa* shows even more tolerance to disturbance than *C. stagnalis*.

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