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**Autor:** Landolt, Elias

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## 2. MORPHOLOGICAL CHARACTERISTICS: VARIABILITY AND FUNCTION

### 2.1. GENERAL

The basis of the following statements is the monograph of HEGELMAIER (1868) which, due to the exactness of his observations, is still considered to remain a standard work. Many supplements from the literature and my own observations also have been added. HEGELMAIER describes morphological characteristics in much more detail; the ontogenic development of the different organs and their homologues are given only cursory treatment here. The main emphasis of this section will be the typical variability of features, whether genetically based or dependent on external factors, and the known or assumed functions of these characteristics. Important elements of the following chapter include observations of living plants, both in the field and in cultures, and of herbarium materials. Many preparations of various fronds served for distinguishing morphological features.

### 2.2. STRUCTURE AND ORGANIZATION OF LEMNACEAE PLANTS; INTERPRETATION OF DIFFERENT ORGANS

#### 2.2.1. Description of the frond

The leaf-like body of the Lemnaceae species, called a frond, is a complex of tissues with only few differentiations. In addition, a reduction in the organizational level within the family of the Lemnaceae can be observed. It begins with Spirodela and leads to Lemna, Wolffiella, and Wolffia.

As figures 2.1a and 2.2 show, Spirodela has a leaf-like body (L) with a membranous scale (P) at the basal part of the body; this scale covers the root primordia on the lower side of the frond with a lobe. The roots are adventitious. In two lateral pouches at the base of the frond (Po),

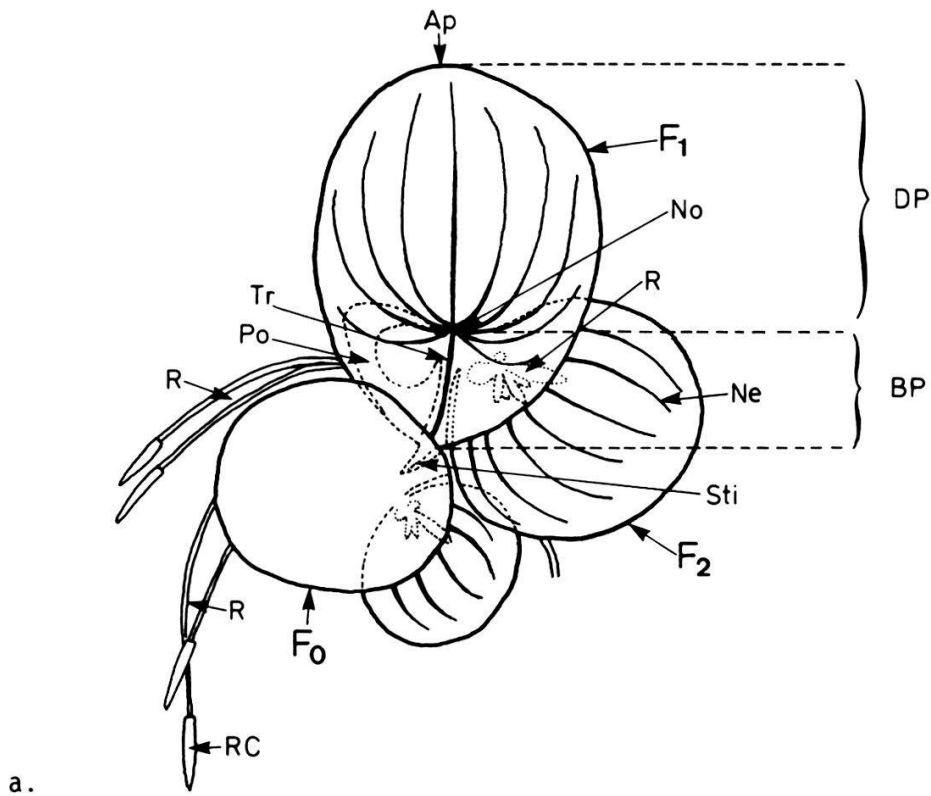
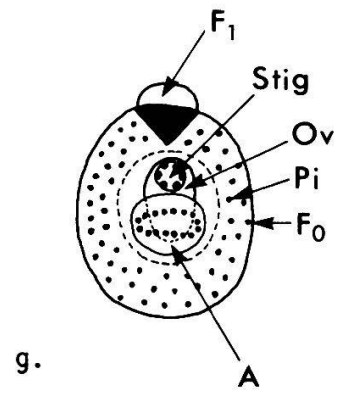
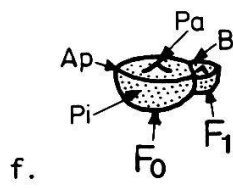
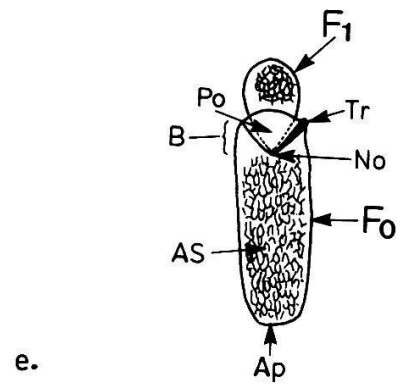
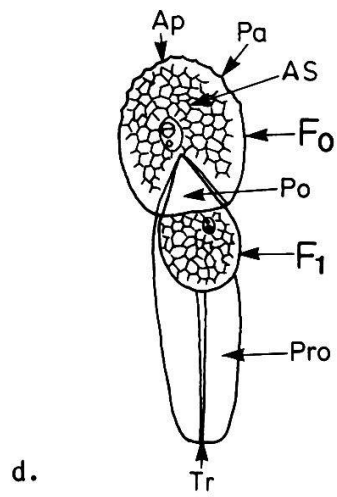
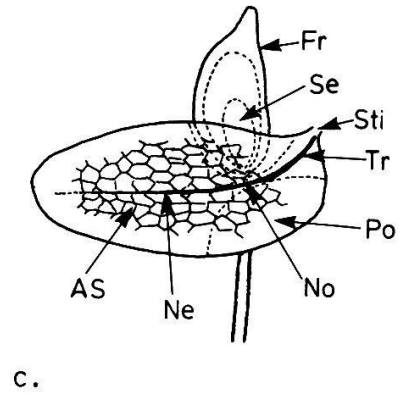
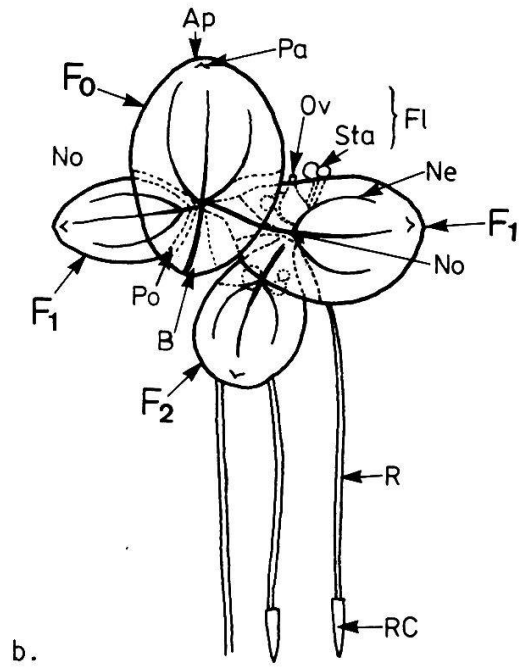


Fig. 2.1. Groups of fronds of various Lemnaceae seen from above or from the side (f.) (after HEGELMAIER 1868)

- |                                |       |                                |       |
|--------------------------------|-------|--------------------------------|-------|
| a. <u>Spirodela polyrrhiza</u> | (x7)  | d. <u>Wolffiella hyalina</u>   | (x7)  |
| b. <u>Lemna aequinoctialis</u> | (x7)  | e. <u>Wolffiella oblonga</u>   | (x7)  |
| c. <u>Lemna valdiviana</u>     | (x12) | f. <u>Wolffia brasiliensis</u> | (x7)  |
|                                |       | g. <u>Wolffia brasiliensis</u> | (x20) |

A	anther	Ov	ovary
Ap	apex	Pa	papule
AS	air spaces	Pi	pigment cells
B	base	Po	pouch
BP	basal part of the frond	Pr	prolongation
DP	distal part of the frond	R	root
F <sub>0</sub>	mother frond	RC	rootcap
F <sub>1</sub>	daughter frond of the first generation	Se	seed
F <sub>2</sub>	daughter frond of the second generation	Sta	stamen
F <sub>1</sub>	flower	Sti	stipe connecting daughter frond and mother frond
Fr	fruit	Stig	stigma
Ne	nerve	Tr	tract of elongated cells connecting stipe and node
No	node		



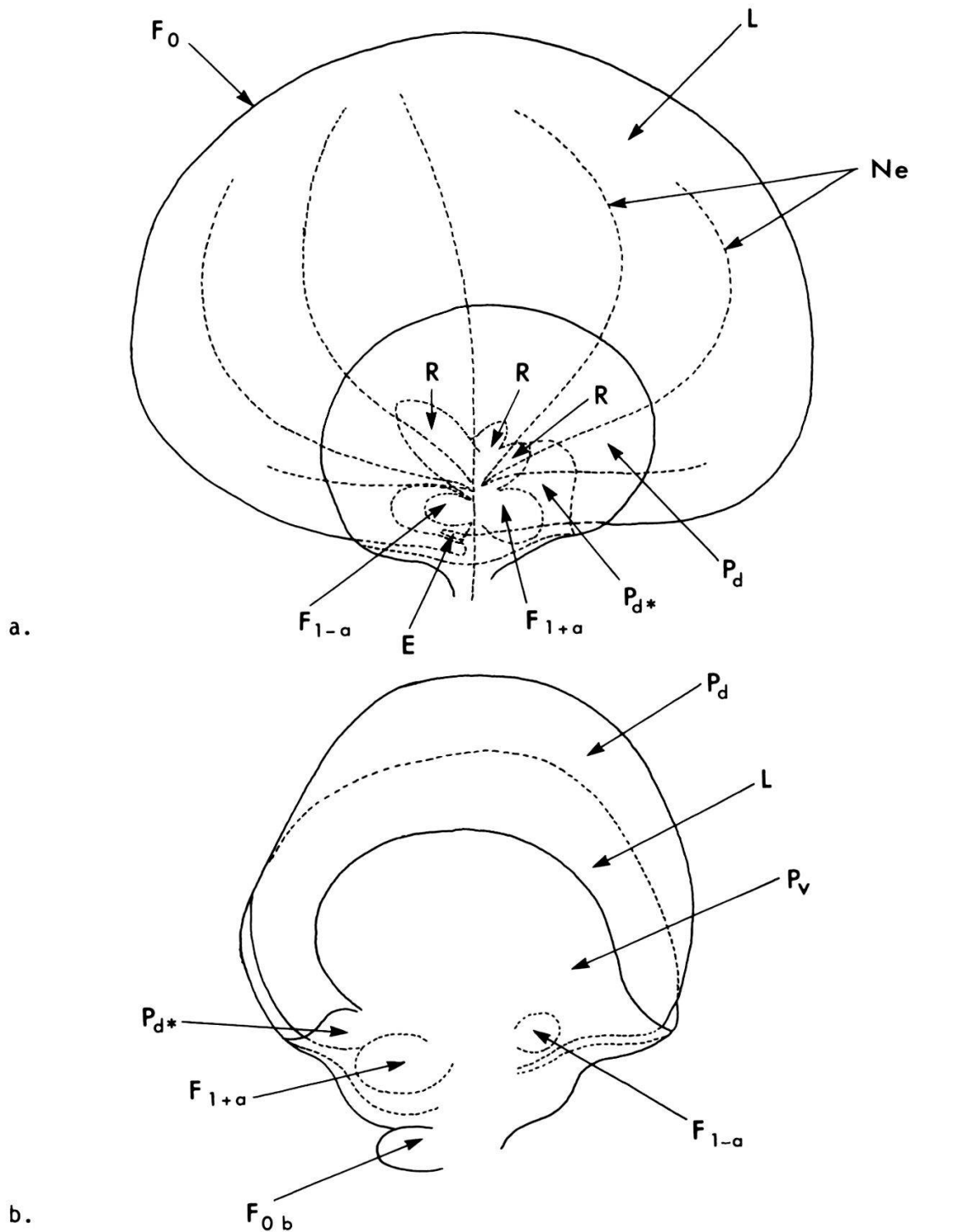


Fig. 2.2. Young fronds of *Spirodela polyrrhiza*

a. Young frond from above (upper surface) (after HEGELMAIER 1868) (x60)

b. Very young frond from below (lower surface, ventral side) (x180)

E	slit-shaped entrance to the pouch	L	leaf-like body
F <sub>1+a</sub>	first daughter frond of the first generation, plus side	Ne	nerves
F <sub>1-a</sub>	first daughter frond of the first generation, minus side	P <sub>d</sub>	prophyllum, dorsal lobe
F <sub>0</sub>	mother frond	P <sub>d*</sub>	prophyllum, corner of the dorsal lobe transgressing to the ventral side
F <sub>0b</sub>	second frond of the mother generation	P <sub>v</sub>	prophyllum, ventral lobe
		R	roots

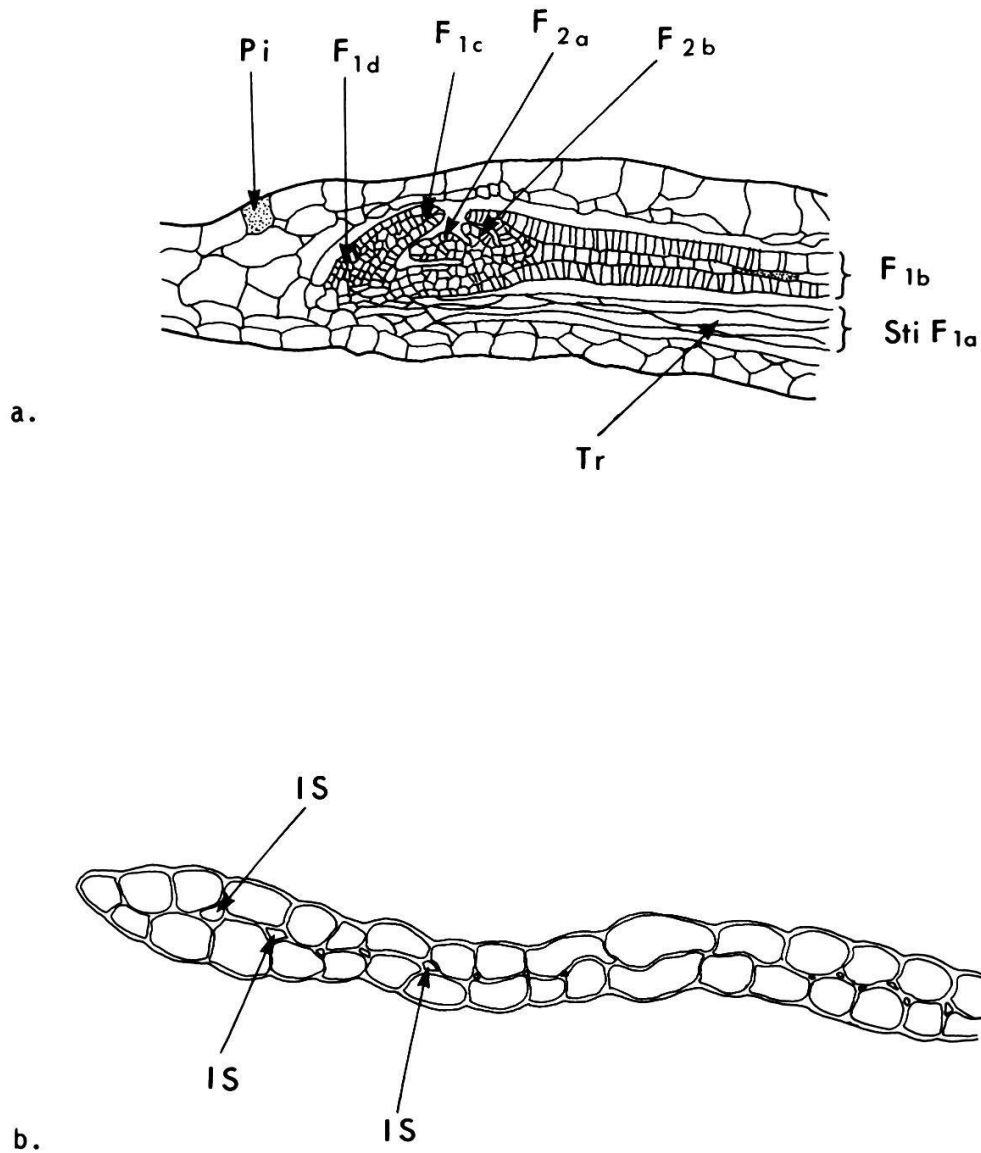


Fig. 2.3. Longitudinal section of Wolffiella lingulata (from GOEBEL 1921)

a. pouch (x80), b. distal part (x120)

F <sub>1a</sub>	first daughter frond of the first generation
F <sub>1b,c,d</sub>	second to fourth daughter frond of the first generation
F <sub>2a,b</sub>	first and second daughter frond of F <sub>1b</sub>
IS	intercellular spaces
Pi	pigment cells
Sti	stipe of the first daughter frond
Tr	tract of elongated cells belonging to the first daughter frond

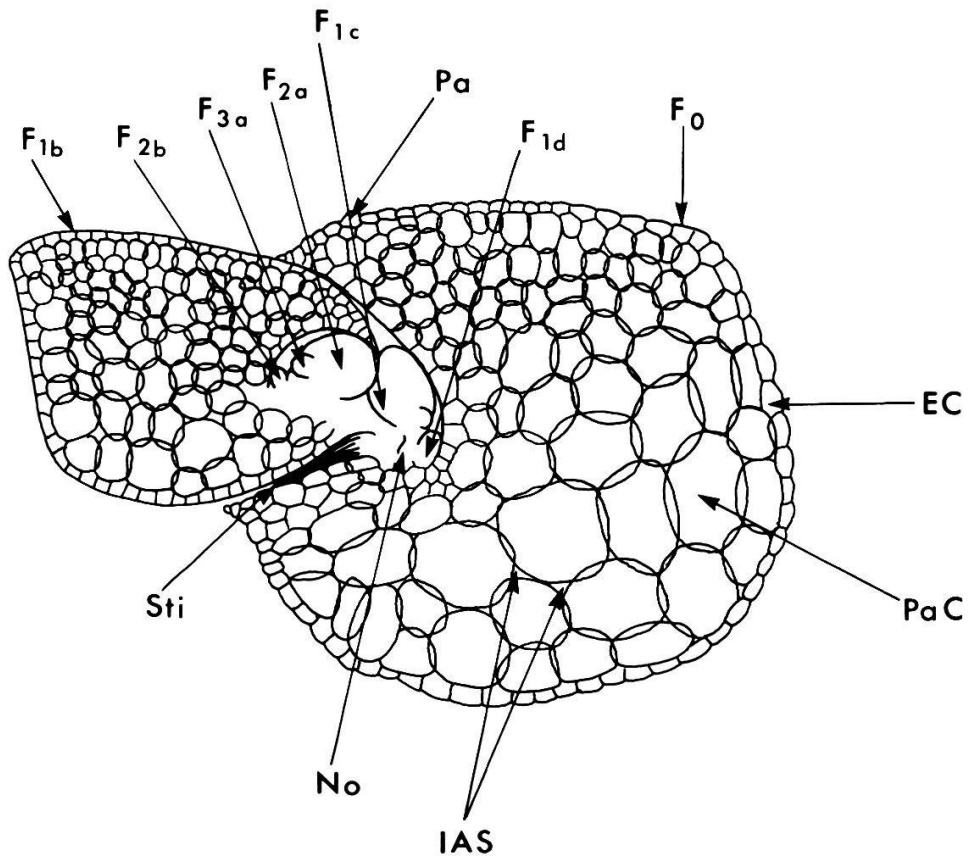


Fig. 2.4. Longitudinal section of Wolffia arrhiza (60x)  
(after HEGELMAIER 1868)

- |                 |  |
|-----------------|--|
| EC              | epidermis cells  |
| F <sub>0</sub>  | mother frond   |
| F <sub>1b</sub> | second daughter frond of the first generation                          |
| F <sub>1c</sub> | third daughter frond of the first generation                           |
| F <sub>2a</sub> | first daughter frond of the second generation                          |
| F <sub>3a</sub> | first daughter frond of the third generation                           |
| IAS             | intercellular air spaces (very small)                                  |
| No              | node   |
| Pa              | papilla  |
| PaC             | parenchyma cells   |
| Sti             | stipe of the first daughter frond (F <sub>1a</sub> ) already separated |

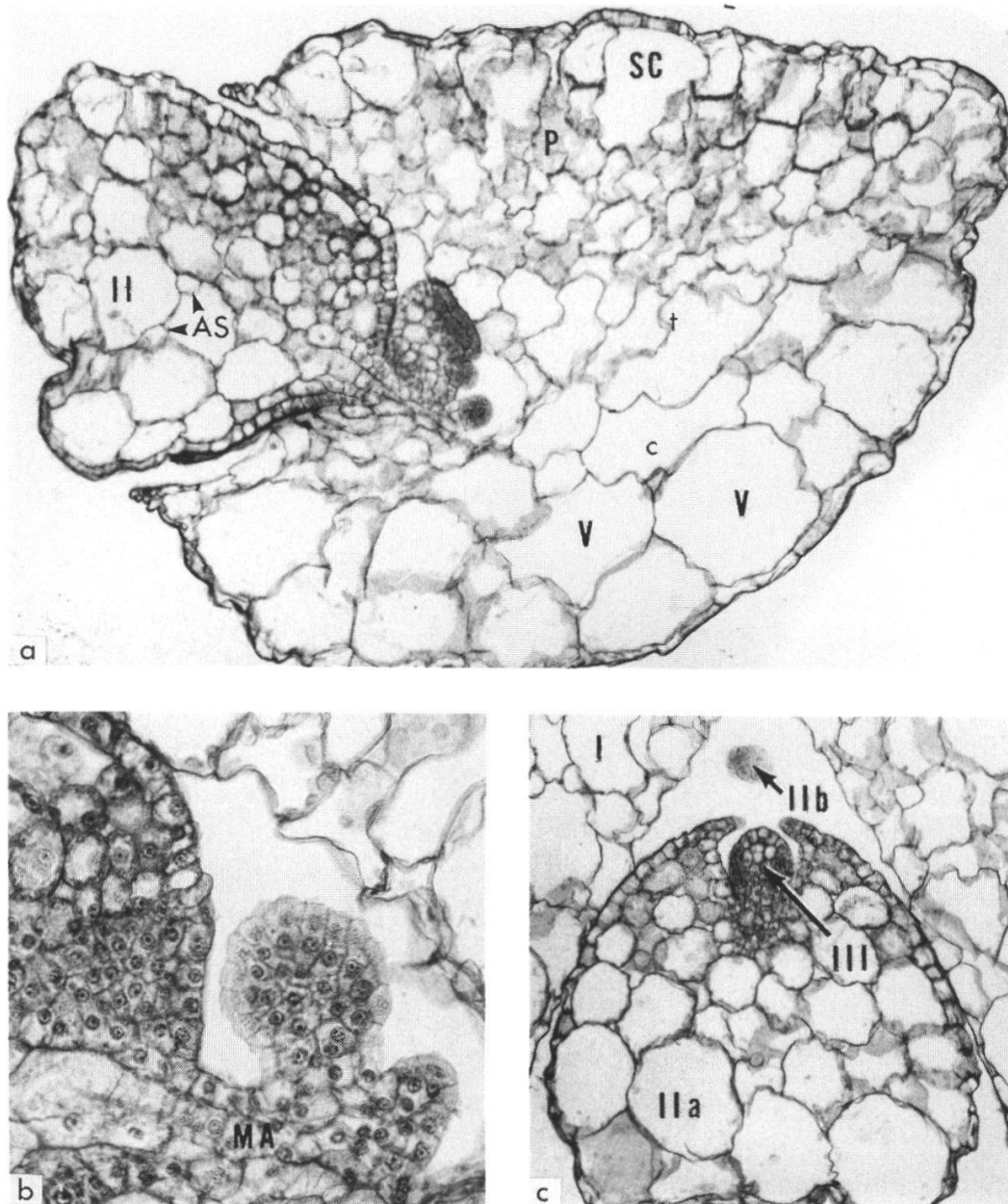


Fig. 2.5. Light micrographs of *Wolffia arrhiza* (No. 7014)  
 (from ANDERSON et al. 1973)

- a. Vertical cross section of mature mother and daughter fronds showing palisade-like photosynthetic area (P), highly vacuolate cells (V), substomatal cavity (SC), intercellular air spaces (AS), and daughter frond (II) (x80).
- b. Placenta-like meristematic area (MA) in the older frond giving rise to successive daughter fronds (x180).
- c. Horizontal cross section of daughter frond in reproduction pouch showing three vegetative generations: mother frond (I), successive daughter fronds  $F_{1a}$ ,  $F_{1b}$  (IIa, IIb) of the first generation, and primordia of daughter frond of the second generation  $F_{2a}$  (III). (x80).

the young daughter fronds ( $F_1$ ,  $F_2$ ) originate. The Spirodela flowers consist of a membranous scale, two stamens and one ovary (with one to few ovules).

Lemna (fig. 2.1b) has only one root, and the scale at the base of the leaf-like frond is missing. The flowers are similar to Spirodela flowers. There are no roots at all in the Wolffiella and Wolffia genera (fig. 2.1d-f); these fronds are thin and nearly transparent in Wolffiella (two-dimensional) (fig. 2.3) and small and gibbous in Wolffia (three-dimensional) (fig. 2.4). The flowers of both genera consist of one stamen and one ovary; these organs are situated in a cavity on the upper side of the frond either laterally of the median line (Wolffiella) or in the median line (Wolffia). The daughter fronds grow to the back of the fronds in a flat pouch (Wolffiella) or in a conical cavity (Wolffia).

The leaf-like part of the fronds consists of (fig. 2.1a):

- a rear or basal section (BP), which is behind the node, where the daughter fronds are formed; from the node to the base runs a tract of elongated cells (Tr) that connects the daughter frond with its progenitor.
- the front or distal section (DP), which contains nerves (Ne) in Spirodela and Lemna.

The two sections are not sharply differentiated from each other. In both, the tissue below the epidermis consists of a photosynthesising parenchyma, partly interrupted by large intercellular air spaces. The air spaces of the Lemnoideae and Wolffiella with the surrounding cells form a special aerenchymatic tissue (figs. 2.1c,d,e; 2.25). In Wolffia the air spaces are situated in the small gaps between the adjoining corners of some parenchymatic cells (figs. 2.4, 2.5).

### 2.2.2. Interpretation of the frond

A morphological interpretation of the frond is difficult. In principle, there are three theories:

1. **The frond corresponds to a leaf** (e.g., HOFFMANN 1840a). This theory is contrary to the fact that leaves in general are not able to form new leaves and flowers.
2. **The frond corresponds to a shoot of leaf-like shape** (e.g. SCHLEIDEN 1844, HEGELMAIER 1868). However, as there is no meristematic tissue at the end of the frond, HEGELMAIER himself abandoned this theory and in

1895 supported the third theory. BRUNAUD (1974a, 1974b) believes that the Lemnaceae shoot ends in a terminal cladodium (fig. 2.6). According to BUGNON (1974), BRUNAUD's interpretation (1974a) of the ramification of the Spirodela fronds corresponds, with some modifications, to the situation in Zostera, Ruppia, Dieffenbachia, Crocus, Hydrocharis, Leucoium, etc.

3. In the basal section, the frond consists of a shoot and in the distal section, of a leaf (e.g., VAN HOREN 1869, ENGLER 1877, 1889, HEGELMAIER 1895, MEUSEL 1951, KANDELER 1979). In general, a shoot is not able to end in a leaf. Therefore, there are many variations of this theory. According to ARBER (1920), the frond corresponds to a phyllodium, with the basal section forming a flat axis and the distal section forming an enlarged petiole; the "leaf" of Pistia is interpreted in a similar way. BROOKS (1940) also tries to compare the frond of the Lemnaceae with the leaf-like body of Pistia; he considers the Pistia plant a sympodium. The frond of Spirodela, as a part of a sympodium, consists of a prophyllum at the base, a leaf (forming the leaf-like area), a bract (the lower

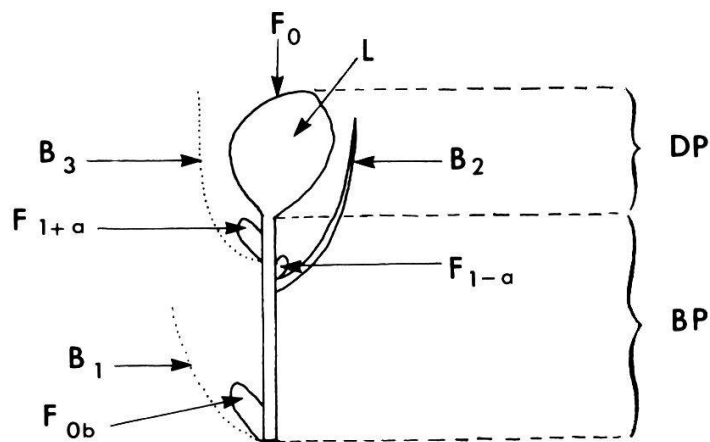


Fig. 2.6. Interpretation of the Spirodela frond, according to BRUNAUD (1974a, 1974b)

$B_1, B_2, B_3$  bracts 1 to 3:  
bracts 1 and 3 supposed  
bract 2 existing  
BP basal part  
DP distal part  
 $F_0$  mother frond  
 $F_{0b}$  second frond of the mother  
generation

$F_1$  daughter frond of the  
first generation  
 $F_{1+a}$  first daughter frond,  
plus side  
 $F_{1-a}$  first daughter frond,  
minus side  
L leaf-like body

wall of the second pouch), and a terminal inflorescence (fig. 2.7). The first daughter frond is formed in the axil of the prophyllum. An interpretation of the following daughter fronds of the same generation is not given by BROOKS. In Lemna, according to BROOKS (1940), there is another bract, instead of the prophyllum, that forms the lower wall of the first pouch. ASHBY et al. (1949) believe that the Lemna minor frond is an axillary shoot from which the daughter frond branches off alternating on both sides with the leaf-like body of the mother frond consisting of a bract. However, it is not possible to explain the prophyllum of Spirodela in this way (fig. 2.8). According to GOEBEL (1921) and LAWALREE (1945), the basal section of the frond consists of a hypocotylous shoot

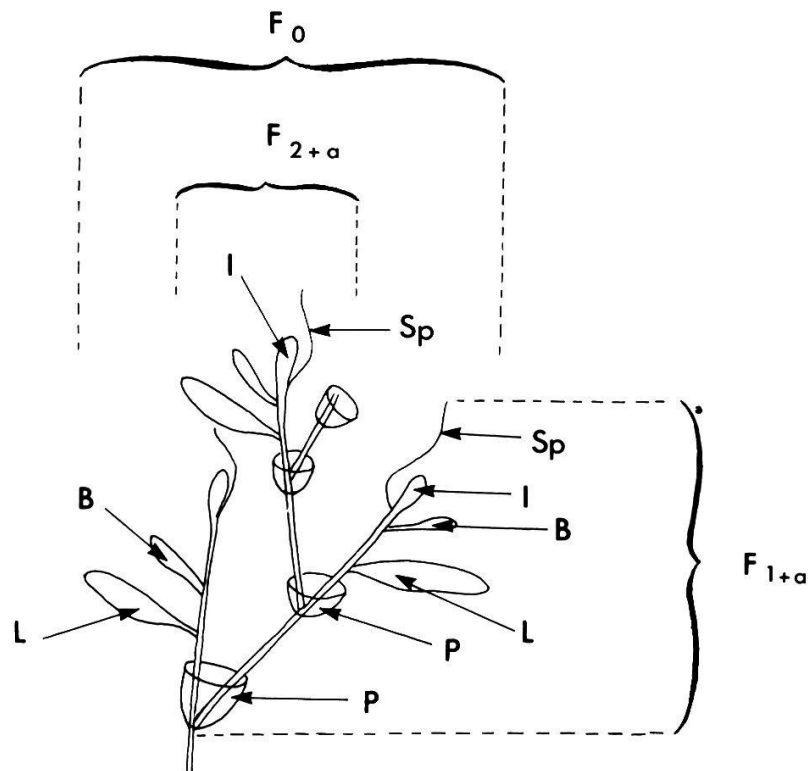


Fig. 2.7. Interpretation of the Lemnaceae frond, according to BROOKS (1940) and DAUBS (1965). The figure represents Pistia; Spirodela is interpreted in the same way.

B	bract	I	inflorescence
F <sub>1+a</sub>	first daughter frond of the first generation, plus side	L	leaf-like body
F <sub>2+a</sub>	first daughter frond of the second generation, plus side	P	prophyllum
F <sub>0</sub>	mother frond	Sp	spathe

and the distal section consists of a cotyledon-like leaf. The Lemnaceae are believed to develop no further than the embryonic stage. There is no true vegetative point; instead, a long-lasting meristematic zone can be observed at the base of the cotyledon and the following cotyledon-like leaves, which later develop into fronds. With this theory it is not possible to explain the presence of the membranous sheets which are interpreted as prophylla in this monograph.

MEUSEL (1951) considers the structure of the Lemnaceae frond homologous to that of Pistia. The distal part of the Spirodela frond corresponds to the foliage-leaf of Pistia, the basal part to a shoot bearing a scale-leaf, an inflorescence and adventitious roots; the first daughter frond on the plus side is homologous to the sympodial shoot of Pistia, the daughter frond on the minus side is homologous to a new stolon of Pistia (fig. 2.9). The daughter frond on the minus side is replacing the inflorescence in non-flowering fronds.

I have not done any extensive work to explain the homologies of the frond but there seems to be possible a somewhat different interpretation

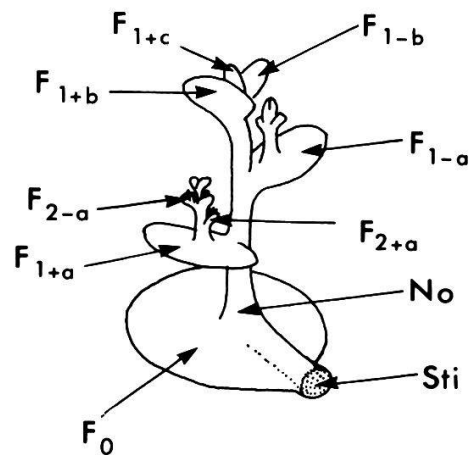


Fig. 2.8. Interpretation of the Lemna frond, according to ASHBY et al. (1949).

$F_0$	mother frond	+a	first daughter frond, plus side
$F_1^0$	daughter frond of the first generation	-a	first daughter frond, minus side
$F_2$	daughter frond of the second generation	+b	second daughter frond, plus side
		+c	third daughter frond, plus side
		No	node
		Sti	stipe

which is more obvious in connection with anatomical investigations in the literature and my own observations (fig. 2.10). The frond of S. polyrrhiza consists of a very short shoot that begins at the base of the stipe (which originally connected the frond with its ancestor) and ends at the node. The shoot of Spirodela forms a partly asymmetric dichasial sympodium, enclosed by a prophyllum. The main shoot ends at the node. There are no bracts at the branching off points. The side shoots end in a terminal flower. If no flower is formed, as usual, in each pouch an axillary daughter frond develops to which further daughter fronds (secondary accessory fronds) of the same generation follow. The flowers, as

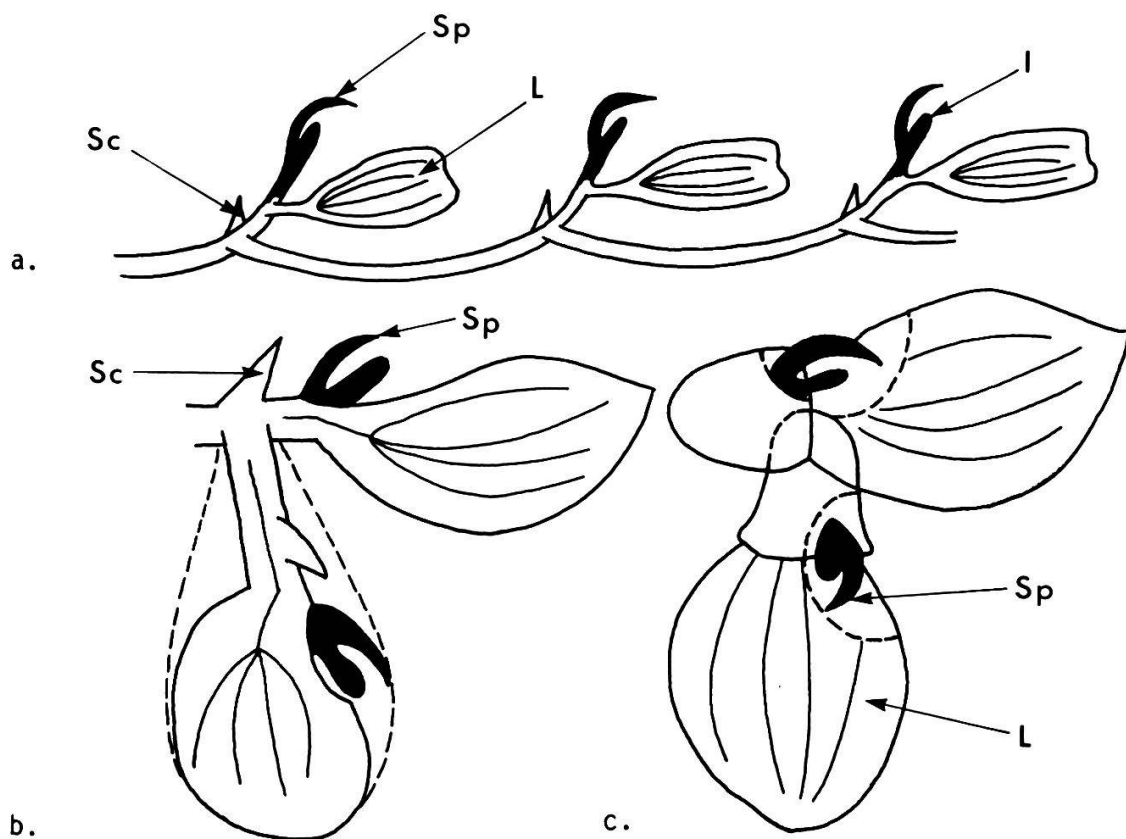


Fig. 2.9. Homology in Pistia and Spirodela, according to MEUSEL (1951)

- a. Scheme of stolon formation in Pistia. It is supposed that always new stolons are formed instead of continuing shoots. The continuing shoot develops sideways of the bract.
- b. Scheme of derivation of the Spirodela frond from a Pistia stolon.
- c. Scheme of the structure of flowering S. polyrrhiza.

I inflorescence  
Sc scale-like leaf

L leaf-like body  
Sp spathe

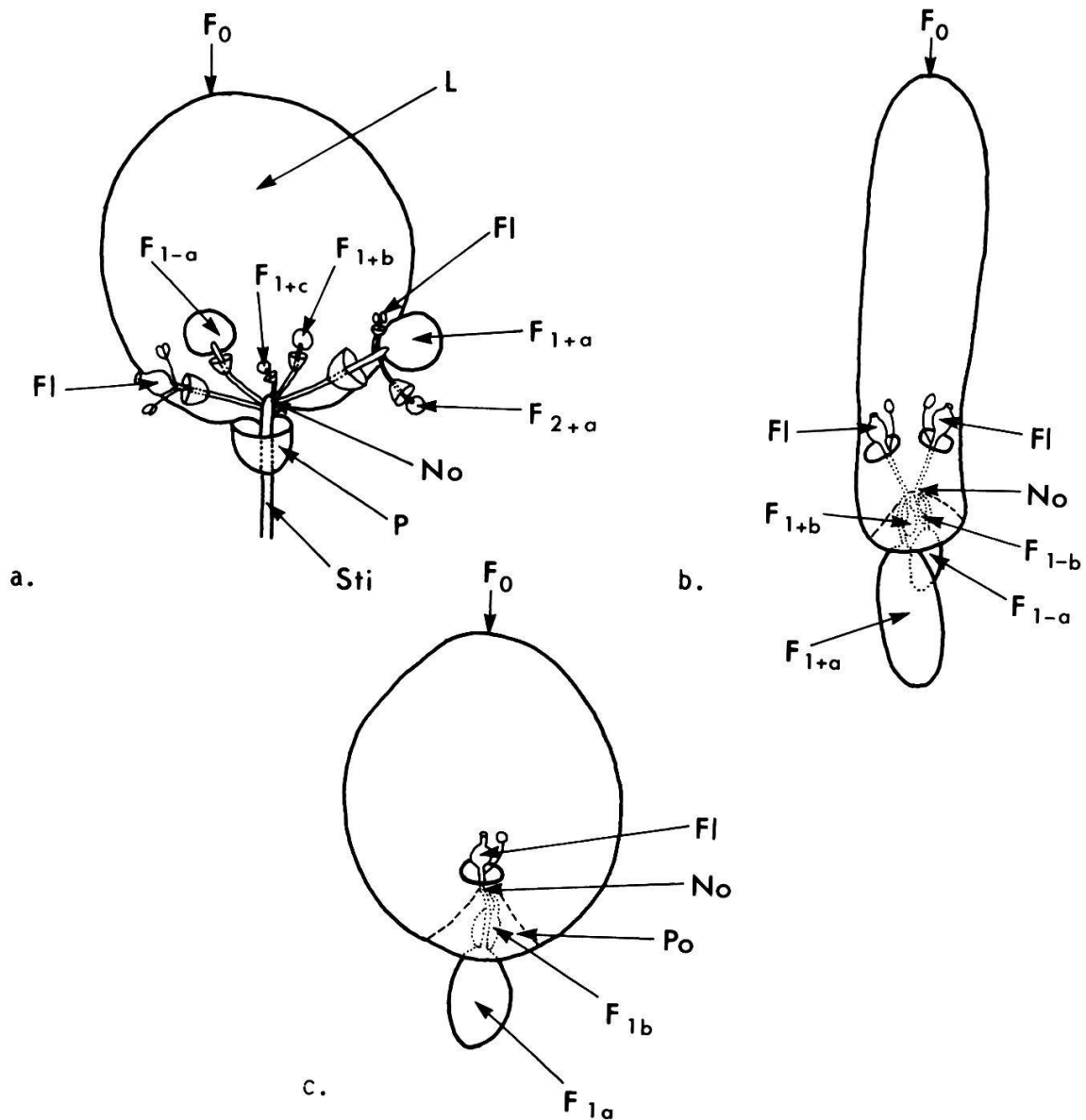


Fig. 2.10. Interpretation of the Lemnaceae frond in the present work.

a. Spirodela, b. Wolffiella, c. Wolffia

The Lemna frond is organized very similarly to Spirodela, but there is no prophyllum at the base of the frond; the prophyllum at the base of the flower is still existing.

F<sub>0</sub> mother frond  
 F<sub>1</sub> daughter frond of the first generation  
 F<sub>2</sub> daughter frond of the second generation  
 a first frond  
 b second frond (accessory frond to a)  
 c third frond (accessory frond to b)

+ plus side  
 - minus side  
 Fl flower  
 L leaf-like body  
 No node  
 P prophyllum  
 Po pouch  
 Sti stipe

well as the daughter fronds of Spirodela, are surrounded by a prophyllum. LACOR (1970) observed at the meristematic basal part of some flowers of S. polyrrhiza one to five roots perforating the prophyllum in a similar way as the roots of the normal frond do. The distal part of the fronds branches off at the end of the main shoot. It probably cannot be determined by the known facts whether this part corresponds to a whole leaf or only to a petiole, as is supposed by ARBER (1920), in analogy to the conditions of Pistia. The leaf tissue grows at the minus side of the frond, where the second daughter frond ( $F_{1-a}$ ) is formed, along the stipe and towards the base, where it forms the lower wall of the pouch (the lower wall of the pouch at the plus side is formed by a lobe of the prophyllum). On the upper side, the tissue of the leaf-like part grows towards the base and, from above, connects with the stipe, thus forming two upper walls of the pouches. In Lemna, there is no prophyllum at the base of the frond. The lower wall of the plus side is formed in a similar way to the formation of the lower wall of the minus side (by leaf tissue). The prophyllum of the flower is still present. In Wolffiella and Wolffia (fig. 2.10b), the tissue growing towards the base of the leaf-like part does not connect with the stipe on the upper side; therefore, only one pouch or one cavity grows towards the back from where daughter fronds emerge backwards. The prophyllum of the flower is also missing in Wolffiella and Wolffia. The flower is directed to the upper side of the frond and is not enclosed in the same pouch as the daughter fronds. It is surrounded by frond tissue and therefore lies in a cavity. In Wolffiella, the flowering shoots are directed laterally from the median line. In Wolffia, there is only one direction for flowers (upwards into the cavity on the median line of the frond) and for daughter fronds (backwards into the cavity). As an exception to this arrangement, I have observed a two-flowered Wolffia columbiana in which both flowers were arranged along the median line, in separate cavities (fig. 2.41). The assymetry of the tract of elongated cells and of the daughter fronds in W. lingulata, W. oblonga, W. gladiata and W. denticulata is secondary. As GOEBEL (1921) already pointed out, the Wolffiella frond is by no means the result of a turning of  $90^{\circ}$  of a Wolffia frond with a successive flattening as proposed by HEGELMAIER (1868). It is just a shifting of the tract of elongated cells from the median line (W. rotunda, W. Welwitschii) to the side. The Wolffia frond probably derived from a symmetric Wolffiella frond.

### 2.3. GROWTH AND AGEING OF FRONDS; FORMATION OF DAUGHTER FRONDS

#### 2.3.1. Mode of growth; growth rate

The growth of Lemnaceae occurs by budding within the pouches or cavities of the basal section of the fronds.

The initiation, elongation, and internal differentiation of the fronds of S. punctata has been described by RIMON (1964) and RIMON and GALUN (1965). Initiation of a new bud (the future frond) takes place in a bud of previous generation which is not more than 18 cells in length. Cell multiplication continues in the initials and in the mother bud. When the bud is 30 cells long, differentiation begins. A typical aerenchym and vascular bundles are produced. The bud is thus converted into a frond, and further cell divisions are restricted to a small portion of the base of the young frond. The normal size of the frond is reached by further cell elongation and by the meristematic activity at the node. The new fronds separate from the mother fronds after a period of time. Each daughter frond emerging from the pouch already contains two new generations of daughter fronds. The distal section of the frond is formed first which is, according to HEGELMAIER (1868) true for all Lemnaceae. HEGELMAIER also reports that the daughter fronds of Lemna are formed in each pouch simultaneously when the mother frond reaches about 0.05 mm in length. Later the development of one daughter frond (on the minus side) is delayed.

Daughter fronds are usually formed by meristematic tissue at the node on the upper side of the mother frond (see e.g. RIMON 1964). However, in the S. polyrrhiza group the daughter frond of the plus side originates laterally from and not on the upper side of the mother frond (see chapter 2.4.8.). The meristematic tissue is dermatogenous in W. arrhiza, according to LAVALREE (1943); in S. polyrrhiza, the subepidermal tissue takes part in the production of frond primordia (VINTEJOUX 1969).

With optimal nutrient composition, the growth rate of Lemnaceae is nearly exponential. However, it should be noted that successive daughter fronds do not appear at regular intervals. Also, the elongation of the cells might be slowed or accelerated by making small changes in culture conditions. This more-or-less means that new daughter fronds appear unexpectedly from the pouch, although in the long run, the number of new

daughter fronds formed remains the same. To obtain a regular growth rate, it is necessary to count only cultures with many fronds. The frond number almost doubles within 24 hours with fast-growing species (e.g., L. aequinoctialis and W. microscopica) and under optimal conditions (LANDOLT 1957, VENKATARAMAN et al. 1970, DATKO et al. 1980a).

### 2.3.2. Lifespan and ageing

During the vegetation period, the fronds have a lifespan of only a few weeks. In L. minor, ASHBY et al. (1949) observed a maximal age of 5-6 weeks; PIRSON and GOELLNER (1954) recorded an age of 7 weeks and, rarely, up to 10 weeks; and WANGERMANN (1952) reported an age of up to 9-10 weeks and HOSSELL and BAKER (1979a) 5 weeks. In L. obscura (named as L. minor) a lifespan of up to 10 weeks was measured (BOSS et al. 1963a). The same authors (1963b) observed different lifespans for different clones and different species under optimal conditions. They reported a lifespan of 33 days for S. polyrrhiza and L. obscura, 29 days for L. gibba, 28 days for L. minuscula, 26 and 22 days for two clones of L. aequinoctialis, and 21 days for S. punctata. According to many authors, the lifespan depends on the temperature. At 30°C, the life span is about half of that at 20°C. However, in a resting stage and at low temperatures, the fronds may survive for several months. Turions of S. polyrrhiza are still viable after 10 months (HENSSEN 1954). Nutritional conditions probably have an influence on lifespan, too. However, available information is contradictory. WANGERMANN and LACEY (1955) observed longer lifespans for L. minor in nutrient solutions lacking nitrogen, whereas BOESZOERMENYI and BOESZOERMENYI (1957) reported shorter lifespans when nitrogen and phosphorus were lacking; the authors suppose that the assimilation of nitrogen and phosphorus is slower with older fronds; therefore, the new daughter fronds are not as well nourished and do not form meristematic tissue at the same rate as under conditions where N and P are present. The difference in the results of the two groups must be explained by the different culture conditions used. WANGERMANN and LACEY (1955) grew L. minor at a constant temperature of 25°C and at relatively low light intensities of 2000 lux (200 ft-c); BOESZOERMENYI and BOESZOERMENYI (1957) cultivated duckweeds in a greenhouse with changing temperatures and light intensities. The nitrogen-deficient

plants probably did not tolerate the stress situation (sometimes high temperatures) as well as optimally nourished plants. According to OSTROW and DIJKMAN (1969) the lifespan of S. punctata declines with increasing day length (from 33.4 days under 8 hours of light daily to 22.8 days under continuous light). On the other hand, the lifespan of L. aequinoctialis fronds cultivated under long-day conditions was distinctly longer (39 days) than of fronds kept under short-day (flowering) conditions (KANDELER 1974). In general, it appears that the lifespan is longer with slower growth rates. Also, as the concentration of certain nutrients falls below a minimum requirement, the fronds become damaged and die earlier. KASINOV (1981) reports a faster propagation of new fronds and a faster ageing of L. minor when the daughter fronds are removed prematurely (at a size of about half the maximal length). However, the number of daughter fronds did not change. The rate of photosynthesis but not the chlorophyll content of a frond of S. polyrrhiza decreases continuously after an age of 15 days (GAPONENKO and STAZHETSKII 1969). For details and interpretations see vol. 2, chapter 2.4.1.4 (LANDOLT and KANDELER 1987).

As the mother frond ages, the successive daughter fronds become smaller and consist of fewer cells of the same size. However, the first daughter fronds of the final fronds of this second generation again grow larger; maximal size of fronds is reached after about four generations (ASHBY et al. 1949, WANGERMANN and ASHBY 1950, 1951, WANGERMANN 1965). Figure 2.11 gives an example of the changes of frond area in the course of the generations. The above cited authors also report a shorter lifespan for the daughter fronds formed later. The same is reported by BOSS et al. (1963b) for L. obscura (named as L. minor): the lifespan of the daughter frond reaches 73 days, the lifespan of the 24th and last one only 41 days. They confirmed that the time between successive daughter fronds increases as parent fronds age. The results show that the maximum size of daughter fronds is reached in the second and third daughter frond and that the later daughter fronds successively become smaller. On the other hand, CLAUS (1972) observed similar lifespans in L. obscura (named as L. minor) and in L. aequinoctialis, independent of position in the sequence of formation; according to CLAUS, each daughter frond gave rise to about the same number of daughter fronds in the next generation. MENDIOLA (1919) demonstrated that, in spite of physiological and morphological differences between early and late daughter fronds of L. minor, the

characteristics of a clone cannot be altered by long-term selection of particular fronds.

ASHBY and WANGERMANN (1950) postulate that a particular substance is transported from the mother frond to the daughter frond, which induces the formation of new cells. According to the authors, the continuing loss of this substance is the cause of the ageing of the mother frond. Another substance is supposed to result in the elongation of the cells. For more recent investigations and interpretations see vol. 2, chapter 2.4.1 (LANDOLT and KANDELER 1987). GUERN (1965) reports that there is a mutual influence between the daughter and mother fronds of S. polyrrhiza and L. trisulca, in which a stimulating effect from the mother frond is transmitted to the first daughter frond. The successive consumption of

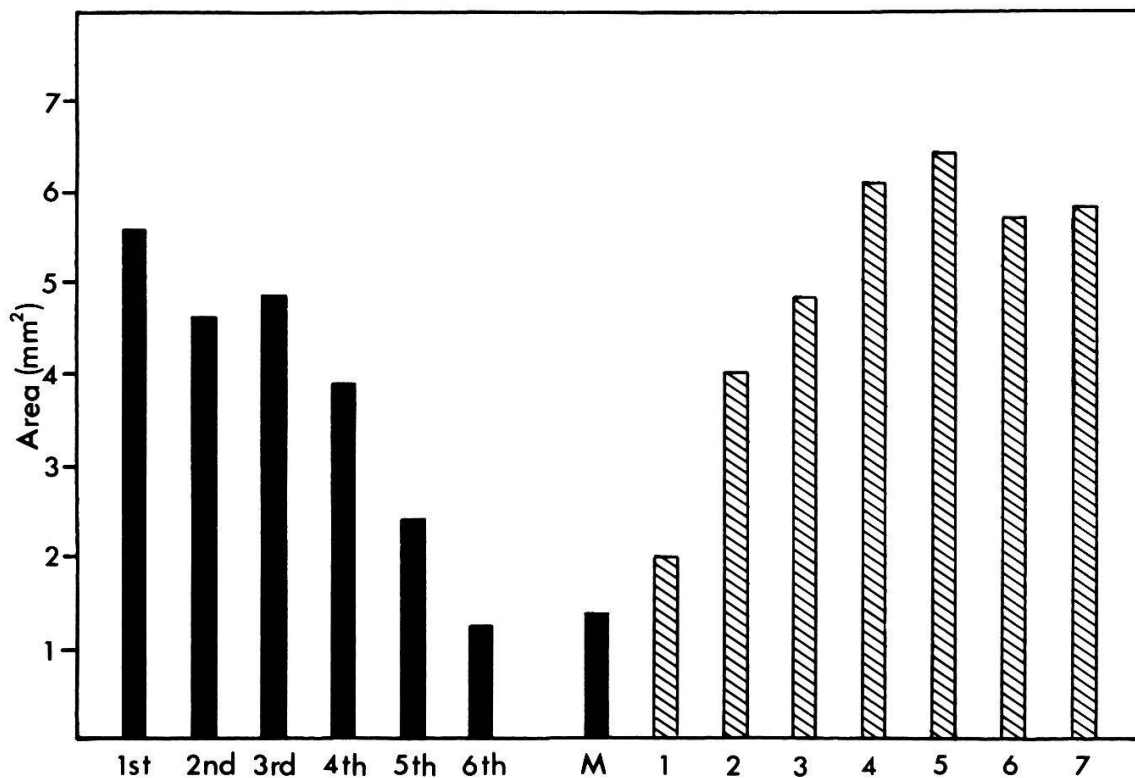


Fig. 2.11. Changes of frond area of Lemna minor grown at 20°C and 5000 lux light intensity in the course of generations

Black columns: reduction of frond area from the first to the sixth daughter frond (all produced from the same mother frond).  
Hatched columns: increase of area in successive generations of first-daughter fronds, originated from a small mother frond (M). (From KANDELER 1983, after data from WANGERMANN and ASHBY 1951).

nutrients and energy by this daughter frond inhibits the nutrient supply and the development of future daughter fronds. If the first daughter frond is removed, a second one develops on the other pouch. The retardation effect of the first daughter frond on the second one can be prevented by the addition of kinetin and other cytokinines, as well as by gibberellins.

### 2.3.3. Number of daughter fronds

The fronds of L. minor are able to form up to 15 daughter fronds, which appear after successively longer intervals with ageing (ASHBY et al. 1949, PIRSON and GOELLNER 1954). KASINOV (1981) reports up to 21 daughter fronds in L. minor and BOSS et al. (1963a) up to 24 in L. obscura. SCHUSTER (1968) observed up to 15 and HILLMAN (1969c, citing POSNER 1962a) 17-20 daughter fronds in L. aequinoctialis (clone 6746). According to KANG and CLELAND (1985), L. gibba G3 develops 10-12 daughter fronds before senescence and death, whether grown on short days (non-flowering conditions) or on long days (flowering conditions). The authors did not succeed in finding any treatment to increase daughter frond production. LAVALREE (1943) counted up to 5 daughter fronds in W. arrhiza. The number of daughter fronds formed by a mother frond is dependent upon the clone and on the culture conditions (WANGERMANN and ASHBY 1951). Under suboptimal light conditions, it is smaller for flowering fronds than for vegetatively reproducing ones. SCHUSTER (1968) counted 14 daughter fronds for L. aequinoctialis (6746) under long day conditions and only 5 under short day conditions (L. aequinoctialis flowers under short day conditions).

### 2.3.4. Position of daughter fronds

The daughter fronds are attached to the mother frond with a connecting piece called the stipe. According to the species, the fronds either separate as soon as the daughter fronds have matured (e.g. many species of Wolffia) or the mother and daughter fronds stay together, connected by the stipes over more than one generation. In the latter case, the connected fronds may form small groups (many species of Lemna, fig. 2.12),

rosettes (*S. polyrrhiza* under certain conditions), long and often branched chains (*L. trisulca*, fig. 2.13), or clusters (*W. gladiata*). In species where the daughter fronds separate, the moment of separation partly depends upon the external conditions. The same species may form groups of one, a few, or many fronds, according to the conditions of their origin and to the season. For instance, cultures grown in the darkness show a tendency to form large groups and clusters (LANDOLT 1957, LEUCHTMANN 1979, and others). A surplus of carbohydrates promotes the bonds between the fronds (YOSHIMURA 1944). For separation of the

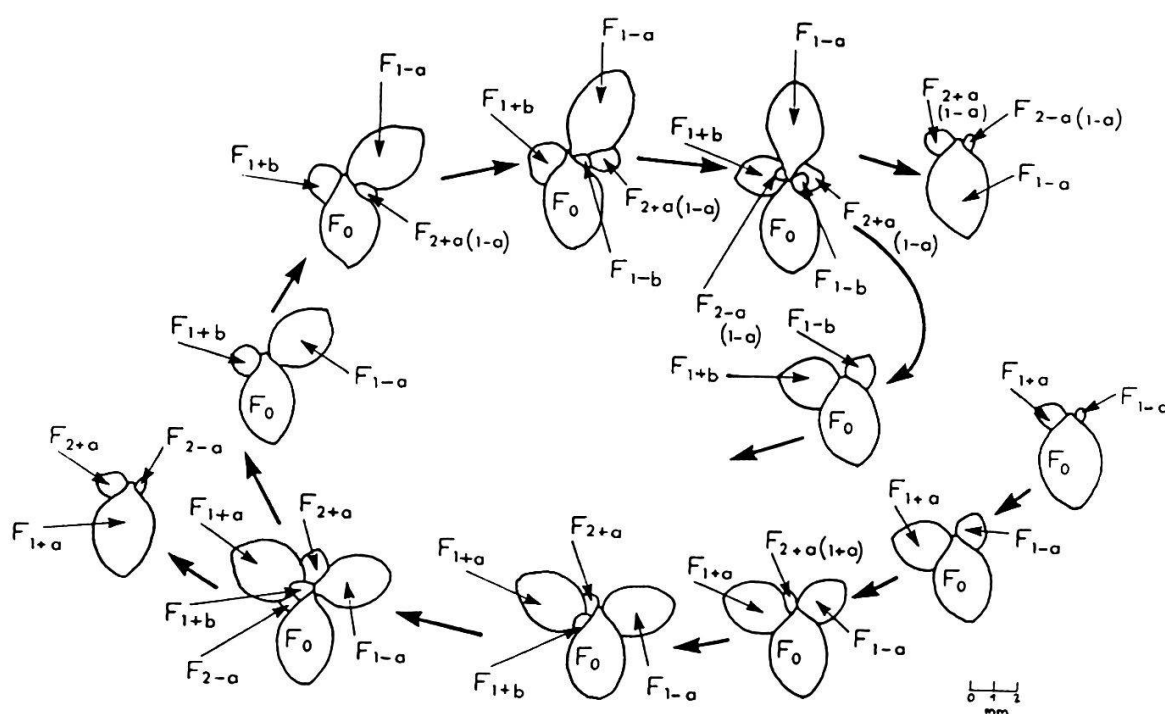


Fig. 2.12. Diagram of the vegetative growth cycle of *Lemna aequinoctialis* (after DATKO et al 1980b)

$F_0$	mother frond	a	first daughter frond
$F_1$	daughter frond of the first generation	b	second daughter frond
$F_2$	daughter frond of the second generation	+	plus side
		-	minus side
$F_{2+a(1-a)}$	first daughter frond of the plus side of the second generation originating from the first frond of the minus side of the first generation		

If the group with daughter fronds has a certain size the successive daughter fronds (with daughter fronds of the second generation) detach.

fronds, the endogenous content of abscisic acid (ABA) is very important, according to WITZTUM and KEREN (1978a). The content of ABA is improved by a short irradiation with ultraviolet light and by increased sugar concentration in the nutrient solution. Salicylic acid and EDDHA are supposed to lower concentration of ABA and therefore promote the formation of large groups of fronds. DATKO et al. (1980b) observed the chronological sequence of the separation of the daughter fronds of L. aequinoctialis. The first daughter colony from one pouch separated after approximately 60 hours; the second colony from the other pouch separated after a further 30 hours; and the third colony from the first pouch separated again after a further 40 hours. The sequence of new daughter fronds and the distribution of frond ages in a culture is described.

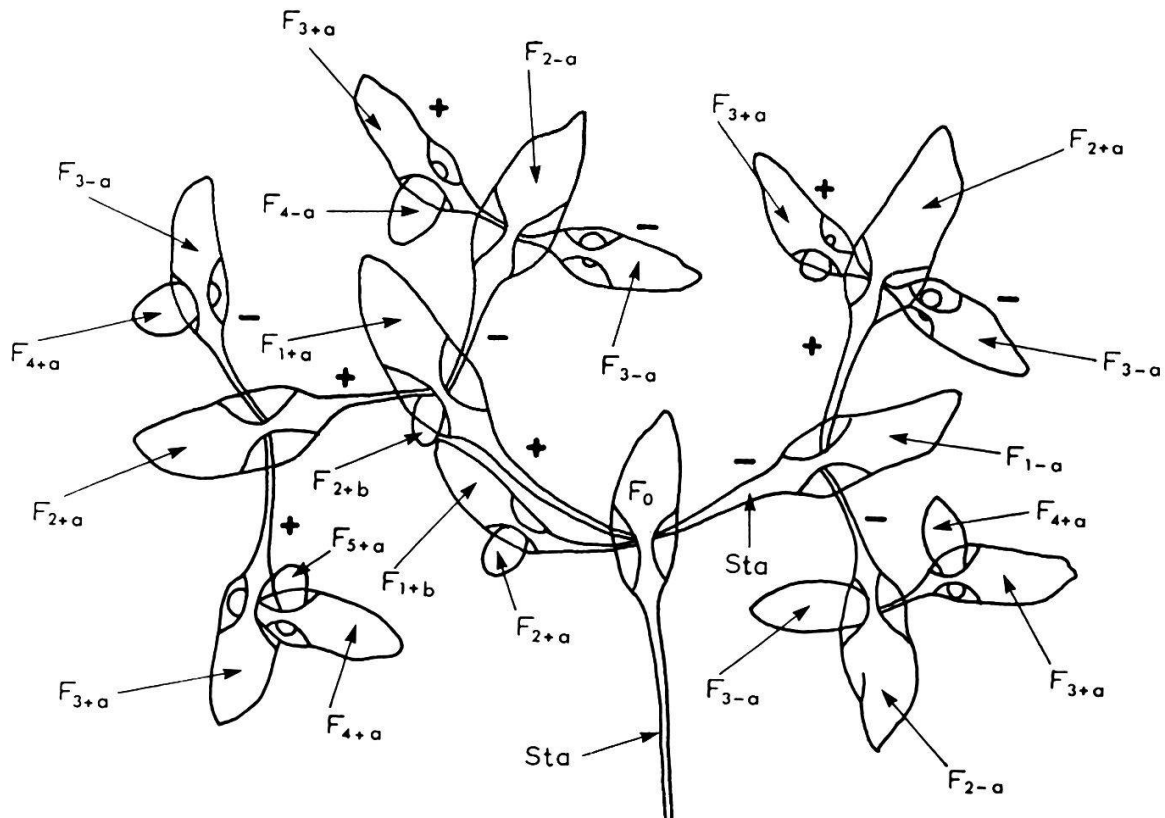


Fig. 2.13. Frond chains in Lemna trisulca (from GUERN 1965) (x4)

F <sub>0</sub>	mother frond	a	first daughter frond
F <sub>1,2,3,4</sub>	daughter frond of the first, second, third, and fourth generation	b	second daughter frond
		+	plus side
		-	minus side
Sta	stalk (basal part of the frond containing the stipe at the end)		

Only one daughter frond forms if the light conditions are unfavourable, the daughter frond always developing on the same side. For example, in the half-shade L. trisulca produces spiral chains instead of reticular groups (GOEBEL 1921) (fig. 2.14). I made the same observations; with the addition of sugar, reticular groups are formed again. ZURZYCKI (1957b) reports simple chains with antidrome branches; he explains this phenomena as due to too-low amounts of carbohydrates; he also found that the addition of sugars produced daughter fronds on both sides.

There is no genetic fixation on which side the first daughter frond of Lemna or Spirodela is formed, although the first fronds of a clone will develop on the same side for many generations. HILLMAN (1961) observed from the offspring of self-fertilized seeds of L. aequinoctialis that about 50% of the clones begin to develop the first daughter fronds on the right side and about 50% on the left side. Our clone 6746 has changed preferences for the side of the first daughter fronds twice since 1953 in agar culture. Sometimes in nature, one can see symmetrical groups of Lemna (e.g. L. valdiviana) which means that the first daughter

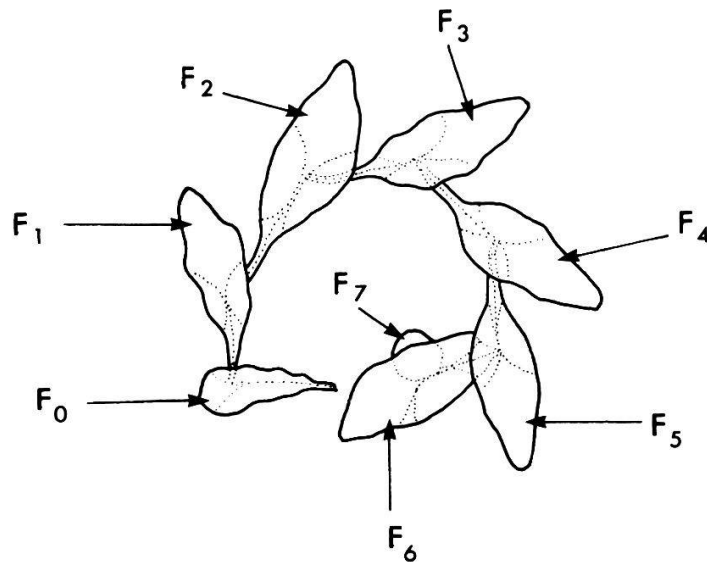


Fig. 2.14. Lemna trisulca producing spiral chains in the half-shade (from GOEBEL 1921) (x4)

F<sub>0</sub> mother frond

F<sub>1</sub> to 7 daughter fronds of the first to the seventh generation

frond from the right side developed the first daughter frond of the second generation on the left side and that the daughter frond from the left side produced its first daughter frond on the right side. Under certain conditions, the preference of the side might change after each generation. BOECKER (1936) made a similar observation with an unidentified Lemna from an aquarium. DOSS (1978) reported similar symmetric groups of L. aequinoctialis which showed a flower on alternating sides. The daughter frond changed the side preference when forming new daughter fronds of the second generation. Side preference may be changed by TIBA (WANGERMANN and LACEY 1953, WITZTUM 1966), X-ray radiation (POSNER and HILLMAN 1960, POSNER 1961) or by the application of 2,4-D (KASINOV 1973, 1978). According to KASINOV and KASINOVA (1974) and KASINOV and PAVLOVA (1977), the side preference of clones of L. minor is relatively stable. However, for a short time, when the daughter fronds reach 20-25  $\mu\text{m}$ , they are easily susceptible to external factors. During this time, the application of 2,4-D can produce a symmetrical arrangement of daughter fronds. Afterwards, the new daughter fronds will again have their own side preferences ("right-handed" or "left-handed"). A spontaneous change in the side preference was observed only in the relation of 1:10-20'000 (KASINOV and KASINOVA 1974). These authors point out, too, that the side preference cannot be transmitted by genes of either the nucleus or of the plasma. Stereoisomers of arginine, aspartic acid and atebine have no influence on the side preference of L. minor (KASINOV 1980). According to WITZTUM (1966, 1979), the development of a daughter frond is dependent on the concentration of certain hormonal substances ("auxins"). These substances are formed in the meristematic tissues. The side that has more of this tissue present (the side of the accessory bud of the mother frond), grows larger and is called the plus side. There, the first daughter frond will develop on this side. After the application of TIBA, the accessory frond might often be suppressed resulting in a reversal of the plus side. If growth conditions are very favourable, there is the possibility that the daughter fronds will develop simultaneously on each side (e.g. L. aequinoctialis).

Normally, the position of the accessory fronds (= successive fronds which are formed after the first daughter frond in each pouch) is on the plus side of the previously formed daughter frond (WITZTUM 1966).

## 2.4. CHARACTERISTICS OF NORMAL VEGETATIVE FRONDS

### 2.4.1. Form, size and weight

In general, the Lemnoideae (Spirodela, Lemna) are thin and leaf-like; some species (e.g., S. intermedia, S. punctata, L. gibba, L. disperma, L. obscura) become inflated (gibbous) due to enlarging of air spaces. In shape, they are orbicular, obovate or lanceolate; they grow up to 1.5 cm long (without stalk, L. trisulca) and 1 cm wide (S. intermedia, S. polyrrhiza). The smallest species of the Lemnoideae reach 3 mm under optimal conditions; in sub-optimal conditions, sometimes they are smaller than 1 mm. Wolffiella is thinner than the Lemnoideae, leaf-like, often transparent, orbicular, obovate, tongue-shaped or sabre-shaped. The largest species (W. neotropica, W. Welwitschii, W. lingulata) become up to 8 mm in length and 5 mm in width; the smallest species reach no more than 3 mm in length (W. repanda) and 0.8 mm in width (W. gladiata, W. denticulata). The fronds of Wolffia are thick and either globular, ovoid, cylindrical, conical, boat-shaped or nutshell-shaped. The fronds grow up to 2.5 mm in length (W. elongata), 1.5 mm in width (W. brasiliensis) and 2 mm in depth (W. microscopica). The smallest species, (W. angusta and W. globosa), which are the smallest species of all flowering plants, reach a length, width and depth of 0.8, 0.4, and 1 mm and 0.8, 0.6, and 0.7 mm, respectively.

The size and shape of fronds are strongly dependent upon external conditions. However, there are also genetic differences between different clones. It is rather difficult to make precise statements on the relationship between external factors and the size of each frond because most factors do not work independently; moreover, the different species and clones do not always react in the same way. Some variations within time under the same culture conditions is also reported (e.g. TILLBERG et al. 1979).

In general the size of fronds increases and the shape may change with the following conditions:

- increased light intensity: for L. minor, up to at least 8000 lux (ROMBACH 1976); for W. arrhiza, between 500 and 5000 lux (GODZIEMBA-CZYŻ 1969).
- increased light duration: bigger fronds of S. intermedia develop in

continuous light than in a 12-hour day (FERNANDEZ and MUJICA 1973).

- addition of sugar: the size of S. polyrrhiza and L. minor is enlarged 1 1/2 to 3 times with the addition of 1% sucrose at temperatures between 20°C and 30°C (LANDOLT 1957). Higher concentrations of sugar (2 to 5%) give rise to smaller fronds in S. polyrrhiza (GORHAM 1945, LANDOLT 1957). The effect of glucose is still greater than that of sucrose; fructose and maltose are less effective than sucrose.
- increased nitrogen, phosphorus, potassium, calcium and magnesium content: however, very high concentrations of these elements will result in a reduction of frond size (WHITE 1936a,b,c, PIRSON and SEIDEL 1950, LUEOEND 1980, ZIMMERMANN 1981, DANN 1982).
- increased temperature: temperatures up to a maximum of about 20°C for L. minor and S. polyrrhiza in solutions without sugar; at higher temperatures, the size is reduced; again, with the addition of sugar, the optimum was reached at higher temperatures (LANDOLT 1957).
- low night temperatures: the size of fronds of S. intermedia was much greater with night temperatures of 5°C than of 25°C, and with a day temperature of 25°C (FERNANDEZ and MUJICA 1973).
- addition of benzimidazole: the size of L. minor fronds nearly doubled when 3.4 mM benzimidazole was added to the solution (HILLMAN 1955).
- addition of IAA: in L. trisulca, an elongation of the frond was observed with addition of IAA (BATA and NESKOVIC (1982a).
- application of lanolin: lanolin applied to the upper frond surface of L. minor increased the frond size (GORHAM 1941).

The size of L. minor fronds was reduced with the addition of gibberellin (LOOS 1962). In S. punctata, frond size was decreased with the addition of EDDHA or salicylic acid (SCHARFETTER et al. 1978).

The length-width ratio and the thickness of fronds depends upon external factors, too. Fronds of L. minor are narrower under 6 hours daylight than under 16 hours (FELDMANN 1975). In L. trisulca the length-width ratio substantially increases with growth under far-red light and to a lesser extent under blue light, when compared with growth under green or yellow light (at low light intensities) (ZURZYCKI 1957b). Blue light at high intensities reduces the length of L. trisulca (BATA and NESKOVIC 1982b). In S. polyrrhiza, DAVIDSON and SIMON (1981b) observed in most clones relatively longer fronds at high temperatures (28°C > 23°C > 18°C). LUEOEND (1980) was able to reduce length-width ratios by increasing the phosphorus content of the nutrient solution up to a certain maximum;

with a high concentration of phosphorus, the ratio increased again. LUEOEND (1980, 1983) made similar observations in relation to the content of nitrogen (except L. minuscula). For the clones used, the ratio varied between: 1.03 and 1.27 (S. polyrrhiza), 1.10 and 1.53 (L. gibba), 1.35 and 1.60 (L. minor), 1.19 and 1.62 (L. minuscula). In the lower concentrations of P and N the length-width ratio of the fronds of L. minuscula was extremely high, in higher concentrations very low. There are also length-width differences between various clones of the same species. L. minor and L. turionifera showed a length-width ratio of about 1.6 when grown on either a balanced or a nitrate-limited medium. The ratio of L. turionifera fell below 1.5 when grown on a phosphate-limited medium while that of L. minor remained near 1.6 (DOCAUER 1984). The use of Hoagland solution in different concentrations did not cause any differences in the length-width ratio of S. polyrrhiza (SPOONER 1967). The same was true in solutions with and without nitrogen. However, it should be pointed out that SPOONER (1967) used relatively low light intensities (3000 lux, as opposed to 18000 lux used in the investigations of LUEOEND 1983). Under conditions of continuous darkness, LEUCHTMANN (1979) noticed no change in the ratio of different species if yeast extract, vitamin B, or amino acids were added.

Although the length-width ratio is variable within a clone and between different clones of one species, there are extreme values which might be characteristic for a certain species. To distinguish L. gibba from L. minor, extreme values of the ratios can definitely be assigned to the two different species, and only the medium values, which unfortunately occur rather frequently, cannot be identified. Fronds of a ratio of 1.0 to 1.2 belong to L. gibba and those of a ratio 1.6 to 2.1 belong to L. minor (DE LANGE and WESTINGA 1979). In a similar way, extreme ratio values are characteristic for species within the groups of L. valdiviana and W. lingulata.

The length-width ratio of Spirodela varies between 1 and 2; of Lemna, between 1 and 5; of Wolffiella, between 1 and 20; and of Wolffia, between 1 and 4.

PIETERSE (1972, 1974b, 1975a), DE LANGE (1974), DE LANGE and PIETERSE (1973), ELZENGA et al. (1980) measured the thickness of L. minor and L. gibba under different external conditions; similar investigations were done by DE LANGE and REVIER (1982) with S. polyrrhiza (see also chapter 2.4.4.). The gibbosity of the lower side of the frond of L. gibba is

caused by the enlargement of air spaces. For a long time, there was much speculation about which factors in nature were responsible for this enlargement. GUPPY (1894) and VAN HOREN (1869) attributed it to high temperatures (because flat forms were mostly seen during winter times, cf. also DE LANGE et al. 1984). REJMANKOVA (1975a) made similar observations. However, DE LANGE and PIETERSE (1973) and PIETERSE (1975a) also reported gibbous fronds at low temperatures. Varying the nitrogen and phosphorus content in the solution, LUEOEND (1983) observed gibbous fronds only in solutions with simultaneously high nitrogen and phosphorus content (8-40 mg N/l and 0.6-80 mg P/l). High nitrogen concentration coupled with medium phosphorus concentration did not stimulate gibbosity. PIETERSE et al. (1970 b,c), PIETERSE (1972, 1975a, 1976), CLELAND (1974c), ELZENGA et al. 1980, and CLELAND et al. (1982) mention that the following chemicals promote the gibbosity of L. gibba: EDDHA, EDTA, Ethrel, and salicylic acid. Gibberellins and copper prevent the enlargement of air spaces, whereas iron has no influence. EDDHA and salicylic acid (SCHARFETTER et al. 1978) are able to make S. punctata gibbous. On the other hand, L. minor only becomes a little thicker with the addition of EDDHA. No enlargement of the air spaces was observed with either S. polyrrhiza by DE LANGE and REVIER (1982) or by me with S. intermedia. ELZENGA et al. (1980) show that the gibbosity of L. gibba is actually caused by ethylene, in nature as well as under culture conditions. The necessary amount of ethylene is 24 nl/l air or more. The authors were able to measure the same amounts of ethylene at places in nature where gibbous L. gibba were found. The cultures grown in a nutrient solution containing EDDHA were able to produce the needed amount of ethylene. The assumption is therefore that gibbosity is caused by a certain endogenous concentration of ethylene.

A high dry weight of Lemnaeae fronds is promoted by high light intensities and by addition of sugar (LANDOLT 1957). At low temperatures, the dry weight of a single frond is higher than that at high temperatures. According to LANDOLT (1957), the dry weight of L. minor is more than doubled at 14°C in comparison to the values at 20°C. TILLBERG et al. (1979) report fluctuations of the average fresh weight of L. gibba (three frond groups) between 25 and 50 mg at different times but under exactly the same conditions.

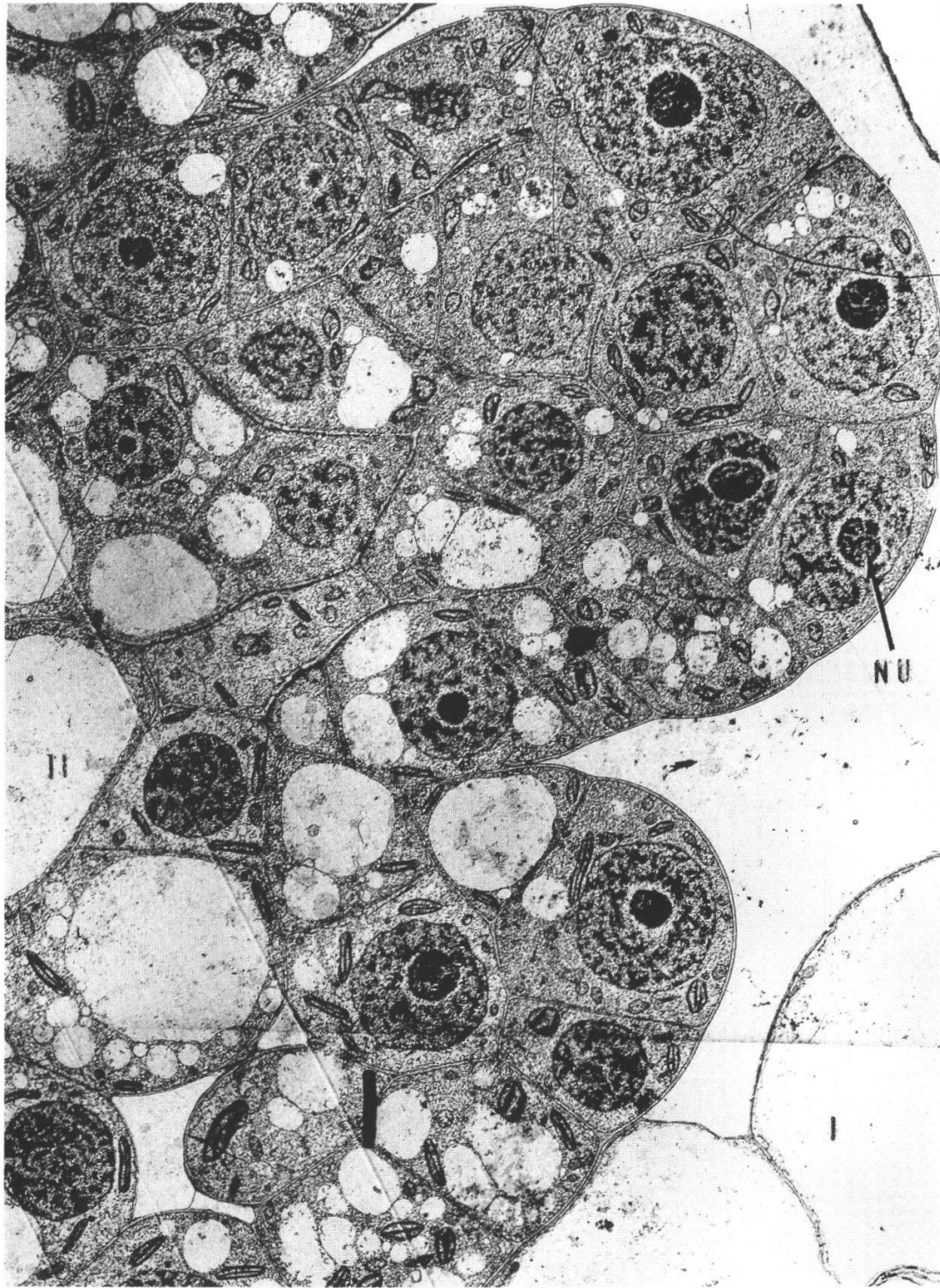


Fig. 2.15. Young frond primordia (II) of Wolffia arrhiza (No. 7014) arising in the reproductive pouch of a mother frond (I). NU = nucleolus. (x2500) (from ANDERSON et al. 1973)

#### 2.4.2. Elements of the cells

The cells of L. gibba contain three main compartments, according to BIELENSKI et al. (1984a,b): cell wall, cytoplasm (incl. nucleus), and vacuole.

The mean relative volumes of the wall, the cytoplasm and the vacuoles in L. gibba are 0.03, 0.32 and 0.65, respectively (GAUDINET et al. 1984).

Fig. 2.15 shows meristematic cells in W. arrhiza (ANDERSON et al. 1973). The early development of vacuolation can be seen. Chloroplast are present in all cells. This presence of chloroplasts and the absence of proplastids in meristematic cells is unusual for vascular plants. BEAMS et al. (1979) showed that Lemna fronds are able to survive ultracentrifugation and to multiply again afterwards.

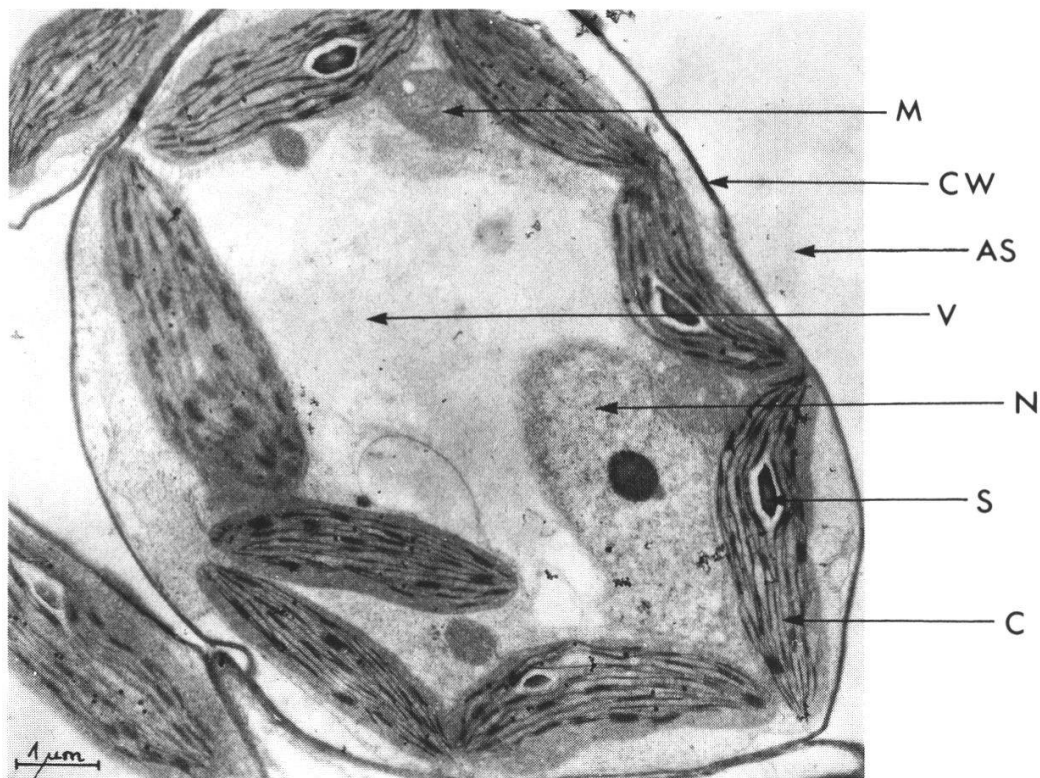


Fig. 2.16. Ultrastructure of a parenchymatic cell of Spirodela polyrrhiza showing cell wall (CW), chloroplast (C), starch grains (S), vacuoles (V), mitochondria (M), nucleus (N), and part of the intercellular air space (AS). (x8500) (from LE PABIC 1972).

The ultrastructure of meristematic cells in S. polyrrhiza is essentially similar to those described for other higher plants with nucleus, mitochondria, Golgi apparatus, plastids, and endoplasmic reticulum (ER) (RAO 1969, LE PABIC 1972). Predominant organelles in the mesophyll cells of S. polyrrhiza are the chloroplasts with well developed grana-fretwork system. Starch granules are often present; the protoplasm was seen to be sparse with respect to ER and Golgi apparatus but contains a large vacuole. Continuity between ER and plasmodesms was noted (RAO 1969, LE PABIC 1982b, ANDERSON et al. 1973).

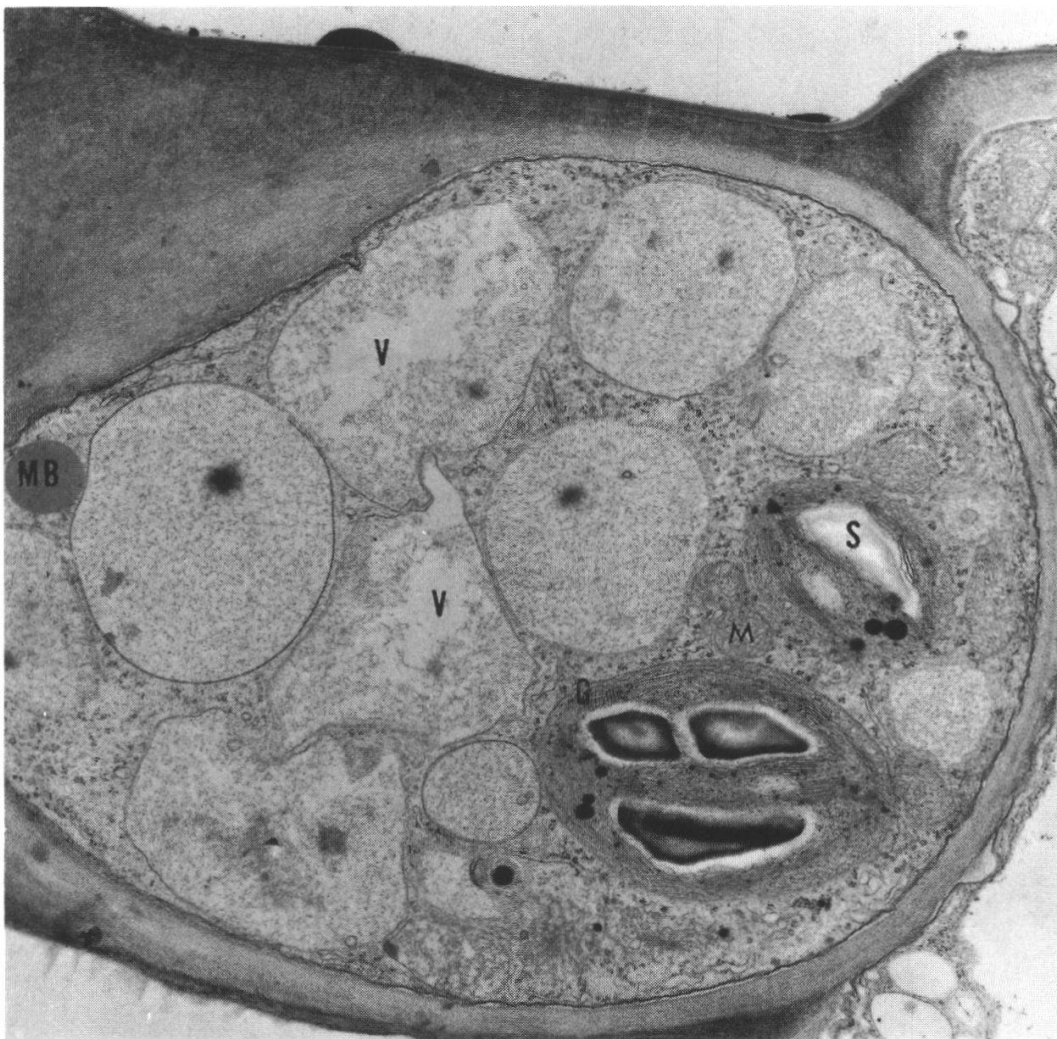


Fig. 2.17. Ultrastructure of a guard cell of a mature frond of Wolffia arrhiza (No. 7014) showing chloroplasts containing starch (S), numerous vacuoles (V), granum (G), microbodies (MB), and mitochondria (M). (x13000) (from ANDERSON et al. 1973).

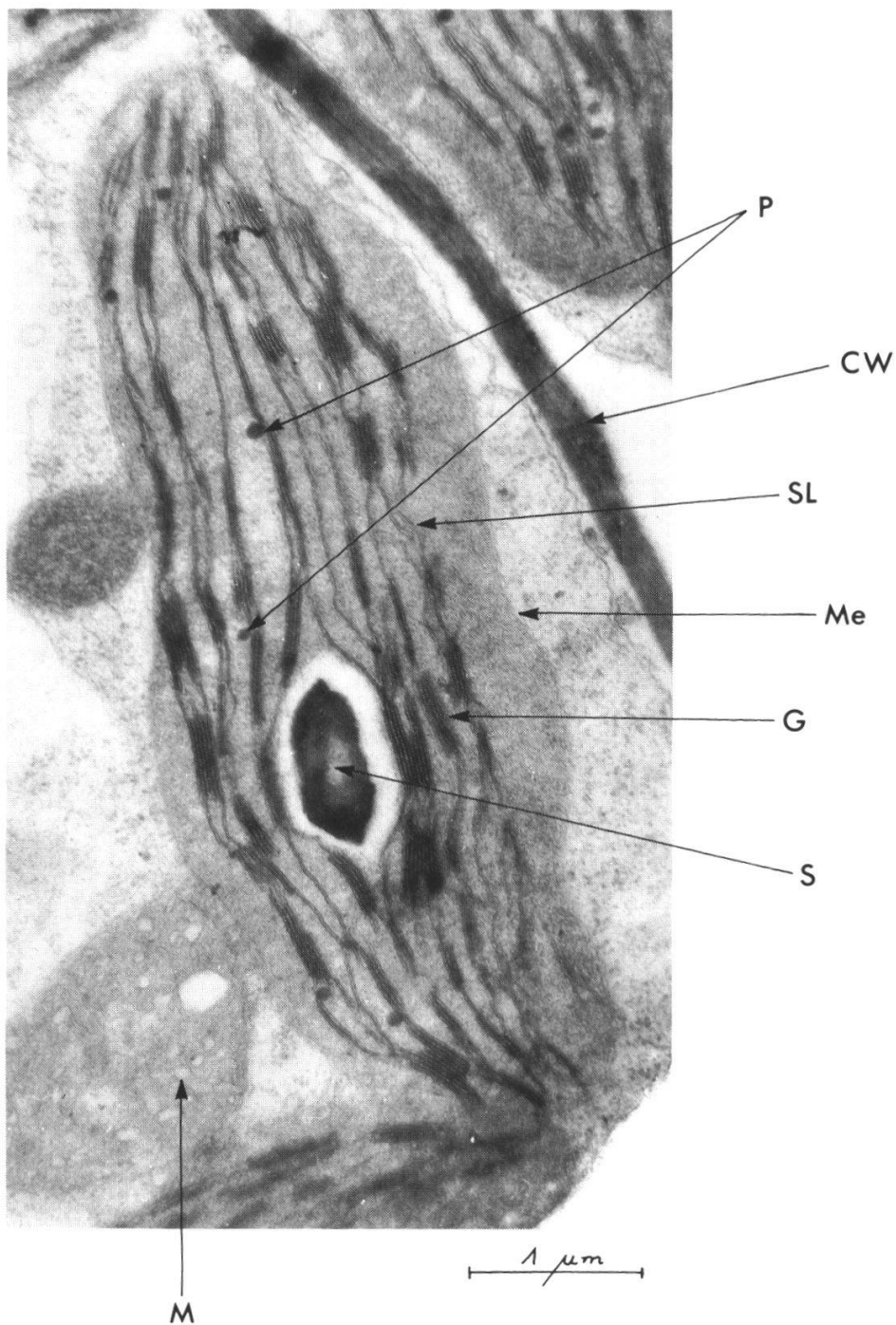


Fig. 2.18. Chloroplast of *Spirodela polyrrhiza*. The membrane limiting the chloroplast is very fine; the grana are formed by 3 to 10 lamellae. The dense stroma contains a starch grain and several globuli. (x16000) (from LE PABIC 1972).

CW	cell wall	P	plastoglobuli
G	grana	S	starch grain
M	mitochondria	SL	stroma lamellae (thylacoids)
Me	membrane of the chloroplast		

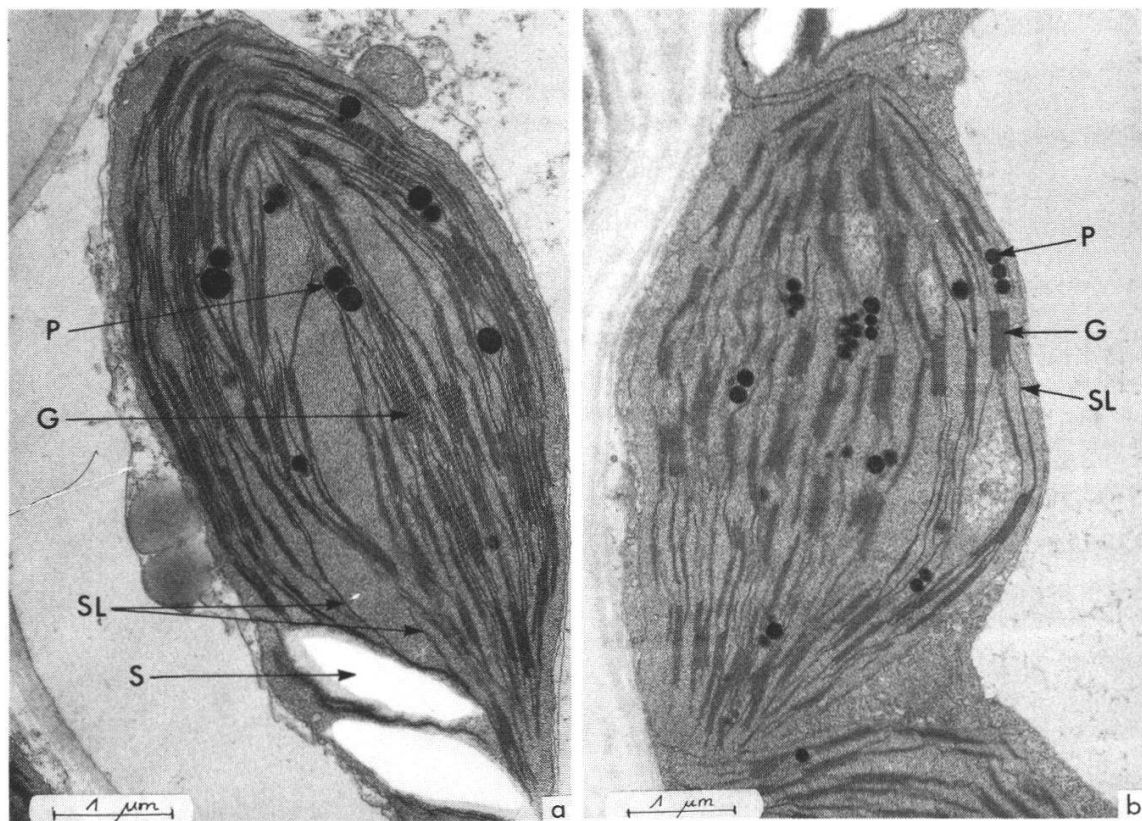


Fig. 2.19. Chloroplasts of *Lemna* sp. (from BEAMS et al. 1979)  
 The chloroplasts are from fronds ultracentrifuged for 1 hr. The plants were then allowed to recover for a period of 6 (a) and 24 (b) hours, respectively.

- The starch grains (S) are still located at one end of the chloroplast, but the plastoglobuli (P), grana (G), and stroma lamellae (SL) have become partially redistributed. (x13000)
- The chloroplast constituents are distributed in a manner similar to those of uncentrifuged fronds. (x12000).

Fig. 2.20 (p. 45)

- Division figure of a cell in the primordium. Note numerous mitochondria and chloroplasts in the dividing cell and abundance of plasmodesmata. (x14000)
- Dictyosomes and ribosomes in a cell adjacent to a) (x25000)
- Young chloroplast in the meristematic proximal end of a daughter frond. (x42000)
- Chloroplasts and mitochondria in an enlarging vacuolate cell. (x9200)

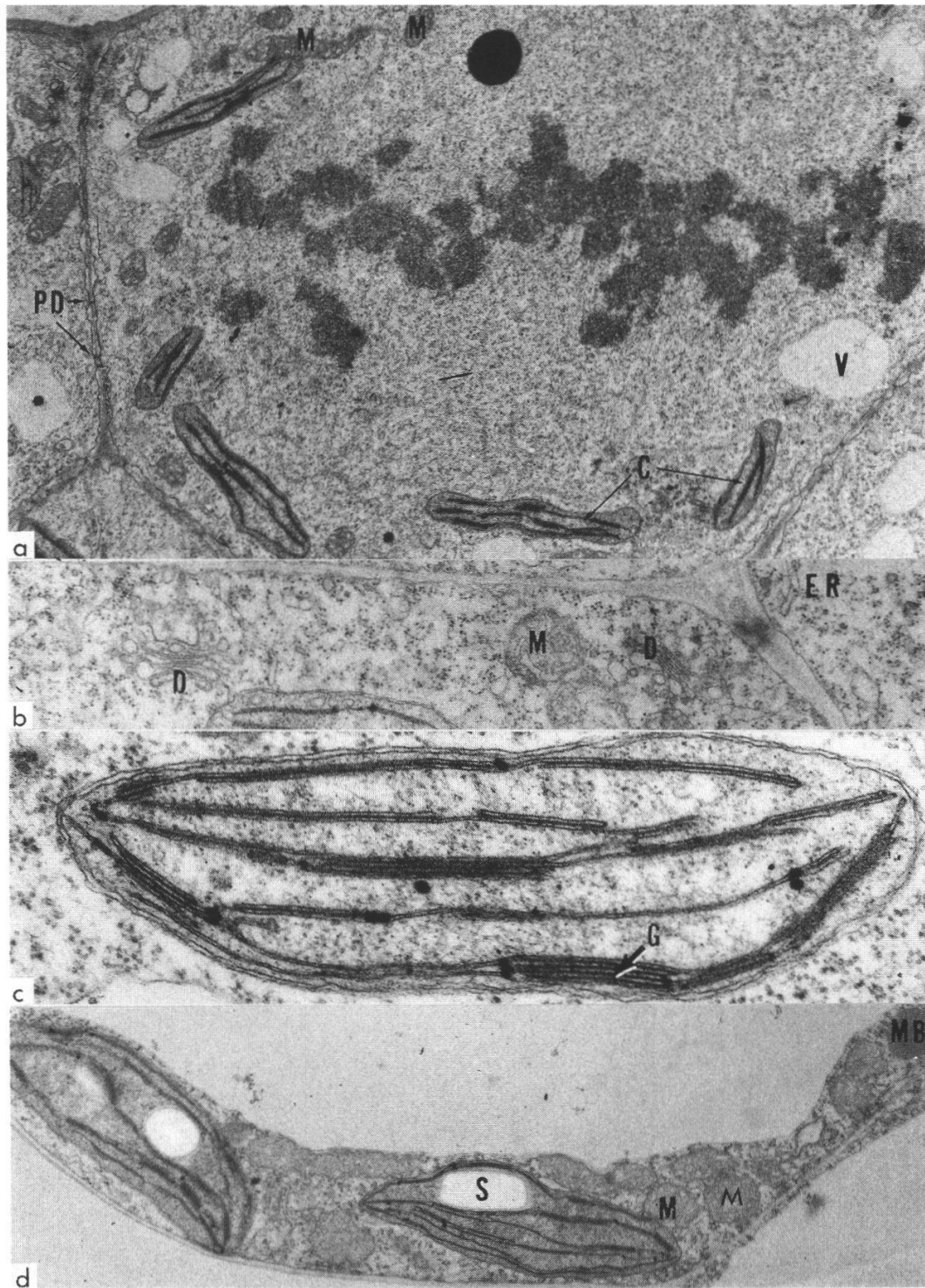


Fig. 2.20. Ultrastructure of young daughter frond cells of Wolffia arrhiza (No. 7014) (from ANDERSON et al. 1973)  
(for further information see p. 44)

C chloroplast	G granum	PD plasmodesmata
D dictyosome	M mitochondrion	S starch
ER endoplasmic reticulum	MB microbody	V vacuole

The chloroplasts of L. minor grown in a weak light show the typical thylakoid network of higher plants with grana usually containing 8 to 9 adpressed thylakoids. In strong light, the internal membrane system was less well developed having longer grana structures with only 3 to 4 adpressed thylakoids (RAO 1982). In the chloroplasts of S. polyrrhiza and of Lemna sp. LE PABIC (1972) and BEAMS et al. (1979) observed a rather typical ultrastructure with well developed thylakoids, and with grana and stroma (figs. 2.18, 2.19). Similar results in W. arrhiza are reported by ANDERSON et al. (1973) (fig. 2.20). The authors recognized chloroplasts which lack starch in the meristematic tissue but had no proplastids in contrast to most other plants. Further investigations of chloroplasts of Lemnaceae were made by WROBLEWSKI (1973) on L. minor and MONTSELVE et al. (1984, 1986) on L. aequinoctialis.

VINTEJOUX (1978, 1982b) studied the endoplasmic reticulum (ER) and the dictyosomes of turions of S. polyrrhiza. In turions formed in the fall, the tubes of ER are much reduced, stand vertically to the wall and are shorter than in normal fronds. During winter time, the tubes get more frequent, often anastomize and form later two parallel rows. The reticulum does not seem to be preferentially associated with the vacuoles. At the moment of germination in the spring, the tubes of the ER get dispersed within the cytoplasm. Some vesicles of ER become hypertrophied. The dictyosomes in the meristematic tissue of newly built turions consist of 5 to 6 tubes of irregular shape which are mostly bigger than in normal fronds. The Golgi vesicles, which are reduced at the beginning of the resting period, get more and more frequent towards germination time. The cell elements of S. polyrrhiza and W. arrhiza are reproduced in figs. 2.16 and 2.17, respectively.

#### **2.4.3. Colour and brightness; pigment cells**

The colour of fronds is influenced by the following conditions:

- chlorophyll content of the different layers
- thickness of the frond and occurrence of air spaces
- occurrence and distribution of anthocyanins within the frond.

Thick fronds with plenty of chloroplasts in the upper layers are intensively green; thin fronds with few, regularly-distributed chloroplasts are light green and often transparent. Just as the frond thickness is

dependent upon external conditions, the intensity of the colour may also vary, according to the given conditions. A small amount of light or other unfavourable conditions results in the production of a small amount of chlorophyll (ROMBACH 1976); yellowish carotinoids and flavonoids may then affect the colour, whereas they are usually not visible under normal conditions. HONDA (1983b) showed that in W. arrhiza the percentage cover of mesophyll cells by chloroplastic matter is constant for a given set of growth conditions, independent of cell size and age. The number of chloroplasts per cell is correlated with the cell size.

Some species are usually intensively green coloured: all Spirodela species, species of L. minor and W. brasiliensis groups, and W. australiana. Very rarely intensively green species include all species of Wolffiella and L. valdiviana, W. microscopica, W. elongata, and W. columbiana. Other species may be intensively green in colour, depending on the growth conditions.

Anthocyanins are restricted to all species of Spirodela, to the L. minor group and to L. trisulca, but they are not always produced. In some species, the anthocyanin colour pattern is very characteristic. Sometimes the fronds have a slightly reddish colour, and sometimes there are definite spot patterns on either the upper or lower sides. Very often the subepidermal layer of the lower side is intensively red coloured. Red colour in the cortex parenchyma of the roots also is possible in the genus Spirodela. Neither chloroplasts nor starch grains are present in cells of S. polyrrhiza with anthocyanin dissolved in the sap contrary to Lemna species (HEGELMAIER 1868). The formation of anthocyanins is promoted by high light intensity, unfavourable temperature and nutrient conditions (e.g., THIMANN and EDMONSON 1949 with S. punctata). LUEOEND (1983) observed more anthocyanin in L. gibba grown in culture solutions with low nitrogen and phosphorus content. More anthocyanins are produced with the addition of fructose (HENSSEN 1954 with S. polyrrhiza). The presence of copper is necessary for the formation of anthocyanins (THIMANN et al. 1951, THIMANN and RADNER 1955b). The synthesis of riboflavin must precede the development of anthocyanin (THIMANN and RADNER 1958). High concentrations of kinetin prevent the formation of both anthocyanin and chlorophyll (GUERN 1965). Phytochrome is involved in the phyto-regulation and anthocyanin production. The most effective spectral regions for anthocyanin production in S. polyrrhiza are red, blue and ultraviolet, with action in the far-red (FR) minimal or nil (MANCINELLI and

RABINO 1984). A more detailed survey of metabolic processes leading to anthocyanin formation is given in volume 2, chapter 2.5.8.5. (LANDOLT and KANDELER 1987).

Brown pigment cells are characteristic of dead cells found in the genera Spirodela (fig. 2.23) and Wolffiella (except W. hyalina, W. repanda and W. rotunda), and in Wolffia brasiliensis (fig. 2.15b) and W. borealis. The cells are predominantly subepidermal in Spirodela and epidermal in Wolffiella and Wolffia (HEGELMAIER 1868, cf. figs. 2.1 f,g). WITZTUM (1966) observed an accumulation of pigment cells in the substomatal chambers and in the abscission region of the stipe of S. punctata. There are also pigment cells surrounding the stigma of Spirodela and some Wolffioideae species and along the opening fissures of anthers of Spirodela, Wolffiella and Wolffia. WITZTUM (1966) suggests growth regulating capacities for the chemical substance in the pigment cells. It is not known whether the pigment cells give some protection against damage by animals. The pigment is believed to be a phlobaphene-like substance which is formed after the frond dies or after treatment with ultraviolet radiation (WITZTUM 1966, 1974a). In a living colourless pigment cell, phenolic compounds (perhaps leucanthocyanins) are present that can be changed to phlobaphene-like pigment by oxydation and polymerisation. HEGNAUER (1963) calls the pigment cells "myriophyllin cells". The pigment cells have no influence on the colour of living fronds. They can get stained by methylene blue, malachite green, gentian violet, methyl red and safranin but not by cresol red, thymol blue, phenol red, bromcresol green, resorcin blue, anilin blue and bromphenol blue (WITZTUM 1966).

The shininess of the upper surface of the frond is probably due to a wax layer of the cuticula; it is, as a rule, most pronounced with fronds that are intensively green coloured. However, there is the possibility of species-specific differences; under the same conditions, fronds of L. gibba are less shiny than those of L. minor and L. obscura.

#### **2.4.4. Epidermis, stomata, and papillae (papules)**

The one-layered epidermis of Lemnaceae consists of panel-shaped cells. The cells may be limited laterally by straight walls (Wolffiella and Wolffia), nearly straight walls (S. intermedia), walls that are slightly

undulated on the upper side and nearly straight on the lower side of the frond (S. polyrrhiza), or walls that are distinctly undulated on both sides (S. punctata, Lemna) (fig. 2.21). The epidermis cells of the cotyledons of all Lemnaceae have straight walls (HEGELMAIER 1868, LAWALREE 1943). The epidermis cells of the flowering fronds of L. trisulca show less undulated walls on the upper side than the vegetative fronds (HEGELMAIER 1868). According to HEGELMAIER, the epidermis of Lemnaceae is protected by a cuticle. The cuticle on the upper surface of Spirodela and species of groups of L. minor and L. perpusilla consists of a wax-like layer. The cuticle is not very effective in preventing water loss. A water surface completely covered by duckweeds has only a 10-15% lower evapotranspiration than a surface without Lemnaceae (BOYD 1975, RYTHER et al. 1980, DEBUSK 1980).

The size of the epidermis cells may vary greatly. On the average, the cells are smallest in Spirodela, larger in Lemna and largest in Wolffia and Wolffia. However, the size is dependent on external factors, too. The number of epidermis cells per mm<sup>2</sup> of S. intermedia is between

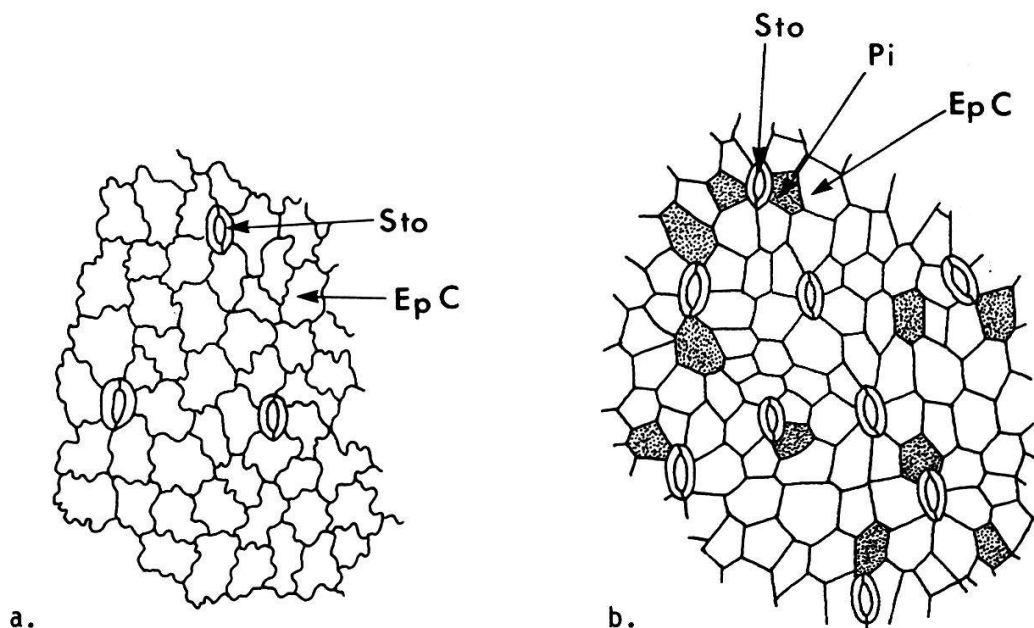


Fig. 2.21. Upper epidermis (x180)  
a. Lemna aequinoctialis, b. Wolffia brasiliensis  
(after HEGELMAIER 1868)

EpC = epidermis cells, Pi = pigment cells, Sto = stomata cells

2483 and 3689, the smaller number (and therefore the larger cells) occurring with cool night temperatures and the larger number occurring with warm night temperatures (FERNANDEZ and MUJICA 1973).

The epidermis cells of Wolffia and Wolffiella contain chloroplasts and starch cells (HEGELMAIER 1868 for different species of Wolffia, MONOD 1949 ANDERSON et al. 1973 for W. arrhiza, LANDOLT unpubl. results for Wolffiella; ANDERSON et al. did not observe starch grains in epidermis cells except in the guard cells (fig. 2.22). In L. gibba, the epidermis cells as well as 1-3 cell layers above the lower epidermis contain only small amount of cytoplasm and very few chloroplasts (GAUDINET et al. 1984). The same is probably true for all species of Spirodela and Lemna with many cell layers in cross section. The ultrastructure of the epidermis cells of S. polyrrhiza seem to be similar to mesophyll cells (RAO 1969).

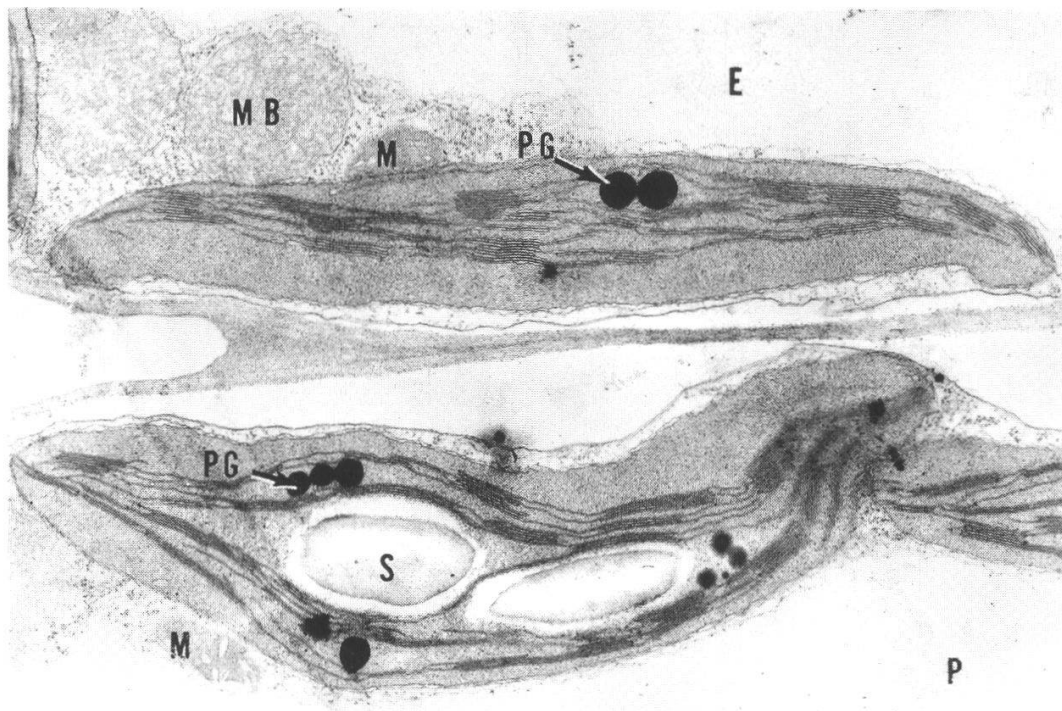


Fig. 2.22. Comparison of chloroplasts of Wolffia arrhiza in epidermal (E) and in palisade-like (P) cells showing starch (S) and plastoglobuli (PG). Note also mitochondria (M) and microbodies (MB) in cells. (x22000) (from ANDERSON et al. 1973)

With the exception of vegetative fronds of L. trisulca , L. tenera and some Wolffiella species which grow totally submerged in water, the fronds of Lemnaceae develop stomata on the upper surface or at least on that part of the frond surface that emerges from the water. The stomata are arranged with the length as opposed to their width parallel to the longitudinal axis. The 4-7 surrounding cells are not different from the other epidermis cells. They are therefore named anomocytic by SANCHEZ et al. (1984). The number of stomata is a characteristic property for different species: 2(0)-10 stomata (rarely up to 20 in flowering fronds) (W. Welwitschii, W. lingulata, W. oblonga, W. gladiata, W. denticulata, W. columbiana and W. elongata) and 5 to 30 stomata (W. globosa and W. angusta). All other species usually have more than 30 stomata (W. arrhiza sometimes has only 15 to 30; flowering fronds of L. trisulca have 30-50 stomata). KAUL (1976) counted 170 stomata per mm<sup>2</sup> on the average for S. polyrrhiza, FERNANDEZ and MUJICA (1973) reported 63 to 81 stomata per mm<sup>2</sup> for S. intermedia, according to the growth conditions (more stomata were produced under long light duration and low night temperatures). The ratio of the number of stomata cells to the number of epidermis cells is also dependent on growth conditions and is smaller with warmer night temperatures as well as with lower light intensities (4000 instead of 7000 lux).

The size of the stomata varies considerably, with cell lengths varying between 0.018 and 0.034 mm. Stomata size may vary up to 0.005 mm within the same frond. However, stomata size is not a species-specific characteristic, and it has not been investigated whether size correlates with chromosome number. The length-width ratio of the stomata amounts to about 1 1/2. Below the stomata there is an air space due to lack of sub-epidermal cells.

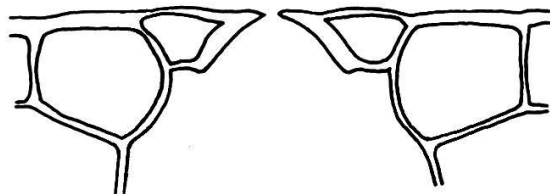


Fig. 2.23. Longitudinal section of stomata cells of Lemna minor (x1100)  
(from HABERLANDT 1887)

According to REUTER (1948, see also HABERLANDT 1887), full-grown stomata cells of L. minor no longer function; the stomata cells stay open and are empty (fig. 2.23). Some are divided by transverse walls. Young stomata cells still have a nucleus and chloroplasts; however, their ability to open and close is significantly limited. The osmotic value is slightly higher than in normal epidermis cells. It reaches 0.25-0.30 M  $\text{CaCl}_2$ . ANDERSON et al. (1973) observed chloroplasts with starch grains in the full-grown stomata of W. arrhiza. HEGELMAIER (1868), too, saw starch-containing chloroplasts in the stomata cells of flowering L. trisulca, as well as of full-grown Spirodela and Wolffia fronds. The stomata of turions or resting fronds are closed. SEVERI and BARONI (1982, 1983b) showed that the ability to control stomatal openings is maintained in S. punctata, but not in L. minor. About half of the open stomata of S. punctata close after treatment with CMU, FCCP, valinomycin or nigericin. In L. minor, the above mentioned substances seem to exert no effect. The difference in stomatal behaviour between Spirodela and Lemna might be explained by differences in stomatal guard cells. In Spirodela, stomatal guard cells show roundish-shaped plastids with large starch grains, clusters of plastoglobuli and a lamellar system more reduced than in mesophyll chloroplasts. Lemna guard cells lack plastids or other cytoplasmic organelles and structures. The authors also found potassium in open stomata cells of Spirodela which was not present in closed ones. In Lemna, potassium is not detectable at all in guard cells except in rare instances and to a limited extent. Potassium is abundant in epidermal cells in both investigated species. ABA (abscisic acid) has no closing effect on stomata of L. gibba and L. minor, contrary to the effect of many other phanerogams (TILLBERG et al. 1981). Due to the presence of plastids and starch grains observed in some species of Spirodela, Wolffiella and Wolffia, it is supposed by the present author, these genera are at least partly able to open the stomata actively. Flowering fronds of L. trisulca might behave the same way.

In many Lemnaceae, small papillae consisting of one to several cells, can be seen either on the surface of the frond or along the edge (fig. 2.1b,f). The point of the papilla is formed by a thickening of the outer walls of the outermost cells. The following species contain papillae along the edge (mostly in the distal section): L. trisulca, W. hyalina (fig. 2.1d), W. repanda, and W. denticulata (only at the tip of the frond). Some Wolffia and Wolffiella species sometimes have a few very

small papilla-like cells grown on the upper frond surface or on the edge. More distinct papillae can be observed on the upper surface of fronds of floating species. Most prominent are the papillae on the node and near the tip of the frond in L. disperma, L. ecuadoriensis, L. obscura, L. perpusilla, L. aequinoctialis (fig. 2.1b), W. hyalina and W. repanda. A very prominent papilla is situated in the middle of the upper frond surface of W. brasiliensis (figs. 2.1f, 2.24). Some smaller, but still prominent papillae are found along the median line of the frond surface of S. punctata and L. turionifera.

The formation of papillae is dependent upon different external factors. Under certain conditions, which are not completely understood, species that normally have very distinct papillae (e.g., L. perpusilla, L. aequinoctialis, L. disperma, W. brasiliensis) do not develop any obvious papilla. Very often papillae are not formed distinctly at high temperatures (30°C) in old cultures. SCHARFETTER et al. (1978) observed more prominent papillae in S. punctata when EDDHA or salicylic acid ( $10^{-5}$ M) was added to the nutrient solution. The ethylene-releasing agent ethephon is able to cause very distinct papillae in S. punctata and L. aequinoctialis (KANDELER, BUECHELE, SCHARFETTER, unpubl. results). The unexpected differences in the formation of papillae according to the culture conditions explain why THOMPSON (1898) distinguished between a

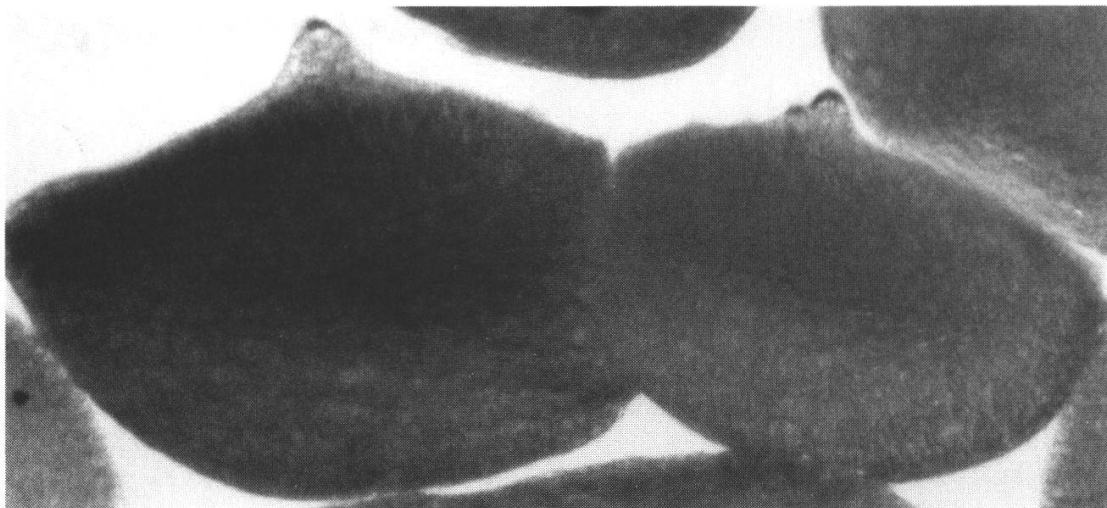


Fig. 2.24. Wolffia brasiliensis with a prominent papilla in the middle of the upper frond surface, consisting of many cells (x40) (photo by W.P. Armstrong, San Marcos, Cal., U.S.A.)

papilla-bearing form of W. brasiliensis as W. papulifera and a form without papilla as W. brasiliensis. In a similar way, L. trinervis without a prominent papilla at the tip was described as a different species from L. perpusilla (or L. aequinoctialis), which has a prominent papilla. It can be shown that these differences were not genetically determined, but were caused by varied external conditions. On the other hand, there are some genetic differences that affect the size of papillae within a species. Plants of L. aequinoctialis with very prominent papillae were distinguished as a separate species (L. angolensis) from those with less prominent papillae (L. paucicostata) by HEGELMAIER (1868). Clones with very prominent papillae are restricted not only to Africa but can be found within all areas where L. aequinoctialis occurs. There is a gradual transition between clones with very prominent papillae and those with less prominent papillae. However, in some species groups the size and location of a papilla may be characteristic for that particular species, especially within the group of L. minor, and can be one of the very few differentiating features between species.

PAN and CHEN (1979) describe a "trichome" in W. globosa (mentioned as W. arrhiza) which is interpreted as rhizoid. According to the published scanning electron micrographs, this "rhizoid" is the place of breaking-off of the stipe (the connection piece to the mother frond).

#### 2.4.5. Air spaces and parenchymatic tissue

The fronds of Lemnaceae contain one to ten layers of parenchymatic tissue between the epidermis of the upper and the lower sides. The tissue cells contain chloroplasts, with the layers of tissues just below the upper epidermis containing many more chloroplasts than the tissues in the lower parts and in the layers between the air spaces (KAUL 1976 with S. polyrrhiza). In Lemna (except L. trisulca) and Spirodela, the upper cells have intercellular spaces between the corners; the lower cells are packed closely together. Otherwise there is not much differentiation within the parenchymatic tissue. In contrast to most other fronds, the intensively green-coloured Wolffia species (W. brasiliensis, W. borealis, W. australiana) have much smaller cells in the parenchymatic tissue with more chloroplasts in the layers directly under the upper epidermis. There is an air space directly below the stoma.

The fronds of Spirodela, Lemna and Wolffiella species contain air spaces within the parenchymatic tissue (called aerenchymatic tissue) either throughout the whole frond or only around the node (figs. 2.1c,d,e, 2.25, 2.26, 2.27). The formation of air spaces begins when the young frond reaches c. 0.2 mm in length (HEGELMAIER 1868). There are fewer air spaces in turions and resting fronds. No aerenchymatic tissue is present in Wolffia.

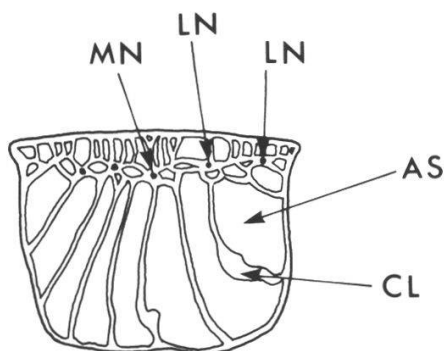


Fig. 2.25. Transverse section of the distal part of Lemna gibba, showing the aerenchymatous tissue (x7.5) (after HEGELMAIER 1868)

AS = air space, CL = layers of cells, surrounding the air space,  
LN = lateral nerve, MN = median nerve



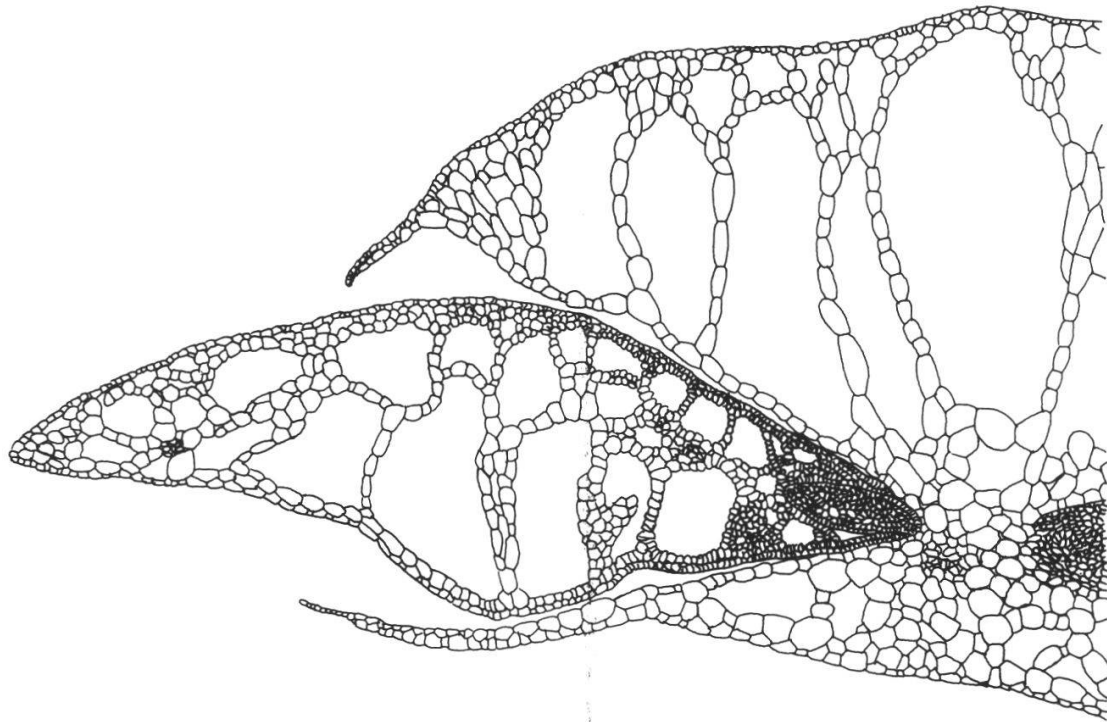
Fig. 2.26. Lemna gibba (No. 7218). Gibbous fronds from below showing air spaces and roots (x3)

The air spaces are ventricles filled with air or with different mixtures of gases and are limited by layers of cells. In gibbous fronds of L. gibba, the gas mixture consists of 78-82% N<sub>2</sub>, 17-21% O<sub>2</sub>, and 0.9-1.1% CO<sub>2</sub>; flat fronds of the same species contain 74-76% N<sub>2</sub>, 21-23% O<sub>2</sub>, and 2.8-3.4% CO<sub>2</sub>. No methane or ethylene was found in the air spaces of L. gibba fronds (BEST et al. 1977). In general, air spaces help to diminish the specific gravity and to give the frond more buoyancy and stability. Gibbous fronds are able to cover neighbouring flat fronds by their margins. They therefore have a competitive advantage.

The size and the number of air spaces within one layer of aerenchymatic tissue, as well as the number of air space layers, may be species-specific; on the other hand, the number may also depend upon external factors. The following species never have more than one layer of air spaces: species of Wolffiella, L. tenera (except flowering fronds), and L. valdiviana. Air spaces of 1 to 3 layers can be found in all other species of Lemna. In S. punctata and S. polyrrhiza, there are usually 2 to 3 layers; and 3 to 4 layers in S. intermedia, although at the edge of the frond, the layers are reduced to one. The following species have air spaces distributed over nearly the whole area of the distal section of the frond: all species of Spirodela, and the groups of L. minor and L. perpusilla, as well as W. gladiata and W. denticulata. Some Wolffiella species contain air spaces only near the node. The extension of the area of the air spaces in L. trisulca, L. tenera, L. minuscula, L. valdiviana and W. oblonga varies, according to the growth conditions, between these extremes. The species of Spirodela and Lemna, except for the submerged vegetative fronds of L. trisulca and L. tenera, additionally have air spaces in the basal section of the frond. The submerged Wolffiella species (sect. Wolffiella) have no air spaces in the basal part of the frond (similar to the submerged Lemna species).

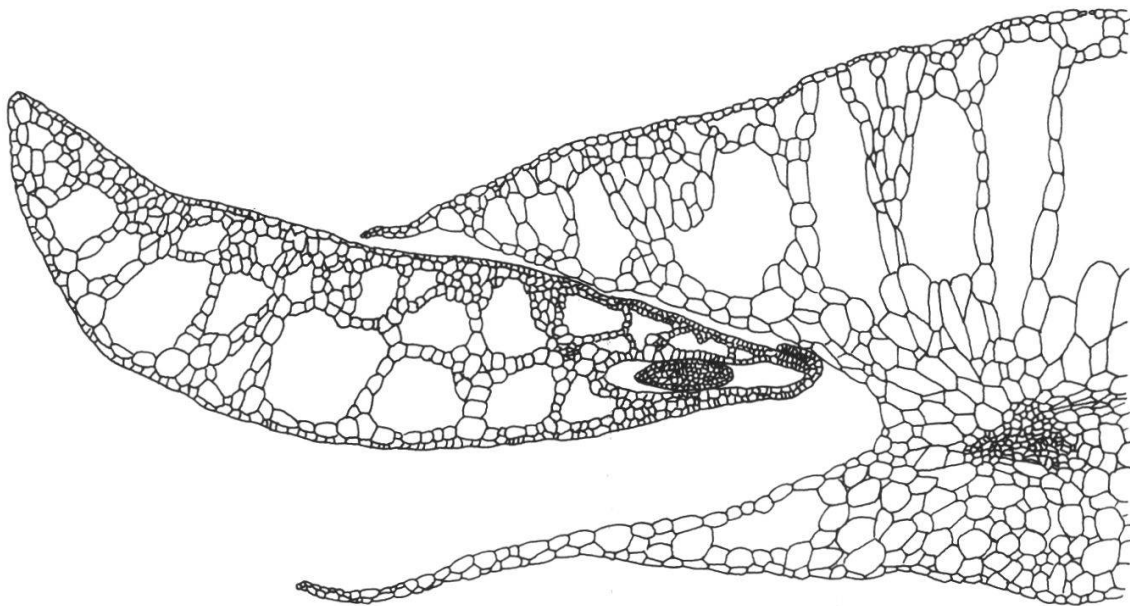
As already mentioned, turions do not have air spaces. Resting fronds of other species develop only very small air spaces (e.g., L. gibba, cf. VAN HOREN 1869). Smaller air spaces also form in L. minor grown in darkness (ROMBACH 1976).

Some species (e.g., S. intermedia, S. punctata, L. gibba, L. disperma, L. obscura and, to a lesser extent, S. polyrrhiza, L. turionifera, L. japonica) have enlarged air spaces and form gibbous fronds under certain growth conditions (cf. chapter 2.4.1.). This enlargement is possible due to the multiplication of cells, which limits the air spaces laterally



a.

0.4 mm



b.

0.1 mm

Fig. 2.27. Transverse sections of *Lemna gibba* fronds (EFRAT et al. 1977)  
a. gibbous frond  
b. flat frond

(EFRAT et al. 1977). The assumption of HEGELMAIER (1868) that the enlargement of air spaces is caused by elongation of the cells is apparently not accurate. Figs. 2.27a and b show cross sections of gibbous and flat L. gibba fronds.

A system of air spaces within the roots, between the cortex parenchyma and the endodermis, has also been reported (figs. 2.35, 2.36, 2.37, cf. VINTEJOUX 1958, FAGERLIND and MASSALSKI 1974). The open space between the rootcap and the root is filled with water.

The Wolffia species have no big air spaces at all. Instead, there are some small intercellular spaces filled with air between the corners of the cells. These spaces are especially apparent in the lower parenchymatic tissue (GODZIEMBA-CZYŻ 1970 in W. arrhiza) (cf. figs. 2.4, 2.5).

#### **2.4.6. Nerves and tracts of elongated cells in the basal section of fronds; vascular and sieve cells**

Nerves are present only in Lemnoideae (Spirodela, Lemna). They originate in the node and run through the distal section. A tract of elongated cells connecting the node through the basal section to the mother frond is found in Lemnoideae, Wolffiella, and, to a lesser extent in Wolffia. A similar tract of elongated cells runs through the center of the roots of Spirodela and Lemna from the tip up to the node. It is supposed that nerves and tracts of elongated cells serve as a transport system for nutrients and sugar.

The nerves and the tracts of elongated cells of Spirodela and most Lemna species contain some tracheids with ring- or spiral-shaped strengthenings in the walls (annular tracheids) (fig. 2.28). No tracheids have been reported as present in the nerves and tracts of L. tenera, L. valdiviana and L. minuscula. In L. perpusilla and L. aequinoctialis, tracheids are restricted to the tract in the basal section and to the lowest part of the central nerve; in L. trisulca, tracheids are visible up to the middle of the central nerve; and in L. gibba, L. minor and Spirodela, the tracheids have been found in all nerves up to the tip (HEGELMAIER 1868, 1895). HEGELMAIER's observations are much more precise than the recent indications of NAVARRO ANDRES et al. (1984).

According to SCHENCK (1886), the nerve of L. trisulca contains an upper row of tracheids and a lower row of sieve cells sustained by a row of

accompanying parenchyma cells on each side. Other rows of accompanying elongated parenchyma cells can often be seen which follow the nerve up to its distal part, where tracheids are absent. From the outer main nerves, which originate in the node, secondary nerves might branch off (e.g., in Spirodela, L. minor, L. japonica and very rarely, in L. aequinoctialis). The secondary branching of L. minor can be stimulated by the addition of TIBA (SARGENT 1957, SARGENT and WANGERMANN 1959). The three main nerves have been ramified into as many as 15 branches. NAA and IAA alone cause only a slight increase of the amount of vascular tissue, but other halogenated benzoic acids showed a similar effect. In S. polyrrhiza, using the same treatment, the authors found an increase of parallel nerves but no branching.

The development of the nerves begins relatively late. In L. gibba for instance, the median nerve becomes visible at a frond length of 0.2 mm, the first pair of lateral nerves at 0.3 mm and the second pair of lateral nerves at 1-1.2 mm (HEGELMAIER 1868). In full-grown fronds of Lemna (contrary to Spirodela) tracheids are very difficult to see.

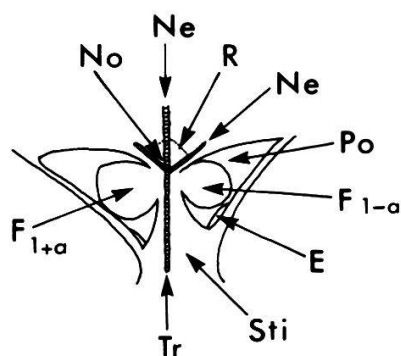


Fig. 2.28. Basal part of the Lemna trisulca frond (x60)  
(after HEGELMAIER 1868)

E	entrance to the pouch	Po	pouch
F <sub>1+a</sub>	first daughter frond of the first generation, plus side	R	root
F <sub>1-a</sub>	first daughter frond of the first generation, minus side	Sti	stipe
Ne	nerve	Tr	tract of tracheids and elongated cells
No	node		connecting the stipe with the node

The number of nerves is species-specific but is also susceptible to the effect of external factors. Up to 16 nerves can be observed within the group of S. polyrrhiza (HEGELMAIER 1868 mentions up to 21 nerves), up to 12 of which are formed at the beginning of development, the rest, which are usually shorter, are formed later on the outside of or between the existing nerves. Three to 7 nerves are recorded for S. punctata and 1 to 5 (very rarely 7 in L. gibba) in Lemna. L. gibba and L. disperma have 3 to 5 nerves originating from the node (fig. 2.29). The other species of the L. minor group as well as L. aequinoctialis, L. perpusilla and L. tenera have 3 nerves beginning at the node. In addition, L. minor and L. japonica sometimes have 1 or 2 secondary nerves that branch off from the lowest part of the outer nerves (fig. 2.29). The number of nerves in L. trisulca might vary between 1 and 3, according to external conditions and genetic predisposition. The clones of L. trisulca of our living collection originating from Australia never formed more than one nerve under experimental conditions; however, some herbarium material from Australia has 3 nerves. L. valdiviana and L. minuscula are characterized by only 1 nerve. In contrast to some reports in floristic literature, I have never observed fronds of these species without nerves. The tract of elongated cells, which runs from the node along the median line of the basal section of the mother frond, contains tracheids in Spirodela and in most Lemna species. Sometimes the tract is asymmetric,

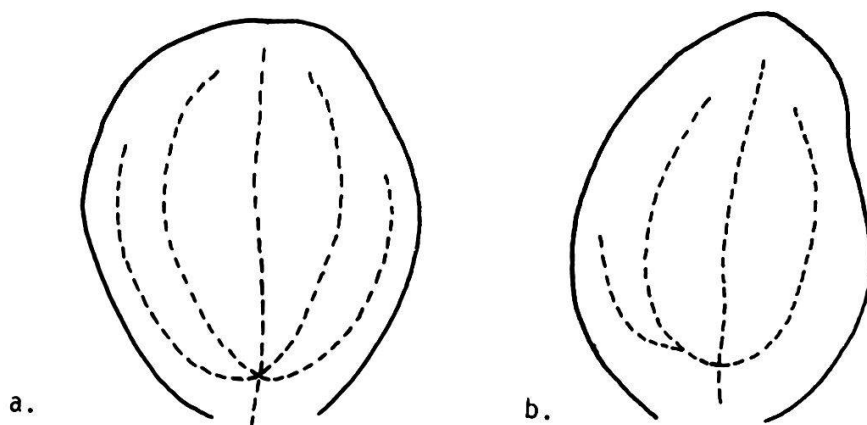


Fig. 2.29. Nerves of Lemna gibba (a) and Lemna minor (b) (x15)  
(after LANDOLT 1975)

especially in L. valdiviana (fig. 2.1c). In Wolffiella (figs. 2.1d,e; 2.3), the tract is distinctly visible, but tracheids are not present. The tract runs near the median line of the lower wall of the pouch in W. hyalina, W. repanda, W. rotunda, W. neotropica, and W. Welwitschii. In W. lingulata it is situated between the median line and the edge of the frond; in W. oblonga (fig. 2.1e), W. gladiata, and W. denticulata it is located near the edge.

In fronds of W. oblonga, W. gladiata, and W. denticulata, due to the lateral position of new fronds, the daughter fronds of the first and second generations do not cover each other and often form stellate groups or clusters. Species with the tract along the median line of the basal part of the frond usually float in two-fronded groups. The tract of asymmetrical species in the daughter fronds of successive generations is nearly always situated at the same side.

Only a very small and indistinct tract of elongated cells is typical for Wolffia. In all species, the tract is situated along the median line of the lower wall of the budding cavity. No tracheids or elongated cells are known from sexual organs of Wolffia.

Tracheids and sieve elements have been investigated more intensively in the roots than in the fronds (cf. chapter 2.4.11). WALSH and MELARAGNO (1974, 1976, 1981) and MELARAGNO and WALSH (1976) studied sieve elements in fronds of L. minor and found that, except for the lack of callose, the lateral walls between sieve elements and contiguous cells are similar in development and mature state to those reported for other species of phanerogams. The open pores are of uniform width, are lined by a plasmalemma, and are almost completely empty. Some plastids have been observed containing protein-like crystalline substances which possibly act as sealing material. Mitochondria persist in mature sieve elements; in many cases an intact tonoplast is also present.

#### 2.4.7. Oxalate-crystal idioblasts

Spirodela and Lemna have certain cells that contain oxalate crystals. In Spirodela, there are two different kinds of crystals: druses and raphides (fig. 2.30). In Lemna, there are only raphide cells, which are distributed throughout the whole body of the plant except in the subepidermal layer and in the cells separating air spaces vertically. The

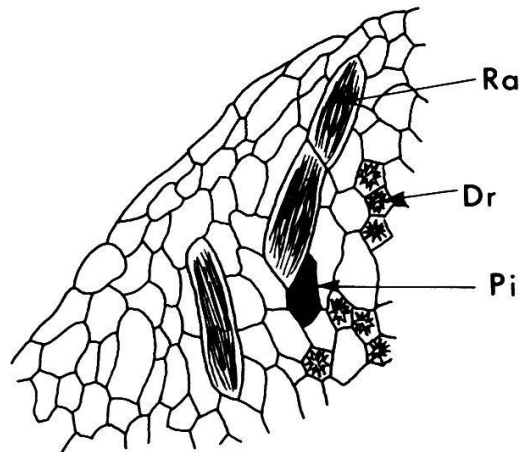


Fig. 2.30. Section of a frond segment of *Spirodela punctata* (x180)  
(after HEGELMAIER 1868)

Dr = druses, Pi = pigment cells, Ra = raphides

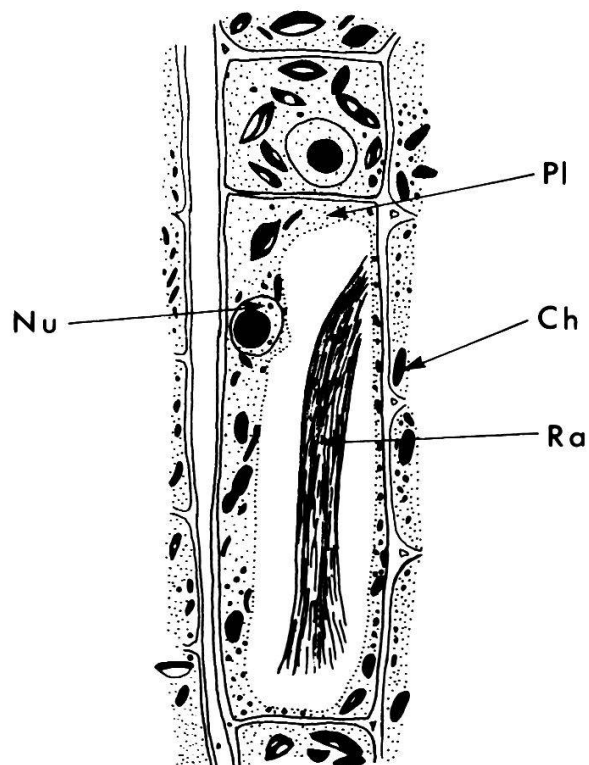


Fig. 2.31. Raphide cell from the root of *Lemna minor* (x1500)  
(after VINTEJOUX 1958)

Ch = chloroplast, Nu = nucleus, Pl = plasma, Ra = raphides

raphide cells are oriented parallel to the margins and nerves of the frond, and are two to three times larger than normal cells. Each cell contains a bundle of needle-shaped crystals. Raphide crystal cells are living and contain some protoplasm for a relatively long time (fig. 2.31). The length of raphides is about 0.03-0.06 mm in L. minor, about 0.04 mm in L. gibba, about 0.13 mm in S. punctata (measured from figures in HEGELMAIER 1868 and VINTEJOUX 1958) and about 0.02-0.05 mm in S. polyrrhiza (VINTEJOUX and SHOAR-GHAFARI 1985). The diameter of the bundle of raphides is 0.006-0.010 mm in S. polyrrhiza. In contrast, druse cells are smaller than normal cells and contain a crystal complex shaped like a morning star (fig. 2.30). They are apparently dead. Whereas the raphides are spread throughout the whole parenchymatic tissue, except the subepidermal layer of the surface, the druses are concentrated mainly in the upper subepidermal layer in the parenchymatic tissue around the node (HEGELMAIER 1868) and around the nerves (VINTEJOUX and SHOAR-GHAFARI 1985). In S. polyrrhiza, VINTEJOUX and SHOAR-GHAFARI (1985) found raphides in the parenchymatic tissue of normal fronds, roots and turions; raphides are especially frequent in the prophyllum (lobe of the lower surface, basal region) where they are oriented parallel to the margin. Within the frond, the orientation is frequently parallel to the radius from the central node to the margins. Many druse cells are located in the perispheric region of the frond; they are often together in groups of 2, 3 or 4. FRANCESCHI (1985) reports that dark-grown fronds of L. minor form four times as many crystal cells as light-grown plants. According to STRASBURGER et al. (1978), the raphides in Lemna are enclosed within a vesicle (raphidosom) formed by a simple membrane and separated from the tonoplast. ARNOTT and PAUTARD (1965, 1970) and CHIU and FALK (1975) investigated the formation of crystals in L. minor and L. aequinoctialis, respectively, and they observed a center of crystallisation within the cell and fibrils connecting the center with the cell walls. It is supposed that the fibrils are able to transport material for crystal formation. AL-RAIS et al. (1971) report that the raphides of L. minor consist of the monohydrate salt of calcium oxalate. The druses (not investigated in Lemnaceae) contain a calcium oxalate salt with 2.25 part H<sub>2</sub>O. Up to 2% magnesium was measured within crystals. WROBLESKI (1976) investigated raphide and tannin idioblasts in L. minor by means of TEM, STEM and X-ray microanalysis on semi-thin sections. He could distinguish two different kinds of raphide idioblasts: cells with large

crystals showing an hourglass profile and cells with small crystals showing hexangular and rectangular profiles. Differences in the elemental composition of the idioblasts may indicate that cells have different metabolic pathways (for details see volume 2, LANDOLT and KANDELER 1987). Since tannin idioblasts and two different kinds of crystal cells are only known in Spirodela, the possibility exists that the author worked with S. punctata instead of L. minor. Then the small crystals would be identical with druses.

The raphide idioblasts of S. polyrrhiza lack the so-called modified plastids. The starch is present in the proplastids, but does not get accumulated in the plastids of raphide cells (RAO 1969).

It is not known if the crystals of calcium oxalate have a biological function apart from removing surplus oxalate out of the protoplasm. These crystals may also serve as protection against damage by snails (STAHL 1883) or other small animals. Contrary to the raphides of Ara- ceae, raphides of S. punctata (LEDBETTER and PORTER 1970) and L. minor (ARNOTT and PAUTARD 1970) do not cause irritation to people. Raphides of Pistia have barbs whereas raphides of Lemnaceae only have grooves (SAKAI et al. 1984).

#### **2.4.8. Pouches (pockets) and cavities protecting daughter fronds**

The daughter fronds originating from meristematic tissue near the node are protected by special pouches, or cavities, during the first stages of life (cf. fig. 2.1). There are two lateral pouches in the basal section of the frond in Spirodela and Lemna (fig. 2.32), which are separated by a tract of elongated cells. In Wolffiella, one single flat basal pouch is present. In Wolffia, there is a similar, but conical-shaped cavity. The upper walls of the pouches are formed by tissue extending towards the back from the distal section of the frond. In Wolffiella, Lemna, and S. punctata, the lower wall corresponds to the basal section of the frond. This lower wall has been the subject of many different interpretations. According to HEGELMAIER (1868) it is the basal part of the shoot. BROOKS (1940) thinks that it is a bract; LAWALREE (1945) considers it an extension of the distal section, similar to the upper wall and homologous to a cotyledonal sheath (cf. chapter 2.2.). In S. intermedia and S. polyrrhiza, the lower wall of the pouch where the first

daughter frond is formed originates in a different way: one lobe of the prophyllum becomes the lower wall (fig. 2.2, Pd\*). The walls of the second pouch (on the minus side) are formed in the same way as in Lemna. The cavity of Wolffia, which is at first ovoid, and later conical in shape, is formed by a ring-shaped extension of meristematic tissue growing towards the back. As in the other genera, the first daughter frond originates at the base of the upper side of the distal section of the mother frond (HEGELMAIER 1868).

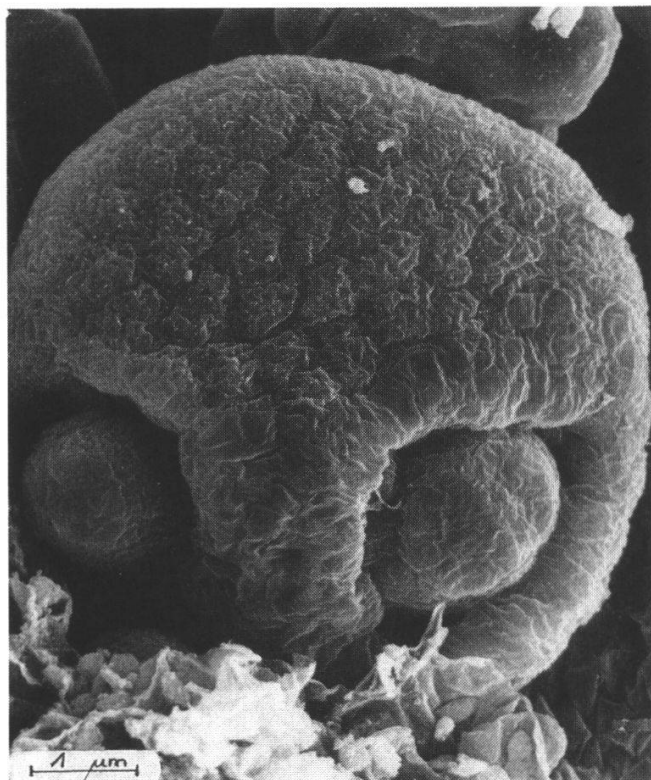


Fig. 2.32. A developing young frond of Lemna aequinoctialis with two daughter fronds, each in a frond pouch (x1000) (from SHIH 1979)

#### 2.4.9. Connection between mother and daughter frond (stipe)

The connection piece between the mother and daughter plant, called a "stipe", originates at the base of the lower pouch wall of the daughter frond by successive divisions of the cells; the dividing cells form new cells which extend towards the mother fronds, and this results in the stipe. An exception is the stalk of L. trisulca, in which the dividing cells form new cells in the direction of the body of the daughter frond. This stalk is not homologous to the stipes of the other Lemnaceae, but rather is a continuation of the basal section of the daughter frond; it is not clearly differentiated from the rest of the frond and is green (with chloroplasts), in contrast to the stipe found in other Lemnaceae. Therefore it is called a stalk. In W. hyalina and W. repanda, the cells at the base of the lower pouch wall form a very short stipe towards the mother frond similar to the stipe formation in other Lemnaceae; but, the cells also divide in the direction of the daughter frond, giving rise to a ribbon-like appendage which attaches to the daughter frond and continues to grow even after separation from the mother frond. This appendage improves the stability of the frond in the water.

The size of stipes or appendages is species-specific. In the group of S. polyrrhiza, the stipe grows up to 5 mm; in the other species (except L. trisulca), it is usually much shorter than 2 mm. The stalk of L. trisulca may be as much as 20 mm long, while the appendages of W. hyalina and W. repanda reach 5 mm and 8 mm, respectively. The lengths of the stalk of L. trisulca and the appendages of W. hyalina and W. repanda definitely depend on culture conditions. The stalk of L. trisulca elongates with far-red irradiation in comparison with white, blue, green or yellow light of the same intensity (ZURZYCKI 1957b). High blue light intensity reduces the length of the stalk of L. trisulca to less than 2 mm. Under red and white light the stalk measured 4-6 mm (BATA and NESKOVIC 1982b). The appendage of W. hyalina becomes longer in diluted nutrient solution (LANDOLT unpubl.). The addition of gibberellin elongates the stipe (stalk) of L. trisulca and S. polyrrhiza (GUERN 1965). Gibberellin, kinetin and indolyl acetic acid (IAA) elongate the stalk of L. trisulca from 9 mm (control) to 12, 16 and 13 mm, respectively (BATA and NESKOVIC 1982a).

The stipe tears near the mother frond when the mother and daughter frond of Spirodela and Lemna separate. In Wolffiella and Wolffia, the tearing

point is near the daughter frond. My own observations have not confirmed the statement of HEGELMAIER (1868) that the stipe of W. lingulata, W. oblonga, W. gladiata and W. denticulata in contrast to the stipe of Wolffia species remains with the daughter frond. After separation the stipe stays within all Wolffioideae species in the pouch or cavity of the mother frond (fig. 2.4). According to HEGELMAIER (1868) and WITZTUM (1966, 1974b), there are two tearing points in Spirodela. The one near the mother frond enables its separation from the daughter frond but leaves the axillary frond with the mother frond; the other serves to separate the stipe from the daughter frond later. In Lemna, there is only one tearing point, which is near the mother frond, according to HEGELMAIER (1868) and WITZTUM (1966). The short stipe connected with the daughter frond withers and becomes invisible. It is possible to promote the separation of the daughter frond by UV-radiation (WITZTUM 1966) or by adding ABA or sucrose to the nutrient solution (WITZTUM and KEREN 1978a,b, OSTROW-SCHWEBEL 1979). The addition of gibberellin (LOOS 1962) and ethylene (NEGBY et al. 1972) also stimulates separation (cf. chapter 2.3; for further details see volume 2, chapter 2.4.1.3, LANDOLT and KANDELER 1987). CHIU and FALK (1975) investigated the tearing point of the stipe of L. aequinoctialis using electronmicroscopy. The abscission zone consists of 4 to 5 rows of rectangular cells. The authors observed myelin strands and long segments of endoplasmic reticulum. Microtubules have been found regularly in cells of this tissue. The loss of orientation of microtubules in abscission cells is supposed to be related to the onset of abscission. According to NEWTON et al. (1978), the tearing point of turions of S. polyrrhiza is sealed by subepidermal idioblasts.

#### **2.4.10. Prophyllum in Spirodela**

In Spirodela, there is a bipartite scale at the base of the frond (fig. 2.2), which HEGELMAIER (1868) interpreted as a prophyllum and a leaf connected together. He described the ontogenesis of this organ in detail and then later changed his opinion, considering both parts as belonging to the same leaf (HEGELMAIER 1871). Most subsequent authors (e.g., BROOKS 1940, LAWALREE 1945, DAUBS 1965) agreed with the opinion that the scale is one single leaf-like organ and does not consist of two connected organs. The most simple assumption is that it is a prophyllum en-

closing the basal section of the frond, since it is attached to the shoot axis below the node. It does not have the right position to be a bract supporting the first daughter frond. In a similar way, the flowers of Spirodela and of Lemna are enclosed in a membranous prophyllum (cf. chapter 2.6.2.). However, many authors (e.g., MEUSEL 1951, BRUNAUD 1974a, KANDELER 1979) regard the scale of Spirodela as a bract.

The prophyllum of S. polyrrhiza and S. intermedia is attached to the base of the frond, laterally on the upper part; from there the prophyllum covers the whole base of the upper part and overlaps to the lower part, where it forms the lower wall of the pouch of the plus side. On the other side, a scale-like lobe reaches to the lower part, covering the point of attachment of the roots. The two lobes are connected at the base on both sides (figs. 2.2a,b). The prophyllum of S. punctata is much smaller than that of S. polyrrhiza. The one lobe covering the base of the upper surface of the frond (which perishes in full-grown fronds) does not overlap to the lower part. Therefore, the lower wall of the pouch on the plus side is formed by an extension of tissue from the distal part, in a similar way to the formation of the pouch on the minus side and of both pouches of Lemna. The second lobe covering the point of attachment of the roots is very similar to that of S. polyrrhiza. The prophyllum does not develop until after the first cell divisions of the new frond have taken place (VINTEJOUX 1969).

The lobe covering the point of attachment of the roots of Spirodela is perforated by roots, the number of which is species-specific. In S. punctata, all roots perforate the lobe (1 to 12); in the S. polyrrhiza group, only some of the roots: 2 to 5 in S. intermedia, and 1, rarely 2, in S. polyrrhiza. In contrast to many reports in the literature, I sometimes observed two perforating roots in clones of S. polyrrhiza from all parts of the world. According to HARRISON (1964) and BEAL (in lit.), all 6 investigated clones of S. polyrrhiza were able to produce 2 perforating roots under certain conditions. However, SPOONER (1967) observed only 2 out of 1296 fronds with 2 perforating roots.

The prophyllum consists of one (along the margins) or two layers of undulated epidermal cells. Towards the base the cells get smaller, have straight walls and enclose one to three layers of parenchymatic tissue. Pigment cells are very frequent within the prophyllum (HEGELMAIER 1868).

The prophyllum cells have fewer chloroplasts per cell than mesophyll

cells. "A wavy envelope and bizarre shape of the chloroplasts along with elongate animal-type mitochondria are considered as distinguishing characteristics of prophyllum cells" (RAO 1969).

#### 2.4.11. Roots

##### 2.4.11.1. Presence of roots

Within the family of Lemnaceae the presence of roots is restricted to Spirodela and Lemna. In Wolffiella and Wolffia, there is no indication of roots at all. Sometimes roots of Spirodela or Lemna may drop off or remain rudimentary (in turions); therefore, these fronds seem to be rootless. The only species of Lemnoideae that does not always form roots is L. trisulca. Under certain conditions and in some regions (e.g. Australia), rootless fronds are frequently found. In the family of Lemnaceae, there are no ramifications of the roots and no root hairs. The roots of Lemnaceae are believed to be adventitious. They originate at the node on the lower part of the frond from the cell layers below the epidermis (HEGELMAIER 1868, VINTEJOUX 1958). They are first visible when the frond reaches 0.035-0.050 mm in length and the first daughter frond appears. The prophyllum in Spirodela becomes perforated in S. polyrrhiza by the first root, in S. intermedia by the first 2 to 5 roots, and in S. punctata by all the roots.

##### 2.4.11.2. Size of the roots

The number of roots in Spirodela is partly dependent on external factors; and partly it is a genetic characteristic. There are up to 21 roots in S. intermedia and S. polyrrhiza. In S. punctata, the addition of EDDHA and salicylic acid to the nutrient solution reduces the usual number of roots of 2-4 to 1 (SCHARFETTER et al. 1978). Under certain unknown conditions, up to 12 roots were observed (VAN DER PLAS 1971). Lemna species have only one root. FURST (1968) observed 2 roots in L. trisulca. However, his figures show so many unclear presentations that an inaccurate observation is possible. Nevertheless, the formation of two roots may occur in Lemna, although this is very rare. I myself observed in L. aequinoctialis (No. 7338) two roots per frond in one out of many

thousands of samples. WITZTUM (1966) reports of a deeply cleft frond of L. aequinoctialis (No. 6746) bearing two roots.

The diameter of the root of L. minor was measured as 0.15-0.2 mm (VINTEJOUX 1958, ECHLIN et al. 1979a, 1980b). Most species of Spirodela and Lemna have roots not longer than 2.5-4 cm. The roots of S. punctata grow up to 7 cm in length; those of the species of the L. minor group reach up to 15 cm. The roots are still able to grow when the frond has already reached final development. The root of L. minor can grow within 40 to 100 hours from 1 to 20 mm (PIRSON and GOELLNER 1953). Growth is more or less linear during this time. The root shows a marked zonation into a meristematic region, a region of extension growth, and a region of mature cells. The cells of the last two regions differ in their plasmolysis form (PIRSON and SEIDEL 1950, SCHAEFER 1956).

The lengths of the roots also depend upon external factors. The roots of L. minor grow longer with higher light intensities (WHITE 1937b, 1938), with higher potassium content (WHITE 1936a, 1938), and with low concentrations of nitrogen (WHITE 1936b, 1937b, WHITE and TEMPELMANN 1937, PIRSON and GOELLNER 1953). The authors observed that the longest roots occurred with high light intensities and low nitrogen content. They explain it with the diffusion gradient determining the supply of carbohydrates to the developing root meristem; carbohydrates and nitrogenous compounds are required in balanced concentrations corresponding to those of utilization in development. Any excess of carbohydrate above that required for development of the frond meristem raises the concentration in the frond and in this way, steepens the diffusion gradient to the developing root meristem. If the nitrogen supply is low the frond meristem grows slower; therefore, more carbohydrates are reaching the root meristem (WHITE 1937b, THORNLEY 1977). The situation seems to be more complex. HENSSEN (1954) observed shorter roots of S. polyrrhiza in solution with sucrose than in solution with fructose. According to PIRSON and GOELLNER (1953) deficiency of nitrogen (and phosphorus) results in a greater elongation of the cells rather than in an increase of the number of cells. LUEOEND (1980) reports an elongation in roots of different species caused by lowering the concentrations of nitrogen from 10 to 1 mg  $\text{NH}_4\text{NO}_3$  per one liter solution. She obtained similar results by lowering the phosphorus content. However, in very low concentrations of nitrogen or phosphorus, the roots became shorter again. In L. gibba, the longest roots develop in solution with a nitrogen content between 0.008

and 0.2 M depending on the clone investigated (DANN 1982). The root/frond ratio increases with decreasing nitrogen supply in L. gibba. The roots comprise about 10% of the frond dry weight in nitrogen-sufficient medium compared with 25% in nitrogen-deficient medium (INGEMARSSON et al. 1984b). The influence of calcium and magnesium on the root length is even more complex (ZIMMERMANN 1981, cf. LANDOLT and KANDELER 1987). According to HENSSEN (1954), the kind of sugars added is also important for root length. With glucose, the roots of S. polyrrhiza grow up to 40 mm long; with maltose and fructose, only 2-4 mm long; with sucrose, a value somewhat between was found. The roots of S. punctata grow longer with the addition of EDDHA or salicylic acid (SCHARFETTER et al. 1978). On the other hand, the addition of gibberellin retards root growth in L. minor (LOOS 1962). CHAMURIS and NIELSON (1983) showed that root growth of L. gibba and L. minor is inhibited by far-red treatment and must therefore be under phytochrome control (see also volume 2, chapter 2.4.1.2, LANDOLT and KANDELER 1987).

#### 2.4.11.3. Elements of the root

At the beginning of root growth, the epidermis below the root tip enlarges by cell division (from a cell ring below the tip of the convexity) and by cell elongation. After being perforated by the root tip, the epidermis forms a one-layered, membranous tube, called root sheath, which is 0.15 mm (Spirodela) to 1 mm (L. minor) long, but varies in length within a species. In L. aequinoctialis and L. perpusilla the root sheath is furnished at the base on each side with a one-layered, wing-shaped appendage consisting of 3-8 cell rows at the base (fig. 2.33).

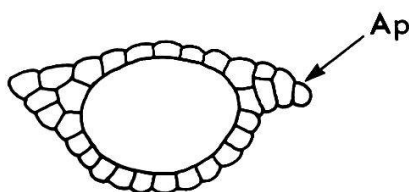


Fig. 2.33. Transverse section of a young root sheath of Lemna aequinoctialis (x180) (after HEGELMAIER 1868)  
Ap = appendix

The epidermis of the root is not supplied with a cuticle. The root tip is enclosed by a rootcap (fig. 2.34) consisting of three layers of cells at the base, which tapers towards the tip to one layer. The tip of the rootcap is 4-7 cells thick. The rootcap originates from the subepidermal layer of the frond; it does not correspond to the root sheath, which is part of the epidermis. It is separated from the inner part of the root by an intercellular space filled with water (FAGERLIND and MASSALSKI 1974). The rootcap in Lemnaceae is never renewed, in contrast to the rootcaps of terrestrial plants where the meristematic tissue forms permanently new cells to replace damaged ones. The rootcap is 0.5-1.8 mm long and exceeds the meristematic zone of the root tip. Even within a species, the length may vary strongly. Longest rootcaps are known from L. gibba (up to 1.8 mm), but because of the great variation, the size of rootcap is of no taxonomical value. The rootcaps of living plants are very acute or furnished with a prominent point in S. intermedia, S. biperforata, L. trisulca, L. tenera, L. perpusilla, and L. aequinoctialis. The rootcaps might be acute or obtuse in S. polyrrhiza, S. punctata, L. turionifera, L. disperma, L. valdiviana, and L. minuscula. In all the other species the rootcaps are usually obtuse, although exceptions are possible in nearly all species. Dried fronds often have much more acute root tips (e.g. S. polyrrhiza, L. gibba). Contrary to the opinion of HEGELMAIER (1868), an acute root tip is not at all typical for L. gibba and therefore is of no value for distinguishing that species from L. minor.



Fig. 2.34. Rootcap of Lemna gibba (x20)

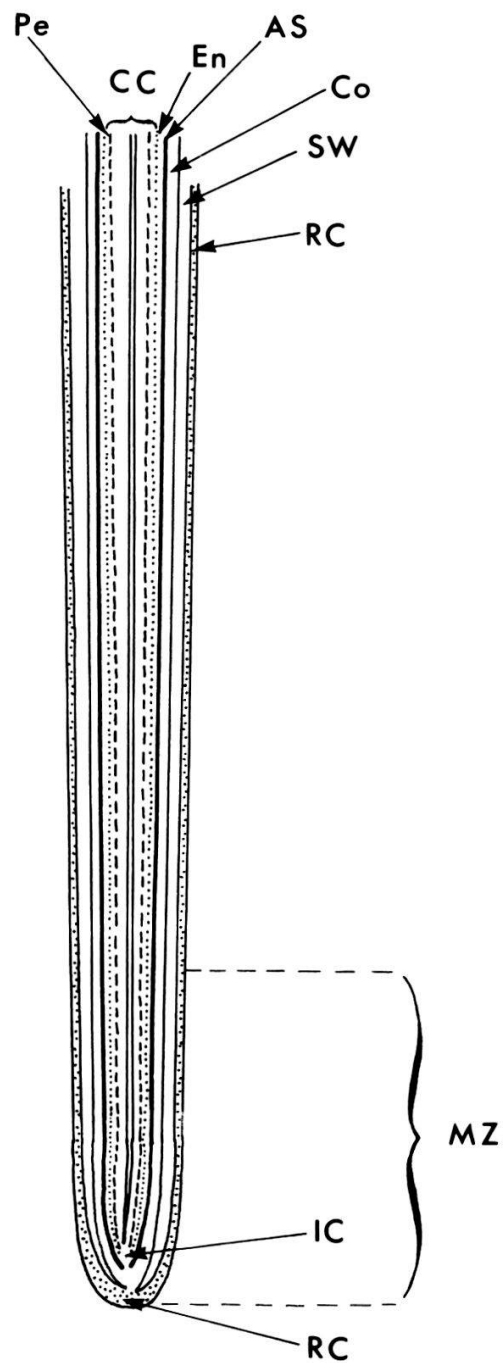
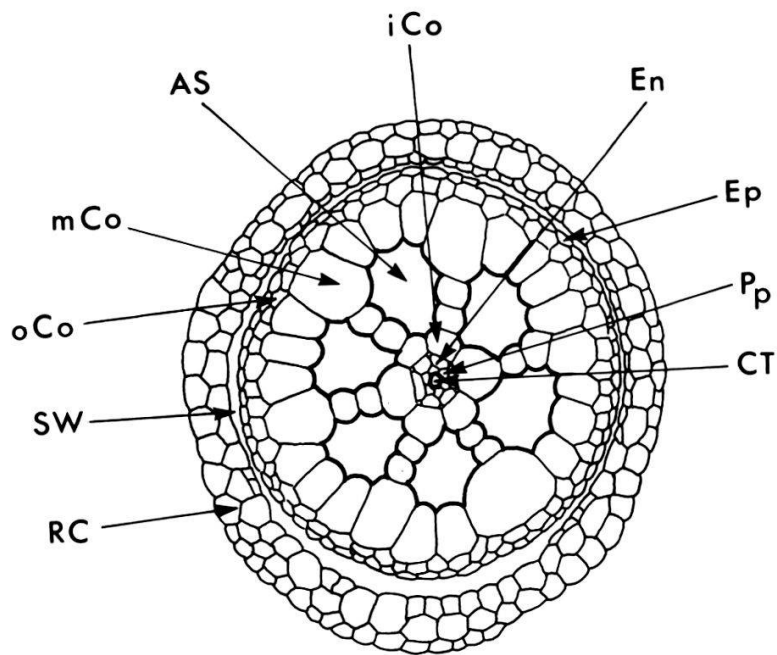


Fig. 2.35. Longitudinal section of the root tip of *Lemna minor* (x75)  
(after VINTEJOUX 1958)

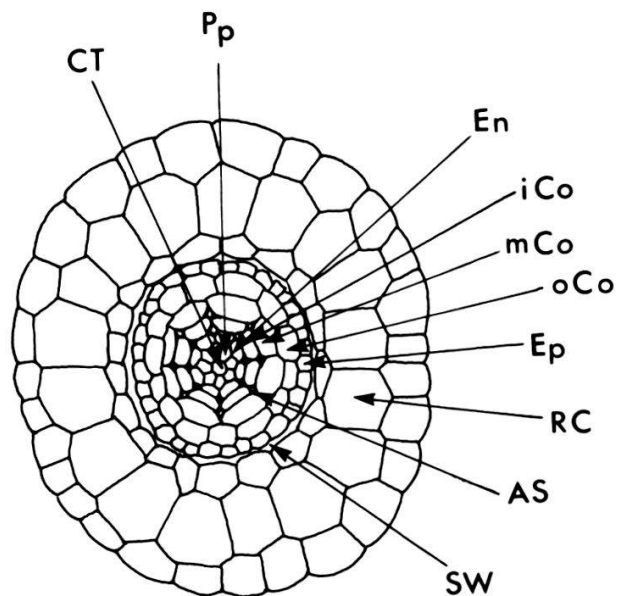
AS	air space	IC	initial cells
CC	central cylinder with phloem cells and axial cell tract working as xylem	MZ	meristematic zones
Co	cortex layer with epidermis	Pe	pericycle
En	endodermis	RC	rootcap
		SW	space system filled with water

#### 2.4.11.4. Structure of the roots

Detailed descriptions of the roots of Spirodela and Lemna are given by HEGELMAIER (1868) and VINTEJOUX (1958, only for L. minor). Inside the rootcap there is the epidermis and a two-layered cortex (in L. trisulca and L. aequinoctialis) or a three-layered cortex (L. minor and L. gibba), followed by a ring of two-layered cells which are interpreted by HEGELMAIER (1868) as additional cortex layers, by VINTEJOUX (1958) as endodermis and pericycle (fig. 2.35), and by NEWTON (1972a) and ECHLIN et al. (1982a) as inner cortex and endodermis. ECHLIN et al. (1982a) observed no Casparian strip in this endodermis, at the beginning of its development. Between the angles of the cells of the three different cell complexes, air spaces are visible. In S. polyrrhiza, one can observe an aerenchymatic system of two cell layers deep between the outer cortex and the inner two-layered ring (fig. 2.36a). Innermost, there is the central cylinder. The central cylinder of S. polyrrhiza contains a tract of tracheids with ring- or spiral-shaped reinforcements in the centre, surrounded by 5-7 thin-walled elongated cells (HEGELMAIER 1868). These cells are homologous to phloem. The detection of tracheids in full-grown roots of S. punctata and Lemna is much more difficult (figs. 2.36, 2.37). HEGELMAIER (1868) recognized tracheids at the base of young S. punctata roots which disappear in older roots. ECHLIN et al. (1980c, 1982a) observed a tracheal element in the centre of the root of L. minor, but they were not sure whether it was functioning. This element was surrounded by 8-10 phloem cells, consisting of 4-6 cells of phloem parenchyma, 2 sieve elements and 2 companion cells. HARRISON (1964) and BEAL (in lit.) reported that root vascularization is partly dependent on nutrient conditions but not on light intensity and photoperiod. One clone (out of 3) of S. punctata showed tracheids in the roots in 1/10 Hoagland solution but not in N-deficient solution. S. intermedia (1 clone studied) had no vascularization in 1/10 Hoagland solution but did have in N-deficient solution. From these results it seems that the development of root vascularization is possible in Spirodela and in the group of L. minor but is dependent on growth conditions. The structure of the sieve elements is similar to that of other monocotyledons (MELARAGNO and WALSH 1976, ECHLIN et al. 1979a, 1980b, 1982a). The development of the phloem is described by WALSH and MELARAGNO (1976). FAGERLIND and MASSALSKI (1974) also observed the development of xylem cells and



a.



b.

Fig. 2.36. Transverse section of the root tip of Spirodela polyrrhiza (a) and Lemna minor (b) (x230) (after HEGELMAIER 1868).  
Explanations according to ECHLIN et al. (1982a)

AS	air spaces	Ep	epidermis
Co	cortex layer (i = inner, m = middle, o = outer layer)	Pp	phloem parenchyma
Com	companion cell	RC	rootcap
CT	axial cell tract (xylem)	Si	sieve element
En	endodermis	SW	space system filled with water

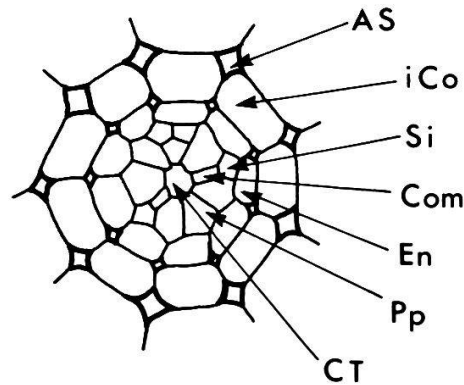


Fig. 2.37. Transverse section of the root tip of Lemna minor, without rootcap and cortex (x460) (after HEGELMAIER 1868). Explanations as in fig. 2.36.

surrounding phloem tissue of the root of L. minor. The sieve cells are eventually converted to vacuoles, whereas the accompanying parenchyma cells maintain their living structure (ECHLIN et al. 1979a). However, WALSH and MELARAGNO (1981) described plastids in L. minor which are able to plug the sieve pores with a protein-like substance.

ECHLIN et al. (1980b,c,d) analyzed the Na, K, Ca, P, S and Cl content of apical meristem, endodermis, xylem, and undifferentiated and differentiated phloem parenchyma in L. minor. The highest values for P and K were found in the apical meristem and the endodermis, which apparently still has meristematic properties. The lowest values for P were in the xylem. Otherwise, the lowest values for all other elements were found in the differentiated phloem parenchyma. In the root tip, the highest value for K was found in the inner cortex and the lowest in the rootcap (ECHLIN et al. 1981). The distribution of Na, Mg, P, S, K, and Ca within the root tip of L. minor was analyzed by ECHLIN et al. (1982a).

#### 2.4.11.5. Function of the roots

The roots of Spirodela and Lemna contain chloroplasts and starch grains, especially in the outer cell layers, in the inner cortex and also in the rootcap (HEGELMAIER 1868, VINTEJOUX 1958, NEWTON 1972b). Therefore, light-exposed roots are green and able to assimilate (PIRSON and GOELLNER 1953). Crystal cells are present in the root and especially in the inner layers of the rootcap. VINTEJOUX (1958) has observed tannins in

the rootcap of L. minor. Anthocyanins have been found in the roots of S. polyrrhiza and S. punctata, which are often red coloured.

The roots of Lemnaceae are not essential for the absorption of nutrients or water. This was demonstrated earlier by GASPARRINI (1856), HEGELMAIER (1868) and SNELL (1907). Covering the lower surface of the frond of L. minor with lanoline resulted in a slower growth rate and elongation of the roots (GORHAM 1941). The application of lanoline on the upper surface had no effect at all. If a frond was lifted above the water surface, with the root tip still in the water, the frond dried up. Therefore, it is obvious that the Lemna frond gets water and nutrients mostly through the lower surface of the frond and not through the root. The upper surface of the frond is also able to absorb water and nutrients, if it is in contact with water (HILLMAN 1961). MUHONEN et al. (1983) also observed that roots of S. polyrrhiza play no important role in nutrient uptake. However, FERNANDEZ et al. (1972) report that the roots of S. intermedia absorb 2,4-D more rapidly than the lower frond surface. There is a possibility that the roots of Spirodela are more efficient in absorbing and transporting water and nutrients than the roots of Lemna, but it seems more probable that the faster absorption is due to a higher pH of the vascular sap in roots which then is acting as a more efficient trap for weak acids (KANDELER, pers.comm.). LEHMAN et al. (1981) report of the catching of minerals (e.g. nitrate) by the roots of Lemna species. Probably the function of the roots of Lemnaceae as stabilization organs is more important (see chapter 2.4.12).

#### **2.4.12. Organs of stabilization**

One of the big dangers for Lemnaceae is the possibility of drifting to land by wind or water movements and drying up. The fronds might also become tangled together in the water and therefore be blocked from light. If a frond is turned upside down, it has difficulties getting CO<sub>2</sub> for assimilation. Therefore, it is not surprising that there are devices to stabilize the position of the fronds. An important function of the roots of Spirodela and Lemna is this stabilizing effect. The roots prevent the frond from turning, and they enable the fronds to stick together and to other waterplants. Long-rooted species such as L. minor are able to interlace with each other to form a big mat and in this way cover wet rocks (fig. 4.1).

Some species of Wolffiella and Wolffia have developed root-like protuberances that produce the same stabilizing effect. The lower walls of the pouches of W. hyalina and W. repanda grow ribbon-shaped appendages which are directed downwards at a right angle (fig. 2.38b). The appendage is 0.5-5 mm long in W. hyalina and 3-8 mm long in W. repanda. It consists of two layers of epidermis cells which enclose a tract of elongated cells along the median line. W. microscopica, the only Wolffia which is more or less flat on the lower side, has a conical appendage in the center of the lower side that is 0.4 to 3 mm long (fig. 2.38c). In young fronds the projection (appendage) is directed somewhat towards the base of the frond. Later it stretches vertically downwards. The stretching of the projection is acropetalous (similar to the root) (HEGELMAIER 1885). Another means of establishing a stable position on the water surface is a nutshell shape, or boat-like shape on the lower side of the frond (e.g., S. intermedia, S. punctata, L. gibba, L. obscura, L. disperma, most Wolffia species). Some Wolffia species (W. australiana, W. angusta) have a keel-shaped lower side (fig. 2.38d). The prominent papillae of L. aequinoctialis (fig. 2.38a), L. perpusilla, L. obscura, W. hyalina and W. brasiliensis might also serve to prevent the turning of the frond. The stability of lemnaeous plants is very often due to the formation of groups of connecting fronds. The rosette-like grouping of S. polyrrhiza

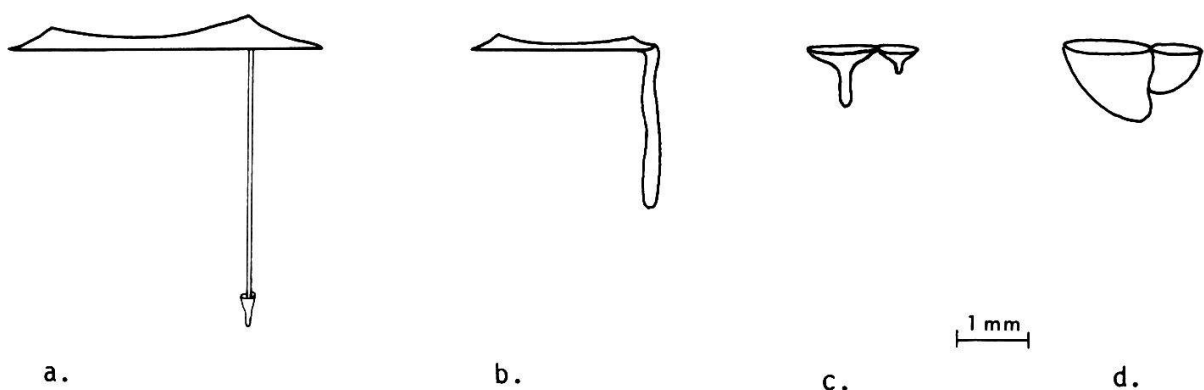


Fig. 2.38. Organs of stabilization (x7). a. Frond of Lemna aequinoctialis with root. b. Frond of Wolffiella hyalina with ribbon-shaped appendage. c. Two fronds of Wolffia microscopica with root-like protuberances. d. Two fronds of Wolffia australiana with keel-shaped lower side.

is much more difficult to turn upside down than a single frond. The submerged living L. trisulca is able to form large, net-shaped, rather stable complexes of fronds which can attach to other water plants. Most of the species of Wolffiella grow with the frond tip or distal section immersed in the water, thus forming small arched or sickle-shaped groups or bigger star-shaped complexes. In the center of the group, there are air spaces concentrated around the node, which give the group more stability. W. elongata behaves in a similar way. The distal section of the cylindrical fronds dips diagonally into the water and nearly forms a right angle to the daughter frond.

Finally, the hydrophobic cuticula of the upper surface of Spirodela, Lemna, and some Wolffia species make it difficult to turn the fronds upside down.

## 2.5. CHARACTERISTICS OF RESTING FRONDS AND TURIONS

### 2.5.1. Different kinds of resting fronds

Many species of Lemnaceae are able to form special "resting fronds", which help them to survive unfavourable conditions. These fronds are smaller than normal fronds, and contain more starch, more anthocyanins (if anthocyanins are formed at all), and fewer air spaces. Three different kinds of resting fronds can be distinguished as follows:

**a. Resting fronds that do not sink to the bottom of the water.** These fronds can be found in S. punctata, L. perpusilla, L. aequinoctialis (most strains), L. gibba, L. minor, and L. japonica (some strains). The roots are small, but visible. These fronds produce new resting fronds or, under very unfavourable conditions, stop reproducing. Since they contain more starch than normal fronds, they are better suited to survive under unfavourable conditions for a long time. They have a higher specific gravity; therefore, the possibility exists that in freezing temperatures they are pressed down under the ice cover, where the temperature is not as extreme (LUDWIG 1909).

The resting fronds of L. minor have been described by HEGELMAIER (1868) and those of L. gibba have been investigated by VAN HOREN (1869), HEGELMAIER (1895), and JUNGNICKE (1978). KANDELER and HUEGEL (1974a) have reported on the resting fronds of L. perpusilla and L. aequinoctialis and more information about the resting fronds of L. perpusilla is given by HUEGEL et al. (1979). Resting fronds of S. punctata are described in a publication of HARRISON (1964). They are only formed under long-day photoperiod.

In some cases, resting fronds remain as buds in the pouch of the mother frond. They sink to the bottom of the water after the mother frond has died and the air spaces of this mother frond have filled with water.

**b. Resting fronds that sink to the bottom and are able to grow further.** Fronds of this type can be observed in L. trisulca and W. gladiata and in species of the W. arrhiza group. These fronds are similar in form to normal fronds; although, in L. trisulca and W. gladiata, they are somewhat shorter and relatively wider. These resting fronds can grow on the

bottom of the water, although rather slowly, forming new, similar fronds. The stomata remain nearly closed. In W. gladiata PIETERSE et al. (1971) observed reduced air spaces, more starch, and partly different gibberellins.

GODZIEMBA-CZYŻ (1969, 1970) described the resting fronds of W. arrhiza which are different from the true turions; PIETERSE et al. (1970d, 1971) and PIETERSE (1972) have reported on the resting fronds of W. gladiata. Submerged growing fronds have often been observed in cultures of W. columbiana. Unlike normal fronds, the resting fronds of W. gladiata are able to survive at temperatures of 5°C for at least five weeks (PIETERSE et al. 1970d, 1971). PIETERSE (1972) was not able to find resting fronds in nature. However, as W. gladiata grows in regions with rather low winter temperatures (far below 0°C), one can assume that, to survive, it sinks in the form of resting fronds down to the warmer water below. The resting fronds of W. arrhiza have about the same sensitivity to low temperatures as normal fronds (GODZIEMBA-CZYŻ 1970). They tolerate temperatures of 1°C for about 10-16 days and temperatures of -2°C for 4-10 days. L. trisulca is not very sensitive to temperatures below 0°C. However, nothing is known about its tolerance level. DALE and GILLESPIE (1976) report that the resting fronds of L. trisulca sink down to 3 m depth during winter time in the continental part of Canada. There, it is possible to survive air temperatures of less than -30°C.

**c. Resting fronds morphologically different from the normal fronds that sink to the bottom of the water but do not grow any further (true turions).** Turions have been reported for S. polyrrhiza, L. turionifera, L. aequinoctialis (N<sub>2</sub>-type of BEPPU et al. 1981c, named 1985 L. aoukikusa ssp. hokurikuensis), W. brasiliensis, W. borealis, W. angusta, W. australiana, W. arrhiza, W. columbiana and W. globosa.

Descriptions of turions of S. polyrrhiza are given by HEGELMAIER (1868), BISCOE (1873), JACOBS (1947), HENSSEN (1954), CZOPEK (1959b), RAO (1969), SMART and TREWAVAS (1983b) and many other authors. Turions of L. turionifera are mentioned by THOMPSON (1898, under the name of L. minor), LANDOLT (1957, under the name of L. minor I), and VAN OVERBECK et al. (1968, under the name of L. minor). HEGELMAIER (1868) and GODZIEMBA-CZYŻ (1970) characterized turions of W. arrhiza.

The turions of S. polyrrhiza (fig. 2.39, plate IIc) are orbicular to reniform in shape, 0.5-3.5 mm in width, olive-green to dark brown in

colour (accumulation of anthocyanins), and have very rudimentary roots that are not visible without magnification. The stomata are closed. The intercellular air spaces are much reduced (no aerenchyma). The cells reach the same final size as the vegetative frond cells. The cell walls of the inner cells are relatively thicker than those of the normal frond mesophyll cells. The cell lumen is almost completely filled with amyloplasts which mask the other cellular components. The cells accumulate numerous starch grains, and large deposits of tannins and anthocyanins at the expense of the vacuolar expansion characteristic of the normal mature vegetative frond. The ratio dry weight to fresh weight achieved 0.27 in turions compared with 0.08 in normal fronds. The chlorophyll a content reached 0.32 mg per g dry weight in turions and 0.89 mg per g dry weight in normal fronds. The data for chlorophyll b are 0.12 mg and 0.25 mg, respectively (BEER 1985). Many enzymes of normal fronds of S. polyrrhiza are essentially absent in turions (PERRY 1963). VINTEJOUX

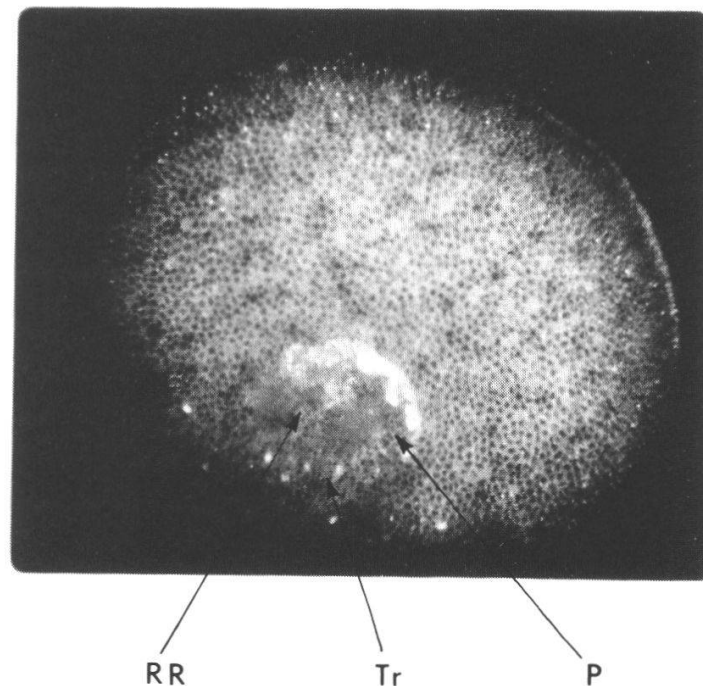


Fig. 2.39. Turion of Spirodela polyrrhiza from below (x25)

P ventral lobe of prophyllum      Tr tract of elongated cells  
RR root rudiments

(1982b) investigated the endoplasmic reticulum (ER), the Golgi apparatus and the dictyosomes of turions of S. polyrrhiza (see chapter 2.4.2). Oxalate crystal idioblasts of turions have been studied by VINTEJOUX and SHOAR-GHAFARI (1985) (see chapter 2.4.7).

The turions of L. turionifera are similar to but smaller than those of S. polyrrhiza (plate IIId); they are orbicular to obovate in shape. Certain clones of L. aequinoctialis from the Northwest of Japan (belonging to 1 out of 4 ecotypes occurring in Japan) form resting fronds which correspond to true turions. They are small, have poorly developed air spaces, abundant starch grains, reduced roots, and sink to the bottom of the water (BEPPU and TAKIMOTO 1981c). In Wolffia, the turions are very small, spherical in shape and light green in colour (plate IIe,f).

In contrast to normal fronds, turions of S. polyrrhiza can tolerate temperatures of  $+4^{\circ}\text{C}$  for more than two years (KRONBERGER, unpubl. results),  $-4^{\circ}\text{C}$  for at least three weeks and  $-8^{\circ}\text{C}$  for several days, although not  $-12^{\circ}\text{C}$  for 24 hours (JACOBS 1947). DAS and GOPAL (1969) observed that turions died after one hour at  $-13^{\circ}\text{C}$  and after 12 hours at  $-5^{\circ}\text{C}$  to  $-7^{\circ}\text{C}$ . These differences in findings of tolerance to low temperatures are probably due to the use of different clones and different experimental conditions. Resting fronds can usually tolerate temperatures up to  $50^{\circ}\text{C}$  for at least a few hours (JACOBS 1947). At that same temperature, the respiration rate is 1/6 to 1/10 of that of normal fronds; photosynthesis is also slower (CZOPEK 1967). The tolerance of the turions of W. arrhiza to low temperatures is about the same as that of normal fronds (less than 6-10 days at temperatures of  $-2^{\circ}\text{C}$ ). The water content of the turion is less than that of the normal frond (79% and 97%, respectively). The turion contains more starch (24 mg) than a normal frond (2 mg) or a resting frond (7 mg); the chlorophyll content is lower (3.9 mg against 17.3 mg); and the respiration and photosynthesis rates are also less (GODZIEMBA-CZYŻ 1970).

Turions, as well as other resting fronds and normal fronds, cannot withstand desiccation. When turions are taken out of the water, they die within a few hours (JACOBS 1947, DAS and GOPAL 1967 for S. polyrrhiza, LANDOLT unpublished observations for L. turionifera, GODZIEMBA-CZYŻ 1970 for W. arrhiza). MALEK (1981) mentions that turions of S. polyrrhiza can tolerate slight drying with 5-20% polyethylene glycol 6000 in a sealed petri dish for up to 12 days. However, turions do not survive complete drying. Contradictory information is given by McCANN (1942), DEN HARTOG

and VAN DER PLAS (1970). McCANN observed germinating turions in an area that was kept dry for two years. Though statements of McCANN have not always proved to be reliable the information was corroborated by GOPAL (pers.comm.) who detected that turions completely covered by mud or silt are able to survive in ponds that dry out. This is a very interesting phenomenon which is also known from some animals (e.g., Amphibia) and which is not completely understood. Further studies on these survival possibilities of S. polyrrhiza turions are desirable. It would be interesting to know if this ability is restricted to turions from Southeast Asia or if it is a general characteristic of turions of Lemnaceae and possibly other water plants. As turions also form in waters low in nutrients, they can survive rainy seasons at the bottom of the water, thus avoiding the danger of being swept away by high water.

There are intermediates between the three kinds of resting fronds. Under certain conditions some clones of L. minor from the eastern part of the U.S.A. and some clones of L. japonica form resting fronds which look like turions and sink to the bottom of the water, but grow slowly (cf. LANDOLT 1957). Intermediate behaviour also exists in other species.

#### 2.5.2. Factors causing formation of turions

To form turions, it is necessary that the plants produce more carbohydrates than they use for growth (JACOBS 1947, HENSSEN 1954, CZOPEK 1959b for S. polyrrhiza). Factors that stimulate the formation of starch (a. to c.) or that reduce growth rate without slowing photosynthesis (d. to e.) favour the formation of turions:

- a. high light intensity
- b. addition of sucrose (HENSSEN 1954, CZOPEK 1963a, NEWTON and DUFFY 1975, NEWTON et al. 1978, SCHEINER et al. 1978)
- c. high concentration of CO<sub>2</sub> (JACOBS 1947, PERRY 1968)
- d. low temperature, especially during night time (PERRY 1963, 1968, SCHEINER et al. 1978, DOCAUER 1983)
- e. shortage of nutrients (JACOBS 1947, HENSSEN 1954, CZOPEK 1963, PERRY 1968, MENSCHICK 1970, NEWTON and DUFFEY 1975, SCHEINER et al. 1978, MALEK and COSSINS 1983, DOCAUER 1983)

A high ratio of carbon to nitrogen in the frond allows the formation of

turions in S. polyrrhiza (MALEK and ODA 1979). In contrast to sucrose, glucose has a stimulating effect only in light, but not in darkness (HENSSEN 1954). Turion formation is possible between 10°C and 35°C (JACOBS 1947). Stimulation of turion growth under short-day conditions has been observed by PERRY (1963, 1968) and SCHEINER et al. (1978). With red light more turions are formed and earlier than with blue light (MALEK and ODA 1979). Apparently, the endogenous content of ABA is also responsible for the formation of turions. The addition of ABA strongly stimulates the production of turions (PERRY 1968, PERRY and BYRNE 1969, STEWART 1969, SAKS et al. 1975, SMART and TREWAWAS 1983a). The necessary ABA concentration in the culture solution for turion formation is between  $7.5 \cdot 10^{-5}$  and  $5 \cdot 10^{-7}$  M. Only fronds shorter than 0.7 mm develop into turions by ABA application. Fronds between 0.7 and 1.3 mm develop into small vegetative fronds with turion-like proximal ends. The process of turion formation appeared reversible for the first two days of exposure to ABA (SMART and TREWAVAS 1983a). A high phytic acid content is characteristic for turions of S. polyrrhiza (SCHEINER et al. 1978). DEUTSCH and RASMUSSEN (1974) report that turion formation in S. polyrrhiza is stimulated by irradiation of fluorescent light (high proportion of red and low proportion of far-red radiation). In India turions of S. polyrrhiza are formed twice a year: in summer under high light intensity, long-day conditions, and high temperatures (up to 44°C during daytime) coupled with low nutrient supply during monsoon rains, and in winter with low temperatures and short-day conditions (DAS and GOPAL 1969).

It is supposed that the formation of resting fronds of other species is stimulated by similar factors. VAN OVERBECK et al. (1968) have shown that the addition of ABA to the nutrient solution gives rise to turion formation in L. turionifera. According to these authors, the formation of turions is dependent upon the ratio of ABA to cytokinin. Sucrose promotes turion formation of L. turionifera similar to that of S. polyrrhiza. According to DOCAUER (1983) turions of L. turionifera can be induced below 25°C if nutrients (especially phosphorus) are limiting. Resting fronds of L. perpusilla are formed under short-day conditions, causing the production of ABA-like substances (KANDELER and HUEGEL 1974, HUEGEL et al. 1979). Sucrose also stimulates the formation of turions in W. arrhiza (GODZIEMBA-CZYŻ 1969). W. gladiata develops resting fronds with 3% sucrose, but not with 1% sucrose. However, the addition of ABA does

not stimulate growth of resting fronds (PIETERSE et al. 1970d). Resting fronds have a different ratio of different endogenous gibberellins than the normal fronds (PIETERSE et al. 1971). (See also volume 2, chapter 2.4.2.1. LANDOLT and KANDELER 1987).

### 2.5.3. Factors causing germination of turions

The two independent investigations of JACOBS (1947) and HENSSEN (1954) both show that, in nature, turions of S. polyrrhiza developed during the fall do not germinate if temperature rises again. The conditions leading to germination are rather complex. The ability to germinate depends upon the conditions under which the turions were formed and stored. In general, turions of S. polyrrhiza need an average temperature of about 15°C or more to germinate (JACOBS 1947, HENSSEN 1954).

Turions germinate in spring from the bottom of the water, even under low light intensities, when the water temperature reaches 15°C. In laboratory conditions, if the turions have been stored for two weeks at 10°C (JACOBS 1947) or two months at 5.5°C (HENSSEN 1954), they begin to germinate within 10 days at higher temperatures. The longer the storage of the turions (up to 4 months), the shorter the period needed for germination after the conditions are changed (CZOPEK 1959b). If storage takes place at temperatures of 20°C (HENSSEN) or 25°C (JACOBS), a resting period of up to 10 and 6 months, respectively, is necessary before germination is possible. The minimum resting period for germination in light is shorter than that for germination in darkness (HENSSEN 1954). CZOPEK (1962) was able to obtain 100% germination after 3 days of turions stored at 0°C-3°C using higher temperatures in lighted conditions; in darkness, germination took place after 8 days, but at a rate no higher than 35%. The stimulating effect of light can be reversed by far red radiation; on the other hand, it is possible to stop the effect of far red by using red light afterwards (CZOPEK 1959b, 1962). DAS and GOPAL (1969) observed in darkness a germination rate of 57% at 23-25°C and of 35% at 32-40°C. A low temperature treatment of eight hours considerably increased the germination of turions stored for 3 1/2 months. At low temperatures, germination is faster under short-day conditions; at high temperatures, long-day conditions are better for germination (DAS and GOPAL 1969).

To break the resting state, turions can be treated with 0.1% potassium

cyanide or 0.2% 2,4-D solution (HENSSEN 1954). Sodium sulfocyanate (3%, for 1-2 hours), IAA (0.1%, for 2 hours), or ethanol (95%, for 1-2 hours) have the same effect (YOSHIMURA 1950). According to MALEK (1981), previous slight drying stimulates the germination rate of turions. Gibberellic acid in light enhances the germination rate of turions, kinetin promotes the germination in light and in darkness (LACOR 1969). Cytokinins (IP, BA) are able to overcome turion dormancy and stimulate germination (MALEK and COSSINS 1983a).

SIBASAKI and ODA (1979) and MALEK and ODA (1980) distinguish between two different kinds of turions in S. polyrrhiza, which show different germination behaviour. In the first kind, so-called "young" turions are produced in new cultures, having a green colour and germinating only after a very long time. Very often they are formed at low temperatures. The "young" turions form normal daughter fronds before developing roots. Light is necessary to break the resting period as well as nitrate in the nutrient solution. Dormancy is also broken when there is a definite period of darkness in the absence of nitrate. In this case, the turions germinate with or without light in the presence of nitrate. In the second kind of turions, so-called "old" turions are produced in old cultures and often in solutions lacking nitrogen. They are brown in colour and germinate rapidly in both light and darkness, provided that nitrate is present. They produce roots before forming new daughter fronds. Under semi-anaerobic conditions, "old" turions germinate only when exposed to light. Sucrose accelerates the "ageing" of turions and enables germination in darkness to occur (cf. also NEWTON et al. 1978). SHELTON (1979) investigated the effect of sugar on turion germination. Turions formed after addition of ABA to the culture medium germinate at once if ABA is removed from the solution (SMART and TREWAVAS 1983a).

Turions of L. turionifera begin to germinate at a low rate at 10°C and reach 100% germination between 15°C and 20°C (DOCAUER 1983). The author observed germination of turions of L. turionifera in the field (Michigan) at a time when the temperature of the lake reached 11.1°C. Turions of W. borealis began germination at 14°C and of S. polyrrhiza at 17.9°C. Turions of W. arrhiza formed at 16°C-28°C are able to germinate in fresh culture solution within a few days, in contrast to S. polyrrhiza under the same conditions. The W. arrhiza turions germinate faster in light than in darkness. Sucrose added to the culture solution retards germination (GODZIEMBA-CZYŻ 1969).

The development of amyloplasts to mature chloroplasts in turions of S. polyrrhiza during germination is completed within 48 hours (RAO 1969). The surfacing of the turion is preceded by a bubble of photosynthetically evolved  $O_2$ . In the dark no turions became buoyant (BEER 1985). For more detailed information see volume 2, chapter 2.4.2.2 (LANDOLT and KANDELER 1987).

## 2.6. CHARACTERISTICS OF FLOWERING AND FRUITING FRONDS

The conditions causing flowering are discussed in detail in chapter 4.3.1 of this volume and in volume 2, chapter 2.4.3 (LANDOLT and KANDLER 1987).

### 2.6.1. Aspects of flowering fronds

Flowering fronds of Spirodela and Lemna (except L. trisulca and L. tenera) are often smaller and thicker than normal fronds, but principally are not different. Very large, flat fronds rarely flower. The fronds of flower-producing L. trisulca and L. tenera rise to the surface of the water and are much shorter than vegetative fronds. The flowering fronds of L. tenera (fig. 9.2q) are pointed but do not taper in a long point as vegetative fronds; the dentation of the frond of L. trisulca is less pronounced in flowering plants (plate VIId). There are more air spaces in flowering fronds, which are also found in the basal section; in contrast to the vegetative frond, which have no stomata, there are 20-50 stomata present on a flowering frond.

Within the group of W. oblonga and in W. denticulata, there are pronounced differences between flowering and vegetative fronds. Other species of the genus Wolffiella are not differentiated. The flowering fronds of W. oblonga group and W. denticulata are less submerged in water and have more stomata than the vegetative fronds. The narrow-shaped fronds of W. gladiata (cf. KURZ and CROWSON 1949) and W. denticulata (cf. OBERMEYER-MAUVE 1966) produce short flowering fronds, which are enlarged and thickened at the base (plate XIId). The flowering fronds of W. lingulata are, in general, somewhat narrower than the vegetative ones (MASON 1938, ESKUCHE and ROMERO FONSECA 1982) (plate Xb), while the flowering fronds of W. oblonga are very similar to the vegetative ones (GIARDELLI 1935) (plate Xd). Flowering fronds of W. neotropica are smaller than the vegetative ones and float with the greatest part of the frond at the surface of the water (LANDOLT 1984) (plate IXb).

In the genus Wolffia, the flowering fronds are similar in shape and size to the vegetative ones, with the flowers covering a large part of the upper surface of the frond. Flowering fronds of W. brasiliensis lack papillae (plate XIIIa,b).



Fig. 2.40. Flowering Wolffiella Welwitschii (x6). Four groups with two flowers per frond and one group (far right) with only one flower. Flowering was achieved in a Hutner solution (1/5) with EDDHA, 26°C, 18000 lux, day of 12 hours.

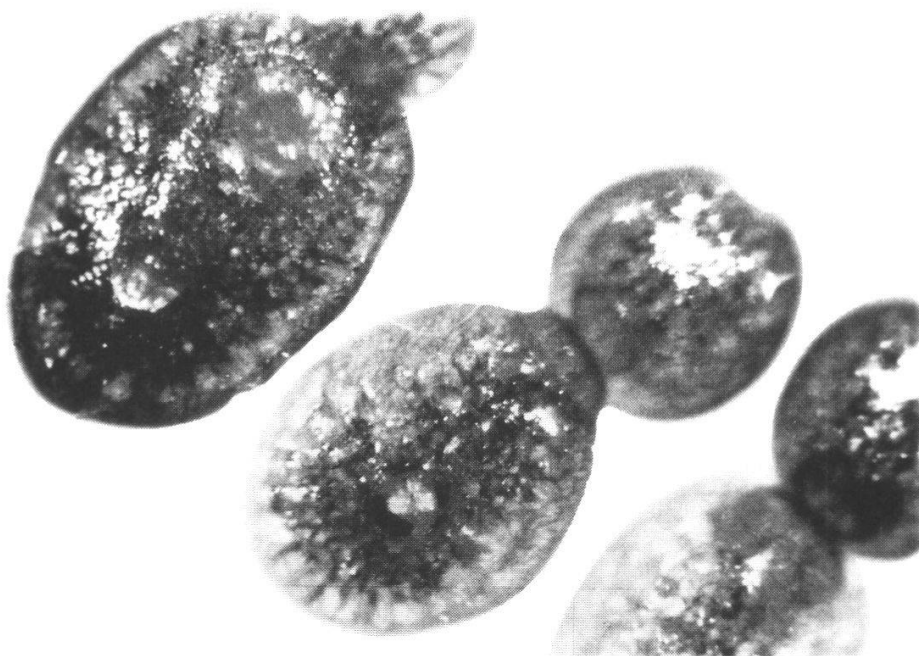


Fig. 2.41. Two flowering fronds of Wolffia columbiana (x40). The lower frond with one flower (anther) near the distal end; the upper frond with two flowers: one flower with stigma in the normal position near the distal end and one flower primordium near the node. Flowering conditions as in fig. 2.40.

In general, there is one flower per frond. However, some species occasionally have two flowers, with each flower in a separate pouch or cavity. In W. Welwitschii, two flowers per frond is the rule (plate IXd) (very rarely only one flower can be observed, fig. 2.40). In L. perpusilla, up to 100% of the fronds have two flowers (KANDELER and HUEGEL 1974a) (plate VIIb); about half of the flowering fronds of W. rotunda have two flowers (type collection, fig. 9.3g) and up to 20% of L. aequinoctialis (POSNER 1961, 1962a, WITZTUM 1966, KANDELER and HUEGEL 1974a, KANDELER 1975, DOSS 1978, SHIH 1979). S. polyrrhiza (HEGELMAIER (1871) and L. trisulca (HOFFMANN 1840a) rarely have two flowers per frond. As an exception, 2 flowers per frond could be observed in Wolffia columbiana (fig. 2.41). WITZTUM (1966) reports three kinds of rare abnormal flowering fronds in L. aequinoctialis: 1) fronds that bear two flowers (one in each pouch) with the base of the two carpels fused to each other; 2) fronds that bear a single-centered carpel containing two ovules, and 3) fronds that bear a single-centered carpel containing a single ovule.

Flowering results in a retardation of vegetative growth. UMEMURA et al. (1963) are of the opinion that a flower consisting of the prophyllum, two stamens and the pistil replaces four daughter fronds (one flower equals four fronds). This probably does not mean that the flowering organs are homologous to daughter fronds. They possibly need about the same amount of assimilates as daughter fronds and replace therefore a frond if light conditions are suboptimal. SCHUSTER (1968) also counted four daughter fronds less (5 compared with 9) in flowering cultures of L. aequinoctialis under short-day conditions than in non-flowering cultures (flowering was prevented by 5 minutes application of blue-red irradiation daily). Under long-day conditions the same clone 6746 developed 14 daughter fronds per mother frond. According to MORI (1979a) one flower of L. aequinoctialis is formed at the expense of two fronds if growth rates of flowering and non-flowering cultures are compared. KANG and CLELAND (1985) who probably worked with higher light intensities observed in fronds of L. gibba G3 10-12 daughter fronds whether grown on short days (not flowering) or on long days (flowering).

The development of unisexual flowers is referred to in chapter 2.6.4.

In rare cases, WITZTUM (1966) observed staminoidal areas within the vegetative tissue of L. aequinoctialis fronds; each sporogenous area had an endothelial layer as in normal stamens, and it also contained pollen grains.

### 2.6.2. Morphology and interpretation of flowering organs

The **flowering organs** of the Lemnoideae consist of the following: 1 membranous scale (prophyllum), 2 stamens and 1 pistil (figs. 2.42, 2.44, 2.46). Rarely, 3 stamens have been observed in S. polyrrhiza (LACOR 1970). If the flower theory (and not the inflorescence theory) is accepted, a flower diagram of Spirodela looks as in fig. 2.45. In Wolffiella and Wolffia, no scale is present and only 1 stamen (figs. 2.43, 2.47). There is much controversy about the interpretation of the flowering organs (see at the end of this chapter and chapters 2.2 and 7.2).

In Spirodela and Lemna, the flowering organs originate in the same pouches in which the daughter fronds are formed. In general, there is only one flower per frond that appears from the pouch on the minus side (where normally the second daughter frond is formed in non-flowering fronds). It is often believed that the flower replaces the first daughter frond of the flowering pouch. But KANDELER (1955, 1968, 1983), CLELAND and BRIGGS (1967) and LACOR (1970) showed clearly that the flower primordium is always formed beside the first daughter frond primordium towards the base of the frond (fig. 2.48).

In Wolffiella and Wolffia, the flowers originate in a cavity on the upper surface of the frond, and not in the same pouch or cavity as the daughter fronds. The flower of Wolffia is situated along in the median line of the frond; the flower of Wolffiella is on the side of the median line.

The **scale (prophyllum)** of flowers of Lemna is mostly a two-layered membrane containing raphide cells and sometimes chloroplasts (HEGELMAIER 1868). Along the margins it consists of two layers in S. intermedia (KLICH and MUJICA 1985) and one layer in S. polyrrhiza (KRAJNCIC and DEVIDE 1979). The rest of the scale in S. intermedia consists of three layers; in S. polyrrhiza three layers are restricted to the base. SHIH (1979) reports only one layer for L. aequinoctialis. In contrast to GIARDELLI (1939), KLICH and MUJICA (1985) saw only pigment cells but no raphides and druses in the scale of S. intermedia. The scale is either an utricle open only at the top (Spirodela, L. trisulca, group of L. minor, cf. fig. 2.44), or it is slit down to the place of attachment (groups of L. perpusilla and L. valdiviana, cf. fig. 2.46). The prophyllum of the latter group is not sack-like in origin and does not rupture at maturity as reported by HEGELMAIER (1868). It is an open cup-like

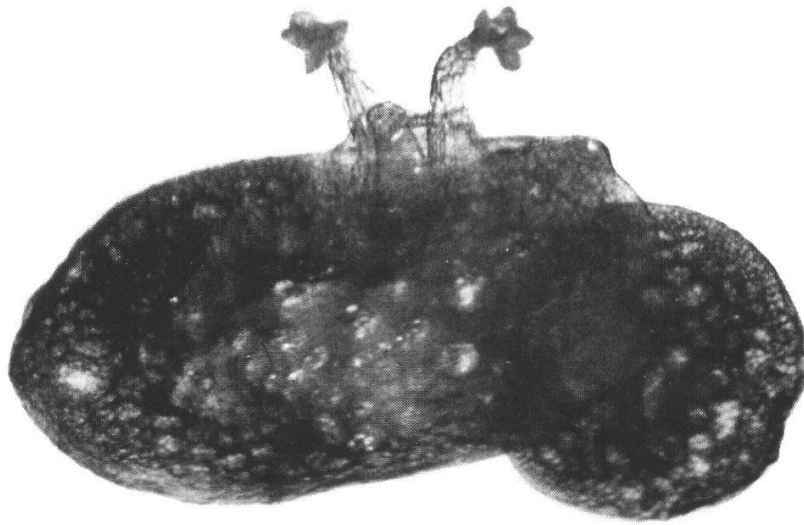


Fig. 2.42. Flower of Lemna disperma: two open anthers with filaments;  
between the anthers the style with the stigma (x20)

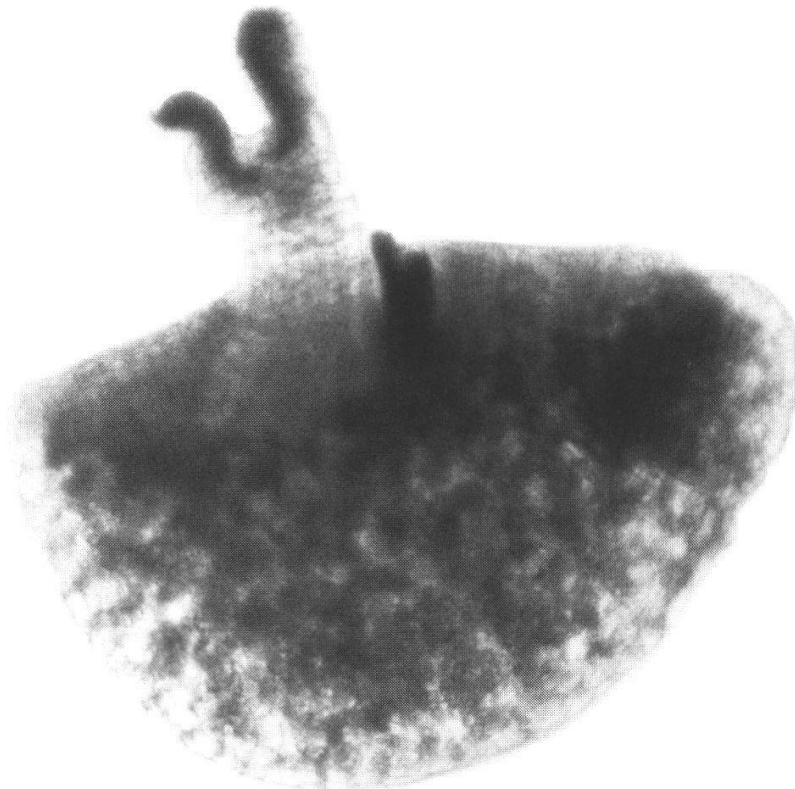


Fig. 2.43. Flower of Wolffia angusta: one anther (open) with filament  
and a style with stigma (right). The locules of the anthers  
are open and the pollen mass emerges (x80)  
(photo by W.P. Armstrong, San Marcos, Cal., U.S.A.)

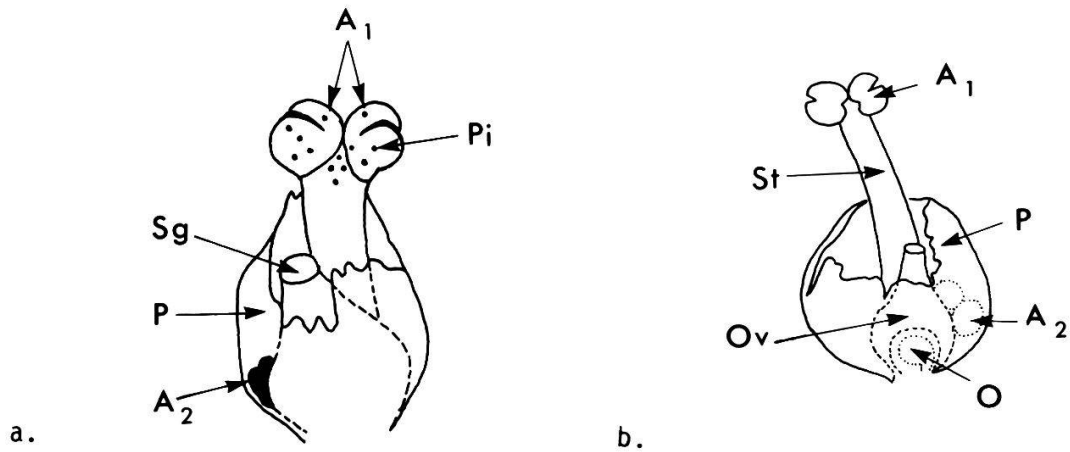


Fig. 2.44. Flowers of *Spirodela polyrrhiza* (a; x30) (after HEGELMAIER 1871) and *Lemna trisulca* (b; x18) (after HEGELMAIER 1868)

A <sub>1</sub>	anthers of the first stamen	P	prophyllum
A <sub>2</sub>	anthers of the second stamen	Pi	pigment cells
Ov	ovary	St	stamen
O	ovule	Sg	stigma

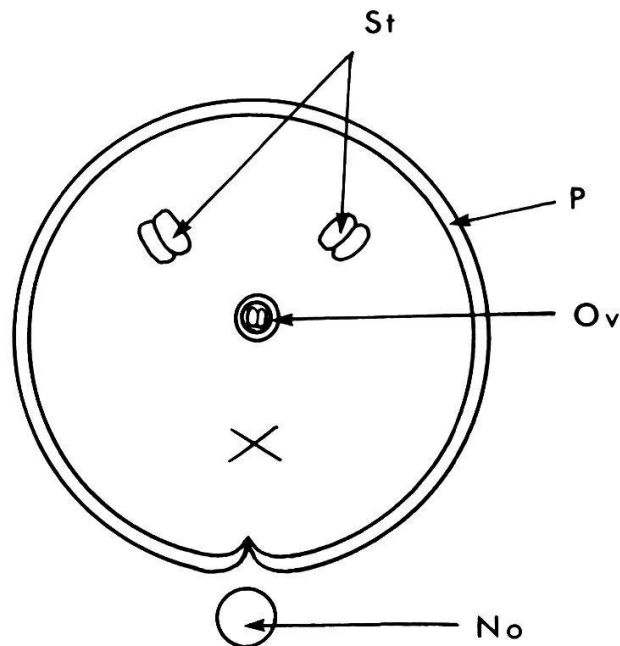


Fig. 2.45. Flower diagram of *Spirodela polyrrhiza*

No	node	St	stamens
Ov	ovary	X	omitted stamen
P	prophyllum		

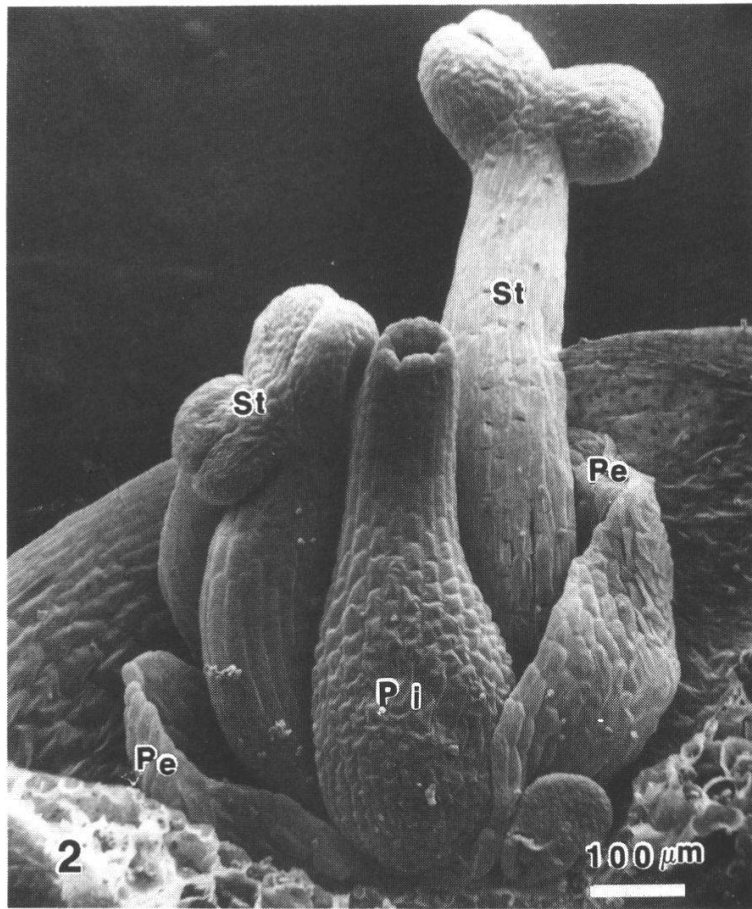


Fig. 2.46. Flower of Lemna aequinoctialis (x100) (from SHIH 1979)  
Pe = prophyllum, Pi = pistil, St = stamen

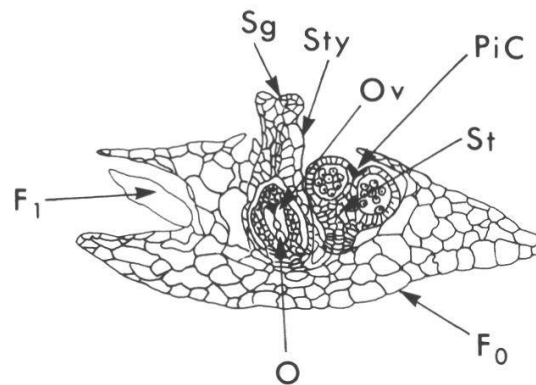


Fig. 2.47. Longitudinal section of a flowering frond of Wolffia microscopica (x35) (after MAHESHWARI 1954)

- 45 -

- F<sub>0</sub> mother frond with 1 flower in a cavity
- F<sub>1</sub> first daughter frond
- Ov ovary with ovule (O), style (Sty), and stigma (Sg)
- PiC pigment cells
- St stamen with 2 one-locular halves

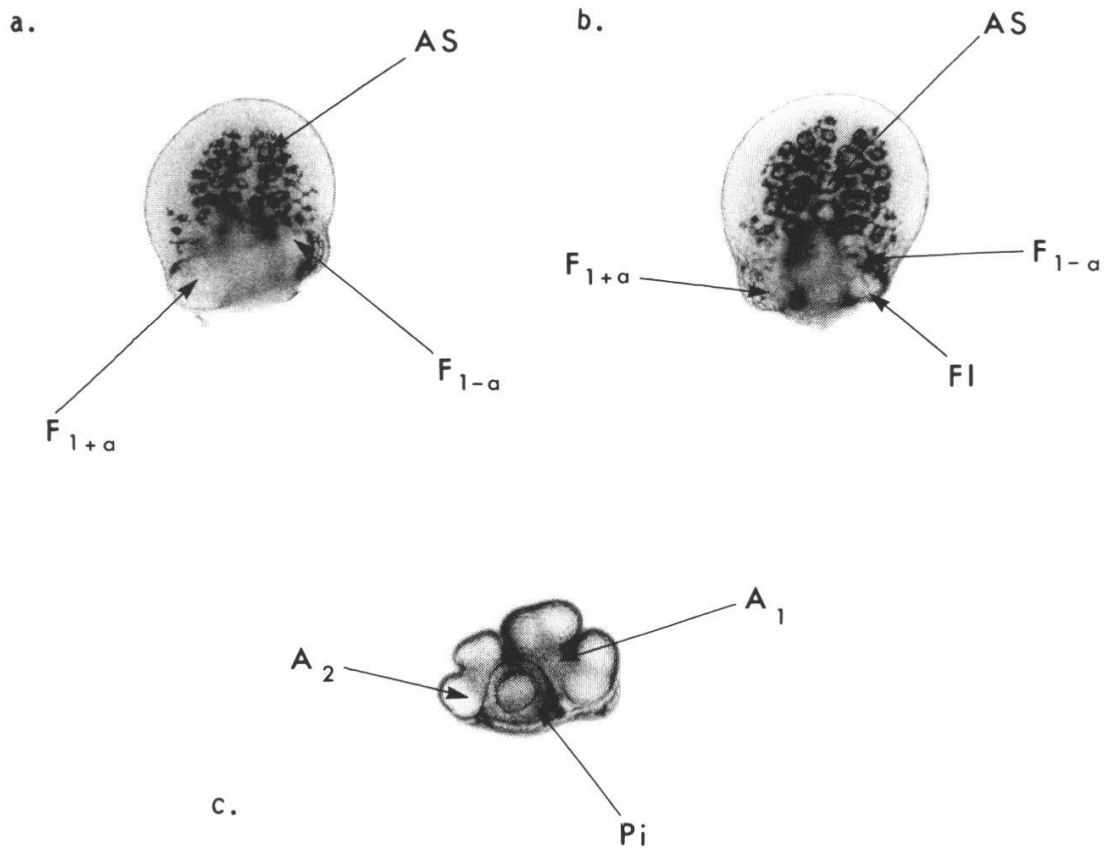


Fig. 2.48. Frond and flower primordia of Lemna gibba (x75)

- a. Frond primordium, 0.3 mm in length, with a vegetative meristem in the pouch on the right side, which is inserted in the basal angle of the pouch.
- b. Frond primordium, 0.3 mm in length, with a flower primordium in the pouch on the right side, which is inserted in the upper part of the pouch.
- c. Flower primordium divided into a greater (right) and a smaller (left) stamen and the pistil (in the center). (From KANDELER 1955, 1983)

A<sub>1</sub> first stamen  
 A<sub>2</sub> second stamen  
 AS air spaces  
 F<sub>1</sub> flower primordium

F<sub>1+a</sub> first daughter frond of the plus side  
 F<sub>1-a</sub> first daughter frond of the minus side  
 Pi pistil

scale in origin (SHIH 1979). LACOR (1970) occasionally observed 1 to 5 adventitious roots at the base of the utricular scale of S. polyrrhiza. The two **stamens** of Spirodela and Lemna do not develop simultaneously, but form one after the other: the distal one first, the basal one later (observations of many authors). A contrary indication of SAEGER (1934) for S. punctata is probably erroneous. The anthers in Lemnaceae contain 2 two-locular halves (Spirodela, Lemna) or 2 one-locular halves (Wolffia, Wolffia). The 2 locules of the anther of S. polyrrhiza are situated side by side (fig. 2.49), whereas the locules of Lemna are placed one on the top of the other, with the external locule at the top (fig. 2.50). In S. punctata, the circumstances are somewhat in between, with the external locule situated slightly higher than the internal (HEGEL-MAIER 1895). Accordingly, the anthers of S. polyrrhiza open horizontally, and those of Lemna, vertically. In Spirodela and in Wolffioideae the one-celled border that surrounds the opening fissures contains pigment cells, even in species that otherwise do not have pigment cells (HEGEL-MAIER 1868). Raphide cells are present in the anther walls of Spirodela and Lemna. Later, the raphides get mixed with the pollen grains. Also pigment cells are present within the epidermis of S. polyrrhiza (HEGEL-MAIER 1871, KRAJNCIC and DEVIDE 1979).

SHIH (1979) counted 20 pollen grains per locule in L. aequinoctialis. The ripe, 3-nucleate pollen grains are 0.011 to 0.052 mm in diameter (most measurements range between 0.015 and 0.026 mm). The smallest diameter has been recorded by GIARDELLI (1935) in W. oblonga (0.011-0.015 mm). The size of the pollen grain is not as dependent upon the species as upon the chromosome number and the method of preparation. URBANSKA (unpubl. results) has measured the pollen grain size of strains of L. turionifera and L. aequinoctialis that had varying chromosome numbers (fig. 2.51). The size of pollen in all of the 40-chromosomal clones is similar. The grains of the 80-chromosomal clones of L. turionifera are 1.3 times the size of the grains of L. aequinoctialis (80-chromosomal clone) and 1.8 times larger than the grains of the 40-chromosomal clones. SHIH (1979) measured an average grain diameter of 0.015 mm, for the (originally) 80-chromosomal clone of L. aequinoctialis (No 6746), as opposed to 0.03 mm determined by URBANSKA for the same clone. These differences might be due to different preparations (dried or soaked in water). Eventually, the clone changed chromosome number from 80 to 40 (see chapter 3).

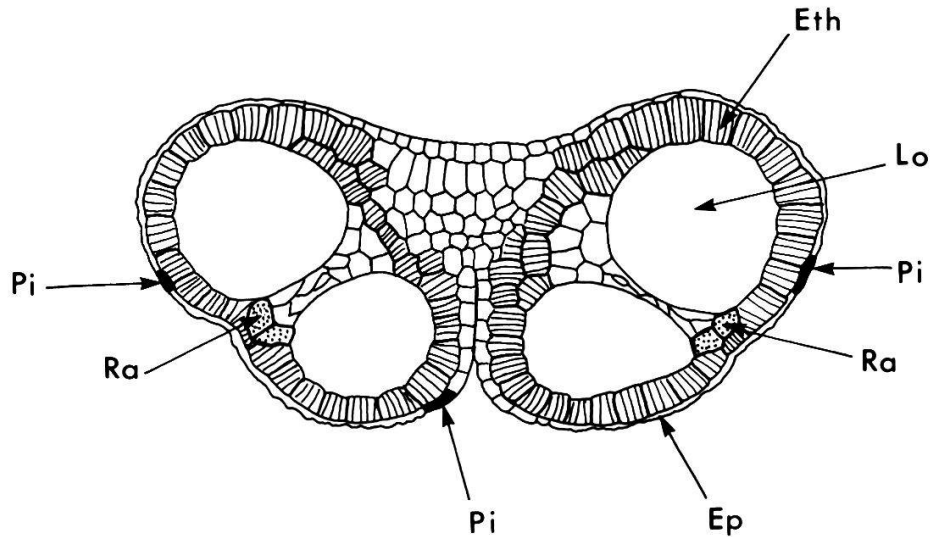


Fig. 2.49. Transverse section of an anther of Spirodela polyrrhiza from above (x180) (after HEGELMAIER 1871)

Ep	epidermis, disintegrating	Lo	locules of the anther
Eth	endothecium consisting of fibrous parenchyma cells	Pi	pigment cells
		Ra	raphide cells

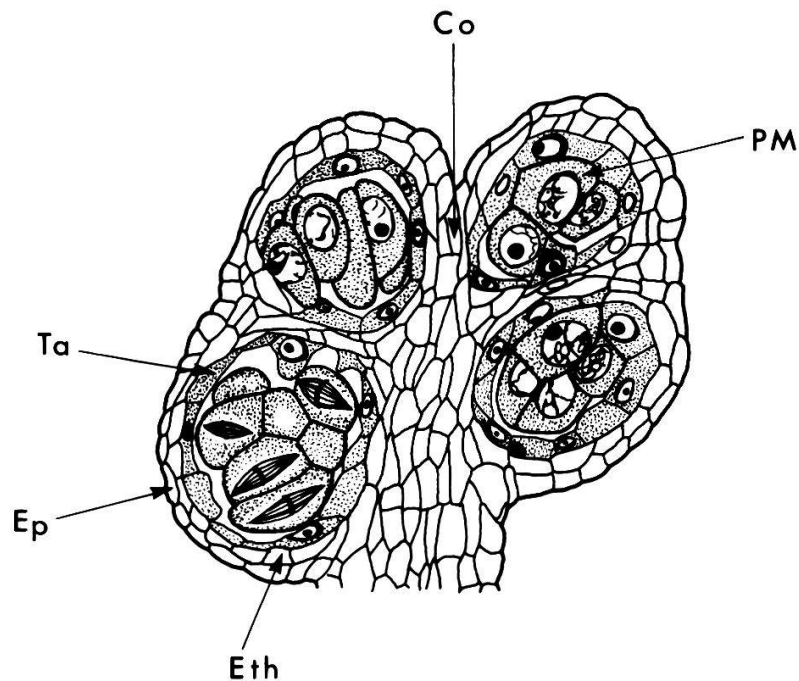


Fig. 2.50. Longitudinal section of an anther of Lemna aequinoctialis during meiosis (x540) (after MAHESHWARI and KAPIL 1963a)

Co	connective	PM	pollen mother cells
Ep	epidermis	Ta	tapetum
Eth	endothecium		

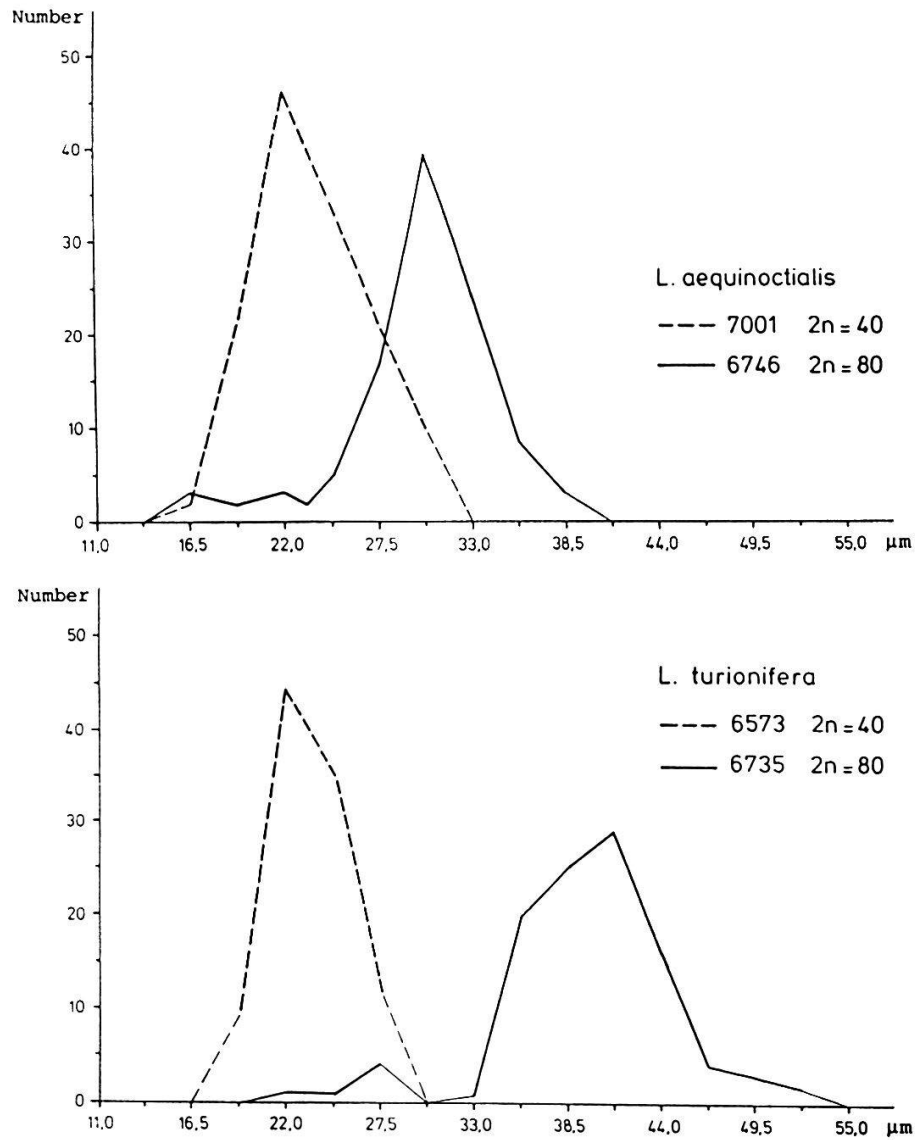


Fig. 2.51. Size of pollen grains of two clones each of Lemna aequinoctialis and Lemna turionifera with varying chromosome numbers ( $2n=40$  and  $80$ ) (from K. Urbanska, Zürich, unpubl.)

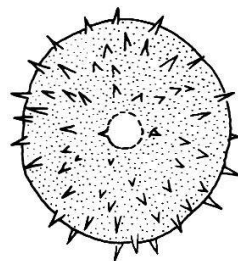


Fig. 2.52. Pollen grain of Lemna minor ( $\times 1000$ ) with protuberances and one pore for germination (after DE SLOOVER 1961)

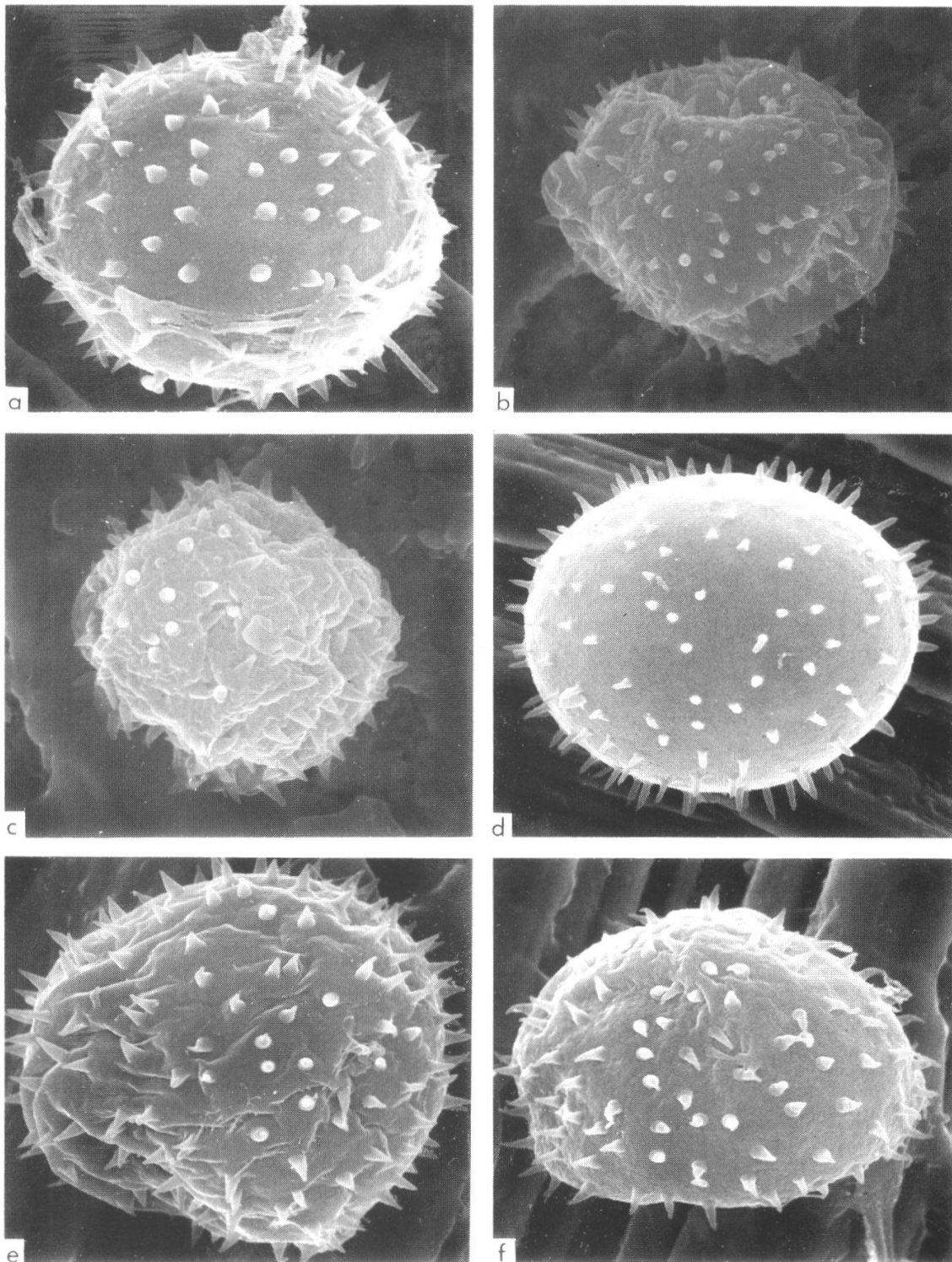


Fig. 2.53. Pollen grains of different Lemnaceae (scanning electron microscopy photographs kindly done by M.H. Grayum, Amherst, Mass. U.S.A.)

- |   |   |
|---|---|
| a. <u>Spirodela punctata</u> No. 7624 (x2800)                 | b. <u>Lemna disperma</u> No. 7782 (x2400)                             |
| c. <u>Lemna minor</u> No. 6591 (x3000)                        | d. <u>Lemna trisulca</u> No. 7579 (x2600)<br>(with aperture at 10:00) |
| e. <u>Lemna perpusilla</u> Ahles and<br>McCrary 58805 (x2800) | f. <u>Lemna aequinoctialis</u> Kiener<br>24866 (x2800)                |

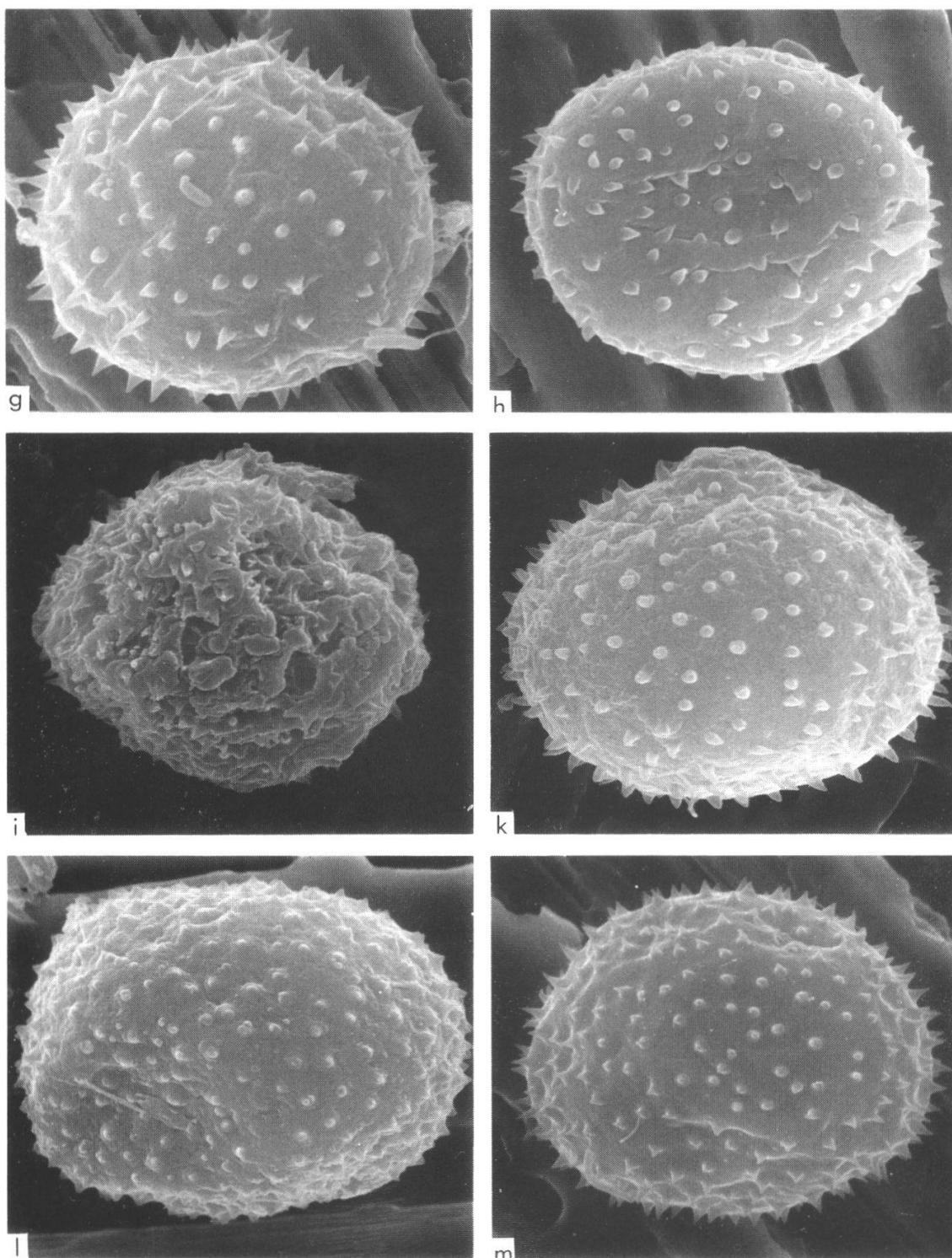


Fig. 2.53 (continued)

- g. Lemna valdiviana No. 7116  
(x2800)
- i. Wolffia microscopica No. 8359  
(x2800).
- l. Wolffia angusta No. 7476  
(x3300)

- h. Wolffiella lingulata No. 7289  
(x3000)
- k. Wolffia brasiliensis No. 7150  
(x3000) (with aperture at 12:00)
- m. Wolffia arrhiza No. 7452 (x2800)  
(with aperture at 1:00)

Each pollen grain contains only 1 pore that has a diameter up to 4  $\mu\text{m}$  (fig. 2.52). The exine is 0.6-1.0  $\mu\text{m}$  thick, and has 0.4-2.5  $\mu\text{m}$  long spiny protuberances (ERDTMAN 1952, DE SLOOVER 1961, THANIKAIMONI 1969, STRAKA in KANDELER 1979, KUPRIYANOVA and TARASEVICH 1984). Contrary to the observations of HEGELMAIER (1868) and many other authors, there is only a very slight difference between the protuberances of Lemnoideae and those of Wolffioideae, the protuberances of Lemnoideae being 1.2-2.5  $\mu\text{m}$  long and those of Wolffioideae, 0.4-1.0  $\mu\text{m}$  (cf. figs. 2.52, 2.53, 2.54). KUPRIYANOVA and TARASEVICH (1984) give values up to 5.1  $\mu\text{m}$  for S. polyrrhiza, but on their figure, the length of the protuberances does not exceed 2.5  $\mu\text{m}$ . W. angusta has the smallest protuberances of the investigated species, S. polyrrhiza the longest ones (LANDOLT, not published results). The width of the protuberances at the base is similar in all species (0.4-0.8  $\mu\text{m}$ ); the length-width ratio ranges from about 1 in W. angusta to 3 in L. trisulca. DE SLOOVER (1961) counted 150-200 protuberances per grain or 0.06-0.19 protuberances per  $\mu\text{m}^2$  grain surface in L. minor. From figure 2.53 one is able to count from about 150 protuberances per grain in S. punctata, up to about 400 in W. angusta. The more reduced the species, the higher the number and the smaller the size of protuberances.

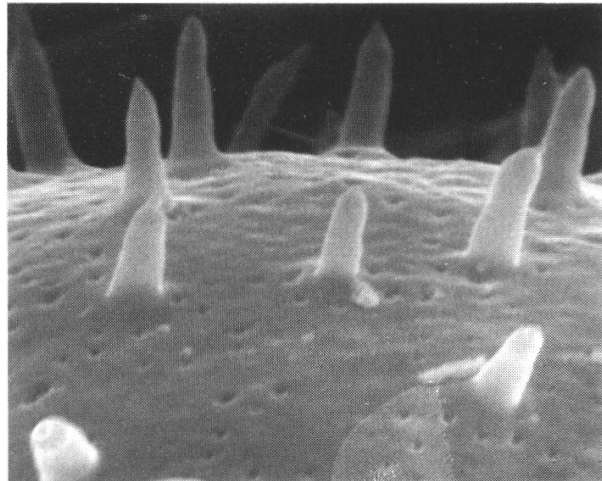


Fig. 2.54. Surface of a pollen grain of Lemna trisulca No. 7579 with protuberances (x9000).  
(Scanning electron microscopy photographs kindly done by M.H. Grayum, Amherst, Mass. U.S.A.)

In all genera, filaments of the stamens contain at the base a row of tracheids with ring-shaped strengthenings in the walls. The row follows into the connective in Spirodela, where it fans out into several rows (HEGELMAIER 1871). In most species of Lemna and in W. hyalina it ends below the connective. In most Wolffioideae and in the group of L. valdiviana, it is only visible at the base or not at all (groups of L. valdiviana and W. arrhiza).

The ovary of both Spirodela and Lemna is inserted above the two stamens (seen from the upper frond surface) and between (seen from the base of the frond); while in Wolffiella and Wolffia it is at the base and proximal of the single stamen (fig. 2.47). The ovary is bottle-shaped, tapering symmetrically (most species of the Lemnaceae) or asymmetrically (groups of L. perpusilla and L. valdiviana and, to a lesser extent, the Wolffioideae) into the short style with the stigma. The length and the diameter of the style is somewhat species-specific, at least for Spirodela and Lemna species. The style of the Wolffioideae is very short. The Lemnaceae stigma is funnel-shaped, tapering at the base in a style duct. At the time of pollination, a spherical secrete droplet shows on the stigma, which contains sucrose (ESKUCHE and ROMERO FONSECA 1982). Pigment cells are present in the stigma of Spirodela and of all species of Wolffioideae having pigment cells in the frond tissue.

Most of the Lemnaceae have only one ovule. Exceptions are S. intermedia with 2-5 (GIARDELLI 1939a, KOCH 1933; KLICH and MUJICA 1985 counted mostly 5), S. polyrrhiza with 1-2 (SCHLEIDEN 1839, GRIFFITH 1851, HEGELMAIER 1871, BROOKS 1940), rarely 3-4 (MAHESHWARI and MAHESHWARI 1963; these authors investigated a strain from Botanical Garden in Delhi; a living strain we got from the same source proved to be S. intermedia), S. punctata with 1-2 (BROOKS 1940, LACOR 1970), L. gibba with 1-7 (HEGELMAIER 1868, GIARDELLI 1970), and L. disperma with 1-2 ovules (HEGELMAIER 1895). The ovules are amphitropous if one-seeded or anatropous if two- or more-seeded in Spirodela, L. trisulca, and the group of L. minor. Almost orthotropous ovules have been found in the groups of L. perpusilla and L. valdiviana. In Wolffiella and Wolffia, the ovules are orthotropous. In the walls of the ovary of Spirodela (HEGELMAIER 1871) and in some species of Lemna (L. trisulca, group of L. minor), there is a row of tracheids (with ring-shaped strengthenings in the cell walls), which connect to the style with a branch towards the chalaza. In all other species, no tracheids are present in the ovary. The fruit wall

contains raphides in Spirodela and Lemna, and also anthocyanins in Spirodela, L. trisulca and the group of L. minor. Therefore, the fruit in these species is sometimes red in colour or has red spots.

**Interpretations of the flowering organs** are controversial. Some authors (HEGELMAIER 1868, EICHLER 1875, LUDWIG 1909, KOCH 1932, 1933, BROOKS 1940, MEUSEL 1951, MAHESHWARI 1954, 1958a,b, MAHESHWARI and MAHESHWARI 1963, DAUBS 1965, GIARDELLI 1970, DEN HARTOG and VAN DER PLAS 1970, and KANDELER 1979), who assume Pistia (Araceae) is the closest relative of Lemnaceae, identify the flowering organs as a reduced inflorescence consisting of 1-2 male flowers and 1 female flower and surrounded by a spathe. Other authors (HEGELMAIER 1871, CALDWELL 1899, LAVALREE 1945, and HILLMAN 1961) identify the organs as a flower having 1-2 stamens and one pistil. HEGELMAIER (1868) was first very convinced of the inflorescence; afterwards (1871) he was as positive about the flower theory; and finally (1895), he was more open about the arguments for the inflorescence theory, although he preferred to stay with the simpler flowering theory. His main argument in favour of the flower definition instead of the inflorescence definition is an angular distance of  $120^{\circ}$  in the position of the two stamens of S. polyrrhiza, suggesting the loss of a third stamen, which is present in other monocotyledons (figs. 2.45, 2.48c). HEGELMAIER looked upon the membranous scale as a bract which is present only below the flower and is missing at the base of the vegetative fronds. BROOKS (1940) and LACOR (1970) rarely observed 3 stamens in S. polyrrhiza. Also, WITZTUM (1966) and SHIH (1979) made photographs of abnormal flowers of L. aequinoctialis that had 3 stamens. In the abnormal flower of SHIH each stamen and the ovary were supported by a small scale. KANDELER (personal communication) observed 3 stamens in L. perpusilla. All three stamens were situated behind the pistil (distally), not distributed around the circle. Occasionally, the 2 stamens are attached to each other at the base (HEGELMAIER 1871, MAHESHWARI and KAPIL 1963a). The strongest argument for the inflorescence theory is the supposed derivation of Spirodela from the superficially similar Pistia, which has an inflorescence. Of course, there are some similarities between Lemnaceae and Araceae, but there are as many similarities to other families of the monocotyledons (chapter 7.2). Another argument is that the two stamens ripe one after the other and not together as is most usual for stamens of the same circle. The present author considers the relationship of the Lemnaceae still open (cf. HEGELMAIER 1895, HILLMAN 1961);

therefore he prefers to use the simpler term flower instead of inflorescence for the flowering organs of Lemnaceae.

If the flower is regarded as terminal, as it is in the monograph, the membraneous scale thus corresponds to a prophyllum; and this prophyllum is assumed to be homologous to the prophyllum at the base of the frond of Spirodela.

IVANOVA (1970) gives a not very convincing interpretation of the membraneous scale at the base of the flowering organs of both Spirodela and Lemna. She thinks of it as a phylloclad that bears the reproductive organs on its upper side and therefore is homologous to the frond of Wolffia. BRUNAUD (1974b) looks upon the stamens and the ovary as three flowers corresponding to three successive daughter fronds of the pouch during vegetative propagation.

### 2.6.3. Development of flowers and fruits

Studies in the development of flowers and embryology within the family of Lemnaceae were done by HEGELMAIER (1868), JOENSSON (1880), CALDWELL (1899), ROSTOWZEW (1905), BLODGETT (1914, 1923), GUPTA (1935), BROOKS (1940), LAWALREE (1952), MAHESHWARI (1954, 1956a,b), SOUEGES (1959), MAHESHWARI and KAPIL (1963a,b), MAHESHWARI and MAHESHWARI (1963) and BRUNAUD (1974).

**Flowering development** begins in fronds still smaller than 0.07 mm (L. gibba) (CLELAND and BRIGGS 1967) and 0.08 mm (L. aequinoctialis) (HILLMAN 1969). In L. gibba the development of a flower starts with the formation of a uniform hemispherical primordium. This primordium is produced in the minus pouch beside the frond primordium and is located closer to the base of the mother frond (fig. 2.48b). Then, the flower primordium is divided into four humps which represent the two thecae of the first stamen, the second stamen and the pistil (fig. 2.48c). After further growth, the pistil shows a ring wall on the upper side. Only at this stage, the first indication of the prophyllum becomes visible in form of a small bulge below the pistil. Later, the prophyllum grows up prematurely enclosing in this way the primordia of the other flower organs. At this stage, the stamens show divisions in anther and filament, and the pistil becomes conical. Next to the prophyllum, the pistil is

the second organ whose development is completed. The style grows out of the pouch curving to the upper side of the frond. Finally, the two stamens are prolonged one after the other curving to the upper side, too (KANDELER 1955).

The **anther** is bordered by a parenchyma cell layer which is missing only around the connective. This layer corresponds to the endothecium (fig. 2.50). Later, as the anther grows, the cells become fibrous (fig. 2.49). The epidermis originally situated outside the endothecium degenerates and, for the most part, is disposed of. Inside the endothecium is a one-layered tapetum (fig. 2.50), which behaves amoeboid at first and later forms a periplasmodium with many nuclei. Whereas most authors (e.g. HEGELMAIER 1868, GUPTA 1935, LAWALREE 1952a, MAHESHWARI and KAPIL 1963a) could not find a middle layer between the endothecium and tapetum, LODKINA (1976) observed one in L. gibba and L. minor; some of the cells within the tapetum of L. aequinoctialis and W. microscopica are supposed to correspond to this middle layer. The microspore mother cells undergo meiotic divisions, leading to tetrads, and finally to 3-nucleate pollen grains. The line of dehiscence of the anther locules can be observed externally as a border line between two rows of cells at the place where the septa between the two locules touch the wall. At that point, just below the wall, there is a double row of raphide cells (fig. 2.49) in Spirodela and Lemna. From here, the opening of the anthers takes place. The oxalate crystals of the raphide cells get mixed with the pollen grains when the anthers open (protection against feeding by arthropods?).

The **ovary** consists of a closed carpel and is one-locular. Most species have only one ovule (cf. chapter 2.6.2.). The ovule (figs. 2.55, 2.56) is at first erect (orthotropous) and contains two pairs of integuments: the outer one is 2- to several-layered and develops into the outer coat of the seed; the inner one is 3- to 6-layered at the top, and otherwise, 2- to 3-layered; it fuses into a very thin, but hard, inner seed coat, the top developing into the operculum. The micropyle has to be formed by the inner integuments, in contrast to Pistia, where the micropyle lies between the outer integuments. The outer integuments are either shorter than the inner ones (Wolffioideae, most Lemna species), or the relatively wide opening is displaced as in S. polyrrhiza, L. gibba, L. minor, and L. trisulca (HEGELMAIER 1868, 1871). An air space is situated between the exostome and the endostome. COCUCCHI (1966) considers this situation

similar to the one in Synandropadix (Araceae). Later, the ovule gets amphitropous (if only one ovule is present) or anatropous (if more than one ovule is present) in Spirodela and in Lemna (species of the group L. minor and L. trisulca). In all other species of Lemna and in the Wolffioideae the ovule stays orthotropous or nearly so. In L. gibba a short row of tracheids with ring-like thickenings can be observed. The nucellus is crassi-nucellate (more than one layer above the embryo sac): it is consumed by the growing embryo and, eventually, only a very

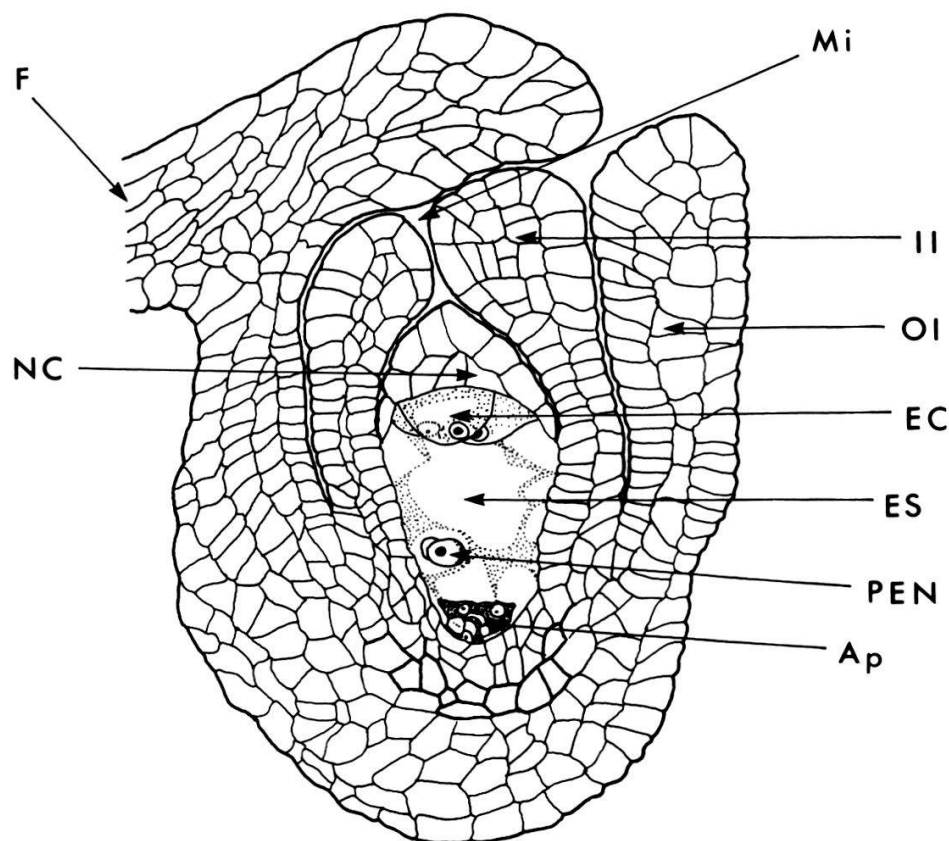


Fig. 2.55. Ovule with mature embryo sac of Spirodela polyrrhiza (x480)  
(from MAHESHWARI and MAHESHWARI 1963)

Ap antipodes  
EC egg-cell  
ES embryo sac  
F funicle  
II inner integument

Mi micropyle  
NC nucellar cap  
OI outer integument  
PEN primary endosperm nucleus

small nucellar cap remains (fig. 2.60). The embryo sac develops monosporically (normal type) in Spirodela (BROOKS 1940, MAHESHWARI and MAHESHWARI 1963), and disporically: Scilla- or Allium-type in Lemna (LAWALREE 1952 in L. minor, MAHESHWARI 1956b, MAHESHWARI and KAPIL 1963a in L. aequinoctialis), and in Wolffia (GUPTA 1935 in W. globosa named as W. arrhiza; MAHESHWARI 1954, 1956a in W. microscopica). In Lemna and Wolffia, the upper cell degenerates and the lower develops into the 8-nucleate embryo sac. The embryo sac of S. punctata is similar to that of S. polyrrhiza (BROOKS 1940); however, it has not been proven to be monosporic. Therefore, it is not certain whether the monosporic embryo sac is a characteristic of the genus Spirodela. In the mature stage, the embryo sac of all species contains 8 nuclei.

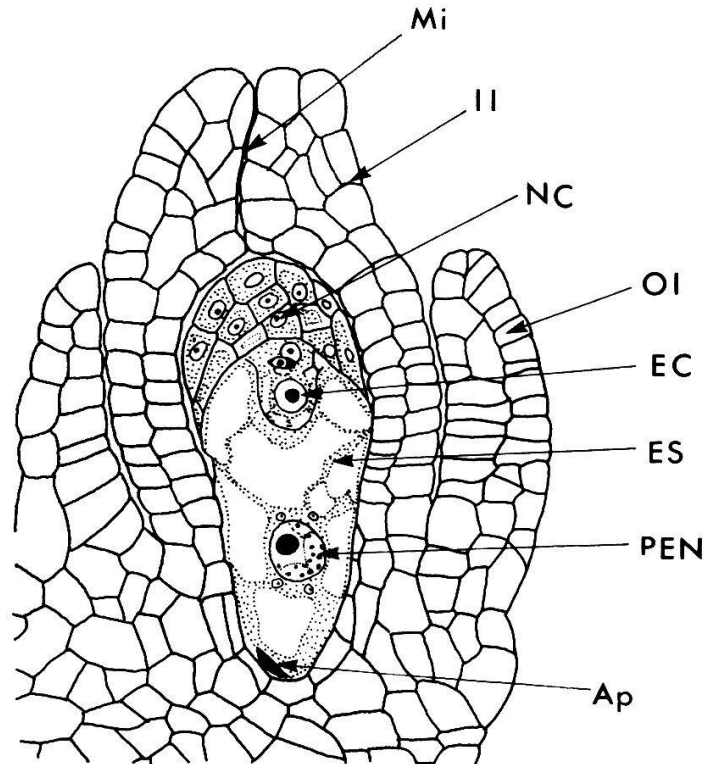


Fig. 2.56. Ovule of Lemna aequinoctialis (x480) (after MAHESHWARI and KAPIL 1963a)

Ap	antipodes (degenerated)	Mi	micropyle
EC	egg-cell	NC	nucellar cap
ES	embryo sac	OI	outer integument
II	inner integument	PEN	primary endosperm nucleus

After **pollination**, the pollen grains germinate on the stigma (fig. 2.57). The pollen tube grows through the style duct and through the micropyle into the ovule (porogam). According to HEGELMAIER (1868), it takes 24 hours for the pollen tube of L. gibba to reach the micropyle after germination. Although fertilization has never been observed, LA-WALREE (1952) assumed that normal, double fertilization takes place.

**Embryonic development** follows the Asterad-type (fig. 2.58): the fertilized egg cell divides into an apical and a basal cell. During maturing of the seed, the apical cell develops into the cotyledon and the basal cell develops into the hypocotyl with the suspensor and the primordium

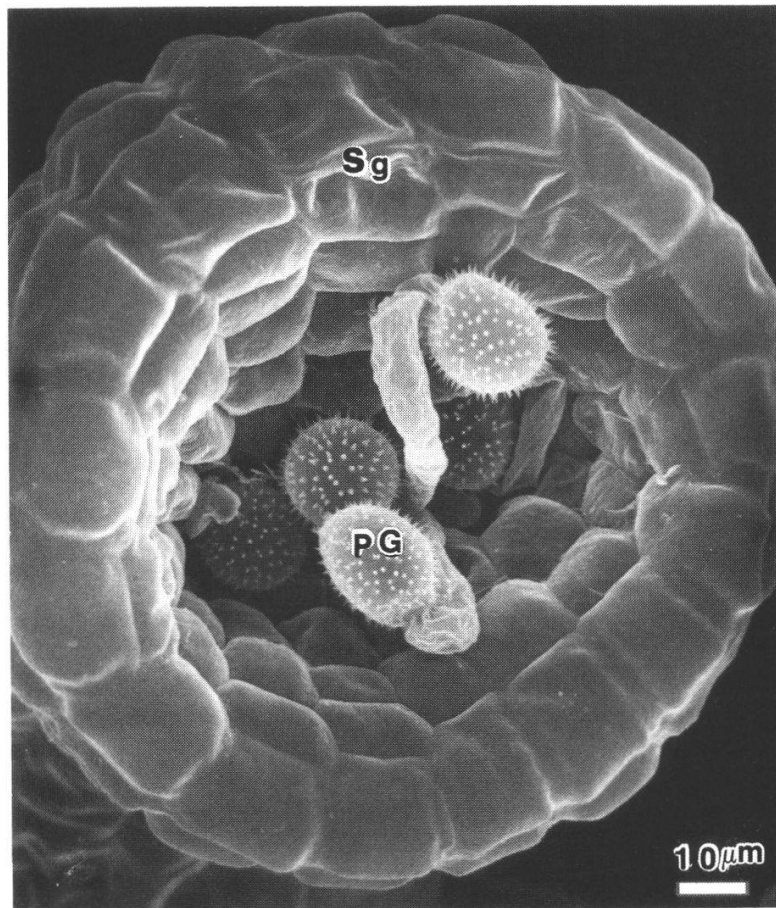


Fig. 2.57. Pollen grains of Lemna aequinoctialis germinating on the stigma (x700) (from SHIH 1979)

PG pollen grain

Sg stigma

of the first frond. In the mature embryo, it is not possible to distinguish sharply between cotyledon, hypocotyl and suspensor (figs. 2.59, 2.60). Therefore, this last organ has been interpreted differently. BLODGETT (1923) and LAWALREE (1952a) deny the existence of a suspensor. On the other hand, JOHANSEN (1950), SOUEGES (1959) and MAHESHWARI and KAPIL (1963b) refer to the cell group below the micropyle as a suspensor. The suspensor does not contain chloroplasts, according to HEGEL-MAIER (1868). Together with the short hypocotyl, the cotyledon forms a pouch containing the first frond. In this first frond of the mature embryo, there are already pouches with further daughter fronds visible; a radicula is missing.

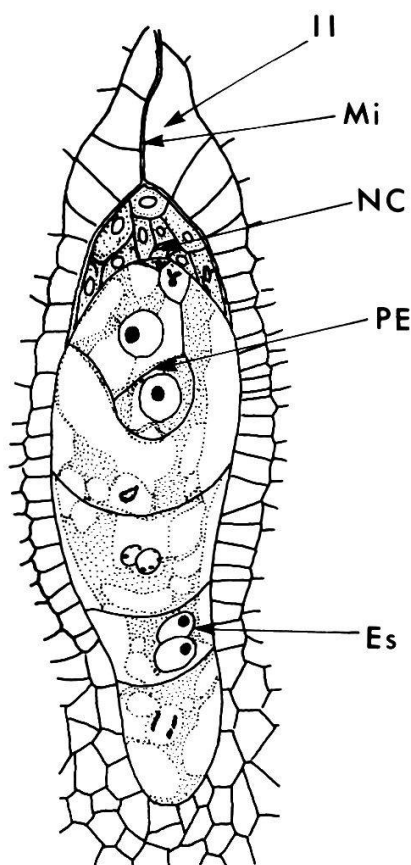


Fig. 2.58. Fertilized embryo sac of Lemna aequinoctialis showing pro-embryo and 8-celled endosperm (x800) (after MAHESHWARI and KAPIL 1963b)

Es	endosperm	NC	nucellar cap
II	inner integument	PE	proembryo
Mi	micropyle		

The **endosperm** develops from the endosperm nucleus, which divides before the zygote divides. The first division segments the embryo sac in a micropylar and a chalazal chamber. In *Spirodela* and *L. minor*, the chalazal chamber does not divide further but grows into a large cell rich in nutrient reserves. The chalazal chamber of *L. aequinoctialis* and *W. microscopica* develops further to an 8-cell state (MAHESHWARI and KAPIL 1963a). Cell walls are formed after each cell division: Therefore, the

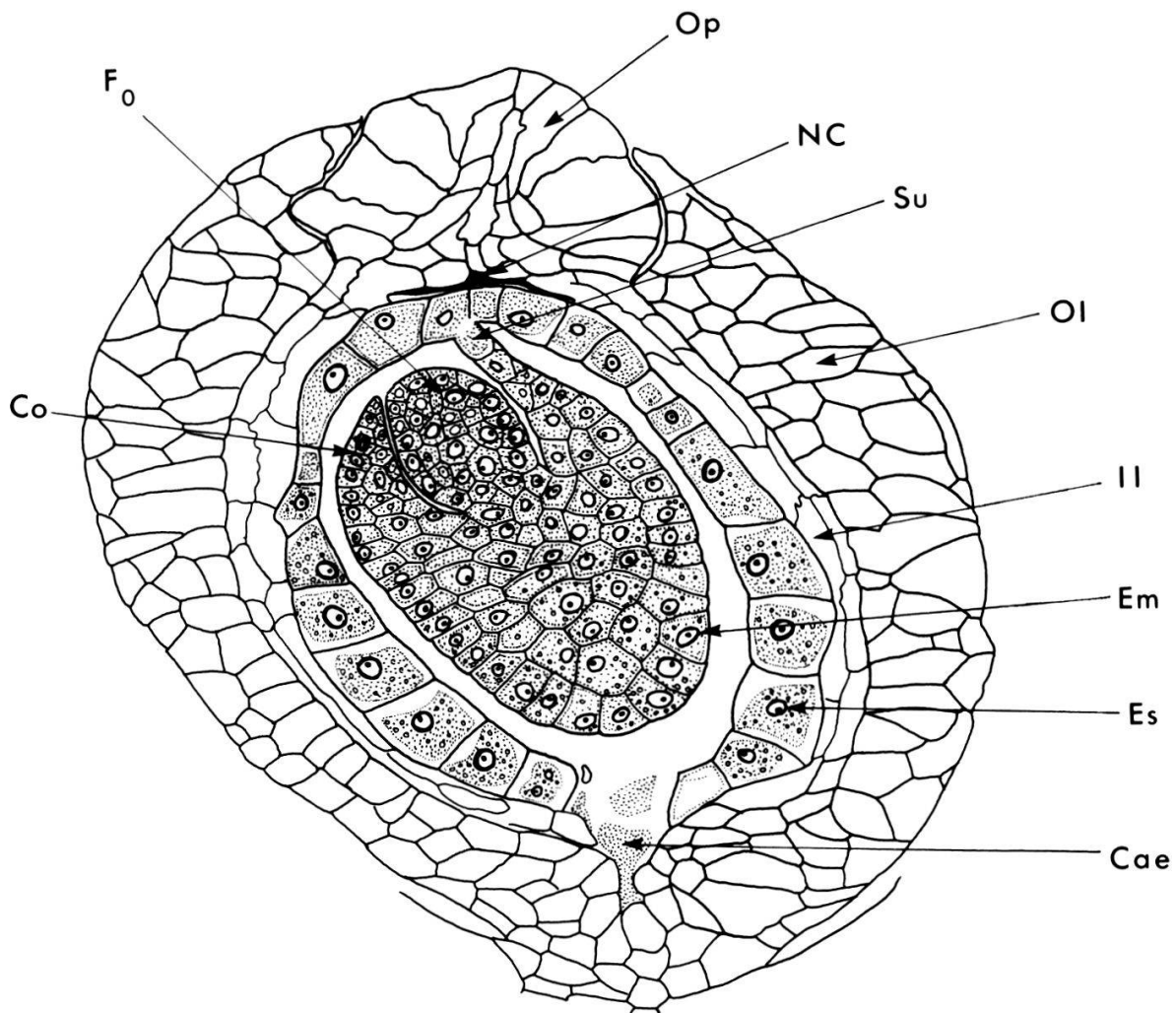


Fig. 2.59. Longitudinal section of a seed of *Wolffia microscopica* (x400)  
(after MAHESHWARI 1954)

Cae	haustorial protuberance (caecum)	II	inner integument
Co	cotyledonar sheath	NC	nucellar cap
Em	embryo	OI	outer integument
Es	endosperm	Op	operculum
F <sub>0</sub>	first frond of the embryo	Su	suspensor-hypocotyl

endosperm is cellular. There are no free nuclear divisions in the chambers, which is typical for Helobial division. Already BROOKS (1940) observed a cellular endosperm in L. minor. According to MAHESHWARI and KAPIL (1963b), LAWALREE (1952a) who reported nuclear endosperm failed to notice these cell walls. The authors assume that there is a cellular endosperm for all species of Lemnaceae. The endosperm of the mature seed consists of only 1-5 cell layers in the Lemnoideae and 1 cell layer in the Wolffioideae along the inner side of the seed coat (figs. 2.59, 2.60). KANDELER (1975) reports 1 layer in L. gibba and 2 to 4 layers in L. minor; MAHESHWARI and KAPIL (1964) found 3 to 4 layers along the longitudinal walls of L. aequinoctialis but only one layer towards the micropyle. It forms a thin haustorial projection towards the back (caecum) which penetrates the chalaza.

The endosperm, as well as the mature embryo, contains many starch grains. Also some droplets of oil could be detected by HEGELMAIER (1868).

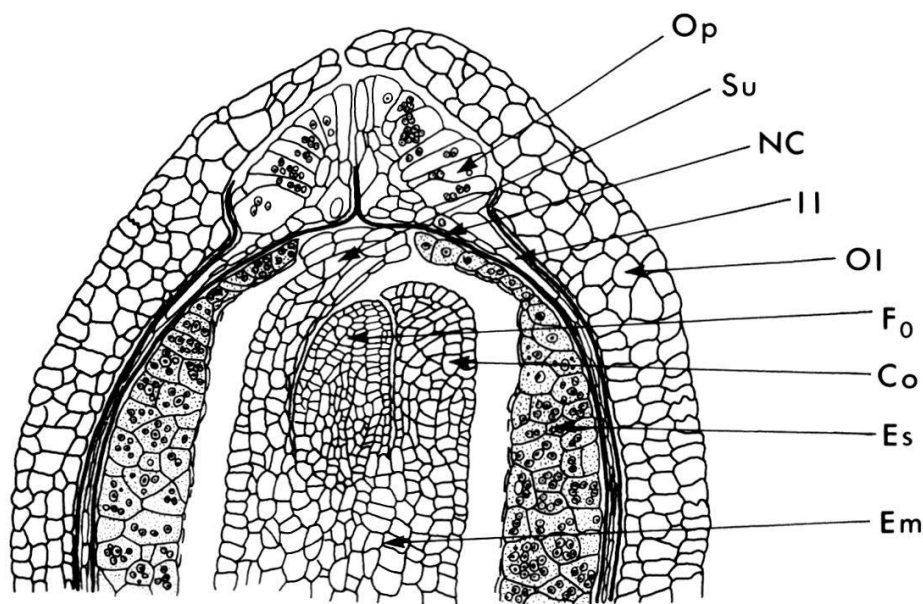


Fig. 2.60. Longitudinal section of the distal part of a seed of Lemna aequinoctialis (x250) (after MAHESHWARI and KAPIL 1963b)

Co cotyledonar sheath  
Em embryo  
Es endosperm  
F<sub>0</sub> first frond  
II inner integument

NC nucellar cap  
OI outer integument  
Op operculum  
Su suspensor

#### 2.6.4. Ripening of flowers

The many observations made of the ripening of male and female parts of the Lemnaceae flower do not always agree with one another. The stamens are considered to be ripe when the walls of the locules open and pollen grains appear. The stigma is thought to be ripe for pollination when the spherical droplet is present (fig. 2.61). The droplet contains sucrose as the only sugar component (ESKUCHE and ROMERO FONSECA 1982). HEGELMAIER (1868) and ENGLER (1877) believed that the stigma is ready for pollination the moment the anthers open. LUDWIG (1909) observed the development of the stamens of L. minor before the appearance of the style. However, most authors agree that the following Lemnaceae species are predominantly protogynous (e.g. KNUTH 1909): S. intermedia (GIARDELLI 1939a), S. polyrrhiza (ENGELMANN 1870, ROSTOWZEW 1905, BROOKS 1940), L. trisulca (KALBERLAH 1895, VUYCK 1895a,b, ROSTOWZEW 1901, 1905, WARNSTORF in LUDWIG 1909), L. gibba (VUYCK 1985a,b, GIARDELLI 1937, RUIZ LEAL 1951), L. minor (KALBERLAH 1895, VUYCK 1895a,b, ROSTOWZEW 1905, WARNSTORF cited in LUDWIG 1909, MARIE-VICTORIN 1931), L. minuscula (JOVET and JOVET-AST

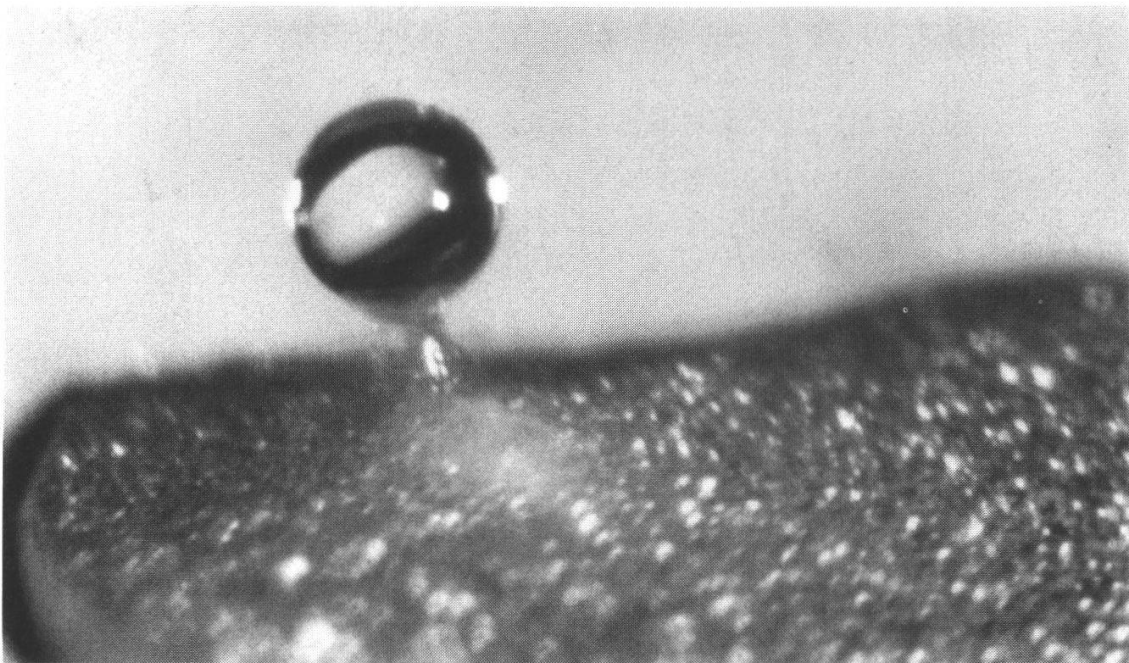


Fig. 2.61. Spherical droplet on the stigma of Wolfffiella lingulata (x80)  
(photo by W.P. Armstrong, San Marcos, Cal., U.S.A.)

1967, named as L. valdiviana), W. Welwitschii (GIARDELLI 1947), W. lingulata (MASON 1938, ESKUCHE and ROMERO FONSECA 1982), W. oblonga (GIARDELLI 1935), W. gladiata (KURZ and CROWSON 1949), W. denticulata (OBERMEYER-MAUVE 1966), W. microscopica (MAHESHWARI 1954), W. brasiliensis (BROOKS 1940, HESS 1986), W. borealis (ARMSTRONG 1982), and W. globosa (HAMASHIMA 1978b, named as W. arrhiza). My own observations confirm the protogyny for S. polyrrhiza, S. punctata, L. turionifera, L. minor, L. gibba, L. disperma, L. obscura, W. Welwitschii, W. lingulata, W. oblonga, W. brasiliensis, W. australiana, W. angusta, and W. arrhiza.

In L. aequinoctialis (e.g. SHIH 1979) and L. perpusilla, the ripening of the stigma very often coincides with the opening of the first anthers or follows it; this might also happen sometimes for W. Welwitschii (MONOD 1949). In L. aequinoctialis, it is known that different clones behave differently. BEPPU and TAKIMOTO (1983), BEPPU et al. (1985) report of protogyny in their S-type (called L. aequinoctialis s.str.) and partly synchronous maturation of stamens and pistil in the  $N_1$ - and  $N_2$ -types (called L. aoukikusa). There is the possibility that other species, too, might open the anthers before or at the same time as the ripening of the stigma. Wrong identifications of the investigated plants are also not to be excluded. External factors possibly have an influence on the ripening of the stigma.

The unisexual development of flowers of Lemnaceae have been studied several times and have been observed to be susceptible to culture conditions. SCHLEIDEN (1844) reports the development of only stamens in flowers of S. polyrrhiza. DEN HARTOG (1968) saw only ovary in some cultures of L. gibba; I have made the same observations in several clones of L. gibba (under certain conditions) but never in L. aequinoctialis (unpublished observations). According to HUEGEL (1974, 1976a,b,c) gibberellic acid stimulates the development of stamens, ethylene (also Ethrel and CCC), the one of the ovary. During in vitro development of explanted flower primordia, the long-day plant L. gibba shows a tendency towards "feminization" and L. aequinoctialis, towards "masculinization", which must be due to a difference in endogenous hormone production in the meristems of both species. A surplus of auxines or ethylene induces the "feminization", a surplus of gibberellin the "masculinization". Small amounts of kinetin and ABA can increase the degree of gender expression in the two species.

ROSTOWZEW (1905), having observed flowering S. polyrrhiza, L. trisulca

and L. minor in nature, gives some detailed information about the development of their flowers. The first (distal) stamen elongates two days after the ripening of the stigma, which by then is already wilting. The second (proximal) stamen develops shortly after the first. Both stamens stay turgescient for 7 to 9 days.

The pollination of flowers and the fruit setting is described in chapter 4.3.2.

#### 2.6.5. Fruits and seeds

It takes 2-5 weeks after fertilization for fruits to ripen. Photographic pictures of the fruits of different Lemnaceae are placed together in fig. 2.62. Each fruit has a dry pericarp and usually contains one seed (1-5 in S. intermedia and L. gibba, 1-2 in S. polyrrhiza, S. punctata, and L. disperma). The membranous pericarp, with remnants of the short style and the stigma, consists in the upper part of 3-5 cell layers and, in the middle and lower part, of 2-3 cell layers (HEGELMAIER 1868). In all species the fruit is nearly symmetrical, except for species of L. valdiviana and L. perpusilla group in which the style is attached laterally of the very top (in direction of the frond base). Fruit length varies between 0.35 mm (in Wolffioideae) and 2.75 mm (in S. intermedia). The fruits of species with anatropous or amphitropous ovules are mostly winged laterally towards the top, with the width of this winged border

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#### Origins of the fruits in fig. 2.62 (p. 116 and 117)

- a. No. 8252 from Surinam
- b. Pedersen 2813 from Corrientes, Argentina
- c. Evans 14.12.1966 from NSW, Australia
- d., e. No. 7219 from Cape, South Africa
- f. s.coll. from Tasmania, Australia
- g. Godfrey 61710 from Florida, U.S.A.
- h., i. Berkheimer 5782 from Pennsylvania, U.S.A.
- k., l. Macoum 76888 from Manitoba, Canada
- m. No. 7703 from Corrientes, Argentina
- n., o. No. 7507 from North Carolina, U.S.A.
- p. Ahlen 1083 from Louisiana, U.S.A.
- q. Hitchcock 20096 from Ecuador
- r. Ahles 56562 from South Carolina, U.S.A.
- s. No. 7267 from Tasmania, Australia



Fig. 2.62. Fruits of different Lemnaceae (from herbarium specimens) (x8)  
(for origins see p. 115)

- a. Spirodela intermedia (unripe fruit from above)
- b. Spirodela intermedia (from above, with a separate fruit)
- c. Spirodela punctata (from above, with a separate seed)
- d. Lemna gibba (from above). e. Lemna gibba (from below)
- f. Lemna disperma (from above)
- g. Lemna obscura (from above)
- h. Lemna minor (from below). i. L. minor (from above)

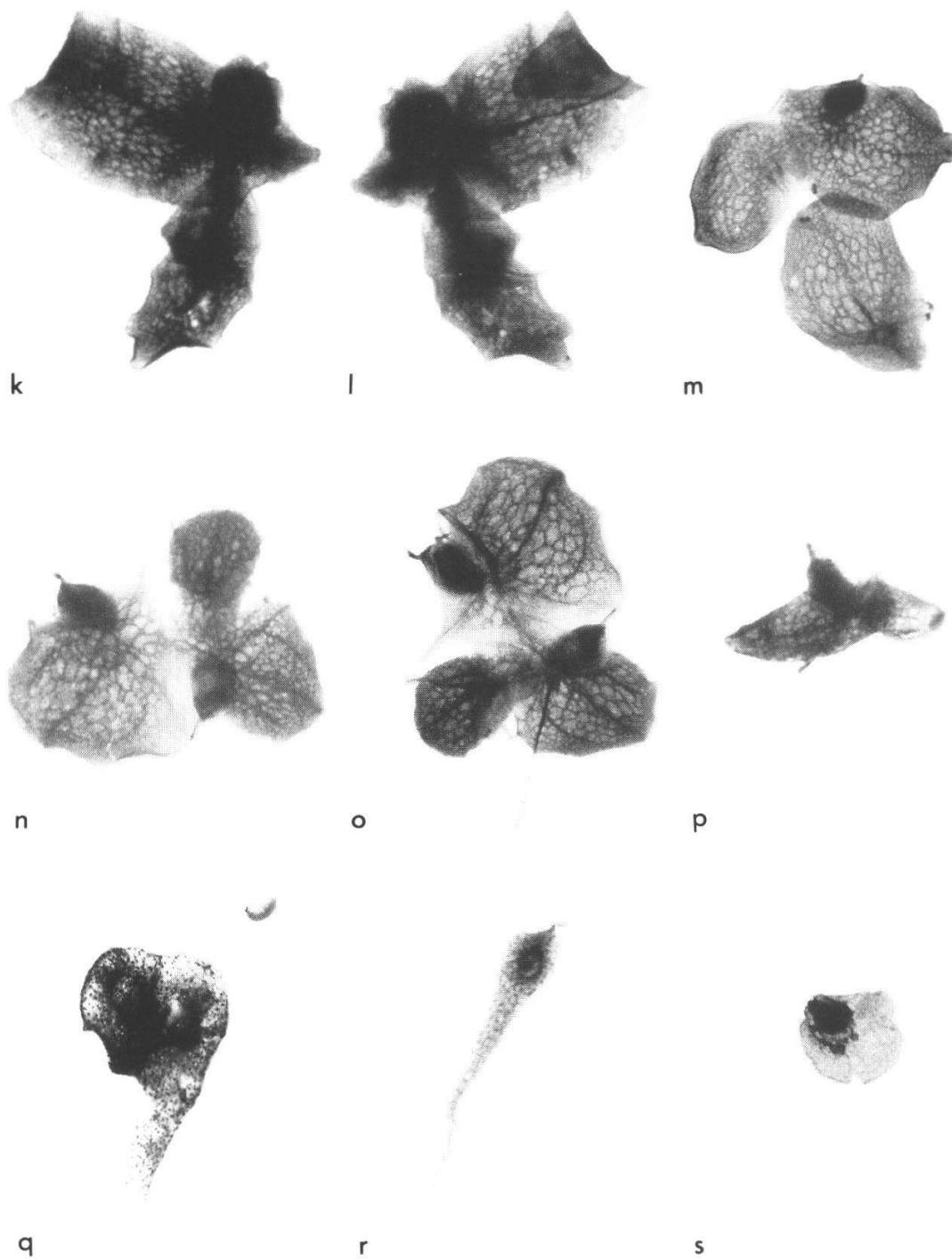


Fig. 2.62 (continued)

- k. Lemna trisulca (from above). l. Lemna trisulca (from below).  
m. Lemna aequinoctialis (from above; the lower frond from below).  
n. Lemna perpusilla (from above). o. Lemna perpusilla (from below).  
p. Lemna valdiviana (from above).  
q. Wolffiella Welwitschii (from above, with a separate seed).  
r. Wolffiella gladiata (from above). s. Wolffia australiana (from the side).

species-specific: S. intermedia (0.3 mm), S. polyrrhiza (0.15 mm), S. punctata (0.1 mm), L. trisulca (0.15 mm), L. gibba (0.1-0.2 mm), L. disperma (0.05-0.1 mm), L. minor (0.05-0.1 mm; the wingless fruits shown by MASON 1957 probably belong to L. turionifera), and L. obscura and L. turionifera (no distinct, winged border). Of the species with orthotropous ovules, only L. perpusilla has a winged border (0.05 mm).

The fruits of Spirodela, L. trisulca and the group of L. minor are often reddish or red-spotted due to the presence of anthocyanins in the sub-epidermal layer.

The length and diameter of the style is specific for many species of Lemnoideae. The style is longer than 0.2 mm in S. intermedia, S. polyrrhiza, L. perpusilla, and L. minuscula; it is between 0.05 mm and 0.1 mm in L. gibba, L. disperma, and L. aequinoctialis. The style of most other species of Lemnoideae is between 0.1 and 0.2 mm long. In Wolffioideae, the style is shorter than 0.05 mm.

Bursting causes the opening of the fruit. This bursting of the pericarp and the release of the seed takes place in L. aequinoctialis just after ripening of the seed. In L. gibba and L. disperma, bursting of the pericarp takes more time, and in L. perpusilla and L. minor, the ripe seed stays within the pericarp for many weeks (cf. KANDELER 1975 for L. perpusilla). There are no precise observations of this phenomena for most species.

The seeds of Lemnaceae are ovoid in shape, with a dark, pointed operculum at the top. In fruits with more than one seed (e.g. L. gibba), the

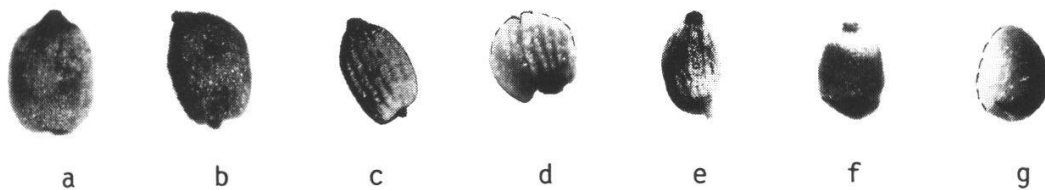


Fig. 2.63. Seeds of some Lemnaceae (x16)

- a. Lemna turionifera (Jennings 2830 from Ontario, Canada)
- b. Lemna perpusilla (Ahles 58805 from North carolina, U.S.A.)
- c. Lemna aequinoctialis (No. 6746 from California, U.S.A.)
- d. Lemna valdiviana (Poppleton et al. 580 from Florida, U.S.A.)
- e. Lemna minuscula (No. 8662 from Corrientes, Argentina)
- f. Wolffiella hyalina (Chevalier 10040 from Chad)
- g. Wolffiella Welwitschii (Hitchcock 20096 from Ecuador)

ovoid form becomes flattened or triangular. The chalaza is recognizable as a dark point at the base. Figs 2.63 and 2.64 show the seeds of different Lemnaceae. The seeds of Spirodela and Lemna are longitudinally ribbed; fine stripes are at right angles between the ribs (fig. 2.65). The number of ribs is species-specific. There are 35 to 70 ribs in L. perpusilla and L. turionifera, and 8 to 22 ribs in all other species of Spirodela and Lemna as far as it is known. The seed surface of Wolffiel-la and Wolffia is nearly smooth (slightly reticulated). It would be worthwhile to investigate the structure of the seed coats of Lemnaceae more closely. In fig. 2.64 some of the variations between different species are shown. Unfortunately, some of the seeds are either too old or not quite ripe. Therefore the following indications are only preliminary. It seems that the structure of S. punctata, L. gibba, L. minor, L. obscura, and L. trisulca are very similar: there are 1-2 rows of stretched fossules between the ribs and 3-4 rows of smaller and shallower more or less square fossules on the ribs. Therefore the ribs look wide and rounded. Differently, the fossules of L. turionifera and L. perpusilla are all similar in magnitude and arranged in many longitudinal rows (sometimes two fossules within a row). The ribs consist of the wall of the fossules and are therefore very narrow. Since the fossules are rather small and not very deep, the ribs look not very distinct. The fossules of L. aequinoctialis are narrower in the longitudinal direction and longer in the tangential direction than those of L. perpusilla. The ribs are less numerous but more pronounced. In L. valdiviana, there are

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**Origins of the fruits in the figures 2.64 (p. 120) and 2.65 (p. 121)**

- a. S. polyrrhiza from N.S.W., Australia (Evans 14.12.1966)
- b. L. gibba from Eem deposits in Poland (Tobolski, sent 1982)
- c. L. minor from Pennsylvania, U.S.A. (Berkheimer 3289)
- d. L. obscura from Florida, U.S.A. (Godfrey 61710)
- e. L. turionifera from Ontario, Canada (Jennings 2.8.1930)
- f. L. trisulca from North Dakota, U.S.A. (Harms and Ward 3643)
- g. L. perpusilla from Arkansas, U.S.A. (ETH No. 8071)
- h. L. aequinoctialis from Oklahoma, U.S.A. (ETH No. 8011)
- i. L. valdiviana from Florida, U.S.A. (Lakela 25940)
- k. W. hyalina from Chad (Chevallier 10040)
- l. W. australiana from Tasmania, Australia (ETH No. 7267)
- m. W. arrhiza from Angola (ETH No. 7452)

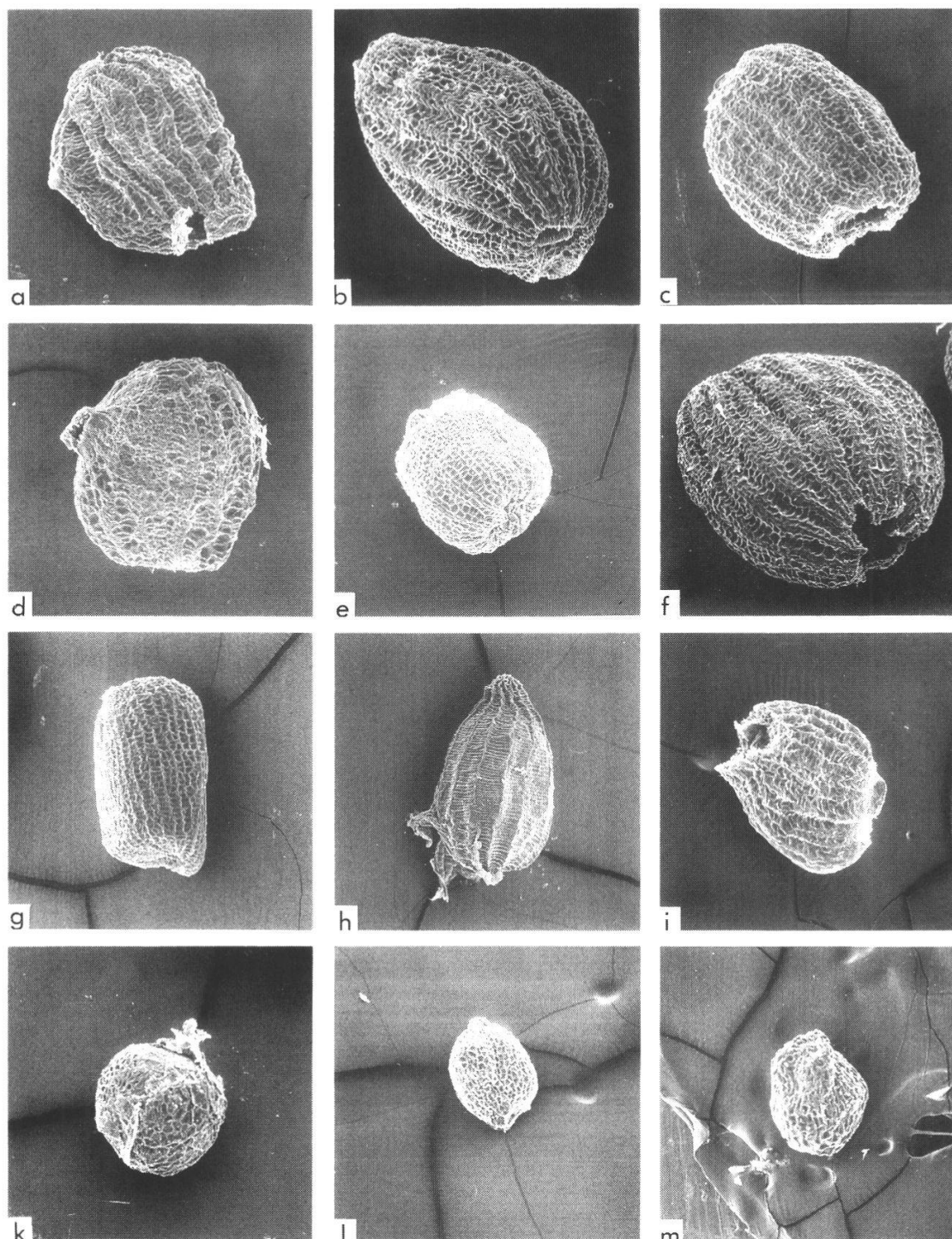


Fig. 2.64. Seeds of different Lemnaceae (scanning electromicroscopic photographs) (the photographs were kindly done by Dr. E. Wehrli, Zürich) (x30) (for origins see p. 119)

- |                              |                                |                               |
|------------------------------|--------------------------------|-------------------------------|
| a. <u>Spirodela punctata</u> | e. <u>Lemna turionifera</u>    | i. <u>Lemna valdiviana</u>    |
| b. <u>Lemna gibba</u>        | f. <u>Lemna trisulca</u>       | k. <u>Wolffiella hyalina</u>  |
| c. <u>Lemna minor</u>        | g. <u>Lemna perpusilla</u>     | l. <u>Wolffia australiana</u> |
| d. <u>Lemna obscura</u>      | h. <u>Lemna aequinoctialis</u> | m. <u>Wolffia arrhiza</u>     |

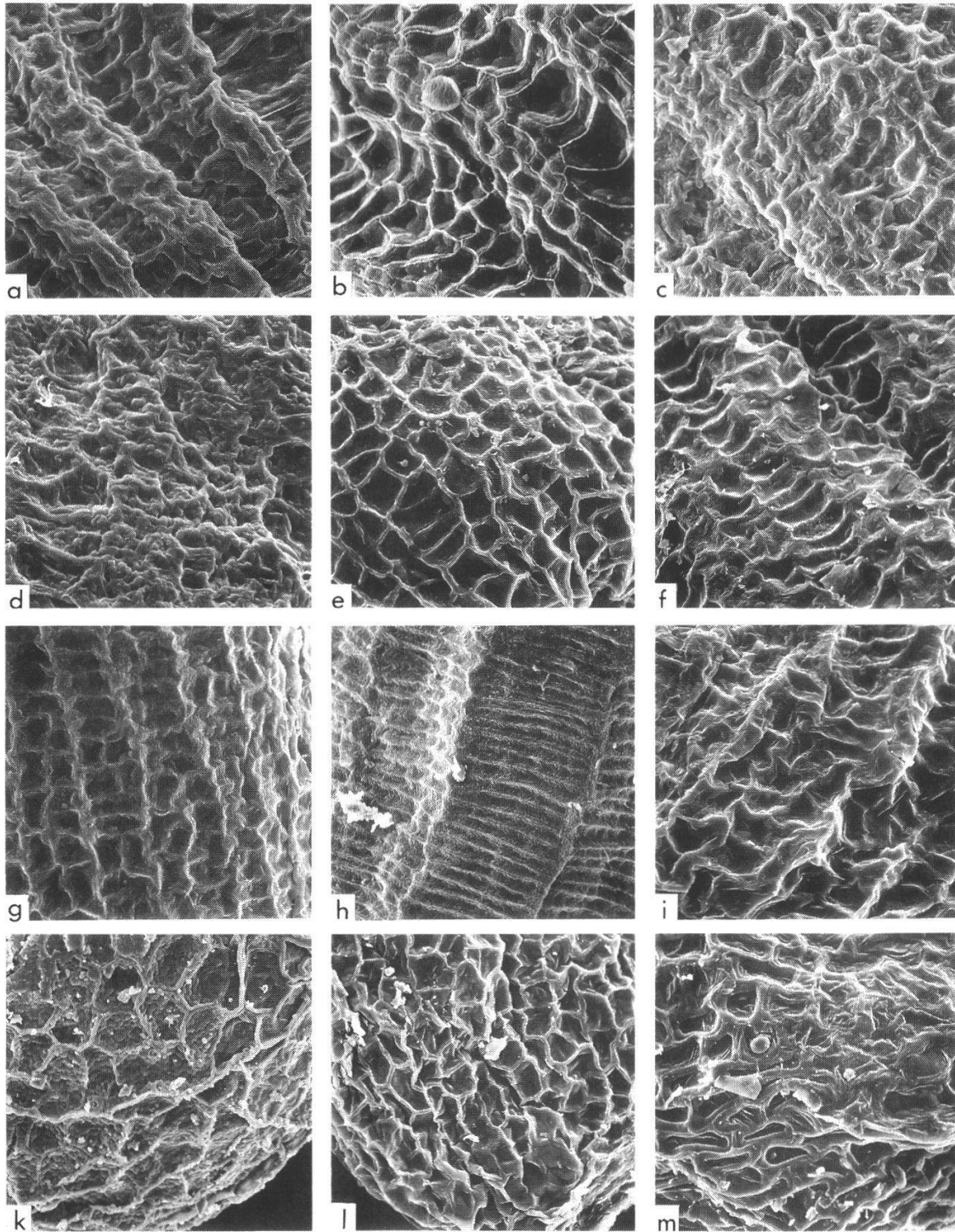


Fig. 2.65. Seed structures of different Lemnaceae (scanning electron-microscopic photographs kindly done by Dr. E. Wehrli, Zürich) (x150) (for origins see p. 119)

- |                              |                                |                               |
|------------------------------|--------------------------------|-------------------------------|
| a. <u>Spirodela punctata</u> | e. <u>Lemna turionifera</u>    | i. <u>Lemna valdiviana</u>    |
| b. <u>Lemna gibba</u>        | f. <u>Lemna trisulca</u>       | k. <u>Wolffiella hyalina</u>  |
| c. <u>Lemna minor</u>        | g. <u>Lemna perpusilla</u>     | l. <u>Wolffia australiana</u> |
| d. <u>Lemna obscura</u>      | h. <u>Lemna aequinoctialis</u> | m. <u>Wolffia arrhiza</u>     |

2-3 rows of fossules between the ribs and, similarly as in L. perpusilla and L. aequinoctialis, no fossules on the ribs. The more or less pentagonal fossules of the Wolffioideae are arranged reticularly and not in rows. On the surface of some of the larger fossules (e.g., in L. gibba and W. hyalina), some small rounded elevations can be observed. It is not known if these structures are specific or not. The size of the seeds varies within a species and between different species from 0.3 mm to

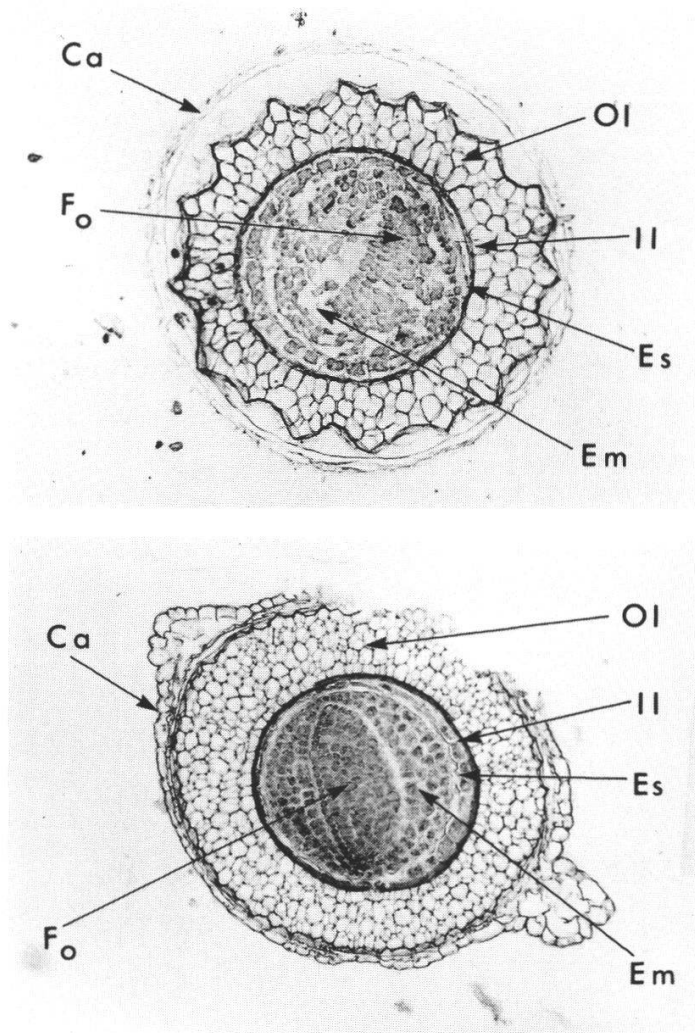


Fig. 2.66. Transverse sections of the fruits of Lemna aequinoctialis (above) and Lemna perpusilla (below) (x110) (from KANDELER and HUEGEL 1974a)

Ca	carpel	II	inner seed coat (corresponds to the inner integument)
Em	embryo	OI	outer seed coat (corresponds to the outer integument)
Es	endosperm		
F <sub>0</sub>	first frond		

2.0 mm in length and from 0.2 mm to 0.8 mm in diameter. The smallest seeds are found in Wolffia and Wolffiella; the largest ones in S. intermedia. The seed consists of an inner and an outer seed coat, an endosperm and an embryo. The outer seed coat is formed by 2 to 11 layers of cells of the outer integument; there are more cell layers towards the top than in the middle or at the base of the seed. The different species are characterized by the following number of layers towards the top of the seed (the epidermis is not counted): 9-11 in S. polyrrhiza (HEGELMAIER 1871), 7 in L. trisulca, L. gibba, L. minor (HEGELMAIER 1868), 5-7 in L. perpusilla (KANDELER and HUEGEL 1974a, fig. 2.66), 2-4 in L. aequinoctialis (HEGELMAIER 1868, MAHESHWARI and KAPIL 1964, KANDELER and HUEGEL 1974a, fig. 2.66), 3-4(5) in W. hyalina, W. brasiliensis (HEGELMAIER 1868), and W. columbiana (BROOKS 1940), 2-3 in W. microscopica (MAHESHWARI 1954), W. Welwitschii, (MONOD 1949), W. repanda (HEGELMAIER 1868), and W. gladiata (KURZ and CROWSON 1949). The upper part of the inner integuments which enclose the micropyle develops into the dark coloured operculum. The region of the chalaza also becomes dark.

#### 2.6.6. Germination of seeds

The germination of seeds is described by HEGELMAIER (1868), CALDWELL (1899), ROSTOWZEW (1905), GOEBEL (1921), BLODGETT (1923), MASON (1938), MAHESHWARI (1956a), MAHESHWARI and KAPIL (1964), POSNER and HILLMAN (1962), GLICKMAN (1966), DE SLOOVER (1966), ROSSI (1969), REJMANKOVA (1976), and HAMASHIMA (1978a).

In germination (fig. 2.67), the operculum is first pushed off the suspensor. Next, the cotyledon appears, followed by the first frond which is enclosed by a cotyledonar sheath (GOEBEL 1921); in a similar way normal fronds are enclosed in the pouch. Already the cotyledon shows stomata in its upper surface: 30-40 in L. gibba, 7-8 in L. minor, 0 in L. trisulca (according to HEGELMAIER 1868). In S. polyrrhiza (HEGELMAIER 1871, BROOKS 1940), L. trisulca (HEGELMAIER 1868, BROOKS 1940), L. gibba (HEGELMAIER 1868, HAMASHIMA 1978a), L. minor (HEGELMAIER 1868, ROSTOWZEW 1905, BROOKS 1940, LAWALREE 1952), the first root develops at the base of the first frond, producing a convexity in the hypocotyl; the root perforates the hypocotyl just before the first frond begins to develop.

In L. perpusilla (BLODGETT 1923, BROOKS 1940, LANDOLT, unpublished observations) and in L. aequinoctialis (MAHESHWARI 1956b) the first root appears from the second frond; according to HAMASHIMA (1978a), the first root comes partly from the second or the third frond. (L. aequinoctialis is mentioned under the name of L. paucicostata and L. perpusilla). The first frond of the embryo grows into a right, horizontal position on the water surface, whereas the heavier seed emerges in the water. At this point, the embryo is already turning green within the closed seed coat (LUDWIG 1909). According to TILLICH (1985) the embryo sac of Lemna is

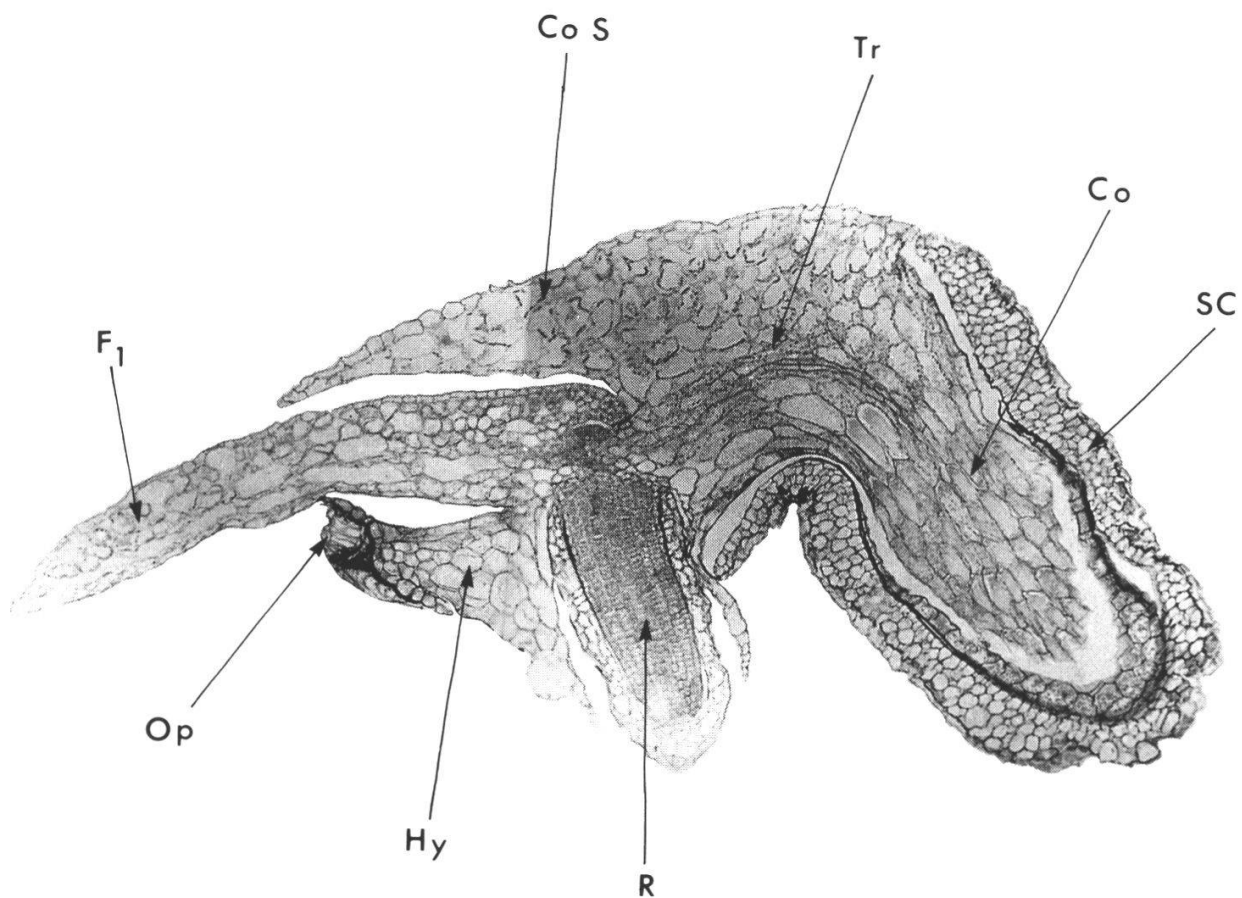


Fig. 2.67. Transverse section through a germinating seed of Lemna gibba (preparation: R. Kandeler, Vienna; photo: R. Wolf, Vienna) (x100)

Co	cotyledon	Op	operculum
CoS	cotyledonar sheath	R	root of the first frond
F <sub>1</sub>	first frond	SC	seed coat
Hy	hypocotyl	Tr	tract of elongated cells

similar in many respects to that of Pistia: no primary root; the adventitious root has the same position on the hypocotyl; the operculum sticks to the hypocotyl for a long time.

L. perpusilla is already able to flower with the appearance of the second or third frond after germination (BLODGETT 1923). In L. aequinoctialis, however, it takes a few generations before flowering is possible (HILLMAN 1975a).

The seeds of the following species are drought-resistant: S. punctata (own observations), L. gibba (WILSON 1830, LANDOLT 1957, REJMANKOVA 1976, WITZTUM 1977), L. disperma (EVANS 1970), L. aequinoctialis (many authors), and L. perpusilla (BLODGETT 1923). It is probable, although not proven, that the seeds of the following species can tolerate desiccation: W. Welwitschii, W. hyalina, and W. microscopica. These species generally grow in areas that dry out periodically. The seeds of L. minor fail to survive drying (ROSTOWZEW 1901, 1905).

The seeds of the following species are able to withstand cold temperatures (around 0°C or a few degrees below) for several weeks: L. gibba (REJMANKOVA 1976), L. minor (ROSTOWZEW 1905), L. aequinoctialis and L. perpusilla (KANDELER 1975, LANDOLT, unpublished observations). ROSSI (1969) stored a seed of S. intermedia at 5°C for 60 days, which afterwards was able to germinate in darkness at 25°C.

In contrast to L. aequinoctialis, L. gibba, L. minor, L. minuscula and other species, seeds of L. perpusilla are not able to germinate immediately after achieving maturity (KANDELER and HUEGEL 1974a). The germination proceeds only after chilling (e.g. by keeping the culture in a refrigerator for one month at 4-6°C (KANDELER 1975). Delivering the embryo from the seed coat, however, makes germination possible without chilling. It is assumed that a substance, presumably ABA, inhibiting germination is located in the seed coat (KANDELER and HUEGEL 1974a). Own experiments (LANDOLT, unpublished observations) corroborate the results of KANDELER (1975); it could be shown that a longer storage period at low temperatures is necessary for germination to proceed later. Dry seeds did not germinate in nutrient solution at temperatures of 25°C nor after storage for three weeks at 3°C. However, after further 10 weeks of storage at temperatures between 7°C and -6°C in the solution, 35 of the 38 seeds germinated within 10 days at 25°C. Four weeks of storage at the same temperatures had no effect. Low temperature treatment stimulates, but is not necessary for, the germination of L. aequinoctialis. Differ-

ent strains show a different reaction (BEPPU pers.comm. 1986).

The seeds of L. gibba can survive drying for more than half a year (WILSON 1830). The first winter after collecting and storing seeds in water at room temperature, REJMANKOVA (1976) achieved 100% germination; during the second winter, 70%, and during the third winter, 1%. Storage at 0°C did not affect the percentage of germination very much. In the second winter, dry seeds had a germination rate between 3 and 7.6% (in the first and the third winter the rate was not measured). The seeds of L. aequinoctialis are able to germinate up to at least 10 months later (stored at 4-7°C or at 23°C) (HILLMAN 1975a).

Light is necessary for the germination of the seeds of L. minor (ROSTOWZEW 1905), L. aequinoctialis (POSNER and HILLMAN 1962a, GLICKMAN 1966, GLICKMAN and POSNER 1966), and L. gibba (REJMANKOVA 1976). However, a dark pretreatment enhanced germination of L. aequinoctialis seeds under continuous white light. The promotive effect of this dark pretreatment was completely inhibited by temperatures lower than about 10°C (GLICKMAN and POSNER 1966).

High temperatures accelerate the germination of seeds of L. gibba (REJMANKOVA 1976). In this study, REJMANKOVA achieved 45% germination after 8 days at 17°C and after 2 days at 25°C and 33°C. Germination in nature occurred in May at temperatures around 14°C. Plants of two different origins showed a somewhat different germination behaviour (REJMANKOVA 1976). L. minor is able to germinate in England when the water temperature reaches 5°C early in February (GUPPY 1894). For further details on germination of seeds see volume 2, chapter 2.4.2.3 (LANDOLT and KANDELER 1987).

## 2.7. USE OF MORPHOLOGICAL FEATURES IN TAXONOMY OF LEMNACEAE; PREPARATION OF TRANSPARENT SLIDES

As has been shown in the preceeding chapters, the morphological features of vegetative fronds are very difficult to use in classifying fronds, considering their many variations due to modifications and genetic differences. A considerable number of the characteristics of different species also overlap. Therefore, it is necessary to consider as many features of the fronds as possible to identify a species. In Lemnoideae, the fruits and seeds are very good specific characteristics, but unfortunately, fruits are very rare in most species. in Wolffioideae, there is almost no differentiation between the fruits.

Herbarium collections of Lemnaceae have to be made very carefully to conserve all important features of the fronds. It is recommended that characteristics of shape and colour be noted before drying. Some researchers prefer to preserve the fronds in 70% ethanol or in some other solutions to maintain the original shape. However, the disadvantage of this method is that colour is not preserved and, in some groups, the anthocyanin pattern is a very important distinctive feature (groups of S. polyrrhiza, L. minor). Other species are also characterized by pigment cells (Wolffiella, Wolffia). Therefore, dry herbarium specimens are indispensable.

The drying of the Lemnaceae fronds has to be done rather quickly. Parts of wood and other small objects that can prevent flattening of the fronds have to be removed before pressing. Well-preserved herbarium material can always be treated using special methods to determine further characteristics as shape, structure, nerves, air spaces, stomata, etc. One method for obtaining transparent slides of fronds is described thereupon. The fronds (living or dried) should be treated in the following manner:

- boiling for 2 minutes in 70% ethyl-alcohol
- immersing for a few minutes in 14% NaOCl (until the fronds were transparent)
- colouring for 12 hours (fresh material) or 2 hours (herbarium material) with Grenachers Alum Carmin (prepared by boiling 1 g Carmin in 5% aqueous solution of  $\text{Al}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2 \text{SO}_4 \cdot 24 \text{H}_2\text{O}$  for 1 hour)
- washing in distilled  $\text{H}_2\text{O}$  and serially immersing in 70% ethanol, 80%

ethanol, 95% ethanol, absolute ethanol, 2/3 absolute ethanol + 1/3 xylene, 1/3 absolute ethanol + 2/3 xylene, absolute xylene for 15-30 minutes each; then placing each frond in an imbedding medium.

These transparent long-lasting preparations permit the observation of the inner structures of fronds, as only the red-coloured cell walls are visible (see figs. 9.1 to 9.4). To see the surface structures, preparations have to be examined with a binocular microscope before immersion in xylene.