7. SYSTEMATIC POSITION, TAXONOMICAL RELATIONS, SPECIES DIFFERENTIATION

7.1. PALEOBOTANICAL RECORDS

Fossil records of Lemnaceae are very rare. Lemnaceae have so few charac-
teristics that it is very difficult and sometimes most speculative to
place rests of frond-like leaflets within the family.

First records date back to the upper Cretaceous and the Paleocene period
of North America (BELL 1949). But, as DAUBS (1965) already pointed out,
the description of this fossil Northamerican species Spirodela scutata
(Dawson) Lesq. does not fit any member of the Lemnaceae. He regarded the
plant as Nelumbo. A Protolemna described by SAPORTA (from VAN DER PLAS
1971) from strata above the later Jura has an axis bearing leaves with
dicotyledon-like venation. A further species described from the early
Tertiary, Spirodela penicillata (Lesq.) Cockerell has, according to
DAUBS (1965), many roots but no nerves. This points to a relationship
outside of the Lemnaceae (Spirodela always has distinct nerves). Another
species from the Eocene ascribed to the family of Lemnaceae is Spirodela
magna MacGinnitie which is bigger than S. polyrrhiza (up to 1.5 cm in
diameter) and shows a complex but even-textured network of veinlets
(MACGINNITIE et al. 1974). Also this last characteristic is quite un-
usual for Lemnaceae so that the position within the family of Lemnaceae
is rather dubious. A similar plant from the Miocene of Europe was
described as Hydromystria expansa (Heer) Hantke (HANTKE 1954). From the
descriptions, and in analogy to the plant of HANTKE, it seems more rea-
sonable to place all mentioned fossil species of Spirodela into the
genus Limnobium (incl. Hydromystria) (Hydrocharitaceae) which looks su-
perficially very similar to Spirodela.

More reliable are records of seeds and pollen grains. DOROFEEV (1963)
described a Lemna tertiaria from the Oligocene of Siberia. The seeds
have 6-10 ribs and are 0.8-0.9 mm long and 0.5-0.8 mm thick. These mea-
surements are just at the lower variation limit of the seeds of L. tri-
sulca or of species of the L. minor group. The lateral position of the
funiculus in the figures would suggest a species with more than one seed
(similar to L. gibba). MAI and WALTHER (1978) found similar seeds in the

lower middle Oligocene of the DDR. Moreover, seeds of a _Lemna_ species are recorded by FRIIS (1985) from the middle Miocene of Denmark and by KNOBLOCH (1981) from the upper Miocene of Moravia (CSSR). Seeds from Eem deposits in Poland (TOBOLSKI in lit. 1982) were checked by the present author and identified as _L. gibba_. SCULTHORPE (1967) collected many seeds of _L. minor_ from the Boreal and Atlanticum periods in Great Britain. More findings of _Lemna_ seeds or pollen grains from the Postglacial time are listed in TUEXEN (1974, p. 37). Seeds of _Lemna_ cf. _minor_ were discovered in Roman ditches (first century A.D.) and in moats of medieval castles by KNOERZER (from TUEXEN 1974 and KANDELER 1979). All seeds attributed to _L. minor_ in paleobotanical publications probably belong to _L. gibba_ which has very similar seeds and fruits much more frequently than _L. minor_.

TRIVEDI and VERMA (1972) identified pollen grains from coal deposits of the Eocene in Malaysia as _Lemnaceae_. But since similar structured pollen grains occur in other families of the monocotyledons (e.g. _Arisaema_), the identity is not quite sure. 27000 year-old pollen grains of _Lemnaceae_ were found by ACCORSI et al. (1982) in a turf layer near Verona. Pollen grains are also reported from the Atlanticum period (BURRICHTER 1969, from KANDELER 1979).

From the few fossil records of _Lemnaceae_ mentioned, it is evident that no conclusions can be drawn in relation to the systematic position of the family.

7.2. POSITION WITHIN THE PLANT KINGDOM

The great reduction of most characteristic organs of the Lemnaceae makes it very difficult to construct genetical relationships to other families and to put the family into a taxonomical group of a higher level. Although I did not investigate this problem particularly, I would like to discuss shortly the different arguments. As we saw already in chapter 7.1, there is scarcely an indication available from fossil records.

There was much discussion if the family of the Lemnaceae is at the end of a reduction line (as proposed by HEGELMAIER 1868, DAUBS 1965, DEN HARTOG 1975 and others) or if it is at the beginning of differentiation and, therefore, at the most primitive level of all the monocotyledons (cf. BOULGER 1881, LAWALREE 1945, CROIZAT 1971). Many arguments tell against the theory of an original position of the Lemnaceae, especially the very pronounced ecological specialization which points to a deductive position and the fact that many plant groups which are much older than the Lemnaceae (Gymnosperms, Pteridophytes) have reached a higher level of organization. In my opinion, it is evident that the low degree of differentiation of the Lemnaceae is due to passive adaptation to the highly specialized way of aquatic life. This adaptation must have originated already at an early stage of the development of the monocotyledons (lower Tertiary or upper Cretaceous) if we can trust the paleobotanical records.

There are mainly two opinions about the relations of the Lemnaceae within the monocotyledons: 1) The Lemnaceae attach to Pistia (Araceae) and must be included in the Arales (and Spadiciflorae) (e.g. HEGELMAIER 1868, ENGLER 1877, VELENOVSKY 1907, ARBER 1919, 1920, BROOKS 1940, MEUSEL 1951, MAHESHWARI 1956b, 1958a,b, DAUBS 1965, KANDELER 1979). 2) The Lemnaceae are associated with the Helobiae (e.g. EICHLER 1875, LAWALREE 1945, DEYL 1955).

The theory of a relationship of the Lemnaceae to the Spadiciflorae is based on the similarity of Spirodela to Pistia almost the only true water plant (hydrophyte) of the Spadiciflorae. Most species of the Spadiciflorae are forest species (including many woody species). Pistia is situated at the end of a long development from forest plant to swamp plant and finally water plant. The deduction of the Lemnaceae from the Spadiciflorae is only possible from Pistia as a link to other Araceae.

The similarity between Pistia and Spirodela is very striking at first sight. Spirodela looks just like a very reduced Pistia. Many publications try to prove this close relation and to show homologies between the organs of Pistia and the Lemnaceae.

MEUSEL (1951) compares the ontogenetic development of Pistia and Spirodela (cf. fig. 2.8 in chapter 2.2). He postulates that the structure and the ramification of the Lemnaceae fronds correspond largely with the organization in Pistia. The same sequence of the leaves in Spirodela as in Pistia is assumed: The prophyllum of Spirodela (in the terminology of the present work) is thought to be a scale leaf, the distal part of the frond a foliage leaf, and the prophyllum of the flowering organs a spathe. The first daughter frond of Spirodela is according to MEUSEL (1951) homologous to the sympodial shoot of Pistia, the second frond (on the minus side) to a stolon. The inflorescences of Pistia as well as of Lemnaceae (here named as flowers) develop at the end of the stolon (replacing the second frond). As KANDELER (1955, 1984) showed, the flowering organs in Lemnaceae are formed laterally to the position of a daughter frond and cannot be replaced exactly by a daughter frond. TILLICH (1985) points to the similarity of seedlings of Lemna with Pistia (cotyledonar sheet with median lobe present, no primary root, persistent operculum). Apparently, an operculum does not occur in the Helobiae. The homology of the different organs of Lemnaceae is discussed in the chapters 2.2, 2.4.10 and 2.6.2. Many arguments in favour of or against the theory of a close relationship between Spirodela and Pistia are not based on clearly recognizable facts but on the interpretation of these facts. They are therefore only indicative but not conclusive. As a matter of fact, most of the common characteristics of Spirodela and Pistia pointing to the same ancestry are so widespread in other families of different taxonomical relationship that they do not provide a definite relationship, e.g. cellular endosperm, monosporic or disporic embryo sac, crassinucellate nucellus (cf. chapter 2.6.3), presence of raphides and druses, presence of flavones, anthocyanins and myriophyllin cells (VAN BEUSEKOM 1967, ZENNIE and McCLURE 1977). Most of these characteristics occur also within the Alismatiflorae (Helobiae) e.g. raphides in Sagittaria (VAN BEUSEKOM 1967). A few characteristics of Lemnaceae seem to be typical for Araceae: no prominent basal cell in the embryo, no micropylar cap (cf. MAHESHWARI 1956b). But, the Monocotyledons and especially the Alismatiflorae embryologically studied are too few to reach

final conclusions. Some differences between _Spirodela_ and _Pistia_ are listed in table 7.1.

Furthermore the chromosome numbers (n=14 in _Pistia_ and n=10,15,20,25 in _Spirodela_: URBANSKA-WORYTKIEWICZ 1980, or n=18,20,23,40: GEBER in lit. 1986) and the chromosome size do not suggest (but do not exclude either) a close relationship between _Pistia_ and the _Lemnaceae_. The DNA content of _Pistia_ does not give any further indication to a relationship with _Lemnaceae_. It is much greater than in _Spirodela_ and contradicts the tendency within the family of _Lemnaceae_ in which the reduction of morphological characeristics is coupled with an increase in DNA content (see table 3.2).

Also some other characteristics typical for _Pistia_ are missing in _Spirodela_: hairs of the leaf-like organs, root hairs, presence of a spadix, stolons. Of course, these differences can be explained by the general reduction of all organs of the _Lemnaceae_. It seems to me that there are too many differences between _Pistia_ and _Lemnaceae_ to make a direct descent of _Spirodela_ from _Pistia_ convincing. There is not a single characteristic which is common to _Pistia_ and the _Lemnaceae_ which is not also present in other water plants. Since _Pistia_ is almost the only water plant (hydrophyte) within the _Ariflorae_ (_Spadiciflorae_), a derivation of the _Lemnaceae_ from any other genus within this superorder is not very obvious. KUPRIYANOVA and TARASEVICH (1984) propose a relationship between the _Lemnaceae_ and _Arisaema_ of the _Araceae_ on account of similar characteristics of pollen grains, but other properties are so different that the similarities between the pollen grains seem more incidental.

The smut genus _Tracya_ has two species, one living on _Spirodela_ (_T. lemnae_), and one living on _Hydrocharis_ (_T. hydrocharidis_) (ZOGG 1985). Though this common occurrence of two genus on members of _Lemnaceae_ and _Hydrocharitaceae_ might be not very conclusive, it certainly shows some relationship between the two families.

It is evident, that there is no close similarity between the _Lemnaceae_ and any living genus of the _Alismatiflorae_ either. But nearly all characteristics of the _Lemnaceae_ are realized somewhere within the _Alismatiflorae_, though in other combination, especially the reduced flowering and vascular system, the slight differentiation of the leaf-like organs and the absence of root hairs which are of course adaptations to life in the water. On the other hand, the presence of endosperm and raphides

Table 7.1. Different characteristics of _Spirodela_ and _Pistia_

Characteristics	Spirodela	Pistia	References
stomata	anomocytic, parallel oriented	tetracytic(?), randomly oriented	SANCHEZ ANTA et al. 1984
embryo sac	Allium type	Polygonum type	MAHESHWARI 1956b
micropyle form-ed by	inner integu-ments	outer integu-ments	BROOKS 1940
form of stigma	funnel-shaped	rounded at the top	DEYL 1955
stamens	independent,with distinct fila-ments	2 joined togeth-er without a filament	LAWALREE 1945
anther tapetum	forming a peri-plasmodium	no periplasmo-dium	LAWALREE 1945
middle layer between endo-thecium and tapetum	present	absent	LODKINA 1976
pollen grain	3-nucleate	2-nucleate	LAWALREE 1945
fruits	dry nut (mem-braneous walls)	berry	LAWALREE 1945
seeds	with endosperm	without endo-sperm	DAHLGREN et al. 1985
surface of pollen grains	with small protuberances ("spiny")	grooved-striate	DEYL 1955, THANIKAIMONI1969 KUPRIYANOVA and TARASEVICH 1984 GRAYUM in lit. 1984
arrangement of raphides in the anthers	in a double row between the 2 locules of each side of the anther	limited to the connective	HEGELMAIER 1871
apiose	present	absent	BEUSEKOM 1967
flavonols	present	absent	ZENNIE and McCLURE 1977
root hairs	absent	present	

distinguishes the Lemnaceae from most members of the Alismatiflorae, but the manifold specialization of the Alismatiflorae to life in the water is very characteristic for this superorder, in contrast to the Ariflorae. I am therefore inclined to place the family of Lemnaceae somewhere in the vicinity of the Alismatiflorae. However, the typical combinations of characteristics and the high ecological specialization are so particular that it is justifiable to place the family in an order of its own (Lemnales). It will be difficult to get much more knowledge about the relationship of the family in the future since the detection of possible intermediate fossil species is, due to the lack of lignified structures, very unlikely. To summarize our knowledge we might quote SCULTHORPE (1967, p. 14) who wrote: "So far as hydrophytes are concerned, much might be gained by admitting, at the outset, that the natural affinities and ancestry of many families are profoundly enigmatic. Botanists are still seriously ignorant, or deeply divided in their interpretation, of many aspects of the morphology, cytogenetics and geological history of aquatic taxa. In this present state of inadequate knowledge, phylogenetic speculation is sometimes provocative and may inspire the pursuit of some hitherto untrodden path of inquiry, but all too often it seems no more than wild intellectual diversion, flourishing precociously on the very lack of factual data and unequivocal evidence."

7.3. TAXONOMICAL RELATIONSHIPS WITHIN THE FAMILY

7.3.1. Differentiation of the genera

Assuming that the phylogenetic development within the family of the Lemnaceae comprises one or more reduction series from a higher to a lower differentiated plant body and an adaptation to a more special way of life in the water, it is evident that the most primitive genus is Spirodela and the most derived Wolffia. LAWALREE (1945) is of the opposite opinion. He thinks that Wolffia is the older genus and Spirodela derived. This is very unlikely since, in the plant kingdom, many families had already reached much higher levels of differentiation at the time of the first appearance of Lemnaceae in the Eocene. Table 7.2 gives a survey of assumed primitive and derived characteristics of the Lemnaceae.

There is obviously a reduction series from Spirodela over Lemna and Wolffiella to Wolffia. From a taxonomical point of view, the family is sharply divided into two subfamilies: Lemnoideae (Spirodela and Lemna) and Wolffioideae (Wolffiella and Wolffia). The Wolffioideae are, as it is evident from table 7.2, much more reduced than the Lemnoideae. The table also shows, that the common ancestor of the Wolffioideae is probably not within the genus Lemna as is supposed by many authors, but within the genus Spirodela. Some species of Spirodela have characteristics in common with the Wolffioideae which cannot be found within Lemna: pigment cells, plastids in the guard cells of the stomata, straight cell walls of the epidermis cells, flavonols. As S. intermedia is the only Spirodela species which has a combination of all these characteristics, it must be the nearest relative to the subfamily of the Wolffioideae. The flavones which can be found in some Wolffia species but are lacking in S. intermedia show up in S. polyrrhiza and S. punctata (as well as in Lemna). The common ancestor of Wolffioideae must therefore be another, probably extinct, species of Spirodela. TURNER (1967) and HARBORNE and TURNER (1984) are of the opinion that the genus Wolffia is of biphyletic origin on account of the different flavonoid content. According to these authors W. microscopica, W. brasiliensis, and W. borealis containing flavonols derived from Wolffiella, W. arrhiza, W. columbiana, and W. globosa from Lemna. As DEN HARTOG (1975) pointed out, this is not very likely. The many common morphological characteristics of the Wolffioi-

Table 7.2. Primitive and derived characteristics within the **Lemnaceae** family

S = **Spirodela**, L = **Lemna**, W1 = **Wolffiella**, W2 = **Wolffia**
Primitive characteristics are valued with the number 4 (important characteristic) or 2 (less important characteristic); derived characteristics are valued with a lower number (down to zero)

Characters	primitive	derived	S	L	W1	W2
size of frond	big(4)	small(0)	3-4	2-4	1-4	0-2
prophyllum (frond)	present(4)	absent(0)	3-4	0	0	0
pouches	two(4)	one(0)	4	4	0	0
number of roots	many(4)	none(0)	2-4	1	0	0
length of root	long(2)	short(0)	0-1	0-2	0	0
aerenchym	many layers(2)	absent(0)	2	1-2	1	0
nerves	many(4)	none(0)	3-4	1-3	0	0
tracheids in the nerve	many(4)	none(0)	3-4	0-3	0	0
tracheids in roots	to the tip(2)	none(0)	1-2	0	0	0
stomata	many(2)	few(0)	2	0-2	0-2	0-2
plastids in stomata guard cells	present(2)	absent(0)	2	0	2	2
raphides	present(2)	absent(0)	2	2	0	0
druses	present(2)	absent(0)	2	0	0	0
pigment cells	present(2)	absent(0)	2	0	1-2	1-2
anthocyanins	present(2)	absent(0)	2	0-2	0	0
flavonols	present(2)	absent(0)	0-2	0	2	0-2
prophyllum (flower)	present(4) side closed(2)	absent(0) side open(0)	4 0-2	4 0-2	0 0	0 0
position of flowers	in vegetative pouch(4)	in separate cavity(0)	4	4	0	0
fruits	many(4) winged(2)	one(0) not winged(0)	1-4 1-2	0-4 0-2	0 0	0 0
ovules	anatropous or amphitropous(2)	orthotropous(0)	2	0-2	0	0
embryo sac	monospore(2)	dispore(0)	2	0	0	0
stamens	two(4)	one(0)	4	4	0	0
anthers	2-loculate(2)	1-loculate(0)	2	2	0	0
length of protuberances	long(2)	short(0)	2	1-2	0	0
total index (potential)	72	0	55-71	26-51	7-13	1-10

deae are strongly against this theory. Also, the flavonoids of too few species of the Wolffioideae have been investigated to come to definite conclusions. Moreover, the flavonols seem to be concentrated in the pigment cells. Except for W. brasiliensis and W. borealis pigment cells are located in flowering organs. Contrary to W. microscopica Wolffia species rarely are flowering in culture. Therefore the flavonols cannot be detected. From the cytological point of view there is a continuous increase of DNA content from Spirodela over Lemna and Wolffiella to Wolffia (GEBER in lit. 1986, see table 3.2) which is parallel to the morphological reduction within the family (fig. 7.1). The chromosome number increases in the genus Spirodela from 2n=36 (S. intermedia) over 2n=40 (S. polyrrhiza) to 2n=46 (S. punctata). In the three other genera the basic
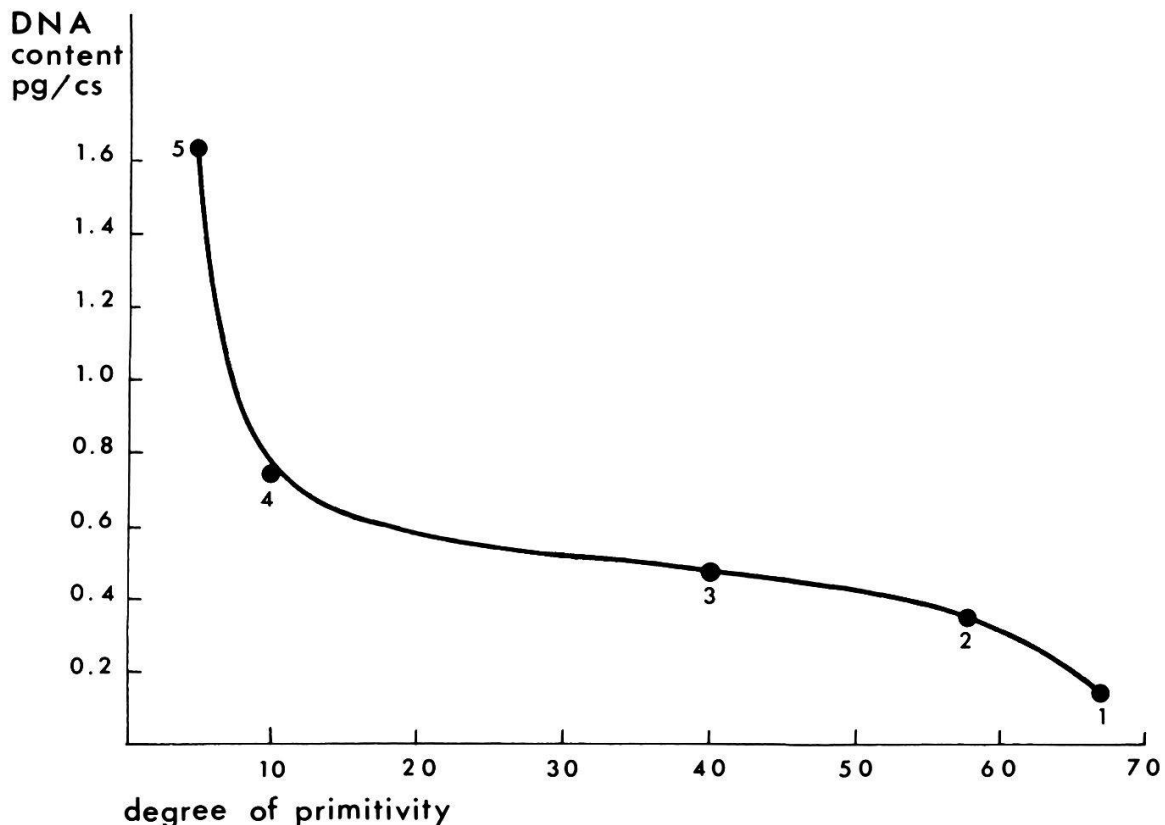

Fig. 7.1. Correlation between the DNA content (in pg) of a chromosome set (CS) of a species (see table 3.2., GEBER in lit. 1986) and the degree of primitivity (according to table 7.3)

1 = S. intermedia, 2 = S. punctata, 3 = L. minor,
4 = W. oblonga, 5 = W. arrhiza

chromosome number seems to be constantly 2n=42 which would point to a common ancestry of Lemna, Wolffiella, and Wolffia from a Spirodela species of 2n=42 which had some characteristics of both S. intermedia and S. punctata and no longer exists.

The connection between Spirodela and Lemna is probably situated near S. punctata which has many features similar to Lemna: undulated epidermis cell walls, transitional position concerning the situation of the external locules of the anthers, similar shape of the fronds, intermediate position concerning the number of nerves and number of roots. The possible phylogenetic situation for the genera of the Lemnaceae is presented in fig. 7.2. IVANOVA (1973) thinks of a diphyletic origin of Lemna which does not seem very convincing.

It is interesting to note that most primitive and probably most ancient species of each genus of Lemnaceae (S. intermedia, L. gibba, W. neotropica, W. brasiliensis) are distributed in the warmer regions of South America. This points clearly to this region as the place of origin of the Lemnaceae.
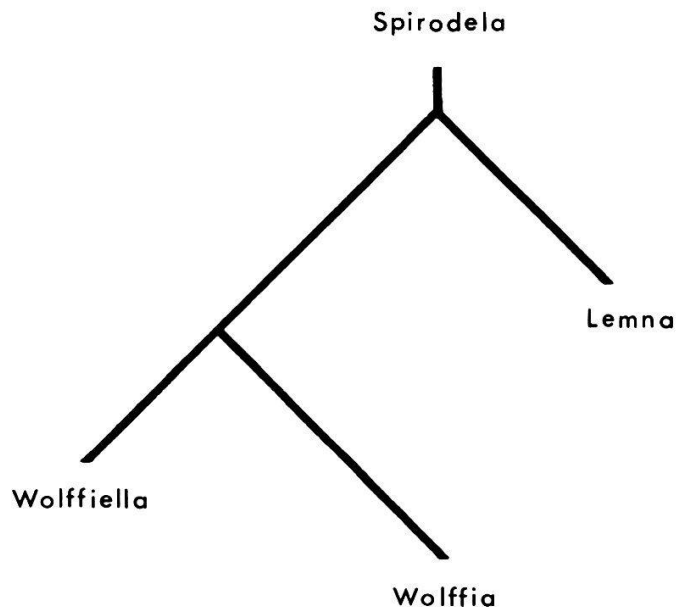
Fig. 7.2. Supposed taxonomical relationship between the genera of the Lemnaceae

7.3.2. Differentiation within the genera

7.3.2.1. General remarks

LANDOLT (1987) gives some examples of ecogeographical differentiation within all four genera. The ecological differentiation follows mainly four different lines:

1) Reduction in size and subsequent simplification of the whole structure: the fronds become more mobile and better adapted to fill gaps within the vegetation cover. Two different growth forms seem to be especially successful: a) flat to gibbous ovate fronds with a stabilization organ (root, appendage) and of a medium size of about 3 mm to 2 mm (1 species of Spirodela, 9 species of Lemna, 1 species of Wolffiella); b) very small ovoid bodies of 0.5-1.5 mm in diameter (7 species of Wolffia). Both types of fronds are distributed all over the world.

2) Adaptations to growing at lower temperatures and to tolerate frosts. This tendency is restricted to Spirodela punctata and the genus Lemna (all species except L. aequinoctialis and possibly L. tenera).

3) Formation of special resting buds (turions) which sink to the bottom of the water. In this way, the plant is able to survive unfavourable conditions of temperature and nutrient content. Turions are achieved in Spirodela polyrrhiza, in Lemna turionifera (and partly in L. aequinoctialis) and in the genus Wolffia (all species except W. elongata and W. microscopica). In S. polyrrhiza and in Lemna the turions are moderately frost resistant, in Wolffia they have the same sensitivity to frost as the normal fronds.

4) Development of a submerged life form. The temperature conditions below the water surface are less extreme, and the nutrient content more favourable due to a nutrient gradient from the soil to the water surface. Submerse life is achieved independently in Lemna trisulca, L. tenera, and partly in L. valdiviana as well as in most species of Wolffiella. Plants growing below the water surface are easily overgrown by species floating on the surface and get less light. To coexist with such plants they are highly shade tolerant and heterotrophically able to use organic substances dissolved in the water. If a nutrient in the water is in short supply, they have the best chance in competition since they are first able to take up the newly released nutrients from the soil.

7.3.2.2. Differentiation within the genus Spirodela

S. intermedia is the species with the highest level of organization within the whole family (table 7.3). It has the greatest number of nerves, roots, and ovules within the genus; the roots partly perforate the prophyllum. S. intermedia has its centre in the climatic zone V (see chapter 6.3.3) where the family has its highest concentration of species, and is distributed within the warmer regions of South America where the family probably has its centre of differentiation (all 4 genera with 16 species, see chapter 6.1.2). From S. intermedia, the differentiation goes in two directions:

1) S. polyrrhiza: slight reduction in average number of roots, nerves, and ovules; usually only 1 root perforating the prophyllum. The newly acquired ability to form turions enabled the species to overwinter in

Table 7.3. Degree of primitivity of the Lemnaceae species according to the characteristic index of table 7.2

Lemnaceae	3-71	Wolffioideae	3-13
Lemnoideae	26-71	Wolffiella	8-13
Spirodela	55-71	sect. Stipitatae	8-10
sect. Spirodela	66-71	W. hyalina	9-10
S. intermedia	69-71	W. repanda	8- 9
S. polyrrhiza	66-68	sect. Rotundae	10
sect. Oligorrhizae	55-60	W. rotunda	10
S. punctata	55-60	sect. Wolffiella	8-13
Lemna	26-49	W. neotropica	11-13
sect. Lemna	38-49	W. Welwitschii	9-11
L. gibba	46-49	W. lingulata	9-11
L. disperma	43-45	W. oblonga	8-10
L. minor	39-41	W. gladiata	8- 9
L. japonica	39-41	W. denticulata	8- 9
L. obscura	38-40	Wolffia	3- 9
L. ecuadoriensis	38-40	sect. Pseudorrhizae	6- 7
L. turionifera	38-40	W. microscopica	6- 7
sect. Hydrophylla	34-36	sect. Elongatae	c.4
L. trisulca	34-36	W. elongata	c.4
sect. Alatae	30-33	sect. Pigmentatae	8- 9
L. perpusilla	31-33	W. brasiliensis	8- 9
L. aequinoctialis	30-32	W. borealis	8- 9
sect. Biformes	27-29	sect. Wolffia	3- 6
L. tenera	27-29	W. australiana	6
sect. Uninerves	26-28	W. angusta	4
L. valdiviana	26-28	W. arrhiza	4- 6
L. minuscula	26-28	W. columbiana	3- 4
		W. globosa	3

cold regions and to spread over the whole world, but the temperatures needed for growth are still high as well as the temperature optimum and heat tolerance.

2) S. punctata: distinct reduction in the number of roots, nerves and ovules; all roots perforate the prophyllum; the prophyllum of the flower opens on one side. The species cannot form true turions and overwinter in regions with cold winters, but became physiologically adapted to grow in cooler regions (relatively low temperature requirements, low temperature optimum, relatively low heat tolerance). The species was able to spread throughout the zones of oceanic climates, especially in the Southern hemisphere.

7.3.2.3. Differentiation within the genus Lemna

The connection between Lemna and Spirodela must be assumed in the vicinity of S. punctata which shows some reductions also found in Lemna: prophyllum reduced (no prophyllum in Lemna); nerves reduced to 3-7 (1-7 in Lemna); tracheids only in the upper part of the roots (no tracheids in the roots of Lemna); 1-7 roots (only 1 root in Lemna); the external
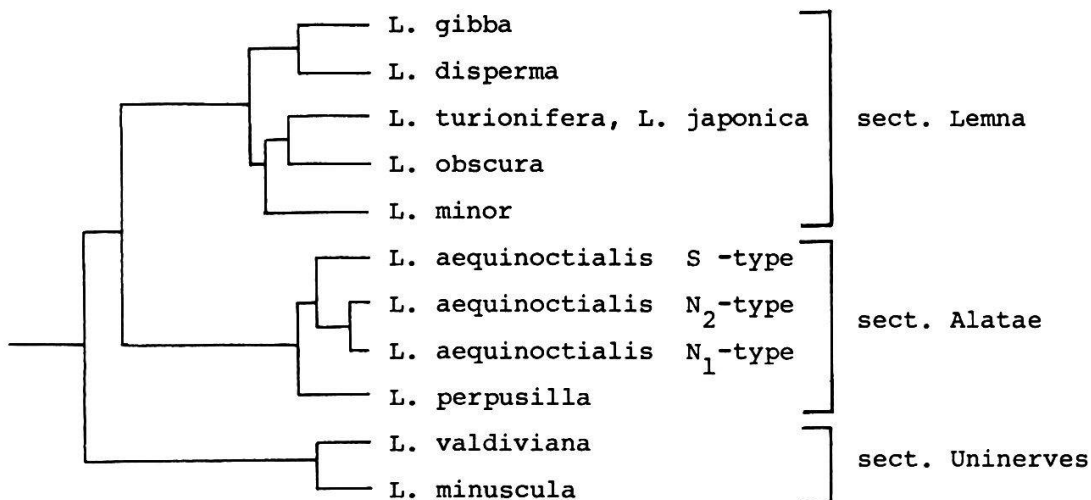

Fig. 7.3. Genetic distance between some species of Lemna (according to BEPPU in lit. 1986). BEPPU names the N_1-type L. aoukikusa ssp. aoukikusa, the N_2-type L. aoukikusa ssp. hokurikuensis, and the S-type L. aequinoctialis (s.str.) (see chapter 7.4.3).

locules of the anthers slightly higher than the internal ones (above the internal ones in Lemna). Furthermore the shape of S. punctata is similar to Lemna. The most developed species of Lemna (section Lemna and Hydrophylla) still have a closed prophyllum as S. intermedia and S. polyrrhiza. Fig. 7.3. shows the genetic distance of some Lemna species according to the banding pattern of the chloroplast DNAs. The good correspondence of the three groups of investigated species with three Lemna sections distinguished in the present monograph is remarkable.

There is a series of reductions within the genus Lemna, from the section Lemna to the section Uninerves (table 7.3, fig. 7.4). The species closest to Spirodela (L. gibba) still has several ovules (up to 7), 3-7 nerves; the wing of the fruit is wider than in any other species of the genus Lemna, the ovule is anatropous or amphitropous and there are 3 layers of aerenchymatic tissue; anthocyanin is still present. L. disperma is reduced in size, but sometimes still has more than 1 ovule and up to 5 nerves. Although it is intermediate between L. gibba and L. minor, it probably originated through a differentiation within L. gibba as a consequence of a long isolation in Australia (and New Zealand). All the other species of the section are situated more or less on the same level
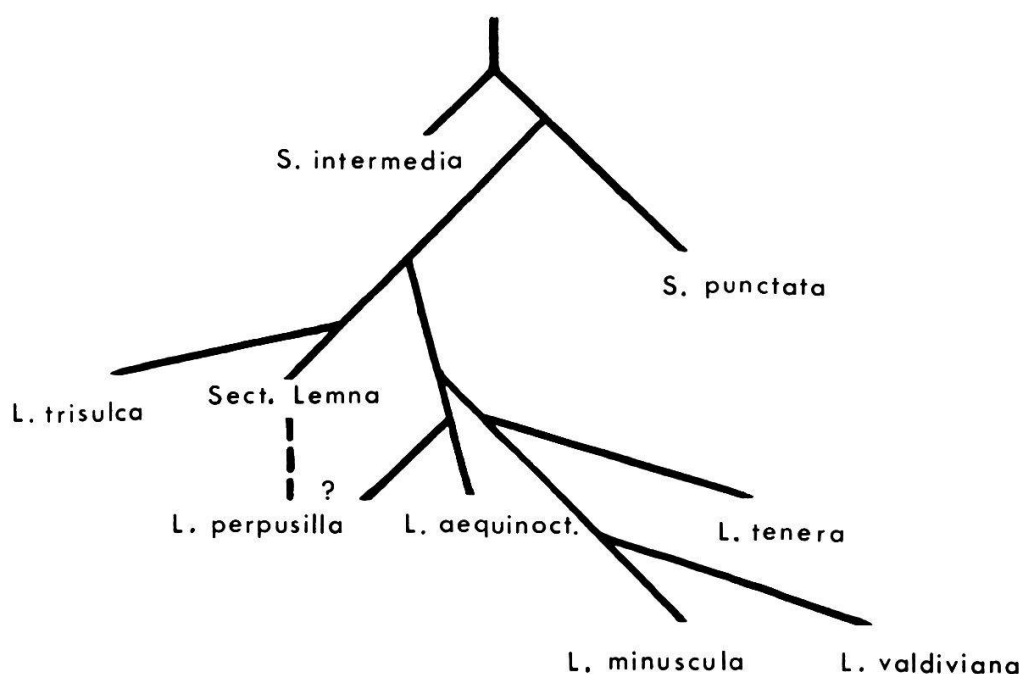

Fig. 7.4. Supposed taxonomical relationship within the genus Lemna

of organization, having only 1 ovule (from <u>L. ecuadoriensis</u>, flowers and fruits are not known) and usually only 3 nerves (table 7.3; fig. 7.5). Fruits are narrowly (<u>L. minor</u>) or not winged. Differences exist mainly in the size and number of papules, in the coloration pattern, in the structure of the seed coat, and in the ability to form turions. <u>L. japonica</u> seems to be of hybrid origin (between <u>L. minor</u> and <u>L. turionifera</u>) and replaces the two presumable parent species in parts of eastern Asia.

The section <u>Hydrophylla</u> with the only species <u>L. trisulca</u> is in many respects on the same level as most species of the section <u>Lemna</u>: similar fruits and seeds, presence of anthocyanins, utriculous bract of the flower closed, but, in some characteristics, it is more reduced: 1-3 nerves, middle nerve with tracheids only at the base, aerenchymatic tissue more reduced, roots short. Some characteristics are typical for the section: fronds submerged, dentate, in long groups connected together by a long green stalk.

The section <u>Alatae</u> (group of <u>L. perpusilla</u>) is distinctly at a more reduced level than the section <u>Lemna</u>; short roots, 3 nerves, middle nerve with tracheids only at the base, 1-3 layers of air spaces, no anthocyanins present, utriculous leaflet of the flower open on one side, ovule
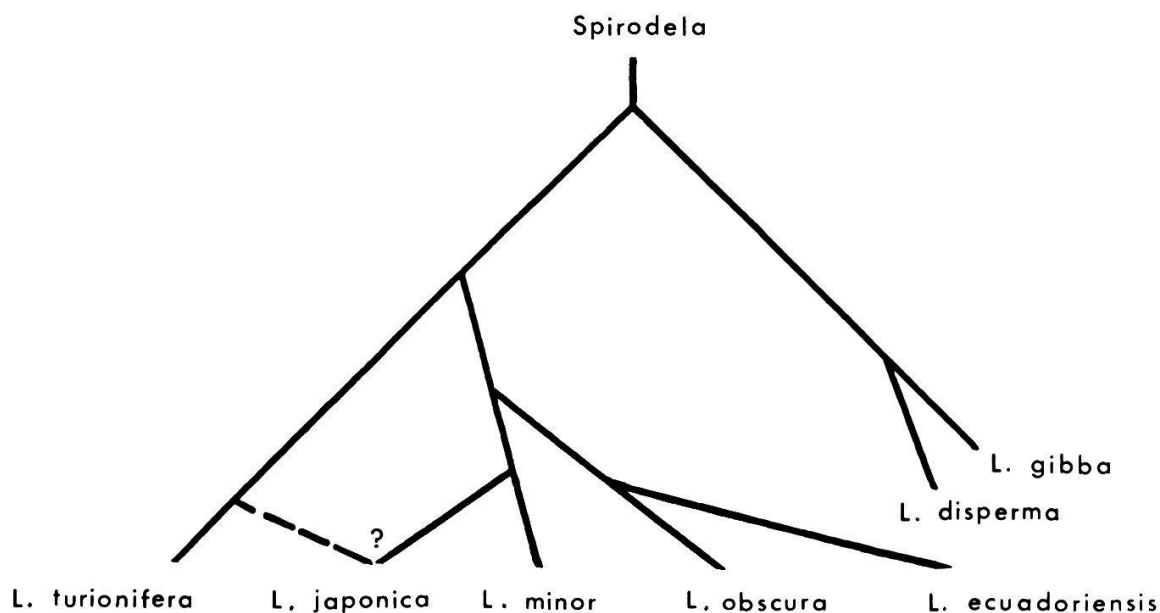

Fig. 7.5. Supposed taxonomical relationship within the section <u>Lemna</u>

orthotropous. The winged root sheath is a special characteristic of this section. L. perpusilla which has the structure of the seed coat in common with L. turionifera might be of hybridogenous origin, L. aequinoctialis as one parent, L. turionifera as the other. The relatively restricted distribution area is situated between the areas of the assumed parent species. The similarity to L. trisulca is only superficial (submerged life form).

The section Biformes consists only of L. tenera and is distinguished from the section Alatae mainly by root sheath without wing and by different growth habit. There seem to be no tracheids present at all. The fruits are not known.

The section Uninerves (group of L. valdiviana), finally, shows the most reduced level of the genus: 1 nerve, no tracheids, 1-2 layers of air spaces. Of the two species in this section, L. valdiviana has a more reduced aerenchymatic tissue and is able to grow submerged, and L. minuscula is smaller and has a shorter nerve.

7.3.2.4. Differentiation within the genus Wolffiella

Though some Wolffiella species at first sight look similar to L. valdiviana which is able to grow submerged, as many Wolffiella species, a
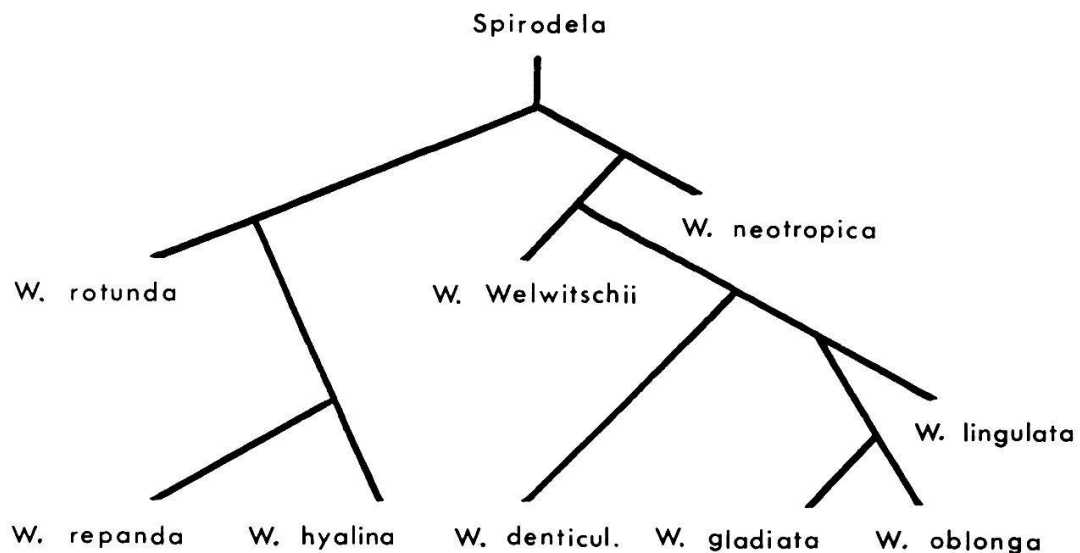
Fig. 7.6. Supposed taxonomical relationship within the genus Wolffiella

common descent is not very probable. Also there is no reason for connection with <u>L. trisulca</u> (as supposed by IVANOVA 1973) but the submerged habit. It has been shown earlier that some characteristics of <u>Wolffiella</u> (and <u>Wolffia</u>) are in common with <u>Spirodela</u> (pigment cells, straight walls of the epidermis, flavonols) but not with <u>Lemna</u>. Therefore a direct descent from an ancestor of <u>S. intermedia</u> is more plausible. Anyway, the step between <u>Spirodela</u> and <u>Wolffiella</u> is a rather big one. The most original species within the genus <u>Wolffiella</u> seems to be <u>W. neotropica</u> (fig. 7.6), which has many stomata, pigment cells in the plant body, and the track of elongated cells along the middle line of the lower wall of the pouch. It floats on the surface of the water or is submerged according to the conditions of growth. <u>W. rotunda</u> differs in having pigment cells only in the flowering organs, in its smaller size, and in not being able to live submerged. In <u>W. hyalina</u> and <u>W. repanda</u>, the lower wall of the pouch is prolonged basewards into a ribbon-like appendage serving as a stabilizing organ. A separate differentiation can be observed from <u>W. neotropica</u> over <u>W. Welwitschii</u>, <u>W. lingulata</u>, <u>W. oblonga</u> to <u>W. gladiata</u> and <u>W. denticulata</u>. <u>W. Welwitschii</u> differs from <u>W. neotropica</u> by the fewer stomata and the permanent submerged life habit. From <u>W. Welwitschii</u>, a successive shifting of the track of elongated
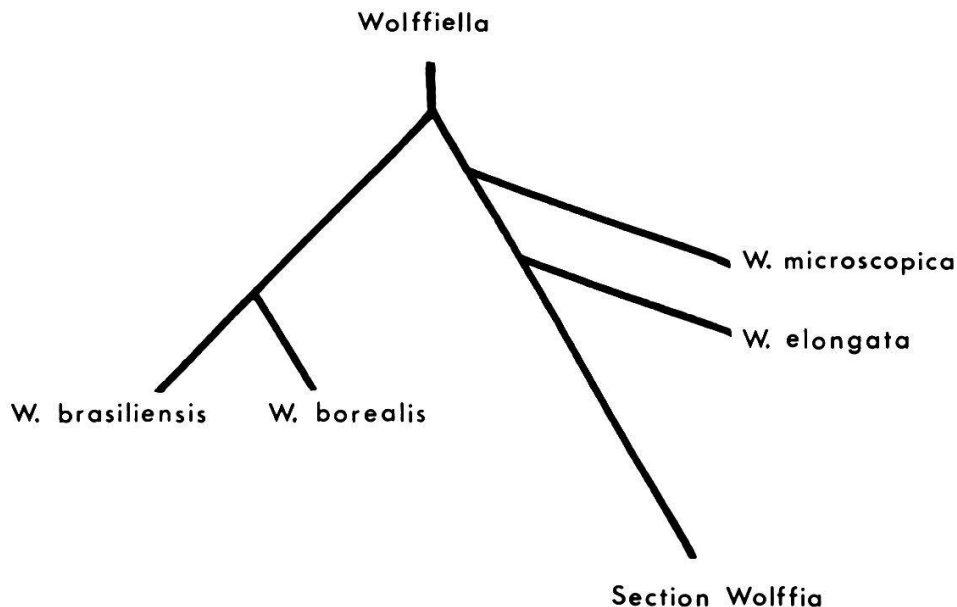
Fig. 7.7. Supposed taxonomical relationship within the genus <u>Wolffia</u>

cells from the middle to the angle of the pouch is evident, a narrowing of the frond and an extension of the aerenchymatic tissue from the area around the base towards the tip of the frond. A successive shifting of the habitat of the species from warmer to cooler regions goes hand in hand.

7.3.2.5. Differentiation within the genus Wolffia

The possible differentiation within the genus Wolffia is shown in figs. 7.7 and 7.8. The most original species are W. brasiliensis and W. punctata with pigment cells throughout the plant body. They derived from Wolffiella from an ancestor similar to W. neotropica which still temporarily floats on the surface with many stomata and a rather reduced aerenchymatic tissue near the base. All other species of Wolffia have pigment cells only in the flowering organs. The section Wolffia partly comprises species with similar shape and size. The other two Wolffia species have one very special characteristic each: a conical appendage on the lower side of the frond in W. microscopica, and a narrow cylindrical frond in W. elongata. In the section Wolffia, there are two groups with different shapes: boat-shaped in W. australiana and W. angusta, and spherical to ellipsoid in W. arrhiza, W. columbiana, and W. globosa. W. australiana and W. arrhiza are on the average bigger than
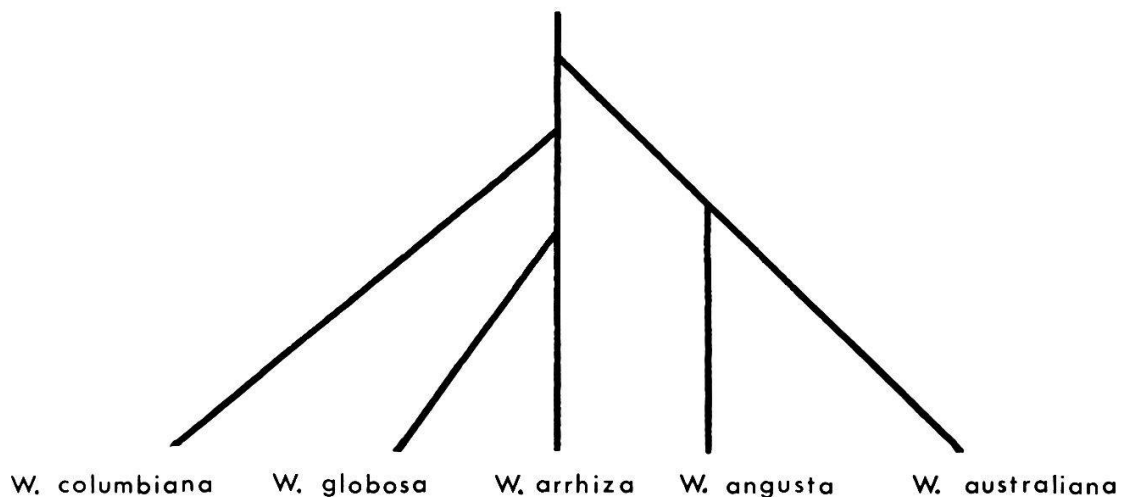

W. columbiana   W. globosa   W. arrhiza   W. angusta   W. australiana

Fig. 7.8. Supposed taxonomical relationship within the section Wolffia

the other species and have more stomata. In the further evolution a re-
duction of the number of stomata (<u>W. angusta</u> and <u>W. columbiana</u>, respec-
tively) and in addition a reduction in size (<u>W. angusta</u> and <u>W. globosa</u>)
has taken place.

7.3.3. Differentiation within the species

Although most species of <u>Lemnaceae</u> usually propagate vegetatively, and
therefore gene flow is probably rather rare, great differentiation with-
in a species can be observed.

7.3.3.1. Differentiation of certain characteristics

MENDIOLA (1919) was one of the first scientists to be aware of the great
variation in the size and shape of the fronds, and rate of growth within
<u>L. minor</u>. A rather intense study on the differences in morphology and
physiology of clones of 13 species of <u>Lemnaceae</u> was made by LANDOLT
(1957). The physiological differences measured included growth rates un-
der different temperatures, light and nutritional conditions, as well as
maximum growth rates, flowering responses etc. The differences between
clones could partly be attributed to different climates at the place of
origin and partly to the geographical origin with apparently no rela-
tionship to the climate. In the last twenty years much work has been
done to detect different races within species of <u>Lemnaceae</u>. Especially
differences in morphological characteristics such as size, shape, and
number of fronds connected together, position of the first daughter
frond, dry weight, number and length of roots and number of nerves were
observed between clones of different origin: SPOONER (1967) worked with
<u>S. polyrrhiza</u>, PIETERSE (1974a) with <u>L. minor</u>, YUKAWA and TAKIMOTO
(1976) and BEPPU and TAKIMOTO (1981b,c) with <u>L. aequinoctialis</u>, WOZA-
KOVSKA-NATKANIEC (1977b) with <u>S. polyrrhiza</u> and <u>L. minor</u>, PORATH et al.
(1980) with <u>S. polyrrhiza</u> and <u>L. gibba</u>, DAVIDSON and SIMON (1981b, 1983)
with <u>S. polyrrhiza</u>, DUDLEY (1983) with <u>L. turionifera</u>, LANDOLT and DANN
(1983) with <u>L. gibba</u>, WOLEK (1984) with <u>S. polyrrhiza</u>.
In most works, also the growth rate was measured. DAS and GOPAL (1969)
observed better resistance to cold in turions of <u>S. polyrrhiza</u> clones
from northern compared with southern regions of India. WOLEK (1984)

reports different competitive ability of 14 clones of S. polyrrhiza from
Europe (the dry weight was measured in mixed cultures with L. minor
under four different conditions). Differences in flowering behaviour of
L. aequinoctialis, L. gibba, and L. turionifera were observed by LANDOLT
(1957), of L. aequinoctialis by HILLMAN (1979a) and by YUKAVA and
TAKIMOTO (1976). Of 13 clones of L. aequinoctialis used by HILLMAN
(1979a), only 2 behaved in the same way, YUKAVA and TAKIMOTO (1976) were
able to group the 22 investigated clones from Japan into four types with
partly allopatric distribution (cf. section 7.4.3). The malate dehydro-
genase activity was measured in L. minor by JEFFERIES et al. (1969a,b),
and the malate dehydrogenase temperature stability in S. polyrrhiza (11
clones investigated) by DAVIDSON and SIMON (1981a,b, 1983). Both groups
of scientists discovered clonal differences. DAVIDSON and SIMON showed
up a general tendency in enzyme activity of clones acclimatized to high-
er temperatures; however, their results indicate a lack of adaptive
trends.

Different chromosome numbers of different clones of the same species
were counted by BANERJEE (1971), BEPPU and TAKIMOTO (1981a), URBANSKA
(1980) and GEBER (in lit. 1986). According to URBANSKA, there are in
nearly all investigated species clones of up to five different ploidy
levels (n=10), and, in addition, clones with aneuploid numbers. There is
no distinct correlation between chromosome number and climatic or geo-
graphic region. Of the four ecotypes of L. aequinoctialis from Japan
distinguished by BEPPU and TAKIMOTO (1981a), the type N_1 has mostly
2n=70 (11 clones, 1 clone has 2n=66, 1 clone 2n=72 and 1 clone 2n=84).
The types N_2 (5 clones) and S (4 clones) show 2n=40 and the type K (1
clone) 2n=50. For further details on chromosome numbers see chapter 3.
BEPPU (TAKIMOTO in lit. 1985) investigated the restriction fragment pat-
tern of DNA in L. aequinoctialis from 21 stations outside of Japon. He
found nine different patterns which are each restricted to certain regi-
ons of the world.

7.3.3.2. Differentiating factors

In most investigations, the influence of temperature was studied. Dif-
ferences in temperature behaviour between clones of the same species are
widespread (e.g. LANDOLT 1957, DAVIDSON and SIMON 1981a,b, 1983, BEPPU
and TAKIMOTO 1981c). DAVIDSON and SIMON could not find any correlation

between growth rate of 11 clones under different temperatures with environmental temperature conditions at the original site of their geographical origin. Differences found by LANDOLT (1957) and by BEPPU and TAKIMOTO (1981c) showed, at least partly, a connection with the climatic conditions of the original habitat.

Different response to light intensity of clones of the same species are reported by HICKS (1934) for L. minor and by LANDOLT (1957) for many species. Different behaviour of clones under different day length was observed in L. aequinoctialis by HILLMAN (1979a) and BEPPU and TAKIMOTO (1981b), and in L. gibba by CLELAND (1979) and KANDELER (1983). HILLMAN (1979a) noted deviating asparagin response of a clone of L. aequinoctialis (no inhibition as in the other investigated clones). LUKINA (1983b) detected infraspecific differences between 3 clones of L. gibba in response to UV radiation. Different tolerance to radiation of X rays in 2 populations of S. polyrrhiza and 14 populations of L. minor was found by WOZAKOVSKA-NATKANIEC (1977a). There was some correlation between the tolerance of X rays and natural radiation intensity at the place of origin (partly high radon content of waters).

The responses of different clones of S. polyrrhiza from North Carolina to conditions of limited nitrogen was studied by SPOONER (1967). He detected differences in growth rates and morphological characteristics between different clones from the same pond as well as from different geographical regions. LANDOLT and DANN (1983) investigated the behaviour of 10 clones of L. gibba in relation to different nitrogen content of the medium. The clones originated from different regions in North and South America, Europe, Africa, and Asia. Each clone was in some feature different from the others (growth rate at different nitrogen concentrations, maximal growth rates, size of frond, length of root, anthocyanin formation were measured). Clones from the same geographical region were more similar in looks and behaviour than clones from different regions. BEYER (1983 and unpubl.) investigated the tolerance of 5 clones of 30 species of Lemnaceae towards calcium and magnesium content of the nutrient solution. He observed some differences between different clones of the same species. But, the differences between different species were greater. The different tolerances could partly be correlated to differences in climate at the place of origin. BOLGIANO (1979) did not find any difference in need of Ca between two clones of L. minor from a soft-water spring and a hard-water spring in Virginia.

Variation in tolerances of 6 L. minor populations from Poland to heavy metals (Cr, Cu, Pb, Zn) is reported by SAROSIEK and WOZAKOVSKA-NATKANIEC (1980), and SAROSIEK et al. (1982). POLAR (pers.comm. 1982) detected different responses in heavy metal uptake (Co, Zn) in L. minor clones from the European part of Turkey.

7.3.3.3. Ecotypic variation

LANDOLT (1957) showed that ecotypic variation occurs within the Lemnaceae. The first and only detailed description of ecotypic differentiation was made by a Japanese group of workers with L. aequinoctialis (YUKAWA and TAKIMOTO 1976, BEPPU and TAKIMOTO 1981a,b, 1983). One hundred clones were collected in Japan and studied in detail at Kyoto University. Four ecotypes could be distinguished.

1. **N_1-type** (named by BEPPU et al. 1985 as L. aoukikusa ssp. aoukikusa): 82 clones collected, 14 investigated in detail. Most common in Japan, mainly distributed in central and northern Japan (from Hokkaido to Kyushu). Behaving as short-day plants, the further north the origin the shorter the critical dark period for flowering. Fronds narrow elliptic, small, 3-5 connected together (in Hutner's medium). Hibernating in Kyoto with seeds. Relatively low growth rate at 27°C, very low growth rate at 17°C. Chromosome number 2n=70 (66, 72, 84).

2. **N_2-type** (named by BEPPU et al. 1985 as L. aoukikusa ssp. hokurikuensis): 5 clones collected, 3 clones studied in detail. Distributed along the west coast of central Honshu. Behaving as short-day plants, the critical dark period for flowering being longer than in the N_1-type clone of similar latitude. Fronds narrow elliptic, large, more than 15 connected together (in Hutner's medium). Hibernating in Kyoto with true turions. Relatively low growth rate at 27°C, very low growth rate at 17°C. Chromosome number 2n=40.

3. **K-type**: Only one clone studied in detail. From Campus of Kyoto University, probably introduced. Behaving as day-length neutral. Fronds narrow elliptic, small, 3-5 connected together (in Hutner's medium). Hibernating in Kyoto with normal fronds. Fast growth rate at 27°C, relatively fast growth rate at 17°C. Chromosome number 2n=50.

4. **S-type** (named by BEPPU et al. 1985 as L. aequinoctialis): 12 clones collected, 5 clones studied in detail. Distributed in southern Japan (Kyushu, Amani, Ryukyu Islands). Behaving as long-day plant (flower-

ing only under high light intensities). Fronds wide elliptic, small, 3-5 connected together (in Hutner's medium). Hibernating in Kyoto with seeds and with normal fronds. Fast growth rate at 27°C, relatively fast growth rate at 17°C. Chromosome number 2n= 40.

L. aequinoctialis in Japan is found mostly in rice fields. Since rice is very widespread in Japan and has been cultivated for several thousand years, a possible differentiation has taken place under a long lasting influence of different climates and human activities. The preconditions for differentiation within L. aequinoctialis in other parts of the world are probably nowhere as favourable. Only a thorough investigation of clones from all over the world would help to decide on the taxonomic rank of the 4 different ecotypes. Since L. aequinoctialis is often self-pollinating, an effective isolation of new ecological races is possible. However, HILLMAN (1975a) showed that clones of the first generation of selfed L. aequinoctialis (No. 6746) behave differently in flowering responses. Clone 6746 therefore is not homozygotic. BEPPU and TAKIMOTO (1983) were able to hybridize two clones of L. aequinoctialis and showed that the flowering behaviour of the hybrids is somewhere between that of the parent clones.

7.3.3.4. Conclusions

The cited literature might be summarized as follows.
1) A great genetical variation within Lemnaceae species is evident regardless whether propagating sexually or not.
2) The genetical variation comprises morphological, cytological, as well as physiological and phytochemical characteristics.
3) A selection of certain ecologically important characteristics in relation to some external factors seems to be widespread, but a geographical differentiation often without any evident correlation to the specific climate prevails.

The results show that a gradual development of local, regional, and zonal races of Lemnaceae species takes place and that Lemnaceae are still in a very dynamic developmental stage in spite of the extreme specialization of life habit and of the mainly vegetative reproduction. In this way the family behaves similarly to most other taxonomic groups. It has to be taken into account that the fast and specific short-distance distribution by birds mixes populations steadily and therefore counteracts

the selection. The mutual pressure of competition between different clones at one place must be big enough to allow a fast selection of relatively small differences in ecological behaviour. The fact that even in the same pond, genetically different clones of vegetatively propagating species occur, may be explained by a steady mutation rate. The fast propagation makes mutations probable: One frond may give rise to 10^{20} descendant fronds within half a year under favourable conditions. The relatively balanced climatic conditions in water compared with conditions on land makes it understandable that climatic differentiation is less evident than in land plants.

It is supposed that the formation of new races in Lemnaceae takes place mainly by slow differentiation and isolation. L. disperma which lives isolated from the closely related L. gibba in Australia is a good example of this type of speciation. Other examples are W. arrhiza (mainly Africa and Europe), W. columbiana (America), and W. globosa (mainly E Asia). That the morphological differentiation is very slow, is demonstrated by the very large distribution of some species (e.g. S. polyrrhiza) without the development of new morphological characteristics or combination of characteristics. This must be attributed either to a steady intermixing rate (distribution by birds and, more recently by human activities) or to the fact that the most reduced morphological characteristics and the great phenotypic variation makes it very difficult to recognize possible differences.

The rare sexual propagation (except in some few species such as L. aequinoctialis and L. gibba) prevents a recombination of features and the formation of new successful types. This does not exclude that hybrids may occur in rare cases. The possible hybrid origin of L. japonica (from L. minor and L. turionifera) and of L. perpusilla (from L. aequinoctialis and L. turionifera) may give some indications. Hybrids and some transitional forms between species of Lemnaceae (especially between L. minor and L. gibba) were reported several times (e.g. GIUGA 1973, DE LANGE et al. 1981), but never proved experimentally. The author of the present work knows overlapping of the morphological features from the following species in addition: L. turionifera and L. minor; L. turionifera and L. japonica; L. valdiviana and L. minuscula; W. lingulata and W. oblonga; W. arrhiza, W. columbiana and W. globosa. Whether in these groups of species occasional hybridization occurs, or if the species are only not yet so clearly separated cannot be decided with the present

knowledge. The only known experimental hybrid was produced by BEPPU and TAKIMOTO (1983) between a clone of <u>L. aequinoctialis</u> from California (6746, 2n=80) and one of the same species from Japan (371, N_1-type, 2n= 70). The 8 hybrid offspring behave intermediately between the two parental clones in flowering response, growth rate, and frond shape. The hybrids did not set seeds. Own preliminary attempts to cross <u>L. minor</u>, <u>L. obscura</u>, <u>L. turionifera</u>, and <u>L. japonica</u> (50 crosses) were not successful, but also crosses between different clones of the same species and selfcrossings failed, except for two crosses between clones from north and south of the Alps. Taking into account that flowering is anyway very rare within this group, the probability of hybrid offspring is very low. Further investigations and crossing on a much larger scale are needed.