3. KARYOLOGY

3.1. CHROMOSOME NUMBERS

There are only few counts of chromosome numbers within the Lemnaceae family. As URBANSKA-WORYTKIEWICZ (1980) pointed out, the Lemnaceae "represent a very difficult material, the chromosomes of numerous taxa being exceedingly small and often tending to stick together in metaphases plates". First counts originated from BLACKBURN (1933, 5 species), further work has been done by TISCHLER (1935, 1 species), ROHWEDER (1937, 1 species), BROOKS (1940, 1 species), LAWALREE (1943, 1 species), EHRENBERG (1945, 1 species), DELAY (1947, 1 species), MOORE in DORE (1957, 1 species), DAUBS (1965, 4 species), ROY and DUTT (1967, 1 species), WCISLO (1970, 5 species), BANERJEE (1971, 2 species), MURIN and MAJOVSKY in

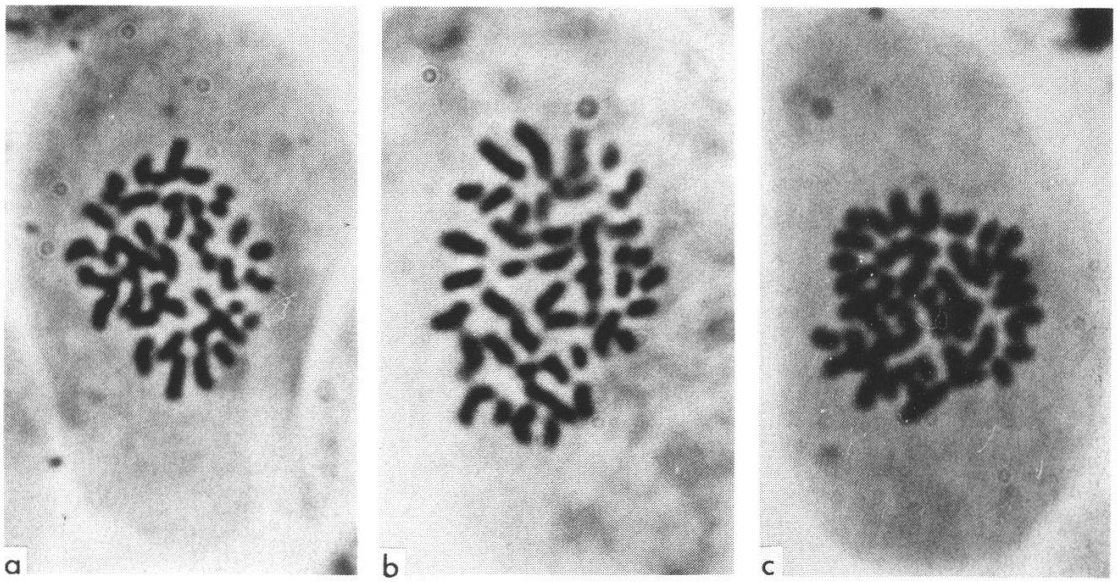

Fig. 3.1. Chromosome preparations by URBANSKA (pers.comm.) (x2600)

a. Wolffiella hyalina (No. 8640) (2n=40)
b. Wolffiella lingulata (No. 7292) (2n=50)
c. Wolffia columbiana (No. 8018) (2n=50)

LOEVE (1978, 1 species), KWANYUNEN (in URBANSKA-WORYTKIEWICZ 1980, 4 species), BEPPU and TAKIMOTO (1981a, 1 species), HONDO (in lit. 1981, 4 species). The most intensive study on chromosome numbers in Lemnaceae was accomplished by URBANSKA-WORYTKIEWICZ (1980) who investigated more than 1500 clones of 30 species. A further cytological investigation has been done in Vienna by GEBER (in lit. 1986). Examples of chromosome preparations are given in figs. 3.1 to 3.3.

The known chromosome numbers of Lemnaceae are surveyed in table 3.1. 2n= 40 is by far the most prevalent number within the family, according to URBANSKA-WORYTKIEVICZ (1980). She found besides the euploid chromosome numbers of 2n=20, 30, 40, 50, 60, 70, 80 aneuploid numbers within many populations. In table 3.1, these aneuploid numbers of URBANSKA-WORYTKIEWICZ are not mentioned except the relatively often occuring number of 2n=42. GEBER (in lit. 1986) only counted 2n=40 for S. polyrrhiza. According to his investigations the basic number for Lemna, Wolffiella, and Wolffia is 2n=42.

URBANSKA-WORYTKIEWICZ (1980) recognizes three levels of cytological variation:

1) Intraindividual variation (aneusomaty and/or mixoploidy)
2) Intrapopulational variation (aneuploidy and/or polyploidy)
3) "Racial" variation (various cytotypes).

This cytological variation might be partly responsible for the differences in chromosome numbers by different authors. Even counts of the same clone did not always correspond if counted at different times or by different authors. URBANSKA-WORYTKIEWICZ (1980) mentions different chromosome numbers counted by KWANYUNEN and by herself (in brackets): No. 63/7121/8216 of W. columbiana, 2n=42(40,50), and No. 8218 of W. globosa, 2n=46(40). The clone No. 7014/8217 of W. arrhiza was counted from one source as 2n=40, 50,74 and from another source uniformly 2n=40. Differences in chromosome numbers of two clones of L. aequinoctialis were found by TAKIMOTO and by URBANSKA (see URBANSKA-WORYTKIEWICZ 1980): 2n= 78 and 81 (TAKIMOTO) and 2n=50 and 50, respectively (URBANSKA); GEBER

Fig. 3.2 (p. 131). Chromosome preparations of Lemna aequinoctialis by BEPPU (pers.comm. 1986)

a. S-type (No. 331) (2n=40) (x2600)
b. N_1-type (No. 7384) (2n c. 70) (x2000)
c. N_2-type (No. 382). Pollen mother cell (2=20) (x1500)

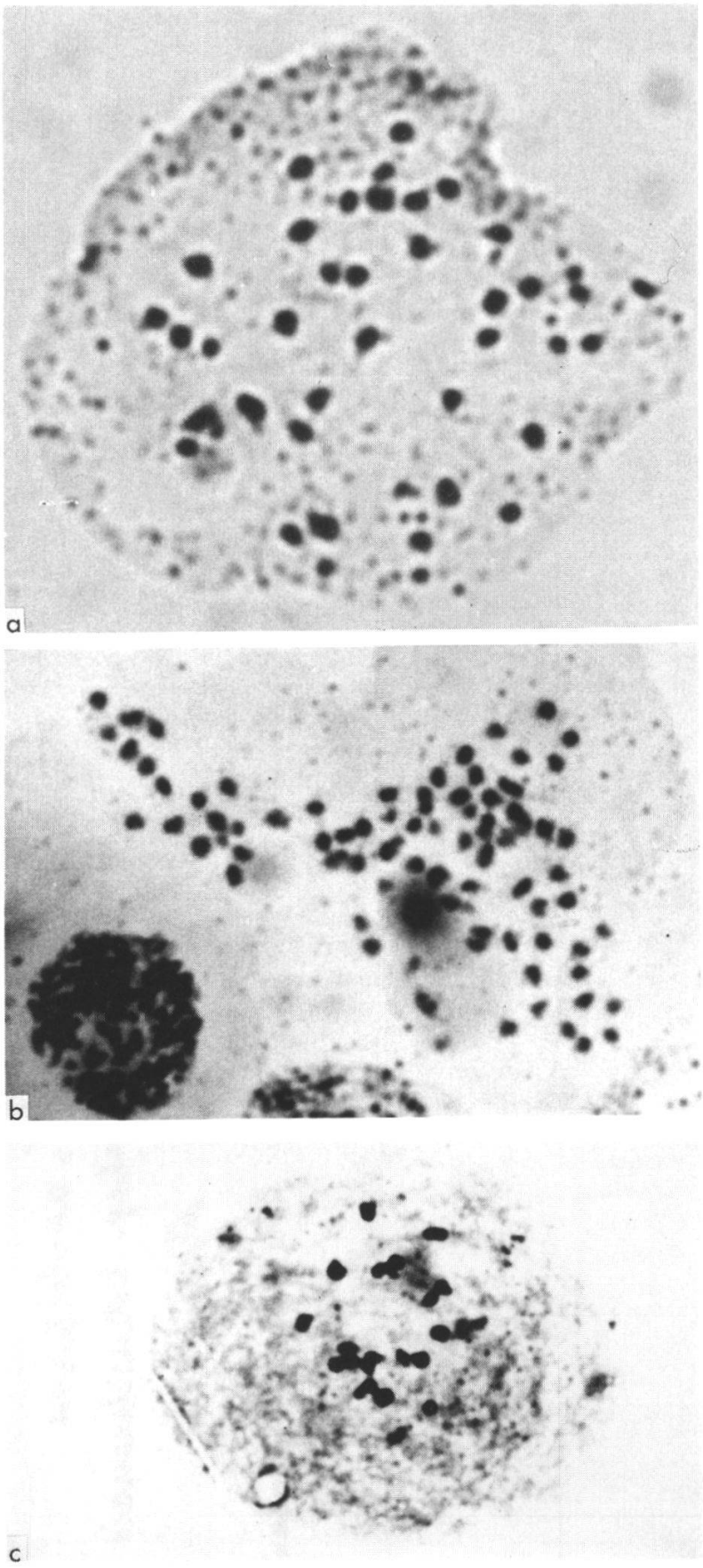

Fig. 3.2.

(in lit. 1986) counted 2n=84 for one of these clones. There are also differences between the chromosome numbers counted by URBANSKA-WORYTKIEWICZ (1980) and by GEBER (in lit. 1986) for the same clones and for some species. For instance, GEBER counted invariably 2n=36 for S. intermedia (6 clones), whereas URBANSKA's numbers are 2n=20 and 30, respectively. It seems that the preparation techniques of the different authors is responsible for the differences, at least partly. BLACKBURN (1933) made cross sections of material fixed with Langlet's modification of Navashin's fixative, embedded in paraffin. A similar method was used by WCISLO (1970). The staining was done with Newton's gentian violet. LAWALREE (1943), too, made microtome sections, but fixed with Benda or Regaud II fixative and stained with Haidenhain's iron hematoxylic solution. ROY and DUTT (1967), BANERJEE (1971), URBANSKA-WORYTKIEWICZ (1980) and BEPPU and TAKIMOTO (1981a) used squash technique. The material was fixed in acetic alcohol (1:3) with a small addition of ferric acetate or with propionic alcohol (BANERJEE 1971) and stained with aceto-carmin (ROY and DUTT 1967, WCISLO 1970, BEPPU and TAKIMOTO 1981a), propionic-carmin (BANERJEE 1971) or lacto-propionic-orcein (URBANSKA-WORYTKIEWICZ 1980). The last author writes that in chromosomes of Lemnaceae several heterochromatic segments might occur which were not visible with her preparations. This makes it difficult to demarkate some of the chromosomes. GEBER (in lit. 1986) used a special technique with application of DNA specific fluorochromes. He was able to recognize the heterochromatin with this method. First he fixed the material in acetic alcohol 1:3 after having prepared the plants with 0.002 M 8-hydroxychinol for 4 to 5 hours. To spread the chromosomes he developed an air drying technique in a similar way as has been done in chromosome preparation of human chromosomes. A protoplast suspension of the meristematic cells was prepared by destroying the cell walls and the middle lamellae enzymatically

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Fig. 3.3 (p. 133). Chromosome preparations by GEBER (pers.comm. 1986) (x2000)

a. Spirodela intermedia (No. 8410) (2n=36)
b. Spirodela polyrrhiza (No. 7551) (2n=40)
c. Spirodela punctata (No. 7461) (2n=46)
d. Lemna gibba (No. 7245) (2n=84)
e. Lemna japonica (No. 8653) (2n=63)
f. Lemna aequinoctialis (No. 7737) (2n=42)

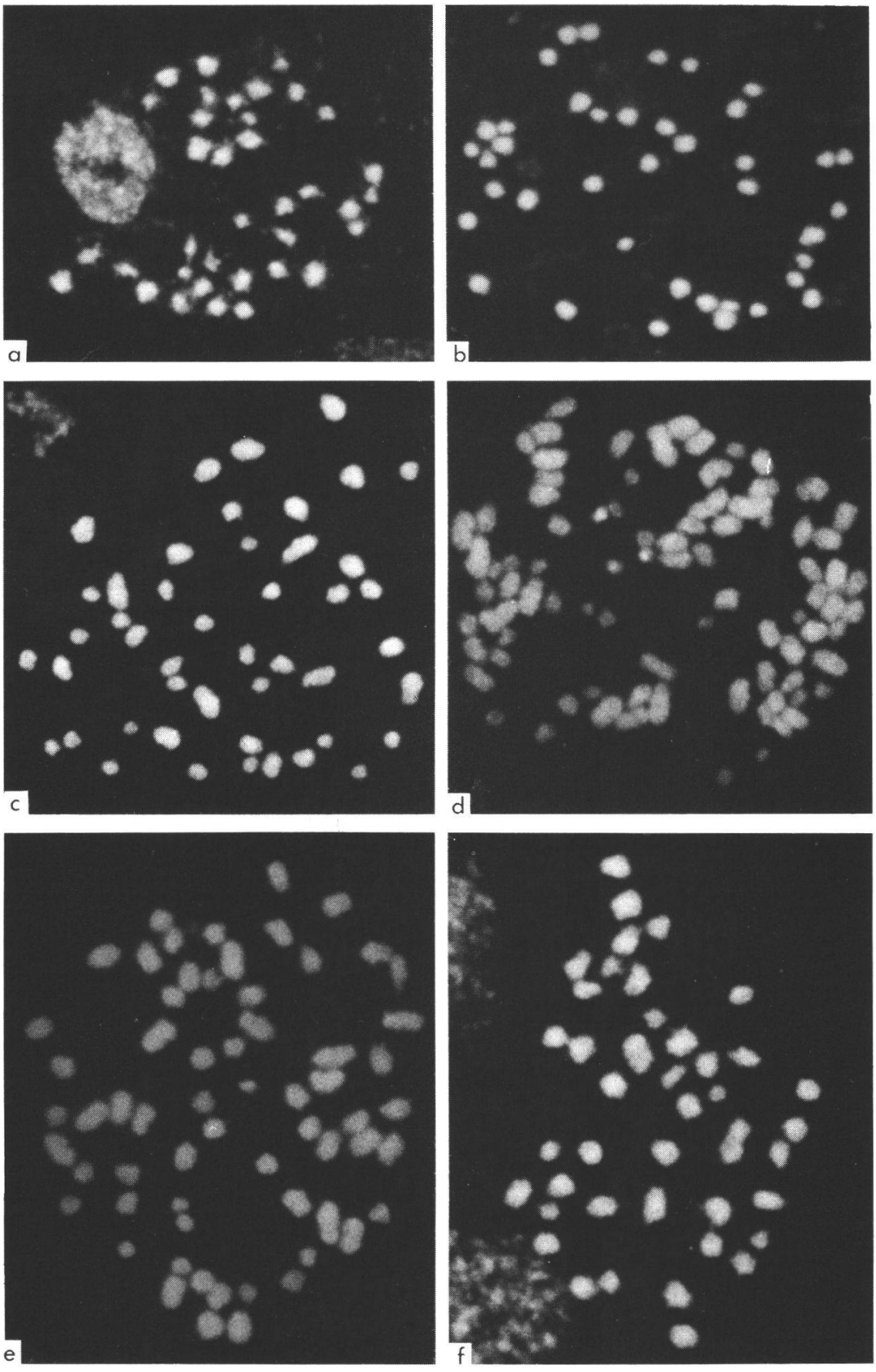

Fig. 3.3.

Table 3.1. Chromosome numbers of Lemnaceae

Spirodela

S. intermedia : 2n=20 (URBANSKA 1980, 2 clones), 2n=30 (URBANSKA 1980, 14 clones), 2n=36 (GEBER in lit. 1986, 6 clones).

S. polyrrhiza : 2n=30 (URBANSKA 1980, 11 clones), 2n=32(30+2) (BANERJEE 1971, 1 clone), 2n=38 (URBANSKA 1980, 1 clone), 2n=40 (BLACKBURN 1933, 1 clone; EHRENBERG 1945, 1 clone; WCISLO 1970, 3 clones; BANERJEE 1971, 1 clone; URBANSKA 1980, 171 clones; GEBER in lit. 1986, 6 clones), 2n=50 (URBANSKA 1980, 1 clone), 2n=80 (GEBER in lit. 1986, 1 clone).

S. punctata : 2n=40 (URBANSKA 1980, 59 clones), 2n=46 (GEBER in lit. 1986, 7 clones), 2n=50 (URBANSKA 1980, 22 clones).

Lemna

L. gibba : 2n=40 (URBANSKA 1980, 96 clones), 2n=42 (GEBER in lit. 1986, 3 clones), 2n=44 (GEBER in lit. 1986, 2 clones), 2n=50 (URBANSKA 1980, 11 clones), 2n=60 (WCISLO 1970, 1 clone), 2n=64 (BLACKBURN 1933, 1 clone), 2n=70 (URBANSKA 1980, 2 clones), 2n=80 (URBANSKA 1980, 4 clones), 2n=84 (GEBER in lit. 1986, 2 clones).

L. disperma : 2n=40 (URBANSKA 1980, 17 clones), 2n=44 (GEBER in lit. 1986, 2 clones).

L. minor : 2n=20 (URBANSKA 1980, 1 clone), 2n=30 (URBANSKA 1980, 3 clones), 2n=40 (BLACKBURN 1933, 1 clone; TISCHLER 1935, 1 clone; ROHWEDER 1937, 1 clone; DELAY 1947, 1 clone; WCISLO 1970, 11 clones; URBANSKA 1980, 271 clones), 2n=42 (BROOKS 1940, 1 clone; URBANSKA 1980, 24 clones; GEBER in lit. 1986, 4 clones), 2n=50 (URBANSKA 1980, 5 clones; MURIN and MAJOVSKY in LOEVE 1978, 1 clone), 2n=63 (GEBER in lit. 1986, 2 clones), 2n=126 (GEBER in lit. 1986, 1 clone).

L. japonica : 2n=40 (URBANSKA 1980, 5 clones), 2n=50 (URBANSKA 1980, 1 clone), 2n=63 (GEBER in lit. 1986, 2 clones).

L. obscura : 2n=40 (URBANSKA 1980, 29 clones), 2n=42 (GEBER in lit. 1986, 2 clones), 2n=50 (URBANSKA 1980, 1 clone).

L. turionifera : 2n=40 (URBANSKA 1980, 46 clones), 2n=42 (URBANSKA 1980, 9 clones; GEBER in lit. 1986, 2 clones), 2n=50 (URBANSKA 1980, 1 clone), 2n=80 (URBANSKA 1980, 1 clone).

L. trisulca : 2n=20 (URBANSKA 1980, 1 clone), 2n=40 (WCISLO 1970, 11 clones; URBANSKA 1980, 52 clones), 2n=42 (GEBER in lit. 1986, 3 clones), 2n=44 (BLACKBURN 1933, 1 clone; GEBER in lit. 1986, 1 clone), 2n=60 (URBANSKA 1980, 6 clones), 2n=63 (GEBER in lit. 1986, 2 clones), 2n=80 (URBANSKA 1980, 5 clones).

L. perpusilla : 2n=40 (URBANSKA 1980, 9 clones), 2n=42 (GEBER in lit. 1986, 2 clones).

L. aequinoctialis: 2n=20 (URBANSKA 1980, 1 clone), 2n=40 (URBANSKA 1980, 158 clones; BEPPU and TAKIMOTO 1981a, 8 clones), 2n=42 (GEBER in lit. 1986, 4 clones), 2n=50 (URBANSKA 1980, 7 clones; BEPPU and TAKIMOTO 1981a, 1 clone), 2n=60 (URBANSKA 1980, 4 clones), 2n=70(66,72,84) (BEPPU and TAKIMOTO 1981a, 14 clones), 2n=80 (URBANSKA 1980, 4 clones), 2n=84 (GEBER in lit. 1986, 2 clones).

Table 3.1 (continued)

L. valdiviana	: 2n=40 (URBANSKA 1980, 65 clones), 2n=42 (GEBER in lit. 1986, 2 clones).
L. minuscula	: 2n=36 (URBANSKA 1980, 3 clones), 2n=40 (URBANSKA 1980, 42 clones), 2n=42 (GEBER in lit. 1986, 2 clones).
Wolffiella	
W. hyalina	: 2n=40 (URBANSKA 1980, 5 clones).
W. neotropica	: 2n=40 (URBANSKA 1980, 4 clones).
W. Welwitschii	: 2n=40 (URBANSKA 1980, 3 clones).
W. lingulata	: 2n=20 (URBANSKA 1980, 2 clones), 2n=40 (URBANSKA 1980, 9 clones), 2n=42 (DAUBS 1965, 1 clone?), 2n=50 (URBANSKA 1980, 2 clones).
W. oblonga	: 2n=40 (URBANSKA 1980, 17 clones), 2n=42 (DAUBS 1965, 1 clone?; GEBER in lit. 1986, 1 clone), 2n=70 (URBANSKA 1980, 1 clone).
W. gladiata	: 2n=40 (URBANSKA 1980, 18 clones), 2n=42 (DAUBS 1965, 1 clone?).
W. denticulata	: 2n=20 (URBANSKA 1980, 1 clone), 2n=40 (URBANSKA 1980, 1 clone).
Wolffia	
W. microscopica	: 2n=40 (URBANSKA 1980, 1 clone), 2n=42 (KWANYUNEN in URBANSKA 1980, 1 clone), 2n=70 and n=35(ROY and DUTT 1967, 1 clone), 2n=80 (URBANSKA 1980, 1 clone).
W. brasiliensis	: 2n=20 (URBANSKA 1980, 1 clone), 2n=40 (URBANSKA 1980, 46 clones), 2n=42 (KWANYUNEN in URBANSKA 1980, 1 clone), 2n=50 (URBANSKA 1980, 10 clones), 2n=60 (URBANSKA 1980, 1 clone), 2n=80 (URBANSKA 1980, 1 clone).
W. borealis	: 2n=20 (URBANSKA 1980, 1 clone), 2n=22(22-24) (HONDO in lit. 1981, 1 clone), 2n=30 (URBANSKA 1980, 6 clones), 2n=40 (MOORE in DORE 1957, 1 clone; URBANSKA 1980, 9 clones).
W. australiana	: 2n=20 (URBANSKA 1980, 2 clones), 2n=40 (URBANSKA 1980, 10 clones).
W. angusta	: 2n=40 (URBANSKA 1980, 3 clones).
W. arrhiza	: 2n=30 (URBANSKA 1980, 1 clone), 2n=40 (URBANSKA 1980, 19 clones), 2n=42 (GEBER in lit. 1986, 3 clones), 2n= 42(33-49) (HONDO in lit. 1981, 1 clone), 2n=44-46 (LAWALREE 1943, 1 clone), 2n=50 (BLACKBURN 1933, 1 clone; WCISLO 1970, 1 clone; URBANSKA 1980, 3 clones), 2n=60 (URBANSKA 1980, 5 clones), 2n=62 (KWANYUNEN in URBANSKA 1980, 1 clone), 2n=63 (GEBER in lit. 1986, 3 clones), 2n=70 (URBANSKA 1980, 1 clone), 2n=80 (URBANSKA 1980, 1 clone).
W. columbiana	: 2n=30 (URBANSKA 1980, 7 clones; HONDO in lit. 1981, 1 clone), 2n=40 (URBANSKA 1980, 60 clones), 2n=42 (DAUBS 1965, 1 clone?), 2n=50 (URBANSKA 1980, 12 clones), 2n=70 (URBANSKA 1980, 1 clone).
W. globosa	: 2n=16 (BANERJEE 1971, 1 clone named as W. arrhiza), 2n=23(22-31) (HONDO in lit. 1981, 1 clone), 2n=30 (URBANSKA 1980, 1 clone), 2n=40 (URBANSKA 1980, 22 clones), 2n=46 (KWANYUNEN in URBANSKA 1980, 1 clone), 2n=50 (URBANSKA 1980, 3 clones), 2n=60 (BANERJEE 1971, 1 clone named as W. arrhiza; URBANSKA 1980, 5 clones).

with pectinase and cellulase. The fluorochrome staining was done with D 287/170 (4'-6-diamidino-2-phenylindol or DAPI) which shows a yellowish green fluorescence when irradiated by UV rays. Additional staining was applied with chromomycin A_3 and distamycin A or with actinomycin D.
All authors counted the chromosomes in mitosis; ROY and DUTT (1967) and BEPPU and TAKIMOTO (1981a) investigated also meiosis in W. microscopica and L. aequinoctialis, respectively.
The ploidy level of the species is expressed by the size of the pollen grains. Measurements made by URBANSKA (not published results) show that in L. turionifera and in L. aequinoctialis the diameter of pollen grains in a clone of each species with 2n=80 are 1.3 and 1.8 times larger than those of clones with 2n=40 (see fig. 2.51). In a preliminary experiment with W. brasiliensis (LANDOLT, not published) an inverse correlation could be observed between the chromosome number of the clone and the size of the biggest fronds (the clones were grown on 1/5 Hutner solution, 25°C and 16 hours day light of 15000 lux). 18 clones with 2n=40 showed a biggest frond length of 1.33 mm (± 0.11), 3 clones with 2n=50 1.13 mm (± 0.10) and 1 clone with 2n=60 0.90 mm, respectively. This results in a ratio of 1:0.85:0.67. Since the clone numbers of higher ploidy levels are very low, the results have to be checked on more strains.

3.2. MORPHOLOGICAL CHARACTERISTICS AND DNA CONTENT OF CHROMOSOMES

URBANSKA-WORYTKIEWICZ (1980) showed that chromosomes are smallest in Spirodela (length between 0.1 µm and 0.5 µm) and largest in Wolffia (mean length about 1.7 µm). Mean length of chromosomes in Lemna reaches 1.2 µm and in Wolffiella 1.4 µm. BANERJEE (1971) gives the total length of chromosomes for S. polyrrhiza to about 20 µm, for W. globosa (named as W. arrhiza) to nearly 60 µm. In contrast to S. polyrrhiza, he was able to distinguish between seven different chromosome types in W. globosa. The types varied from rather long (2 µm) to short (0.5 µm) and from median to subterminal positions of constriction.

GEBER (in lit. 1986) determined the DNA content of five different Lemnaceae species and of Pistia, cytophotometrically. As a standard of a known chromosome value, Pisum sativum was used. The results are put together in table 3.2.

The small amount of DNA in Spirodela is remarkable. Also the increase of DNA content with successive reduction of morphological structures within the family is quite surprising. It shows the unique position and the special behaviour of this small isolated family of extremely specialized water plants (see also chapter 7).

Table 3.2. DNA content of the chromosomes of Lemnaceae and Pistia (from GEBER in lit 1986)
C = chromosome set

	2n	pg DNA	pg DNA/1C	pg DNA/ chromosome
Pistia stratiotes	28	1.02/ 4C	0.25	0.036
Spirodela polyrrhiza 7110	80	1.19/ 8C	0.15	0.015
Spirodela punctata 7461	46	1.48/ 4C	0.37	0.032
Lemna minor 7115	126	5.82/12C	0.49	0.046
Wolffiella oblonga 79 (7166)	42	3.03/ 4C	0.76	0.072
Wolffia arrhiza 7347	42	6.53/ 4C	1.63	0.155