Discussion

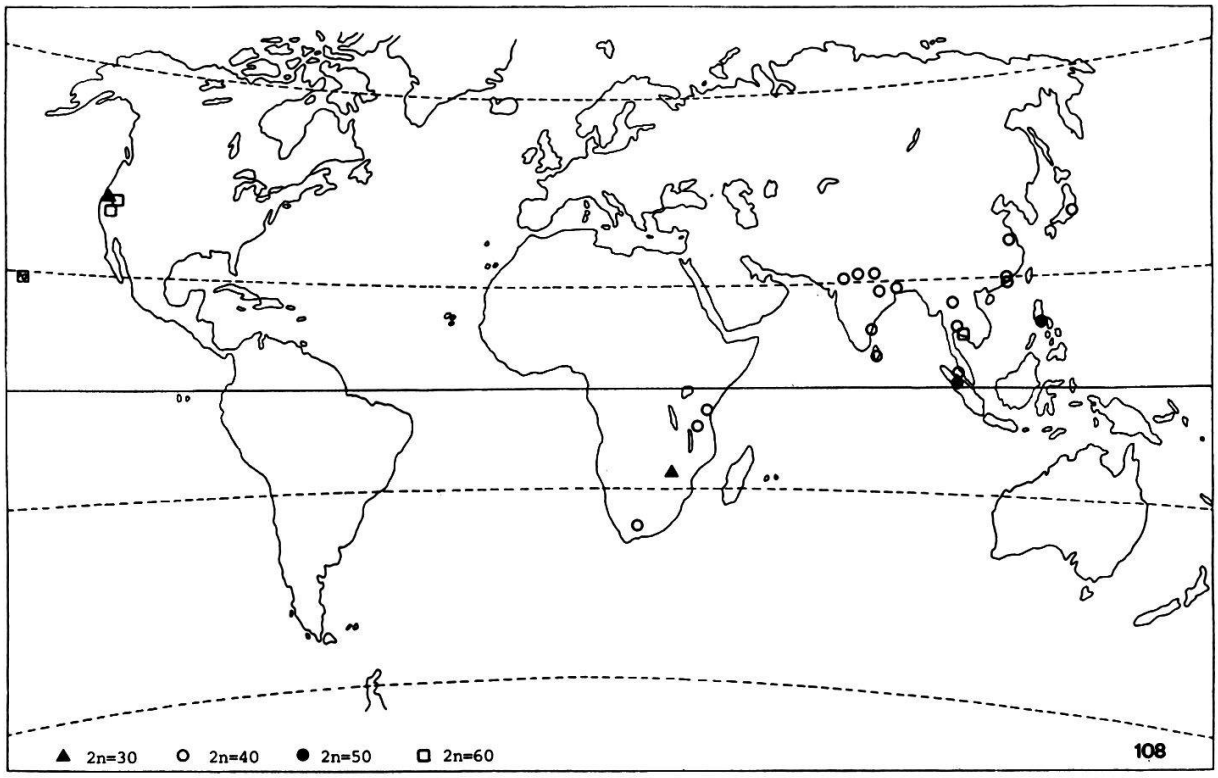

Fig. 108. *Wolffia globosa*: geographical distribution of the studied material. Numerous tetraploid stations are not indicated.

Discussion

Vegetative propagation in flowering plants represents a low-risk strategy of proliferating the genotype but avoiding all hazards involved in the sexual reproduction. The family of *Lemnaceae* offers an excellent example of such strategy, the predominant vegetative propagation accompanied by the spontaneous fragmentation of clones and reinforced by the production of turions in some taxa being a great advantage for survival and dispersion. However, a scientist studying variation within the duckweed family is faced with the dilemma of the sample choice. On the one hand, the genotype identification in wild populations of the *Lemnaceae* is practically impossible with current methods; for this reason, the useful suggestions recently made by HARPER and

WHITE (1974) and HARPER (1977) about distinguishing between genets and ramets do not seem applicable. On the other hand, a study carried out in a sterile clonal culture does give some information on an individual variation, but has an indicative rather than a definitive value, the laboratory conditions being obviously quite different from the natural environment of the duckweeds; furthermore, it cannot be decided whether the observed details are representative of a given population or represent fortuitous aberrations, only a single genotype being frequently investigated. In the present study we tried, in some cases, to compare both these aspects, but further investigations are indispensable to elucidate some of the results obtained on cytological variation within the *Lemnaceae*. The explanations offered here are thus admittedly speculative.

Clonal multiplication results in an increased longevity of given genotypes; it allows therefore for the maintenance of particular gene combinations, the genetic variation being accordingly rather limited in most cases (e.g. GRANT 1971, WILLIAMS 1975, ABRAHAMSON 1980). The duckweeds conform to this trend; as far as the cytological variation is concerned, intra-individual and intra-populational variation was only occasionally observed in the course of the present study, the respective frequencies being 3.3.% and 1.5%. The *Lemnaceae* correspond in this respect to the concept of WILLIAMS (1975) who distinguished mitotically standarized asexual offspring from meiotically diversified sexual progeny.

The stabilizing effect of vegetative propagation embraces as well its influence upon occasionally appearing mutations. Of a particular interest are in this respect mutations of the genome; as vegetative propagation permits to by-pass the detrimental effects of low fertility and/or vigour known in aneuploids and odd polyploids, the deviating chromosome numbers may be transferred to next generations. The recurrent pattern of cytological variation occurring within the whole family of *Lemnaceae* strongly suggests that the asexual reproduction plays an important rôle in differentiation mechanisms operating within this group. Intra-individual variation being apparently carried through numerous cell generations, the daughter fronds may receive various chromosome number. An aneusomatic frond could thus occasionally give rise to both eu- and aneuploid groups; mixoploid fronds could in turn contribute to the formation of groups representing different levels of polyploidy. It is also con-

ceivable that, depending on current environmental conditions, population can contract or expand; some clones might then become locally abundant and eventually colonize the whole areas, whereas the other ones disappear. The occurrence of cytologically uniform clones and colonies of the *Lemnaceae* that represent chromosome numbers deviating from the habitual for the duckweeds 2n=40 points out towards the importance of vegetative propagation to the development and maintenance of populations; in this respect, the present paper can be considered as yet another contribution to the ample documentation existing on the subject (e.g. COTTAM 1954, HARBERD 1961, ANDERSON and LOUCKS 1973, WIGHAM 1974, STEWARD and ORNES 1975, URBANSKA-WORYTKIEWICZ 1977, 1977a, 1979, 1980).

The cytological variation occurring within the family of *Lemnaceae* offers some interesting aspects. Aneusomaty was frequently considered as the result of some mitotic disturbances occurring with a particular frequency in taxa with a strong vegetative propagation (e.g. SNOAD 1955, SHARMA 1956, SINHA 1962, MEYER 1965, RYCHLEWSKI 1967). As far as the *Lemnaceae* are concerned, pronounced mitotic deviations were but exceptionally observed. It cannot be excluded that some minor deviations or/and elimination of a few chromosomes might sometimes have passed unnoticed; it seems, however, that mitotic aberrations are rather ancillary to some other factors influencing the aneusomaty in the duckweeds. Another explanation could be structural rearrangements of chromosomes, in particular fragmentations and/or translocations (BROOKS 1940, SHARMA and DAS 1954). Numerous taxa of the *Lemnaceae* manifest some differences in chromosome size within their sets; there are also indications that some chromosome sectors might be heterochromatic. It is therefore conceivable that some structural rearrangements might indeed occur; however, a positive evidence in this respect is still missing, chromosomes of the *Lemnaceae* being highly unsuitable for karyotype analysis that calls perhaps for more refined preparation techniques than those used in the present study.
Aneuploidy occurring within the family of *Lemnaceae* might as well result from a selective endoduplication, only a part of the nucleus being involved in the multiplication process. DUNCAN (1954) who studied aneusomaty in *Paphiopedilum wardii* found out that only three chromosome types were particularly liable to endoduplication, the remainder of the chromosome complement being much more

stable. Aneuploid plants occurring within diploid populations of *Cardamine pratensis* (URBANSKA-WORYTKIEWICZ and LANDOLT 1974) proved to be polysomic, the smallest pair of chromosomes being involved in this variation. GLAESS (1957) observed aneusomaty in the liver cells of rats and suggested that deviating chromosome numbers resulted from a selective endoduplication operating in chromosome groups that accounted for the viability of particular aneuploid nuclei. Results of BREMER (1949, 1959, 1961) concerning some *Saccharum*-hybrids point out that a preferential endoduplication may not only appear in vegetative tissues but also influence occasionally reproductive cells.

A preferential endoduplication as the mechanism accounting for the aneuploidy within the *Lemnaceae* remains, for the time being, an alternative open to verification. On the other hand, it seems to be a sole plausible explanation for the peculiar pattern of euploid differentiation occurring within the family.

Euploid chromosome numbers found within the *Lemnaceae* form on the whole a continuous range i.e. 2n=20, 30, 40, 50, 60, 70, 80; differences between particular levels of polyploidy correspond to a single genome, the number ten being regarded as basic for the family. As far as the intraspecific differentiation is concerned, 17 out of 21 taxa that possessed more than one euploid chromosome number corresponded, at least partly, to the single-genome-difference pattern. Mixoploid individuals carried chromosome numbers that most frequently differed from each other by a single genome; in addition, odd genome differences were sometimes observed (e.g. 2n=40, 60; 2n=40, 70; 2n=50, 80). Intra-individual polyploid differentiation is obviously well-known both in plants and animals; however, the type represented by the *Lemnaceae* seems to be rather infrequent. A comparable case represents *Zephyranthes mesochloa* propagating principally by bulbs; SHARMA and GOSH (1954) observed in somatic cells of this Amaryllid a wide range of euploid chromosome numbers (2n=42, 48, 60, 66, 72) and supposed that the multiplication of chromosomes possibly involved only members of a single genome (x=6). SHARMA and DE (1956) proposed the analogous explanation for the peculiar differentiation observed in somatic tissues of *Dioscorea alata* (2n=30, 40, 50, 70). It should be added that later on, MARTIN and ORTIZ (1963) found in *Dioscorea alata* a still wider range of euploid differentiation identical with that occurring within the family of *Lemnaceae*.

We are inclined to think that endoduplication might influence the polyploid differentiation occurring within the duckweeds. Patterns of variation observed in the mixoploid individuals suggest that a selective endoduplication might occur, sometimes repeatedly, in some cells of tetraploid plants. On the other hand, the octoploid chromosome number might result from a complete doubling of the 40chromosomic set. As mentioned before, nearly all mitoses were apparently normal and no pronounced deviations that might lead to a restitution were observed; they could not have remained undetected in our ample material originating from 1500 habitats and comprising very numerous fronds. It seems hardly acceptable anyway that either mitotic aberrations or structural rearrangements of chromosomes should regularly raise chromosome numbers just for a single genome. A detailed study on the *Lemnaceae* dealing with intervening phases of the nuclear cycle should be most desirable, as conclusive data are lacking.

Origin and significance of euploid chromosome numbers lower than 2n=40 remain ambiguous. It might be that 2n=20 and 2n=30 partly represent issues of some reversion process, reduction of chromosome numbers being sometimes observed in somatic tissues of plants (e.g. HUSKINS 1948, KITANI 1963, FELDMAN et al. 1966, BROWN and STACK 1968, STACK and BROWN 1969, SEGMEN 1971); however, no structures corresponding to a somatic reduction were found in the studied material of the duckweeds. Each of the clones with 2n=20 or 2n=30 that represented only 3.9% of the whole examined material originated from a different locality, so that the results offer no information as to the actual structure of the populations; it should be noted in this context that in two populations fixed in the wild that respectively comprised 2n=20, 40 and 2n=30, 60, fronds with the lower chromosome number represented the minority. On the other hand, 55.9% of all diploid and triploid clones were found within the genus *Spirodela*; it cannot therefore be excluded that, in some cases, chromosome numbers 2n=20 and 2n=30 might indicate some ancestor types. This suggestion, however, requires further verifications; studies on cytological variation within populations of *Spirodela* from South and Central America and especially those on *S. intermedia* should be particularly important in this respect.

Some aspects of cytological variation observed in the studied material suggest that the *Lemnaceae* may sometimes be cytologically instable. In our previous paper (URBANSKA-WORYTKIEWICZ 1975) we reported intriguing fluctuations in chromosome numbers observed in a clonal culture of *Lemna minor* over a few-year-period; comparable cases were occasionally found in the course of the present work. For instance clonal material of *Wolffia columbiana* sent us from two independent sources in 1968 and 1973 revealed a different chromosome number viz. 2n=50, 2n=40 in either series; the cultures of *Wolffiella oblonga* first received in 1968 from one source and then twice (1971, 1973) from another laboratory, had in the first series 2n=70, in both others 2n=40. Two strains of *Lemna aequinoctialis*, studied by the present author in 1970 and 1973, were invariably pentaploid (2n=50); this year, they were sent on request to Japan, where chromosome numbers representing a higher level of polyploidy i.e. 2n=78, 81 were found (TAKIMOTO, personal communication). Cytological instability in plants is little known, well-documented cases being very rare in the literature (e.g. BRITTON and HULL 1956, LEWIS 1962, 1970, 1970a, LEWIS et al. 1971, FAVARGER 1975, 1978); it should be noted that these reports deal mostly with more or less continuous variation, whereas in the *Lemnaceae* different polyploidy levels were usually revealed. No long-range study, except the outstanding contribution of LEWIS (1970) has included so far the effect of time upon chromosomal variability within a single wild population; investigations in clonal material of some *Lemnaceae* carried out over a longer time-span should be most interesting.

In conclusion, a comment upon a general philosophy of the chromosome counts in cytologically difficult groups should be offered. In his excellent review, FAVARGER (1978) pointed out towards two principal causes of discrepances appearing in the literature viz. 1) possible errors and 2) infraspecific differentiation comprising not only geographical but also less known intra-individual and intra-populational variation. Our results obtained on the *Lemnaceae* differ partly from other reports. We don't qualify those data as erroneous, preferring to consider them as indications of the complex cytological variation occurring within the family of *Lemnaceae*. The duckweeds are not only exceedingly difficult as far as a chromosome study is concerned, but also present great problems when their morphology is examined. According to LANDOLT

(1980, see p. 13 of the present volume) "... it is sometimes difficult to distinguish between particular species without having observed living plants at various developmental stages". This dynamic approach seems advisable in further studies on cytological variation in the *Lemnaceae*, for it gives more chance of coming to satisfactory conclusions when we ask how much of what we find is accident or design.

Summary

Cytological variation was studied in 30 taxa of the family of *Lemnaceae*, material from 1500 localities being examined.
The duckweed family offers an excellent example of a low-risk life strategy based upon a predominant or exclusive vegetative reproduction, its genetic variation being accordingly limited. The recurrent pattern of cytological variation observed within the *Lemnaceae* strongly suggests that the asexual reproduction plays an important and stabilizing rôle within this group.
Three levels of cytological variations were observed:
1) intra-individual variation occurring in form of aneusomaty and/or mixoploidy;
2) intra-populational variation (aneuploidy or polyploidy);
3) "racial" differentiation corresponding to clones and populations that were cytologically uniform yet represented various cytotypes.

Euploid chromosome numbers of the *Lemnaceae* formed a continuous range viz. 2n=20, 30, 40, 50, 60, 70, 80, the tetraploid number 2n=40 being positively the most frequent. Mixoploid individuals as well as populations and taxa possessing more than one euploid chromosome number conformed most frequently to a curious single-genome-difference pattern; it is supposed that a selective endoduplication might influence this variation.
Origin and significance of euploid chromosome numbers lower than 2n=40 remain ambiguous. 2n=20 and 2n=30 might partly result, on the one hand, from some reversion processes; on the other hand, particular frequency of these numbers observed within some taxa of the genus *Spirodela* might indicate some ancestor types. The problem remains open to further verifications.
Some aspects of cytological variation suggest that the duckweeds may sometimes be cytologically instable; long-range studies, comprising ageing clonal cultures, are advised.
In conclusion, a comment upon a general philosophy of the chromosome counts in cytologically difficult groups is offered.

Zusammenfassung

An 30 Arten der Familie der Lemnaceen wurde die zytologische Variation untersucht. Insgesamt fanden 1500 Proben verschiedener Herkünfte Berücksichtigung.