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SPECIATION IN DUCKWEEDS (LEMNACEAE): PHYLOGENETIC AND ECOLOGICAL INFERENCES

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ABSTRACT

Species of duckweeds (Lemnaceae) that were resolved as sister taxa in a phylogeny based on combined molecular and non-molecular data were compared for morphological, physiological, and ecological attributes to infer factors important in the initial divergence leading to speciation. The ability to survive extreme conditions such as desiccation and cold temperatures is the most common difference identified between species. Two morphological characters facilitating survival in extreme environments are production of special resting buds called turions and increased seed production. The prevalent geographic pattern for species pairs consists of one restricted species occurring on the periphery of a more widespread taxon; this pattern indicates that divergence of peripheral isolates is a common geographical mode of speciation in duckweeds. Other species differ in optimal environmental conditions for growth, and these species are largely sympatric. In only one instance does it appear that divergence and speciation occurred following long-distance dispersal. Sympatric species pairs have the lowest molecular divergence; widespread primarily allopatric, and distantly allopatric species have the highest molecular divergence. Despite infrequent sexual reproduction, some degree of detectable variation (molecular, physiological, etc.) occurs within populations and among populations of the same species. Molecular evidence indicates that variation within duckweeds extends from the population and intraspecific levels to very different levels of divergence among recognized species. Contrary to the appearance of morphological and ecological uniformity implied by their reduced morphology and restricted occurrence in fresh water habitats, duckweeds are not a group in evolutionary stasis. Rather, these smallest of all flowering plants are dynamic evolutionarily, and comprise a model system for studying plant evolution and speciation.

Key words: duckweeds, ecology, Lemnaceae, molecular, phylogeny, speciation.

INTRODUCTION

The family Lemnaceae (duckweeds) comprises highly reduced aquatic monocots in which there has been extreme reduction in both the size and presence of organs (Landolt 1986). The family consists of 37 species in five genera (Landolt 2000; Kimball et al. 2003). The highly reduced morphology of duckweeds has caused difficulties in species recognition and in hypothesizing relationships in the family because few characters and character states are available for analysis. Molecular data, particularly plastid DNA sequences, have been utilized to refine species limits and to gain insights into phylogenetic relationships within the family (Crawford and Landolt 1993, 1995; Crawford et. al. 1996, 1997; Les et al. 2002; Kimball et al. 2003). Molecular data, combined with morphology and secondary chemistry, produce a well-resolved phylogeny for the family (Les et al. 2002; Fig. 1) in which all sister species and nearly all groups have strong internal (bootstrap) support. Here we interpret this phylogeny in terms of prior taxonomic and phylogenetic concepts for the duckweeds (e.g., Landolt 1986, 1992, 1994*a*, *b*, 1998). Furthermore, plastid sequences and allozymes have been used to calculate divergence times, something that is essentially impossible to estimate with morphology alone in most flowering plants, let alone in a group so reduced as duckweeds.

The extreme reduction of the duckweed body, while causing taxonomic problems, allows members of the family to be cultured easily, and this feature, combined with their clonal growth, makes them excellent subjects for experimental studies (Landolt 1986). Several investigations have elucidated physiological, morphological, ecological, and other differences, not only between species, but also between strains of the same species. In addition to experimental laboratory studies, field investigations have been directed at identifying factors that limit species distributions and at explaining how these organisms have adapted to environmental conditions (Landolt 1975, 1992, 1994*a*, *b*, 1997, 1998; Landolt and Zarzycki 1994). Landolt (1986, 1987) provided overviews of eco-geographical variation within Lemnaceae and suggested that ecological divergence has been extremely important in duckweed speciation. He hypothesized that the ability to survive extreme conditions, such as cold temperatures and desiccation, was especially important in the initial divergence of lineages.

Although there are few detectable morphological characters by which divergence within and between species may be evaluated, there are other genetically determined characters such as growth rates, response to different nutrient conditions, and flowering behavior, that readily differentiate duckweed strains (Landolt 1986, 1987). Molecular variation

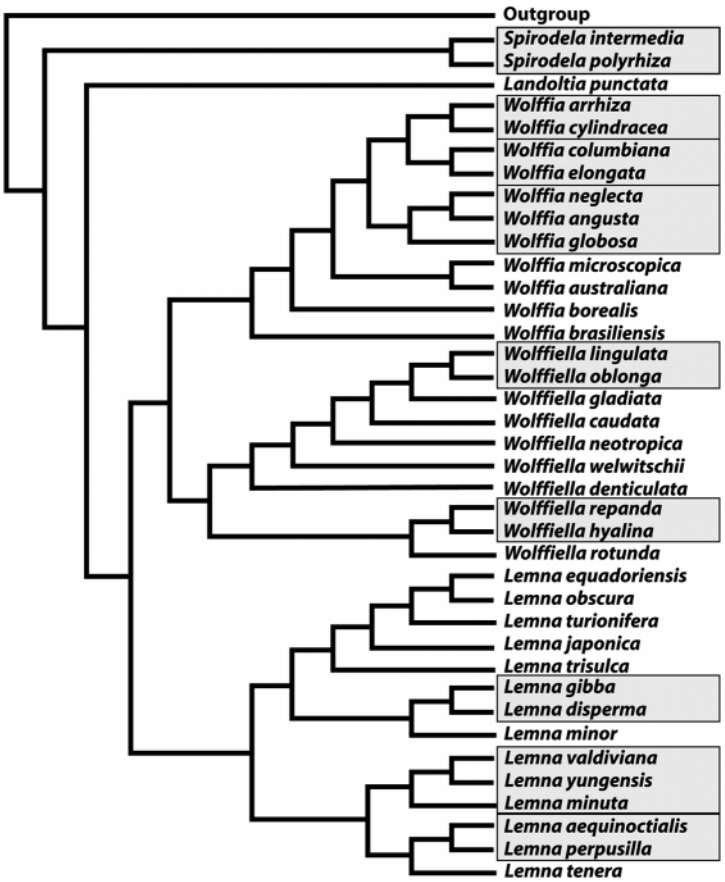

Fig. 1.—Interrelationships among duckweed species indicated by a maximum parsimony cladogram resulting from combined analysis of morphological, flavonoid, allozyme, and plastid DNA sequence data (redrawn and simplified from Les et al. 2002). Taxa discussed in text are shaded, and all have bootstrap support of 96% or higher. All but two remaining clades in the tree have support higher than 70%.

also has been found among clones of duckweed species isolated from the same as well as from different localities (Vasseur et al. 1993; Jordan et al. 1996; Crawford et al. 2001). Landolt (1987) argued further that infrequent sexual reproduction in Lemnaceae, due to low frequency of flowering and fruit set, does not retard differentiation; on the contrary, the very rapid rate of vegetative propagation would facilitate the generation of somatic mutations and the rapid fixation of mutants in clones, even at a local level. Differences arising between strains of duckweed species while growing in culture provide support for Landolt's (1987) hypothesis that generation and maintenance of variation do occur despite the extreme rarity of sexual reproduction in most species.

The existence of a well-resolved and well-supported phylogeny, along with a wealth of other information available for duckweeds, has prompted the present study where we compare closely related species for a variety of ecological, life history, and other attributes. Our primary purpose is to infer features associated with divergence and speciation in Lemnaceae. We also use divergence times calculated from molecular data to estimate when ecological divergence, and thus presumably speciation, was initiated.

MATERIALS AND METHODS

The phylogeny of Les et al. (2002), which is redrawn in Fig. 1, was used to identify sister species or species groups in strongly supported clades. In order to increase the chances of identifying differences associated with speciation instead of features that diverged subsequent to speciation (Templeton 1982), only those taxa exhibiting low divergence in plastid sequences and at allozyme loci (when available) were selected for comparisons (Fig. 1; Table 1). Divergence times for species were estimated from chloroplast DNA sequences for coding (*mat*K and *rbc*L) and noncoding (*rpl*16 and *trn*K introns) regions. GenBank accession numbers for the sequences are given in Table 1 of Les et al. (2002) and in Kimball et al. (2003). Allozyme data used in the calculations were from Crawford and Landolt (1993, 1995) and Crawford et al. (1996, 1997, 2001).

To estimate divergence times, we calculated pairwise distance between sister species, and in a few cases, among three closely related species. This two-and-three taxon approach was used (rather than estimating distances from branch lengths off the entire phylogeny) to avoid different rates of evolution that may have occurred between different genera (e.g., Les et al. 2000, Fig. 2). In a preliminary analysis, we estimated pairwise distances using different methods for sequence correction, including p-distances (uncorrected), Tamura-Nei 93 (TN93) distances, Tamura-Nei 93 plus gamma distances, and Tajima-Nei distances as calculated in MEGA 2.1 (Kumar et al. 2001). Because standard deviations among the different sequence correction methods were very mini-

Table 1. Ecological divergence among closely related species in Lemnaceae. Structures or other features facilitating survival in extreme habitat conditions and estimated divergence times are given for various species groups exhibiting ecological divergence in survival attributes; mybp = million years before present.

Extreme habitat condition	Survival factor	Divergence time (mybp) ± sd
1. Survival of cold season		
Lemna aequinoctialis–L. perpusilla	seed dormancy	2.38 ± 2.50
Spirodela intermedia–S. polyrhiza	turion production	8.70 ± 2.25
2. Survival of desiccation and growth in seasonal water		
Wolffia angusta–W. neglecta	seeds and/or turions	2.02 ± 2.02
Wolffia globosa–W. angusta–W. neglecta		0.22[a]
Wolffia arrhiza–W. cylindracea	turion production	3.72 ± 1.56
Wolffia columbiana–W. elongata	high seed production	2.08 ± 1.32
Wolffiella hyaline–W. repanda	high seed production	1.14 ± 0.16
3. Growth in colder–warmer temperatures		
Lemna minuta–L. valdiviana and *L. yungensis*	physiological	0.93[a]
Wolffiella lingulata–W. oblonga	physiological	0.87 ± 0.82
4. Growth in nutrient-poor water		
Lemna valdiviana–L. yungensis	physiological	1.2 ± 1.76
5. Similar habitats–allopatric distribution		
Lemna gibba–L. disperma	n.a.	5.5 ± 3.5

[a] Divergence time between first species and common ancestor of other two species.

mal (much lower than between different data sets), the uncorrected values (p-distances) were employed for the final analysis. For *rbc*L, the synonymous substitution rate of 0.12% per million years was employed for calculating divergence times, a rate chosen because it is similar to rates calculated for a variety of different plant groups (Xiang et al. 2000). Synonymous substitution rates two to six times higher for *mat*K than for *rbc*L have been reported (Johnson and Soltis 1995), with rates toward the lower end of the spectrum more common (Johnson and Soltis 1994; Xiang et

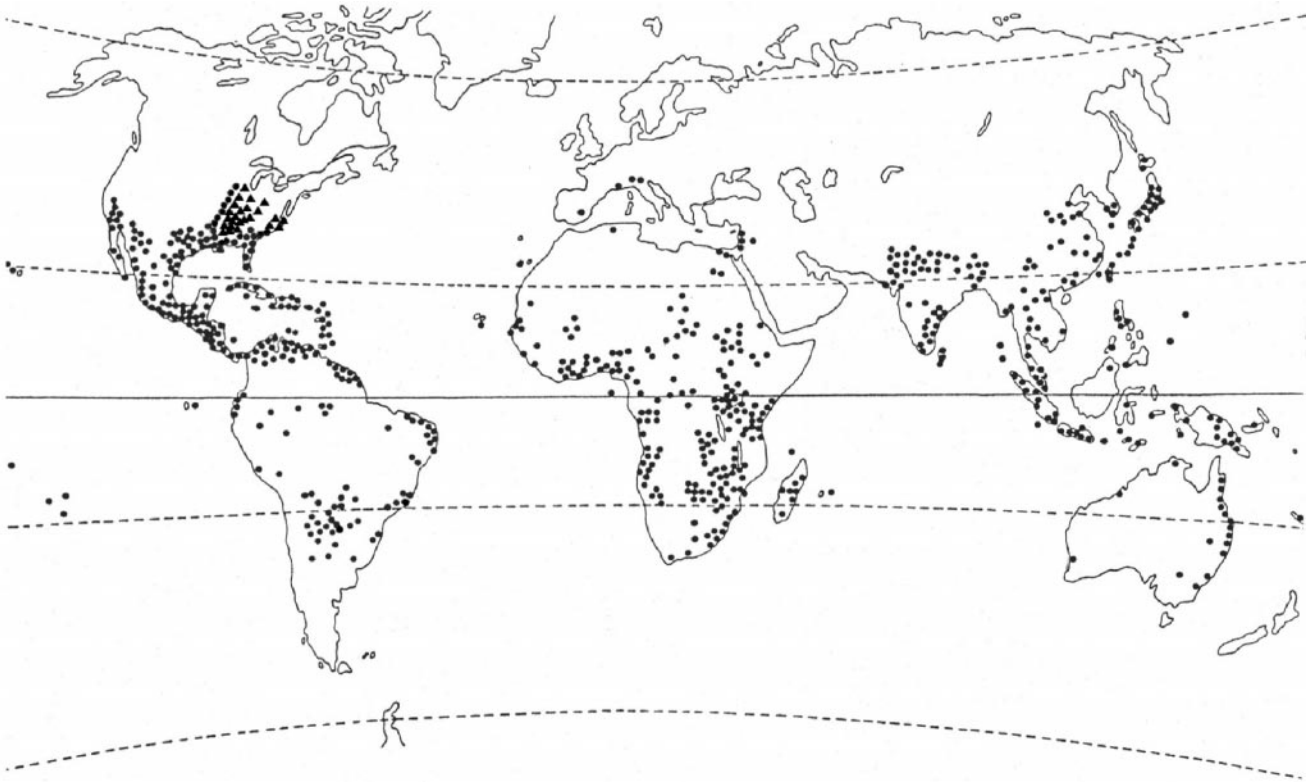

Fig. 2.—Geographic distributions of *Lemna aequinoctialis* Welw. (circles) and *L. perpusilla* Torr. (triangles), with the latter species restricted to eastern North America. (Modified from Landolt 1986).

al. 1998). Accordingly, we chose the value of 0.24% per million years for *mat*K synonymous substitutions. For the noncoding regions *rpl*16 and *trn*K, our estimated rate of 0.198% per million years was calculated from the rate for *mat*K in Lemnaceae (Les et al. 2003).

Divergence times were calculated between each pair of sequences using equation 4.1 of Li and Graur (1991: 67), that is, one half of the p-value for two species divided by the substitution rate for the sequences being compared. When comparisons involved divergence between a species (C) and the common ancestor of two species (A, B), divergence times were calculated by subtracting the divergence time between the two sister species (T_{AB}) from the mean divergence time for the species and the two sister species, $(T_{AC} + T_{BC})/2$.

Two methods were employed to calculate divergence times from allozyme data. One used Table 9.2 of Nei (1987: 237) and the other employed the method of Sarich (1977) that was discussed by Thorpe (1982). In all comparisons using allozymes, both values were included in the calculations. The estimated divergence times for species given in Table 1 represent the means for sequences from chloroplast regions (four in most cases) and, when available, allozyme data were included in the calculations.

Data on ecology, morphology, and geographic distributions were taken from the literature (Landolt 1975, 1986, 1987, 1992, 1994*a*, *b*, 1997, 1998, 2000; Landolt and Zarzycki 1994). Landolt (1986) presented an exhaustive summary and synthesis of the literature up to the mid 1980s. Additional information on the duckweed species is from the unpublished field and laboratory observations of E. Landolt made during the past half century.

RESULTS AND DISCUSSION

Ecological Divergence

There are two sister species pairs in which one species can survive a cold season (overwinter) and the other cannot, and this may have been the key factor in their initial divergence leading to speciation (Table 1; Fig. 1). The sister species *Lemna aequinoctialis* and *L. perpusilla* (Crawford et al. 2001), which at one time were treated as a single species (Landolt 1986), are unlike most other *Lemna* L. species in having frost sensitive fronds. Therefore, they require some survival mechanism in order to occur in areas with cold winters. *Lemna aequinoctialis,* which is almost totally restricted to warmer environments (Fig. 2), has seeds that are released from the fruit and germinate as soon as they are mature. The resulting fronds would be killed by the onset of freezing temperatures. By contrast, seeds of *L. perpusilla* require a cold period to break dormancy and germinate. These seeds are retained within the fruits that overwinter by sinking to the bottom of the water along with the dead fronds. The seed dormancy of *L. perpusilla* enables it to grow in temperate areas of eastern North America that extend just beyond the range of *L. aequinoctialis* (Fig. 2). The evolution of *L. perpusilla* may have involved the origin of cold tolerant ecotypes (i.e., seeds requiring a cold treatment) in *L. aequinoctialis* similar to the situation now seen in northern Japan (see discussion below). Seed dormancy is unknown elsewhere in *Lemna* (Landolt 1986).

The two species comprising the genus *Spirodela* Schleid. are *S. intermedia,* which occurs only in warm temperate climates of Central and South America, and *S. polyrhiza,* which is found nearly worldwide except for most of South America (Fig. 3). Both species have frost sensitive fronds and thus are restricted to areas with mild growing seasons. Overall, *Spirodela polyrhiza* is reduced and hence derived morphologically compared to *S. intermedia* (Landolt 1986, 1987). However, the ''key'' ecological difference between the two species is the development of turions in *S. polyrhiza*; mapping of turions onto the phylogeny of Lemnaceae showed that *Spirodela polyrhiza* represents one of four separate origins for the structures. Turions withstand colder temperatures than the normal fronds (Landolt 1986). In addition, turions are filled with starch, sink to the bottom of the water, and are able to survive there for many months at temperatures approaching 4°C, thereby allowing the species to grow in areas with colder winter temperatures where *S. intermedia* cannot survive. Turions provide *S. polyrhiza* with an effective means of dispersal and colonization (Landolt 1987), but as mentioned above, its frost-sensitive fronds limit it to areas with warm growing seasons. Landolt (1987) proposed that the range of *S. polyrhiza* expanded initially from South America to North America, then to Europe, and subsequently south and eastward into Asia and Africa. Although there has been no explicit phylogenetic test of this hypothesis, Crawford and Landolt (1993) detected distinct multilocus allozyme genotypes in North America, in Europe and Asia, and in Africa, thereby indicating a corroborating pattern of geographical differentiation.

Whether a duckweed species grows in permanent or seasonal water represents a fundamental difference in the ecology and life history of the species. In order to inhabit seasonal water, species must have some mechanism for surviving the dry season. Four species pairs in two different genera differ in whether they grow in permanent or seasonal waters (Table 1; Fig. 1). Landolt (1997) demonstrated experimentally that the turions of the southern African species *Wolffia cylindracea* (Fig. 4), in contrast to the turions produced by other species of Lemnaceae, resist desiccation for time periods adequate to survive dry seasons in their natural habitats. Because *W. cylindracea* does not produce seeds, turions apparently are its sole means of colonization and for survival of seasonal droughts. *Wolffia arrhiza,* the more widespread sister species of *W. cylindracea* (Fig. 1, 4), also produces turions. However, the turions of *W. arrhiza* are not able to survive dry soils, the species has very low seed set, and it is therefore limited to permanent waters (Table 1; Landolt 1997).

Wolffia angusta and *W. neglecta* are quite similar morphologically (even for duckweeds), with the latter species described recently (Landolt 1994*b*) to accommodate subtly distinct populations of *W. angusta* from India, Pakistan, and Sri Lanka (Fig. 5). The two species are divergent at allozyme loci (Crawford and Landolt 1995) and in plastid DNA sequences (Les et al. 2002). Field observations (Landolt unpubl. data) indicate that *W. neglecta* occurs in seasonal water whereas *W. angusta* occupies only permanent water. It is not clear how *W. neglecta* survives desiccation; both seeds and turions are possibilities (Landolt 1994*b*). However, because the turions of *W. neglecta* are not as resistant to desiccation

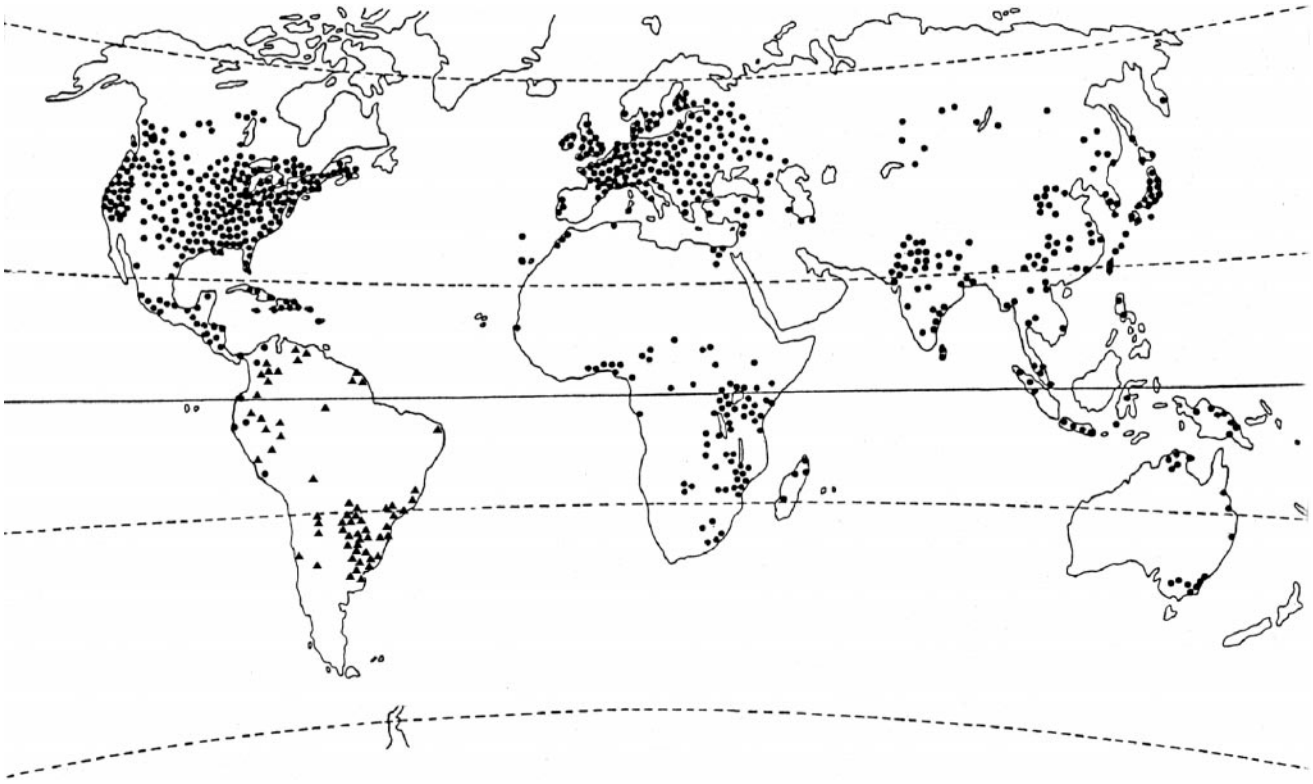

Fig. 3.—Distribution of *Spirodela intermedia* W. Koch (triangles) and *S. polyrhiza* (L.) Schleid. (circles). (Modified from Landolt 1986).

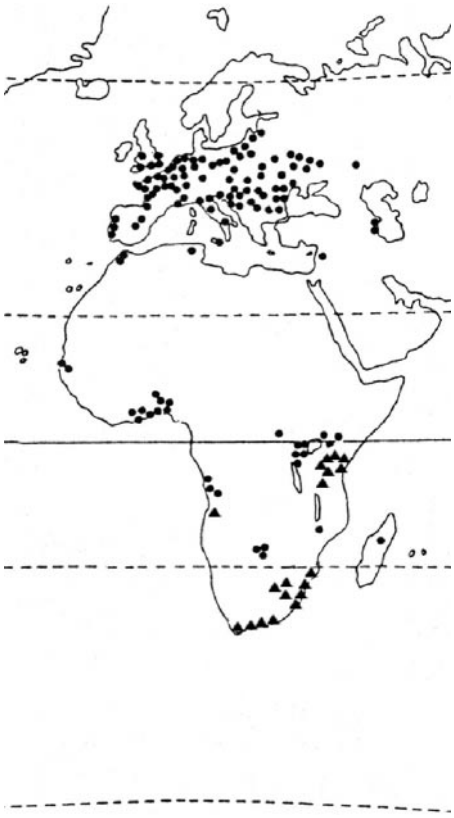

Fig. 4.—Distribution of *Wolffia arrhiza* (L.) Horkel ex Wimm. (circles) and *W. cylindracea* Landolt (triangles). (Modified from Landolt 1986, 1994*b*).

as the turions of *W. cylindracea* (Landolt 1997), and seed set in *W. neglecta* is higher than in *W. angusta* (Landolt unpubl. observ.), it appears that seeds may be more critical for inhabiting seasonal water.

Wolffia columbiana and *W. elongata* are sister species (Fig. 1), with the former distributed widely in permanent waters of the temperate and tropical Americas, and *W. elongata* restricted to seasonal waters in a small area of northern South America (Fig. 6; Landolt 1986, 1994*b*). In culture, *W. elongata* flowers much more frequently than *W. columbiana* (Landolt unpubl. observ.), and fruiting in natural populations of *W. columbiana* is very rare (Landolt 1986). Thus, the frequency of seed set in *W. elongata* appears to be a key factor in its ability to inhabit seasonal water, and increased seed set arguably was important in providing the ecological isolation that initiated divergence and facilitated speciation.

Wolffiella hyalina is widely distributed in Africa in both permanent and seasonal waters, whereas its sister species, *W. repanda* is much rarer and restricted to small bodies of seasonal water south and west of the range of *W. hyalina* (Fig. 7; Landolt 1994*a*). *Wolffiella repanda* is derived (reduced) morphologically relative to *W. hyalina*. Both species can flower with the onset of dry conditions, but unlike *W. hyalina, W. repanda* cannot compete with other species of Lemnaceae in large bodies of permanent water because it has a very reduced appendage that is not effective in stabilizing fronds in larger bodies of water (Landolt 1986, pers. observ.). *Wolffiella repanda,* with its smaller fronds and reduced appendages, can survive in small, seasonal, shaded ponds because the water is calm and the fronds are not blown to shore where they would become desiccated and die

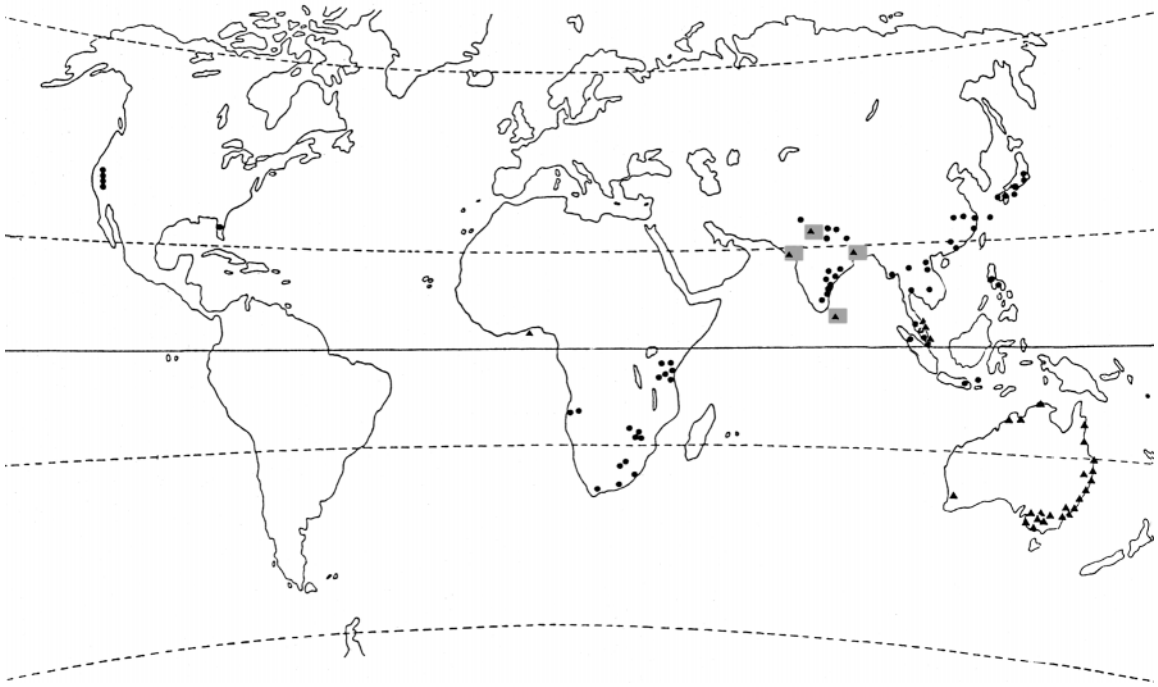

Fig. 5.—Distribution of *Wolffia globosa* (Roxb.) Hartog & Plas (circles), *W. angusta* Landolt (triangles), and *W. neglecta* Landolt (triangles within shaded squares). (Modified from Landolt 1986, 1994*b*).

Fig. 6.—Distribution of *Wolffia columbiana* H. Karst., (circles) and *W. elongata* Landolt (triangles), with the latter species restricted to northern South America. (Modified from Landolt 1986).

(Landolt 1994a). High seed production allows *W. repanda* to survive the dry season and to produce large populations quickly with the onset of rains (Landolt 1994a). *Wolffiella hyalina* and *W. repanda* presently have weakly allopatric distributions (Fig. 7), and *W. repanda* may have originated from dispersal and colonization by seeds that produced plants with smaller fronds and higher seed production that now characterize the species.

There are several examples where differences in temperature tolerances or optimal temperatures for growth and survival of fronds may help explain spatial divergence. *Lemna* sect. *Uninerves* Hegelm., with the most highly reduced fronds in the genus, is a monophyletic group consisting of the three species *Lemna minuta, L. valdiviana,* and *L. yungensis* (Fig. 1). The species appear to be closely related based on their morphological similarity (Landolt 1986, 1998) and low molecular divergence (Table 1; Crawford et al. 1996; Les et al. 2002). *Lemna minuta* and *L. valdiviana* are two widely distributed species in the section (Fig. 8, 9). The indigenous distribution of *L. minuta* is in the warm temperate areas of North and South America; where its distribution extends into the tropics it occurs at higher elevations in the mountains and in drier areas than *L. valdiviana*. *Lemna minuta* has been introduced into central and southern Europe and Japan (Fig. 9; Landolt 1986). *Lemna valdiviana* typically occurs in warm temperate and tropical regions of the Americas, and is found in more humid areas than *L. minuta*. In addition to temperature and humidity differences, *L. minuta* is more of a generalist than *L. valdiviana,* and upon dispersal it can colonize a variety of habitats, which facilitates introductions beyond its original distribution. *Lemna valdiviana* occurs in more stable water, and in addition to growing on the water surface, it can grow submerged when nutrients become scarce (Landolt 1998).

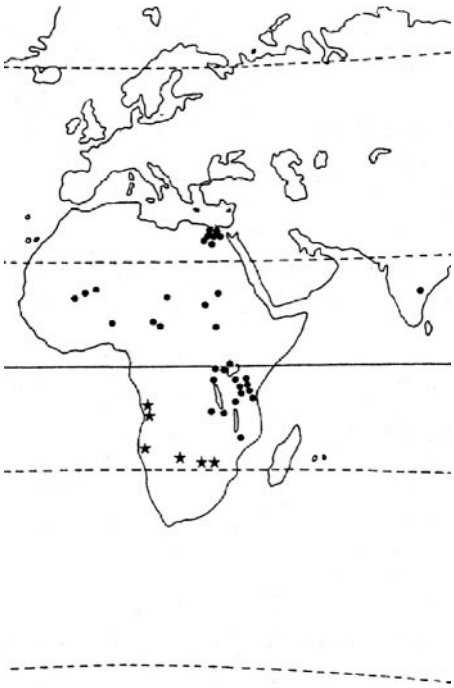

Fig. 7.—Distribution of *Wolffiella hyalina* (Delile) Monod (circles) and *W. repanda* (Hegelm.) Monod (stars). (Modified from Landolt 1986).

Fig. 8.—Distribution of *Lemna valdiviana* Phil. (circles) and location of *L. yungensis* Landolt designated by star. (Modified from Landolt 1986, 1998).

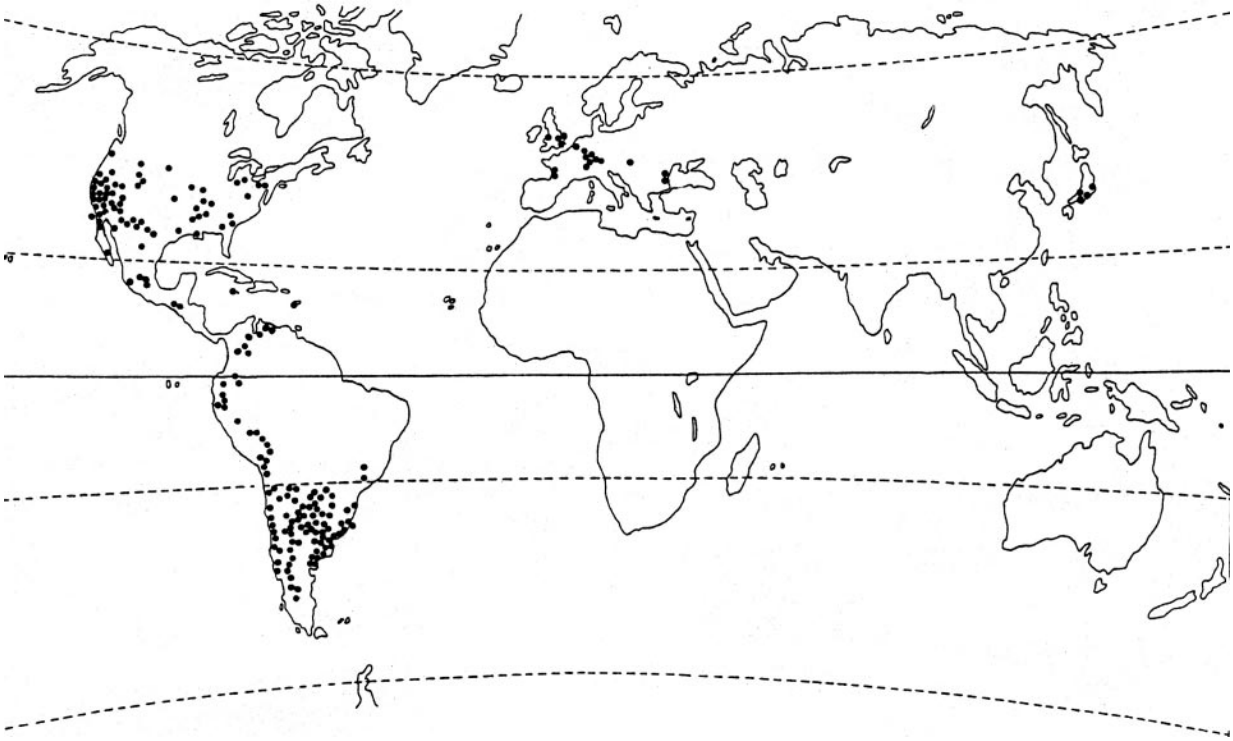

Fig. 9.—Distribution of *Lemna minuta* Kunth (From Landolt 1986).

Fig. 10.—Distribution of *Wolffiella lingulata* (Hegelm.) Hegelm. (From Landolt 1986).

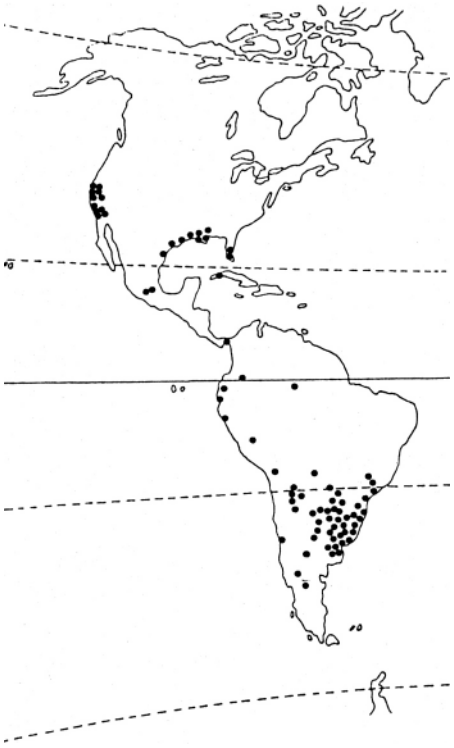

Fig. 11.—Distribution of *Wolffiella oblonga* (Phil.) Hegelm. (From Landolt 1986).

Recently, Landolt (1998) described the new species *L. yungensis* in sect. *Uninerves* and hypothesized that it was most closely related to and derived from *L. valdiviana*. Indeed, the phylogenetic study of Les et al. (2002) showed *L. valdiviana* and *L. yungensis* to be sister species (Fig. 1). *Lemna yungensis* occurs within the range of *L. valdiviana*; it is known from several localities within a small section of the tropical Bolivian rainforest between 1400 and 2600 m (Fig. 8; Landolt 1998). *Lemna yungensis* differs ecologically from *L. valdiviana* by growing on the surface of steep wet rocks over which nutrient-poor water flows; the low-nutrient water would not support the occurrence of most duckweed species (Landolt 1998). Landolt (1998) opined that *L. yungensis* survives in these unusual habitats because it is capable of filtering out the necessary nutrients as the water moves over its fronds. The mechanism by which *L. yungensis* has adapted to its unusual habitat in a localized area is worthy of additional study.

Wolffiella lingulata and *W. oblonga* are sister species that are difficult to distinguish and are distributed widely in the Americas (Fig. 1, 10, 11). *Wolffiella lingulata* occurs in tropical and subtropical regions of the Americas with mild winters. *Wolffiella oblonga* is more common in warm temperate and subtropical regions with mild winters and cool summers, and in South America it extends three degrees further south and more than 1800 m higher in elevation than *W. lingulata*. The two species overlap in several areas such as Argentina (Landolt and Zarzycki 1994), California, Louisiana, Mexico, and Colombia (Fig. 10, 11), and distinguishing them in these areas may be particularly problematical (Landolt 1986; Crawford et al. 1997; Kimball et al. 2003). The morphological distinctions are technical (even by duckweed standards), and include such subtle features as the angle of the pouch, where elongated cells occur on the frond, and the relative length–width of the air spaces in the fronds (Landolt 1986). In contrast to most other congeneric species of Lemnaceae, molecular divergence is minimal to nonexistent between *W. lingulata* and *W. oblonga* (Crawford et al. 1997; Les et al. 2002; Kimball et al. 2003). *Wolffiella lingulata* and *W. oblonga* may represent incipient species in which ecological divergence has been initiated, but there has not been sufficient time for divergence in other characters. These two species are worthy of additional study.

Geographic Distribution and Divergence Times

Generally in duckweeds, those species with largely sympatric distributions have the lowest divergence times; a pattern with one widespread and one restricted peripherally distributed species characterizes relatively more divergent species, and the most highly divergent species are those that are widespread and largely allopatric or are distantly allopatric. The three highly sympatric species *Lemna minuta, L. valdiviana,* and *L. yungensis* have estimated divergence times of about one million years (Table 1; Fig. 8, 9). The broadly overlapping and doubtfully distinct (see above) species pair *Wolffiella lingulata–W. oblonga* likewise exhibits very low divergence times (Table 1; Fig. 10, 11). In several comparisons involving one species of restricted distribution on the periphery of a much more widely distributed species, estimated divergence times are mostly two million years or less (Table 1); these include *Wolffia angusta–W. neglecta* (Fig.

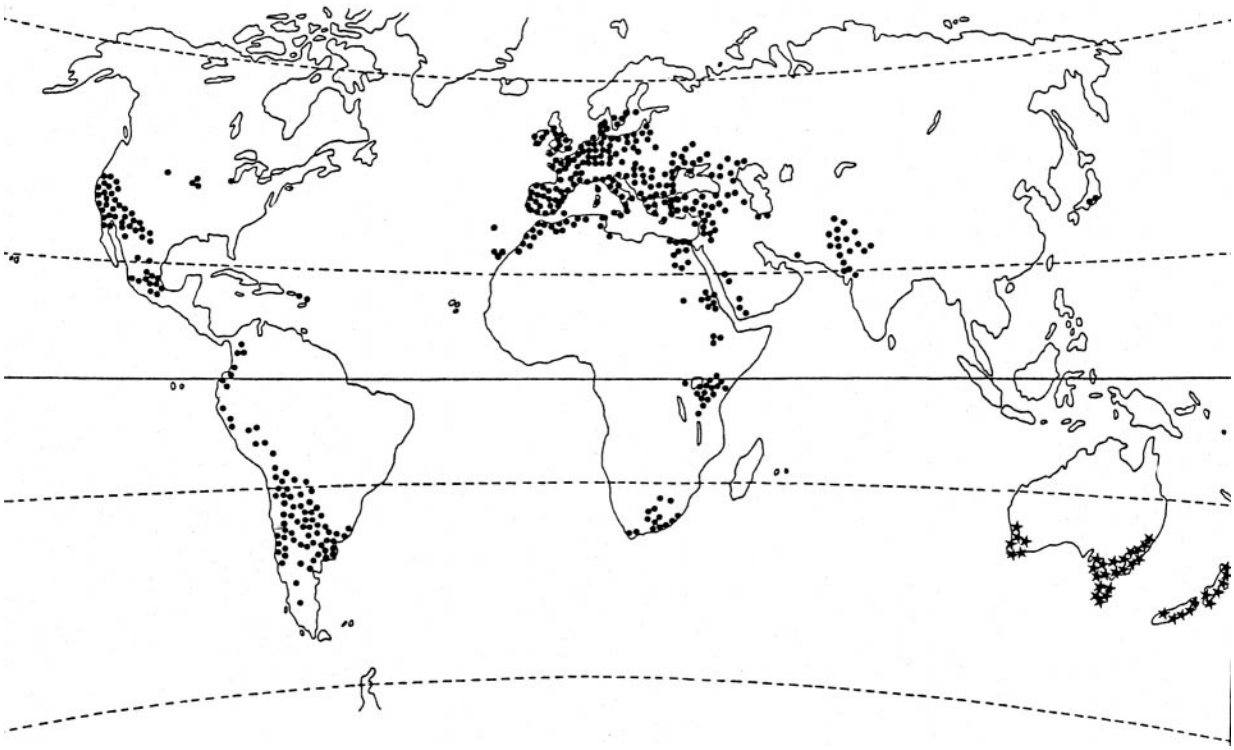

Fig. 12.—Distribution of *Lemna gibba* L. (circles) and *L. disperma* Hegelm. (stars). (From Landolt 1986).

5), *Wolffia columbiana–W. elongata* (Fig. 6), and *Wolffiella hyalina–W. repanda* (Fig. 7). The two very similar species *Wolffia angusta* and *W. neglecta* are in turn sister to and distributed on the periphery of the even more widely distributed *Wolffia globosa* (Fig. 1, 5), with the divergence time between *W. globosa* and the common ancestor of *W. angusta–W. neglecta* estimated to be less than one-half million years (Table 1). *Lemna perpusilla* is rather widely distributed over eastern North America but is much more restricted than the nearly cosmopolitan *L. aequinoctialis* (Fig. 2); the estimated divergence time for these sister species is over two million years (Table 1). The two most divergent species showing the pattern of a widespread and a restricted peripheral species are *Wolffia cylindracea* and *W. arrhiza,* which have estimated divergence times of over three million years (Table 1).

The two most divergent pairs of species are those that are rather distantly allopatric or are widely distributed with contact only along their geographical margins. The two distantly allopatric sister species *Lemna disperma* and *L. gibba* (Fig. 1, 12), with an estimated divergence time of over five million years (Table 1), occupy similar habitats (Landolt 1975) and are similar but distinguishable morphologically (Landolt 1975, 1986). These factors, combined with their similar ecology, indicate that *L. disperma* is a geographical vicariant of *L. gibba*. That is, the former originated from the latter (or a common ancestor) via long-distance dispersal with subsequent morphological divergence. The disjunction of *L. disperma* in Australia and New Zealand from the nearest occurrences of *L. gibba* in any direction (South Africa, Northern India, South America) is nearly 10,000 km. *Spirodela intermedia* and *S. polyrhiza* are both widely distributed (especially the latter), largely allopatric (Fig. 3), and have the highest estimated divergence times of any species included in this study (Table 1).

Speciation in Lemnaceae: General Conclusions

Ecology and geography of duckweed speciation.—Schemske (2000) commented that there has been too little attention paid to the importance of ecological isolation in speciation. Comparison of sister species of duckweeds in the present study has shown that most species differ ecologically, and that these ecological differences limit their distributions. In several cases, species differ in the ability to survive extreme conditions such as cold temperature and desiccation. While recognizing that it is very difficult to distinguish differences associated with speciation from those features resulting from evolution subsequent to speciation (Templeton 1982), we suggest that these basic differences between closely related duckweeds may have been ''key'' factors in the initial isolation and divergence of lineages leading to speciation, as hypothesized by Landolt (1986). While there appear to have been ''key'' factors in the initial divergence of some lineages, additional features likely evolved subsequently during the ''fine tuning'' of species and in maintaining species integrity.

Some duckweed species do not exhibit differences in survival of extreme conditions, but have different conditions for optimal growth or different environmental tolerances. Many experimental studies in the laboratory (Landolt 1975; summarized by Landolt 1986), and correlations between field measurements of temperature, light, nutrients, etc., and the occurrence of particular species, provide strong indirect evidence for the role of these environmental variables in

determining the distribution of species (Landolt 1994*a*, 1997, 1998, 2000; reviewed in Landolt 1986). Divergence in these species may have followed the model of Landolt (1986: 433) of . . .''a gradual development of local, regional and zonal races of Lemnaceae . . .''

The prevalent geographic pattern for those species differing in their ability to survive extreme conditions is to have one restricted species distributed on the periphery (either with contact on the margins or occurring just beyond the geographical boundary) of another more widespread species (Fig. 2, 4–7). This distribution pattern indicates that parapatric and peripatric speciation (Levin 2000) have been common in the duckweeds. The process of divergence and speciation may have involved environmental selection on a local scale for certain variants that occur on the edge of the range of a species or the dispersal of variants just beyond the range of a species. In fact, this process appears to have been detected in ''mid-stride'' in a series of investigations of *Lemna aequinoctialis*. Studies of this species (under the name *L. paucicostata*) in Japan by Yukawa and Takimoto (1976) and by Beppu and Takimoto (1981*a*, *b*, *c*, 1983) demonstrated differentiation for a variety of features including flowering behavior and whether seeds or turions are the means of surviving the cooler winters of northern Japan. In Japan, *L. aequinoctialis* attains its most northern distribution, where it occurs primarily in rice fields; there has been selection for different ''northern'' ecotypes since the cultivation of rice began several thousand years ago.

Duckweed species differing in conditions for optimal growth, including growth in nutrient poor water, have largely sympatric distributions (Fig. 8–11). In the case of *L. yungensis,* habitat divergence has occurred at a very local level within the distribution of its sister (progenitor?) species *L. valdiviana* (Fig. 8). In these species of *Lemna,* as well as with *Wolffiella lingulata and W. oblonga,* ecological divergence and speciation have occurred without geographical segregation of populations. The low molecular divergence and morphological similarity between these sympatric sister species (Table 1) suggest that they may be relatively ''young'' compared to other duckweed species.

The only putative example of disjunct speciation (Levin 2000) identified in this study involves *Lemna gibba* and *L. disperma,* in which the species have distantly allopatric distributions (Fig. 12), but exhibit no apparent ecological differences (Table 1; Landolt 1986). Although dispersal is the most plausible explanation for the disjunct distributions of many Lemnaceae and other hydrophytes (Les et al. 2003), the distribution patterns of closely related sister species of duckweeds provide little evidence that divergence following long-distance dispersal has been a common mode of speciation in Lemnaceae.

Molecular variation and evolution in duckweeds.—Landolt (1986, 1987) suggested that duckweeds, contrary to their superficial appearance of morphological and ecological uniformity resulting from their reduced morphology and occurrence only in aquatic habitats, are not a group in evolutionary stasis. The molecular data support Landolt's (1987) hypothesis that variation is generated and maintained within populations of duckweeds despite very infrequent sexual reproduction (Vasseur et al. 1993; Jordan et al. 1996; Crawford et al. 2001). Given that the number of *Wolffia* individuals within a single pond can exceed the current human population of North America (Clark and Thieret 1968), the extent of somatic mutations that might arise during vegetative reproduction should not be underestimated in this group. In addition to intrapopulational variation, interpopulational geographical variation has been detected within species. As indicated earlier, geographic allozyme variation was detected in *Spirodela polyrhiza* (Crawford and Landolt 1993), although no sequence variation was found in plastid *rpl*16 sequences (Les et al. 2002). Allozyme studies of the very widespread *Lemna trisulca* L. indicate geographic divergence between Old and New World populations (Crawford et al. unpubl. data), and there is nearly one percent sequence divergence in *rpl*16 sequences between clones from North America and Australia (Table 2 in Les et al. 2002). Landolt (1987) observed that some, but not all, clones of *L. trisulca* from Australia exhibit certain morphological features not found elsewhere in the species. The molecular data support Landolt's (1987) view that the Australian populations are differentiated from others, even though the lack of ''fixed'' morphological characters precludes recognition of these populations as a new species. It seems safe to assume that there are a number of cryptic species within Lemnaceae, where the level of morphological differentiation is not sufficient for their recognition as distinct species.

Molecular studies have revealed a wide range of divergence among congeneric species of duckweeds. For example, within the genus *Lemna* divergence at synonymous substitutions in the plastid gene *rbc*L is 34 times greater in the most than the least divergent pair of species, with a range of intermediate values for other species in the genus (Les et al. 2002). The genera *Wolffia* and *Wolffiella* also show extensive variation in levels of molecular divergence among species (Kimball et al. 2003). The totality of evidence, molecular and otherwise, indicates that variation within duckweed genera extends from the population and intraspecific levels to recognized species displaying various levels of divergence. Molecular data indicate that recognized congeneric species range from incipient species to those that diverged tens of millions of years ago.

Landolt (1987) hypothesized that morphological divergence may be slow in Lemnaceae. The high allozyme divergence among sister species or closely related species of duckweeds compared to other congeneric species of flowering plants (Gottlieb 1977, 1981; Crawford 1989), as well as high plastid sequence divergence (Les et al. 2002), would seem to support the hypothesis. However, the extensive reduction and miniaturization of plant organs in duckweeds make it difficult to detect differences, and indeed the point may be reached where further loss or reduction of structures is not possible without reduction in fitness. For example, *Wolffia,* which is the most reduced extant genus of duckweeds, consists of plants with no roots, stems or typical leaves; rather the vegetative structure is a globular ''thallus'' less than 2.5 mm in size. There is a single flower per frond that lacks a perianth entirely and consists of a single 2-loculed stamen and a single, unicarpellate pistil containing only one ovule (Landolt 1986).

A wealth of molecular and ecological data has established Lemnaceae as a model system for studying evolution and

speciation. Duckweeds are particularly appropriate for examining the role of ecological factors as isolating mechanisms because they are grown easily in culture, where differences within and among populations of the same (and different) species growing under the same and varied environmental conditions can be identified. Differences in flowering responses, optimal growth conditions, and environmental tolerances may then be compared to environmental parameters that characterize localities where the plants occur in nature. Molecular data are useful, in combination with ecological studies, because they provide direct evidence of genetic differentiation between ecological variants in cases where morphological differences are imperceptible in these highly reduced flowering plants. By combining ecological and molecular approaches, it is feasible to identify specific factors of potential importance in the initial stages of isolation leading to speciation.

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