

BIOGEOGRAPHY OF DISCONTINUOUSLY DISTRIBUTED HYDROPHYTES: A MOLECULAR APPRAISAL OF INTERCONTINENTAL DISJUNCTIONS

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The extraordinarily wide distributional ranges of aquatic flowering plants have long stimulated phytogeographical discussion. Although aquatic plants occur rarely among the angiosperms, they represent a disproportionately large number of taxa with broad distributions including various intercontinental disjunctions that are manifest even at the species level. Throughout the nineteenth and early twentieth centuries, long-range dispersal by waterfowl was the prevailing explanation for widespread aquatic plant distributions. This explanation gradually fell into disfavor as biologists raised doubts as to the ability of waterfowl to transport propagules across the extensive transoceanic distances between the continents on which an assortment of aquatic taxa now reside. During the twentieth century, the development of biogeographical displacement theory, i.e., “continental drift,” steadily began to supplant dispersal as the preferred explanation for discontinuous angiosperm distributions. Our study assesses the dispersal/displacement hypotheses from a temporal standpoint using molecular estimates of divergence time for a diverse sample of phylogenetically related aquatic taxa that exhibit discontinuous intercontinental distributions. With few exceptions, we found divergence times that are far too recent to implicate continental drift as a major determinant of discontinuous distributions in aquatic plants. We suggest that long-distance dispersal by birds should continue to be regarded as a viable explanation for widely disjunct aquatic plant distributions, although such dispersal is likely to have involved a combination of overland as well as transoceanic migratory routes.

Keywords: biogeography, hydrophyte, aquatic plant, intercontinental disjunction, dispersal.

With respect to plants, it has long been known what enormous ranges many freshwater and even marsh-species have, both over continents and to the most remote oceanic islands. (Charles Darwin [1859], *On the Origin of Species*)

Introduction

As early as the mid-nineteenth century, de Candolle (1855) observed that the geographical distributions of aquatic plants generally are quite widespread, especially for a group comprising relatively so few species. De Candolle explained this phenomenon by suggesting that different occurrences of a species in remote geographical areas might represent incidences of multiple origins. He also attributed aquatic plants with having poor dispersal mechanisms that precluded their transport by most animals. Darwin (1859) was impressed that a few aquatic members of otherwise large terrestrial plant genera had acquired far wider distributional ranges; however, unlike de Candolle, he ascribed this result to their “favourable means of dispersal.” Specifically, Darwin related the extensive distributions of water plants almost exclusively to dispersal by water birds. He demonstrated that a large number of viable prop-

agules typically resides in the mud of aquatic habitats from which they are easily dislodged and transported by birds (Darwin 1859).

Schenck (1886) also observed that similar aquatic plant species occurred frequently in disparate geographical areas that in contrast showed quite distinctive terrestrial floras. Although he credited this pattern to aquatic habitats having greater uniformity in different geographical localities than terrestrial habitats, he also believed that widespread aquatic plant distributions must be a consequence of bird dispersal (Schenck 1886). Höch (1893) similarly attributed extensive aquatic plant distributions to bird dispersal, reasoning that their perennial habit was ideally suited for this dispersal method and that the reduced flowers of many aquatic species indicated a relatively long existence, thus enhancing opportunities for far-reaching dispersal.

Twentieth-century aquatic plant specialists such as Arber (1920), Sculthorpe (1967), and Hutchinson (1975) all implicated birds as prominent dispersal agents of aquatic plants. Arber (1920) suggested that birds were capable of transporting

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aquatic plant propagules for several hundreds of kilometers but avoided discussing distributions of greater magnitude. Sculthorpe (1967, p. 332) accepted animal dispersal, mainly by birds, as the explanation for broad distributions of aquatic plants within geographical regions but also concluded that it was “unlikely to be effective in long-range dispersal between continents.” However, Hutchinson (1975, p. 262) believed that seed dispersal by migratory birds was “the only reasonable explanation of such very long transoceanic migration.”

Although bird dispersal over extreme distances was typically associated with plants having large, fleshy fruits (uncommon in aquatics) (Campbell 1926), long-distance dispersal by migratory water birds prevailed well into the twentieth century as the preferred explanation for the widespread distributions of aquatic plants (Ridley 1930; Camp 1947). Some geographers suggested the possibility of former physical connections or land bridges between continents, a hypothesis perhaps less germane to hydrophyte distributions and one severely discredited by the mid-1960s (Good 1964). Interest also focused increasingly on “displacement theory” as an innovative explanation for long-distance angiosperm dispersal (Good 1927, 1964). Amid much controversy, Wegener (1912) hypothesized that some continents were once contiguous landmasses that subsequently separated, a concept now known as “continental drift.” He provided fossil and geological evidence to theorize that all modern continents had once been joined as a single landmass and that many organismal disjunctions occurred as formerly contiguous continental areas drifted slowly apart.

Although Wegener’s hypothesis provided a novel explanation for widely disjunct plant populations such as those distributed across transoceanic barriers, his mechanistic explanations were weak and his ideas were criticized severely. Yet, Koch (1931) endorsed continental drift as a more reasonable alternative to the then-prevailing concept of land bridges to explain the continentally disjunct distributions of certain plants and animals. Good (1951) summarized a number of phytogeographical observations that he believed could be explained only by land bridges or by continental displacement. Good originally believed that continental drift might be episodic (glacial-like), with the first major separation not occurring until the early Tertiary (Eocene) when most angiosperms would already have been distributed broadly. He continued to favor the idea of continental drift because the theory could “explain the details and sequence of distribution in a way quite beyond the power of any reasonable theory of land bridges or of the theory of distribution entirely by dispersal” (Good 1964, p. 407). Camp (1947, 1952) also was convinced that a large, united continental land mass existed before its sundering during the Mesozoic, and he attributed the disjunct distributions of many angiosperm groups (e.g., Haloragaceae, Mayacaceae) to its subsequent breakup. As new geological facts increasingly revealed that many transoceanic distances were not always vast but were considerably less in the past, continental drift gained widespread acceptance as a major explanatory factor for modern angiosperm distributions (Raven and Axelrod 1974).

Meyen (1836), Guppy (1917), Willis (1914), and others observed that wider distributions generally characterize the more primitive plants, i.e., that plant distributions are often proportional to their age. Because several aquatic taxa (e.g., Ceratophyllaceae, Nymphaeaceae, subclass Alismatidae) are re-

garded as relatively basal angiosperm clades (Judd et al. 2002), this hypothesis is clearly of interest with respect to aquatic plant geography. Following similar reasoning, Koch (1931) proposed as a “general rule” that the more ancient a species, the more “disjunct” would be its modern distribution. This idea is also similar to Cain’s (1944) “theory of generic cycles,” whereby discontinuous distributions arise as old taxa show decline in different parts of their range. Because it seems reasonable that continental drift is more likely to have influenced the geography of older lineages than those of relatively recent origin, it is not difficult to envision that the geographical distributions of at least some aquatic groups could reflect patterns imposed by continental drift.

The previous discussion raises an important question. Did the many continentally disjunct occurrences of aquatic plants arise as an outcome of long-distance dispersal, or rather, are they relicts of continental drift? One way to evaluate this question is by considering relative divergence times. If the specific time of divergence is known for any two disjunct entities, then it should at least be possible to determine whether continental drift could have been involved in their disjunction. If continental drift created a disjunction, then the divergence time of taxa now occupying different isolated continental areas should approximate (or precede) the time when the continental masses were separated physically beyond their effective dispersal distance. In contrast, relatively recent divergence times would indicate that a disjunction probably occurred as the result of recent long-distance dispersal or possibly through introduction. Thus, a comparison of divergence times for continentally disjunct aquatic plant species should provide some insight into the question of mechanisms involved in establishing their current distributional patterns.

Here we provide a phytogeographical perspective for closely related, intercontinentally disjunct aquatic plant taxa (species and genera) that is based on their temporal relationships. Specifically, we use molecular data to estimate divergence times for closely related, intercontinentally disjunct aquatic plant taxa. In turn, these estimates are used to reconstruct approximate continental positions that would have existed at their presumed divergence time. By looking at a large number of taxa with similar modern disjunct distributions, we hope to identify common patterns, indicating whether transoceanic dispersal of aquatic angiosperms has occurred recently (thus implicating long-distance dispersal) or during times that are of sufficient age to implicate continental drift as a potential factor involved in molding their present-day geographical distributions.

Material and Methods

We evaluated 87 different comparisons involving 71 aquatic angiosperm species from 32 genera in 15 families with DNA sequence data obtained from five regions: ITS, *matK*, *rbcL*, *rpl16*, *trnK* (table 1). Sequence data were obtained from studies published previously by the authors (GenBank accessions in Les et al. 1997a, 1997b, 1999, 2002a, 2002b; Padgett et al. 1999; Philbrick and Les 2000; Moody and Les 2002) or from newly generated sequences (GenBank accessions: AY335952–AY336007) that were obtained using similar techniques. All comparisons involved closely related pairs of species, as in-

dicated by phylogenetic analyses showing them to be either sister species (i.e., each other's closest extant relative) or at least members of the same general clade (fig. 1). New sequences were analyzed phylogenetically using approaches similar to those presented in the studies published previously. All comparisons considered taxa whose distributions represented intercontinental disjunctions (fig. 1). Specifically, our estimates were based on the origin of the specimens used to obtain DNA sequence data for analysis. Species whose disjunct distributions are known to have arisen through human activity (e.g., introductions) were not included in our study.

To calculate time of divergence (T) between each pair of sequences, we used equation 4.1 of Li and Graur (1991): $r = K/(2T)$, where r = rate of nucleotide substitution (number/site/year) and K = number of substitutions between two homologous sequences. We rearranged this equation as $T = K/(2r)$ and used previously determined substitution rates with empirical values of K to solve for T . For *rbcL*, we used the synonymous substitution rate of 0.12% per million years, which is similar to rates calculated for a variety of organisms (Xiang et al. 2000). Synonymous substitution rates for *matK* range anywhere from two to six times higher than for *rbcL*, (Johnson and Soltis 1995), with values of two to three times higher being most common (Johnson and Soltis 1994; Xiang et al. 1998). We used the rate of 0.24% per million years for *matK* in this study. Our estimated rate for *trnK/rpl16* (0.198% per million years) was calculated from the rate for *matK* in the Lemnaceae (R. T. Kimball, D. J. Crawford, D. H. Les, and E. Landolt, unpublished data). A wide range of rates has been reported for ITS sequence divergence (Suh et al. 1993; Wendel et al. 1995). We used the rate of 0.27% per million years because it falls within the narrower range that is estimated frequently for the ITS region (Sang et al. 1994, 1995; Malcomber 2002).

Because there are variable estimates of evolutionary rates for ITS and *matK* in the literature, we performed a sensitivity analysis to determine how the rate we used affected our conclusions. For ITS, we used rates ranging from 0.035% per million years (Suh et al. 1993) to 0.78% per million years (Sang et al. 1995), as well as some intermediate rates that are often cited (0.39% and 0.53% per million years; Sang et al. 1994; Wendel et al. 1995). The initial rate of 0.24% per million years that we selected for *matK* was at the lower end of published values, so we compared it to the upper end of 0.72% per million years (Johnson and Soltis 1994, 1995; Xiang et al. 1998).

Initially, we performed preliminary analyses using different methods of sequence correction including *p*-distances (uncorrected), Tamura-Nei 93 distances, Tamura-Nei 93 + gamma distances, and Tajima-Nei distances as implemented in MEGA 2.1 (Kumar et al. 2001). We focused on the more rapidly evolving noncoding regions (ITS, *trnK*, and *rpl16*), as these were most likely to be problematic. Corrected ITS distances indicated a possible age underestimate of ca. 14%, whereas the noncoding cpDNA regions were underestimated by ca. 4%. Because we found the standard deviation among these different sequence correction methods to be minimal (and far less than that between different data sets), we relied on the uncorrected values (*p*-distances) to provide representative estimates of K .

Means and standard deviations were calculated for com-

parisons where multiple data sets existed. We also combined different taxon comparisons that reflected similar intercontinental disjunctions (e.g., Africa vs. South America), calculating an overall mean and standard deviation for divergence time estimates relevant to that particular disjunction. These "regional means" include all data from every different estimate available for the regions (different taxa and different loci) but exclude data from any intraspecific comparisons. The overall mean values were summarized on a global map to depict overall divergence time estimates associated with various intercontinentally disjunct aquatic taxa.

Once divergence times were calculated for disjunct taxon pairs, maps showing the approximate positions of the relevant continents at those times were generated using a program provided by the Ocean Drilling Stratigraphic Network (ODSN; established by GEOMAR, Research Center for Marine Geosciences/Kiel, and the Geological Institute of the University Bremen; see <http://www.odsn.de>) that is based on data used by Hay et al. (1999).

Wolffia species illustrate our approach (fig. 1). In this case, the relative position of Africa and South America at the estimated divergence times of disjunct species provides a means of evaluating the importance of continental drift in establishing the disjunction. The distant proximity of the continents in both comparisons indicates that the disjunctions did not originate at a time when Africa and South America were close to one another physically. Thus for this example we would conclude that recent long-distance dispersal across the Atlantic Ocean would more likely account for the observed disjunctions. Next, we pooled data from other taxon pairs that showed similar disjunct distributions to arrive at mean divergence time estimates associated with particular continental areas (table 2). These mean divergence times are summarized in figure 2, and the corresponding continental reconstructions at those times are reproduced in figure 3.

Results

Estimated divergence times in millions of years before present (mybp) ranged from less than 1 mybp for *Nuphar microphylla* versus *Nuphar pumila* (ITS), *Nuphar luteum* versus *Nuphar variegatum* (*trnK* intron), and four intraspecific comparisons to 104.6 mybp for *Tristicha* versus *Mourera* (*rbcL*) (table 1). Generally, the most recent divergence times were associated with intraspecific disjunctions, with all 10 intraspecific comparisons estimating times less than 2.3 mybp (table 1). Standard deviations for taxon pairs derived from multiple estimates of different loci ranged from ± 0.2 to ± 17.5 mybp, with the poorest agreement among estimates for *Astonia* versus *Wiesneria* (18.3 [± 17.5] mybp) and the best agreement among estimates for *Lagarosiphon* versus *Apalanthe* (40.5 [± 0.3] mybp) (table 1).

Mean divergence times associated with different, geographically isolated regions ranged from 3.9 mybp (Asia vs. South America) to 44.0 mybp (Australia vs. South America) (table 2). Standard deviations for geographical regions derived from multiple estimates of different taxa ranged from ± 0.3 to ± 37.8 mybp, with the poorest agreement among estimates for Australia versus eastern Asia (10.7 [± 14.9] mybp) and the best agreement among estimates for Europe versus South

Table 1

Values for Uncorrected Sequence Divergence (p) and Estimated Divergence Time (T ; millions of years before present) for Intercontinentally Disjunct Aquatic Plant Taxa

Taxa compared	Regions compared	p	T (mybp)
Alismataceae:			
<i>Astonia/Wiesneria</i>	Australia/India	0.0234 ^a	5.9
<i>Astonia/Wiesneria</i>	Australia/India	0.1652 ^b	30.6
<i>Astonia/Wiesneria</i>	Australia/India	Mean (SD)	18.3 (± 17.5)
Aponogetonaceae:			
<i>Aponogeton distachyos/Aponogeton eurypermus</i>	Africa/Australia	0.1255 ^b	23.3
<i>A. distachyos/Aponogeton madagascariensis</i>	Africa/Madagascar	0.0825 ^b	15.3
<i>A. eurypermus/Aponogeton crispus</i>	Australia/India	0.0725 ^b	13.5
<i>A. eurypermus/A. madagascariensis</i>	Australia/Madagascar	0.0157 ^a	4.0
<i>A. eurypermus/A. madagascariensis</i>	Australia/Madagascar	0.1095 ^b	20.3
<i>A. eurypermus/A. madagascariensis</i>	Australia/Madagascar	Mean (SD)	12.2 (± 11.5)
<i>Aponogeton rigidifolius/Aponogeton longiplumosa</i>	India/Madagascar	0.0852 ^b	15.8
Callitrichaceae:			
<i>Callitriche cophocarpa/Callitriche deflexa</i>	Europe/North America	0.0454 ^c	18.9
Ceratophyllaceae:			
<i>Ceratophyllum demersum</i> (intra)	Asia/Australia	0.0015 ^b	0.3
<i>C. demersum</i> (intra)	Asia/North America	0.0124 ^b	2.3
<i>C. demersum</i> (intra)	Australia/North America	0.0030 ^c	1.3
<i>C. demersum</i> (intra)	Australia/North America	0.0108 ^b	2.0
<i>C. demersum</i> (intra)	Australia/North America	Mean (SD)	1.7 (± 0.6)
<i>Ceratophyllum submersum/Ceratophyllum echinatum</i>	Europe/North America	0.0246 ^c	10.3
<i>C. submersum/C. echinatum</i>	Europe/North America	0.0587 ^d	12.3
<i>C. submersum/C. echinatum</i>	Europe/North America	0.0236 ^a	6.0
<i>C. submersum/C. echinatum</i>	Europe/North America	0.0807 ^b	15.0
<i>C. submersum/Ceratophyllum muricatum</i>	Europe/North America	Mean (SD)	10.9 (± 3.8)
<i>C. submersum/Ceratophyllum muricatum</i>	Europe/North America	0.0246 ^c	10.3
Cymodoceaceae:			
<i>Syringodium isoetifolium/Syringodium filiformis</i>	Australia/ North America	0.0140 ^c	5.8
Elatinaceae:			
<i>Elatine triandra/Elatine minima</i>	Europe/North America	0.0248 ^c	10.3
Haloragaceae:			
<i>Myriophyllum dicoccum/Myriophyllum heterophyllum</i>	Australia/North America	0.0415 ^d	8.7
<i>M. dicoccum/M. heterophyllum</i>	Australia/North America	0.0224 ^a	5.7
<i>M. dicoccum/M. heterophyllum</i>	Australia/North America	0.1293 ^b	24.0
<i>M. dicoccum/M. heterophyllum</i>	Australia/North America	Mean (SD)	12.8 (± 9.8)
<i>Myriophyllum pedunculatum/Myriophyllum aquaticum</i>	Australia/South America	0.0825 ^d	17.2
<i>M. pedunculatum/M. aquaticum</i>	Australia/South America	0.0368 ^a	9.3
<i>M. pedunculatum/M. aquaticum</i>	Australia/South America	0.2004 ^b	37.1
<i>M. pedunculatum/M. aquaticum</i>	Australia/South America	Mean (SD)	21.2 (± 14.4)
<i>Myriophyllum spicatum/Myriophyllum exalbescens</i>	Europe/North America	0.0186 ^b	3.5
<i>Myriophyllum variifolium/Myriophyllum ussuricense</i>	Australia/Japan	0.0102 ^d	2.2
<i>M. variifolium/M. ussuricense</i>	Australia/Japan	0.0077 ^a	2.0
<i>M. variifolium/M. ussuricense</i>	Australia/Japan	Mean (SD)	2.1 (± 0.2)
Hydrocharitaceae:			
<i>Hydrocharis/Limnobium</i>	Europe/North America	0.0280 ^c	11.7
<i>Hydrocharis/Limnobium</i>	Europe/North America	0.0671 ^b	12.5
<i>Hydrocharis/Limnobium</i>	Europe/North America	Mean (SD)	12.1 (± 0.6)
<i>Lagarosiphon/Apalanthe</i>	Europe/South America	0.0976 ^c	40.7
<i>Lagarosiphon/Apalanthe</i>	Europe/South America	0.2172 ^b	40.2
<i>Lagarosiphon/Apalanthe</i>	Europe/South America	Mean (SD)	40.5 (± 0.3)
<i>Maidenia rubra/Vallisneria americana</i>	Australia/North America	0.0549 ^b	10.2
<i>Thalassia hemprichii/Thalassia testudinum</i>	Australia/North America	0.0350 ^c	14.6
<i>Vallisneria nana/V. americana</i>	Australia/North America	0.0234 ^b	4.4
<i>Vallisneria spiralis/V. americana</i>	Europe/North America	0.0523 ^c	21.8
<i>Vallisneria triptera/Nechamandra alternifolia</i>	Australia/India	0.1447 ^b	26.8
Juncaginaceae:			
<i>Cycnogeton/Tetroncium</i>	Australia/South America	0.2792 ^b	51.7
Leptomitaceae:			
<i>Lemna disperma/Lemna gibba</i>	Australia/North America	0.0247 ^c	10.3
<i>L. disperma/L. gibba</i>	Australia/North America	0.0262 ^d	5.5
<i>L. disperma/L. gibba</i>	Australia/North America	0.0154 ^a	3.9
<i>L. disperma/L. gibba</i>	Australia/North America	0.0095 ^c	2.4
<i>L. disperma/L. gibba</i>	Australia/North America	Mean (SD)	5.5 (± 3.5)
<i>Lemna minor</i> (intra)	Europe/North America	0.0000 ^c	0.0
<i>Lemna tenera</i> (intra)	Australia/Southeast Asia	0.0047 ^c	1.2
<i>Lemna trisulca</i> (intra)	Australia/North America	0.0091 ^c	2.3

Table 1

(Continued)

Taxa compared	Regions compared	<i>p</i>	<i>T</i> (mybp)
<i>L. trisulca</i> (intra)	Australia/eastern Asia	0.0091 ^c	2.3
<i>L. trisulca</i> (intra)	Europe/North America	0.0023 ^c	0.6
<i>Lemna turionifera</i> (intra)	Europe/North America	0.0000 ^c	0.0
<i>Wolffia arrhiza/Wolffia columbiana</i>	Africa/South America	0.0120 ^c	5.0
<i>W. arrhiza/W. columbiana</i>	Africa/South America	0.0321 ^d	6.7
<i>W. arrhiza/W. columbiana</i>	Africa/South America	0.0262 ^a	6.6
<i>W. arrhiza/W. columbiana</i>	Africa/South America	0.0236 ^c	6.0
<i>W. arrhiza/W. columbiana</i>	Africa/South America	Mean (SD)	6.1 (\pm 0.8)
<i>Wolffia australiana/Wolffia microscopica</i>	Australia/India	0.0816 ^c	34.0
<i>W. australiana/W. microscopica</i>	Australia/India	0.1064 ^d	22.2
<i>W. australiana/W. microscopica</i>	Australia/India	0.0689 ^a	17.4
<i>W. australiana/W. microscopica</i>	Australia/India	0.0547 ^c	13.8
<i>W. australiana/W. microscopica</i>	Australia/India	Mean (SD)	21.9 (\pm 8.8)
<i>Wolffia cylindracea/Wolffia elongata</i>	Africa/South America	0.0107 ^c	4.5
<i>W. cylindracea/W. elongata</i>	Africa/South America	0.0292 ^d	6.1
<i>W. cylindracea/W. elongata</i>	Africa/South America	0.0355 ^a	9.0
<i>W. cylindracea/W. elongata</i>	Africa/South America	0.0214 ^c	5.4
<i>W. cylindracea/W. elongata</i>	Africa/South America	Mean (SD)	6.3 (\pm 2.0)
<i>Wolffia denticulata/Wolffia welwitschii</i>	Africa/South America	0.0215 ^c	9.0
<i>W. denticulata/W. welwitschii</i>	Africa/South America	0.0368 ^d	7.7
<i>W. denticulata/W. welwitschii</i>	Africa/South America	0.0241 ^a	6.1
<i>W. denticulata/W. welwitschii</i>	Africa/South America	0.0256 ^c	6.5
<i>W. denticulata/W. welwitschii</i>	Africa/South America	Mean (SD)	7.3 (\pm 1.3)
<i>Wolfiella rotunda/W. welwitschii</i>	Africa/South America	0.0308 ^c	12.9
<i>W. rotunda/W. welwitschii</i>	Africa/South America	0.0956 ^d	19.9
<i>W. rotunda/W. welwitschii</i>	Africa/South America	0.0516 ^a	13.1
<i>W. rotunda/W. welwitschii</i>	Africa/South America	0.0502 ^c	12.7
<i>W. rotunda/W. welwitschii</i>	Africa/South America	Mean (SD)	14.7 (\pm 3.5)
<i>W. welwitschii</i> (intra)	Africa/South America	0.0047 ^c	1.2
Nymphaeaceae:			
<i>Euryale ferox/Victoria amazonica</i>	Asia/South America	0.0069 ^c	2.9
<i>E. ferox/V. amazonica</i>	Asia/South America	0.0229 ^d	4.8
<i>E. ferox/V. amazonica</i>	Asia/South America	Mean (SD)	3.9 (\pm 1.3)
<i>Nuphar luteum/Nuphar variegatum</i>	Europe/North America	0.0143 ^d	3.0
<i>N. luteum/N. variegatum</i>	Europe/North America	0.0020 ^a	0.5
<i>N. luteum/N. variegatum</i>	Europe/North America	0.0232 ^b	4.3
<i>N. luteum/N. variegatum</i>	Europe/North America	Mean (SD)	2.6 (\pm 1.9)
<i>Nuphar microphylla/Nuphar pumila</i>	Europe/North America	0.0018 ^b	0.3
Podostemaceae:			
<i>Torrenticola/Cladopus</i>	Australia/Japan	0.0671 ^c	28.0
<i>Tristicha/Mourera</i>	Australia/South America	0.2510 ^c	104.6
Posidoniaceae:			
<i>Posidonia australis/Posidonia oceanica</i>	Australia/Europe	0.0192 ^c	8.0
<i>P. australis/P. oceanica</i>	Australia/Europe	0.1370 ^b	25.4
<i>P. australis/P. oceanica</i>	Australia/Europe	Mean (SD)	16.7 (\pm 12.3)
Zannichelliaceae:			
<i>Lepilaena/Zannichellia</i>	Australia/North America	0.0918 ^c	38.3
Zosteraceae:			
<i>Zostera capricornii/Zostera marina</i>	Australia/North America	0.0458 ^c	19.1
<i>Z. capricornii/Z. marina</i>	Australia/North America	0.0585 ^a	14.8
<i>Z. capricornii/Z. marina</i>	Australia/North America	0.1627 ^b	30.2
<i>Z. capricornii/Z. marina</i>	Australia/North America	Mean (SD)	21.4 (\pm 7.9)
<i>Z. capricornii/Zostera noltii</i>	Australia/Europe	0.0071 ^c	3.0
<i>Z. capricornii/Z. noltii</i>	Australia/Europe	0.0055 ^a	1.4
<i>Z. capricornii/Z. noltii</i>	Australia/Europe	0.0217 ^b	4.0
<i>Z. capricornii/Z. noltii</i>	Australia/Europe	Mean (SD)	2.8 (\pm 1.3)

Note. Regions indicate geographical origin of DNA samples compared. Means and standard deviations are provided where multiple data sets exist. Intra = intraspecific comparison.

^a *trnK* intron.^b ITS-1,2.^c *rbcL* (synonymous).^d *matK* (synonymous).^e *rpl16* intron.

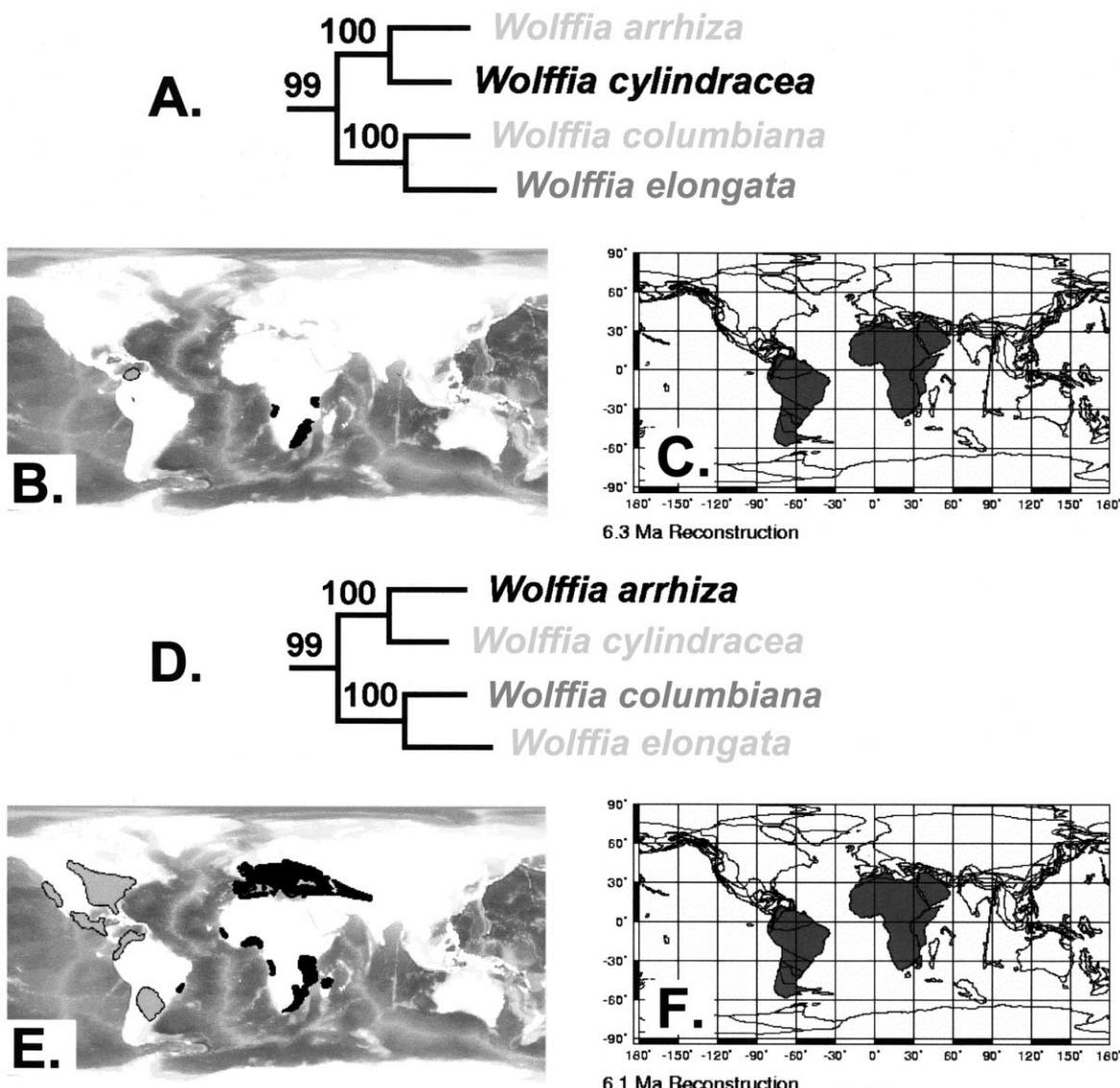


Fig. 1 Example of approach used to study discontinuous intercontinental distributions in aquatic angiosperms (illustrated by *Wolffia*, Lemnaceae). The African distributions (shown in black) of *Wolffia cylindracea* (B) and *Wolffia arrhiza* (E) are disjunct from the South American distributions (shown in gray) of *Wolffia elongata* (B) and *Wolffia columbiana* (E) (from Landolt 1994). These species are closely related, as evidenced by a strongly supported sister relationship in cladograms derived from multiple data sets (A, D) (data from Les et al. 2002a; relative branch lengths shown; numbers above branches represent bootstrap support for clades). The intercontinental disjunction of these *Wolffia* species between Africa and South America was assessed by comparing DNA sequence divergence between specimens obtained in natural populations from Africa (*W. arrhiza*, *W. cylindracea*) and South America (*W. columbiana*, *W. elongata*) to estimate their divergence times (table 1). For each comparison, estimated divergence times were used to reconstruct respective continental positions (C, F) by means of a GEOMAR computer model (see “Material and Methods”).

America (40.5 [\pm 0.3] mybp) (table 2). Only two divergence time estimates exceeded 50 mybp, in both cases involving disjunctions between Australia versus South America (table 2).

Discussion

Intercontinental Dispersal of Aquatic Plants—Is It for the Birds?

Extraordinary vagility is characteristic of many aquatic angiosperms (Philbrick and Les 1996). The various sexual and

vegetative propagules capable of dispersal confer to this biological group a remarkable ability to establish and spread quickly across a broad geographical range (Hutchinson 1975). The successful widespread dispersal and establishment of aquatic plants has been linked to the relatively greater uniformity of aquatic habitats (Schenck 1886; Les 1986), to their broad ecological tolerances (Les 1986; Santamaría 2002), to their enhanced survivorship because of clonal growth (Grace 1993; Santamaría 2002), to the abundance of easily dislodged propagules found in aquatic habitats (Darwin 1859), and to

Table 2
**Mean Divergence Times for Isolated Regions Based on Values for Aquatic Taxa
Having Similarly Disjunct Distributions**

Disjunction (regions)	Estimated divergence time in mybp (\pm SD)
Asia/South America:	
<i>Euryale ferox; Victoria amazonica</i>	3.9 (\pm 1.3)
Indo-Pacific/Mediterranean (seagrasses):	
<i>Posidonia australis; Posidonia oceanica</i>	16.7 (\pm 12.3)
<i>Zostera capricorni; Zostera noltii</i>	2.8 (\pm 1.3)
Mean	8.4 (\pm 9.8)
Africa/South America:	
<i>Wolffiella rotunda; Wolffiella welwitschii</i>	14.7 (\pm 3.5)
<i>Wolffiella denticulata; W. welwitschii</i>	7.3 (\pm 1.3)
<i>Wolffia cyindracea; Wolffia elongata</i>	6.3 (\pm 2.0)
<i>Wolffia arrhiza; Wolffia columbiana</i>	6.1 (\pm 0.8)
<i>W. welwitschii</i> (intra)	1.2 (n.a.)
Mean	8.6 (\pm 4.1)
Europe/North America:	
<i>Vallisneria spiralis; Vallisneria americana</i>	21.8 (n.a.)
<i>Callitricha cophocarpa; Callitricha deflexa</i>	18.9 (n.a.)
<i>Hydrocharis; Limnobium</i>	12.1 (\pm 0.6)
<i>Ceratophyllum submersum; Ceratophyllum echinatum</i>	10.9 (\pm 3.8)
<i>C. submersum; Ceratophyllum muricatum</i>	10.3 (n.a.)
<i>Elatine triandra; Elatine minima</i>	10.3 (n.a.)
<i>Myriophyllum spicatum; Myriophyllum exalbescens</i>	3.5 (n.a.)
<i>Nuphar luteum; Nuphar variegatum</i>	2.6 (\pm 1.9)
<i>Lemna trisulca</i> (intra)	0.6 (n.a.)
<i>Nuphar microphylla; Nuphar pumila</i>	0.3 (n.a.)
<i>Lemna minor</i> (intra)	0.0 (n.a.)
<i>Lemna turionifera</i> (intra)	0.0 (n.a.)
Mean	9.4 (\pm 6.4)
Australia/eastern Asia:	
<i>Torrenticola; Cladopus</i>	28.0 (n.a.)
<i>L. trisulca</i> (intra)	2.3 (n.a.)
<i>Myriophyllum variifolium; Myriophyllum ussuriense</i>	2.1 (\pm 0.2)
<i>Lemna tenera</i> (intra)	1.2 (n.a.)
<i>Ceratophyllum demersum</i> (intra)	0.3 (n.a.)
Mean	10.7 (\pm 14.9)
Australia/North America:	
<i>Lepilaena; Zannichellia</i>	38.3 (n.a.)
<i>Zostera capricorni; Zostera marina</i>	21.4 (\pm 7.9)
<i>Thalassia hemprichii; Thalassia testudinum</i>	14.6 (n.a.)
<i>Myriophyllum dicoccum; Myriophyllum heterophyllum</i>	12.8 (\pm 9.8)
<i>Maidenia rubra; V. americana</i>	10.2 (n.a.)
<i>Syringodium isoetifolium; Syringodium filiformis</i>	5.8 (n.a.)
<i>Lemna dispersa; Lemna gibba</i>	5.5 (\pm 3.5)
<i>Vallisneria nana; V. americana</i>	4.4 (n.a.)
<i>L. trisulca</i> (intra)	2.3 (n.a.)
<i>C. demersum</i> (intra)	1.7 (\pm 0.6)
Mean	13.2 (\pm 10.6)
Africa/Madagascar:	
<i>Aponogeton distachyos; Aponogeton madagascariensis</i>	15.3 (n.a.)
Madagascar/India:	
<i>Aponogeton longiplumosa; Aponogeton rigidifolius</i>	15.8 (n.a.)
Australia/India:	
<i>Aponogeton euruspermus; Aponogeton crispus</i>	13.5 (n.a.)
<i>Astonia; Wiesneria</i>	18.3 (\pm 17.5)
<i>Wolffia australiana; Wolffia microscopica</i>	21.9 (\pm 8.8)
<i>Vallisneria triptera; Nechamandra alternifolia</i>	26.8 (n.a.)
Mean	20.5 (\pm 9.6)
Europe/South America:	
<i>Lagarosiphon; Apalanthe</i>	40.5 (\pm 0.3)
Australia/South America:	
<i>Tristicha; Mouraria</i>	104.6 (n.a.)
<i>Cycnogeton; Tetrancium</i>	51.7 (n.a.)
<i>Myriophyllum pedunculatum; Myriophyllum aquaticum</i>	21.2 (\pm 14.4)
Mean	44.0 (\pm 37.8)

Note. Means were calculated using data from all available individual estimates except intraspecific comparisons (excluded). Regional comparisons are presented in order of increasing mean values. n.a. = not available (single estimate); mybp = millions of years before present.

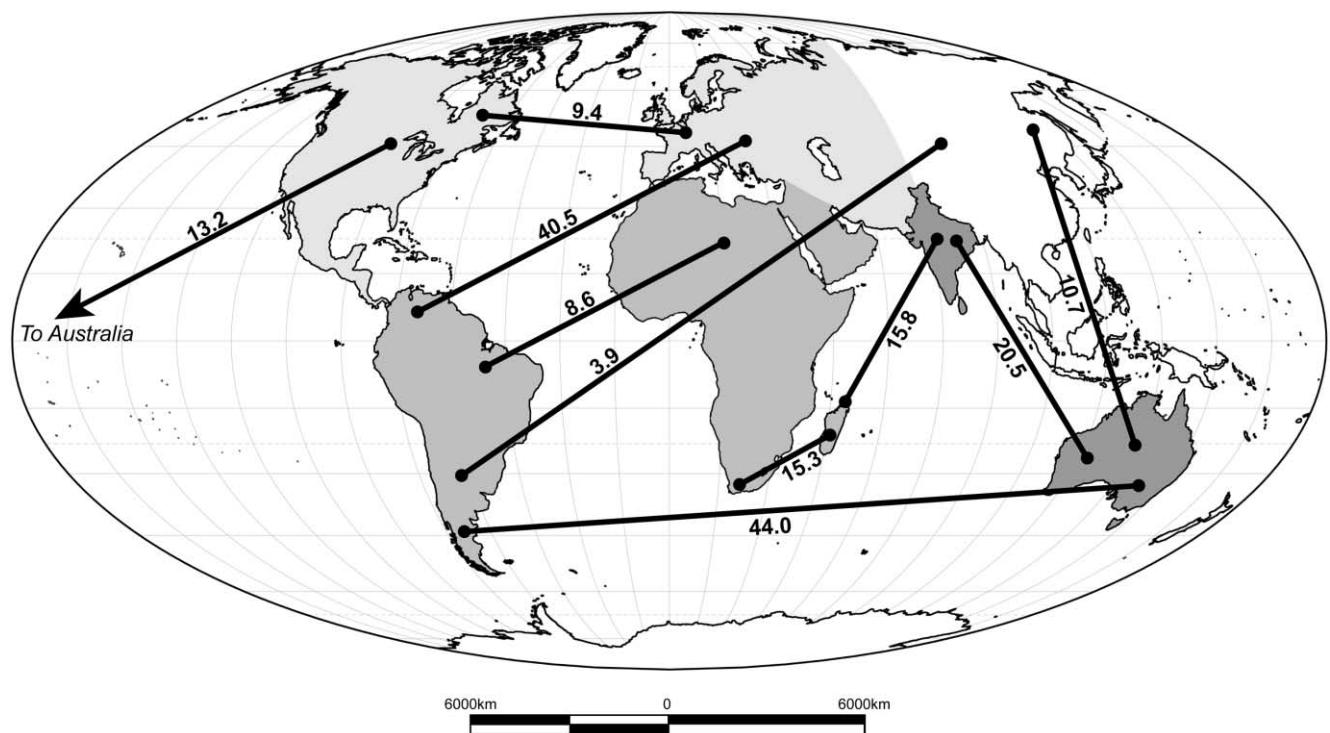


Fig. 2 Global map of mean divergence time estimates (numbers represent millions of years before present) for aquatic plant taxa showing similar, discontinuous intercontinental distributions (estimates, ranges, and standard deviations are reported in table 2). Shading approximates geographical limits of DNA samples used to compare various discontinuous regions (illustrated as solid lines).

the presence of dispersal agents (mainly waterfowl) capable of transporting propagules both exo- and endozoically for considerable distances (Sculthorpe 1967; Charalambidou and Santamaría 2002; Santamaría 2002). Together, these factors that facilitate both dispersal and establishment help to explain why aquatic plants are among the most widely distributed angiosperms.

Evidence for the rapidity of spread by aquatic angiosperms within a contiguous region is indicated by postglacial colonization and also by recent introductions. Canada, which was essentially ice covered 18,000 years ago, presently possesses a diverse aquatic plant flora (Lamoureux 1987; Newmaster et al. 1997) that has recolonized the area since the last glacial retreat (Les 1986). Introduced aquatic angiosperm species are notorious for their rapid spread. The small duckweed *Landoltia punctata* (= *Spirodela oligorrhiza*), first reported in the U.S.A. from Missouri (Saeger 1934), now ranges from Massachusetts to Oregon (Landolt 1986), having spread more than 2000 km in less than 70 yr. In Britain, *Elodea canadensis* appeared near Berwick in 1842 and was found near Market Harborough in 1847 (Sculthorpe 1967), achieving a range expansion of roughly 365 km within just 5 yr. Other instances of rapid range expansion have been documented for many introduced aquatic plants in the United States (Les and Mehrhoff 1999). The movement of introduced aquatic plants is facilitated to some degree by human intervention; yet, these examples demonstrate that their dispersal across vast contiguous landmasses can be achieved fairly easily and quickly. However, the issue more pertinent to this discussion is whether

dispersal of aquatic plants across vast uninhabitable regions (such as oceanic barriers for freshwater species) is possible.

Several authors have expressed reservations regarding the ability of waterfowl, mainly ducks, to transport aquatic plant propagules over extended distances. Löve (1963) and Sculthorpe (1967) doubted that waterfowl could retain propagules long enough during flight to achieve successful intercontinental dispersal. Sculthorpe (1967) also felt that their "fastidious preening" would remove external propagules and that the timing of migrations in the spring and fall comprised periods either before propagules had formed or after they had already ripened and been shed. A recent review by Clausen et al. (2002) echoed similar arguments, namely that aquatic plant reproduction tends to be out of phase with migration, that bird gut contents are likely to be discarded within 300 km of their departure site, and that seeds arriving in a new site would likely encounter ecological conditions quite unlike those of their origin, making establishment difficult. However, discussion of such issues often is biased toward a temperate perspective, whereas greater dispersal opportunities exist in nontemperate regions (Figueroa et al. 2002; Green et al. 2002). Charalambidou and Santamaría (2002) concluded that insufficient experimental evidence exists to resolve conclusively the limits of long-distance dispersal.

Hutchinson (1975) viewed the issue of intercontinental dispersal as a question of probability. He believed that dispersal events as unlikely as "one bird in a million" would suffice to explain the modern intercontinental distributions of aquatic plants. Furthermore, Green et al. (2002) argued that aquatic

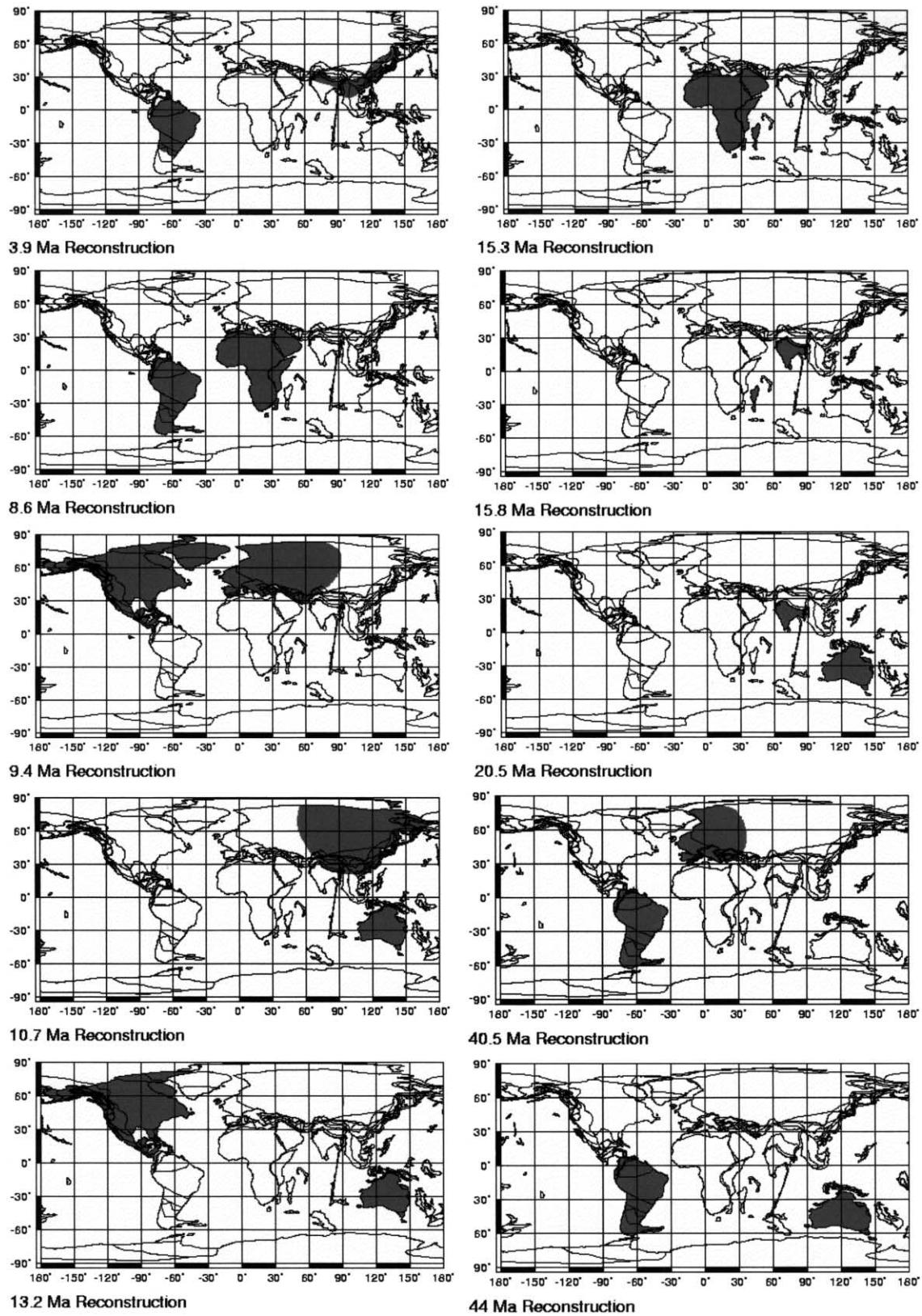


Fig. 3 Reconstructions of continental positions at the mean divergence times (Ma = millions of years before present [mybp]) shown in fig. 2 (see “Material and Methods”). Shading approximates geographical regions compared (see table 2). Most reconstructions show continental positions not substantially closer together than at present (similar to the 3.9 mybp estimate).

plant seed dispersal via endozoochory could occur not only by ducks, geese, and swans (as commonly accepted) but also by shorebirds and other water birds, thus increasing further the opportunities for successful long-distance dispersal. Certainly, the ability of birds to migrate across large global distances is well known. Bird migratory routes “encompass most of the earth’s surface like a mesh,” and migrating birds “cross all our oceans, deserts, mountains, and icefields” (Berthold 2001). Clearly, these opposing views on the possibility of avian intercontinental dispersal of aquatic plants illustrate a question that remains controversial.

The preceding question can be assessed comparatively. If aquatic plants, which occur in ca. 3% of angiosperm genera (Cook 1996), do possess significantly greater vagility than terrestrial species because of some aspect unique to migratory waterfowl, then one would anticipate a disproportionately high incidence of discontinuously distributed aquatic taxa. However, such a relationship is not evident. In Good’s (1927) summary of discontinuously distributed angiosperms, roughly 31 of the 717 genera (only 4.3%) are aquatic, a value only slightly higher than what might be expected. Even though Good’s tabulation inexplicably excluded several discontinuously distributed aquatic genera (e.g., *Ceratophyllum*, several Lemnaceae, *Myriophyllum*, *Tristicha*, etc.), the large number of disjunct terrestrial genera indicates that discontinuous distribution is by no means a phenomenon exclusive to aquatic plants.

However, the occurrence of cosmopolitan species shows greater bias toward aquatics. Of the 18 angiosperm species designated by de Candolle (1855) as the most widespread, only one (*Potamogeton natans*) is truly aquatic; however, of those 117 species listed as occupying a third or more of the earth’s surface, 15 (13%) are submersed aquatics, another 23 (20%) are emergent aquatics, and 9–10 others (8%–9%) are hydrophilous species (helophytes). Together, these aquatic and wetland taxa represent 41% of the widespread species, a proportion anomalous with their relatively rare occurrence in angiosperms, estimated by Cook (1996) to be only 1%–2% of all angiosperm species. Furthermore, de Candolle also stated that the widespread aquatic and semiaquatic plants showed “all the characters of an ancient geographical extension independent of the activity of people” (de Candolle 1855, p. 583). He remarked that none of the aquatics deserved designation as “transported” (i.e., introduced) but that most of the widespread terrestrial species were naturalized in at least a portion of their present range. Höch (1893) also pointed out the disproportionately high number of cosmopolitan species in small aquatic families such as Lemnaceae and Najadaceae. He emphasized that most cosmopolitan species were either aquatic plants (dispersed widely by birds) or agricultural weeds whose distributions were strongly influenced by human activities. Similarly, Good (1964) included *Callitricha*, *Ceratophyllum*, *Myriophyllum*, *Vallisneria*, and *Wolffia* as part of a conspicuous group of aquatic and semiaquatic plants of small but very widespread genera.

The distributional comparisons above indicate hierarchical differences between terrestrial and aquatic plants. At the generic level, the number of discontinuously distributed aquatic and terrestrial taxa is proportional to their relative occurrence in the angiosperms. However, at the species level, there is a

far higher proportion of widespread aquatic than terrestrial plants. Assuming that species would usually have diverged more recently than genera, one could hypothesize that any distributional influence of continental drift would be more likely to characterize angiosperms at the generic rather than species level. If this presumption is valid, then cosmopolitan species, prevalent in aquatic plants, would be more likely to reflect recent dispersal events, whereas discontinuously distributed genera would more likely reflect the influence of continental drift.

A Molecular Appraisal of Geographic Hypotheses for Aquatic Plants

Although his focus was not limited to aquatic plants, Camp (1947) succinctly stated the question most germane to this discussion. Camp asked rhetorically whether discontinuous angiosperm genera such as those distributed in Africa and South America, or in South America, Australia, and New Zealand, had achieved this pattern by overland migration (i.e., across the Bering Strait) or whether their distributions indicated that the continents possessed a “direct connection” in the past (Camp 1947). Camp’s comments can be interpreted as two alternative hypotheses for explaining discontinuous distributions, namely displacement versus dispersal.

The essence of the continental-drift hypothesis assumes that during times past taxa would have dispersed more readily between continents that were physically closer in proximity. As continental distances increased over time, opportunities for dispersal would diminish and eventually cease. This hypothesis assumes that a lineage would be of sufficient age for continental motions to be pertinent. Presumably, cessation of migration would establish an effective isolating mechanism that would initiate divergence of the disjunct populations. Accordingly, the more ancient the instance of isolation, the greater is the level of divergence that should exist between extant populations. Therefore, this hypothesis lends itself to a temporal test whether the divergence time of two disjunct taxa is known. Hence, only relatively ancient divergence times (ideally dating to the time when a land mass was believed to first break apart) would support the displacement hypothesis. However, recent divergence times (coinciding with epochs when continents were separated widely) would implicate intercontinental dispersal as the more reasonable factor in establishing the disjunction. This reasoning applies equally to any plant group, whether terrestrial or aquatic.

What Do Molecular Divergence Times Indicate about Aquatic Angiosperm Distribution?

Our results indicate that discontinuously distributed aquatic angiosperms are characterized by comparatively recent divergence times. Only two of our 87 estimates exceeded 41 mybp, with the majority indicating divergence times of less than 21 mybp (table 1). If we consider the continental positions relative to these times (fig. 3), then it is apparent that the divergence of many intercontinentally disjunct aquatic plants was initiated when substantial barriers to migration (i.e., large transoceanic distances) already existed.

Consequently, these results offer little support for the displacement hypothesis as an overriding explanation for the dis-

continuous modern distributions of aquatic plants. Rather, it appears that the majority of aquatic plants included in our study must have traversed significant transoceanic distances through dispersal. However, this is not to say that continental drift has played no role in establishing these geographical incongruities. We are unaware of any study that has determined an absolute “threshold” (i.e., maximum) dispersal distance for any aquatic plant species; establishment of a maximum distance for any species would be exceptionally difficult because of the variety of conditions under which dispersal events could occur. Furthermore, if successful long-distance dispersal occurs only in a minute fraction of instances, as Hutchinson (1975) assumed, it would be virtually impossible to argue for circumstances under which dispersal was impossible.

Despite the idiosyncratic and unpredictable nature of dispersal, we reason that continental drift probably has played an adjunct role in producing disjunct distributions. Increasing transoceanic distances could place certain continents beyond the usual specific threshold dispersal distances or might induce alterations of the typical migratory routes followed by waterfowl. By reducing the probability of successful dispersal through wide transoceanic distances, chance dispersal events still could occur on occasion; however, it would be highly unlikely that sufficient gene flow would occur to prevent the eventual divergence of the same disjunct taxa.

Certainly, there are many taxa that strongly indicate recent dispersal events. In Lemnaceae (duckweeds), the genera *Wolffia* and *Wolfiella* include closely related species that are disjunct between Africa and South America (fig. 1). The different interlocus estimates of divergence times for comparisons in these genera are relatively uniform with small standard deviations (table 1). The pooled comparisons also show fairly close agreement with the mean time estimated for the African/South American disjunction of these duckweeds (table 2). None of these estimates exceeds 20 mybp, and the mean is considerably less (tables 1, 2). *Lemna disperma* (Australia and New Zealand) and its phylogenetic sister species *Lemna gibba* (Europe, Africa, and the Americas) (Landolt 1986; Les et al. 2002a) also indicate low divergence times (<10.5 mybp) for comparisons made between populations in Australia and North America (table 1). These estimated divergence times indicate that dispersal over considerable geographical distance has occurred within a relatively short period of time. In *Wolffia* and *Wolfiella* species, their dispersal is assumed to have occurred across the Atlantic Ocean. However, for *L. gibba* and *L. disperma*, the dispersal presumably occurred through Southeast Asia and across the North Atlantic Bering Sea area, as the direct transoceanic distance between Australia and North America is much too excessive to represent a reasonable expectation of dispersal limits.

One might argue that duckweeds represent a special case among aquatic angiosperms because of their minute size. Size is important because smaller propagules (e.g., seeds) typically can be transported greater distances than larger ones (e.g., vegetative propagules). In the duckweeds, entire plants function as propagules because seeds are produced rarely (Höch 1893; Landolt 1986). The duckweed family comprises five genera, which in decreasing order of relative size are *Spirodela*, *Landoltia*, *Lemna*, *Wolfiella*, and *Wolffia*. Size reduction of duckweed genera coincides with their phyletic relationships;

i.e., the genera with the largest plants are basal in the family and those with the smallest are the most derived (Les et al. 2002a). *Wolffia* comprises the world’s smallest angiosperms, with some species less than 0.5 mm in size (Les et al. 2002a).

If size influences duckweed dispersal, then one would expect the smallest species to be more widespread than larger species. However, species in the smallest genera (*Wolffia*, *Wolfiella*) possess the most restricted distributions, whereas those in the largest genera (*Spirodela*, *Landoltia*) are the most widespread geographically (Les et al. 2002a). Put another way, the most basal duckweed species have wider distributions than the derived species. Therefore, duckweed distributions may better illustrate age/area relationships (Willis 1922) than influences proportional to propagule size.

Unlike duckweeds, *Aponogeton* (Aponogetonaceae) species are seed dispersed (Cook 1996). The genus is restricted to, but widely distributed throughout, the Old World tropics (Africa, Madagascar, India, Southeast Asia, Australia). Here the estimated divergence times between species currently residing in Australia, India, Madagascar, and Africa (fig. 2) are intermediate among our estimates and are quite similar (15–20 mybp). Although some influence of continental drift on the distribution of these *Aponogeton* species is possible, their divergence times still place continental positions fairly distant from one another, indicating that dispersal must have occurred over fairly large distances, whether overland or transoceanic. Also, it is notable that similar divergence times (~15 mybp) exist between Madagascar and either continental Africa (presently separated by a transoceanic distance of only 370 km) or India (presently separated by a transoceanic distance of roughly 2700 km). In this case, the lack of correlation between divergence time and distance indicates the influence of factors other than distance in initiating isolation.

The water lily genera *Nymphaea* and *Euryale* (Nymphaeaceae) comprise a sister group phylogenetically (Les et al. 1999) but have modern distributions that are considerably disjunct. *Victoria* is restricted entirely to South America, whereas *Euryale* occurs in eastern Asia, with extirpated localities known from fossil sites in northern Europe (Sculthorpe 1967; Cook 1996). Although these genera are now separated by the Atlantic Ocean and spatially by more than 9000 km, their estimated divergence time is less than 5 mybp (table 1; fig. 2). No extant or fossil localities of *Euryale* are known for Africa. It is likely that divergence of these water lily genera resulted from dispersal across the Bering Sea and migration between Asia and South America.

Several of the aquatic sister taxa presently disjunct between Europe and North America exhibit similar divergence times around 10 mybp (table 2). It is possible that a threshold to dispersal distance was reached at that time by this group of aquatic plants. However, there are also a number of taxa whose divergence times are less than 3.5 mybp, which indicates more recent long-distance dispersal (table 2). As in many of the other comparisons between any two discontinuous continental areas, these results indicate that aquatic plant species differ considerably in their relative ability to achieve long-distance dispersal. However, the results might also indicate that the overall probability for long-distance dispersal is very low, as Hutchinson (1975) suggested, and that these disjunct taxa are simply those that happened to succeed in dispersal. In any event, the

results do not implicate continental drift as the primary determining factor in the establishment of the modern distributions of these species, with some degree of long-distance dispersal necessarily involved in most instances.

Most aquatic taxa disjunct between Australia and eastern Asia exhibit divergence times less than 2.5 mybp (table 2). These examples indicate that dispersal between these regions has continued even when intercontinental distances differ little from those existing presently. The one exception involves the genera *Cladopus* and *Torrenticola* of the unusual family Podostemaceae, whose members are lotic river-habitat specialists (Sculthorpe 1967). Most species in this family have narrow distributions and poor dispersal mechanisms, leading to assumptions that the few widespread taxa are relatively ancient (Sculthorpe 1967). This supposition is supported by data illustrating a substantial level of molecular divergence associated with the family (Les et al. 1997b). Comparisons of other discontinuously distributed Podostemaceae (*Mourera* and *Tristicha*) show an even greater estimated divergence time (table 2). These genera, discontinuous between South America and Australia, represent relatively basal lineages in the two major subfamilies of Podostemaceae. We suggest that, given the apparent age and poor dispersal mechanisms in Podostemaceae, the intercontinental disjunctions of widespread Podostemaceae taxa are more likely to have been influenced, at least to some degree, by continental drift.

Cosmopolitan Species

The observation that many aquatic plant species have achieved nearly global distributions has been of phytogeographical interest for centuries (Höch 1893). Unlike other discontinuously distributed taxa, the recognition of a single species across vast regions of the earth raises the question of how such taxa persist across formidable geographical barriers without undergoing phenotypic divergence. Thus, cosmopolitan species either possess widespread modern distributions because they are ancient and have existed since continents were more contiguous and dispersal was readily achieved or their global dispersal has occurred relatively recently. If the former hypothesis is correct, then some mechanism must exist to account for their phenotypic stasis; otherwise the taxa would surely have diverged and would not be recognized as comprising a single species.

Ceratophyllum demersum (Ceratophyllaceae) represents a classic cosmopolitan distribution. It is distributed nearly across the globe except for the Antarctic continent (Les 1989). The genus *Ceratophyllum* is regarded as ancient, shows geographical distributional patterns consistent with hypotheses of continental drift, and appears to experience prolonged morphological stasis (Herendeen et al. 1990; Les et al. 1991). However, when we compared populations of *C. demersum* from North America, Asia, and Australia, we estimated their divergence times to be less than 2.5 mybp (table 1), thus indicating recent dispersal rather than a paleodistribution among these continents. Supporting the idea that isolated populations of widespread species should inevitably exhibit divergence is the pantropical species *Ceratophyllum muricatum*, which has differentiated into recognizable subspecies relative to its Ne-

otropical, paleotropical, and temperate Asian localities (Herendeen et al. 1990).

In fact, none of our intraspecific comparisons showed divergence times exceeding 2.5 mybp (tables 1, 2), indicating that all of these very widespread aquatic species have achieved their distributions relatively recently, arguably without any major influence due to continental drift. In retrospect, this observation is reasonable, given that isolated taxa of ancient divergence would probably exhibit a level of phenotypic divergence sufficient enough to warrant unique taxonomic status, thus precluding their consideration as cosmopolitan species.

Seagrasses

The discontinuously distributed marine angiosperms (seagrasses) deserve some special discussion because they represent an interesting variation of intercontinental dispersal, involving localities in the oceans themselves rather than on the continental landmasses. For seagrasses, the oceans provide barriers to dispersal by virtue of their excessive depth and different habitat characteristics. Continental drift is also a relevant factor to consider for seagrass geography because continental motions have created openings and closures in marine corridors such as the Tethys seaway, which opened during the late Cretaceous, arguably near the time of seagrass origins (Ivany et al. 1990). Seagrasses have a rather poor fossil record, but some Eocene fossils are known for localities that no longer support certain extant taxa.

Several divergence estimates (based on presumed geological influences) have been made for discontinuously distributed seagrass species. Philipps and Menez (1988) have estimated that the Australian *Posidonia australis* and the Mediterranean *Posidonia oceanica* (Posidoniaceae) diverged during the late Eocene (ca. 40 mybp). Our mean estimate for their divergence is more recent (16.7 [\pm 12.3] mybp), placing their initial separation some time during the Miocene (table 1). Philipps and Menez (1988) estimated that other disjunct seagrass species such as the Australian *Zostera capricorni* and Mediterranean *Zostera noltii* (Zosteraceae), the Australian *Thalassia hemprichii* and Caribbean *Thalassia testudinum* (Hydrocharitaceae), and the Australian *Syringodium isoetifolium* and Caribbean *Syringodium filiformis* (Cymodoceaceae) diverged sometime during the Miocene (i.e., 6–23 mybp). Again, our divergence time estimates are more recent, with 2.8 (\pm 1.3) mybp for the *Zostera* species and 5.8 mybp for the *Syringodium* species, placing their initial separation in the Pliocene. Our estimated divergence for the *Thalassia* species (14.6 mybp) provides a Miocene date in agreement with Philipps and Menez (1988).

Our findings for discontinuously distributed seagrasses are similar to those for most of the freshwater taxa; i.e., relatively recent dispersal is indicated in most instances, with some older divergence times possibly indicating distributions that could have been influenced at least minimally by continental drift.

Credibility of Molecular Divergence Time Estimates

The validity of conclusions reached in this analysis depends entirely on the accuracy of the divergence times estimated from the molecular data. We acknowledge that a number of as-

sumptions made in the calculations of divergence times could influence the accuracy of these estimates. One of these assumptions is a molecular clock for each locus across all species comparisons. The substitution rates used for the different sequences were chosen because they are similar to ones commonly reported for flowering plants; at the same time it is recognized that a range of rates has been estimated for these sequences (Suh et al. 1993; Johnson and Soltis 1994, 1995; Sang et al. 1994, 1995; Wendel et al. 1995; Xiang et al. 1998, 2000; Malcomber 2002). The “more average” values were used for all taxa because no attributes of the plants suggest that especially slow or fast rates would be expected, and there are no conspicuous differences between species, e.g., long-lived perennials versus short-lived annuals (Gaut 1998), leading *a priori* to expectations of different rates.

Sensitivity analyses using different evolutionary rates for ITS and *matK* indicated that, with one exception, the use of different rates did not alter our general conclusions. The ITS value from Suh et al. (1993) is 10 times slower than most estimates of ITS evolution and generated values that were seven times greater than those obtained using 0.27% per million years and up to 22 times greater than those obtained from the most rapid rate we used (0.78% per million years; Sang et al. 1995). Also, the ITS rate from Suh et al. (1993) is about an order of magnitude greater than those obtained from other loci. Therefore, it appears that this value may not be applicable to the rate of ITS evolution in most taxa. Use of other estimates (Sang et al. 1994, 1995; Wendel et al. 1995) yielded results that were only slightly more recent than our ITS results (table 1) and were comparable to the estimates obtained from other loci. Increasing the rate of *matK* to six times that of *rbcL* yielded divergence times that were one-third of those estimated using the slower rate that we adopted conservatively.

It might appear at first glance that the use of uncorrected distances would produce underestimates of divergence times, particularly for rapidly evolving loci and more distant comparisons. However, tests using corrected distances for several more divergent sequences showed that the corrected values did not substantially influence the estimates. If uncorrected distances indeed were having an effect, it would be expected that the faster-evolving ITS region would underestimate divergence times, and one would not obtain the higher times actually observed for most comparisons (table 1). In addition, one would expect that this underestimation would be greatest for the most divergent taxa. However, when we calculated how many times greater the ITS estimate of divergence time is than the *trnK* estimate (for those taxa with both estimates) and plotted them against average divergence times, the relationship was flat rather than increasing, as would be indicative of greater disparity between ITS and *trnK* as divergence time increased.

These results indicate that saturation *per se* is not the key explanation for *trnK*-ITS differences. One notable aspect of our results is the generally higher estimates of divergence for ITS than for plastid sequences (table 1). It is possible, given that estimated substitution rates for ITS vary 10–15-fold (Malcomber 2002), that the ITS calibration we used was too slow to be accurate for most comparisons, which would account for the large disparities in some cases. However, for some species of Hydrocharitaceae, comparisons of divergence times es-

timated from ITS are comparable to or slightly lower than those of the plastid sequences (table 1). Rates of ITS sequences do vary among the species included in the analyses, with some having rates lower than those used in our calculations. However, as indicated above, the taxa included in our analyses do not differ in life-history attributes that sometimes have been shown to correlate with substitution rates (Gaut 1998), and thus there was no justification to assume different values.

We also opted to err on the conservative side for ITS substitution patterns, using a rate that was consistent with many studies but also that fell among the slowest reported for this region. Thus even if materially incorrect, our divergence time estimates associated with ITS comparisons would be biased toward greater-than-actual divergence times and would yield results more supportive of the displacement hypothesis. Because most ITS-based estimates yielded recent divergence times anyway, the use of a faster rate would have only provided even stronger support for the dispersal hypothesis. Therefore, our results, even in light of the potentially problematic ITS data, remain soundly in favor of the dispersal hypothesis.

Other factors must be considered when interpreting our results. It may be tenuous, as we have done, to generalize the commonality of a particular intercontinental disjunction by assuming that different taxa should indeed exhibit similar divergence time estimates if a common mechanism was operating. Thus mean values associated with a particular disjunction are less informative if individual distributions are due instead to a variety of factors. Where multiple taxonomic comparisons existed for a particular disjunction, we typically observed a combination of similar and different divergence time estimates for the same disjunction. Nevertheless, the standard deviations associated with these comparisons still enabled us to draw conclusions about the relative times of divergence associated with different geographical disjunctions that allowed a reasonable assessment of dispersal versus displacement hypotheses.

Our approach also assumes that divergence between presently disjunct taxa was initiated mainly as a result of the event (dispersal or displacement) that created their physical separation, i.e., that geographical isolating mechanisms prevail. We actually know little about the precise nature of isolating mechanisms in these species. If in some instances initial isolation was not geographic (e.g., genetic), then some taxa may already have diverged before the time when they became separated physically. Because divergence time would not equal the time of disjunction, this factor could lead to overestimation of disjunction time. However, in that case, the outcome would bias our results not toward dispersal but in favor of the displacement hypothesis.

Finally, it is possible that some undetermined mechanism for molecular stasis might operate in aquatic plants, thus imparting a bias toward underestimation of divergence times in our comparisons. Micromolecular characters are thought to be influenced by the reduced levels of ultraviolet (UV) radiation at increased water depths (Les and Sheridan 1990), and it is conceivable that reduced UV penetration underwater could also result in lower mutation rates. However, our survey included plants whose predominant leaf surface occurs above the water surface (e.g., Lemnaceae, Nymphaeaceae), as well as those whose leaves are entirely submersed (many others), with no

discernable rate differences associated with these different morphological groups.

Another hypothesis is that longer generation times, perhaps associated with clonal reproduction, which characterizes most aquatic plants, has led to slowed rates of divergence at all sequenced loci (Page and Holmes 1998) and thus to underestimated divergence times. Although we cannot dismiss the influence of generation time on our overall estimates, two factors argue against it constituting a major bias. First, generation time is not always correlated with, and thus a cause of, slower sequence divergence (Gaut 1998). Second, it seems unlikely that such bias, even if it is a factor, could materially influence a significant number of our comparisons involving very recent divergence times. Our use of rate averaging for specific disjunctions helps to overcome this potential problem (see table 2). Our Australia/North America comparison includes both annuals (*Lepilaena*, *Maidenia*, *Zannichellia*) and perennials (other taxa). Indeed, one comparison involving annual genera (*Lepilaena*, *Zannichellia*) yielded a divergence time estimate higher than for the perennial taxa. However, if we assume that the actual rate for these annuals is somewhat lower (e.g., 20%) and for the perennials is somewhat higher (e.g., 20%) and we correct the values accordingly, the resulting average is similar to but even less than the original estimate. Thus despite the many factors that might influence our estimates of divergence time, we believe that none would be so overwhelming as to invalidate our general conclusions.

Conclusions

Although continental drift undeniably has resulted in the large physical separations now existing between many of the continents, various aquatic angiosperms currently occupying these isolated landmasses appear to have dispersed among them after the continents already had become separated by considerable transoceanic distances. Estimates of divergence times from molecular data for a taxonomically diverse group of aquatic plants indicate that relatively recent long-distance dispersal has been important in establishing their presently disjunct distributions. While recognizing potential problems with the various assumptions involved in estimating divergence times from DNA sequences, with rare exceptions our results from comparisons of many different disjunct aquatic plants portray divergence times too recent to indicate that these taxa diverged during geological times when continents were still relatively contiguous. Furthermore, molecular divergence times estimated for aquatic plants with similar intercontinental disjunctions are variable and do not indicate a single common isolation time that could be attributable to continental drift.

However, even for aquatic plants, we believe that continental drift probably has influenced dispersal patterns to some degree by greatly reducing the probability of successful dispersal across large transoceanic distances. For some groups (e.g., Podostemaceae), continental drift may even have initiated the disjunction of species populations that once had more widespread ancient distributions among relatively contiguous con-

tinental areas. Similarly, the divergence of aquatic plants between South America and Australia (e.g., *Myriophyllum*, *Cycnogeton/Tetroncium*) or Europe (*Lagarosiphon/Apalanthus*) also appears to have occurred relatively long ago with respect to other regions we evaluated, indicating more ancient dispersal between these regions.

Given the rapidity with which aquatic plants spread across contiguous continental areas, it is not necessary to assume that their dispersal between continents always proceeds across the most direct, often transoceanic, route. A migratory route encompassing Australia, eastern Asia, North America, and South America characterizes a number of aquatic plants and does not involve excessive transoceanic distances even at present. Camp (1952) identified this route as one showing a "phyletic trend" in angiosperms but to much less a degree than that proceeding from Australia, India, and Africa to South America. Nevertheless, our results indicate that dispersal of aquatic plants between Australia, eastern Asia, and the Americas has continued during relatively recent times and comprises an important migratory route. Similarly, dispersal across the north Atlantic Ocean between Europe and North America also has taken place quite recently for various aquatic species, and it represents another important migratory route for aquatic plants.

Recent dispersal of aquatic plants from Africa to South America has also occurred but principally within the family Lemnaceae. Divergence times of aquatic taxa disjunct between Africa, Madagascar, India, and Australia are more moderate, indicating their dispersal among these regions occurred when continental positions were somewhat closer than at present. Thus these disjunct distributions may have been influenced somewhat by continental drift.

Persuasive arguments against routine long-distance dispersal by birds have been presented (Löve 1963; Sculthorpe 1967; Clausen et al. 2002), and we would agree that successful dispersal and establishment events are rare. Our results indicate that, even granting the high likelihood that any one dispersal event would be unsuccessful, at least one such improbable event has occurred for a variety of aquatic plants through evolutionary time. Although long-distance dispersal events to small remote oceanic islands have little chance of success, it is clear that dispersal accounts for the origin of diverse insular floras (Carlquist 1974). Thus, perhaps Hutchinson's (1975) "one in a million" hypothesis presents an accurate, albeit unpredictable appraisal of aquatic plant dispersal and distribution over broad geographical distances.

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