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HABITAT, EVOLUTION, AND SPECIATION IN *ISOETES*[1,2]

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ABSTRACT

Species of *Isoetes* are notorious for the difficulties they present in identification. These difficulties are attributable to a series of habitat adaptations that have resulted in morphological simplicity, homoplasy, and reticulate evolution. Internal air chambers in the leaves of all taxa indicate that primitive *Isoetes* was aquatic. During the breakup of Gondwana, ancestors of modern taxa appear to have passed through a terrestrialization phase, which was accompanied by development of several morphological novelties and reductions including the evolution of sclerotic phyllopodia. This adaptation appears to have evolved after separation of the Indian subcontinent. A new section of the subgenus *Isoetes* centered in India and possessing non-sclerified, persistent leaf bases is described. Following a terrestrialization phase, several lineages became secondarily aquatic and, in some instances, once again evolved a terrestrial habitat. As a result, the genus now occupies a variety of niches, from wholly aquatic to wholly terrestrial. Most terrestrial species, found as isolated populations of basic diploids, appear to be the result of gradual speciation via isolation and genetic divergence. Some aquatic species, often found in mixed populations containing taxa of different ploides, appear to have evolved abruptly via interspecific hybridization and chromosome doubling. Evidence from distribution patterns, megaspore morphology and viability, chromosome numbers, and electrophoretic profiles of leaf enzymes supports a hypothesis of allopolyploid speciation.

Isoetes L. is a cosmopolitan genus of heterosporous lycopsids comprising approximately 150 species. The generic limits of *Isoetes* are sharp, and while there is no difficulty in recognizing a plant as a member of the genus, there are difficulties with the identification of species and species relationships within it. Our studies indicate that these difficulties are attributable to (1) simplicity of form, (2) morphological convergence, and (3) allopolyploidy. Simplicity of form confounds species identification. Plants of *Isoetes*, often resembling tufts of grass, reveal few characters that can be used to distinguish species. Morphological convergence hinders phylogenetic reconstruction and obscures evolutionary relationships among species. Allopolyploidy results in a subtle mosaic of character state distributions, thus limiting our perception of both species and species relationships.

As a result of these difficulties, taxonomists of *Isoetes* traditionally have employed a simplistic approach to classification and phylogeny. Often single characters have been used as the primary basis for understanding the genus. Because students of *Isoetes* have had different points of view, their classification systems and species alliances have varied widely. For example, Pfeiffer (1922) revolutionized

Isoetes taxonomy by proposing a classification system based primarily on megaspore morphology. Her scheme replaced the earlier systems of Baker (1880) and Motelay & Vendryès (1882), which were based on habitat preference and concomitant morphological adaptations. Pfeiffer (1922) pointed out that many species are highly variable in their habitat preference. Furthermore, she argued that there is often little correlation between morphology (e.g., presence or absence of stomata and fibrous bundles in leaves) and habitat. Since many early collections of *Isoetes* lacked adequate habitat data, species were often assigned to these habitat sections based solely on their morphology, a situation that led to an artificial suite of relationships. Although Pfeiffer's system improved upon earlier classification schemes, it is still fraught with problems such as convergence, reduction, and polymorphisms (Hickey, 1986a).

The replacement of earlier classification systems based on habitat preference by Pfeiffer's classification based on spore morphology resulted in de-emphasizing habitat preference as a feature of taxonomic value. Today, not only is habitat preference again recognized as a valuable diagnostic character, but there is also considerable evidence indi-

cating habitat preference has played a major role in the evolution of *Isoetes* at two levels: major phylogenetic change and recent speciation. In the first part of this paper we will show that habitat shifts have been responsible for a number of major morphogenetic changes. Many of these changes are quite ancient and the current geographic distribution of the various morphological states can best be understood using a vicariance model. In addition to these morphological changes, numerous reversions to the ancestral, aquatic habitat have resulted in homoplasy. The second part of this paper outlines the relationships between a species habitat preference and its gregariousness in nature, which in turn dictates the dominant mode of speciation, either divergence or allopolyploidy.

MAJOR PHYLOGENETIC CHANGE

The Isoetaceae (sensu Reed, 1953) can be characterized by the following synapomorphies: foliar embedded sporangia, labia, vela, indehiscent sporangia, trabeculae, multiflagellate sperm, lacunate leaves, and reduced axial growth. Based on these synapomorphies, the family represents a monophyletic assemblage that can be construed as primitively aquatic (Hickey, 1986b). Evidence supporting this contention comes from the presence of air chambers in the leaves of extant taxa and the fossil genus *Isoetites*. Such air chambers are generally regarded as providing buoyancy and an aeration mechanism for aquatic plants (Keeley, 1982). Certainly, the occurrence of air chambers in the leaves of all extant terrestrial *Isoetes* indicates that these plants had aquatic ancestors.

Isoetes includes species with alate leaves that are fully laminate and species with nonalate leaves that are nearly without blades. Alate leaves are universal in the Lepidodendrales, Lycopodiaceae, and Selaginellaceae, which are all close outgroups of the Isoetaceae. Therefore, alate leaves are inferred to be plesiomorphic in *Isoetes*. *Isoetites* may have arisen during the generally wetter conditions associated with the late Carboniferous and early Triassic. Species of *Isoetites* were alate. While the cosmopolitan distribution of this genus through the lower Tertiary suggests that it was rather successful, only three alate species have survived into modern times: *Isoetes baculata*, *I. gigantea*, and *I. bradei*. Of these, the latter two are probably now extinct. Together these three alate species of *Isoetes* and *Isoetites* represent *Isoetes* subgenus *Euphyllum* (Hickey, 1990). The other and far larger part of the genus, subgenus *Isoetes*, is characterized by a distal, three-dimensional subula and by alae that are restricted to the proximal portion of the leaf. In addition, many species of subgenus *Isoetes* have leaves with supporting fibrous bundles in the leaf. Such bundles are not found in species of subg. *Euphyllum*. Both the subulate condition and the presence of peripheral fibrous bundles are potentially adaptive because they provide stability and mechanical support for leaves in habitats that are ephemerally aquatic or terrestrial (Hickey, 1986b). Radiation from aquatic habitats may have occurred in response to the generally warmer and drier environmental conditions since the Cretaceous. Nearly all extant species of *Isoetes* have retained this essentially terrestrial habit, although some have again reverted to an aquatic habitat.

The development of a terrestrial habit resulted in an early morphological and systematic radiation, which apparently occurred subsequent to the fragmentation of Pangea. The transition from homogeneous, stable, aquatic environments to seasonally dry, terrestrial habitats resulted in additional problems for *Isoetes*, in particular that of desiccation during arid periods. Species of *Isoetes* have adapted to this situation in two ways, both of which involve protecting the apical meristem during drought conditions. In one group of species, unmodified leaf bases are retained throughout the dry season and form a scarious protective layer about the apex of the corm. Such modifications in *Isoetes* appear to be uniquely derived and thus represent a synapomorphy for the associated species. In general, this clade is restricted to the Indian subcontinent (Hickey, 1986b).

The second manner of protecting the corm apex during drought involves the sclerification of leaf tissue. In some species, leaf primordia become sclerified and their growth is arrested. This results in scales similar to those found on branches of temperate deciduous angiosperms. *Isoetes nuttallii*, of western North America, produces such scales (Figs. 1, 2). In other species, portions of the leaf bases become sclerotic and remain after the nonsclerotic portions of the leaves have eroded. This produces a distinctive ring of sclerotic phyllopodia covering the apex. Such phyllopodia are most extensively developed in certain European and African species, but sclerotic phyllopodia are found also in the North American species *I. howellii* and *I. melanopoda* (Figs. 3, 4). The geographic and evolutionary relationships of the species involved strongly suggest that scales and phyllopodia represent two independently derived states for resistance to desiccation. Furthermore, the absence of species with sclerified scales and phyllopodia on the Indian subcontinent suggests that these synapomorphies originated in Gondwana after separation of the Indian subcontinent.
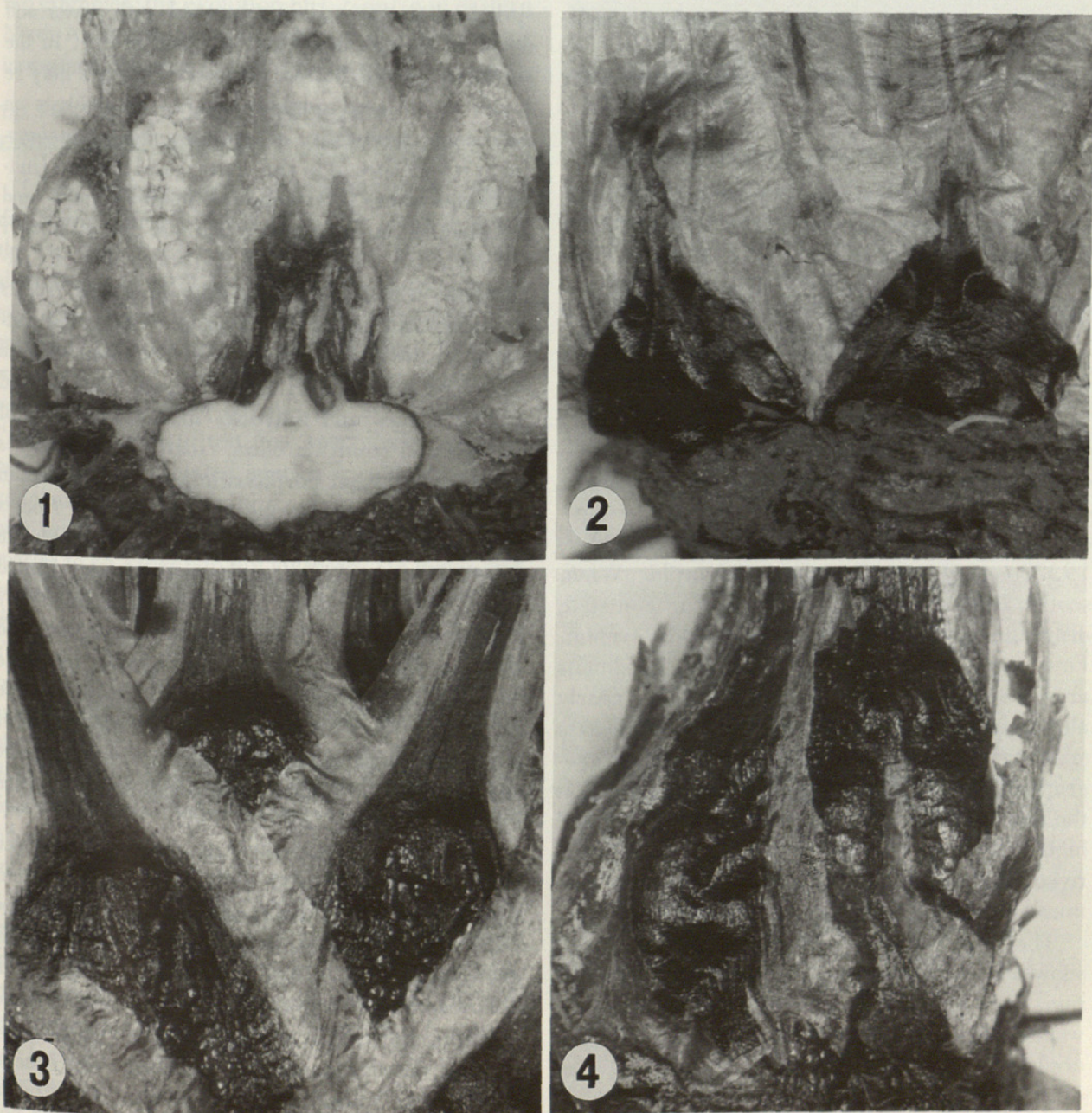
FIGURES 1–4. Scales and phyllopodia in *Isoetes*. —1. Transection through corm and leaf bases of *I. nuttallii*. Scales surround corm apex during summer dormancy.—2. Leaf bases of *I. nuttallii* surrounded by scales. Near end of growing season, scales have been displaced to peripheral position by current season's growth.—3. Sclerified leaf bases of *I. howellii* near end of growing season.—4. Phyllopodia (remnants of sclerified leaves) surround corm apex during summer dormancy.

This group of nonsclerotic species, defined by scarious, persistent leaf bases, is centered in India and represents a distinctive element within subgenus *Isoetes*:

Isoetes sect. **Coromandelina** Hickey & Taylor, sect. nov.

TYPE: *I. coromandelina* L.f., Suppl. Pl. 447. 1781.

Species foliorum basibus scariosis, persistentibus.

The remaining species of subgenus *Isoetes* comprise section *Isoetes*, which is here redefined as those subulate taxa that have, or were presumably

derived from taxa that had, the ability to produce sclerotic pigmentation in leaf primordia, leaf bases, or sporangial tissue. As so defined, section *Isoetes* contains most of the extant species of the genus. It is an extremely diverse assemblage including both terrestrial and aquatic species, which are found throughout Africa, Australia, Europe, Asia, and the New World. By comparing the morphology of these species with that found in subgenus *Euphyllum* and section *Coromandelina* of subgenus *Isoetes*, we can polarize several included characters (Watrous & Wheeler, 1981). Notable among these are velum and labium development. Analyses in-

TABLE 1. Polyploid series for North American aquatic species of *Isoetes*.

Species	Chromosome number (2n)
I. lacustris	110
I. occidentalis	66
I. maritima, I. riparia, I. tuckermanii	44
I. bolanderi, I. echinospora,	
I. engelmannii, I. prototypus	22

dicate that the absence of a velum and the presence of a large, well-developed labium are plesiomorphic conditions (Hickey, 1985). In addition, the generalized condition of tuberculate (sensu Pfeiffer, 1922) megaspores is likewise plesiomorphic. When section *Isoetes* is analyzed for these character state distributions, two facts are immediately evident. First, species retaining all plesiomorphic conditions are terrestrial or amphibious; and second, nearly all aquatic species have derived or intermediate states for these characters, regardless of their geographic location. These data strongly suggest that the aquatic condition seen in most temperate and high-altitude tropical species is a secondarily derived condition that has evolved independently in unrelated lineages (Hickey, 1985).

The more plesiomorphic taxa of section *Isoetes* show a distinctly southern or Gondwana distribution, most evident in Africa and South America, whereas derived members of the section are generally Laurasian, but with close affinities to species of proximate Gondwana landmasses. These observations, coupled with data on section *Coromandelina*, suggest that subgenus *Isoetes* had its origin within Gondwana and subsequently radiated northward into Laurasia. Such hypotheses are strongly corroborated by the distribution of other character states, including the persistence of leaves during cold and drought periods, megaspore surface morphology, and electrophoretic anomalies such as the migrational location of TPI-2 (triosephosphate isomerase-2) and the presence or absence of anomalous TPI-3 activity (Hickey et al., 1989a).

RECENT SPECIATION

In North America, species of *Isoetes* vary in habit from ephemeral terrestrials to evergreen aquatics. Terrestrial *Isoetes* are found in seasonally wet soils where, in general, plants are active in spring when the soil is saturated with water and dormant in summer when the soil is dry. In the southern United States, terrestrial species may be found as isolated populations in soil pockets on sandstone, limestone, or granite. *Isoetes melanopoda* is widespread in the central and eastern United States where it is found in meadows, fields, and soil pockets on sandstone outcrops. *Isoetes butleri* occurs in the south-central and southeastern United States in calcareous soils over limestone. *Isoetes piedmontana* grows in soil pockets on granite outcrops in the southeastern United States. *Isoetes melanospora* inhabits shallow pools on isolated granite domes and flatrocks in the Piedmont of Georgia and South Carolina. *Isoetes tegetiformans* occurs in pools on porphyritic granite flatrocks in the Piedmont of Georgia (Rury, 1985). *Isoetes lithophila* is found in temporary pools on granite domes in central Texas. In the western United States, *I. howellii* and *I. nuttallii* inhabit seasonally wet meadows, lake margins, and vernal pools and streams. All of these terrestrial species have the lowest diploid chromosome number found in the genus ($2n = 2x = 22$). These basic diploids appear to represent examples of gradual speciation due to spatial isolation of ancestral populations followed by genetic divergence.

Aquatic *Isoetes* occur mostly in glacially formed lakes, ponds, and streams. In contrast to the terrestrial species, which occur mainly as isolated populations, two or more aquatic species frequently grow together. For example, in the northeast, *I. echinospora, I. engelmannii, I. riparia, I. tuckermanii*, and *I. lacustris* may all grow in the same body of water. Likewise, in the northwest, *I. echinospora, I. bolanderi, I. maritima*, and *I. occidentalis* could occur in the same lake. Such assemblages, which might have resulted as divergent species of *Isoetes*, were brought together by foraging water fowl, which carried spores into bodies of water left by retreating glaciers. Because gametes of different species can readily mingle in these aquatic habitats, the potential exists for interspecific hybridization. The existence of a polyploid series among the aquatic taxa (Table 1) implies that some species could be allopolyploids, which have evolved through interspecific hybridization and chromosome doubling. In addition to cohabitation and chromosome numbers, further evidence for hybridization and allopolyploidy come from spore morphology and viability, *in vitro* hybridizations, and electrophoretic profiles of leaf enzymes. How these sources of evidence support an allopolyploid mode of evolution in aquatic *Isoetes* are shown by

two examples, the evolution of *I. riparia* in eastern North America and the evolution of *I. brochonii* in western Europe.

THE EVOLUTION OF *ISOETES RIPARIA* IN EASTERN NORTH AMERICA

In the early summer of 1895, A. A. Eaton discovered unusual plants of *Isoetes* growing along the Powwow River in southeastern New Hampshire. Dodge (1897) described these unusual plants as a new species, *I. eatonii*. Dodge noted his new species to be "peculiar" in several ways. First, he mentioned that *I. eatonii* "seldom is found growing very near another of its species" and that plants "are from a foot to ten feet apart." By contrast, he noted the associated species *I. echinospora*, *I. engelmannii*, and *I. riparia* grow "for the most part gregariously." Dodge also reported his new species to be exceptionally vigorous. He mentioned vernal leaves attaining a length of 28 inches and the plants producing from 50 to nearly 200 leaves. Further, Dodge noticed the "straightness" of the radial ridges on the proximal side of the megaspore and "the low angle they form with the equatorial plane." Such a configuration imparts a proximally flattened, nearly lenticular form to the megaspore. Species of *Isoetes* typically have curvilinear radial ridges conforming to a rounded proximal hemisphere and a globose megaspore.

Taylor et al. (1985) reported that the megaspores of *I. echinospora*, *I. engelmannii*, and *I. riparia* readily germinated and formed megagametophytes in culture, but megaspores of *I. eatonii* did not germinate. They also showed that *I. eatonii* had been found almost entirely within the overlapping ranges of *I. echinospora* and *I. engelmannii* and that hybrids between these two species are readily produced in culture. They concluded that *I. eatonii* ($2n = 2x = 22$) represented a sterile, basic diploid hybrid between the basic diploids *I. echinospora* and *I. engelmannii* (both $2n = 2x = 22$).

Although the megaspores of *I. eatonii* are described most often as small and flattened, with a labyrinthiform-convolute (brain coral) surface texture, larger, globose megaspores are produced occasionally (Fig. 5). The cristate surface texture of the larger, globose megaspore of *I. eatonii* appears intermediate between the echinate megaspore of *I. echinospora* (Fig. 7) and the reticulate spore of *I. engelmannii* (Fig. 8). Furthermore, the larger, cristate megaspore of *I. eatonii* resembles the even larger, globose megaspore of *I. riparia* (Fig. 6), a fertile tetraploid ($2n = 4x = 44$) occurring in

northeastern North America, throughout the range of *I. eatonii*. If *I. eatonii* is the sterile, diploid hybrid between *I. echinospora* and *I. engelmannii*, could *I. riparia* be its fertile, allotetraploid derivative?

Electrophoretic profiles of triosephosphate isomerase (TPI) from *Isoetes* leaves help to answer this question. Leaves of *I. echinospora*, *I. engelmannii*, *I. eatonii*, and *I. riparia* were crushed and ground in Tris-HCl buffer-PVP solution. The resulting mixtures were absorbed onto filter paper wicks and subjected to horizontal starch gel electrophoresis. Electrophoretic procedure, composition of grinding, gel, and electrode buffers, and staining method follow Soltis et al. (1983). Electrophoresis was conducted using electrode and gel buffer system 7 in a 12% starch gel at 4°C with a constant current of 35 mamp for 8.5 hr. Wicks were removed from the gel after 15 min. The gel was sliced, incubated in substrate at 37°C for 1 hr. in the dark, rinsed in distilled water, and photographed.

Electrophoretic profiles of *I. echinospora*, *I. engelmannii*, *I. eatonii*, and *I. riparia* support the hypothesis that *I. eatonii* is a sterile hybrid between *I. echinospora* and *I. engelmannii* and that *I. riparia* could be an allotetraploid derivative of *I. eatonii*. In Figure 9, TPI, a dimeric enzyme, requiring the combination of two subunits to form an active enzyme, is expressed by two loci, TPI-1 and TPI-2. TPI-1 appears single-banded, and invariant for the taxa sampled. TPI-2, composed of subunits designated "a" and "b," shows variability that distinguishes *I. echinospora* and *I. engelmannii* and relates *I. eatonii* to *I. riparia*. *Isoetes echinospora* expresses the band "aa," whereas *I. engelmannii* expresses the band "bb." *Isoetes eatonii* and *I. riparia* express bands "aa" and "bb" in addition to the heterodimeric band "ab," which is not present in either parent. The profiles of TPI-2 for *I. eatonii* and *I. riparia* are similar and additive for *I. echinospora* and *I. engelmannii*. Lighter bands cathodal to bands "aa" and "ab" may represent subbands. A subband is formed when an additional, charged component attaches to an enzyme thereby changing its charge, possibly its size and shape, and thus its migration (Buth, 1990). Subbanding for "bb" is not evident in Figure 9.

Evidence from distribution patterns, spore morphology and viability, *in vitro* hybridizations, and electrophoretic profiles of leaf enzymes supports the hypothesis that *I. echinospora* has crossed with *I. engelmannii* to form the sterile hybrid *I. eatonii*,
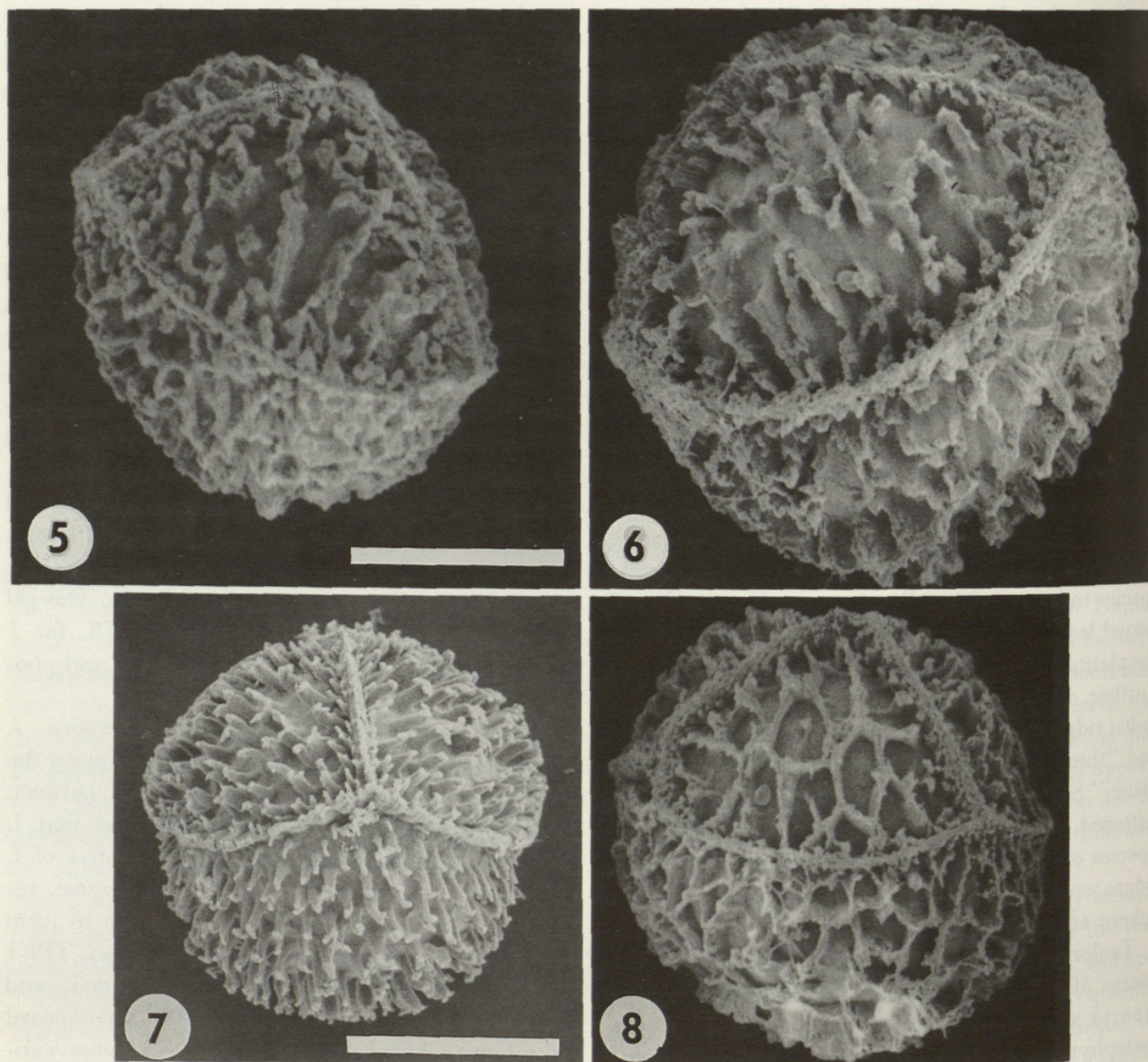
FIGURES 5–8.   Scanning electron micrographs of *Isoetes* megaspores.—5. *I.* × *eatonii.*—6. *I. riparia.*—7. *I. echinospora.*—8. *I. engelmannii.* Scale bar = 0.25 mm.

which then doubled its chromosome number to form the fertile allotetraploid *I. riparia.*

THE EVOLUTION OF *ISOETES BROCHONII* IN WESTERN EUROPE

In October 1983, a mass collection of *I. echinospora* and *I. lacustris* was made from Neva Lake in northern Wisconsin to obtain spores for germination and hybridization experiments. Neva Lake is a small, glacially formed, soft-water lake with a sand, gravel, and muck bottom. Examination of plants from this collection revealed several individuals bearing megaspores that were far more variable in size, shape, and surface texture than typical megaspores of *I. echinospora* or *I. lacustris.*

Isoetes echinospora and *I. lacustris* grow together in many lakes in northeastern North Amer-

ica and Europe. Although they can look alike vegetatively, these two species are readily distinguishable by their spores, which differ in size and surface texture. *Isoetes echinospora* produces echinate megaspores about 400–500 µm in diameter, whereas *I. lacustris* bears cristate megaspores mostly 600–800 µm in diameter. Scanning electron photomicrographs show that megaspores from the unusual Neva Lake plants bear a spine and ridge texture that seems to combine the patterns of *I. echinospora* and *I. lacustris* (Fig. 10).

Megaspores from these plants are not viable. Using the procedure described by Taylor & Luebke (1986) for germinating spores of aquatic *Isoetes*, *I. echinospora* and *I. lacustris* megaspores germinated and formed megagametophytes in culture, but megaspores from the unusual Neva Lake plants did not germinate.
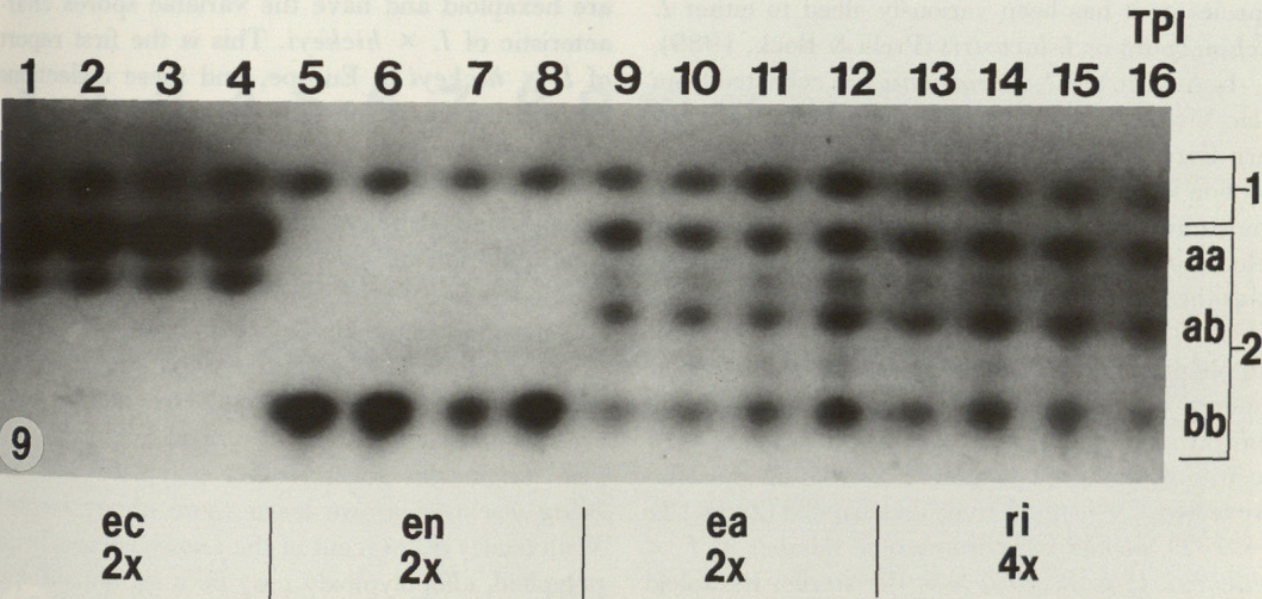
FIGURE 9. TPI (Triosephosphate isomerase) zymogram of *Isoetes* leaves.—Lanes 1–4. ec = *I. echinospora*.—Lanes 5–8. en = *I. engelmannii*.—Lanes 9–12. ea = *I.* × *eatonii*.—Lanes 13–16. ri = *I. riparia*. x = 11, chromosome base number for *Isoetes*.

A hybrid between the basic diploid ($2n = 2x = 22$), *I. echinospora*, and the decaploid ($2n = 10x = 110$), *I. lacustris*, should have a chromosome number of $2n = 6x = 66$, the sum of 11 chromosomes from a haploid gamete of *I. echinospora* and 55 chromosomes from a haploid gamete of *I. lacustris*. Root tip squashes of the unusual Neva Lake plants show cells containing the predicted 66 chromosomes for a hybrid between a diploid and a decaploid (Taylor & Luebke, 1988).

Based on megaspore morphology, production of nonviable megaspores, and chromosome number, it appears that these unusual Neva Lake plants are sterile, interspecific hybrids between *I. echinospora* and *I. lacustris*. This hybrid has been named *I.* × *hickeyi* (Taylor & Luebke, 1988).

Isoetes brochonii, a poorly known taxon, has been collected from several lakes in the Pyrenees of France where it grows with *I. echinospora* and *I. lacustris*. Since its description by M. L. Motelay (1892), the taxonomic status of *I. brochonii* has been debated, and it has been treated as a distinct
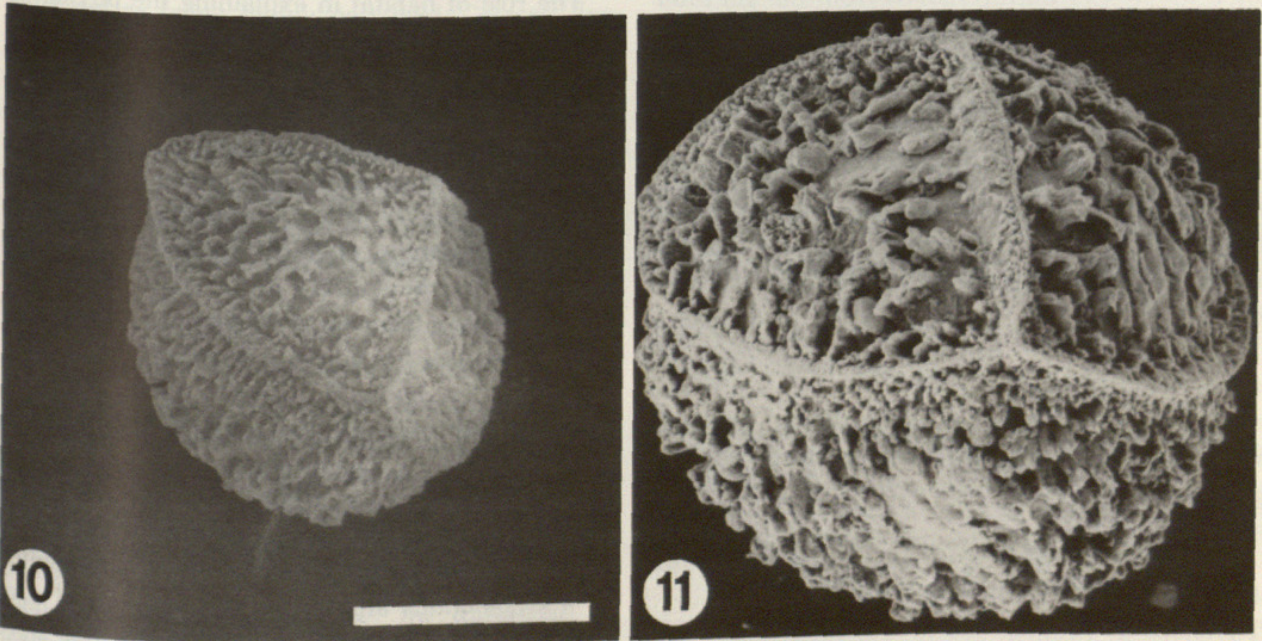
FIGURES 10, 11. Scanning electron micrographs of *Isoetes* megaspores.—10. *I.* × *hickeyi*.—11. *I. brochonii*. Scale bar = 0.25 mm.

species or it has been variously allied to either *I. echinospora* or *I. lacustris* (Prelli & Bock, 1989).

In August 1987, *I. brochonii* was collected from Lac Vivé, just west of Lac Bouillouses in the western Pyrenees of France. Specimens from this collection were cultured to provide root tips for chromosome counts and leaves for enzyme electrophoresis. Of initial interest in this collection was the surface texture of *I. brochonii* megaspores (Fig. 11). Although they were globose, and uniform in shape, size, and surface texture, these megaspores had a surface texture like many of the megaspores of *I. × hickeyi* from Neva Lake, Wisconsin. Chromosome counts from root tip squashes revealed *I. brochonii* to be dodecaploid ($2n = 12x = 132$), double the chromosome number of *I. × hickeyi*. If *I. × hickeyi* is the sterile, hexaploid hybrid between *I. echinospora* and *I. lacustris*, could *I. brochonii* be the fertile, allododecaploid derivative?

Electrophoretic profiles of malate dehydrogenase (MDH) from *Isoetes* leaves help to answer this question. Leaves of *I. echinospora, I. × hickeyi, I. brochonii*, and *I. lacustris* were crushed and ground in phosphate-PVP buffer solution. The resulting mixtures were absorbed onto filter paper wicks and subjected to horizontal starch gel electrophoresis. Electrophoretic procedure and composition of grinding buffer and staining method follow Soltis et al. (1983). Electrophoresis was conducted using an electrode and gel buffer system by Clayton & Tretiak (1972) adjusted to pH 6.0 with N-3 (3-aminopropyl)-morpholine in a 12% starch gel at 4°C at a constant current of 40 mamp for 6 hr. Wicks were removed from the gel after 30 min. The gel was sliced, incubated in substrate at 37°C for 30 min. in the dark, rinsed in distilled water, and photographed.

Electrophoretic profiles of *I. echinospora, I. × hickeyi, I. brochonii*, and *I. lacustris* support the hypothesis that *I. × hickeyi* is a sterile hybrid between *I. echinospora* and *I. lacustris* and that *I. brochonii* could be an allododecaploid derivative of *I. × hickeyi*. In Figure 12, MDH profiles of *I. × hickeyi* and *I. brochonii* appear similar and additive for their suspected parents *I. echinospora* and *I. lacustris,* but it is impossible to precisely interpret the MDH band patterns until variation in parental species is identified and segregation studies are conducted.

Isoetes × hickeyi has recently been collected from Lac Long and Lac Font Vivé in the western Pyrenees of France, within the range of *I. brochonii* (Carmen Prada, pers. comm.). Prada's plants

are hexaploid and have the variable spores characteristic of *I. × hickeyi*. This is the first report of *I. × hickeyi* in Europe, and these collections represent a geographically closer link to *I. brochonii*.

Evidence from distribution patterns, spore morphology and viability, chromosome numbers, and electrophoretic profiles of leaf enzymes supports the hypothesis that *I. brochonii*, like *I. riparia*, is an allopolyploid species formed through interspecific hybridization and chromosome doubling.

In addition to the allopolyploid evolution of *I. riparia* and *I. brochonii* described here, allopolyploid origins for several other polyploid taxa are being disclosed as we learn more about *Isoetes*. With nearly 60 percent of the known species being polyploid, allopolyploidy may be a significant speciation mechanism in this genus.

Thus, species of *Isoetes* appear to have evolved in two ways. First, species have developed gradually by isolation and genetic divergence as taxa adapted to terrestrial and aquatic habitats. Second, species have formed through interspecific hybridization and chromosome doubling as divergent species migrated into the same aquatic habitats. Interspecific hybridization produces plants of more or less intermediate morphology and the inclusion of such hybrids within species descriptions has confused species distinctions. Allopolyploidy fixes this intermediate, hybrid morphology in fertile, polyploid species (Hickey et al., 1989b).

CONCLUSIONS

The role of habitat in explaining the phylogeny of *Isoetes* has only recently been recognized, but there is a growing body of evidence indicating that the major clades of the genus radiated subsequent to environmental shifts. Initially *Isoetes* species were evergreen aquatics with lacunate, laminate, nonsclerified leaves without peripheral fibrous bundles. The evolution of novel character states occurred as members of the genus radiated into terrestrial habitats following the Cretaceous. Peripheral fibrous bundles for support, sclerified leaves and leaf bases for protection, subulae, and ephemeral leaves to reduce water loss were derived as plants adapted to more xeric environments. Subsequently, many of these novel states were lost as plants reverted to aquatic habitats perhaps during wetter periods. These serial environmental shifts resulted in a tremendous amount of parallelism and convergence as characters or character states were gained, lost, or modified.
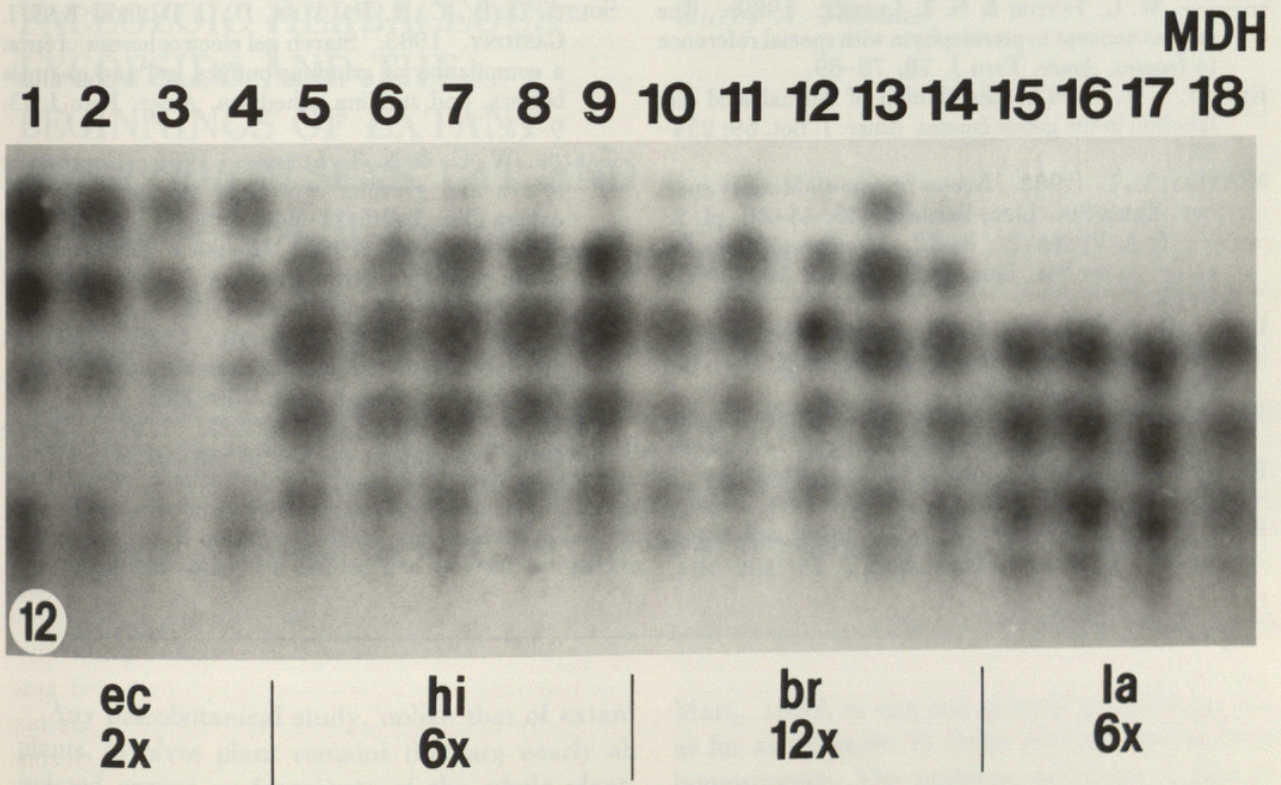
MDH

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

| ec 2x | hi 6x | br 12x | la 6x |

FIGURE 12. MDH (Malate dehydrogenase) zymogram of *Isoetes* leaves.—Lanes 1–4. ec = *I. echinospora.*—Lanes 5–9. hi = *I.* × *hickeyi.*—Lanes 10–14. br = *I. brochonii.*—Lanes 15–18. la = *I. lacustris.* x = 11, chromosome base number for *Isoetes*.

Because of their terrestrial habit these species had little or no long-range dispersal ability during continental movements, and their geography can be explained largely on the basis of vicariant events.

Extant species can be viewed loosely as being either terrestrial or aquatic. The assemblage (non-phylogenetic) of extant aquatic species can be characterized as being highly polyploid, "social" (co-existing with one or more additional taxa), and promiscuous. The terrestrial taxa are quite the opposite. These taxa are generally diploid, often exist in monospecific populations, and rarely hybridize. These differences appear to be tied to the mode and frequency of dispersal events. Ponds and lakes may be good "visual targets" for migrating waterfowl that carry spores of *Isoetes*. This increases the opportunity both for long-distance dispersal and for commingling of species. This sociality of aquatic *Isoetes* species in turn leads to a greater opportunity for hybridization and evolution via subsequent polyploidy. Terrestrial species, by contrast, produce mature spores as or when their habitats, ephemeral ponds or moist glades, are drying out. The habitats of terrestrial species may then be poor "visual targets" for waterfowl, at least during the time of year necessary for dispersal. This would lead to a reduced number of dispersal events and a decrease in sociality of species.

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