

Molecular Evolution and Adaptive Radiation

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7 ADAPTIVE RADIATION IN THE AQUATIC PLANT FAMILY PONTEDERIACEAE: INSIGHTS FROM PHYLOGENETIC ANALYSIS

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The invasion of aquatic environments from land has occurred repeatedly during the evolutionary history of the flowering plants. The precise number of transitions from land to water is not known with certainty, although Cook (1990) recently estimated that it had taken place at least 50 times. Approximately 33 diverse families of monocotyledons and dicotyledons are exclusively aquatic, and numerous aquatic genera are found in predominantly terrestrial plant families. Aquatic plants constitute only 1–2% of angiosperms but they have received considerable attention from botanists and ecologists, primarily because of the high degree of ecological specialization that they exhibit. Adaptation to life in water has demanded the evolution of a distinctive array of morphological, anatomical, physiological and biochemical attributes that have developed on multiple occasions among the lineages that have invaded aquatic habitats.

Depending on the degree to which the life cycle of an aquatic plant is spent in water, individual taxa show increasing divergence from their terrestrial ancestors. At one extreme are species that spend their entire lives submersed below the water surface and are most distinct from land plants, to amphibious taxa that are equally at home on land or in water and that closely resemble their strictly terrestrial relatives. Aquatic groups often display considerable evolutionary diversification resulting from adaptation to the wide range of ecological conditions that occur in wetland habitats. This diversity offers excellent opportunities for relating form to function (reviewed in Arber 1920; Sculthorpe 1967; Crawford 1987; Barrett et al. 1993).

While the concept of adaptive radiation is central to evolutionary theory, there is a wide range of viewpoints as to what it entails. Futuyma (1986) states that adaptive radiation is simply diversification into different ecological niches by species derived from a common ancestor. According to Simpson (1953), however, such diversification is a direct response to a novel ecological or geographic circumstance experienced by the common ancestor of species involved in the radiation. More recently, the idea that increased species richness may (or must) be associated with adaptive radiation has become prevalent among phylogenetic systematists (e.g., Brooks and McLennan 1991). In this paper however, we follow Simpson's perspective that adaptive radiation occurs through character diversification among different lineages in response to a novel set of ecological circumstances or a key innovation. This process may involve an increase in speciation rate, no change in speciation rate, or even a reduction in speciation rate. In our view a radiation in slow motion is still a radiation – the number of lineages arising from an adaptive radiation is of secondary importance to the patterns of character diversification among lineages.

Aquatic plant groups have rarely been investigated from the perspective of adaptive radiation. While in part this is undoubtedly associated with the paucity of phylogenetic data available for most angiosperm families, it may also have been because of a widespread belief that aquatic environments are relatively homogeneous compared with those on land, thus providing less opportunity for evolutionary diversification. Indeed, such arguments have frequently been used to explain the apparently conservative macroevolutionary patterns found in certain aquatic groups (Sculthorpe 1967; Hutchinson 1975; Les 1988; Cook 1990). Of the 33 strictly aquatic families, 30 include fewer than 10 genera, 17 contain only one genus and three consist of a single species (Sculthorpe 1967). Increasing commitment to an exclusively aquatic existence appears to be associated with reduced taxonomic differentiation, as groups containing primarily amphibious and emergent aquatics show little evidence of reduced species diversity. The suggestion that some aquatic radiations are associated with reduced species richness (via decreased speciation rates or increased extinction rates) is intriguing, but requires detailed phylogenetic analysis of the sort suggested by Sanderson and Donoghue (1994) and Nee and Harvey (1994).

The wide spectrum of life-forms and diversity of reproductive strategies found in aquatic plants suggests that extensive character diversification has occurred in response to the novel ecological opportunities afforded by possession of the aquatic habit. The breadth of adaptations implies that the habitats occupied by aquatic plants are far from ecologically uniform, as is often supposed. Because of their many specialized features we believe that aquatic plant groups can provide outstanding opportunities for studies of adaptive radiation and character evolution, as has been undertaken in many animal groups that are restricted to aquatic environments (see Chapters 5 and 12).

Pontederiaceae is a small monocotyledonous family of exclusively freshwater aquatics, composed of approximately six to nine genera and 35 to 40 species, most of which are native to the New World tropics (Barrett 1978a). Members of the family display a remarkable diversity of life history and reproductive strategies ranging from highly clonal, long-lived taxa that inhabit permanent marshes and river systems to exclusively sexual species that are annual and occur in ephemeral pools, ditches and ricefields. Linking these extremes are species with various combinations of sexual and asexual reproduction and a variety of different pollination and mating systems. Evolutionary studies of the family over the past two decades have focused primarily on the floral biology and sexual systems of selected taxa (reviewed in Barrett 1988, 1993; Barrett et al. 1992). More recently, phylogenetic reconstructions using both morphological (Eckenwalder and Barrett 1986) and molecular data (Graham and Barrett 1995; Kohn et al. 1996; Graham et al., unpubl. data) have been employed to investigate character evolution and the systematic relationships of taxa within the family and its close relatives.

The diversity of life history traits in Pontederiaceae suggests that this family might provide a valuable opportunity for investigating the processes of adaptive radiation in an aquatic plant group. Carson (1985) and Johnson (1996) suggested two major modes of adaptive radiation in plants: habitat-driven and pollinator-driven.

Below we review the patterns of character variation and ecological differentiation in vegetative traits (with a particular focus on traits important for growth under aquatic conditions) and reproductive characters in Pontederiaceae. These lines of evidence suggest that selection acting on both reproductive and vegetative characters has contributed to the radiation of taxa in this family.

We begin by providing a brief review of the taxonomy and natural history of Pontederiaceae, emphasizing the diversity of life history and morphological adaptations to life in and out of water that occur in the family. We then perform phylogenetic reconstructions to examine the origins and evolution of a range of life history and reproductive attributes, including aquatic life-form, life-cycle duration, patterns of leaf development, types of clonality, floral form and self-incompatibility system. Throughout our chapter two particular issues form the basis of much of the discussion: (1) What is the ecological evidence that the various morphological characters we consider are adaptations in response to an aquatic life-style? (2) What is the phylogenetic pattern of diversification in these characters and which traits have evolved on multiple occasions within the family?

Taxonomy and natural history

Taxonomy

Pontederiaceae is composed of four main genera: *Eichhornia* (8–9 spp.), *Pontederia* (6 spp.), *Heteranthera* (10–12 spp.), and *Monochoria* (7–8 spp.), and several smaller segregate genera: *Eurystemon* (1 sp.), *Hydrothrix* (1 sp.), *Scholleropsis* (1 sp.), and *Zosterella* (1–2 spp.) allied with *Heteranthera*, and one segregate genus, *Reussia* (2–3 spp.) allied with *Pontederia*. The taxonomic affinity of this family to other monocotyledons is not clear-cut (see Dahlgren and Clifford 1982; Dahlgren et al. 1985; Rosatti 1987; Simpson 1987; Goldberg 1989) but recent treatments suggest a close affinity to Philydraceae and Haemodoraceae (Hamann 1966; Huber 1977; Simpson 1990; Thorne 1992a,b).

Biogeography

Members of Pontederiaceae are largely tropical in distribution with the primary concentration of species occurring in the Neotropics, particularly lowland South America and especially Brazil. Several taxa occur in North America, with some reaching as far north as Canada (e.g., *Pontederia cordata* [Figure 7.1A] and *Heteranthera* [*Zosterella*] *dubia* [Figure 7.1F]). In common with many other aquatic plants (see Ridley 1930; Sculthorpe 1967; Cook 1987), most species of Pontederiaceae have widespread distributions, often involving strikingly disjunct areas (e.g., *Eichhornia paradoxa*, *Eichhornia paniculata*; Barrett 1988). All members of the family occur in freshwater habitats frequented by waterfowl and wading birds that are capable of mediating long-distance dispersal. Aside from *Pontederia* and *Reussia* which are relatively large-seeded, all species have small diaspores that are likely to adhere to mud and be easily transported on the feet of water birds. In some cases long-distance dispersal can be achieved by stem fragments acting as floating vegetative propagules. The occurrence

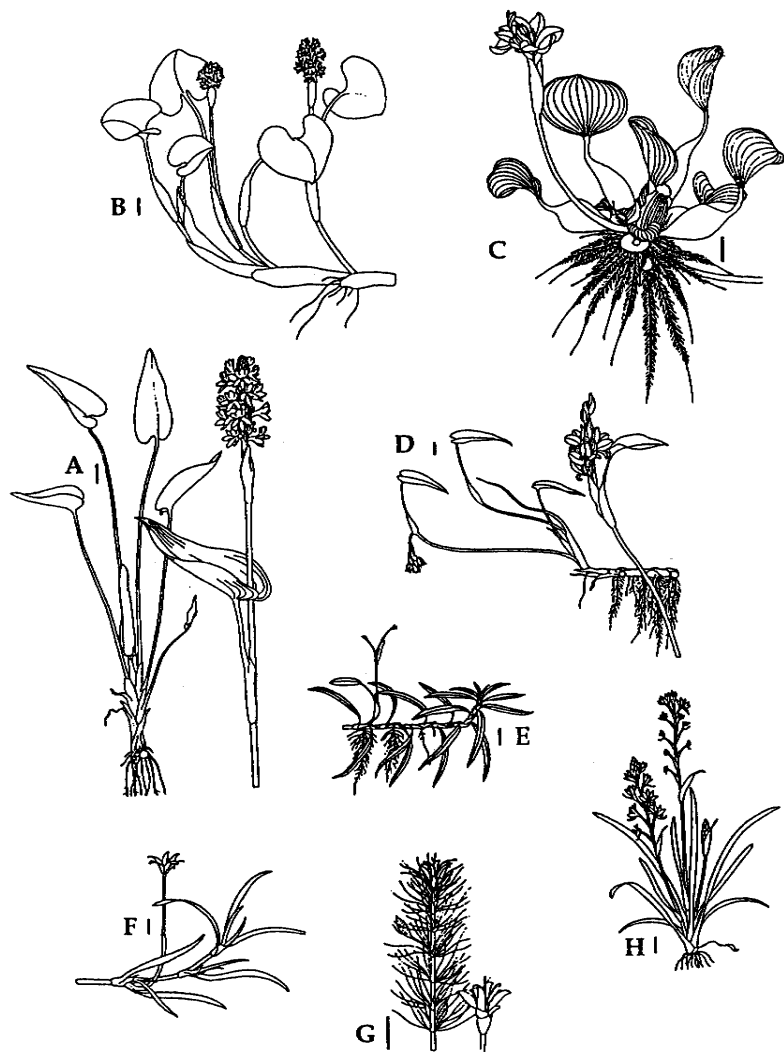


Figure 7.1. Pontederiaceae: diversity of aquatic life-forms and range of leaf types. (A) *Pontederia cordata*; (B) *Pontederia (Reussia) rotundifolia*; (C) *Eichhornia crassipes*; (D) *Monochoria vaginalis*; (E) *Heteranthera zosterifolia*; (F) *Heteranthera (Zosterella) dubia*; (G) *Hydrothrix gardneri*; (H) *Heteranthera (Eurystemon) mexicana*. All species except *H. mexicana* are represented in the phylogenetic reconstructions. Illustrations are from Cook (1990), with permission. All scale bars are 1 cm (except for C, where it is 3 cm).

of *Eichhornia crassipes* (Figure 7.1C) and *Eichhornia azurea* throughout the large river systems of South America and also on some Caribbean islands may have been largely the result of dispersal by vegetative means (Barrett 1978b; Barrett and Forno 1982). The natural distributions of a handful of Pontederiaceae have been extended over the past century due to human influences. Several New World *Heteranthera* species (*Heteranthera limosa*, *Heteranthera rotundifolia*, *Heteranthera reniformis*) occur as weeds of rice in Europe and Asia (C. Horn, pers. comm.; S. W. Graham, pers. obs.). *Monochoria vaginalis* (Figure 7.1D), a noxious weed of Asian rice, has also been introduced to Californian rice fields, probably as a seed contaminant (Barrett and Seaman 1980). The most widespread and economically important member of the family is the notorious clonal weed water hyacinth (*E. crassipes*). Originally native to lowland South America, vast floating mats of this species now infest lakes, rivers, reservoirs, and drainage canals in many parts of the warmer regions of the world (Barrett 1989).

Aquatic habitats and ecological differentiation

Members of Pontederiaceae can be found in a wide variety of natural and man-made habitats provided by lakes, rivers, streams, permanent marshlands, bogs and fens, seasonal pools, drainage ditches, low-lying pastures, and rice fields, indicating a wide range of habitat preferences within the family. Aquatic habitats can be exceptionally diverse and therefore provide considerable opportunities for ecological differentiation by aquatic plants. Extensive field observations of the family over the past two decades by the first author indicate that the most significant features of aquatic environments that determine whether a particular species of Pontederiaceae can persist relate to the permanency of the habitat, depth of water, extent of water-level fluctuations, amount of nutrient loading, and the degree of interspecific competition from other aquatic plants. The overall depth of water, the predictability of the habitat, and the degree of interspecific competition are of particular importance in determining the aquatic life-form, duration of the life cycle and degree of clonality of individual species.

We outline below the variation in growth forms, life histories and reproductive strategies found in members of the Pontederiaceae and discuss their likely ecological and evolutionary bases. In order for phylogenetic reconstructions of character evolution to be conducted, it is necessary to classify the range of observed variation in traits. This exercise can be difficult, particularly where apparently continuous variation occurs or where detailed morphological and developmental information on the homologies of differing structures are not available. Nevertheless, we present such classifications below to begin exploration of the patterns of adaptive radiation in life history traits in the family.

Aquatic life-forms

Life-form classifications of aquatic plants are many and varied (reviewed in Raunkiaer 1934; Den Hartog and Segal 1964; Sculthorpe 1967; Hutchinson 1975). Here we adopt the classification of Sculthorpe (1967) which distinguishes four main classes of aquatic life-forms: emergent, floating-leaved, free-floating, and submergent. All occur in Pontederiaceae (Figure 7.1) and are closely associated with the habitat

preferences of individual taxa. The emergent life-form, in which the plant is rooted in soil below water, but grows above the water surface to varying degrees, is the most common aquatic form and occurs in the majority of taxa in the family. Populations with this habit can be found over a broad range of aquatic conditions from temporary pools to more permanent wetland habitats. Leaf-blades of emergent taxa are held by self-supporting petioles. This allows them to overshadow and outcompete floating-leaved and submerged species in shallow waters. In deeper waters such petioles would be too costly to produce (Givnish 1995). The emergent life-form is therefore restricted to shallow locations at the edges of ponds, lakes, or rivers. In this study we distinguish two subclasses of emergents depending on whether the growth form is largely erect or procumbent. This dichotomy is somewhat artificial since a few species occur that link the two extremes (e.g., *Monochoria vaginalis*, *Pontederia (Reussia) subovata*) and considerable plasticity in the degree of erectness is evident depending on water depth (e.g., *Heteranthera seubertiana*; Horn 1988) and stand density. Nevertheless, we believe that this is a useful distinction because most taxa are distinguished by whether or not internodal elongation is extensive, producing plants that have either a creeping stem or a compact, erect rosette (Figure 7.1).

The only members of the family exhibiting the floating-leaved life-form in mature plants are *Eichhornia diversifolia*, *Eichhornia natans*, and *Scholleropsis lutea*. Individuals of these species are rooted to the substrate, with the stems and leaves floating on the water surface. We distinguish this growth form from the procumbent class of emergents by the predominance of truly floating leaves possessed by species in this category. While species such as *Pontederia (Reussia) rotundifolia* (Figure 7.1B), *E. azurea*, and *H. reniformis* frequently grow out from land over the surface of water, the majority of leaves that they produce are held erect as a result of upturned petioles and laminae. Many species of Pontederiaceae with emergent life-forms produce a small number of floating leaves as they emerge from below the water surface, following seed germination or perennation (Horn 1988). However, these leaves can be viewed as transitional, since the majority of mature leaves produced by these forms are adapted for terrestrial rather than aquatic conditions.

Eichhornia crassipes is the only species in the family that is truly free-floating (Figure 7.1C). The free-floating life-form is characterized by only a brief dependence on solid substrate to enable seed germination and establishment. Once established, young seedlings sever their connection with the sediments in which they germinated and float to the water surface. Floating is accomplished via swollen, aerenchymous petioles. Subsequent growth, clonal propagation, and dispersal occur entirely independently of land. While many taxa of Pontederiaceae with procumbent or floating-leaved growth forms can form floating mats, these are incapable of extensive growth and regeneration unless rooted to the substrate.

The final aquatic life-form in Pontederiaceae is the submersed life-form, represented by *Heteranthera zosterifolia* (Figure 7.1E), *H. dubia* (Figure 7.1F), and *Hydrothrix gardneri* (Figure 7.1G). In these species the entire plant body is submersed below the water surface, except during flowering when reproductive parts may be elevated just above the water (Wylie 1917; Rutishauser 1983). *Heteranthera dubia* and *H. zosterifolia*

can tolerate partial emergence (e.g., mud-flat ecotypes of *H. dubia*; Horn 1983). Apart from occasional flowers above water, *Hydrothrix gardneri* is obligately submersed. The four main aquatic life-forms therefore appear to show different degrees of adaptation to the aquatic environment and could be thought of as involving an evolutionary transition from a terrestrial ancestor through an amphibious existence to a fully aquatic habit. Phylogenetic reconstruction may assist in evaluating this hypothesis by determining the direction and sequence of evolutionary change within Pontederiaceae.

Life-cycle duration

The adaptive basis of life-cycle duration in flowering plants has been the subject of much discussion, with a variety of ecological and demographic factors invoked as important selective agents (reviewed in Harper 1977; Grime 1979). Members of Pontederiaceae display a spectrum of life histories that are frequently associated with the permanency of the aquatic habitat occupied. These range from annual species that occur in ephemeral aquatic habitats such as seasonal pools, ditches, and rice fields (e.g., *E. diversifolia* and *Heteranthera* spp.) to very long-lived taxa that are largely restricted to permanent marshlands (*Pontederia* spp.), or to large river and lake systems (*E. azurea*) such as those found in Amazonia and the Pantanal region of South America.

We distinguish three categories of life-cycle duration: annual, short-lived perennial, and long-lived perennial. Annual species are those in which the majority of populations of a species complete their life cycle within a year. Short-lived perennials may persist for up to five years and long-lived perennials often live for considerably longer time periods. These categories are not rigid, because altered ecological conditions may modify patterns of longevity in any species. For example, several of the species that we classify as annuals (e.g., *E. paniculata*), because they usually cannot persist vegetatively in their native habitats from season to season as a consequence of severe desiccation, can continue growing almost indefinitely in the glasshouse if provided with suitable conditions. In contrast, populations of some annual species (e.g., *Eichhornia meyeri*, *H. limosa*) display obligate annualness, undergoing programmed senescence regardless of growing conditions. Among several of the species we classify as short-lived perennials are populations that appear to be annual when grown under glasshouse conditions (e.g., *M. vaginalis* from Californian rice fields).

Clonality

A considerable literature has been devoted to addressing questions concerned with the ecology and evolution of clonal versus sexual reproduction (Williams 1975; Maynard Smith 1978; Bell 1982). Valuable perspectives on the adaptive basis of clonality in plants have been provided by Abrahamson (1980), Leakey (1981), and Cook (1985). Aquatic plants are of particular interest in these discussions because of their heavy reliance on asexual methods of propagation (Arber 1920; Hutchinson 1975), and it has often been suggested that cloning may be favored in aquatic environments where regular seed reproduction is difficult in deep or turbulent water (Sculthorpe 1967). However, in a recent review Grace (1993) drew attention to the variety of clonal

strategies found in aquatic plants and argued that at least six major selective forces may be involved in the evolution of clonal growth in aquatics: (i) numerical increase, (ii) dispersal, (iii) resource acquisition, (iv) storage, (v) protection, and (vi) anchorage.

Clonality in members of the Pontederiaceae appears to be closely linked with the life-form and longevity of individual taxa. Propagation in annual species is typically entirely sexual but most perennials in this family possess some form of clonal growth. This includes local colony expansion through rhizome growth in erect, emergent taxa such as *Pontederia sagittata* and *Monochoria hastata*, fragmentation of creeping stems in procumbent taxa with extensive internodal elongation [e.g., *P. rotundifolia* (Figure 7.1B) and *E. azurea*], fragmentation of stems in submersed taxa (e.g., *H. dubia*) and the formation of slender stolons with daughter rosettes in the free-floating *E. crassipes*. As in many other perennial aquatics (see Eckert and Barrett 1993) the balance between sexual and asexual reproduction in Pontederiaceae can vary with habitat conditions and the combination of growth form and clonality that occurs (Richards 1982). Seed reproduction is common in most emergent taxa with rhizomatous growth or stem fragmentation because they usually occupy habitats suitable for seed germination and seedling establishment. In contrast, in submersed and free-floating taxa sexual recruitment probably occurs less often, despite seed formation, because of deep water conditions that restrict seedling establishment, and in some taxa there are populations that regenerate exclusively through clonal propagation (e.g., *E. crassipes*; Barrett 1980a,b).

The various clonal strategies displayed by members of the Pontederiaceae serve different functions. One of these is numerical increase (i.e., reproduction via ramet formation), which is most obvious in taxa with regular stem fragmentation or stolon production. Dispersal of these vegetative structures by water currents also enables exploitation of new environments, with the free-floating daughter rosettes in *E. crassipes* representing the most specialized adaptation for vegetative dispersal in the family. For species that experience long periods with unfavorable growing conditions, such as during winters in eastern North America, rhizomes and stem fragments are also used as perennating structures (e.g., *P. cordata* and *H. dubia*; Lowden 1973, Horn 1983). However, these structures are also capable of withstanding considerable desiccation and in tropical habitats prone to drought can contribute to persistence during dry periods. Finally, in taxa of Pontederiaceae with creeping stems or stolons, the structures involved in clonal growth are also photosynthetic and produce roots. Thus they are highly effective in resource acquisition, the exploitation of suitable habitat patches, and competition with coexisting aquatic species.

Patterns of leaf development

Aquatic plants display striking foliar plasticity involving continuous variation in leaf shape and the formation of discrete leaf types with very distinct morphologies on a single individual. The latter condition has been referred to as heterophylly and a considerable literature exists on the proximate ecological, physiological, and developmental mechanisms that control changes in leaf shape in heterophyllous species (Arber 1919; Sinnott 1960; Sculthorpe 1967; Lee and Richards 1991). Less attention has

been paid to the genetic and evolutionary basis of such patterns (although see Bradshaw 1965; Cook and Johnston 1968). It is usually assumed that in aquatic plants the formation of flaccid, ribbon-shaped (Figure 7.1E,F), or highly dissected leaves or leaf whorls (Figure 7.1G) represents an adaptive response to submersed conditions. Heterophylly often has been considered to be a manifestation of heteroblastic leaf development – the ontogenetic sequence in which early-formed “juvenile” leaves are markedly different in appearance from later “adult” ones. However, because the distinction between leaf types often is not clear-cut and so-called “juvenile” leaves often can be retained throughout the life cycle by neoteny (Sculthorpe 1967, and see below), it is important to realize that considerable diversity exists in the patterns of leaf development found in aquatic plants and that any attempt at classification is likely to be somewhat artificial.

With the exception of a detailed investigation of leaf ontogeny in *E. crassipes* (Richards 1983) and descriptions of heterophylly in *Heteranthera* (Horn 1988), there has been little work on the developmental basis of leaf-shape variation in Pontederiaceae. For the purpose of our study we tentatively recognize five basic classes (referred to as patterns A–E) that differ primarily in the duration of the “juvenile” phase. In the first and most common type (pattern A) plants first produce a small number (one to four) of juvenile, linear, strap-shaped leaves, the width and size of which vary with species and degree of submersion, before producing “adult” aerial leaves (Figure 7.1A–D) with distinct petioles and laminas. In *Heteranthera* the juvenile leaves can be very narrow, whereas in *Pontederia* they can be up to several centimeters in width. Species with this type of leaf development are usually amphibious with seedlings commonly developing in shallow water or on wet mud. The important feature of this leaf development strategy is a rapid transition to the formation of aerial leaves, the characteristics of which vary according to species.

In the second class (pattern B), this transition is much slower and a greater number (more than twenty) of submersed ribbonlike leaves are produced before the transition to aerial or floating leaves. This pattern of leaf development is quite restricted in the family and occurs only in *Eichhornia azurea*, *E. diversifolia*, *E. heterosperma*, and *E. natans*, all species that commonly germinate in deep water and experience extended periods of seedling development under water. While ribbon-shaped leaves are always the first leaves to be produced by species in class B, damage to the shoot apex through herbivory or disease in adult plants can result in a temporary reversion to the “juvenile” phase, indicating that heteroblastic development is not necessarily developmentally fixed.

The next two classes involve taxa in which all foliar leaves retained throughout the life-cycle are sessile, linear, and ribbon-shaped and are similar in appearance to the “juvenile” leaves initially produced by taxa in the first two categories. We chose to recognize two separate classes of these (presumably) “retained juvenile” or paedomorphic forms, because it seems probable that they have different developmental origins (neotenic versus progenetic; J. H. Richards, pers. comm.) and different ecological significances. Petiolate leaves are never produced in these taxa (the lower spathe in inflorescences of *H. zosterifolia* being the sole exception).

Pattern C occurs in the perennial, submersed aquatics *Heteranthera zosterifolia* (Figure 7.1E) and *H. dubia* (Figure 7.1F), and presumably reflects their predominantly submersed existence. The pattern appears to have arisen through neoteny (i.e., through slower somatic development relative to the onset of reproductive maturity), analogous to but much more accentuated than in pattern B. Such a neotenic shift may be a consequence of direct selection for retention of the juvenile leaf form throughout the life cycle. Ribbon-shaped leaves have been interpreted as a mechanical (anti-drag) adaptation to moving water (Sculthorpe 1967), but may play a role in counteracting diffusive limitations on photosynthesis underwater (see below). Pattern D is found in two annual taxa, *Heteranthera seubertiana* and *Heteranthera (Eurystemon) mexicana* (Figure 7.1H). These two species are primarily emergent, and are found in ephemeral pools. This pattern could have arisen through an earlier onset of reproduction (i.e., progenesis; see Alberch et al. 1979). Such precocious reproduction may be an adaptive response to the ephemeral nature of some aquatic environments (Arber 1920; Van Steenis 1957; Sculthorpe 1967). Emerged leaves from the four species exhibiting these two patterns are not flaccid but are stiff and erect and clearly adapted for terrestrial conditions.

The final class of leaf development (pattern E) is restricted to the submersed *Hydrothrix* (Figure 7.1G). This monotypic genus is characterized by whorls of small, threadlike leaves whose developmental origin is highly unusual (Rutishauser 1983). The leaf whorl, which has analogues in many plant groups (including some algae), is an example of the "Hippuris syndrome" (Cook 1978). The occurrence of this syndrome in *Hydrothrix* is virtually unique within monocotyledons (Cook 1978).

The functional significance of the Hippuris syndrome may be to reduce self-shading in deep water (Arber 1920). It has also been interpreted as a means of reducing drag in flowing water (Arber 1920, but see Sculthorpe 1967) or for increasing total assimilating area (Sculthorpe 1967, but see Arber 1920). A major function of ribbon- or threadlike leaves in submersed aquatics may be to decrease the impedance of leaf-surrounding boundary layers on CO₂ diffusion, which is far slower in water than in air (Givnish 1987). The more effectively "narrow" a leaf or leaf division is, the smaller its boundary layer will be, and the less it will impede diffusion and limit photosynthesis (Givnish 1987). More slowly flowing waters should therefore favor narrower leaves. This prediction is supported by comparative data on aquatic plants in ponds vs. streams (see Madsen 1986; Givnish 1987). The cordate bases seen in the petiole of several emergent taxa may represent the minimization of vein construction costs in lamina atop erect petioles (Givnish and Vermeij 1977).

Floral ecology, pollination, and mating systems

Angiosperms display a spectacular array of floral diversity associated with the pollination biology and mating systems of individual species. Indeed, reproductive adaptations associated with different pollen vectors are among the few plant traits that have been considered explicitly in the context of adaptive radiation (Grant and Grant 1965; Stebbins 1970). In addition to descriptive studies of pollination syndromes, recent functional interpretations of floral radiation have emphasized the

importance of individual selection for fitness gain through female and male reproductive function (e.g., Bell 1985; Campbell 1989; Devlin and Ellstrand 1990). Using this approach it is important to distinguish aspects of floral display and design that reduce the incidence of self-pollination and inbreeding depression (Charlesworth and Charlesworth 1987) from those that promote outcrossed siring success through more effective pollen dispersal (Harder and Barrett 1996). This is of particular importance in species that possess physiological self-incompatibility systems (Lloyd and Webb 1986; Bertin and Newman 1993; Harder and Barrett 1995).

The flowers of Pontederiaceae are showy and blue, mauve, yellow, or white and can be solitary or displayed in inflorescences. They exhibit a broad range of morphological specializations associated with a variety of pollination mechanisms and mating systems. Despite being an entirely aquatic family, the floral syndromes of Pontederiaceae involve either animal pollination or self-pollination, with no evidence of the kinds of adaptations towards hydrophily that occur in many other exclusively aquatic taxa (Cox 1988). Flowers in the family are largely pollinated by bees, and to a lesser extent by butterflies. In *Pontederia* and *Eichhornia*, the flowers are tubular and moderately zygomorphic and pollination is largely achieved through the services of long-tongued bees that feed primarily on nectar (Wolfe and Barrett 1988; Husband and Barrett 1992). In *Heteranthera* and *Monochoria*, floral visitors are mostly pollen-collecting bees (Iyengar 1923; Wang et al. 1995; S. C. H. Barrett, pers. obs.). In most species of *Heteranthera*, *Hydrothrix gardneri*, *E. diversifolia*, *E. natans*, and *M. vaginalis*, flowers are also produced which develop underwater and hence are completely self-fertilized. This phenomenon is known as pseudo-cleistogamy and is commonly reported in other aquatic groups (Sculthorpe 1967). Amphibious flowers with underwater ovaries but aerial pollination organs are found in *Heteranthera dubia* (Wylie 1917; Horn 1985). Floral tubes in this species are very variable in length (Horn 1985) and can reach over 11 cm long (R. Rutishauser, pers. comm. in Endress 1995). Amphibious flowers and pseudocleistogamy represent the only obvious examples of shifts in reproductive characters in response to the aquatic habit of the family.

Two conspicuous floral polymorphisms (tristyly and enantiostyly) occur in Pontederiaceae (Figure 7.2). The genetic polymorphism tristily occurs in all species of *Pontederia* s. lat. except *Pontederia parviflora*, and in three species of *Eichhornia* (*E. azurea*, *E. crassipes*, and *E. paniculata*). Tristyly species possess a reciprocal arrangement of style and anther heights (Figure 7.2A) and an associated syndrome of ancillary characters exhibiting polymorphisms of pollen and stigmas (reviewed in Barrett 1992). The remaining taxa in both genera are small-flowered and monomorphic for style and stamen length. A self- and intramorph-incompatibility system accompanies the floral heteromorphism in all tristylous species, except for *E. crassipes* and *E. paniculata*, which are tristylous but highly self-fertile (Barrett 1979; Barrett and Anderson 1985; Barrett 1988). Experimental studies of *Pontederia* and *Eichhornia* support Darwin's (1877) original hypothesis that the tristylous polymorphism functions to promote proficient cross-pollination among plants through the reciprocal arrangement of male and female sex organs (Barrett and Glover 1985; Kohn and Barrett 1992; Lloyd and Webb 1992).

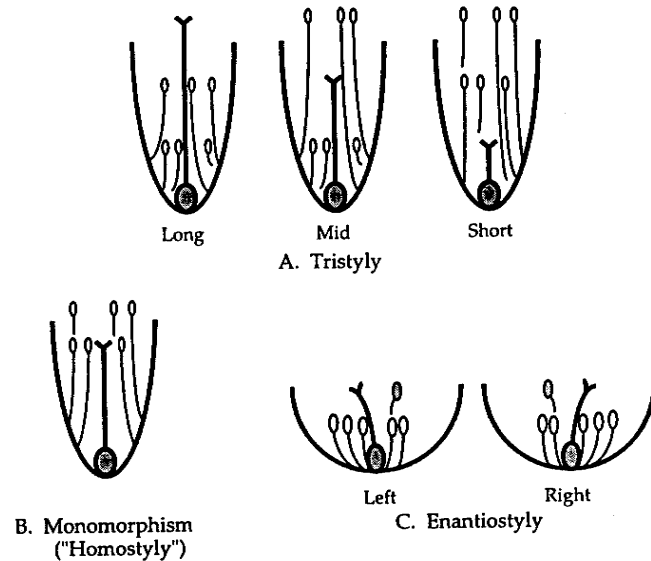


Figure 7.2. Schematic representation of stamen and style configurations in the three most common floral forms in Pontederiaceae (after Graham and Barrett 1995). (A) Tristyly – individuals produce either long-, mid- or short-styled flowers, depending on their genotype at two diallelic loci controlling this genetic polymorphism. (B) Floral monomorphism (referred to as “homostyly” when evolutionarily derived from heterostyly) – in *Eichhornia*, monomorphic species have three or six stamens at the same level as the stigma (Barrett 1988). Typically, homostylous variants found *within* tristylous species of *Eichhornia* have only one stamen at the same level as the stigma (referred to as “semi-homostyly” [not shown]). (C) Enantiostyly – flowers have either left- or right-bending styles, with a single stamen (one of six in *Monochoria*, one of three in *Heteranthera*) bending in the opposite direction. In contrast with heterostyly, individuals can produce both floral forms (after Graham and Barrett 1995).

The second floral polymorphism, enantiostyly (Figure 7.2C), occurs in species of *Heteranthera* s. lat. and *Monochoria* (Iyengar 1923; Eckenwalder and Barrett 1986; Wang et al. 1995). The outward-facing flowers possess either left- or right-bending styles with a single stamen reflexed in a lateral position opposite the stigma. This condition rarely occurs as a true genetic polymorphism with populations composed of plants with either right- or left-handed flowers, but not both (e.g. in *Wachendorfia*; Ornduff and Dulberger 1978). More commonly, however, it exists as a somatic polymorphism with individual flowers possessing both flower types. All enantiostylous members of Pontederiaceae have the somatic form of the polymorphism. They also display a striking anther dimorphism with the reflexed stamen cryptically colored and larger than the remaining stamens. Such dimorphism is termed heteranthery and represents a functional division of labor into attractive “feeding” anthers and a cryptically colored “pollinating” anther (Vogel 1978; Buchmann 1983; Lloyd 1992). Enantiostyly has

most often been interpreted as an adaptation to increase the effectiveness of cross-pollination, in a manner analogous to heterostyly. However, there is little empirical evidence to support this hypothesis (e. g., Fenster 1995) and where the polymorphism is somatic other factors must be involved in its origin and maintenance (see Graham and Barrett 1995 for further details).

While mating patterns have not been quantified in the majority of Pontederiaceae, some inferences can be drawn from information on floral biology and experimental studies conducted by the first author over the past two decades. All species of *Monochoria* and *Heteranthera* s. lat. are highly self-compatible. Undisturbed flowers of species in these two groups can usually achieve full seed-set through autonomous self-pollination. Although pollen-collecting bees visit flowers, it seems likely that populations of these taxa experience considerable self-pollination, particularly in taxa with pseudo-cleistogamous flowers. In contrast, tristylous species of *Pontederia* and *Eichhornia* with self-incompatibility must be largely outcrossing because of their physiological barrier to self-fertilization. Even where tristyly is associated with self-compatibility, marker-gene studies indicate that populations can exhibit high outcrossing rates (Barrett et al. 1993). Among non-heterostylous species of *Pontederia* and *Eichhornia*, self-fertilization is likely to predominate, since these taxa are self-compatible and homostylous, with anthers and stigmas close together within each flower (Figure 7.2B; see Barrett 1988).

Phylogenetic systematics of Pontederiaceae

Morphological and molecular evidence of phylogenetic relationships

Molecular evidence from the chloroplast gene *rbcL* strongly supports the monophyly of Pontederiaceae (Graham and Barrett 1995). Three highly congruent data sets derived from the chloroplast genome (based on restriction-site variation and sequence data from *rbcL* and *ndhF*) yield robust and well-resolved estimates of the phylogenetic history of the family (Graham et al., unpubl. data; Figure 7.3). These estimates indicate that *Monochoria*, *Pontederia* s. lat., and *Heteranthera* s. lat. are monophyletic, but that *Eichhornia* is not. Morphological evidence concerning the phylogenetic history of Pontederiaceae also rejects the monophyly of *Eichhornia* (Eckenwalder and Barrett 1986), but is largely insufficient for estimating a robust phylogenetic history of the family (Graham et al., unpubl. data). In combined analyses with the molecular evidence, the morphological evidence has almost no impact on phylogenetic reconstructions (Graham et al., unpubl. data).

Despite being swamped by the molecular evidence, there is statistically detectable incongruence between the molecular and morphological data (Graham et al., unpubl. data). Eckenwalder and Barrett (1986) hypothesized that a “selfing syndrome” in the family (involving multiple parallel shifts in reproductive characters during the origin of predominantly self-fertilizing species) could result in incorrect phylogenetic reconstructions using the morphological data. However, as this hypothesis is not actually supported by the morphological evidence and is contradicted by the molecular data, it cannot be the source of the incongruence between these two

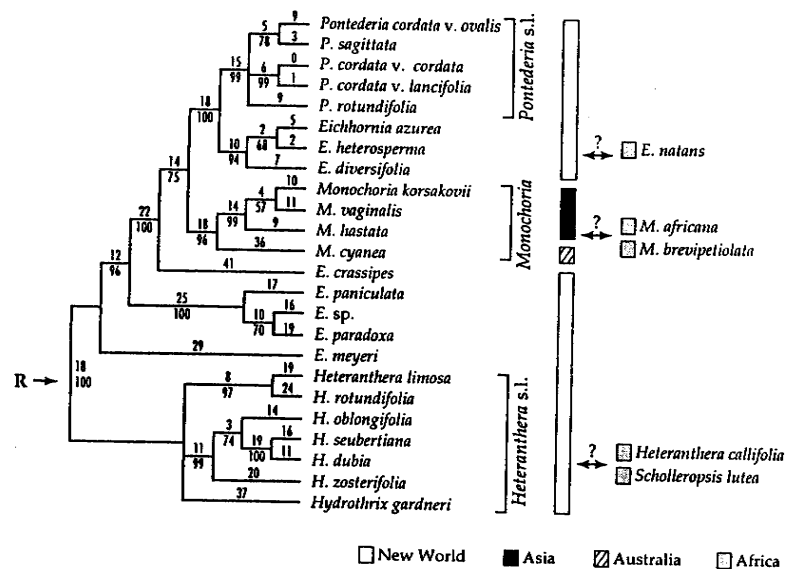


Figure 7.3. Reconstructed phylogenetic history of Pontederiaceae using combined evidence from DNA sequence variation in the chloroplast genes *rbcl* and *ndhF*, and restriction-site variation in the chloroplast genome (Graham et al., unpubl. data). The tree is a strict consensus of four shortest unrooted trees. Branch lengths were determined using ACCTRAN optimization for one of the four shortest trees and are indicated above each branch. Bootstrap values (from 100 bootstrap replicates) are below branches. The root is indicated R was found by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets (see text). For the four most parsimonious trees found with the combined chloroplast data, tree length (including autapomorphies) = 609 steps, CI (including autapomorphies) = 0.637, CI (excluding autapomorphies) = 0.525, RI = 0.775 (Graham et al., unpubl. data). The bars indicate whether each taxon is found in the Old World (Asia, Australia, or Africa), or the New World. Question marks indicate possible long-distance dispersal events involving several taxa missing from this analysis (see text).

major lines of evidence (Graham et al., unpubl. data). There is little evidence of hybridization among modern species of Pontederiaceae, but it is not impossible that undetected ancient hybridization events have contributed to the observed incongruence between the morphological and chloroplast data (see Doyle 1992). An improved morphological data base (or new molecular evidence from the nuclear genome) is needed to pinpoint the precise source and extent of the incongruence between these two sources of data. In the meantime, the chloroplast evidence is our only substantial source of evidence concerning the phylogenetic history of Pontederiaceae, and we use it here to investigate the radiation of morphological characters in the family.

Given the trees found with the morphological data set of Eckenwalder and Barrett (1986) as modified by Graham et al. (unpubl. data), the morphological data have levels of homoplasy very close to that expected for this number of taxa. The observed CI

(excluding autapomorphies) for the 24 ingroup taxa in Figure 7.3 is 0.474. The expected CI for this number of taxa is 0.495, based on Sanderson and Donoghue's (1989) survey of 60 data sets. However, the trees derived from the molecular data indicate that the amount of homoplasy in the morphological data is substantially higher than this. The CI (excluding autapomorphies) of the morphological data on the four shortest trees found with the combined chloroplast evidence ranges from 0.404 to 0.410. This lower value may partly be a reflection of the incongruence between the morphological and molecular sources of data, and given the low number of informative morphological characters (only 33), it is possible that these estimates of homoplasy in the morphological data are a quite imprecise reflection of the real levels of homoplasy in morphological characters. The patterns of homoplasy among subsets of the morphological characters are nonetheless intriguing. On the molecular trees, the CI for 17 informative floral characters (excluding autapomorphies and post-anthesis characters) ranges from 0.446 to 0.453; for 11 informative vegetative characters, the CI (excluding autapomorphies) ranges from 0.310 to 0.316. The trend toward high levels of homoplasy relative to that expected for this number of taxa (Sanderson and Donoghue 1989) in both classes of data suggests that they have been subject to elevated levels of character divergence in this family, at least compared to unrelated groups.

The location of the family's root was one of the few elements of the phylogenetic structure of Pontederiaceae left unresolved by the molecular studies of Kohn et al. (1996) and Graham et al. (unpubl. data). The local position and membership of Pontederiaceae within a cluster of superorders consisting of Arecanaceae, Bromelianaceae, Commelinaceae and Zingiberaceae (*sensu* Dahlgren et al. 1985) also was not well supported by evidence from *rbcl* (Duvall et al. 1993, Graham and Barrett 1995) or surveys of chloroplast restriction-site variation (Davis 1995). A range of taxa in this complex of superorders was surveyed for variation in the chloroplast genes *ndhF* and evidence from this gene was employed in tandem with the *rbcl* evidence to determine which taxa are most closely related to the family, and to establish the position of the root of the family (Graham and Barrett, unpubl. data). Using equal weighting of all characters and a variety of different unequal weighting schemes to correct for among-site rate variation, combined analyses of these two genes indicated that the sister-group of the family is a clade consisting of Commelinaceae and Haemodoraceae, and that the next most closely related clade is Philydraceae (Graham and Barrett, unpubl. data). These three most closely related families (Commelinaceae, Haemodoraceae, and Philydraceae) also converged upon a single most parsimonious root location of Pontederiaceae (Graham and Barrett, unpubl. data), which is the *a posteriori* rooting employed in character reconstructions here (Figure 7.3).

Implications of the phylogenetic data and fossil evidence for the biogeography of the family

As discussed earlier, the modes of dispersal of taxa in Pontederiaceae provide substantial opportunities for long-range dispersal. With a few isolated exceptions, the basal clades in the family are all currently limited to the New World, and the exclusively Old World genus *Monochoria* is located quite far from the base of the tree,

suggesting an ancient inter-continental dispersal (Figure 7.3). The fossil record of the family reaches back into the Eocene (ca. 50 Mya) when Africa, Australia, and South America were no longer in direct contact (Briggs 1987). *Monochoria* is the only genus in Pontederiaceae currently restricted to the Old World (Figure 7.3). Fossilized seeds and leaf material similar to modern *Eichhornia*, and seeds like those of modern *Monochoria*, are known from the upper Eocene onwards in Europe (see Collinson et al. 1993). The presence of *Eichhornia*-like fossils in Europe raises the intriguing possibility that the current limitation of this genus to the New World may be a consequence of ancient extinctions in the Old World. Fossilized root and stem fragments of Pontederiaceae are known from the Eocene in India (Patil and Singh 1978). However, the uncertain generic affinity of this material (Eckenwalder and Barrett 1986) means that it is not clear whether this represents a lineage that arose before or after the divergence of the extant members of the family. Other fossils ascribed to the family (Bureau 1892; Knowlton 1922; Fritel 1928) are from North American and European sites, but as these reports are based solely on leaf material, they must also be treated as being of unclear affinity to modern genera. Philydraceae is currently limited to Australia and Asia (Dahlgren et al. 1985; Adams 1987), but the two families constituting the sister-group (Commelinaceae and Haemodoraceae) are distributed throughout the Old and New World (Dahlgren et al. 1985; Simpson 1990). Aside from the *Monochoria*, the only other species in Pontederiaceae with Old World distributions are *Heteranthera callifolia*, *Scholleropsis lutea*, and *Eichhornia natans*. These three species, together with two species of *Monochoria*, are limited to Africa. All of these taxa are missing from the current study, but their taxonomic affinities and positions in the morphology-based analysis of Eckenwalder and Barrett (1986) suggest that their current distributions are a consequence of several long-range dispersal events (Figure 7.3). However, the possibility that some of these species are relicts from a more cosmopolitan distribution of these genera cannot be ruled out, although this is unlikely for *E. natans* given its probable close relationship to *E. diversifolia* (see below). Multiple long-range dispersal events probably also contributed to the modern distributions of the various New World taxa. North American taxa in general seem to be more morphologically apomorphic than those in tropical South America, suggesting that they may have migrated north after intercontinental contact in the Miocene (Eckenwalder and Barrett 1986).

Character diversification and adaptive radiation in vegetative and reproductive characters

Outgroups and their effect on character reconstruction in Pontederiaceae

The inclusion of outgroup taxa in phylogenetic analysis serves two major purposes: locating the position of the root of ingroup, and polarizing character-state transformations within this group. Although these analytical goals are often addressed simultaneously, they need not be if the characters used to reconstruct a phylogeny (as in this study) are not the ones in whose evolutionary transformation we are interested (e.g., Brooks and McLennan 1991; Maddison and Maddison 1992).

The sister-group plays a major role in polarizing character reconstructions, but other less closely related outgroups also play an important role in this (Maddison et al. 1984; Nixon and Carpenter 1993).

We employed the three most closely related families to Pontederiaceae to provide information concerning the polarity of the character transformations discussed below: Commelinaceae and Haemodoraceae (which together constitute the sister-group) and Philydraceae (the next most closely related taxon). In cases where there was character-state variation among the constituent taxa of individual outgroups, knowledge of the internal phylogenetic structure of each outgroup would be valuable for obtaining "globally parsimonious" reconstructions of the evolution of such characters (Maddison et al. 1984; Maddison and Maddison 1992, p. 47). Simpson (1990) provided a phylogeny of Haemodoraceae based on morphological data, but there are no published phylogenies for Commelinaceae or Philydraceae. It was consequently often necessary in this study to code individual families as polymorphic for character-states for which there was known to be variation among different species within each family. Using polymorphic coding to account for this variation is a less-than-ideal solution to lack of knowledge concerning the phylogenetic structure within individual outgroup families (Nixon and Davis 1991; Maddison and Maddison 1992). However, the reconstructions obtained here, while conditional on increased knowledge of the phylogenetic structure of these groups and improved knowledge of character distributions in them, are nonetheless the most parsimonious ones given our current state of knowledge (Maddison and Maddison 1992, p. 47).

The reconstructions of character diversification were performed using MacClade version 3.0 (Maddison and Maddison 1992) and employed the four most parsimonious unrooted trees of the family found in the combined analysis of the three chloroplast sources of evidence (Graham et al., unpubl. data), with the rooting determined using combined evidence from *rbcl* and *ndhF*. Only minor differences existed among the four trees concerning the placements of *Pontederia rotundifolia* and *Hydrothrix gardneri* in *Pontederia* s. lat. and *Heteranthera* s. lat., respectively. All reconstructions were performed using MacClade version 3.0 (Maddison and Maddison 1992) and used Fitch optimization (Fitch 1971), in which all character-state changes were treated as equally likely events (i.e., unordered or equally weighted), apart from an analysis of reproductive characters in which "relaxed Dollo" schemes of character evolution (Swofford and Olsen 1990) were also assessed (see below). The results are indicated in legends on each figure. With the aid of the "equivocal cycling" tool in MacClade, we obtained counts of the number of gains of each character-state for each character within Pontederiaceae, and determined the primitive state of the family, for all most parsimonious reconstructions of each character on the four trees. The character reconstructions in Figures 7.4–7.8 exemplify much of the diversification observed for each character: the tree used in these figures is one of the four most parsimonious ones, and is the most highly converged-upon tree found in analyses of several different combinations of the available chloroplast evidence (Graham et al., unpubl. data).

Character codings

A total of 24 species were surveyed, representing approximately two-thirds of the family and including all major taxonomic groups (for source and voucher information see Kohn et al. [1996]; Graham 1997). Except for leaf developmental pathway, the character codings for the taxa of Pontederiaceae considered here are derived from Eckenwalder and Barrett (1986), Graham and Barrett (1995), and Graham et al. (unpubl. data). The codings for the three outgroup taxa we included are presented here. Of the 50 genera in Commelinaceae, a few are found in wet places, but only *Murdannia* possesses aquatic species (at least two of 50 species; Cook 1990). Cook (1990) lists only one species of Philydraceae as being helophytic (*Philydrum lanuginosum*), but all six species in the family are found in marshes and wet rain forest habitats (Adams 1987). Species of Haemodoraceae are almost all xeric (M.G. Simpson, pers. comm.), although *Tribonanthes* is found in similar habitats (low, winter-wet flats) to *Philydrella* (Philydraceae) (Simpson 1990). Commelinaceae, Haemodoraceae, and Philydraceae are therefore almost exclusively terrestrial groups, and most closely approach the "emergent" condition in Pontederiaceae. Procumbent and erect life-forms are known in Commelinaceae (Faden 1988), and so this outgroup is coded as polymorphic for these two forms. Haemodoraceae and Philydraceae contain only erect taxa (Adams 1987; Cook 1990; M. G. Simpson, pers. comm.) and these families are coded accordingly for life-form.

For life-cycle duration, Commelinaceae and Philydraceae both are coded as polymorphic for annuality and short- and long-lived perenniality (Dahlgren et al. 1985; Faden 1988; Cook 1990). A "long-lived perennial" coding is appropriate for Haemodoraceae (M. G. Simpson, pers. comm.). Non-clonal species are found in Commelinaceae (Faden 1988), and some species in this family express clonality via rhizomes, stolons, or spreading stem fragmentation (Faden 1988). Commelinaceae is coded as polymorphic for "non-clonality," and for these three kinds of clonality ("via rhizomes," "via stolons," and "via stem fragmentation"). There is no direct evidence of clonality in Haemodoraceae, but extensive underground rhizome/stolon systems are known in this family (M. G. Simpson, pers. comm.). Haemodoraceae is provisionally coded as polymorphic for "non-clonality," "clonality via rhizomes," and "clonality via stolons." Species of Philydraceae are rhizomatous or cormous (Adams 1987), but it is not known if these structures are involved in regeneration. Philydraceae is provisionally coded as polymorphic for "non-clonality" and "clonality via rhizomes."

Information on the timing of the transition to adult leaves, and on the homology of such pathways among the outgroup families and Pontederiaceae is mostly lacking. However, Tillich (1994, 1995) noted that the seedlings of Pontederiaceae and Philydraceae are very alike and that the primary leaves in both families are ribbonlike. The homology of adult leaf-form among these families is uncertain. Members of Commelinaceae possess bifacial leaves, but Haemodoraceae and Philydraceae possess unifacial, ensiform leaves (Dahlgren and Rasmussen 1983). Anatomical data suggest that the bifacial leaves typical of taxa in Pontederiaceae have a unifacial origin (see Arber 1925), and Simpson (1990) hypothesized that an origin of the bifacial leaf was associated with the shift to an aquatic environment in Pontederiaceae. We provisionally coded the three families as "unknown" for leaf developmental pathway.

Showy, insect-pollinated flowers are typical of species in Commelinaceae, Haemodoraceae, and Philydraceae. Species lacking either somatic or genetic polymorphisms in stylar class (referred to here as "monomorphic flowers") predominate in Commelinaceae and Haemodoraceae and in most monocotyledons. Tristylous is only known in Pontederiaceae, but enantiostyly is found in some species of Commelinaceae (Faden 1991) and Haemodoraceae (Simpson 1990) and all species of Philydraceae (Simpson 1990). We did not count as enantiostylous those species that possess flowers with bent styles but that lack a "handedness," such as *Hydrothrix gardneri* (Rutishauser 1983) and *H. dubia* in Pontederiaceae. Only two genera in Haemodoraceae (*Schiekia* and *Wachendorfia*) possess flowers with bent styles that have true left-versus right-handedness, i.e., with zygomorphic, outward-facing flowers (Simpson 1990). The non-basal position of these taxa in morphology-based cladograms of Haemodoraceae (Simpson 1990) suggests that monomorphic flowers were ancestral in this family. Haemodoraceae was therefore coded as "monomorphic" for floral form, Philydraceae as "enantiostylous," and Commelinaceae as polymorphic for these two conditions.

Owens (1981) reported self-compatible and self-incompatible species in Commelinaceae. The form of self-incompatibility in Commelinaceae is gametophytic and non-heteromorphic and consequently is highly unlikely to be homologous to that in Pontederiaceae (Graham and Barrett 1995). Commelinaceae was therefore coded as polymorphic for gametophytic SI and self-compatibility. The self-incompatibility status of species in the two other outgroup families is largely unknown. *Philydrum lanuginosum* is fully autogamous (S. C. H. Barrett, pers. obs.). Hamann (1966) also reported autogamy in the family, but no explicit surveys for self-incompatibility have been performed. Philydraceae is therefore coded as "unknown" for this character. There is a single report of a weakly developed incompatibility system in *Wachendorfia paniculata* that appears to be associated with true genetic enantiostyly, in a manner analogous to distylous or tristylous self-incompatibility systems (Ornduff and Dulberger 1978; see also Wilson 1887). However, we are aware of no other data concerning the self-incompatibility status of species in Haemodoraceae, and so code it as "unknown" for this character.

Reconstructions of character evolution

AQUATIC HABIT – Is the capacity to thrive in an aquatic habitat a synapomorphy for the taxa in Pontederiaceae? Of the taxa that are closely related to Pontederiaceae, the two families that constitute its sister-group (Haemodoraceae and Commelinaceae; see above) are almost exclusively terrestrial. For each of these families, it is almost certain that the primitive forms were adapted to a completely terrestrial existence (see above). However, the next most closely related clade (Philydraceae) is semi-aquatic. Whether the aquatic habit is homologous in Philydraceae and Pontederiaceae depends partly on the distribution of aquatic versus terrestrial taxa in clades more distant to Pontederiaceae than its sister-group and Philydraceae. If we assume that these more distant taxa are exclusively terrestrial, then the parsimony criterion indicates that the aquatic habit either arose independently in the two families, or it arose prior to the origin of

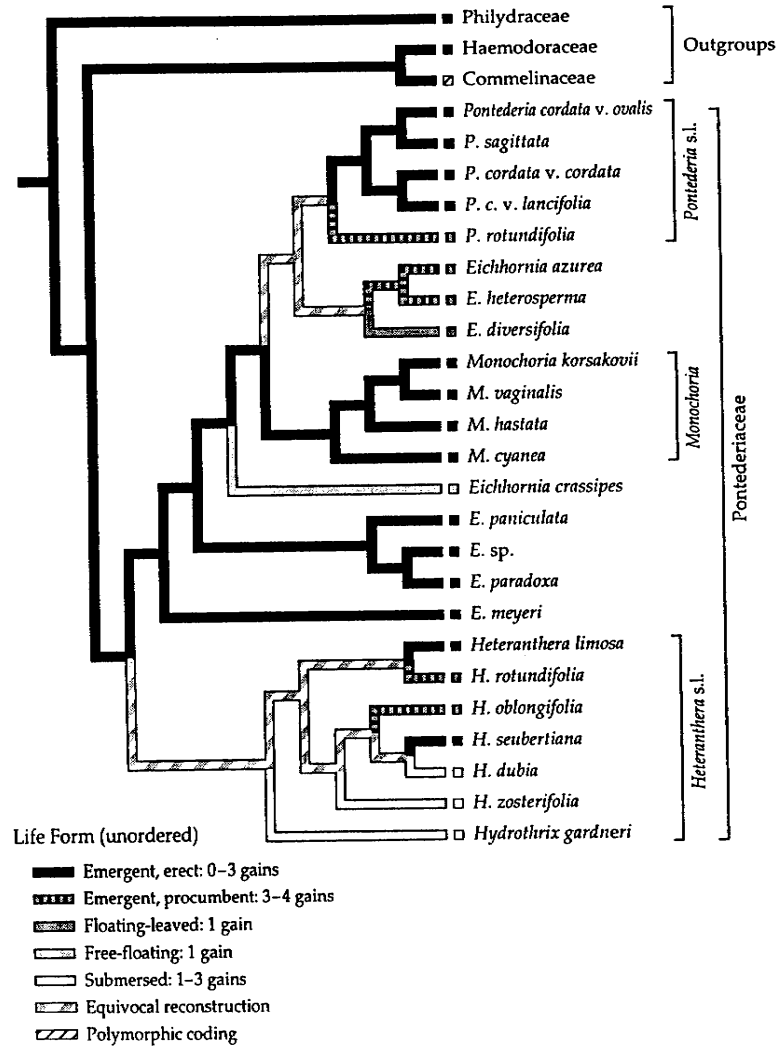


Figure 7.4. Reconstruction of diversification in aquatic life-form in Pontederiaceae and its closest relatives. The ingroup tree is one of four most parsimonious trees found using the combined chloroplast evidence from *rbcL*, *ndhF*, and a survey of restriction-site variation. The root of the ingroup tree is that indicated by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets. See text for a description of the character-states and codings. Commelinaceae was coded as polymorphic. The reconstructed numbers of gains in each character-state are indicated in the legend. They refer to changes within Pontederiaceae (not the whole tree). These values were the same across all four shortest trees.

Commelinaceae, Haemodoraceae, Philydraceae, and Pontederiaceae, with a subsequent loss prior to the split of Commelinaceae and Haemodoraceae. If more distant clades than Philydraceae are aquatic, then homology of the aquatic habit between the two families is more parsimonious than non-homology. In either case, homology of the aquatic habit between Philydraceae and Pontederiaceae is a definite possibility. However, aquatic adaptations in Pontederiaceae are much more complete and diverse than in Philydraceae, indicating that the majority of the character diversification constituting this radiation has taken place in this family alone.

LIFE-FORM – An emergent, erect habit is reconstructed as the primitive condition in Pontederiaceae for all four shortest trees (see the branch that connects the outgroup taxa to Pontederiaceae; Figure 7.4). The free-floating form typical of *Eichhornia crassipes* arose directly from the emergent, erect habit, and the floating-leaved form arose from an emergent, erect or an emergent, procumbent form. In *Eichhornia* and *Pontederia* the emergent, procumbent form arose from an emergent, erect form on one or two occasions, although an origin of the former condition from a floating-leaved form in *Eichhornia* is also possible. Surprisingly, there was no phylogenetic record of any transitional forms between the emergent, erect, and submersed life-forms in *Heteranthera*. There were up to three independent origins of the submersed life-form from the emergent, erect form in this genus. There were no parsimonious reconstructions for any of the trees in which the emergent, procumbent habit was homologous between *Heteranthera* versus *Pontederia* and *Eichhornia* (Figure 7.4). There were two independent origins of this life-form in *Heteranthera* s. lat. under all reconstructions, and up to two independent origins of this form in species of *Pontederia* and *Eichhornia*. Under some of the most parsimonious reconstructions, the emergent, erect life-form within *Heteranthera* and *Pontederia* represented a reversion from a submersed or emergent, procumbent form. The emergent, erect habit in *Heteranthera* is thus potentially not homologous with the occurrence of this life-form outside the genus (Figure 7.4).

A number of taxa that are currently missing from the phylogenetic estimate of Pontederiaceae are likely to make an impact on future reconstructions of this character. These include several emergent, procumbent taxa in *Heteranthera* (*H. reniformis* and allies) and two emergent, erect species (*Heteranthera spicata* and *H. mexicana*). One missing species with a floating-leaved life-form is *Scholleropsis lutea*. This species probably belongs in *Heteranthera* s. lat. (Eckenwalder and Barrett 1986) and consequently may well represent an additional origin of the floating-leaved form in the family. Inclusion of these taxa may also indicate that some or all of the submersed taxa in *Heteranthera* did not arise directly from emergent, erect forms, a finding that in any case seems to us to be biologically implausible. The other missing floating-leaved form is *Eichhornia natans*, but as this species appears to be very closely related to *E. diversifolia* (Verdcourt 1968; Eckenwalder and Barrett 1986), it probably does not represent a novel origin of this life-form.

LIFE-CYCLE DURATION – Despite uncertainty at the base of the tree, with a variety of most parsimonious reconstructions of shifts in life history among the outgroup taxa, an annual life history was reconstructed as the primitive condition of Pontederiaceae for all shortest trees (Figure 7.5). Under all most parsimonious reconstructions on

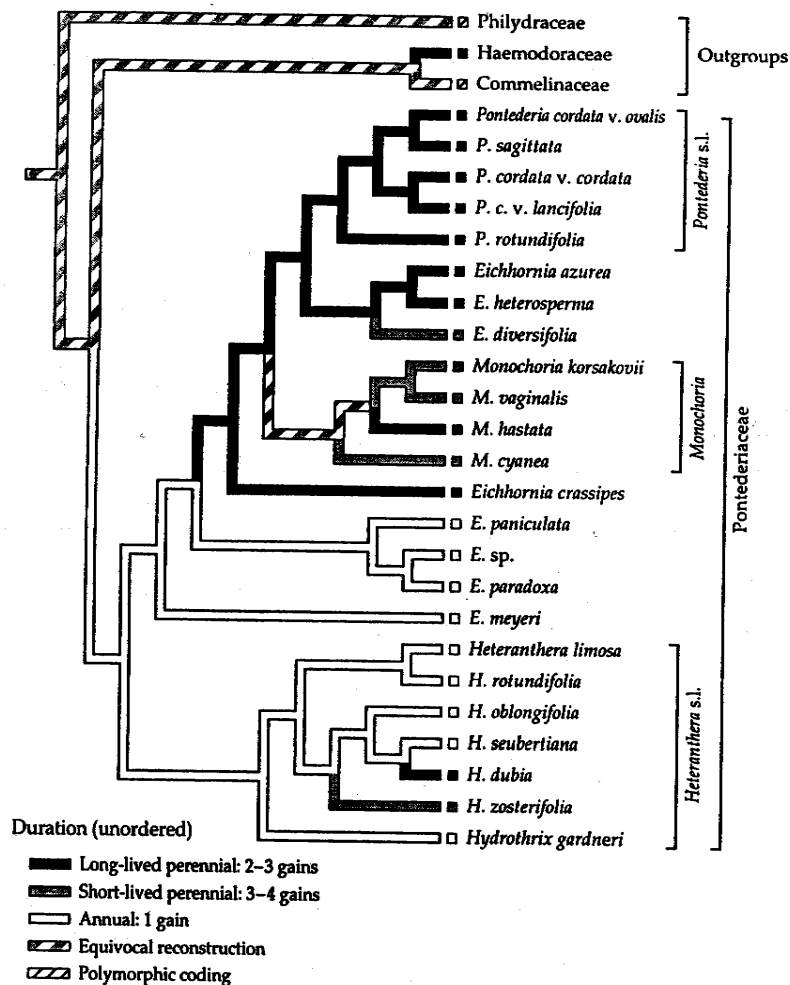


Figure 7.5. Reconstruction of diversification in life-cycle duration in Pontederiaceae and its closest relatives. The ingroup tree is one of four most parsimonious trees found using the combined chloroplast evidence from *rbcL*, *ndhF*, and a survey of restriction-site variation. The root of the ingroup tree is that indicated by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets. See text for a description of the character-states and codings. Commelinaceae and Philydraceae were coded as polymorphic. The reconstructed numbers of gains in each character-state are indicated in the legend, and refer to changes within Pontederiaceae. These values were the same across all four shortest trees.

these trees, the annual life history was homologous for all species exhibiting this life history. It should be borne in mind, however, that many of the species that we coded as annual are capable of growing as short-lived perennials and are therefore "facultative" annuals. In contrast, species coded as short-lived perennials are incapable of an annual existence. Most workers assume that in herbaceous groups annuals are derived from perennials (Stebbins 1974), a shift in life history that has normally been invoked in the context of adaptive radiations from mesic to arid environments. However the reverse shifts may well have occurred in Pontederiaceae during invasion of permanent aquatic habitats. Such environments require specialized adaptations and may have represented relatively unsaturated niches. Under these circumstances, lack of species competition could have aided an evolutionary transition that for terrestrial groups occurs less frequently, a situation that may be analogous to the evolution of perenniality in island floras (see Carlquist 1974).

Long-lived perennials arose two or three times in the family, and long-lived perenniality in *Heteranthera dubia* was not homologous with other species in the family for any most parsimonious reconstruction. Instances of long- and short-lived perenniality in *Monochoria* were homologous with the occurrences of these forms in *Eichhornia* and *Pontederia* in some reconstructions, but not in others (Figure 7.5). Short-lived perenniality in *E. diversifolia* and *H. zosterifolia* arose uniquely in the terminal lineages leading to these species. Missing perennial taxa from this study include *H. reniformis* and its allies in *Heteranthera*, and several species of *Monochoria* and *Pontederia* s. lat.

CLONALITY – A non-clonal form is reconstructed as the most primitive condition in Pontederiaceae. Vegetative reproduction via stolons (typical only of *Eichhornia crassipes*) arose directly from this form, as did the instance of clonal reproduction via rhizomes in *Monochoria hastata*, which consequently must have arisen independently from the other instances of this clonal form in *Pontederia*. Species of *Pontederia* and *Eichhornia* that express clonality via stem fragmentation can also clone via rhizomes (we scored them using the former coding only), so the question of the number of origins or interconversions between these two clonality modes in the local part of the tree containing these taxa is somewhat moot. Examination of the most parsimonious reconstructions on the four shortest trees indicates that clonality via stem fragmentation arose independently in *Heteranthera* versus *Pontederia* and *Eichhornia*, and may have arisen from one to three times (Figure 7.6) in the former genus. Several missing taxa of Pontederiaceae in this study that are clonal include *H. reniformis* and its allies (which all express clonality via stem fragmentation) and several species in *Pontederia* s. lat. that can reproduce via rhizomes or stem fragmentation. The only taxa to reproduce via stem fragmentation are those with a growth form that could broadly be described as procumbent (emergent, submersed, or floating-leaved).

LEAF DEVELOPMENTAL PATHWAY – Pattern A, with a rapid transition to adult leaves, is the primitive form in the family, although it is possible that it also arose once by reversion from pattern C or D in the terminal lineage leading to *Heteranthera oblongifolia* (Figure 7.7). Pattern B, in which the production of petiolate leaves is more delayed than in pattern A, evolved once from pattern A in *Eichhornia*, supporting the idea that this represents a neotenic shift. The suggestion that the other two patterns (C and D,

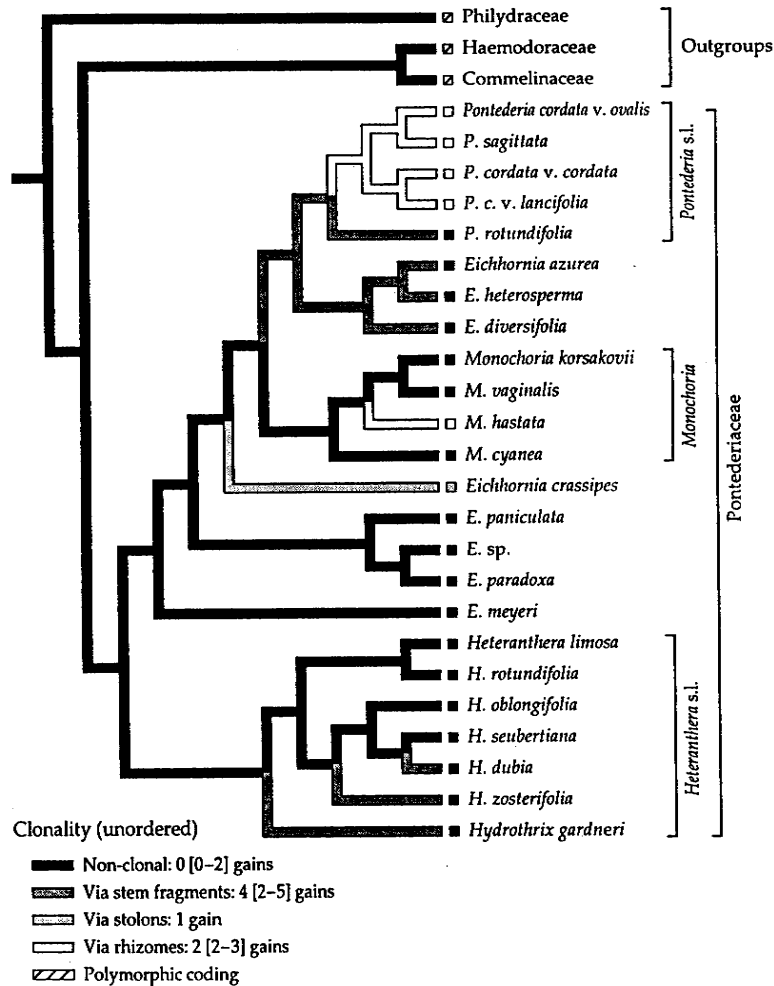


Figure 7.6. Reconstruction of diversification in clonality in Pontederiaceae and its closest relatives. The ingroup tree is one of four most parsimonious trees found using the combined chloroplast evidence from *rbcl*, *ndhF*, and a survey of restriction-site variation. The root of the ingroup tree is that indicated by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets. See text for a description of the character-states and codings. The three outgroup taxa were coded as polymorphic. The reconstructed numbers of gains in each character-state are indicated in the legend. They refer to changes within Pontederiaceae. Where numbers of gains differ across the four shortest trees, this is indicated in square brackets.

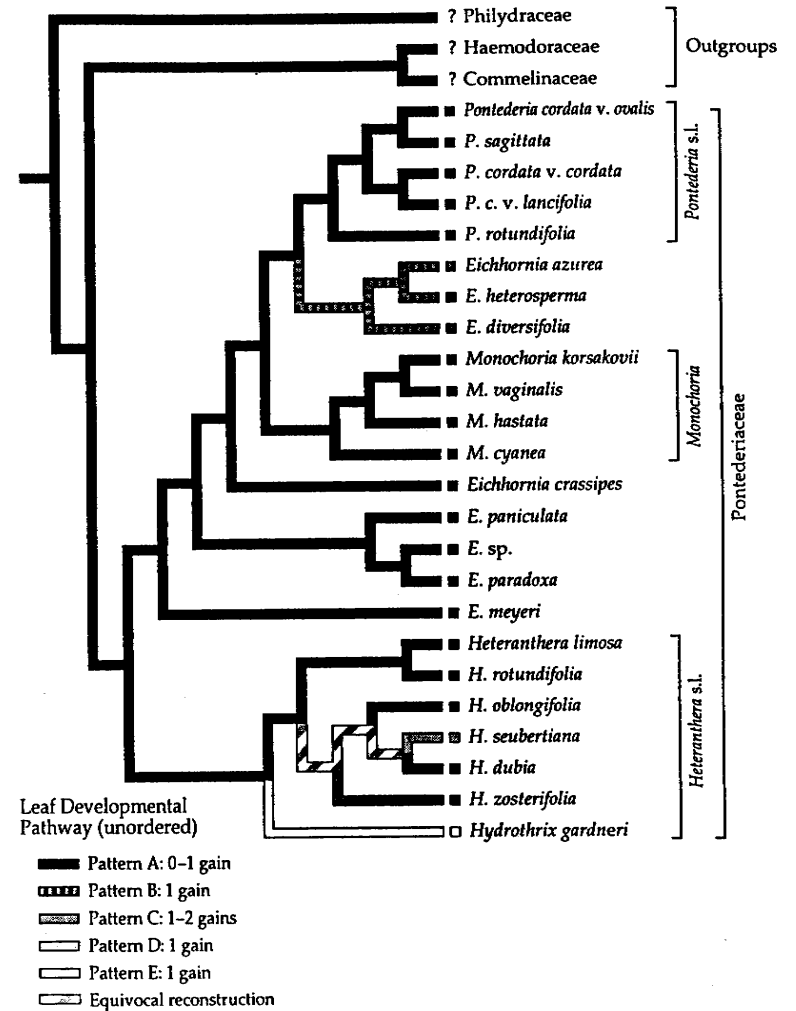


Figure 7.7. Reconstruction of diversification in leaf developmental pathway in Pontederiaceae and its closest relatives. The ingroup tree is one of four most parsimonious trees found using the combined chloroplast evidence from *rbcl*, *ndhF*, and a survey of restriction-site variation. See text for a description of the character-states and codings. The root of the ingroup tree is that indicated by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets. The three outgroup taxa were coded as "unknown" (missing data) for this character (see text). The reconstructed numbers of gains in each character-state are indicated in the legend. They refer to changes within Pontederiaceae (not the whole tree). These values were the same across all four shortest trees.

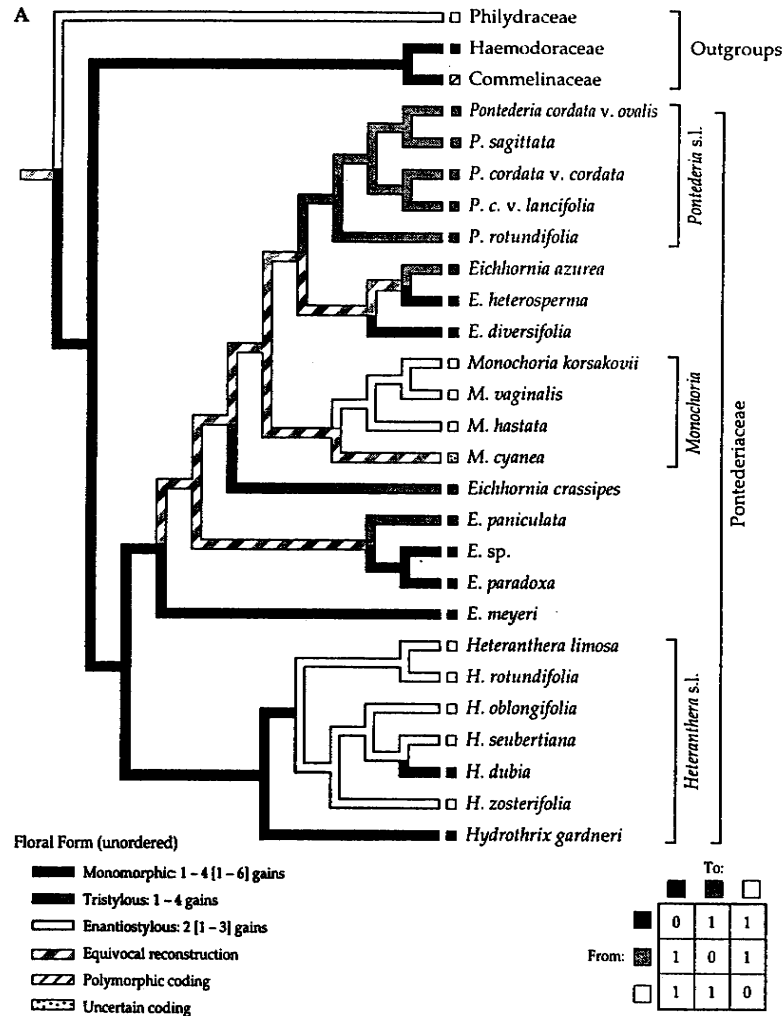
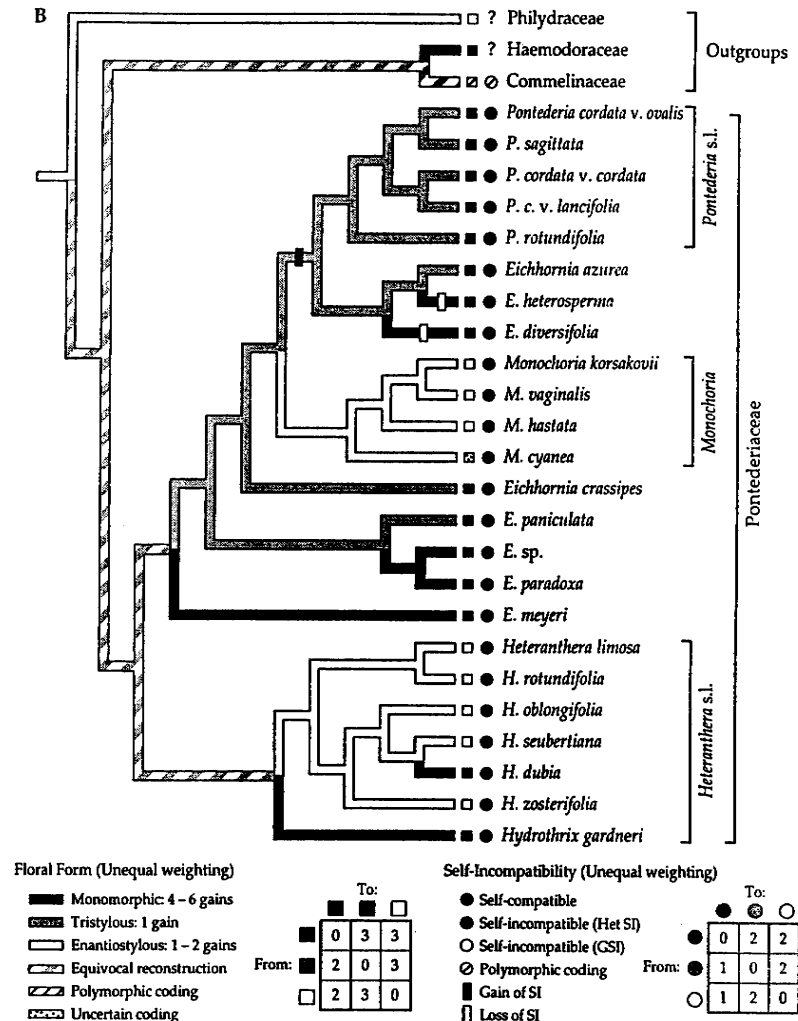


Figure 7.8. Reconstruction of diversification in floral form in Pontederiaceae and its closest relatives (Comelinaceae, Haemodoraceae, and Philydraceae). The ingroup tree is one of four most parsimonious trees found using the combined chloroplast evidence from *rbcL*, *ndhF*, and a survey of restriction-site variation. The root of the ingroup tree is that indicated by a variety of closely related taxa using the two chloroplast DNA sequence-based data sets. *Monochoria cyanea* was coded as "enantistylous or monomorphic," and Commelinaceae was coded as polymorphic for this character (see text for descriptions of the character-states and codings). The reconstructed numbers of gains in each character-state are indicated in the legend. They refer to changes within Pontederiaceae (not the whole tree). Where numbers of gains differ



across the four shortest trees, this is indicated in square brackets. (A) Reconstructions of shifts in floral form, performed using Fitch optimization. (B) Reconstructions of shifts in floral form and self-incompatibility (SI) status performed using a "relaxed Dollo" weighting scheme described in the step matrix below the tree. Gains of either polymorphic form (tristyly or enantistylous) were coded more heavily (3:2 weighting, see step matrix) than reversions to floral monomorphism. Gains of SI (solid bars) were coded more heavily (2:1, ACCTRAN optimization, see step matrix) than reversions to self-compatibility (hollow bars). CSI = gametophytic self-incompatibility; Het SI = heteromorphic, sporophytic self-incompatibility; see section on character codings.

which possess adult leaves resembling the juvenile leaves of pattern A) arose by various pedomorphic processes was supported by only some of the reconstructions on the shortest trees. A variety of shifts between patterns A, C, D, and E were seen in different most parsimonious reconstructions on the four shortest trees: pattern D arose from patterns A and C in different reconstructions, pattern C arose from patterns A, D, or E (with either one or two origins), and the leaf form unique to *Hydrothrix gardneri* (pattern E, with whorls of highly reduced leaves) could have arisen from patterns A or C on some shortest trees. To our knowledge, all of the taxa missing from the current study exhibit pattern A, apart from *Heteranthera mexicana* (pattern D) and *E. natans* (pattern B; probably homologous with the other three instances of this pattern). An increased sampling of taxa within *Heteranthera* s. lat. would be valuable for obtaining a less equivocal reconstruction of the evolution of patterns C to E, since so much of the variation in leaf developmental pathway is in this genus.

FLORAL FORM AND SELF-INCOMPATIBILITY – We considered two types of evolutionary schemes for the shifts in floral form and self-incompatibility status: (i) Fitch optimization, in which all character-state shifts are equiprobable and there is no implied order of change; and (ii) “relaxed Dollo” schemes that weight against the origin of self-incompatibility and the two polymorphic floral forms in the family, enantiostyly and tristily. A large body of comparative and microevolutionary evidence indicates that evolutionary gains of tristily are much more difficult than their loss (reviewed in Graham and Barrett 1995; Kohn et al. 1996), and it seems likely that the same is also true for self-incompatibility systems. Schemes that disfavor the origin of such complex characters are probably more biologically and historically accurate than those that weight all character-state shifts equally.

The choice of weighting scheme can have a profound influence on reconstructions of character evolution (compare Figures 7.8A,B). The scheme that weights all character shifts equally (Fitch optimization) indicates a wide possible range of gains in each floral form, including up to four origins of tristily (Figure 7.8A). Not surprisingly, the scheme that weights gains of the floral polymorphisms more heavily than their reversion to monomorphism indicates a single origin of tristily, up to two origins of enantiostyly, and multiple (four to six) origins of floral monomorphism in the family (Figure 7.8B). Enantiostylous flowers in *Monochoria* and *Heteranthera* were not homologous under either scheme, for any of the most parsimonious reconstructions on the shortest trees. Floral monomorphism and enantiostyly were the primitive floral forms in the family in different most parsimonious reconstructions (using the Fitch or relaxed Dollo optimization schemes). Of course, the precise weights that correspond to the actual probabilities of change in these floral forms are unknown, but only very small weighting biases (around 3:2 to 2:1; Graham and Barrett 1995; Kohn et al. 1996) were required to reconstruct a single origin of tristily in the family.

The reconstructed shifts in self-incompatibility (SI) status indicates that it arose at most twice in the clade consisting of *Pontederia* s. lat. and the several species of *Eichhornia* associated with *E. azurea*. One most parsimonious reconstruction is shown in Figure 7.8B under a small weighting bias. Under equal weighting (Fitch optimization), or unequal weighting (2:1 bias against the origin of SI with DELTRAN opti-

mization), two origins of SI are indicated on the tree (see Kohn et al. 1996). Under unequal weighting (2:1 bias with ACCTRAN optimization), a single origin and two losses of SI are implied (Figure 7.8B, and see Kohn et al. 1996). In either case, SI must have arisen subsequent to the origin of tristily, given reconstructions of floral form that indicate a single origin of tristily.

This evolutionary sequence casts doubt on models of the evolutionary origin of heterostyly (Charlesworth and Charlesworth 1979; Charlesworth 1979) in which an origin of self-incompatibility is required prior to the origin of floral heteromorphisms (Graham and Barrett 1995; Kohn et al. 1996). Floral shifts between the three floral types are undoubtedly associated with shifts in pollination mode. Tristylous species of *Eichhornia* and *Pontederia* are predominantly pollinated by nectar-collecting bees, enantiostylous species of *Monochoria* and *Heteranthera* are pollinated by pollen-collecting bees, and monomorphic species throughout the family are predominantly self-pollinating (see above). The reconstructions also indicate that at least some predominantly selfing lineages of *Eichhornia* have existed for substantial evolutionary periods and were even capable of speciation (see also Kohn et al. 1996), a finding at odds with Stebbins' (1957) view that selfing species are evolutionary dead ends. Missing taxa that could influence reconstructions of character evolution include *Eichhornia natans* and *Pontederia parviflora* (both monomorphic and presumably self-compatible).

Conclusions

There is substantial ecological evidence that a number of life-history traits in Pontederiaceae are involved in or affected by an aquatic existence, and that a variety of reproductive characters have undergone diversification in response to shifts in their mode of pollination. We used currently available molecular evidence of phylogenetic relationships within this family to reconstruct patterns of character diversification associated with adaptive radiations in vegetative and floral characters. Shifts in pollination mode, particularly those resulting in predominant self-pollination, occurred on multiple occasions in the family. Vegetative characters in the family are particularly prone to convergence (see also Eckenwalder and Barrett 1986). The extensive homoplasy in vegetative characters suggests that aquatic habitats are far from ecologically uniform, as has often been supposed. As with all phylogenetic analyses, these findings are liable to new interpretations when more taxa are sampled inside and outside the family, and when new sources of phylogenetic evidence become available. More detailed developmental and ecological work is also needed to determine how plastic some of our character classes are in different taxa. Several of the reconstructions of character diversification in our studies of Pontederiaceae challenge widely held views on the course of plant evolution. These include the shift from the annual to the perennial habit, the evolutionary longevity of some predominantly selfing lineages and the sequence in which morphological and physiological traits became associated in the heterostylous syndrome. In challenging these orthodox views, we hope that our analyses may help to provoke future research on these topics in Pontederiaceae and other aquatic plant families.

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