

**Monocotyledons:
Systematics and Evolution**

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**PHYLOGENETIC SYSTEMATICS OF PONTEDERIALES:
IMPLICATIONS FOR BREEDING-SYSTEM EVOLUTION**

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Abstract

Using currently available morphological- and molecular-phylogenetic evidence, we discuss the systematics of Pontederiales and the evolution of breeding systems in this monofamilial order. Data from the chloroplast gene *rbcL* indicate affinities with other orders among Commelinanae, Bromelianae and Zingiberanae, but are shown to be of limited value in determining precise sister-group relationships. Morphological and molecular evidence concerning monophyly of genera within Pontederiaceae are summarised. Both lines of evidence indicate that *Eichhornia* is an unnatural genus as currently circumscribed. In contrast with evidence from morphology, the molecular evidence indicates multiple losses of the sexual polymorphism tristylous within *Eichhornia*. The single loss implied in the morphological analysis is probably a consequence of the confounding effects of a 'selfing syndrome' on phylogenetic reconstruction. The significance of breeding-system variation and evolution in tristylous, homostylous and enantiostylous taxa of Pontederiales is discussed in a broader evolutionary and phylogenetic context.

Introduction

Pontederiales (*sensu* Dahlgren & Clifford, 1982) is a monofamilial order of monocotyledons. The family Pontederiaceae is composed of six to nine genera and about 35 to 40 species of freshwater aquatics, the majority of which are native to the Neotropics. Members of the family are most readily distinguished by a sympodial growth pattern, herbaceous stems with sheathing leaf bases and petiolate leaves, often multi-flowered showy inflorescences subtended by a single bract, six petaloid tepals (blue, mauve, yellow or white) which are variously basally connate and in two series of three, variously dimorphic stamens which are adnate to the perianth, and superior ovaries with a single style. While plants are rarely misclassified as to family, there have been a variety of opinions concerning the local placement of Pontederiaceae within the monocotyledons. The family has been allied with a number of families in a variety of combinations (reviewed in Dahlgren and Clifford, 1982; Dahlgren *et al.*, 1985; Simpson, 1987; Rosatti, 1987; Goldberg, 1989). Recent treatments suggest a close affinity of the family with Haemodoraceae and Philydraceae (e.g., Hamann, 1966; Huber, 1969, 1977; Simpson, 1990; Thorne, 1992a, 1992b).

Adaptive radiation to the multitude of ecological niches associated with aquatic environments has given rise to a diversity of life-histories and reproductive systems among members of Pontederiaceae (e.g. Barrett, 1988). Life-history variation is governed largely by the duration, predictability and depth of flooding. Annual life-histories are characteristic of

ephemeral habitats, while perenniality is more commonly found associated with permanent water bodies. Annual species are largely self-pollinating, whereas perennial species are more frequently insect-pollinated and outbreeding. The reproductive ecology of populations is thus closely linked to their life-histories.

Of particular interest to evolutionary biologists is the occurrence of tristylly in the family. This sexual polymorphism has evolved on only a handful of occasions in the angiosperms (Charlesworth, 1979; Barrett, 1993), and its origin and adaptive significance are still the subject of debate. Tristylous breeding systems appear to be particularly susceptible to evolutionary modification, giving rise to a range of derivative conditions, particularly involving autogamy (Ganders, 1979; Weller, 1992). The occurrence of variation in breeding systems and life-histories among members of Pontederiaceae provides opportunities for application of the comparative method for analysing character evolution and the origin of adaptations (Brooks and McLennan, 1991; Harvey and Pagel, 1991). Such approaches, however, are contingent upon the availability of sound phylogenetic information. A major objective of this review is therefore to evaluate current evidence provided by morphological and molecular data concerning the phylogenetic relationships of taxa within Pontederiaceae and its closest relatives.

This review has two major sections. Using available morphological and molecular evidence, we begin by evaluating contrasting schemes concerning the affinities of the family with other monocotyledonous taxa and provide a brief description of the systematic features of Pontederiaceae and its constituent genera. We then discuss molecular evidence concerning the relationships of taxa within the family and use this evidence to examine the major pathways of breeding-system evolution; in particular the evolutionary build-up and breakdown of tristylly. We also demonstrate how convergent floral evolution associated with multiple shifts from outbreeding to inbreeding can be difficult to detect when only morphological data are available for phylogenetic reconstruction. Finally, we discuss the evolutionary significance of the enantiostylous floral form in this and other orders.

Suprafamilial Systematics

A. Morphological Evidence

Dahlgren and Rasmussen (1983) used a cladistic approach in their morphologically-based study of suprafamilial systematics in the monocotyledons. Except for Zingiberales, they did not attempt intensive cladistic analyses. They presented what they felt were probable phylogenetic arrangements of taxonomic units. Dahlgren and Clifford (1982) and Dahlgren and Rasmussen (1983) discussed a range of morphological characters within the monocotyledons and provided argumentation concerning plesiomorphic *versus* apomorphic conditions. The degree of support for their phylogenetic groupings varied in terms of the number and quality of their proposed synapomorphies. Their study provides a useful framework for discussing morphological evidence concerning monocotyledon systematics. We discuss their phylogenetic groupings below with special reference to the local placement of Pontederiales. We employ the superordinal ending '-anae' throughout the discussion.

Dahlgren and Rasmussen (1983) proposed a major clade within the monocotyledons consisting of the members of Commelinanae, Zingiberanae and Bromelianae (and possibly Arecanae) based on three proposed synapomorphies; UV-fluorescent cell walls, copiously starchy endosperm, and the *Strelitzia*-type of epicuticular wax. The *Strelitzia*-type of epicuticular wax has a scattered occurrence throughout the Commelinanae-Zingiberanae-Bromelianae complex and is also present in Arecanae. Copiously starchy endosperm is present in other monocotyledons but its occurrence is concentrated and probably synapomorphic within the complex. The possession of UV-fluorescent cell walls is a highly

consistent feature of this complex and is also present in Arecanae. Their superorder Bromelianae includes Pontederiales, which although it lacks the *Strelitzia*-type of epicuticular wax, has UV-fluorescent cell walls (Harris and Hartley, 1980) and a starchy endosperm (Dahlgren and Clifford, 1982).

The further sub-clades within this complex that include Pontederiales were solely defined on the basis of single synapomorphies (Dahlgren and Rasmussen, 1983). A number of exceptions and ambiguities weaken Dahlgren and Rasmussen's argumentation concerning relationships among the orders. A Zingiberanae-Bromelianae complex was defined on the basis of a single synapomorphy; the possession of a showy petaloid perianth. The utility of this character in delimiting this group is somewhat dubious given the existence of showy petaloid tepals within Commelinanae. The superorder Bromelianae was further defined on the basis of a single synapomorphy; possession of helobial endosperm with a small, starch-free, and sometimes haustorial chalazal chamber. A further sub-clade within their Bromelianae consisting of the orders Pontederiales, Haemodoraes, Philydrales and Typhales was also defined by one synapomorphy; the possession of distichous leaves. However, this condition also has a widespread distribution in Commelinanae and Zingiberanae and has variable expression within Pontederiales, where the more broad-leaved taxa tend to have spiral phyllotaxy. Possession of an amoeboid tapetum was used to define a group consisting of Pontederiales, Haemodoraes and Typhales (Philydrales has a glandular-secretory type tapetum). The precise status of the tapetum in Pontederiaceae is, however, uncertain (Dahlgren *et al.*, 1985).

Earlier treatments of Pontederiaceae, Haemodoraes and Philydraceae emphasised the liliaceous character of these families (Takhtajan, 1969; Dahlgren, 1975; Dahlgren and Clifford, 1982; Cronquist, 1988). Dahlgren and Clifford's study listed eleven (*versus* four) attributes that reflected the stronger liliaceous than commelinaceous character of Pontederiaceae. However, five of the liliaceous characters mentioned (sulcate pollen grains, presence of oxalate raphides, several to many ovules, axile placentation and dehiscent fruit) were later considered by Dahlgren *et al.* (1985) to be primitive within the monocotyledons and therefore cannot be used as an indication of phylogenetic affinity.

The possession of oligosulcate pollen (disulcate pollen in Simpson, 1987) and girdle-type endothelial thickening were considered by Dahlgren and Rasmussen to constitute synapomorphies of taxa in Pontederiales. Both are apomorphic conditions within the monocotyledons (Dahlgren and Rasmussen, 1983). Other features of Pontederiales which may constitute apomorphies for the order include its aquatic habit (but note that Philydrales also inhabits semi-aquatic habitats), possession of petiolate leaves with stipule- or ligule-like structures, possession of hairs on the stamen filaments (Dahlgren and Clifford, 1982), and possibly also their bifacial leaf anatomy, which Simpson (1990) suggested may be secondarily derived from a unifacial form. None of these features are unique to Pontederiales but may still represent synapomorphies of the order if their occurrence in other groups is found to be homoplasious.

Several characters shared among Pontederiales, Haemodoraes and Philydrales may constitute synapomorphies for a clade consisting of these three orders. Of the taxa he investigated palynologically, Simpson (1987) proposed that similarities in pollen exine sculpturing and architecture between Haemodoraes and Pontederiales constitute synapomorphies of these two orders. Dimorphic stamens are present in some members of Haemodoraes and most Pontederiales. However, only a single stamen is found in Philydrales. Possible synapomorphies of the three orders include possession of placental sclereids, perianth tannin cells (Simpson, 1990) and a form of herkogamy (the spatial separation of stigmas and anthers within a flower) known as enantiostyly, where flowers possess either right- or left-bending styles. Enantiostyly is present in all four genera of Philydraceae, in most genera of the tribe Haemodoraes of Haemodoraes (Simpson, 1990)

and in two of the four main genera (*Monochoria* and *Heteranthera*) of Pontederiaceae (Eckenwalder and Barrett, 1986). These latter characters in particular require further investigation in closely related orders to determine if they represent evidence for monophyly or are instead retained plesiomorphies. For example, it is unclear how widespread enantiostyly is in other orders of monocotyledons -- the presence and type of herkogamy are not regularly recorded in taxonomic descriptions (Webb and Lloyd, 1986). Enantiostyly is reported in Tecophilaeaceae (Dulberger and Ornduff, 1980) and appears to be present in some species of *Aneilema* (Commelinaceae) (Faden, 1991).

B. Molecular Evidence

Chase *et al.* (1993) used the chloroplast gene *rbcL* to investigate phylogenetic relationships within the seed plants. Their study included a broad range of monocotyledons (see their Figs 5 and 6). Here we further analyse evidence from this molecule concerning monocotyledon relationships and attempt to measure the degree of compatibility of this evidence with several recent suprafamilial taxonomical treatments.

Phylogenies were reconstructed using sequence data from 88 monocotyledon taxa: 85 from the study of Chase *et al.*, and an additional three from Pontederiaceae (S.W. Graham, B.R. Morton and S.C.H. Barrett, unpubl. data). All analyses were performed using PAUP version 3.1.1 (Swofford, 1993). A two-tier heuristic search strategy was used during each analysis. NNI (nearest-neighbour interchange) branch swapping was used, with twenty five random-addition replicates employed to help uncover further islands of parsimony (Maddison, 1991). The shortest trees found with these searches were then used as the starting point for a second round of searching using TBR (tree bisection-reconnection) branch-swapping. MULPARS and STEEPEST DESCENT options were activated in both tiers of searching. Analyses were performed both with and without topological constraints imposed on the search process. Topological constraints were defined using the treatments of Dahlgren *et al.* (1985), Cronquist (1988), and Thorne (1992b), under the assumption that taxonomical units therein represent monophyletic groups. Sequences from the taxa were constrained both by superorder and order (by subclass and order for Cronquist, 1988). Thorne (1992b) treated *Acorus* as a taxon of uncertain affinity. However, it was not possible to fully 'unconstrain' the phylogenetic position of the *rbcL* sequence of this species for the Thorne analysis. Instead, the constraints employed allow it to freely associate with other taxa only at the superordinal level. Thorne's (1992b) Philydrales (i.e., Pontederiaceae, Haemodoraceae and Philydraceae) and Dahlgren and Rasmussen's (1983) Bromelianae (their Bromeliiflorae) were both used to delimit topological constraints for separate analyses involving these as the sole constraints. In the case of the Bromelianae constraint set, additional topological structure was imposed based upon the cladistic arrangement of the orders within this superorder (i.e., Typhales, Velloziales, Bromeliales, Pontederiales, Haemodorales and Philydrales) proposed by Dahlgren and Rasmussen (1983). The monocotyledon portion of the cladogram presented from search 2 of the study of Chase *et al.* (see their Figures 5B and 6B) was also reconstructed using MacClade version 3.0 (Maddison and Maddison, 1992) in order to derive tree statistics for purposes of comparison with the searches performed here. The few taxa not shared between studies were cut from the Chase *et al.* tree, except that the three additional sequences from Pontederiaceae were added onto the terminal branch leading to *Pontederia sagittata* in the order found in the unconstrained analysis. A bootstrap analysis was also performed to determine the relative robustness of clades in the unconstrained analysis.

Table 1 lists tree statistics resulting from the various analyses. The degree of incongruence between molecular- and taxonomically-based treatments of monocotyledon affinities was taken as the increase in the number of steps and amount of homoplasy (as measured by CI and RI statistics; Table 1) of shortest trees found in analyses employing the constraint sets, as compared to those found in the unconstrained analysis. It is not possible to represent any

TABLE 1. Shortest trees found in the analyses of 88 monocotyledon taxa using constrained and unconstrained heuristic searches. (see text for further details).

Analysis	Number of steps	Consistency Index ¹	Retention Index	Number of trees found	Sister group to Pontederiaceae
Chase <i>et al.</i> (1993) ²	3205	0.285	0.572	(1) ²	Commelinaceae
Unconstrained ³	3194	0.286	0.575	64	Comelinaceae
Constrained ³ :					
Cronquist (1988)	3332	0.273	0.547	192	Velloziaceae
Dahlgren <i>et al.</i> (1985)	3288	0.277	0.556	24	Velloziaceae
Thorne (1992b)	3278	0.278	0.558	194	unresolved ^{4,5}
Bromelianae (Dahlgren & Rasmussen, 1983)	3231	0.282	0.567	40	Haemodoraceae ⁵
Philydrales (Thorne, 1992b)	3198	0.285	0.574	320	Philydraceae ⁵
'Not local'	3197	0.285	0.574	120	Velloziaceae

¹Excluding uninformative characters. ²Portion of the single tree shown in Chase *et al.* (1993). ³See text. ⁴Unresolved trichotomy between Pontederiaceae, Haemodoraceae and Philydraceae. ⁵Highly constrained local topology.

non-explicit taxonomical concepts of 'affinity' between or within groups, such as the relative location of groups depicted in 'Dahlgrenograms'. It should also be noted that this analysis does not determine which subsets of groups within constrained sets are relatively more incongruous with the historical signal present in molecular data, since it only compares the gross schemes.

The unconstrained analysis resulted in the shortest trees found overall (3194 steps). Imposing topological constraints on the tree-searching algorithm resulted in shortest trees of between 3 steps to 138 steps longer than this (Table 1). The greatest increase in tree length was observed using constraints based on Cronquist's (1988) scheme (4.32% more steps than the shortest unconstrained tree). Constraints based on the schemes of Dahlgren *et al.* (1985), and Thorne (1992b) yielded trees longer than the shortest unconstrained tree by 2.94% and 2.62%, respectively. One of the greatest distinctions between Cronquist's scheme and the others is his Liliidae. This subclass contains a number of families (Haemodorales, Philydraceae and Pontederiaceae) treated quite differently by Thorne and Dahlgren and co-workers. Thorne (1992b) places these families in the order Philydrales of his Commelinanae, a superorder that strongly resembles the Commelinanac-Bromelianac-Zingiberanac complex of Dahlgren *et al.* (1985) in terms of its constituent families. Constraints employed using only the Bromelianac (*sensu* Dahlgren and Rasmussen, 1983) resulted in trees longer than the shortest unconstrained tree by 37 steps (1.15% longer), indicating that there is also some incompatibility between their morphology-based scheme in comparison to the arrangement implied by the *rbcL* data.

Fig. 1 is a portion of the strict consensus tree computed from the 64 shortest trees found in the unconstrained analysis. No outgroup was defined in the analysis, but the tree is presented such that *Acorus* would be placed most basally (not included in the figure). It is notable that this large *rbcL* clade contains taxa only found in the Commelinanac-Bromelianac-Zingiberanac complex of Dahlgren *et al.*, although one member of this complex (Velloziaceae) falls outside this section of the tree. Apart from Commelinales, all orders in this group are monophyletic, at least with respect to the taxa included. Neither Bromelianae nor Commelinanae (*sensu* Dahlgren and Rasmussen, 1983) are monophyletic here. Many of the deeper branches in this section of the monocotyledon tree are unsupported or only moderately supported by the bootstrap analysis. In particular, relationships between the orders are not robust. The arrangement shown here is similar to that depicted in Figs 6A, 6B of Chase *et al.* (1993).

The monophyly of Pontederiaceae is strongly supported (bootstrap proportion, BP = 100%) based on the representative taxa from the four main genera employed in the unconstrained analysis, but the local position of the family within the complex is problematical. Commelinaceae is depicted as the immediate sister group of Pontederiaceae in all the shortest unconstrained trees found in the unconstrained analysis, but this association was not supported by the bootstrap analysis (BP < 50%). The shortest 192 trees from the NNI tier of the unconstrained search were one step longer than those found after TBR branch swapping. In all of these trees the clade consisting of Pontederiaceae and Commelinaceae was transposed relative to that illustrated in Fig. 1 such that it constituted the immediate sister-group of *Anigozanthos* (Haemodoraceae). Furthermore, the constraint set that denied sister group status to Commelinaceae, by enforcing a monophyletic clade uniting Haemodoraceae, Philydraceae and Pontederiaceae (Philydrales *sensu* Thorne, 1992b), resulted in shortest trees only four steps (0.13%) longer than the shortest unconstrained trees. Trees not uniting Commelinaceae and Pontederiaceae were thus not substantially longer than the shortest unconstrained trees. An additional constraint analysis was performed to examine the robustness of the membership of Pontederiaceae in the Commelinanac-Bromelianac-Zingiberanac complex. This analysis enforced a clade consisting of the taxa found in the portion of the monocotyledon tree shown in Fig. 1, corresponding to this complex, with the

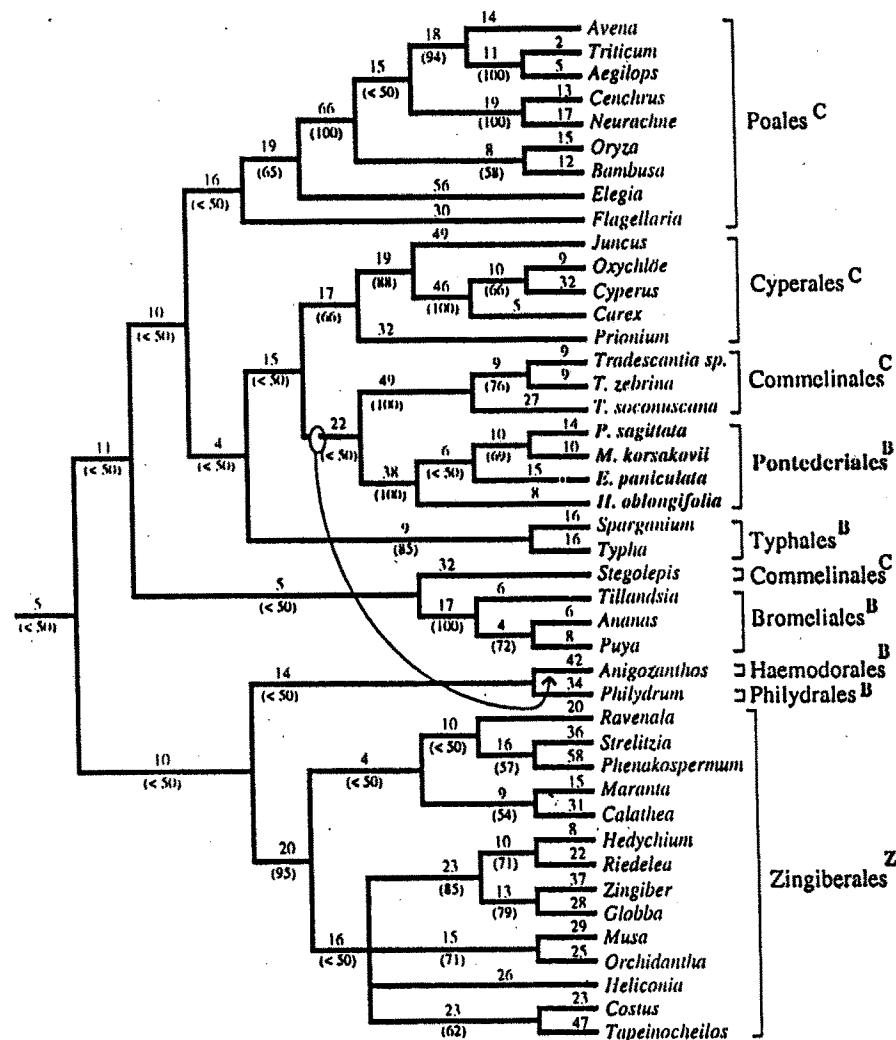


FIG. 1. A portion of a strict consensus of the 64 shortest trees found in the unconstrained analysis of *rbcL* sequences from 88 monocotyledons. In terms of its constituent families, this clade strongly resembles Thorne's Commelinanae (1992a, 1992b) and the Commelinanac-Bromelianac-Zingiberanac complex of Dahlgren *et al.* (1985). Branch lengths, as projected onto the consensus tree (using ACCTRAN optimisation), are indicated above branches. Bootstrap proportions (the percentage of bootstrap replicates supporting each branch from 110 replicates) are indicated in parentheses below branches. C = Commelinanae, B = Bromelianae, Z = Zingiberanae (after Dahlgren *et al.*, 1985). The arrow indicates an arrangement of Pontederiaceae-Commelinaceae observed in trees one step longer than the shortest trees (see text). Representatives of Pontederiales are: *Eichhornia paniculata*, *Pontederia sagittata*, *Monochoria korsakovii* and *Heteranthera oblongifolia*.

exception that species from Pontederiaceae were constrained to lie outside this clade (the 'not local' constraint set in Table 1). The most parsimonious trees from this analysis were only three steps (0.09%) longer than those found in the unconstrained analysis, and showed Pontederiaceae as being sister to Velloziaceae. Since a very low penalty in the number of tree steps is needed to shift Pontederiaceae to a dispersed range of positions within the monocotyledons, it is apparent that the present evidence from the *rbcL* locus is not suitable for providing a strong indication of the local phylogenetic placement of Pontederiaceae within the monocotyledons.

Infrafamilial Systematics

A. Morphological Evidence

Six to nine genera have been recognised in Pontederiaceae, with the majority of species in only four: *Eichhornia*, *Pontederia*, *Monochoria* and *Heteranthera*. Eckenwalder and Barrett (1986) treated 32 species and three varieties in their phylogenetic analysis of the family. Of the 42 morphological characters they examined, 35 were potentially informative within the family. Eckenwalder and Barrett's analysis did not fully resolve intergeneric relationships (see their Fig. 2), but they presented a range of character states considered to be synapomorphic for the genera and other clades in the family; the brief discussion below makes special reference to these character states. See also Lowden (1973), Horn (1985), Rosatti (1987), Barrett (1988), and Cook (1989) for more complete discussions of individual genera.

The native distribution of *Eichhornia* is centred in the Neotropics (including the West Indies), with the African *E. natans* being the sole non-New World member of the genus. The genus is composed of 8 to 9 species and can be broadly subdivided into two groups on the basis of life-history, habit and chromosome number. This separation is reflected in a classification of the genus by Schwartz (1927). One group of species is clonal to various degrees, procumbent or free-floating and polyploid (*E. azurea*, *E. crassipes*, *E. diversifolia*, *E. heterosperma* and *E. natans*). The other has an erect and non-clonal habit, inhabits ephemeral aquatic habitats demanding a more amphibious existence and is diploid (*E. meyeri*, *E. paniculata* and *E. paradoxa*). Tristyly and non-tristyly taxa are found in both groups.

Eckenwalder and Barrett's (1986) cladistic analyses indicated a monophyletic group of non-tristyly *Eichhornia* species, but did not clearly resolve whether this clade was closely associated with tristyly species of *Eichhornia*. However, in the full cladogram presented by these authors (one of the shortest trees linking tristyly and non-tristyly species) a paraphyletic *Eichhornia* was depicted, with *Pontederia* being derived from within *Eichhornia*. No characters were synapomorphic for *Eichhornia* alone. However, three characters were synapomorphic for a clade consisting of *Eichhornia* and *Pontederia*: a long-lived, perennial life-history, a geniculate infructescence attitude associated with submerged fruit maturation, and a zygomorphic perianth divided into upper and lower lips. Not all species of *Eichhornia* exhibit these character states. Only *E. azurea* and *E. crassipes* are long-lived perennials (the other taxa are short-lived perennials or annuals; these character states were treated as derived), and *E. meyeri*, *E. paniculata*, and *E. paradoxa* have an erect infructescence. Tristyly is probably uniquely derived within the family (see later) and serves as a further synapomorphy uniting *Eichhornia* and *Pontederia* (Eckenwalder and Barrett, 1986), since homostyly species are usually interpreted as being derived from tristyly taxa (Barrett, 1988). However, it should be noted that this character was not employed in their analysis. The morphologically more advanced taxa of *Eichhornia*, as measured by advancement indices assigned in Eckenwalder and Barrett (1986), approach *Pontederia* in their overall morphology and perenniality.

Pontederia is composed of six species and has a primarily Neotropical distribution with extensions into cooler temperate regions (Canada and Argentina) at the boundaries of the range of the genus. All taxa are long-lived perennials with an erect to procumbent habit. Clonal propagation is achieved through trailing stems and rhizomes, with these organs additionally facilitating perennation under harsh conditions. The single-seeded fruits in *Pontederia* are utricles enclosed by a hardened residual perianth base. The utricle is light and the surrounding perianth-remainder is aeriferous. The consequent buoyancy of the fruit facilitates long-range dispersal (Lowden, 1973). Lowden also noted that animal-mediated fruit dispersal may occur, especially in subgenus *Reussia*, where the fruit has prominently curved spines.

As measured by the five synapomorphies presented in Eckenwalder and Barrett's (1986) study, *Pontederia* is the best-supported genus in the family. Synapomorphies for *Pontederia* include the possession of a single fertile locule (the other two locules are non-fertile), a single ovule per fruit (with terminal pendulous placentation), seed lengths exceeding 1.5 mm, an indehiscent fruit, and a non-smooth fruit wall. However, it is possible that the first three character states are evolutionarily correlated with each other. They could be interpreted as being part of a packaging strategy for single-seeded fruits, with large seed size a consequence of an energetic trade-off between seed size and number.

The perianth in *Pontederia* is strongly zygomorphic and consists of two lips. Various interpretations of perianth structure have been made in *Reussia* (Endlicher, 1836; Solms-Laubach, 1883; Lowden, 1973), and this character has been used as a basis for generic segregation of *Reussia* from *Pontederia* (Endlicher, 1836). Lowden (1973) concluded that earlier interpretations are somewhat confused and that this character represents a weak basis for generic segregation. He divided *Pontederia* into two subgenera. Subgenus *Pontederia* is supported by four synapomorphies; the possession of a pulvinus, a derived paniculate inflorescence type, more than 100 flowers per inflorescence, and a smooth-ribbed or tooth-ribbed fruit wall. Subgenus *Reussia* has two supporting synapomorphies; its procumbent habit and a spiny fruit wall.

Monochoria is composed of seven to eight species (Cook, 1989) and is the only genus of Pontederiaceae restricted to the Old World. It has representatives in Australia and Africa and a centre of diversity in tropical Asia. Life-histories range from short-lived perennial to annual, with plants most commonly emergent in habit. The fruit is a capsule enclosed in a withered perianth and it is dispersed as a unit, later releasing the numerous seeds for water-mediated dispersal. Eckenwalder and Barrett (1986) found that two character states lend support for a monophyletic *Monochoria*: a geniculate infructescence and a poorly fused perianth (10-20% fusion) resulting in a nearly bowl-shaped flower. In contrast with the rest of the family, anther dehiscence in *Monochoria* is poricidal (Dahlgren and Clifford, 1982; Cook, 1989). This may represent a further synapomorphy of this genus.

Heteranthera and its allied genera comprise some 15 species and inhabit ephemeral aquatic habitats throughout the New World tropics, with two species in Africa (*H. callifolia* and *Scholleropsis lutea*). Most taxa are annuals or short-lived perennials, although *Zosterella* is a long-lived clonal perennial. *Heteranthera* in the broadest sense encompasses *Zosterella*, *Eurystemon mexicanum*, *S. lutea* and *Hydrothrix gardneri* (see the analysis of Eckenwalder and Barrett, 1986). Synapomorphies grouping these taxa are: regular possession of cleistogamous flowers, 40-60% fusion of the perianth (10-25% in *Hydrothrix*), and possession of three or one stamen(s). *Hydrothrix* and *Heteranthera* species also have parietal placentation, in contrast to the rest of the family where placentation is axile (Dahlgren and Clifford, 1982). This placentation type is rare in Bromeliales (Dahlgren *et al.*, 1985).

While genera of Pontederiaceae can be effectively divided into a clade of tristyly and homostyly taxa composed of *Eichhornia* and *Pontederia*, versus a largely enantistyly clade composed of *Monochoria* and *Heteranthera* (Fig. 1 in Eckenwalder and Barrett, 1986),

the morphological evidence supporting such a phylogenetic division is not strong (Table 5 and Fig. 2 in Eckenwalder and Barrett, 1986). Only two synapomorphies (basifixed anthers and dimorphic stamens) support the clade composed of *Monochoria* and *Heteranthera*, although to these might also be added the enantiostylous floral morphology. Four synapomorphies support a clade consisting of *Eichhornia* and *Pontederia* (see earlier). Uncertainty concerning phylogenetic relationships among genera, and the restricted numbers of morphological synapomorphies in Pontederiaceae, motivated us to obtain new sources of phylogenetic information to further clarify intergeneric relationships within the family.

B. Molecular Evidence

We reconstructed the phylogeny of Pontederiaceae with a combined data set based upon partial sequences from the chloroplast genes *rbcL* and *ndhF*. Twenty-five taxa (23 species, including three varieties of *P. cordata*) of Pontederiaceae were examined (S. W. Graham & S. C. H. Barrett, unpubl. data). Within the family 120 characters were potentially informative. Heuristic searches were performed as described earlier, except that 1000 random addition replicates were performed with TBR branch-swapping. *Philydrum lanuginosum* (Philydraceae) was used as an outgroup. A single tree with a length of 464 steps was found (CI = 0.552, excluding uninformative characters; RI = 0.775). The tree is shown in Fig. 2.

This chloroplast-based tree indicates that three of the four main taxonomic groups in the family are monophyletic: *Monochoria*, *Heteranthera* s.l. (including *Zosterella dubia* and *Hydrothrix gardneri*) and *Pontederia* s.l. (including one representative of subgenus *Reussia*, *P. rotundifolia*). *Heteranthera* is the sister group to the rest of the family. Two phylogenetically-distinct clades of *Eichhornia* each consist of a tristylous species (*E. paniculata* or *E. azurea*) together with two selfing species of *Eichhornia*. The clade consisting of *E. azurea*, *E. diversifolia* and *E. heterosperma* is sister to *Pontederia*. *Eichhornia crassipes* and *E. meyeri* are in neither of these groups of *Eichhornia*. The former is situated basally in a clade consisting of *Monochoria*, *Pontederia* and the *Eichhornia* group that includes *E. azurea*. The latter is basal to the clade consisting of *Pontederia*, *Monochoria* and all other species of *Eichhornia*.

Both morphological and molecular data sets thus support the monophyly of three of the four main genera of Pontederiaceae (*Pontederia*, *Monochoria* and *Heteranthera*). Significantly, however, the monophyly of *Eichhornia* is supported by neither the molecular nor morphological data sets. The unnaturalness of *Eichhornia* has taxonomic implications and raises the issue of whether the genus should be maintained as currently circumscribed. The non-monophyly of *Eichhornia* and the dispersed positions of the homostylous species of *Eichhornia* on the tree complicates phylogenetic reconstruction of the gain and loss of tristily. However, as discussed below, it seems likely that this breeding system arose only once within the family.

Breeding-System Evolution in Pontederiales

A diversity of floral syndromes associated with the pollination biology and breeding systems of individual taxa are found within Pontederiaceae. Here we briefly review some of the major issues concerned with the evolution of breeding systems in Pontederiaceae, and focus particularly on the role of phylogenetic data and models in informing our understanding of the origin and evolutionary relationships of the three primary floral conditions (Fig. 3) that occur within the family: tristily, homostily and enantiostily.

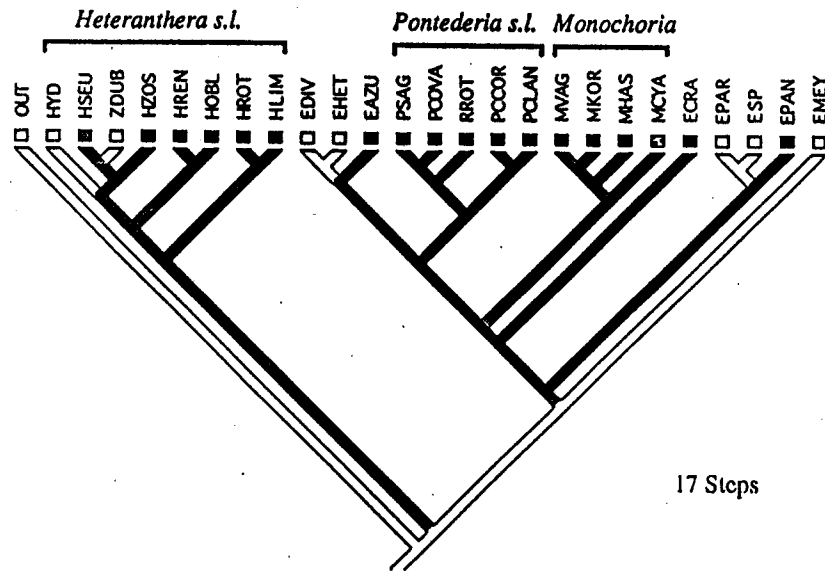
A. Origin and Evolution of the Tristylous Syndrome

Tristily is a floral syndrome of animal-pollinated plants that functions to increase the proficiency of cross-pollen transfer (Darwin, 1877; Lloyd and Webb, 1992a, 1992b; Kohn and Barrett, 1992). Three main components usually constitute the tristylous syndrome: reciprocal positioning of stigma and anther heights among the three floral morphs (reciprocal herkogamy), a self- and intramorph incompatibility system in which only pollen from the same level as the stigma is compatible, and a range of ancillary polymorphisms, primarily of pollen and stigmas. Each of the three floral morphs in tristylous species possesses a stereotypical combination of floral form and incompatibility type. The breeding system is controlled by a simple genetic system involving two diallelic loci with dominance, and with epistasis operating between the loci (Lewis and Jones, 1992).

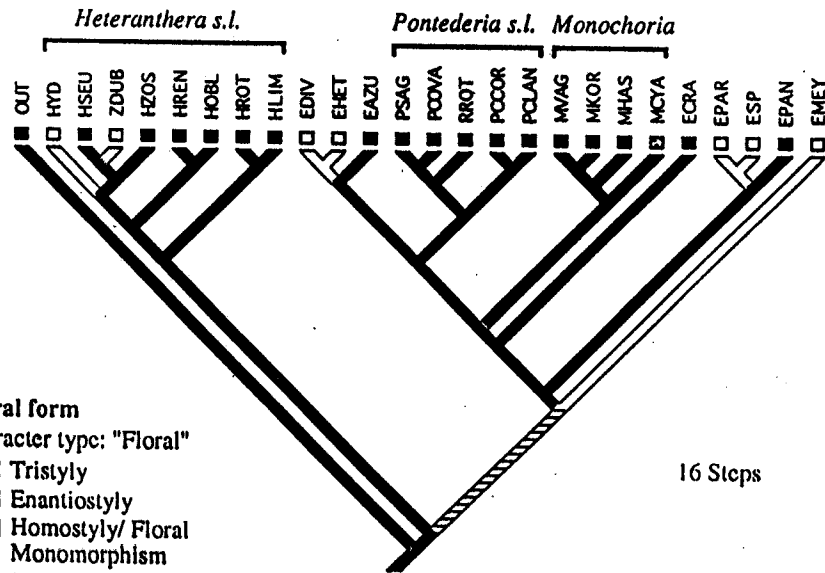
In optimising the three floral conditions (tristily, enantiostily and homostily) onto the chloroplast-based tree, we used a weighting scheme (see below) that favours the loss of tristily or enantiostily over their gain (Fig. 2A, 2B). An optimisation (not shown) that treats all shifts in floral form as equally weighted indicates an independent origin of enantiostily in *Monochoria* (as is also the case with the optimisations presented in Fig. 2A and 2B), but is equivocal with regard to the origin of tristily. This alternative optimisation requires between one and four independent origins of tristily within the family; the number of origins of tristily depends partly on how the trichotomy involving *E. azurea*, *E. heterosperma* and *E. diversifolia* is resolved. However, a range of microevolutionary and genetic evidence (described in the next section) indicates that the breakdown of tristily to homostily is a relatively simple process that occurs frequently. In addition, a number of lines of evidence suggest that the evolution of tristily is likely to be a very infrequent event. The overall rarity of this breeding system argues against it arising twice or more within the same genus. Tristily is known to have evolved within only four or five angiosperm families (Pontederiaceae, Lythraceae, Oxalidaceae, Amaryllidaceae and possibly Connaraceae; see Barrett, 1993), only two of which are monocotyledons and all of which are phylogenetically distant from one another. Moreover, if the relative frequency of the two basic forms of heterostily is any indication, tristily appears to have much more difficulty evolving than distily. The latter is believed to have evolved on at least 23 separate occasions in the flowering plants (Lloyd and Webb, 1992a). Finally, striking differences in the developmental basis of the polymorphism exist among the tristylous families (Richards and Barrett, 1992). In contrast, there is a high degree of morphological consistency in the polymorphism within *Eichhornia* and *Pontederia* species, lending further support to the hypothesis that tristily had a single origin within the family.

The weighted optimisation of floral conditions onto the tree indicates a single origin of tristily in the family (Figs 2A, 2B). There is a certain danger of circularity in using a weighting scheme that favours a single origin of tristily and then using the resulting optimisation as further evidence of this fact. However, we agree with Maddison and Maddison (1992; chapter 4) that workers should feel compelled to use the available biological evidence concerning a process when reconstructing the history of that process using local phylogenetic data. Finally, we should point out that the weighting scheme employed in our analysis (a gain: loss weighting ratio of 3:2; see Fig. 2) only marginally favours the loss of tristily or enantiostily over their gain during the optimisation process.

In an evolutionary scheme proposed by Lloyd and Webb (1992a, 1992b), heterostily evolves from a uniformly herkogamous taxon, i.e., an ancestor in which all individuals possess the same type of stigma-anther separation. Herkogamy serves to reduce self-interference during mating (Webb and Lloyd, 1986). The reciprocal herkogamy that characterises heterostylous plants represents a functional improvement upon the monomorphic condition because it acts to increase the efficiency of pollen transfer among individuals, by more precisely matching pollen dispatch-receipt points on the pollinator's



17 Steps



16 Steps

Floral form
Character type: "Floral"

- Tristyly
- ▤ Enantioistyly
- Homostyly/ Floral Monomorphism
- ▨ Uncertain
- ▧ Equivocal

body. A body of empirical evidence in Pontederiaceae lends support to this interpretation of the functional significance of heterostyly (Price and Barrett, 1982; Barrett and Glover, 1985; Wolfe and Barrett, 1989; Lloyd and Webb, 1992b).

Under the evolutionary model of Lloyd and Webb (1992a, 1992b), heteromorphic incompatibility, the class of self-incompatibility associated with the heterostylous syndrome, arises after the floral heteromorphism. It arises either as a passive consequence of (co)adaptation of each class of pollen to the stylar morph to which it is most proficiently transferred, an hypothesis first suggested by Darwin (1877), or as an actively selected anti-selfing device. This hypothesis stands in opposition to the other major theoretical model for the evolution of heterostyly (Charlesworth and Charlesworth, 1979) which presupposes that self-incompatibility arises as an anti-selfing device prior to the origin of reciprocal herkogamy.

The Lloyd and Webb model potentially permits different origins and evolutionary histories of self-incompatibility (SI) in each morph and among the different lineages of heterostylous species. Differences in the site and strength of action of SI are well documented in tristylous species of Pontederiaceae. For example, the different illegitimate pollen classes fail at different, but characteristic points in the stylar tract and ovary of *P. cordata* (Anderson and

FIGS. 2A and B. Phylogenetic reconstruction of breeding-system evolution in Pontederiaceae. The tree is the single shortest one found in an analysis based on sequence data from the chloroplast genes *rbcL* and *ndhF* (see text). Reconstruction of character evolution was performed using MacClade version 3 (Maddison & Maddison, 1992). *Philydrum lanuginosum* was used to root the tree. A trichotomy involving *E. azurea*, *E. heterosperma* and *E. diversifolia* was arbitrarily resolved to permit character optimisation using user-defined character types in MacClade. Figs 2A and 2B: Evolution of tristily, enantioistyly and homostyly in Pontederiaceae. The "Floral" user-defined character type employed in these reconstructions gives a slightly smaller weight to the loss of tristily or enantioistyly (a shift to homostyly or floral monomorphism) than to the gain or interconversion between these two flower types. Weights employed: loss of tristily or enantioistyly = 2 steps; gain of enantioistyly or tristily or shift between them = 3 steps. Alternative resolutions of the trichotomy involving *E. azurea*, *E. heterosperma* and *E. diversifolia* lead to an optimisation with two, rather than one, loss(es) of tristily in this clade (not shown). *Philydrum lanuginosum* is enantioistylyous, but other potential sister-groups to Pontederiales have some enantioistylyous taxa (see text), or are florally monomorphic. Two different codings of the outgroup's floral state were therefore examined; the outgroup was coded as either monomorphic (Fig. 2A) or enantioistylyous (Fig. 2B). *Monochoria cyanea* was coded as uncertain for floral form (i.e. enantioistylyous or monomorphic).

Abbreviations: OUT = Outgroup; HYD = *Hydrothrix gardneri*; HSEU = *Heteranthera seubertiana*; ZDUB = *Heteranthera (Zosterella) dubia*; HZOS = *H. zosterifolia*; HREN = *H. reniformis*; HOBL = *H. oblongifolia*; HROT = *H. rotundifolia*; HILIM = *H. limosa*; EDIV = *Eichhornia diversifolia*; EHET = *E. heterosperma*; EAZU = *E. azurea*; ECRA = *E. crassipes*; EPAR = *E. paradoxa*; ESP = *Eichhornia* sp.; EPAN = *E. paniculata*; EMEY = *Eichhornia meyeri*; PSAG = *Pontederia sagittata*; PCCOR = *P. cordata* var. *cordata*; PCLAN = *P. cordata* var. *lancifolia*; PCOVA = *P. cordata* var. *ovalis*; RROT = *Pontederia (Reussia) rotundifolia*; MVAG = *Monochoria vaginalis*; MKOR = *M. korsakovii*; MHAS = *M. hastata*; MCYA = *M. cyanea*. An undescribed species of *Eichhornia* (referred to here as *Eichhornia* sp.) was incorrectly identified in Eckenwalder and Barrett (1986) as *E. paradoxa* (Mart.) Solms-Laub.

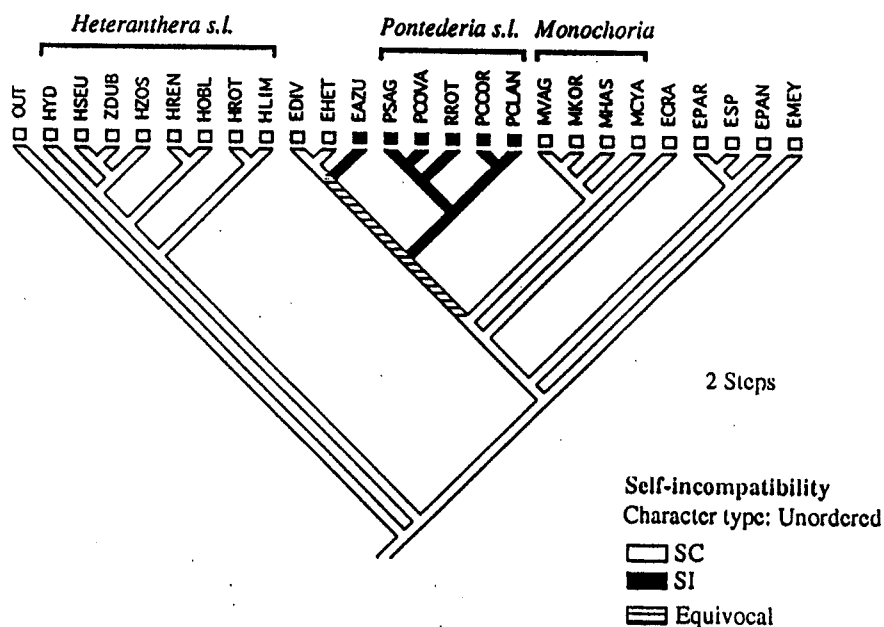
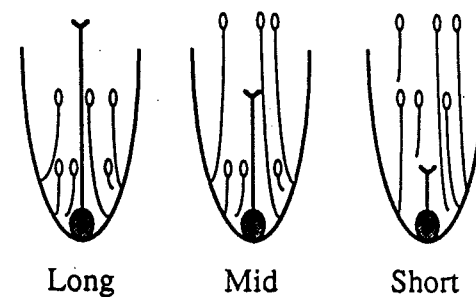
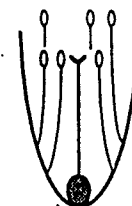


FIG. 2C: Evolution of heteromorphic self-incompatibility (SI) in Pontederiaceae. The outgroup was coded as self-compatible (SC) (see text). Transitions between SI and SC were equally weighted and unordered. Depending on the resolution of the trichotomy involving *E. azurea*, *E. heterosperma* and *E. diversifolia*, SI arises either once or twice in the clade containing *Pontederia* and *E. azurea* and associated homostyles. With the resolution of *E. azurea* and associated homostyles shown here, the origin of SI is equivocal (i.e., one or two origins of SI are possible). Regardless of how this equivocality is viewed, SI arises after the origin of tristily in the family. For abbreviations see caption for Figs. 2A and B.

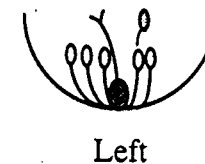
Barrett, 1986) and *P. sagittata* (Scribailo and Barrett, 1991b). Self-incompatibility is stronger overall in tristylous *Pontederia* species than in *Eichhornia* species, where it is found only in *E. azurea*. In all tristylous *Pontederia* species examined, SI is much stronger in the long- and short-styled morphs than in the mid-styled morph, where illegitimate pollination results in abundant seed set (Barrett and Anderson, 1985). In tristylous *Eichhornia* species only *E. azurea* has appreciable SI, although only data from the long-styled morph is available for this species. Even here, the strength of incompatibility differs among illegitimate anther levels (Barrett, 1978). *Eichhornia paniculata* is completely self-compatible, in the traditional sense of this expression (i.e., full seed set upon application of self-pollen). However, differences in the prepotency of the pollen types of this species have been observed when different classes of pollen are forced to compete for access to ovules (Cruzan and Barrett, 1993). Typically, legitimate pollen performs better than illegitimate pollen and the species can therefore be viewed as possessing a cryptic trimorphic incompatibility system.



A. Tristily



B. Homostyly



C. Enantiostyly

FIG. 3. Schematic representation of stamen and style configurations in the three most common floral forms in Pontederiaceae. A. Tristily. Individuals produce either long-, mid- or short-styled flowers, depending on their genotype at two diallelic loci controlling this genetic polymorphism (see text). B. Homostyly. In homostylous species, populations are usually composed of a single floral form with either one ("semi-homostyly") or two sets of anthers adjacent to the stigma. In Pontederiaceae this most commonly involves the mid-styled morph. The phenotype illustrated is a semi-homostylous mid-styled flower, with short-level anthers adjacent to the stigma (see Barrett, 1988). 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 flower types simultaneously.

Issues of homology make phylogenetic interpretation of the evolution of incompatibility systems difficult. Should cryptic SI in *E. paniculata* be viewed as homologous with full heteromorphic SI? Under the Lloyd and Webb evolutionary scheme (1992a, 1992b) both physiological systems can arise from the same evolutionary force, i.e., pollen-style adaptation. However, as traditionally defined, *E. paniculata* 'functions' as a fully self-compatible species. If cryptic SI is homologous with full SI, then the optimisation of SI depicted in Fig. 2C would by this interpretation be misleading; its point of origin would indicate when full heteromorphic SI evolved, presumably from a version weak enough to masquerade as self-

compatibility. It is also not clear if the trait we call heteromorphic SI is a single unified character, since under Lloyd and Webb's model, morph-specific pollen-style interactions leading to pollen failure can have different evolutionary and phylogenetic trajectories among different heterostylous lineages.

An additional problem concerns the difficulty of accurately determining root position in phylogenetic reconstructions. The underlying structure of an ingroup phylogeny can be sturdy, but the precise location of the root of the tree still remain unclear. Different root placements can lead to different optimisations of the origins of tristylly and SI onto the tree (not shown). This issue is explored by Graham *et al.* (MS) using a phylogeny of the family based on three chloroplast data sets.

Fig. 2C illustrates the optimisation of self-incompatibility and self-compatibility onto the chloroplast-based tree. Transitions between self-incompatibility and self-compatibility were equally weighted. The outgroup is coded as self-compatible in Fig. 2C, but coding it as self-incompatible does not produce a different reconstruction of the evolution of self-incompatibility within Pontederiaceae. Excluding Pontederiaceae, sporophytic self-incompatibility systems are unknown in the monocotyledons (Charlesworth, 1985; Weller *et al.*, 1995). Some taxa in Commelinaceae possess a gametophytic self-incompatibility system (reviewed in Owens, 1981), but it seems highly unlikely that this system is homologous with the heteromorphic sporophytic system found in Pontederiaceae.

The optimisation of SI is equivocal and is also dependent on how the trichotomy involving *E. azurea* and its associated homostylous species is resolved. Depending on how these ambiguities in optimisation are disentangled, SI either originates at the base of the clade containing *Pontederia*, *E. azurea*, *E. heterosperma* and *E. diversifolia* (with one or two losses along the branch(es) leading to the two homostylous species), or it arises twice within this clade; once along the branch leading to *Pontederia*, and once along the branch leading to *E. azurea*. In either case, SI arises after the origin of tristylly. These conclusions concerning the evolutionary history of SI in Pontederiaceae also serve as a caution against assumptions that the existence of self-compatibility in heterostylous taxa (or morphs) always represent a degenerate condition (cf., Ornduff, 1972; Weller, 1992). In the optimisations presented here, self-compatibility can be the more primitive condition, a pattern consistent with Lloyd and Webb's model (1992a, 1992b) for the evolution of heterostylly.

B. Effects of the Selfing Syndrome on Phylogenetic Reconstruction

In virtually every heterostylous group, multiple shifts to predominant self-fertilisation have occurred via the evolution of homostylly. Homostyles possess anthers and stigmas at the same position within a flower, and as a result are largely self-fertilising. It has generally been assumed, following Darwin (1877), that homostyles are evolutionarily derived from heterostylous ancestors. In many cases the evolution of homostylly in heterostylous groups is closely associated with the development of reproductive isolation and speciation (Baker, 1961).

Phylogenetic reconstruction is problematical in groups where such repeated transitions to predominant self-fertilisation (autogamy) have occurred, since this evolutionary shift is typically accompanied by multiple parallel changes in a broad range of floral characters (a 'selfing syndrome'), as well as changes in life-history (Lloyd, 1965; Ornduff, 1969; Eckenwalder and Barrett, 1986; Wyatt, 1988; Morgan and Barrett, 1989). The evolution of multiple, correlated morphological changes associated with shifts to autogamy violates the critical assumption of character independence that is implicit in phylogenetic reconstruction. Although floral characters represent some of the most important and numerous morphological data employed in phylogenetic reconstruction, and the shift to predominant self-fertilization from predominantly outcrossing breeding systems constitutes one of the most pervasive themes in floral evolution (Stebbins, 1970; Jain, 1976), the effect of this breeding-system shift

on phylogenetic reconstruction is not well documented (although see the studies on *Leavenworthia* (Lloyd, 1965), *Limnanthes* (Arroyo, 1973; McNeill and Jain, 1983), *Arenaria* (Wyatt, 1988), and *Amsinckia* (Schoen, 1993)).

Four of the seven taxa of *Eichhornia* and one of the eight taxa of *Pontederia* included in the morphology-based phylogenetic analysis of Pontederiaceae by Eckenwalder and Barrett (1986) are homostylous. Their analysis indicated only two origins of homostylly within the family; one in *Pontederia* (*P. parviflora*) and the other in *Eichhornia*. They suggested that the finding of a single origin for homostylly in *Eichhornia* is a consequence of the distorting effects of the selfing syndrome on phylogenetic reconstruction.

Several lines of evidence indicate that selfing variants evolve readily in tristylous *Eichhornia* populations (Barrett, 1988; Barrett *et al.*, 1989). For example, relationships inferred among Brazilian populations of *E. paniculata* using genetic distance estimates from isozyme data (Husband and Barrett, 1993) imply that populations possessing selfing variants arise repeatedly from outcrossing populations in different parts of the geographical range of the species. Although it is difficult to assess mutational *versus* migratory hypotheses for the origins of selfing in such populations from isozyme data alone, data on the genetic architecture of floral traits causing selfing are consistent with the multiple origin hypothesis (Fenster and Barrett, 1994). Theoretical models and computer simulations (Eckert and Barrett, 1992; Husband and Barrett, 1992a) also demonstrate the inherent instability of the tristylous genetic polymorphism in the face of the kinds of levels of genetic drift observed in natural populations of species of *Eichhornia* (Husband and Barrett, 1992b). These population-level studies indicate that the number of origins for homostylly may be considerably greater than can be revealed through phylogenetic analysis using species as OTUs. In the future, genealogical studies at the population level may enable more refined estimates of the number of evolutionary events that are occurring below the species level.

This range of microevolutionary and genetic evidence strongly suggests that evolutionary shifts to homostylly occur readily. The phylogenetic reconstruction based on molecular evidence from the chloroplast (Figs 2A, 2B) indicates that tristylly evolved near the base of the family and was subsequently lost on at least three occasions, with at least two losses giving rise to homostylous species. One loss was associated with a shift to an enantiostylous floral form in *Monochoria*. *Pontederia* includes one species lacking tristylly (*P. parviflora*). This was not available for the current molecular analysis, but probably represents another case of the loss of tristylly.

The conflict between molecular and morphological phylogenetic analyses concerning the evolution of selfing in Pontederiaceae suggests that either molecules or morphology (or both) are not telling the whole truth concerning phyletic descent in the family. A variety of phenomena can cause erroneous reconstruction of phylogenetic history when using molecular data based on single genetic linkage groups, e.g. lateral gene transfer, mistaken genetic orthology (Doyle, 1992) and ancestral polymorphism (Pamilo and Nei, 1988). We feel, however, that the simplest interpretation of the systematic evidence from Pontederiaceae is that the selfing syndrome has distorted phylogenetic reconstruction using morphological data. This conflict among different data sets highlights the need to use a variety of sources of data in phylogenetic reconstruction.

C. Evolution and Adaptive Significance of Enantiostylly

Outside Pontederiales, heterostylly does not occur in any putatively related order of monocotyledons (e.g., Philydrales, Haemodorales, Commelinales). A report of heterostylly in *Anellema aequinoctiale* (Commelinaceae) by Vogel (1955) is almost certainly a misinterpretation of the true nature of the polymorphism (Ornduff, 1974; Faden, 1991; S. Vogel, pers. comm.). Heterostylly has only reliably been reported from two other monocotyledonous taxa: *Nivenia* of Iridaceae (Mulcahy, 1965; Goldblatt and Bernhardt,

TABLE 2. Occurrence of heterostyly in the monocotyledons and general features of the syndrome.

Taxa	Number of heterostylous species (total number in genus)	Type of heterostyly	Incompatibility Type	Expression	Ancillary polymorphisms	
					Stigmas	Pollen
Pontederiaceae <i>Pontederia</i>	5(6)	Tristyly	TSI	Strong, with different sites of inhibition	Strong	Strong
<i>Eichhornia</i>	3(9)	Tristyly	TSI	Variable, from absent or cryptic to weak	Weak	Weak
Amaryllidaceae <i>Narcissus</i>	1(30)	Tristyly ¹	?	Late-acting SI, and/or inbreeding depression	Weak	Absent
Iridaceae <i>Nivenia</i>	5(9)	Distyly	Absent	Plants apparently fully self-compatible	Absent	Weak

TSI = Trimorphic self-incompatibility. Sources: *Pontederia*; Barrett & Anderson (1985), Scribailo & Barrett (1991 a,b). *Eichhornia*; Barrett (1988), Cruzan & Barrett (1993). *Narcissus*; Fernandes (1935, 1964), Lloyd, Webb & Dulberger (1990), Barrett, Lloyd & Arroyo (1995). *Nivenia*; Mulcahy (1965), Goldblatt & Bernhardt (1990). ¹ Reports of distyly in *Narcissus* are erroneous and involve only a stigma-height dimorphism (Barrett, Lloyd & Arroyo, 1995).

1990) and *Narcissus triandrus* of Amaryllidaceae (Fernandes, 1935; Lloyd *et al.*, 1990; Barrett *et al.*, 1995). The distinctive nature of heterostyly in *Nivenia* and *Narcissus* (Table 2) compared with its expression in Pontederiaceae, and the fact that molecular evidence indicates that Iridaceae and Amaryllidaceae are distantly related to this family (Chase *et al.*, 1993), lends strong support to the hypothesis that heterostyly is apomorphic within Pontederiaceae.

In contrast, enantiostyly is reported from three orders of monocotyledons with possible close affinities to Pontederiales: Philydrales (Simpson, 1990), Haemodorales (Wilson, 1887; Ornduff and Dulberger, 1978; Simpson, 1990) and Commelinales (Faden, 1991). This raises several issues concerning the evolution and phylogenetic origins of enantiostyly in these related orders. What are the features of enantiostyly in these groups? Has the floral polymorphism originated independently in each order? What are the evolutionary relationships between heterostyly and enantiostyly in Pontederiales, and are the two conditions independent responses to pollinator-mediated selection for increased mating efficiency?

Several floral traits (e.g., heteranthery, zygomorphy, the absence of nectar secretion, and poricidal anther dehiscence) are commonly associated with enantiostyly in a variety of unrelated angiosperm taxa (Bowers, 1975; Ornduff and Dulberger, 1978; Dulberger and Ornduff, 1980; Dulberger, 1981; Buchmann, 1983). These assemblages of floral characters are found to varying degrees in the enantiostylous monocotyledons (Table 3). These traits are discussed with respect to their distribution among enantiostylous taxa and the possible evolutionary significance of their associations with the enantiostylous floral form.

In the sense used here, enantiostyly is the possession of flowers with left- and right-bending styles, typically with a single stamen reflexed in a lateral position opposite the stigma. While this condition can apparently exist as a true genetic polymorphism (e.g., in *Wachendorfia paniculata* (Haemodoraceae), Ornduff and Dulberger, 1978), with individual plants possessing either right- or left-bending styles, it more commonly occurs as a somatic polymorphism with both right and left-handed flowers occurring within the same individual. In Pontederiales the polymorphism is of this latter type and is usually associated with a clear stamen dimorphism. In *Monochoria* and *Heteranthera*, the reciprocally reflexed stamen tends to be larger than the other stamens and cryptically coloured. Such dimorphism is known as heteranthery when it represents a functional division of labour among the stamens into predominantly attractive 'feeding' stamens and one or more cryptically-coloured 'pollinating' stamens (Vogel, 1978; Buchmann, 1983; Lloyd, 1992).

Enantiostyly and heteranthery are reported in a few phylogenetically unrelated angiosperm groups, and are commonly found associated together. Most taxa in *Monochoria* and *Heteranthera* are enantiostylous and heterantherous. In *H. reniformis* and *M. vaginalis*, it is reported that pollinators ignore the single pollinating anther and are instead attracted to the feeding anthers (Müller, 1883; Iyengar, 1923). However, this division between attractive and fertilising functions is probably not absolute, since pollen from the feeding anthers is capable of fertilisation (S. C. H. Barrett, unpubl. data). *Zosterella dubia*, *Hydrothrix*, and *M. cyanea* all lack stamen dimorphisms (Eckenwalder and Barrett, 1986) and at least the first two are also not enantiostylous. Apart from Pontederiaceae and *Cyanella* (Dulberger and Ornduff, 1980), it is not clear how many of the instances of stamen dimorphism indicated in Table 3 represent true heteranthery.

Faden (1991) was hesitant about calling the floral heteromorphism found in certain taxa of *Aneilema* true enantiostyly, since reciprocal deflection of a single stamen against the left- or right-bending style is not found. However, as is the case with the floral heteromorphism in *Cassia didymobotrya* (Caesalpinaceae) (Dulberger, 1981) flowers in these species possess two laterally-placed stamens such that whether the style is left- or right-bending, it is always

TABLE 3. Occurrence of enantiostyly in the monocotyledons and general features of the syndrome.

Taxa	Perianth Symmetry		Floral orientation		Stamen dimorphism		Anther dehiscence		Nectaries	
	Zygo-	Actinomorphic	Outward	Upward	Present	Absent	Poricidal	Longitudinal	Present	Absent
Pontederiaceae										
<i>Heteranthera</i>	✓	-	✓	-	✓	-	-	✓	-	✓
<i>Monochoria</i>	-	✓	✓	-	✓	-	✓	-	✓(?)	-
Haemodoraceae										
<i>Wachendorfia</i>	✓	-	✓	-	-	✓	-	✓	✓	-
<i>Schiekia</i>	✓	-	✓	-	✓	-	-	✓	✓	-
Other genera ¹	two species	most species	-	✓	most species	two species	one species	most species	most species	two species
Philydraceae										
<i>Philydra</i>	✓	-	✓	-	(Single stamen only)	-	-	✓	-	✓
Tecophilaeaceae										
<i>Cyanella lutea</i> , <i>C. alba</i>	-	✓	✓	-	✓	-	✓	-	-	✓
Commelinaceae										
<i>Anellema</i> (some species ²)	✓	-	✓	-	✓	-	-	✓	-	✓

¹*Xiphidium*, *Barbaretta*, *Dilatris*, *Haemodorum*, *Lachnanthes*; see text and Simpson (1990, 1993) for further details. Sources: Haemodoraceae; Ornduff and Dulberger (1978); Simpson (1990, 1993). Philydraceae; Simpson (1990), Dahlgren, Clifford & Yeo (1985). Cyanella; Dulberger & Ornduff (1980). ²*Anellema* (section *Lamproditityros*); Faden (1991).

reflexed against one of these stamens. We feel less hesitant about calling this floral heteromorphism enantiostyly.

Enantiostyly has most often been interpreted as an adaptation for increasing the proficiency of cross-pollination (Todd, 1882; Iyengar, 1923; Ornduff and Dulberger, 1978; Webb and Lloyd, 1986). In a manner analogous to heterostyly, the consistent spatial positioning on the pollinator's body of the sites of pollen dispatch and receipt may promote pollen transfer between individuals. One problem with this hypothesis is that this consistency in pollen transfer may also actively promote geitonogamy (between-flower selfing) when regular visitation of opposite-form flowers takes place within the same plant. Promotion of geitonogamy may, however, be low if only one or a few flowers are open on a given day, or if the flight path of the pollinator is such that few flowers are visited per individual (Dulberger, 1981). Geitonogamous matings will also be reduced if an SI system is present, as is the case with *Cyanella alba* and *C. lutea* (Tecophilaeaceae) (Dulberger and Ornduff, 1980), but obviously SI by itself cannot act to increase the proficiency of cross-pollen transfer.

Dulberger (1981) suggested that the primary role of enantiostyly in species of *Cassia* is to provide pollinators with unobstructed access to feeding anthers, while protecting the gynoeceum during vibrational collection of pollen (buzz pollination) from poricidal anthers. It is unclear if this explanation holds for enantiostyly species in general, since it is not certain that all enantiostyly species are buzz pollinated. For example, species of *Heteranthera* and most enantiostyly Haemodoraceae have longitudinal anther dehiscence and are therefore probably not buzz pollinated, since this pollen-collecting behaviour is strongly associated with poricidal anther dehiscence (Buchmann, 1983).

Simpson (1990) suggested that in species with actinomorphic flowers, enantiostyly serves only to reduce the amount of self-pollination, by increasing stigma-anther separation. When the flower is zygomorphic, pollinators will be positioned consistently with respect to the pollinating anther and style. In actinomorphic flowers there may be no consistency in pollinator approach to the flower and hence in the sites of pollen dispatch and receipt on the pollinator's body. Thus, enantiostyly may not function to increase the proficiency of cross-pollen transfer in radially-symmetrical flowers. Among the enantiostyly taxa of Haemodoraceae, only *Wachendorfia*, *Schiekia*, *Xiphidium xanthorrhizon* and *Barbaretta aurea* possess zygomorphic perianths (Simpson, 1990; Simpson 1993, Fig. 10). Species of Philydraceae have enantiostyly flowers with only a single stamen, and strongly zygomorphic perianths. Within Pontederiaceae, species of *Heteranthera* are mostly zygomorphic while species of *Monochoria* are actinomorphic. Flowers borne on *Monochoria* inflorescences are outwardly facing, so that pollinators are likely to approach them in a consistent orientation. Because of this feature and their enantiostyly-heterantherous morphology, the flowers of species of *Monochoria* may be functionally zygomorphic. A parallel case is found in Tecophilaeaceae, where flowers of *Cyanella alba* and *C. lutea* are actinomorphic, but are enantiostyly, outwardly-facing and apparently heterantherous (Dulberger and Ornduff, 1980).

Pollen from the feeding anthers of heterantherous species serves to attract pollinators in place of nectar. Secretion of nectar may not be present in *Monochoria* (Cook, 1989) and nectaries are absent from *Heteranthera* (Van Heel, 1988; Simpson, 1990) and Philydraceae (Dahlgren and Clifford, 1982). All species of *Monochoria* have poricidal anthers, and are therefore probably buzz-pollinated by pollen-collecting bees (Buchmann, 1983). Although it is not a universal association, poricidal anthers are a well documented feature of enantiostyly taxa (*Solanum rostratum*: Bowers, 1975; *Cyanella*: Dulberger and Ornduff, 1980; *Cassia*: Dulberger, 1981). Enantiostyly is also not always associated with an absence of nectar secretion. Apart from *Xiphidium*, all enantiostyly Haemodoraceae species have septal nectaries (Simpson, 1993).

Repetition in elements of the enantiostylous syndrome among phylogenetically disjunct taxa is probably indicative of similar selective pressures operating on floral morphology. The regular association of enantiostyly with outwardly-facing, zygomorphic flowers (Table 3) suggests that consistent positioning of the pollinator is usually an important part of the functional operation of enantiostyly. Heteranthery, poricidal anther dehiscence and absence of nectaries are all likely to be associated with pollen-collection by pollinators, so their frequent co-occurrence may not be surprising. It would be particularly valuable to use a phylogenetic approach to determine if traits associated with pollen-collection by pollinators are truly more commonly associated with enantiostyly than might be expected by chance, and to perform experimental studies to examine the functional significance of the different components of the enantiostylous syndrome, in much the same way as has been conducted for heterostylous plants (e.g., Ganders, 1974; Kohn and Barrett, 1992).

The precise evolutionary relationships of enantiostyly to heterostyly (if any) are unknown, but it is intriguing to note that both conditions involve forms of reciprocal herkogamy and dimorphic stamens. The primitive floral form in Pontederiaceae may be homologous with that found in Haemodoraceae and Philydraceae, if these are indeed the sister groups of Pontederiaceae. The reconstructions presented in Fig. 2A and 2B indicate an independent origin of enantiostyly in *Monochoria*. Depending on how the outgroup is coded, enantiostyly in *Heteranthera* represents either a second independent origin of the floral form in the family (Fig. 2A) or the primitive floral condition of the family (Fig. 2B). In both optimisations, enantiostyly is lost in two lineages within *Heteranthera s.l.*

Conclusion

Studies of floral evolution have largely been performed using contemporaneous, population-level evidence. However, in recent years the importance of adding an historical component to such studies has become widely appreciated (Donoghue, 1989; Cox, 1990; Sytsma *et al.*, 1991; Reiseberg *et al.*, 1992; Weller *et al.*, 1995). Phylogenetic systematics can provide this historical perspective. The addition of new phylogenetic data from a variety of different sources serves to strengthen our confidence in reconstructions of the evolutionary history of organisms and of their constituent character complexes. It can also function to highlight deficiencies in the capacity of any particular class of data to permit the accurate reconstruction of historical events. This paper brings together a range of phylogenetic evidence from morphological and molecular sources to examine the systematics of Pontederiales and the evolutionary history of polymorphic breeding systems present in the family Pontederiaceae. We argue that tristylly probably evolved once in the family and that there have been multiple breakdowns of the syndrome to self-fertilisation via the evolution of homostyly. Given the diversity in form of enantiostyly in this and putatively related orders, we suggest that these taxa provide excellent opportunities for phylogenetic, as well as functional, investigations of the evolutionary significance of this floral syndrome. Future systematic studies of Pontederiales should concentrate on providing more robust evidence concerning its local placement within the monocotyledons and on collecting phylogenetic data from a greater range of morphological and molecular sources.

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