Monocotyledons: Systematics and Evolution

VOLUME II

Editors
P.J. RUDALL, P.J. CRIBB, D.F. CUTLER & C.J. HUMPHRIES


PHYLOGENETIC SYSTEMATICS OF PONTEDERIALES: IMPLICATIONS FOR BREEDING-SYSTEM EVOLUTION

SEAN W. GRAHAM AND SPENCER C.H. BARRETT

Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

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, Bromeliaceae 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 tristyly 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 tristylistic, homostylistic and enantiotylistic 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 tristyly 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. Tristyly in breeding systems thus 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 McNennan, 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 tristyly. 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 enantiostyly 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 argument concerning pleiomorphic 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 superfamilial ending -‘anae’ throughout the discussion.

Dahlgren and Rasmussen (1983) proposed a major clade within the monocotyledons consisting of the members of Commelinaceae, Zingiberaceae and Bromeliaceae (and possibly Arecales) 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 Commelinaceae-Zingiberaceae-Bromeliaceae complex and is also present in Arecales. 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 Arecales. Their superorder Bromeliaceae 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 Zingiberanaceae-Bromeliaceae complex was defined on the basis of a single synapomorphy; possession of showy petaloid tepals. The utility of this character in delimiting this group is somewhat dubious given the existence of showy petaloid tepals within Commelinaceae. The superorder Bromeliaceae was further defined on the basis of a single synapomorphy; possession of helobial endosperm with a small, starchy-free, and sometimes hestorial chalazal chamber. A further sub-clade within their Bromeliaceae consisting of the orders Pontederiales, Haemodorales, Philydrades and Typhales was also defined by one synapomorphy; the possession of distinctos leaves. However, this condition also has a widespread distribution in Commelinaceae and Zingiberaceae and has variable expression within Pontederiaceae, where the more broad-leaved taxa tend to have spiral phylloxy. Possession of an amoeboeid tapetum was used to define a group consisting of Pontederiales, Haemodorales and Typhales (Philydrades 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, Haemodoraceae and Philydraceae emphasised the lillaceous character of these families (Takahijan, 1969; Dahlgren, 1975; Dahlgren and Clifford, 1982; Cronquist, 1988). Dahlgren and Clifford's study listed eleven (versus four) attempts to identify papilionaceous than commelinaceous characters of Pontederiaceae. However, five of the lillaceous characters mentioned (sulfate 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 oligosulate pollen (dilicate 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 Philydrades also inhabits semi-aquatic habitats), possession of petiole leaves with stipule- or ligule-like structures, possession of hairs on the stamen filaments (Dahlgren and Clifford, 1982), and possibly also their bisexual 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 homoplasic.

Several characters shared among Pontederiales, Haemodorales and Philydrates 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 sculpture and architecture between Haemodorales and Pontederiales constitute synapomorphies of these two orders. Dimorphic stamens are present in some members of Haemodorales and most Pontederiales. However, only a single stamen is found in Philydrades. 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 stamens. Enantiostyly is present in all four genera of Philydraceae, in most genera of the tribe Haemodoraeae of Haemodoraceae (Simpson, 1990)
Monocotyledons: systematics and evolution

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 Anelieima (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 Acrocl is 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) Phyllydrae (i.e., Pontederiaceae, Haemodoraceae and Phyllydraeaceae) and Dahlgren and Rasmussen's (1983) Bromeliaceae (their Bromeliilfiae) were both used to delimit topological constraints for separate analyses involving these as the sole constraints. In the case of the Bromeliaceae 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 Phyllydrae) 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 Ponederia 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>
<thead>
<tr>
<th>Analysis</th>
<th>Number of steps</th>
<th>Consistency index</th>
<th>Number of trees found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chase et al. (1993)</td>
<td>3205</td>
<td>0.285</td>
<td>199</td>
</tr>
<tr>
<td>Contrarian</td>
<td>3304</td>
<td>0.286</td>
<td>200</td>
</tr>
<tr>
<td>Dahlgren et al. (1985)</td>
<td>3322</td>
<td>0.277</td>
<td>201</td>
</tr>
<tr>
<td>Thorne (1992b)</td>
<td>3278</td>
<td>0.288</td>
<td>202</td>
</tr>
<tr>
<td>Phyllydrae (Thorne, 1992b)</td>
<td>3231</td>
<td>0.289</td>
<td>203</td>
</tr>
<tr>
<td>Not local</td>
<td>3198</td>
<td>0.290</td>
<td>204</td>
</tr>
</tbody>
</table>

1Excluding uninformative characters. 2Portion of the single tree shown in Chase et al. (1993). 3Highly constrained local topology.
Monocotyledons: systematics and evolution

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 incongruent 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 (Hymenocorales, Philydracaceae and Pontederiaceae) treated quite differently by Thorne and Dahlgren and co-workers. Thorne (1992b) places these families in the order Philydreales of his Commelinanae, a superorder that strongly resembles the Commelinanae-Bromeliaceae-Zingiberales complex of Dahlgren et al. (1985) in terms of its constituent families. Constraints employed using only the Bromeliaceae (sensu Dahlgren and Rasmussen, 1983) resulted in trees longer than the shortest unconstrained tree by 37 steps (1.15% longer), indicating there is also some incompatibility between their morphology-based scheme and morphological 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 cladette contains taxa only found in the Commelinanae-Bromeliaceae-Zingiberales complex of Dahlgren et al., although one member of this complex (Velloziaceae) falls outside this section of the tree. Apart from Commelinanae, all orders in this group are monophyletic, at least with respect to the taxa included. Neither Bromeliaceae 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. Commelinanae 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 of the unconstrained search were one step longer than those found after TBR branch swapping. In all of these trees the cladace of Pontederiaceae and Commelinanae was transposed relative to that illustrated in Fig. 1 such that it constituted the immediate sister-group of Anigozanthos (Haemorhoidaceae). Furthermore, the constraint set that denied sister group status to Commelinanae, by enforcing a monophyletic cladette uniting Haemorhoidaceae, Philydraceae and Pontederiaceae (Philydreales sensu Thorne, 1992b), resulted in shortest trees only about 4 steps (0.13%) longer than the shortest unconstrained trees. Trees not uniting Commelinanae 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 Commelinanae-Bromeliaceae-Zingiberales complex. This analysis enforced a cladace 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 tree computed from the 64 shortest trees found in the unconstrained analysis of rbcL sequences from 88 monocotyledons. In terms of its constituent families, this cladace strongly resembles Thorne's (1992a, 1992b) and the Commelinanae-Bromeliaceae-Zingiberales 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 = Bromeliaceae, Z = Zingiberales (after Dahlgren et al., 1985). The arrow indicates an arrangement of Pontederiaceae-Commelinanae observed in trees one step longer than the shortest trees (see text). Representatives of Pontederiaceae are: Elachnornia paniculata, Pontederia sagittata, Monochoria vomitoria and Heiranthera oblata.](image-url)
Monocotyledons: systematic and evolution

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 genus 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 polyplody (E. azurea, E. crassipes, E. diversifolia, E. heteropetala 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. paradoxo). Tristylos and non-tristylos taxa are found in both groups.

Eckenwalder and Barrett’s (1986) cladistic analyses indicated a monophyletic group of non-tristylos Eichhornia species, but did not clearly resolve whether this clade was closely associated with tristylos species of Eichhornia. However, in the full cladogram presented by these authors (one of the shortest trees linking tristylos and non-tristylos 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 gonochoric infructescence attitude associated with submergent fruit maturation, and a gymnospermous 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. paradoxo 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 homostylos species are usually interpreted as being derived from tristylos 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 remains 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 locale (the other two locules are non-fertile), a single ovule per fruit (with terminal pendulous placenta), seed lengths exceeding 1.5 mm, an indiscernible 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 pulvini, a derived panicle 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 inflorescence 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 (I. callifolia and Scholleropis lutescens). Most taxa are annuals or short-lived perennials, although Zosteraella is a long-lived clonal perennial. Heteranthera in the broadest sense encompasses Zosteraella, Eurytemon mexicanum, S. lutescens and Hydrotrichis 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 Hydrotrichis), and possession of three or one stamen(s). Hydrotrichis and Heteranthera species also have parietal placenta, in contrast to the rest of the family where placenta are axile (Dahlgren and Clifford, 1982). This placenta type is rare in Bromeliaceae (Dahlgren et al., 1985).

While genera of Pontederiaceae can be effectively divided into a clade of tristylos and homostylos taxa composed of Eichhornia and Pontederia, versus a largely enantiostylos 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 these might also be added the enantioystylous 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 Hydrocharis 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 tristylosous species (E. paniculata or E. azurea) together with two selfing species of Eichhornia. The clade consisting of E. azurea, E. diversifolia and E. heteroasperma 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 tristyly. However, as discussed below, it seems likely that this breeding system arose only once within the family.

Breeding-System Evolution in Pontederiaceae

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: tristyly, homostyly and enantioystyly.

A. Origin and Evolution of the Tristylos Syndrome

Tristyly is a floral syndrome of animal-pollinated plants that functions to increase the propensity of cross-pollen transfer (Darwin, 1877; Lloyd and Webb, 1992a, 1992b; Kohn and Barrett, 1992). Three main components usually constitute the tristylos 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 tristylos 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 (tristyly, enantioystyly and homostyly) onto the chloroplast-based tree, we used a weighting scheme (see below) that favours the loss of tristyly or enantioystyly 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 enantioystly 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 tristyly. This alternative optimisation requires between one and four independent origins of tristyly within the family; the number of origins of tristyly depends partly on how the trichotomy involving E. azurea, E. heteroasperma and E. diversifolia is resolved. However, a range of microevolutionary and genetic evidence (described in the next section) indicates that the breakdown of tristyly to homostyly is a relatively simple process that occurs frequently. In addition, a number of lines of evidence suggest that the evolution of tristyly 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. Tristyly is known to have evolved within only four or five angiosperm families (Pontederiaceae, Lythraceae, Oxlidaceae, Amaryllidaceae and possibly Conaraceae; 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 heterostyly is any indication, tristyly appears to have much more difficulty evolving than distyly. 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 tristylos 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 tristyly had a single origin within the family.

The weighted optimisation of floral conditions on the tree indicates a single origin of tristyly in the family (Figs 2A, 2B). There is a certain danger of circularity in using a weighting scheme that favours a single origin of tristyly 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 tristyly or enantioystyly over their gain during the optimisation process.

In an evolutionary scheme proposed by Lloyd and Webb (1992a, 1992b), heterostyly 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
Monocotyledons: systematics and evolution

Under the evolutionary model of Lloyd and Webb (1992a, 1992b), heteromorphic incompatibility, the class of self-incompatibility associated with the heterostylosous syndrome, arises after the floral heteromorphism. It arises either as a passive consequence of (co)adaptation of each class of pollen to the styrar 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 heterostylosous species. Differences in the site and strength of action of SI are well documented in tristylos species of Pontederiaceae. For example, the different illegitimate pollen classes fall at different, but characteristic points in the styrar 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. heteroperma and E. diversifolia was arbitrarily resolved to permit character optimisation using user-defined character types in MacClade. Figs 2A and 2B: Evolution of tristyly, enantioistyly and homostyly in Pontederiaceae. The "Floral" user-defined character type employed in these reconstructions gives a slightly smaller weight to the loss of tristyly or enantioistyly (a shift to homostyly or floral monomorphism) than to the gain or interconversion between these two flower types. Weights employed: loss of tristyly or enantioistyly = 2 steps; gain of enantioistyly or tristyly or shift between them = 3 steps. Alternative resolutions of the trichotomy involving E. azurea, E. heteroperma and E. diversifolia lead to an optimisation with two, rather than one, loss(es) of tristyly in this clade (not shown). Philydrum lanuginosum is enantioistylos, but other potential sister-groups to Pontederiaceales have some enantioistylos taxa (see text), or are floral monomorphic. Two different codings of the outgroup's floral state were therefore examined; the outgroup was coded as either monomorphic (Fig. 2A) or enantioistylos (Fig. 2B). Monochoria cyanea was coded as uncertain for floral form (i.e. enantioistylos or monomorphic).

Abbreviations: OUT = Outgroup; HYD = Hydrorhiz gardneri; HSEU = Heteranthera seubertiana; ZDUB = Heteranthera (Zosterella) dubia; HZOS = H. zosterifolia; HREN = H. reniformis; HOBIL = H. oblongifolia; HROT = H. rotundifolia; HLM = H. limosa; EDIV = Eichhornia diversifolia; HET = E. heteroperma; EAZU = E. azurea; ECRA = E. crassipes; EPAR = E. paradoxa; ESP = Eichhornia sp.; EPAN = E. paniculata; EMYE = Eichhornia meyeri; PSAG = Pontederia sagittata; PCCOR = P. cordata var. cordata; PCLAN = P. cordata var. lancifolia; PCOVA = P. cordata var. ovalis; RROT = Pontederia (Ruevia) rotundifolia; MAVG = Monochoria vaginalis; MOKOR = M. korakovi; 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.
Monocotyledons: systematics and evolution

![Diagram showing the evolution of heteromorphic self-incompatibility (SI) in Pontederiaceae](img)

Self-incompatibility
Character type: Unordered
- SC
- SI
- Equivocal

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. azuera*, *E. heterosperma* and *E. diversifolia*, SI arises either once or twice in the clade containing *Pontederia* and *E. azuera* and associated homostyles. With the resolution of *E. azuera* 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 tristyly in the family. For abbreviations see caption for Figs. 2A and B.

Barrett, 1986 and *P. sagittata* (Scribello and Barrett, 1991b). Self-incompatibility is stronger overall in tristylosous *Ponederia* species than in *Eichhornia* species, where it is found only in *E. azuera*. In all tristylosous *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 tristylosous *Eichhornia* species only *E. azuera* 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 preponderance 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.

![Diagram showing stamen and style configurations in the three most common floral forms in Pontederiaceae](img)

A. Tristyly

B. Homostyly

C. Enantioistyly

Fig. 3. Schematic representation of stamen and style configurations in the three most common floral forms in Pontederiaceae. A. Tristyly. Individuals produce either long-, mid- or short-styled flowers, depending on their genotype at two allelic 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. Enantioistyly. 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 adaption. 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-
Monocotyledons: systematics and evolution

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 tristyly 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 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 homostyly 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 branches along the branch(es) leading to the two homostyly 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 tristyly. These conclusions concerning the evolutionary history of SI in Pontederiaceae also serve as a caution against assumptions that the existence of self-compatibility in heterostyly taxa (or morphs) always represent a degenerate condition (cf., Ornduff, 1972; Weller, 1992). In the optimisation 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 heterostyly.

B. Effects of the Selfing Syndrome on Phylogenetic Reconstruction

In virtually every heterostyly group, multiple shifts to predominant self-fertilisation have occurred via the evolution of homostyly. Homostyle 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 homostyly are evolutionarily derived from heterostyly ancestors. In many cases the evolution of homostyly in heterostyly 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-fertilisation 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), Aneraria (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 sources of homostyly within the family; one in Pontederia (P. parviflora) and the other in Eichhornia. They suggested that the finding of a single origin for homostyly 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 tristyly 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 migrationary 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 tristylistic 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 homostyly 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 homostyly occur readily. The phylogenetic reconstruction based on molecular evidence from the chloroplast (Figs 2A, 2B) indicates that tristyly evolved near the base of the family and was subsequently lost on at least three occasions, with at least two losses giving rise to homostylyous species. One loss was associated with a shift to an enantostylous floral form in Monochoria. Pontederia includes one species lacking tristyly (P. parviflora). This was not available for the current molecular analysis, but probably represents another case of the loss of tristyly.

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 (Famili 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 Enantostyly

Outside Pontederiaceae, heterostyly does not occur in any putatively related order of monocotyledons (e.g., Phylloclades, Haemodorales, Commelineae). A report of heterostyly in Anelisena aquinociale (Commelineae) by Vogel (1955) is almost certainly a misinterpretation of the true nature of the polymorphism (Ornduff, 1974; Faden, 1991; S. Vogel, pers. comm.). Heterostyly has only reliably been reported from two other monocotyledonous taxa: Nivenia of Iridaceae (Mulcahy, 1965; Goldblatt and Bernhardt,
<table>
<thead>
<tr>
<th>Monocotyledons: systematics and evolution</th>
</tr>
</thead>
</table>

### Table 2: Occurrence of heterostyly in the monocotyledons and general features of the syndrome.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Pontederiales</th>
<th>Eichhornia</th>
<th>Narcissus</th>
<th>Irisacea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of heterostyly species (total number in genus)</td>
<td>5(6)</td>
<td>3(3)</td>
<td>1(3)</td>
<td>5(9)</td>
</tr>
<tr>
<td>Type of heterostyly</td>
<td>Triphyllly</td>
<td>Triphyllly</td>
<td>Triphyllly</td>
<td>Distyly</td>
</tr>
<tr>
<td>Expression Type</td>
<td>TSI</td>
<td>TSI</td>
<td>TSI</td>
<td>Absent</td>
</tr>
<tr>
<td>Incompatibility Type</td>
<td>Triphyllly</td>
<td>Triphyllly</td>
<td>Triphyllly</td>
<td>Distyly</td>
</tr>
<tr>
<td>Pollen Type</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
<td>Absent</td>
</tr>
<tr>
<td>Auxiliary polymorphisms</td>
<td>Strong</td>
<td>Weak</td>
<td>Weak</td>
<td>Absent</td>
</tr>
</tbody>
</table>

In contrast, enantiostyly is reported from three orders of monocotyledons with possible close affinities to Pontederiales: Philydrales (Simpson, 1990), Haemodorales (Wilson, 1887; Ornduff and Dalberger, 1978; Simpson, 1990) and Commeliniales (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 Dalberger, 1978; Dalberger and Ornduff, 1980; Dalberger, 1981; Buchmann, 1983). These assemblages of floral characters are found to varying degrees in the enantiostyloous monocotyledons (Table 3). These traits are discussed with respect to their distribution among enantiostyloous taxa and the possible evolutionary significance of their associations with the enantiostyloous 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 Dalberger, 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 reflexically 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 enantiostyloous 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, 1922). 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). *Zasterella dubia*, *Hydrothrix*, and *M. cyanea* all lack stamen dimorphisms (Eckenwalder and Barrett, 1986) and at least the first two are also not enantiostyloous. Apart from Pontederiales and *Cyperus* (Dalberger 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 *Anelena* 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* (Caesalpiniaceae) (Dalberger, 1981) flowers in these species possess two laterally-placed stamens such that whether the style is left- or right-bending, it is always...
reflected against one of these stamens. We feel less hesitant about calling this floral heteromorphism enantiostrły.

Enantiostrły has most often been interpreted as an adaptation for increasing the efficiency of cross-pollination (Todd, 1882; Iyengar, 1923; Ornduff and Dumbleton, 1978; Webb and Lloyd, 1986). In a manner analogous to heterostóry, the consistent spatial positioning on the pollinator’s body is that 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 by individual (Dumbleton, 1981). Geitonogamous matings will also be reduced if an SI system is present, as is the case with *Cyanella alba* and *C. lutea* (Tecophilaceae) (Dumbleton and Onduff, 1980), but obviously SI by itself cannot act to increase the efficiency of cross-pollen transfer.

Dumbleton (1981) suggested that the primary role of enantiostrły in species of *Cassia* is to provide pollinators with unobstructed access to feeding anthers, while protecting the gynostegium during vibrational collection of pollen (buzz pollination) from poricidal anthers. It is unclear if this explanation holds for enantiostrły species in general, since it is not certain that all enantiostrły species are buzz pollinated. For example, species of *Heterantha* and most enantiostrły 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, enantiostrły 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, enantiostrły may not function to increase the efficiency of cross-pollen transfer in radially-symmetrical flowers. Among the enantiostrły taxa of Haemodoraceae, only *Wachendorfia*, *Schiekia*, *Xiphidium santoninum*, and *Barbretia aurea* possess zygomorphic perianths (Simpson, 1990; Simpson 1993, Fig. 10). Species of Philydraceae have enantiostrły flowers with only a single stamen, and strongly zygomorphic perianths. Within Pontederiaceae, species of *Heterantha* are mostly zygomorphic while species of *Monochoria* are actinomorphic. Flowers borne on *Monochoria* inflorescences are outward-facing, so that pollinators are likely to approach them in a consistent orientation. Because of this feature and their enantiostrły-heterantherous morphology, the flowers of species of *Monochoria* may be functionally zygomorphic. A parallel case is found in Tecophilaceae, where flowers of *Cyanella alba* and *C. lutea* are actinomorphic, but are enantiostrły, outward-facing and apparently heterantherous (Dumbleton and Onduff, 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 *Heterantha* (Van Heel, 1988; Simpson, 1990) and Philydraceae (Dahlgen 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 enantiostrły taxa (*Solomon rotundatus: flowers*, 1975; *Cyanella: Dumbleton and Onduff*, 1980; *Cassia: Dumbleton*, 1981). Enantiostrły is also not always associated with an absence of nectar secretion. Apart from *Xiphidium*, all enantiostrły Haemodoraceae species have sepals nectaries (Simpson, 1993).
Monocotyledons: systematics and evolution

Repetition in elements of the enantiostylo syndrome among phylogenetically disjunct taxa is probably indicative of similar selective pressures operating on floral morphology. The regular association of enantiostyly with outward-facing, zygomorphic flowers (Table 3) suggests that consistent positioning of the pollinator is usually an important part of the functional operation of enantiostyly. Heteranthory, 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 enantiostylo 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 Phylldraceae, 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; Systma 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 Pontederiaceae and the evolutionary history of polymorphic breeding systems present in the family Pontederiaceae. We argue that tristyly 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 Pontederiaceae 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.

Acknowledgements

We thank Michael Donoghue, Jim Eckenwalder, Bob Faden, Josh Kohn, Michael Simpson and Wendy Untereiner for fruitful discussion, and the many workers responsible for the rbcl sequence data employed in the monocotyledon analysis. Gerard Zurawski (DNAX Research Institute) kindly provided rbcl sequencing primers. We gratefully acknowledge the financial support of research grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to SCHB, and a Connaught Scholarship from the University of Toronto, an Ontario Graduate Scholarship from the Province of Ontario, and an NSERC post-graduate fellowship to SWG.

References

Monocotyledons: systematics and evolution


