Amer. J. Bot. 74(12): 1831–1841. 1987.

DEVELOPMENT OF TRISTYLY IN PONTEDERIA CORDATA (PONTEDERIACEAE). I. MATURE FLORAL STRUCTURE AND PATTERNS OF RELATIVE GROWTH OF REPRODUCTIVE ORGANS[1]

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ABSTRACT

Pontederia cordata is a tristylous, self-incompatible emergent aquatic. Measurements of reproductive organs in mature flowers and developing buds and analysis of relative growth rates of the styles, filaments, and floral tube were used to analyze the developmental processes that result in reciprocal positioning of reproductive parts among the style morphs. Flowers are trimerous with two series of three tepals, two series of three stamens, and a tricarpellate ovary with a single ovule. Each flower has stamens of two lengths, but the two lengths correspond to upper vs. lower position in the zygomorphic flowers, not to the two series initiated in the primordium. Morph-dependent variation in stigma height depends on differences in style length, not ovary length. Differences among morphs in anther height result from differences in the position of filament insertion on the floral tube and differences in filament length. Styles of the three morphs develop to different lengths as a result of two distinct processes. Styles of L and M morphs develop at different rates with the L style growing more rapidly than the M style, whereas styles of the S morph have a brief period of rapid growth followed by early inhibition. Stamen growth in the S morph also differs qualitatively from that in the L and M morphs, which are distinguished from each other by quantitative differences in growth rates. Results indicate that the developmental processes that result in the complementary arrangements of organs in different morphs are morph-specific. An attempt is made to integrate the findings of this study with the model for genetic control of tristyly.

ANALYSES of allometric growth have been used to formulate models of the evolution of floral form in several angiosperm species (Guerrant, 1982; Lord, 1982; Kirchoff, 1983; Smith-Huerta, 1984; Lord and Hill, 1987). These studies have compared development of flowers of related species (Guerrant, 1982; Kirchoff, 1983; Smith-Huerta, 1984) or development of different kinds of flowers produced by one individual (Lord, 1982; Lord and Hill, 1987). Changes in growth rate (acceleration or retardation) and variation in the onset or completion of a common developmental event are frequent causes of floral variation. In some cases a single type of heterochronic change is responsible for major divergences in form (Guerrant, 1982; Lord, 1982; Kirchoff, 1983), while in other cases a variety of heterochronic processes account for morphological differences in mature flower structure (Smith-Huerta, 1984).

The genetic bases for heterochronic changes in floral development are largely unknown. The floral polymorphism tristyly provides a useful system in which to study the genetic controls on heterochrony since tristyly has a relatively simple genetic basis which results in discrete morphological differences among flowers of a species. The most common model for the inheritance of tristyly involves two gene loci, *S* and *M*, which control the relative positions of anthers and stigmas in the floral morphs. Each locus has two alleles, and *S* is epistatic to *M* (Fisher and Mather, 1943; Weller, 1976; S. C. H. Barrett, unpublished data). Plants with genotype *ssmm* are long-styled with anthers positioned below the stigma at mid and short levels. Plants with genotypes *ssMm* or *ssMM* are mid-styled with long level anthers above the stigma and short level anthers below the stigma. Plants with genotypes *Ssmm, SsMm,* or *SsMM* are short-styled with mid and long level anthers positioned above the stigma. This pattern of inheritance is found in the three families in which tristyly is known to occur (Lythraceae, Oxalidaceae, and Pontederiace-

[1] Received for publication 25 September 1986; revision accepted 17 March 1987.

We thank Petra Donnelly, Martin Morgan and David Storch for technical assistance and acknowledge financial support to JHR from the Florida International University Faculty Development Fund and McKnight Programs and to SCHB from an operating grant from the Natural Sciences and Engineering Research Council of Canada.

ae). Differences in the linkage relationships among the *S* and *M* loci and polyploidy result in variations of the basic two-locus model.

In the Pontederiaceae tristyly occurs in two genera, *Eichhornia* and *Pontederia*. Four of the five species of *Pontederia* (*P. cordata* L., *P. rotundifolia* L., *P. sagittata* Presl, and *P. subovata* (Seub.) Lowd.) are tristylous; the remaining species, *P. parviflora* Alex., is monomorphic for style length (Lowden, 1973). In species that have been studied experimentally (*P. cordata, P. rotundifolia,* and *P. sagittata*), tristyly is associated with a physiological self-incompatibility system (Barrett and Anderson, 1985). We initiated a study of floral development in the pickerelweed, *P. cordata,* in order to define the developmental processes that result in differences in form among the floral morphs, as well as to provide data for comparison with the related tristylous, self-compatible *Eichhornia paniculata* (Spreng.) Solms (Richards and Barrett, 1984). In this paper we address the following questions: 1) What is the general organography of reproductive shoots and how does mature floral organization differ among the three morphs? 2) What are the heterochronic processes that result in differences in floral structure? and 3) Are the developmental processes that result in structural differences morph specific and/or organ specific? In a subsequent paper we will discuss initiation and early development of reproductive parts in the floral morphs.

MATERIALS AND METHODS—Plants were collected from two natural populations of *Pontederia cordata* representing the two taxonomic varieties present in N. America: *P. cordata* L. var. *cordata* was obtained from the shoreline of Paugh Lake, near Barrys Bay, Ontario, Canada; and *P. cordata* var. *lancifolia* (Mohl.) Torrey was collected from a marsh adjacent to the Tamiami Trail, 1 mi. west of Krome Ave., Miami, FL. Mature flower structure was examined in flowers of both varieties. Since the two varieties do not differ significantly in details of floral structure, our discussion does not distinguish between them. Cell sizes were measured in flowers of *P. cordata* var. *lancifolia,* whereas allometric studies were done on buds of *P. cordata* var. *cordata.* All measurements were made on material which had been fixed in either FAA or CRAF III (Berlyn and Miksche, 1976). Material for scanning electron microscopy was fixed in CRAF III, dehydrated in a graded alcohol series, CO_2 critial point dried, and viewed with an ISI SUPER-IIIA scanning electron microscope.

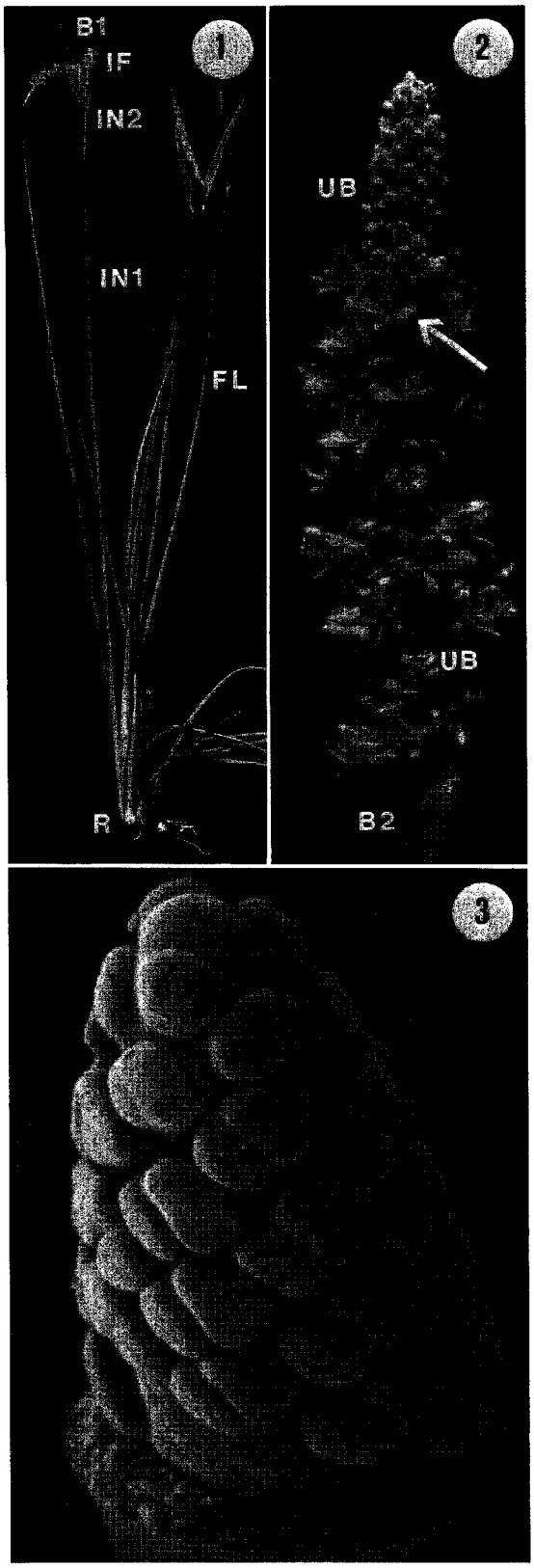
Mature flower structure—To define the contribution of different floral parts (e.g., ovary vs. style, filament length vs. insertion position) to stigma and anther height, measurements of mature flowers were made using a dissecting microscope equipped with a calibrated ocular micrometer. Twenty flowers of each morph in both varieties were measured for 1) length of the flower, 2) length of the free filament and tube below filament insertion for all stamens, and 3) length of the style and ovary.

Measurements of epidermal cell length were used to assess the relative contributions of cell length to final organ length for styles and filaments of each morph. Measurements of ovary and style length were made in 10 mature flowers of each morph of *P. cordata* var. *lancifolia.* Fifty epidermal cells were measured on these styles, using a light microscope equipped with a calibrated ocular micrometer.

Filament epidermal cell sizes were determined for five mature flowers of each morph of *P. cordata* var. *lancifolia.* In all flowers measurements of the length of the free filament and of cell size were made on an outer stamen from each stamen level within a flower. Fifty cells were measured per filament.

Relative growth rates—The relative growth rates of reproductive organs were investigated by dissecting 60 flowers per morph ranging in size from buds 2 mm long to expanded flowers. Bud length and width, ovary and style length, the length of an outer filament from each stamen level, and the length of the floral tube below the filament were measured using a dissecting microscope equipped with a calibrated ocular micrometer. These data were used to construct allometric growth plots for the different flower parts of each morph. Comparison of the plots of bud length vs. bud width showed no significant differences among morphs in overall growth patterns. Hence, bud length was used as a standard for comparing growth among morphs. In studies of style growth for each morph 20 additional buds between 1 and 2.5 mm were cleared in 5% alcoholic NaOH and examined with the light microscope.

Cell sizes were compared using Kruskall-Wallis nonparametric tests. Growth rates were examined with regression analysis and nested analysis of covariance. Data were analyzed on a Zenith Z-151 microcomputer using the STAT-PRO Statistical Package (Wadsworth Publishing Co.). Curve-fitting and estimation of nonlinear models were done on an Apple MacIntosh Plus using Statview 512 (Brain Power, Inc.).



Throughout this paper the abbreviations L, M, and S designate the long-, mid-, and short-styled morphs or their respective styles, while l, m, and s refer to long-, mid-, and short-level stamens, filaments, or floral tubes below stamens, respectively. When referring to the stamen level of a particular morph, the stamen level abbreviation is separated from the morph abbreviation with a slash. Thus, the mid-level stamens of the short morph are designated m/S.

RESULTS—*General organography*—*Pontederia cordata* grows sympodially. A horizontal rhizome with short internodes produces foliage leaves which are 0.5–1.5 m long and are arranged distichously. Each leaf has a sheathing base, an elongated petiole, and a lamina which varies from cordate in *P. cordata* var. *cordata* to lanceolate in *P. cordata* var. *lancifolia* (Fig. 1). After a period of vegetative growth, the shoot apex produces a terminal, spicate inflorescence subtended by two inflorescence bracts (Fig. 1, 2). The internode below the first inflorescence bract elongates, elevating the inflorescence slightly above the foliage leaves. The first inflorescence bract has a short petiole (1–3 cm), but its lamina resembles the laminae of preceding foliage leaves. This bract's sheathing base initially encloses the next bract and developing inflorescence. The second bract has a greatly reduced lamina (Fig. 2). Its membranous leaf base completely surrounds the inflorescence during development. As the inflorescence expands, the internode between the inflorescence bracts expands 10–20 cm. The internode between the second bract and the first flowers grows 3–5 cm, and the inflorescence axis also elongates, freeing the inflorescence from the second bract's sheath (Fig. 1, 2). The inflorescence axis continues to grow as flowers expand.

The inflorescence consists of a series of spirally arranged floral branch primordia which are produced acropetally (Fig. 3). Each branch

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Fig. 1–3. *Pontederia cordata* var. *lancifolia*. **1.** A ramet with horizontal rhizome (R), erect foliage leaves (FL), and inflorescence with elongated first internode (IN1), foliate first bract (B1), shorter second internode (IN2), membranous second bract (not clearly visible), and flowers (IF). **2.** Close-up of inflorescence; flowers, as shown at arrow, are trimerous, slightly zygomorphic, and have a prominent nectar guide on the banner petal; UB = unopened buds; B2 = second inflorescence bract. **3.** SEM of inflorescence primordium with acropetally initiated branch primordia (BR) which produce flower primordia (FP) in scorpioid cymes; A = inflorescence apex.

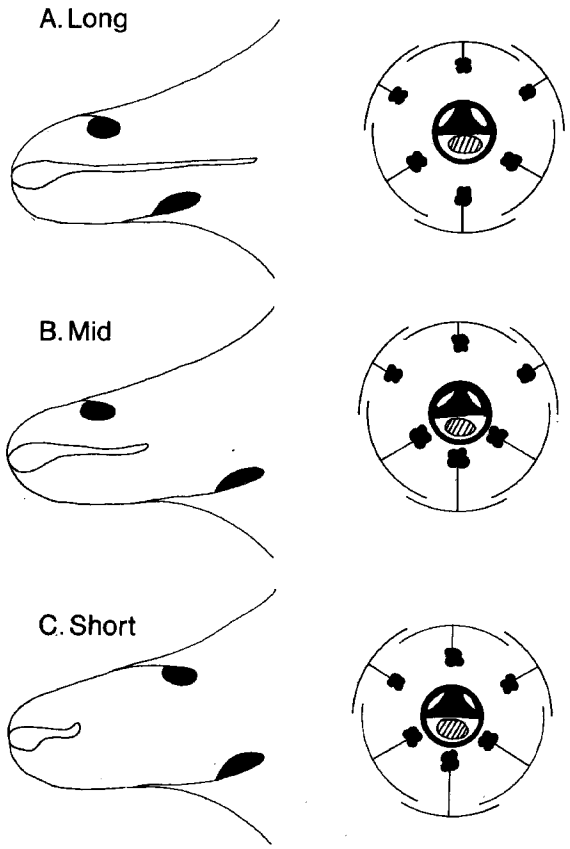


Fig. 4. Diagram of flower structure in longitudinal and cross section in the three morphs of *Pontederia cordata*. The longitudinally sectioned diagrams show the reciprocal positioning of anthers and stigmas and differences in stamen insertion between the upper and lower levels within flowers. The cross sections show stamen insertion patterns on the tepals and position of the fertile carpel.

primordium is a condensed scorpioid cyme. Flower expansion proceeds from the base of the inflorescence to the top and from the first-produced flower on each cyme to successively-produced flowers. This inflorescence structure results in mature flowers occurring throughout the length of the inflorescence during much of its flowering phase (Fig. 2).

When the main shoot apex terminates in an inflorescence, a renewal shoot bud axillary to the last foliage leaf expands. The bud in the axil of the next-to-last foliage leaf may also grow out, resulting in clonal ramification. Each axillary shoot produces a prophyll, zero to many foliage leaves, and a terminal inflorescence.

Mature flower structure—Flowers of *P. cordata* are trimerous, having two series of three tepals, two series of three stamens, and a tricarpellate ovary (Fig. 4). A single ovule is produced in the carpel on the flower's lower side (Fig. 4). The tepal series are joined into a floral tube on which the stamens are inserted. Flowers are 12–16 mm in length, moderately zygomorphic, and blue to purple with a prominent yellow and green nectar guide on the banner petal (Fig. 2).

Depending on morph, a flower has either a long (L), medium (M) or short (S) length style and two sets of stamens which correspond in height to the stigma levels of the other morphs. Variation in stigma height results from differences in style length alone, since ovary length does not vary significantly among the morphs (Table 1). Stylar cell length differs among morphs, with S styles possessing the shortest cells, L styles the longest, and M styles with intermediate cell lengths (Table 1). When differences among morphs are compared by calculating ratios of the S style and stylar cell lengths to those of the other two morphs, however, the ratios of style lengths are greater than the ratios of cell lengths. This implies that differences occur among styles in cell number as well as cell length (Table 1). A similar pattern is evident in *E. paniculata* (Richards and Barrett, 1984).

In *P. cordata* stamens of each level within a flower are developmentally heterogeneous. The two stamen levels within each flower do not correspond to the two stamen series initiated

TABLE 1. *Measurements of organ and cell sizes for gynoecium in the morphs of* Pontederia cordata *var.* lancifolia

	Floral morph		
	L	M	S
Ovary length[a]	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1
Style length[a]	11.1 ± 0.6	6.1 ± 0.2	1.3 ± 0.1
Stigma height[a]	12.6 ± 0.7	7.6 ± 0.3	2.7 ± 0.1
Stylar cell length[b]	129.1 ± 9.7	84.8 ± 5.2	28.6 ± 1.5
Ratio of style lengths[c]	8.5	4.7	1
Ratio of cell lengths[c]	4.5	3.0	1

[a] In mm.
[b] In μm.
[c] Ratio of short to other morphs.

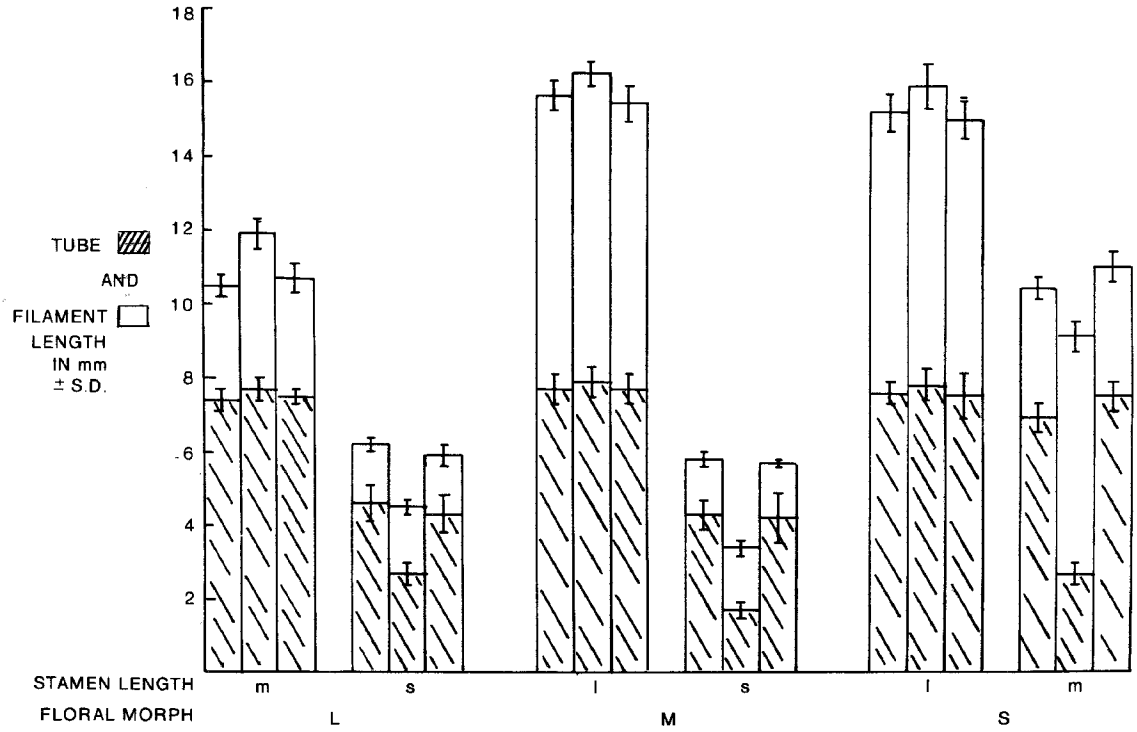


Fig. 5. Diagram of tube and filament length in the three stamens of both the upper and lower stamen levels within each flower of the three morphs of *Pontederia cordata* var. *cordata*; data were similar for *Pontederia cordata* var. *lancifolia*. The central stamen within each group of three stamens is the central stamen in that stamen level. Patterns of within-level stamen variation are correlated with stamen level, not absolute stamen length. Anther height is a function of both filament length and position of insertion on the floral tube.

in the primordium but comprise an upper, shorter level of stamens (s/L, s/M or m/S) and a lower, longer level (l/M, l/S, or m/L) (Fig. 4, 5). The shorter filaments are narrower than the longer filaments within a given flower.

The three stamens in each stamen level show consistent differences in length when compared among themselves. In upper stamens the central stamen is longer than the outer stamens, whereas in lower stamens, the central stamen is shorter. These patterns occur regardless of whether stamens are m or s stamens in the upper level or m or l stamens in the lower level (Fig. 5). The variation in stamen length within each level is correlated with the tepal series on which the stamens are inserted. The two outer stamens in the upper level are inserted on members of the outer tepal series, while the central upper stamen is inserted on one of the inner tepal series. Similarly, the two outer stamens in the lower stamen level are inserted on tepals in the inner series, while the central lower stamen is inserted on a tepal in the outer series (Fig. 4, 5). If the 3-dimensional structure of the flower is considered, there is a gradient in stamen length from the upper, central shortest to the lower, central longest, with a major discontinuity occurring between the two stamen levels.

Differences among morphs in anther height are a product of position of filament insertion on the floral tube and of differences in filament length (Fig. 5). Short level stamens have both shorter filaments and are inserted further down the floral tube than l level stamens. The difference between l and m level stamens, however, results primarily from differences in fil-

TABLE 2. *Filament cell lengths in μm in the floral morphs of* Pontederia cordata *var.* lancifolia

Filament level	Floral morph L	*M*	*S*
l	—	119 ± 12	125 ± 9
m	50 ± 5	—	103 ± 8
s	25 ± 2	28 ± 4	—
Ratio of filament lengths[a]	2.25	5.08	1.77
Ratio of cell lengths[b]	2.00	4.25	1.21

[a] Ratio of average filament lengths between levels in flowers used for cell measurements.

[b] Ratio of average cell lengths for filaments within morph.

TABLE 3. *Regression equations for relative growth rates of style length, floral tube length below stamen insertion, and filament length against bud length for the three floral morphs of* Pontederia cordata *var.* cordata. *Correlation coefficients are given in parentheses below each equation. F values were derived from a nested analysis of covariance which tested first for the difference between regression line slopes; if slopes were not significantly different, then regression intercepts were compared;* $* P \leq 0.05$; $*** P \leq 0.01$

	Floral morph			
	Long	Mid	Short	*F*
Bud length vs. style length	−2.69 + 1.62 log x (0.985)	−2.04 + 1.42 log x (0.977)	0.73 + 0.54 log x (0.717)	35.13***
Bud length vs. tube length				
Long	—	−2.56 + 1.54 log x (0.975)	−2.31 + 1.47 log x (0.987)	3.47, 5.76*
Mid	−2.61 + 1.55 log x (0.972)	—	−2.25 + 1.45 log x (0.961)	4.21*
Short	−1.90 + 1.31 log x (0.935)	−1.73 + 1.26 log x (0.927)	—	0.58, 0.65
F	17.79***	24.29***	0.27, 26.03***	
Bud length vs. filament length				
Long	—	−2.85 + 1.60 log x (0.971)	−2.43 + 1.48 log x (0.952)	4.68*
Mid	−1.14 + 1.10 log x (0.955)	—	−1.29 + 1.13 log x (0.892)	0.36, 1.92
Short	0.08 + 0.74 log x (0.832)	0.22 + 0.71 log x (0.888)	—	0.34, 3.38

ament length (Fig. 5). Whether the m level is the lower stamen level, as in the L morph, or the upper stamen level, as in the S morph, insertion level and filament length of the outer stamens are similar (Fig. 5). The central stamens of the two mid levels, however, are very different, since the central m/S stamen is inserted lower on the floral tube and has a longer filament than the central m/L stamen (Fig. 5).

Filament cell size differs among stamen levels. Filament cells in s level stamens are significantly smaller than filament cells in m or l level stamens, while filament cells in m level stamens are smaller than cells in l level stamens (Table 2). Filament cell sizes are the same for corresponding stamens at the l and s levels. In m level stamens, however, filament cell size differs between the L and S morphs, with m/S filament cells twice as long as m/L filament cells (Table 2).

When the ratio of filament length is compared to the ratio of filament cell lengths for a given flower, the ratio of filament lengths always exceeds the ratio of cell lengths. This implies that differences in cell division, as well as in cell elongation, occur among morphs (Table 2).

Relative growth rates of reproductive organs—Styles of the three floral morphs develop to different lengths as a result of two distinct developmental processes. Styles of the L and M morphs develop at significantly different rates, with L styles growing more rapidly than M styles (Table 3; Fig. 6). In contrast, styles of the S morph undergo a brief period of rapid growth early in development, but their growth slows sharply in buds approximately 3 mm (log 3.5 in μm) long (Fig. 6).

In each morph growth of the floral tube below stamen insertion is linear in log-log plots against bud length (Table 3; Fig. 7). The floral tube of s level stamens, however, displays more variation in growth rate than tubes of l or m level stamens. Relative growth rates of floral tubes associated with l level stamens are not significantly different (Table 3). Relative growth rates of floral tubes of s level stamens are also not significantly different (Table 3). Intercepts are slightly different between l level tubes but do not differ significantly between s level tubes (Table 3).

Growth rates for tubes of m level stamens are different at the 5% but not at the 1% level of significance (Table 3). The difference probably reflects the difference in position within the flower of the two m stamen levels, as discussed above. In evaluating this data, it should be noted that only the outer stamens of a level were measured and, thus, differences in growth between the central stamens of the m levels are not included in the analysis.

Since floral tubes below filaments of the same level of different morphs either do not differ

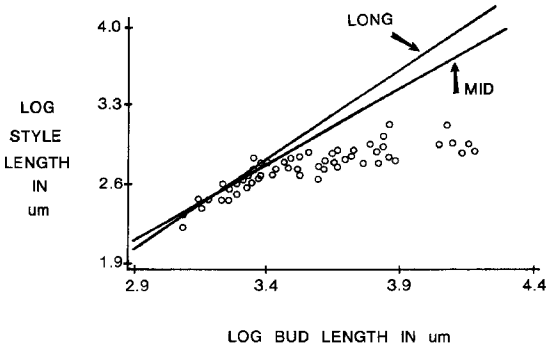


Fig. 6. Logarithmic plot of bud length vs. style length from buds 1 mm in length to anthesis for the three morphs of *Pontederia cordata* var. *cordata*. Growth of the L and M morphs are summarized by regression lines (see Table 3 for equations), while data for the S morph are plotted (O). Styles of L and M morphs have different growth rates, while styles of the S morph are inhibited in buds approximately 3 mm long.

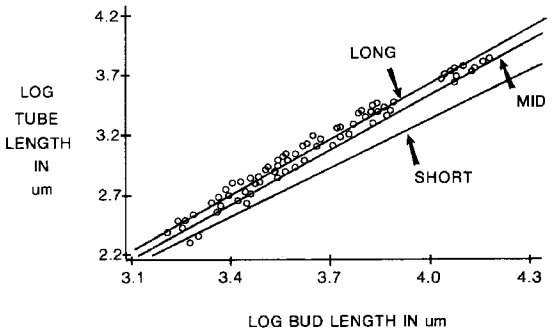


Fig. 7. Logarithmic plot of bud length vs. length of floral tube below stamen insertion for buds 2.5 mm in length to anthesis for the three stamen types of *Pontederia cordata* var. *cordata*. Data from different morphs were combined by stamen length. Growth of all three tubes are summarized by regression lines (see Table 4 for equations); data for floral tubes below long stamens are shown to illustrate number and distribution of points. The lines for long and mid length floral tubes have similar slopes but different intercepts, while the line for the short length tubes has a different slope.

(L, S morphs), or differ slightly (M morph), data from different morphs were combined by stamen level to compare growth among levels (Table 4, Fig. 7). The differences in floral tube length among stamen levels do not arise from variations in one growth pattern. Instead, different types of developmental processes cause the differences among levels. Tubes associated with l and m level stamens have a significantly greater growth rate than tubes below s level stamens (Table 4, Fig. 7). Allometric plots of tubes below l and m level stamens have similar slopes ($F = 0.01$, ns). The allometric plot of the m level tube, however, has a smaller intercept ($F = 16.56$, $P \leq 0.01$; Table 4, Fig. 7), implying differences at origin or in early development between l and m level tubes.

Filaments of the three stamen levels have more variable growth rates than do floral tubes, as indicated by lower values of r^2 for the same level in each morph (Table 3). In addition, growth of the upper stamen level within each morph is not well described by a log-linear plot (Table 3, Fig. 8–11). Growth of s level filaments is inhibited in buds longer than approximately 6 mm (log 3.8 in μm, Fig. 8–10). Short level filaments from the L and M morphs do not exhibit major differences in this pattern (Fig. 9–10). Filaments of m level stamens of the S morph (the upper stamen level in that morph) have a more complex pattern of growth than filaments of s level stamens. Growth analyses of these filaments indicate a double sigmoid curve, with two phases of rapid growth separated by a slower phase (Fig. 11). The data are better described by a 4th order polynomial ($r^2 = 0.937$) than by a linear equation ($r^2 = 0.892$). This complex growth pattern is not apparent in filaments of the m level stamens of the L morph (Fig. 9). When combined data for filaments of l and m level stamens are compared, the two levels have significantly different growth rates (Table 4, Fig. 8).

Stamen growth compared among morphs—Stamen growth in the S morph differs qualitatively from that in the L and M morphs, while stamens in the latter morphs differ primarily in developmental rates. In the L and M morphs the floral tube grows at different rates on the

TABLE 4. *Regression equations for floral tube length below stamen insertion and filament length against bud length for data combined by stamen level among the three floral morphs of* Pontederia cordata *var.* cordata. *Correlation coefficients are given in parentheses below each equation. F value derived from an analysis of covariance; *** $P < 0.01$*

	Long-level stamens	Mid-level stamens	Short-level stamens	*F*
Bud length vs. floral tube length	−2.41 + 1.51 log x (0.980)	−2.43 + 1.50 log x (0.965)	−1.81 + 1.29 log x (0.931)	21.50***
Bud length vs. filament length	−2.64 + 1.54 log x (0.958)	−1.21 + 1.11 log x (0.919)	0.14 + 0.72 log x (0.855)	

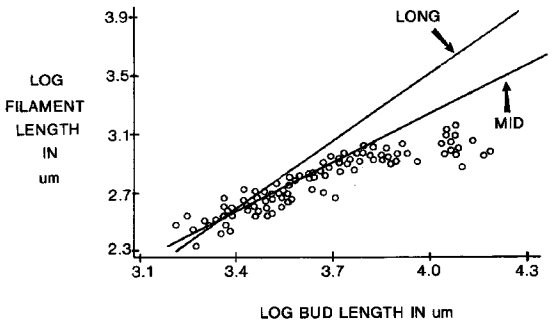


Fig. 8. Logarithmic plot of bud length vs. stamen filament length for buds 2.5 mm in length to anthesis for the three stamen types of *Pontederia cordata* var. *cordata*. Growth of the long and mid length filaments are summarized by regression lines (see Table 3 for equations) while data for the short length filaments are plotted (O). The lines for long and mid length filaments differ in slope, while the short length filaments are inhibited in buds approximately 6 mm in length.

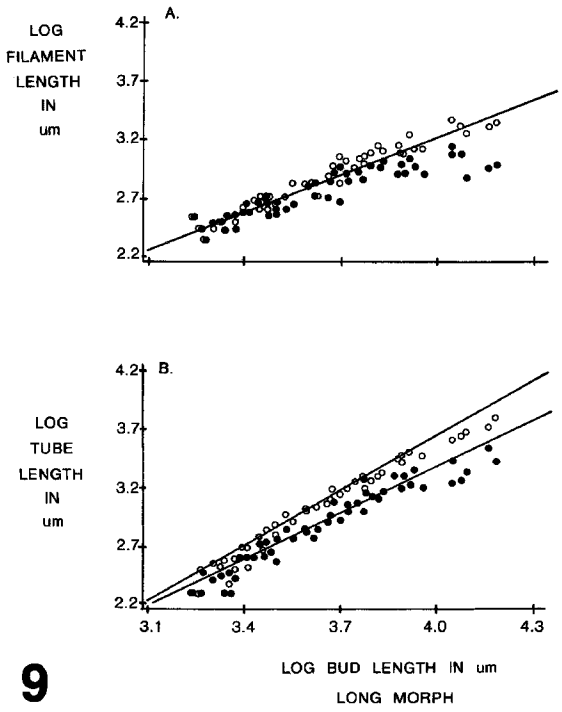
upper and lower sides of the flower (Fig. 9B, 10B; Table 3), whereas in the S morph, the floral tube grows at the same rate on both sides (Fig. 11B; Table 3), but the lines describing the growth have different intercepts (Fig. 11B; Table 3).

Filaments in the L and M morphs differ only in the growth rate of the lower filament level. The upper filaments are at the same relative level and have similar growth curves (Fig. 9A, 10A). Filament growth in the S morph differs qualitatively from that in the other morphs. This difference is most marked in the upper, m filaments, as described above, but some elements of the complex pattern of m/S filament growth are also seen in l/S filaments (Fig. 11A). When fitted with a 4th order polynomial, the r^2 for the long filament level vs. bud length increases to 0.970. The similarities in the growth patterns between S morph filament levels are most pronounced in the acceleration of growth seen in buds approximately 5 mm (log 3.7 in μm) long (Fig. 11A). In the S morph growth on the flower's upper side does not show the marked filament inhibition and decreased floral tube growth rate seen on the upper side of the flower in the L and M morphs.

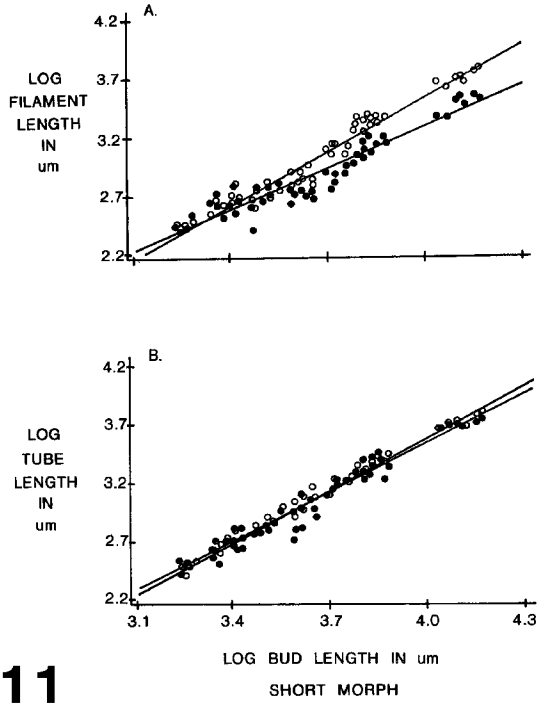
DISCUSSION—*Differences among morphs in relative growth rates of reproductive organs*—The allometric analyses suggest that at least four distinct growth processes control the precise, reciprocal positioning of reproductive organs in *P. cordata*. The processes involve 1) early cessation or inhibition of growth, as occurs in S styles and s filaments; 2) differences in growth rate, as between the L and M styles, floral tube growth below s filaments as compared to l or m filaments, and l and m/L filaments; 3) differences in origin or early development, as between floral tube growth below l and m filaments; and 4) the complex growth processes of the m/S filaments which appear to involve inhibition followed by acceleration of growth. These processes, with the exception of the fourth, have all been found to regulate various aspects of floral development in other plant species (Guerrant, 1982; Lord, 1982; Kirchoff, 1983; Smith-Huerta, 1984; Lord and Hill, 1986). In *P. cordata,* however, differences among the same organs (stamens or styles) result from contrasting processes in different morphs. For example, styles in the L and M morphs differ in length because they have different growth rates, whereas S styles are shorter than these two because their growth is inhibited relatively early in development. The developmental differences among filaments provide another example.

Both the allometric analyses and data on filament cell length indicate that the developmental processes that result in the complementary arrangements of organs in different morphs are specific to the morph, not to the organ position (upper vs. lower) or level (long, mid or short). The L and M morphs have similar developmental patterns: in each morph upper and lower sides of the flowers have different growth rates; upper and lower filament growth curves have similar shapes. Differences between these morphs arise from differences in growth rates, e.g., the differences in style length and lower level filament length. The relative positions of reproductive parts in the S morph arise by fundamentally different growth processes than are evident in the L and M morphs. The upper and lower sides of the floral tube in the S morph grow at similar relative rates and differ only slightly in intercepts. Growth of the upper m filament slows at the same stage that the s filaments of the other two morphs become inhibited, but then increases rapidly, resulting in a double-sigmoid growth curve. This secondary increase in filament growth rate may result from a rapid increase in cell elongation and result in the longer cells of the m/S filaments.

Several other features of the S morph of *P. cordata* are distinct from the L and M morphs. The self-incompatibility system tends to be strongest in the S morph and weakest in the M morph (Ornduff, 1966; Barrett and Anderson, 1985). Observations of pollen tube growth in incompatible pollinations indicate that while



9



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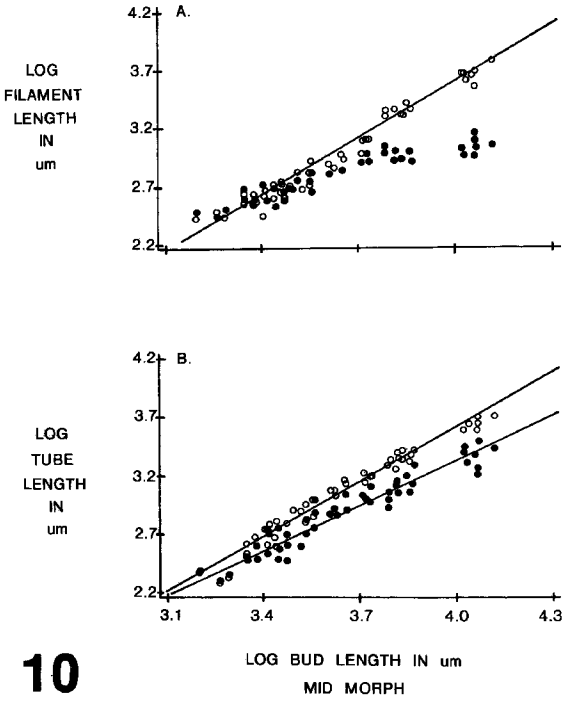

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Fig. 9–11. Logarithmic plots of filament length vs. bud length (A) and length of the floral tube below the filament vs. bud length (B) for the three morphs of *Pontederia cordata* var. *cordata*. See Table 3 for equations and text for discussion. **9.** Long-styled morph; ● = lower, mid length stamen level; ○ = upper, short length stamen level. **10.** Mid-styled morph; ● = lower, long length stamen level; ○ = upper, short length stamen level. **11.** Short-styled morph; ● = lower, long length stamen level; ○ = upper, mid length stamen level.

most pollen tubes are arrested in the style in the L and M morphs, inhibition takes place in the ovary in the S morph (Anderson and Barrett, 1986). This difference suggests that the overall properties of the incompatibility mechanism in the S morph may differ from the other morphs. Striking differences in pollen production are also evident among the floral morphs of *P. cordata*. The S morph produces less than one-half of the pollen produced by the L and M morphs (Price and Barrett, 1982). This difference is largely attributable to a reduction in the amount of pollen produced by the upper anthers of the S morph compared with the upper anthers of the L and M morphs. In the S morph these anthers are m anthers whereas in the L and M morphs they are s anthers.

Positional- and morph-related differences in filament cell size—Regardless of morph, s level filaments have relatively short cells and l level filaments have relatively long cells. In contrast, m level filament cell length differs between morphs, as does m stamen position within the flower. If degree of filament cell elongation is determined by stamen position within the flower, cell length of m filaments should be shorter when the filaments are on the upper

side of the flower (m/S) and longer when they are on the lower side (m/L). Our data shows that the opposite condition occurs. When m level stamens are the upper stamen level (m/S), they have longer cells, whereas when they are the lower stamen level (m/L), they have shorter cells (Table 2). This finding demonstrates that filament cell size is not solely dependent on position within the flower. When cell size is related to morph type, however, a pattern emerges. Filaments in the S morph have relatively long cells, while filaments in the L morph have relatively short cells (Table 2). Filaments in the M morph, in contrast, have either long or short cells, and thus a greater range of cell size from the upper to the lower side of the flower. Filament cell length, therefore, may be governed by the genes determining style morph, as well as by filament position within the flower.

Comparison of floral structure among tristylous species—Despite superficial differences in floral display and inflorescence architecture, the inflorescence of *Pontederia cordata* is very similar to that of the related tristylous species, *Eichhornia paniculata.* The major difference is that the scorpioid cymes of *E. paniculata* elongate (Richards and Barrett, 1984), whereas those of *P. cordata* remain condensed. The two species also resemble one another in details of flower structure both within and between morphs (Richards and Barrett, 1984). Thus, the dorsiventral organization of the tristylous syndrome is similar in both species, and the pattern of variation in anther height within stamen levels is the same. Differences between levels within a morph and among morphs in lengths of floral parts, in arrangement of these parts within the flower, and in cell size among parts also resemble each other in the two species.

The similarities of inflorescence and flower structure in *P. cordata* and *E. paniculata* reflect a basic developmental pattern that underlies the tristylous syndrome of the Pontederiaceae. Although stamens in the two species arise in two whorls or series of three (Richards and Barrett, 1984; J. H. Richards, unpublished data), mature anther height does not depend on this order of initiation. Instead, anther height is correlated with position (upper vs. lower) in the zygomorphic flower. Mature flowers of other tristylous species of *Pontederia* and *Eichhornia* have a similar zygomorphic organization.

These observations suggest that the development of tristyly in the Pontederiaceae may be different from that in the Oxalidaceae and Lythraceae, where stamen levels are organized in whorls rather than as upper and lower positions (Barlow, 1923; Stirling, 1933, 1936; fig. 1 in Ornduff, 1972). In addition, variation in anther height in *Lythrum* and *Oxalis* results primarily from differences in filament length, although slight differences in insertion level have been reported for *Lythrum* (Stirling, 1933, 1936). These contrasting patterns make it unlikely that the genes controlling tristyly in the three families regulate mature floral structure through similar developmental pathways.

In discussing the rarity of tristyly, Yeo (1975) hypothesizes that a necessary precondition for its evolution is the arrangement of stamens in two whorls. Although stamens in the Pontederiaceae arise in two series, each stamen level within a flower has members derived from both series. The level of a stamen within a flower is *not* determined by the series to which that stamen belongs. Thus, the development of tristyly is not dependent on the presence of these stamen series, and their occurrence is probably not important in the evolution of tristyly in this family.

Genetic control of floral development in P. cordata—Our studies of the genetics of tristyly in the genus *Pontederia* are not complete, but the genetic model for tristyly discussed above, which involves two loci (*S, M*), each with two alleles, occurs in the related *Eichhornia paniculata* (S. C. H. Barrett, unpublished data). Given the rarity of tristyly in the angiosperms and the limited number of tristylous taxa in the Pontederiaceae, it seems reasonable to assume a monophyletic origin and common inheritance pattern for the polymorphism in *Pontederia* and *Eichhornia.* Cladistic analysis of the family provides evidence in support of this assertion (Eckenwalder and Barrett, 1986). Following this assumption, we can integrate our heterochronic analysis of floral form in *P. cordata* with the putative genetic model for the inheritance of tristyly in an attempt to provide insights into the developmental system that gives rise to the polymorphism.

If we assume that genotypes for the L, M, and S morphs are *ssmm, ssM-,* and *Ss–,* respectively, then dominance and recessiveness at the *S* and *M* loci are the important genetic factors regulating floral phenotype. Thus, the *M* allele may affect floral development primarily by altering rates of development, increasing the relative growth rate of the lower stamen level and decreasing that of the style. These changes in growth rate could result from differences in timing and duration of cell division and elongation in styles and filaments.

The *S* allele appears to act differently than

the *M* allele, causing inhibition of stylar growth, an increase in the rate of floral tube growth below the upper stamen level, and a relatively late increase in filament growth rate. The *S* allele would appear to promote cell elongation in the upper filaments, and we hypothesize that this occurs relatively late in development and results in the secondary increase in filament growth rate which we observed. The *S* allele, following this model, would also inhibit style elongation and pollen production in the upper stamen level.

As discussed above, in *Pontederia* and *Eichhornia* the difference between stamen levels does not result from a switch between levels at a specific point in organ initiation and does not depend on the timing of initiation. Instead, our data are consistent with a developmental model which is spatially regulated, with stamen level being determined by the position of stamen primordia on the floral primordium. The *S* and *M* genes then regulate specific organ sizes (both stamen and style) within this spatial gradient. A similar spatially-controlled developmental model has been advanced by Bachmann (1983) to explain the development of hairy and hairless achenes in *Microseris*.

This attempt to link genetic and relative growth data should be regarded as a tentative first step in unravelling the complex developmental organization of tristyly. Experimental manipulation of floral form and the study of semi-homostylous variants may enable us to proceed further. Heterochronic analyses do not themselves answer the question of what controls a developmental process, but they do indicate the type of regulatory change which has occurred.

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