Chapter 4

The Development of Heterostyly

J.H. RICHARDS[1] and S.C.H. BARRETT[2]

1 Introduction

Heterostyly is a genetic polymorphism in which the two (distyly) or three (tristyly) mating types in a population differ in floral morphology. The principal feature that distinguishes the floral morphs is that they differ in stigma and anther heights. The sex organs are reciprocally positioned with anthers in flowers of one morph at the same level as stigmas in flowers of the other morph(s). This structural difference is usually accompanied by a physiological self- and intramorph incompatibility that limits mating to crosses between organs at the same level. In this chapter we describe the diversity of organization in heterostylous flowers, consider the developmental bases for this diversity, and examine the genetic and environmental components of phenotypic variation in heterostylous breeding systems. We review the developmental implications of models for the genetic control and evolution of heterostyly and conclude with recommendations for future research.

2 Structure of Mature Heterostylous Flowers

2.1 Constraints on Morphology of Heterostylous Flowers

The evolution of heterostyly appears to occur within some general constraints on floral morphology (Ganders 1979a; Chaps. 3, 5 and 6). Heterostylous flowers are generally moderate sized and have a floral tube, a limited number of stamens, and a syncarpous ovary with few carpels. The reasons for these limitations are unclear but may depend on both developmental and functional constraints.

The length of heterostylous flowers is usually between 5 and 30 mm. In their study of breeding systems in *Cordia* species (Boraginaceae), Opler et al. (1975) found that dioecy is present in the smallest flowers and heterostyly in medium to large flowers. Similarly, the smallest-flowered species of *Melochia* (Sterculiaceae) in the Caribbean lack distyly or derived homostyly, conditions found in three larger-flowered species in the region (Martin 1966).

[1]Department of Biological Sciences, Florida International University, Miami, FL 33199, USA, and Fairchild Tropical Garden, 11935 Old Cutler Road, Miami, FL 33156, USA
[2]Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

Monographs on Theoretical and Applied Genetics 15
Evolution and Function of Heterostyly (ed. by S.C.H. Barrett)

Possible reasons for these size limits include both restrictions on pollination efficiency and developmental constraints imposed by the need for reciprocity in organ position. For example, the lower end of the size range may be determined by how precisely stigma-anther separation can be controlled developmentally, as well as whether pollen from small flowers can be effectively separated by morph on the bodies of pollinators. Small flowers usually have short stamens and styles. If distances of a few mm are all that separate stigmas and anthers within heterostylous flowers, selection must severely limit variation in sex organ level both within and between flowers of the morphs in order to preserve stigma-anther separation. The inherent variation in developmental processes may preclude maintenance of such small differences and thus set a lower limit on the size of heterostylous flowers.

The absence of heterostyly in large-flowered species may also have both ecological and developmental explanations. The feeding behavior of long-tongued moths, bats, and birds, which are common pollinators of large flowers, may make these pollinators less effective at mediating disassortative pollination in comparison with smaller pollinators, such as bees and butterflies that visit moderate-sized flowers. Some distylous plants, however, are hummingbird-pollinated, e.g., *Cephaelis* (Rubiaceae) (Bawa and Beach 1983) and *Palicourea* (Rubiaceae) (Feinsinger and Busby 1987). Upper limits on floral size could also result if variability in stigma-anther height exceeds the constraints imposed by the need for reciprocity among morphs in organ position. Long styles and stamens may show more absolute variability in stigma and anther levels because minor variations in early development are magnified during growth. The organs of large flowers may therefore be less efficient at promoting disassortative pollination.

In addition to size constraints, heterostylous species show limitations on floral architecture. Heterostylous flowers usually have few stamens. Distylous flowers commonly produce 2–12 (Ganders 1979a), while tristylous flowers have from 6 to 12. An increased number of stamens prolongs the time between the beginning and end of stamen initiation and increases the complexity of positional effects in development (cf. Hufford 1988). Both of these factors could increase the amount of developmental variability. The variability caused by timing and position of stamen origin probably accounts for variation in stamen length within flowers of distylous species that have two whorls or series of stamens, such as *Byrsocarpus coccineus* (Connaraceae) (Baker 1962) and *Erythroxylum coca* (Erythroxylaceae) (Ganders 1979b). Differences in length between stamen series of these species caused early researchers to speculate that these taxa are tristylous (Baker 1962; Ganders 1979b).

Because tristylous species have two stamen levels in each flower, Yeo (1975) proposed that two series of stamens are a necessary precondition for the evolution of tristyly. He assumed that one stamen series developed into one anther level, while the other produced the second level. Although all tristylous species have two series of stamens, in the Pontederiaceae each stamen level has members from both the first and second series (Richards and Barrett 1984, 1987). The organization of heterostyly in this family therefore negates the reason for assuming that two stamen series are a precondition for the evolution of tristyly, although a within-flower stamen dimorphism probably is a prerequisite (see Sect. 6).

Hypericum aegypticum and *Cratoxylum formosum* in the Guttiferae are unusual among heterostylous species because they have numerous stamens arranged in

bundles (Ornduff 1975; Lewis 1982). In both species stamen length differs within a flower, and some overlap between stamen levels is found in *C. formosum*. Distyly is achieved in the latter species by an ephemeral bending of the filaments at anthesis in the long-styled morph (Lewis 1982). This bending places the stamens at the level of the short style. The occurrence of heterostyly in species of *Cratoxylum* and *Hypericum* indicates that a limited number of stamens is not a precondition for the evolution of heterostyly. The unusual stamen behavior in *Cratoxylum*, however, emphasizes that a large number of stamens presents different developmental problems in evolution of the syndrome.

The gynoecium of heterostylous species is usually syncarpous and has from one to five carpels [Chap. 6, this Vol.; heterostylous Sterculiaceae have five (*Melochia*) or one (*Waltheria*) carpel(s)]. Styles are free or united and the ovary can be superior or inferior. The floral structure of apocarpous species (many carpels spread over a broad or elongated receptacle, short styles, many stamens, and open flowers) may be inherently unsuitable for evolution of the heterostyly syndrome. Problems in regulating development of separate style lengths would be similar to those of regulating numerous stamens. In species with carpels on an elongated receptacle, the range of stigma heights could compromise legitimate pollen transfer between the floral morphs and lead to considerable pollen wastage.

2.2 Structural Basis for Style and Stamen Heteromorphisms

The characteristic that defines heterostyly is the reciprocal positioning of anthers and stigmas in flowers of different morphs. Stigma height depends on the size of the ovary and length and orientation of the style. Style length is the major source of differences in stigma height among heterostylous morphs, since ovary lengths are usually similar among morphs (Stirling 1932, 1936; Richards and Barrett 1984, 1987). Cases where this is not so have been associated with gender specialization, as in subdioecious *Cordia inermis* (Opler et al. 1975).

Bending of the style can make a significant contribution to final stigma position, especially in the short-styled morph. Darwin (1888) described bending in *Linum grandiflorum* (Linaceae), noting that short styles diverge much more than long styles and that bending allows the short styles to pass between the stamen filaments. A similar condition is found in *Oxalis* (Oxalidaceae) (Darwin 1888), *Piriqueta* (Turneraceae) (JH Richards pers. observ.), *Turnera* (Turneraceae) (Barrett and Shore 1987), and *Melochia* (Martin 1966). The contribution of stylar bending to stigma position has not been quantified nor has variation in this character among morphs been studied in detail. In *Oxalis* stylar bending in the short morph occurs prior to anthesis (JH Richards pers. observ.).

Anther height depends on filament length and orientation. Since many heterostylous plants are sympetalous (Ganders 1979a), both the position of filament insertion on the floral tube and the floral tube length can contribute to anther height. Filament length establishes anther height differences in some species, such as *Jepsonia* (Saxifragaceae) (Ornduff 1971), *Erythroxylum* (Ganders 1979b) and *Oxalis* (Ornduff 1964, 1972; Weller 1976a; and see Sect. 3.1), whereas insertion position determines anther height in other species, e.g., *Primula* spp. (Primulaceae) (Darwin

1888; A.J. Richards 1986) and *Hedyotis caerulea* (Rubiaceae) (Ornduff 1980). In many heterostylous species both filament length and position of insertion vary among morphs (e.g., *Pontederia* and *Eichhornia* (Pontederiaceae), Richards and Barrett 1984, 1987; species of *Psychotria, Cephaelis*, and *Faramea* (Rubiaceae), JH Richards unpubl. data).

In the unusual case of *Cratoxylum formosum*, anther height is not determined solely by filament length or insertion (Lewis 1982). Stamen bending positions the "short" anthers at a height comparable to the short stigma. Unlike stylar bending, this reorientation is reversible – the stamens erect themselves after about 6 h – and the developmental timing and, presumably, mechanism is different.

In studies of heterostylous flowers, stigma and anther height are usually measured from the base of the flower or some similar reference point. The biologically important measurements, however, are stigma-anther separation. Although separation can be calculated from stigma-anther height, this calculated value and the actual separation are not necessarily equal, especially if ephemeral changes in position, bending, or lateral displacement of sex organs are significant. To overcome this problem, careful observations of flowers in situ are needed in addition to the traditional measurements.

2.3 Ancillary Characters of Heterostylous Flowers

Heteromorphism of other reproductive characters is often associated with stigma-anther positions in heterostylous species (Darwin 1888; Ganders 1979a). Differences in anther length, pollen size and number, pollen shape and exine sculpturing, pollen color, stigma size and shape, stigma papillae size and shape, and stigma color have been reported (see Chap. 3). The pattern of heteromorphism is often similar in different heterostylous taxa. Long-level styles, for example, frequently have larger stigmas and longer stigmatic papillae than short-level styles, while long-level stamens usually produce larger but fewer pollen grains than short-level stamens (Dulberger 1975a; Ganders 1979a). Recurrent associations among characters in heterostylous species may reflect common developmental bases for the correlated characters. Larger stigmas with longer papillae, for example, can be a direct result of increased cell length in long styles.

Pollen-size dimorphism is so frequent in distylous taxa that Darwin (1888) considered it a basic feature of heterostyly. Number of pollen grains per anther also often varies among anther levels. Ganders (1979a) showed that the ratio of thrum:pin pollen volume is correlated with the ratio of pin:thrum pollen grain number. This correlation suggests that there is a developmental relationship between pollen size and pollen grain number that results in either many small grains or fewer large grains. Differences between morphs in pollen grain size could arise any time from sporogenous cell origin until the grains mature and could have a number of causes, such as size differences when sporogenous cells differentiate or variations in how long or rapidly the cells grow. The kinetics of pollen growth have not been studied in a heterostylous species, but premeiotic size heteromorphisms have been reported for sporogenous cells of *Primula* (Dulberger 1975a) and *Eichhornia paniculata* (Richards and Barrett 1984).

Differences among anther levels in pollen number probably originate during microsporogenesis. Although post-meiotic pollen abortion could also produce pollen number differences, a substantial number of cell deaths would be required to obtain the observed differences in pollen number [e.g., app. 4000 vs. 21 000 pollen grains in long-level vs. short-level anthers of *Pontederia cordata* (Price and Barrett 1982)], and evidence of such abortion has never been reported.

The developmental basis for pollen number differences may vary among species. Pollen production (P) is usually reported per anther level and can be approximated by the equation

$$P = (ALS)\,(2^n)\,(4),$$

where A is the number of anthers per stamen level, L is the number of locules per anther, S is the initial number of sporogenous cells per locule, and n is the number of sporogenous cell mitotic divisions prior to meiosis, which are multiplied by 4 to account for products of meiosis. Anther number and locule number per anther have not been observed to vary between morphs in heterostylous species, so the variables of interest are S and n. Ratios of short-level to long-level pollen number in most distylous species range from 1.13 to 3.12 (Ganders 1979a). Small differences in S could account for the lower end of this range and such small differences will be difficult to detect in developmental studies. The twofold or greater differences in sporogenous cell number required to achieve the upper end of this range, however, should be obvious.

Alternatively, differences between anther levels in n, caused by one or two additional divisions in some or all of the sporogenous cells, could account for the observed range of pollen number ratios. Morph-specific differences in the cell cycle or in the duration of microsporogenesis would produce different numbers of microspore divisions. The two anther levels within flowers of tristylous Pontederiaceae enter meiosis at different times, and differences in pollen mother cell numbers, as well as size, are present prior to meiosis (Richards and Barrett 1984; JH Richards unpubl. data), which indicates that the number of divisions in the sporogenous tissue contributes to pollen number heteromorphisms in the Pontederiaceae. Developmental studies in other heterostylous taxa are needed to define the processes responsible for pollen number differences and to understand the relation of pollen number to pollen size heteromorphisms.

While certain patterns of ancillary character heteromorphism predominate among heterostylous taxa, none of the correlations between primary and ancillary characters is invariant. Pollen and stigma heteromorphisms are lacking in some heterostylous genera (Vuilleumier 1967; Dulberger 1974). In addition, cases are known where the predominant pattern of heteromorphism is reversed. For example, the stigmas of short styles can be larger than those of long styles (e.g., *Palicourea lasiorrachis*, Feinsinger and Busby 1987), and long-level anthers can have more pollen than short-level anthers, as in *Amsinckia spectabilis* var. *microcarpa* and *A. vernicosa* var. *furcata* (Boraginaceae) (Ganders 1975, 1976). In the two *Amsinckia* species pollen grains from the long-level anthers are larger than grains from short-level anthers, so the increase in pollen number has occurred without change in the usual pollen size relations. The pattern of pollen size dimorphism is variable in *Fauria* (= *Nephrophyllidium*) *crista-galli* (Menyanthaceae), with long-level pollen

larger than short-level pollen in one population but smaller in another (Ganders 1979a).

The diversity of expression in ancillary characters may reflect differences among species in development of the heteromorphisms. A species that produces long styles by increased cell division rather than increased cell elongation, for example, may not have a large stigma with long papillae. More detailed analysis of the structural and developmental basis of heterostyly are needed before the relation of these ancillary characters to the primary stamen-style heteromorphism can be understood.

3 Floral Development in Heterostylous Species

The structural diversity of stamen-style heteromorphisms and taxonomic diversity of heterostylous species suggests that different developmental mechanisms are involved in the evolution of the polymorphism in unrelated angiosperm families. Unfortunately, there are few detailed structural analyses or developmental studies with which to test this prediction. The most complete body of work is on tristylous species, and even among the small group of plants that share this polymorphism, several patterns of development are evident. Below we review what is known about the development of tristylous and distylous flowers.

3.1 Mature Flower Structure in Tristylous *Lythrum*, *Oxalis*, and the Pontederiaceae

Mature flower structure in tristylous members of the Lythraceae, Oxalidaceae, and Pontederiaceae are summarized in Table 1 and Fig. 1. The flowers of *Lythrum* (Lythraceae) have six sepals that are united basally into a tube, six petals inserted just below the sinuses of the calyx tube, twelve stamens, and a two-carpellate superior ovary (Fig. 1A; Table 1). Six outgrowths that have been called sepals (Sattler 1973; Cheung and Sattler 1967) or epicalyx appendages (Mayr 1969) develop in the sinuses between calyx lobes after lobe initiation. The 12 stamens are inserted a short distance from the base of the calyx tube (app. 1 mm in *L. salicaria*), and in each morph the six longer stamens, opposite the sepals, alternate with the six shorter stamens, opposite the epicalyx appendages and petals. Our observations on floral organization agree with those of Cheung and Sattler (1967) for *Lythrum salicaria* and Ornduff (1979) for *Lythrum junceum*. In *L. salicaria* the longer stamens (long-level stamens in the M and S morphs, mid-level stamens in the L morph) are inserted slightly higher on the floral tube than the shorter stamens (short-level stamens of the L and M morphs, mid-level stamens of the S morph), but most of the difference in stamen height within morphs results from variation in free filament length. Mature *Lythrum* flowers extend almost horizontally from the inflorescence and are slightly zygomorphic.

Oxalis (Oxalidaceae) flowers have five free sepals and petals, ten stamens that are united basally into a ring, and a five-parted, syncarpous superior ovary (Fig. 1B). The two stamen levels within a flower alternate on the staminal ring, with the five longer stamens opposite the sepals and the five shorter stamens opposite the petals. Differences in anther height depend primarily on differences in filament length.

Table 1. Structural and developmental features of *Lythrum, Oxalis,* and the Pontederiaceae

Characteristic	*Lythrum*	*Oxalis*	Pontederiaceae
Perianth structure	Sepals + petals	Sepals + petals	Tepals
Perianth fusion	Calyx tube, free petals	Free sepals and petals	Tepals fused 1/2 of length
Floral symmetry	Slightly zygomorphic	Radial	Zygomorphic
No. stamen series × no. stamens/series	2 × 6	2 × 5	2 × 3
Insertion of filaments	On calyx tube	On staminal ring on receptacle	On floral tube
Organization of stamen dimorphism	Radial	Radial	Dorsivental
Position of longer stamen level	Opposite sepals	Opposite sepals	Opposite lower tepals
Position of shorter stamen level	Opposite petals	Opposite petals	Opposite upper tepals
Origin of stamen dimorphism within morphs	At initiation	At initiation	Post-initiation
Origin of differences between morphs	?	?	Post-initiation

Flowers of *Eichhornia* and *Pontederia* (Pontederiaceae) have three narrow outer tepals and three broader inner tepals, six stamens, and a tricarpellate, syncarpous ovary, although in *Pontederia* only one carpel develops an ovule (Lowden 1973; Richards and Barrett 1984, 1987; Fig. 1C; Table 1). The six tepals are united into a floral tube for approximately half their length. Mature flowers are oriented horizontally, and the longer and shorter stamens in each morph occur on opposite sides of the flower. The shorter stamen level is on the upper side of the flower, with the central stamen opposite an inner tepal and the two lateral stamens opposite outer tepals. The longer stamen level is on the lower side, with the central stamen opposite an outer tepal and the lateral stamens opposite inner tepals. Anther height depends on both filament length and position of filament insertion on the floral tube. Longer stamens are inserted higher on the floral tube and have longer filaments, while shorter stamens are inserted lower on the floral tube and have shorter filaments.

Comparison of mature flower structure indicates that the two dicotyledonous families resemble each other but are distinct from the monocotyledonous Pontederiaceae in the organization of tristyly. Intraflower stamen dimorphism is organized on a radial pattern with alternating longer and shorter stamens in *Lythrum* and *Oxalis*. In these two genera stamen height within a flower depends primarily on free filament length. In both *Lythrum* and *Oxalis* the relatively long stamen level arises opposite the sepals, whereas the relatively short stamen level is opposite the petals.

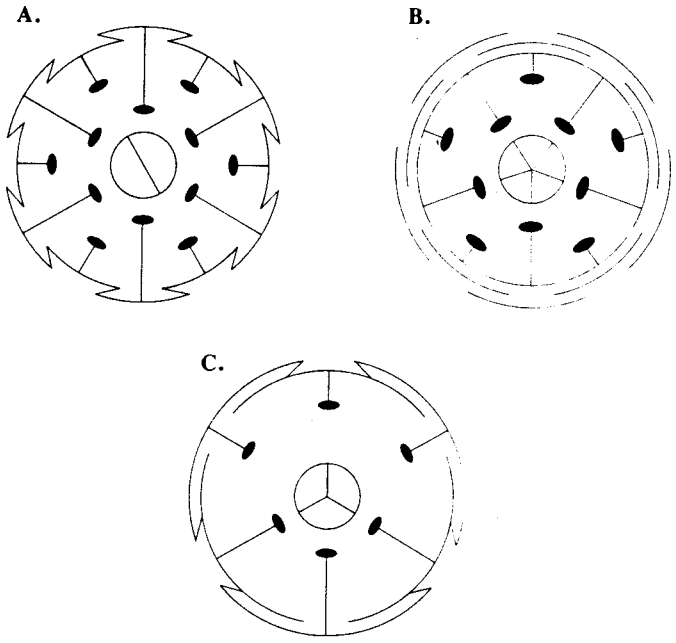


Fig. 1A-C. Floral diagrams of *Lythrum* (**A**), *Oxalis* (**B**), and tristylous Pontederiaceae (**C**). In *Lythrum* stamens are inserted on the floral tube with the longer stamens opposite the sepals and the shorter stamens opposite the petals; in *Oxalis* the stamen bases are united into a ring with the longer stamens opposite the sepals and the shorter stamens opposite the petals; in tristylous Pontederiaceae the stamens are inserted on the floral tube with the shorter stamens on the upper side of the horizontally oriented flowers and the longer stamens on the lower side

Although the perianth in the Pontederiaceae is organized in two series, the within-flower stamen dimorphism is dorsiventral and thus has a fundamentally different architecture from the intraflower stamen dimorphism of the tristylous dicotyledons. Moreover, stamen dimorphism in the Pontederiaceae depends both upon level of insertion on the floral tube and upon free filament length. The differences among families in stamen dimorphism are correlated with perianth structure. The calyx is morphologically distinct from the corolla in the Lythraceae and Oxalidaceae, and each stamen level within a flower is associated with only one perianth series. In the Pontederiaceae, in contrast, distinctions between the inner and outer tepals are less marked, and the within-flower stamen dimorphism is not correlated with tepal series. The lack of differentiation between perianth series in the Pontederiaceae may have provided insufficient developmental cues to the associated stamens to allow for the differentiation of stamen levels from the initial stamen series.

Differences among families in the architecture of tristyly may reflect ancestral floral organizations that provided contrasting developmental contexts for the evolution of tristyly. It is remarkable that the inheritance patterns of the polymorphism are similar, even though the genes controlling tristyly must regulate different developmental processes in these families (see Sect. 5).

3.2 Development of Tristyly in Pontederiaceae

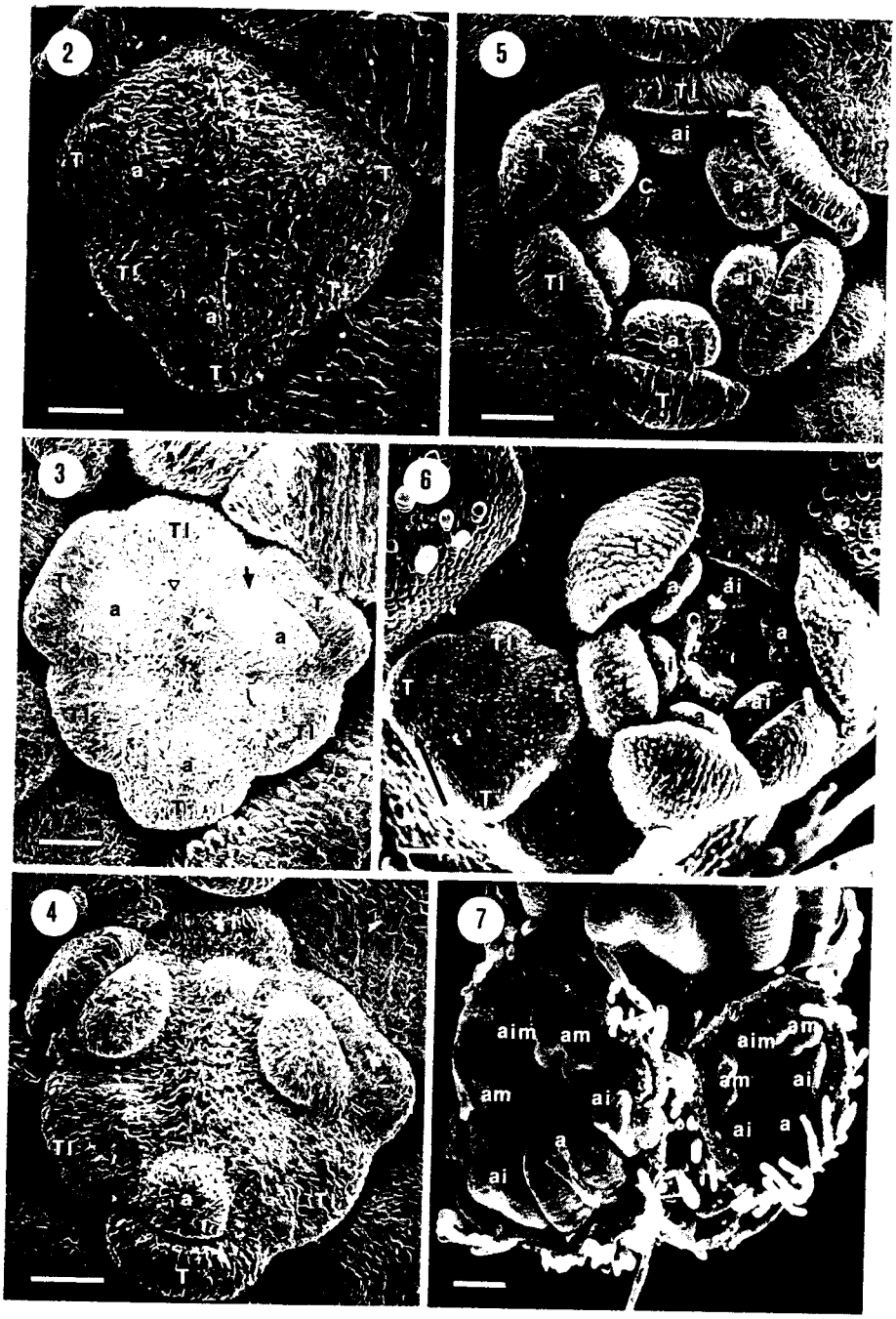
The Pontederiaceae is a small aquatic monocotyledonous family with six to nine genera. Two genera, *Eichhornia* and *Pontederia*, have tristylous species (Eckenwalder and Barrett 1986). These two genera differ in the expression of tristyly. Four out of the five species of *Pontederia* are tristylous, and all species that have been investigated have a strong trimorphic self-incompatibility system. Only three of eight *Eichhornia* species are tristylous, and of these only *E. azurea* has self-incompatible populations (Barrett 1978).

Although flowers of both *Eichhornia* and *Pontederia* have dorsiventral organization when mature (Richards and Barrett 1984, 1987), the floral organs arise in a radial pattern. The six sepals are initiated in two series of three each (Figs. 2, 3), followed by a first stamen series and then a second. The first stamen set is opposite the outer tepal whorl and the second opposite the inner whorl (Figs. 3, 4). Three carpels develop from primordia that arise opposite the outer tepals and first stamen whorl (Figs. 5, 6). Although distinct differences in size between stamen primordia associated with each tepal series are apparent in young flower buds (Figs. 4, 5, 7), these variations are unrelated to differences between stamen levels in mature flowers. The three stamen primordia on the upper half of the flower primordium develop into the shorter stamens within each flower, while the three lower primordia develop into the longer stamens (Fig. 1C, Fig. 7). In both *Pontederia* and *Eichhornia*, size differences between stamen levels become apparent in post-meiotic stamens. Premeiotic differences can be seen, however, in the number and size of pollen mother cells and in time of entry into meiosis (Richards and Barrett 1984 and unpubl. data).

In *Pontederia* and *Eichhornia* differences among morphs in anther and stigma heights arise through differences in both relative growth rates and the duration of growth (Richards and Barrett 1984, 1987, unpubl. data). In *Pontederia* the L and M morphs have similar patterns of growth but differ in the growth rates of the lower, longer filaments and of the styles (Figs. 8, 9). The S morph is qualitatively different from the other two morphs in showing an early inhibition of style and filament expansion, followed by a late acceleration of filament growth (Figs. 8, 9).

Variation in filament length, filament insertion, and style length can arise from differences in cell size and/or cell number. Measurements of *P. cordata* (Richards and Barrett 1987) and *E. paniculata* (Richards and Barrett 1984) indicate that stylar cell length differs among morphs. Long styles have longer cells than mid-length or short styles, and mid-length styles have longer cells than short styles. Cell size differences are not sufficient to account for style length variation, however, so cell numbers must also differ.

Filament cell length also varies among stamen levels. Long filaments have longer cells, short filaments shorter cells, and mid-length filaments have cells of intermediate length. In both *P. cordata* and *E. paniculata*, however, cells of mid-level filaments of the L and S morphs differ in length (Fig. 10). Mid-level filaments of the S morph have longer cells than mid-level filaments of the L morph (Richards and Barrett 1984, 1987, Fig. 10). This is unexpected, since mid-length stamens of the S morph are the upper, shorter stamen level, whereas mid-length stamens of the L morph comprise the lower, longer stamen level. The longer cells of mid-level fila-


2
T
a
a
T
Tl
Tl
a
T
5
Tl
ai
T
a
C
a
Tl
ai
a
T
3
Tl
T
a
a
T
Tl
Tl
a
Tl
6
a
ai
a
T
T
ai
a
T
4
Tl
a
T
7
aim
am
aim
am
am
ai
ai
a
a
ai

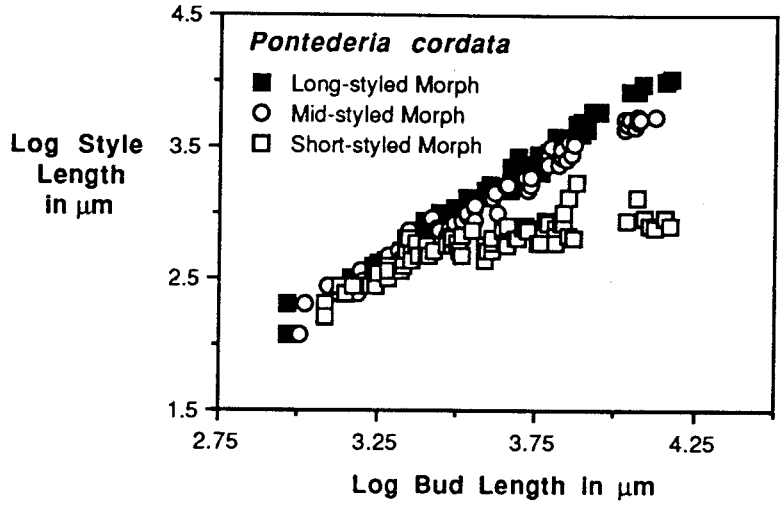


Fig. 8. Log bud length vs. log style length for the three morphs of *Pontederia cordata*. Style relative growth rates are similar in young buds of the three morphs. In later development stylar growth is inhibited in the S morph, while the relative growth rate of the M morph is less than that of the L morph

Figs. 2–7. Scanning electron micrographs illustrating flower development in the S morph of *Pontederia cordata*. *a* stamen primordium in the first-initiated stamen series; *ai* stamen primordium in the second-initiated stamen series; *am* mid-level stamen that develops from a primordium in the first stamen series; *aim* mid-level stamen that develops from a primordium in the second stamen series; *c* carpel; *T* outer tepal; TI inner tepal. **Fig. 2.** Floral primordium that has initiated three outer tepals and stamens and three inner tepals. The two upper stamens, which are more developed than the lower stamen, will belong to the shorter (mid) stamen level. *Bar* 33 μm. **Fig. 3.** Floral primordium with three outer tepals and stamens and three inner tepals. Stamens associated with the inner tepals are just beginning to develop. The upper stamens of both the outer and inner stamen series, which will become the shorter (mid) stamen level, are larger than the lower stamens, which will become the longer (long) stamen level. Within the outer stamen series the upper primordium away from the parent branch (*arrow*) is slightly larger than the other upper stamen primordia, while within the inner stamen series the upper primordium (at *triangle*) is more developed than the two lower stamen primordia. *Bar* 50 μm. **Fig. 4.** A bud that has an exaggerated expression of size differences between the inner and outer stamen series and of earlier development of the upper stamen of the inner stamen series. *Bar* 50 μm. **Fig. 5.** A flower bud in which the tepals have begun to grow around the stamens, and the carpels have been initiated on a radius with the outer tepal and stamen series. The outer stamen series has larger primordia than the inner stamen series. *Bar* 50 μm. **Fig. 6.** Two floral primordia on a branch. In the older primordium the tepals have begun to enclose the bud, while the outer stamen series exceeds the inner in size and has initiated microsporangia. The younger floral primordium is slightly better developed than the bud in Fig. 2. The three outer tepal primordia are distinct, as is the upper, inner tepal primordium. The outer tepal primordium away from the parent branch (*arrow*) is more developed than the other tepal primordia. *Bar* 50 μ. **Fig. 7.** Two older buds with tepals dissected away. In both buds the outer stamen series is larger than the inner stamen series, but in the older bud growth in the long stamen level has begun to exceed that in the mid stamen level, so the lower outer stamen is larger than the upper outer stamens and the lower inner stamens are approximately equal in length to the upper outer stamens. *Bar* 150 μm

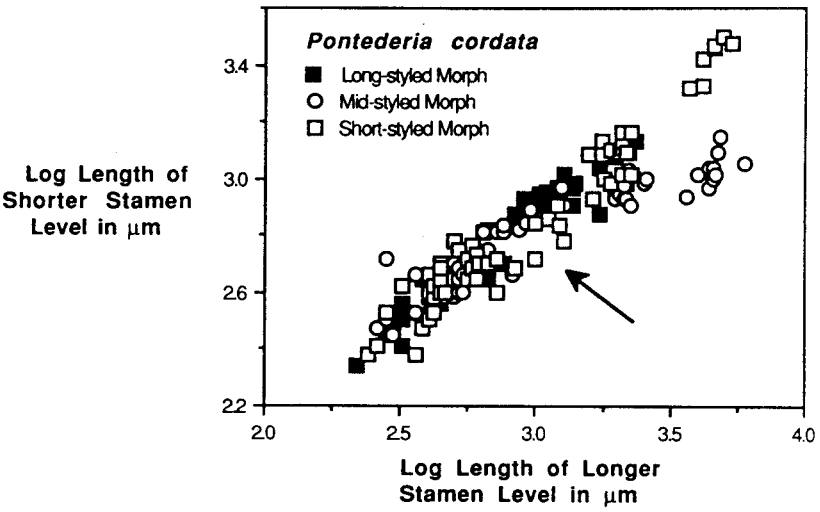


Fig. 9. Log length of the longer vs. the shorter stamen level for the three morphs of *Pontederia cordata*. The L and M morphs have similar patterns of relative growth, while the S morph shows an early inhition (*arrow*) then acceleration in relative stamen growth

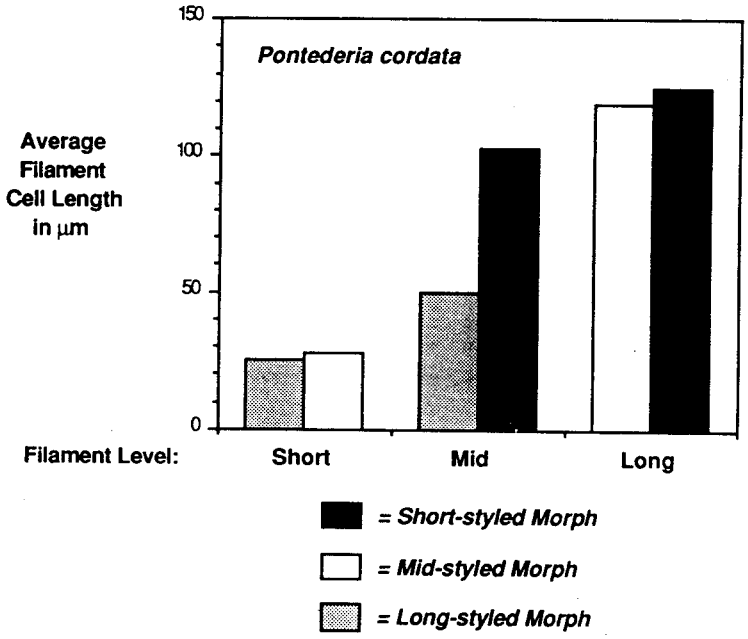


Fig. 10. Average filament cell length for the three morphs of *Pontederia cordata*. Average cell length of long-level filaments is similar, regardless of morph, as is average cell length of short-level filaments. Average cell length of mid-level filaments depends on morph. The S morph, which has mid-level stamens on the upper side of the flower, produces filaments with longer cells than the mid-level filaments of the L morph

ments in the S morph may be a product of the late acceleration in filament relative growth rate observed in this morph (Fig. 9).

3.3 Development of Tristyly in Lythraceae and Oxalidaceae

In common with the Pontederiaceae, the sequence of organ initiation and qualitative aspects of early development are the same in all three morphs of tristylous species of *Lythrum* and *Oxalis*. We have examined development in *Lythrum salicaria* and *Oxalis rubra*.

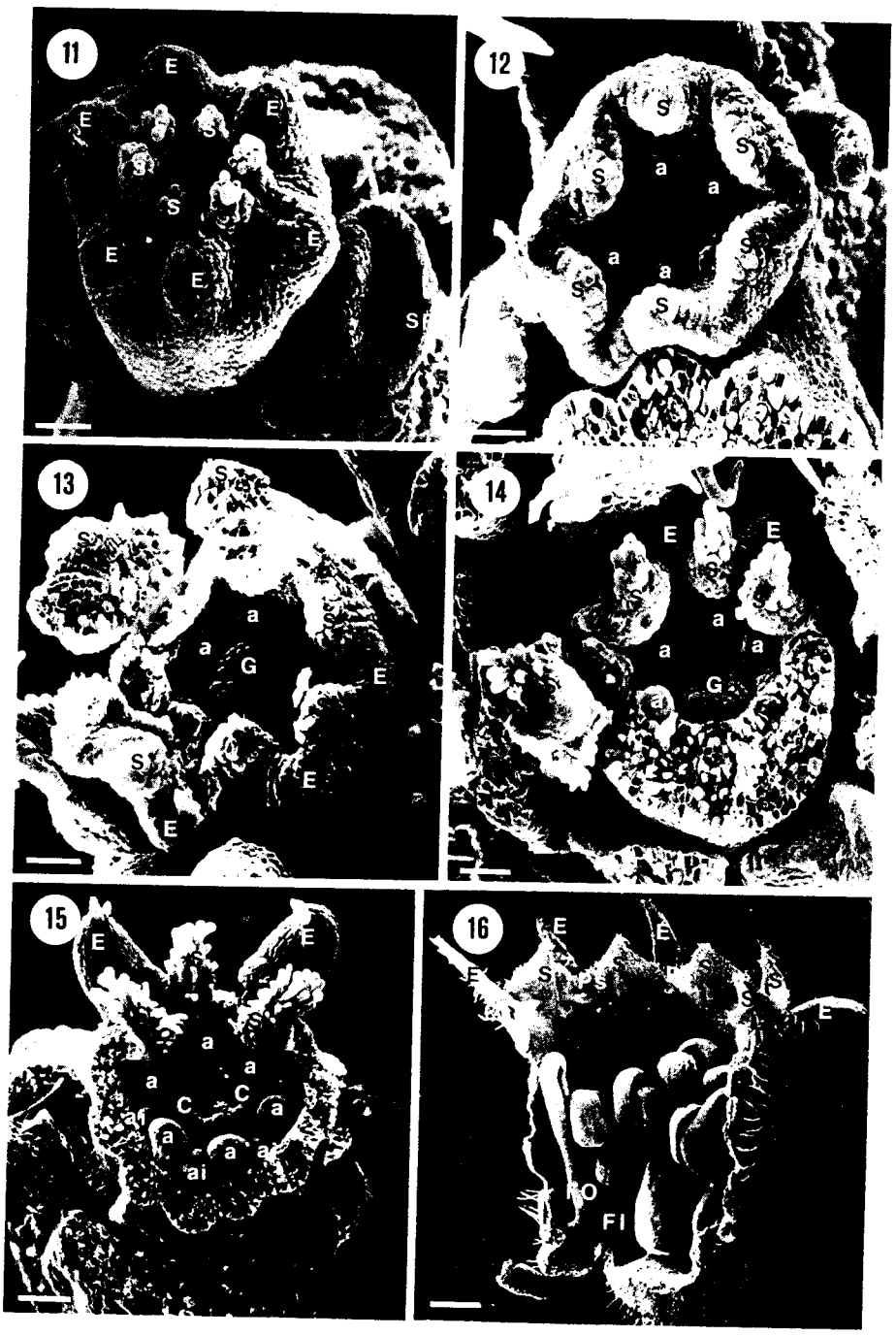
The floral organs of *Lythrum salicaria* arise in five series (Cheung and Sattler 1967; Sattler 1973; JH Richards pers. observ.). The calyx arises as six sepal primordia that are quickly united into a meristematic ring (Fig. 11). The first stamen whorl is initiated next, opposite the sepal primordia (Figs. 12, 13), followed by origin of the gynoecium in the center of the bud (Figs. 12–14). The second stamen whorl, which alternates with the first set of stamens (Fig. 15), arises after the gynoecium primordium is defined. The epicalyx appendages begin to develop at approximately the same time as the carpel (E in Figs. 13, 14). These appendages arise between the calyx lobes and eventually develop into elongated cylindrical outgrowths (Figs. 15, 16). Relatively late in development the petals arise as individual primordia on the upper edge of the calyx tube between the sepal lobes and thus on a radius with the epicalyx appendages and second stamen whorl.

Lythrum salicaria, therefore, has a temporal separation between initiation of the two stamen whorls. The first-initiated stamens, which become the longer stamens within each flower, are always larger than the second-initiated stamens, which become the shorter stamens (Figs. 15, 25, 26). The first stamens precede the second ones in sporangia and filament initiation (Fig. 15) and exceed them in filament growth (Fig. 16).

In *Oxalis rubra* the five sepals arise in a spiral (Fig. 17), then the floral apex broadens and becomes pentagular with the initiation of the petals, which alternate with the sepals (Fig. 18). Growth of the petals is slow, however, relative to growth of the other floral organs – the petals do not exceed the stamens in length until bud length is ca. 3 mm. The first stamen series is initiated opposite the sepals (Figs. 19, 27), and the second series arises opposite the petals (Figs. 20–22, 28). The petal-opposed stamen series is initiated slightly later than the sepal-opposed series and appears to be lower on the floral apex than the sepal-opposed stamens (Figs. 20–22, 27, 28). Carpel primordia originate on a radius with the petals and second stamen series, forming the five-parted gynoecium (Figs. 23, 28–30).

Thus, as in *L. salicaria*, the two stamen levels within a flower of *O. rubra* differ in time of origin. The differences in early development are accentuated as the anthers grow and initiate sporangia (Figs. 23, 29, 30). Differences in filament length are present prior to significant growth of the stamen ring (Fig. 24).

Stirling (1933, 1936) provides the only published studies of later stages of floral development in tristylous species of *Lythrum* and *Oxalis*. His data from measurements of dissected buds of *L. salicaria* (Stirling 1933) and *O. cernua* (= *O. pes-caprae*) (Stirling 1936), which we have regraphed (Figs. 31–34), suggest that growth patterns in these two species differ from those in the Pontederiaceae. Graphs of petal length


11
E
E
E
E
S
S
E
E
E
S
12
S
S
S
a
a
a
a
S
S
S
13
S
S
a
a
G
E
S
E
E
14
E
E
a
a
a
G
15
E
E
a
a
a
C
C
a
a
a
a
ai
16
E
E
E
S
S
S
S
E
FI

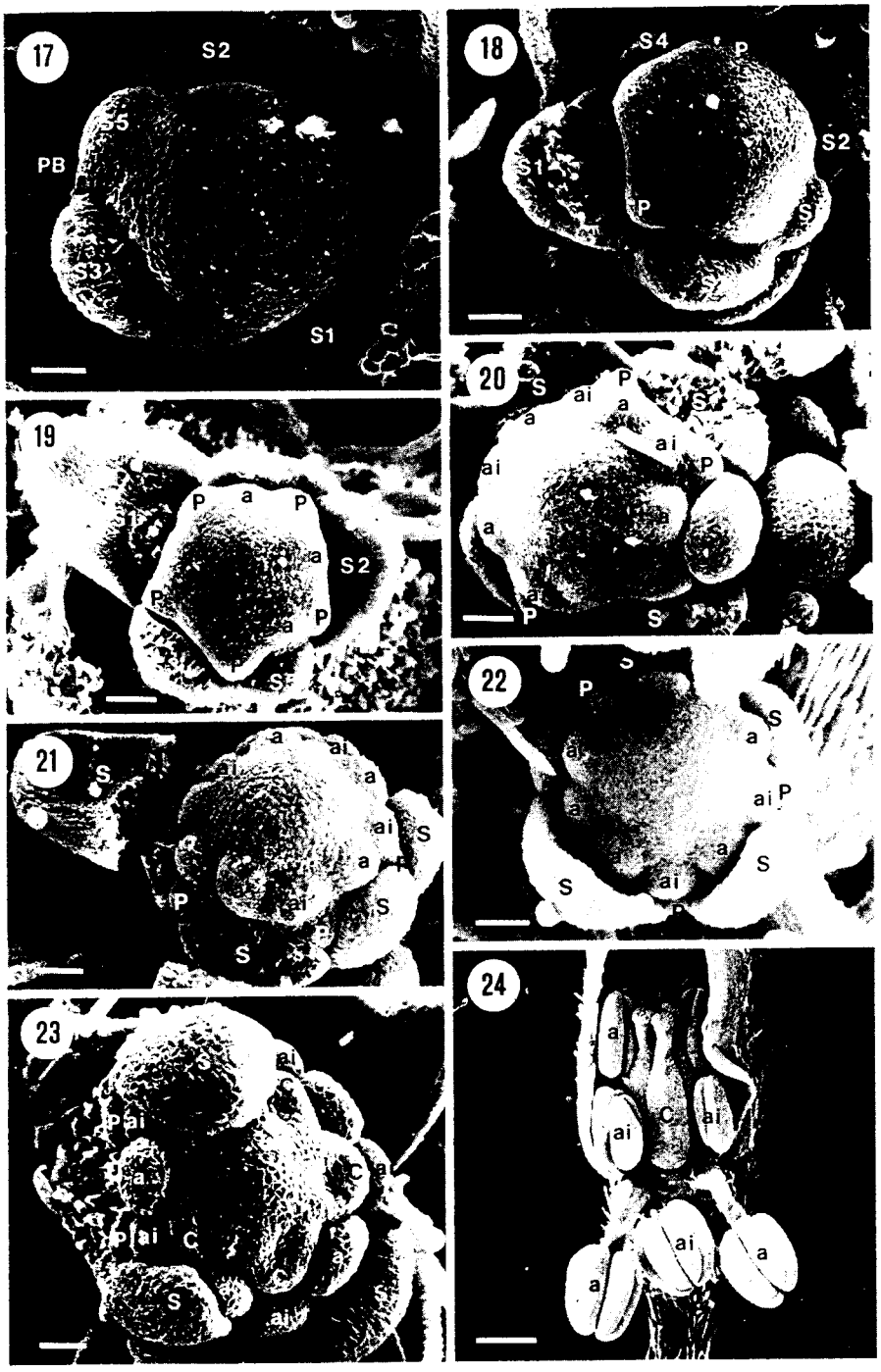
vs. pistil length indicate that in both *L. salicaria* and *O. cernua* the three morphs have different relative growth rates in the later stages of development (Figs. 31, 32). Neither species, however, shows the degree of stylar inhibition that occurs in the S morph of the Pontederiaceae (Fig. 8). Although Stirling provides less information on young buds, the available data on pistil length for both *L. salicaria* and *O. cernua* are consistent with an early similarity in relative growth rates among morphs and an earlier divergence in growth rate of the S morph from the other morphs (Figs. 31, 32).

As in the Pontederiaceae, relative growth of stamens differs among morphs in *Lythrum* and *Oxalis*. In both species graphs of longer vs. shorter filament length show a more divergent pattern in the M morph than in the L and S morphs (Figs. 33, 34). Figures 33 and 34 indicate that in the L and S morphs relative growth rates of stamen levels within a flower are constant and relatively linear. This implies that differences between stamen levels within a morph are established early and maintained throughout development. Development of stamens in the M morph diverges from the other morphs in both *Lythrum* and *Oxalis*, although the pattern of divergence appears to differ in the two species (Fig. 33, 34).

3.4 Comparison of Development Among Tristylous Species

The tristylous species of the Pontederiaceae, Lythraceae, and Oxalidaceae initiate two, temporally separated stamen series. The difference in time of origin establishes a size differential between stamen series that is maintained throughout development. In the Pontederiaceae this difference in time of origin merely produces a subtle variation in height within each stamen level and does not contribute to intraflower stamen dimorphism (Richards and Barrett 1984, 1987). In *Lythrum* and *Oxalis*, in contrast, the first stamen series initiated corresponds to the longer stamen level

Figs. 11–16. Scanning electron micrographs of floral development in the S morph of *Lythrum salicaria*. *a* stamen primordium in the first-initiated stamen series; *ai* stamen primordium in the second-initiated stamen series; *c* carpel; *E* epicalyx appendage; *FO* filament of stamen in first, long-level stamen series; *FI* filament of stamen in second, mid-level stamen series; *G* gynoecium; *Ps* site of petal attachment to calyx tube; *S* calyx lobes; *SR* calyx primordium ring. **Fig. 11.** Two flower buds. In the younger bud the calyx primordia are united into a ring that will form the calyx tube. In the older bud the calyx lobes have grown over to enclose the bud, while the epicalyx appendages are growing between the sepals. *Bar* 50 μm. **Fig. 12.** Bud with first set of stamens initiated opposite sepal lobes. *Bar* 50 μm. **Fig. 13.** Bud with calyx broken away to reveal first stamen set and gynoecial primordium. *Bar* 50 μm. **Fig. 14.** Bud with part of floral tube removed to show first stamen primordia opposite the sepals and the gynoecium primordium, which has begun to form a meristematic ring. *Bar* 50 μm. **Fig. 15.** Older bud broken open to show two sets of stamens and bilocular ovary. Primordia in the first stamen series, opposite the calyx lobes, have initiated anthers and are larger than primordia in the second stamen series. *Bar* 100 μm. **Fig. 16.** Well-developed bud with calyx lobes and epicalyx appendages visible but petals removed. Filaments of both long and mid-level stamens are contorted. The long-level filaments are inserted higher on the floral tube than the mid-level filaments. *Bar* 1 mm

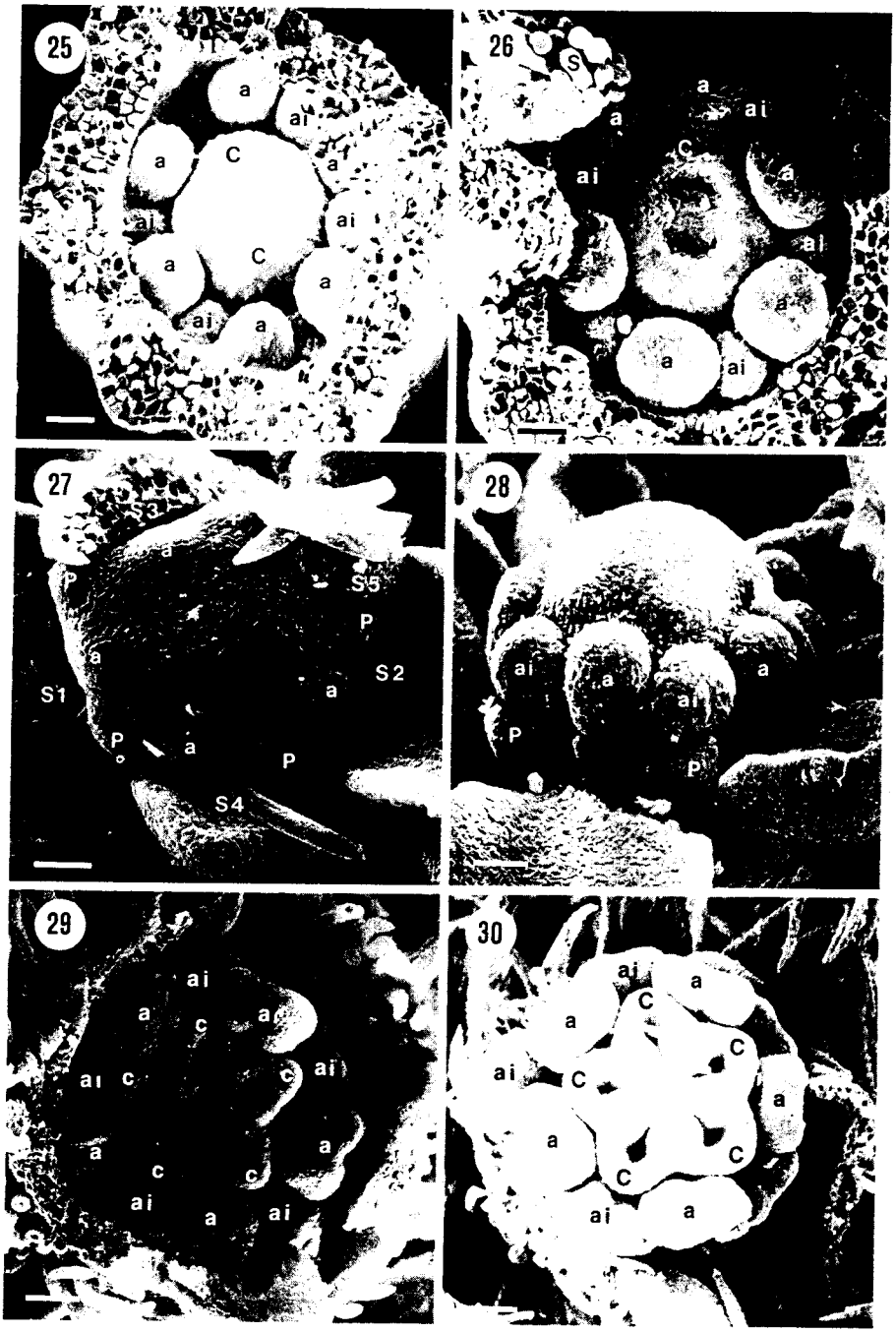

17
S2
S5
PB
S3
S1
18
S4
P
S2
S1
P
19
P
a
P
S1
a
S2
P
P
a
S
20
S
P
ai
a
a
ai
ai
P
a
P
S
21
a
ai
S
a
ai
S
P
ai
S
S
22
P
S
a
ai
P
a
S
S
ai
23
S
ai
Pai
C
a
Pai
C
S
ai
24
a
ai
C
ai
ai
a
a

within each mature flower, while the second stamen series corresponds to the shorter level. The difference between stamen whorls in time of origin is greater for *Lythrum* than *Oxalis*, in which the primordia arise almost simultaneously in some buds.

Our survey of the available data indicates that morph-specific patterns of stamen relative growth differ for *Lythrum* and *Oxalis* as compared to the Pontederiaceae. In both *Lythrum* and *Oxalis* the M morph appears to have the most complex pattern of stamen relative growth and shows relatively late-developing changes in relative growth rates. In the Pontederiaceae, in contrast, the S morph has the most divergent pattern of stamen relative growth when compared to the other morphs. The differences in pattern of stamen relative growth are probably related to the radial architecture of tristyly in the dicotyledonous families vs. the dorsiventral architecture in the Pontederiaceae. Additional developmental data for *Lythrum* and *Oxalis* are needed in order to understand this relationship.

While differences between stamen levels within a flower are present from stamen origin in tristylous species of *Lythrum* and *Oxalis*, these are not necessarily the differences that define stamen levels in tristyly. We do not know whether there are between-level differences in characters other than time of origin. For example, do primordia from different levels have different sizes at origin – e.g., do long-level stamens, which have larger anthers at maturity, have larger primordia than mid- or short-level stamens? This question is important for models of how the tristyly genes affect development. Do the genes regulate some event at primordium origin that results in the tristyly syndrome through developmental amplification or do they act directly on subsequent developmental events? Because many species initiate stamens in two or more whorls but do not develop two stamen levels [e.g., *Silene cucubalus* (Caryophyllaceae) (Sattler 1973) or *Pisum sativum* (Fabaceae) (Tucker 1989)], and because the order of stamen initiation is independent of intraflower stamen dimorphism in the Pontederiaceae, the differences in time of origin between stamen levels in the Lythraceae and Oxalidaceae are not necessarily the first expression of tristyly in these families.

Figs. 17–24. Scanning electron micrographs showing flower development in the S morph of *Oxalis rubra*. *a* stamen primordium in first-initiated stamen series, opposite the sepals; *ai* stamen primordium in second-initiated stamen series, opposite the petals; *C* carpel; *P* petal; *PB* prophyll bud; *S* sepal or sepal position if sepal has been removed; *numbers after S* indicate order of initiation. **Fig. 17.** A floral bud that has initiated sepals. *Bar* 20 μm. **Fig. 18.** A bud with five sepals that has initiated petals, forming a pentangular floral apex. *Bar* 23 μm. **Fig. 19.** A bud that has initiated the first stamens opposite the sepals. *Bar* 30 μm. **Fig. 20.** A bud with some sepals removed. The second set of stamens has been initiated opposite the petal primordia. *Bar* 30 μm. **Fig. 21.** A lateral view of a bud slightly older than in Fig. 20 that shows the relation of sepals, petals, and the two stamen sets. The stamens opposite the sepals are slightly larger than those opposite the petals. *Bar* 30 μm. **Fig. 22.** A surface view of a bud similar to that in Fig. 21. The stamens opposite the petals are lower on the apical dome than those opposite the sepals. *Bar* 30 μm. **Fig. 23.** A bud with the five-carpellate ovary initiated. The carpels arise on a radius with the petals and second stamen set. *Bar* 30 μm. **Fig. 24.** An older bud with some sepals removed to show the two stamen levels, which alternate within the flower and have different filament lengths. *Bar* 400 μm


25
a
ai
C
26
S
27
S3
S5
P
S2
S1
S4
28
29
30

In Fig. 35 we illustrate graphically four developmental relationships between stamen levels that could lead to the stamen differences observed in tristylous species. In all the models one factor that contributes to differences between morphs is the absolute amount of stamen growth. For example, the mid- and long-level stamens of the S morph grow more than the short- and mid-level stamens of the L morph. Such differences could result from variations between morphs either in the time over which growth occurs or in absolute growth rates. Differences in amount of growth can be seen by comparing the length of the line describing relative stamen growth in each model. Such differences, however, cannot alone account for the differences between morphs in relative stamen position within a flower. In the descriptions below, we concentrate on the other factors that contribute to morph-specific differences. In Fig. 35A all stamen primordia are equal in size at initiation, and differences among morphs in mature stamen position result from different relative growth rates, as well as differences in amount of growth. In Fig. 35B differences among morphs arise at stamen initiation because of size differences between each stamen series in a flower. These differentials could result from the amount of growth that occurs prior to initiation of the second stamen level or through differences in the size of stamen primordia at origin. Relative growth rates are equal after stamen initiation, but the S morph grows more than the other morphs. This model predicts that the largest difference between primordia within a flower will occur in the M morph. In Fig. 35C stamen primordia are equal-sized at initiation and have similar relative growth rates during early development, but their relative growth rates diverge later in development. Figure 35D incorporates elements of the three previous models: differences are present between stamen levels at time of origin, but the size differential is the same in all three morphs. Relative growth rates are equal among morphs in early development but diverge subsequently. An interesting aspect of this model is that the L and S morphs differ only in the extent of relative growth, whereas the M morph requires a different relative growth rate at some point in development.

Figs. 25–30. Scanning electron micrographs of flower buds of the M (**Fig. 25**) and L (**Fig. 26**) morphs of *Lythrum salicaria*. Labels as in Figs. 11–16. **Fig. 25.** A bud with calyx removed to show the first- and second-initiated stamen series. The stamens from the first series are larger and higher on the floral tube than the stamens from the second series. The gynoecium has formed a meristematic ring and is beginning to be subdivided into two carpels. *Bar* 30 μm. **Fig. 26.** A bud with calyx removed to show the first- and second-initiated stamen series. Differences between stamen series are as in Fig. 25. The two carpels are distinct. *Bar* 30 μm. **Figs. 27–30.** Scanning electron micrographs of flower buds of *Oxalis rubra*. **Figs. 27** and **29** of M morph; **Figs. 28** and **30** of L morph. Labels as in Figs. 17–24. **Fig. 27.** A bud with sepals, petal primordia, and stamen primordia opposite the sepals but not the petals. *Bar* 20 μm. **Fig. 28.** A lateral view of a bud with sepals bent back to reveal petal primordia and two sets of stamens. The stamens opposite the sepals are larger than the stamens opposite the petals. Carpels have been initiated on a radius with the petals and second stamen series. *Bar* 20 μm. **Fig. 29.** An older bud with sepals removed. The stamens that alternate with the carpels are larger than those opposite the carpels. *Bar* 50 μm. **Fig. 30.** An older bud at a developmental stage comparable to the bud in Fig. 29, with similar differences between stamen levels. *Bar* 50 μm

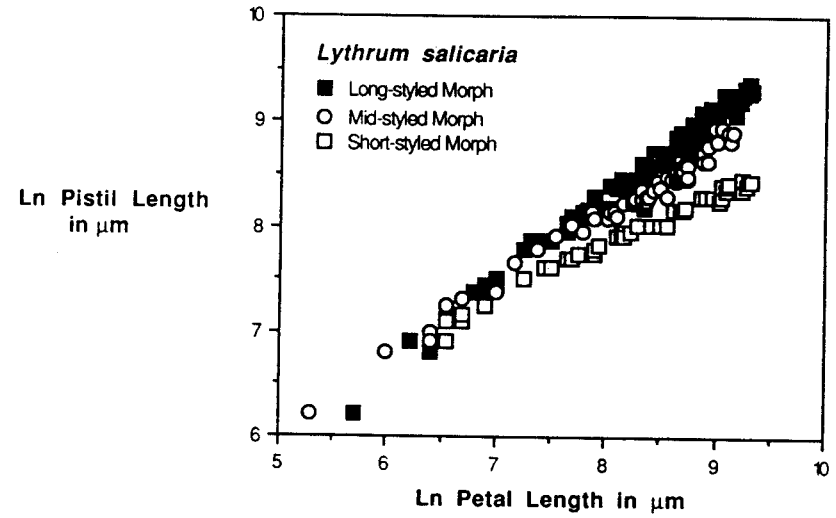


Fig. 31. Ln petal length vs. ln pistil length for the three morphs of *Lythrum salicaria*. The three morphs appear to have similar relative growth rates in early development but diverge in rate subsequently. (Data from Stirling 1933)

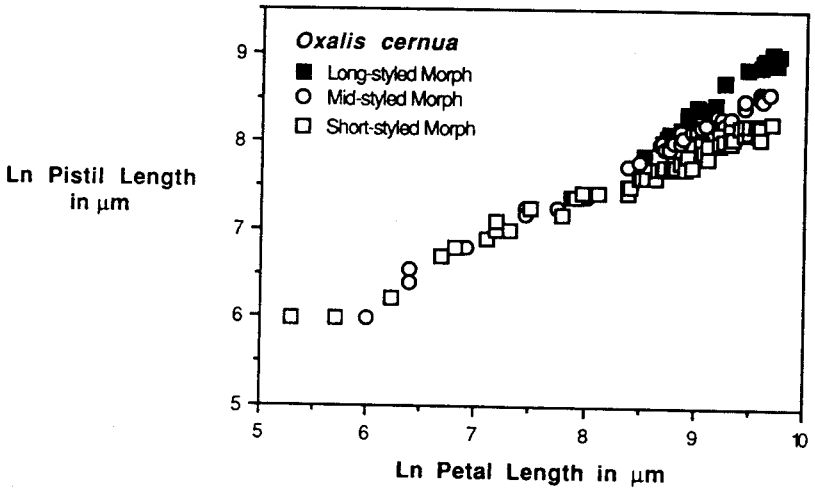


Fig. 32. Ln petal length vs. ln pistil length for the three morphs of *Oxalis cernua* (= *O. pes-caprae*). The data are consistent with a hypothesis of an early similarity but subsequent divergence in relative growth rates. (Data from Stirling 1936)

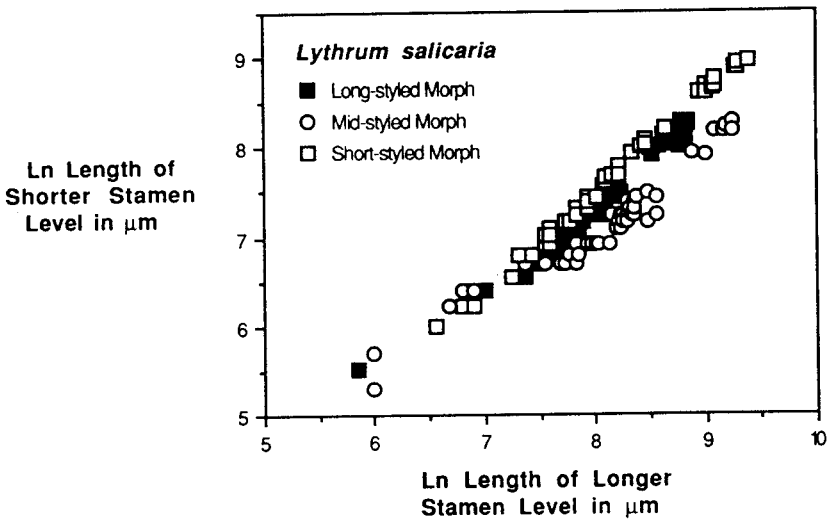


Fig. 33. Ln length of longer stamen level vs. ln length of shorter stamen level for the three morphs of *Lythrum salicaria*. The relative growth rates of all three morphs appear similar in young buds, although more data are needed. The L and S morphs have relatively similar relative growth rates, whereas the M morph diverges distinctly from these two. (Data from Stirling 1933)

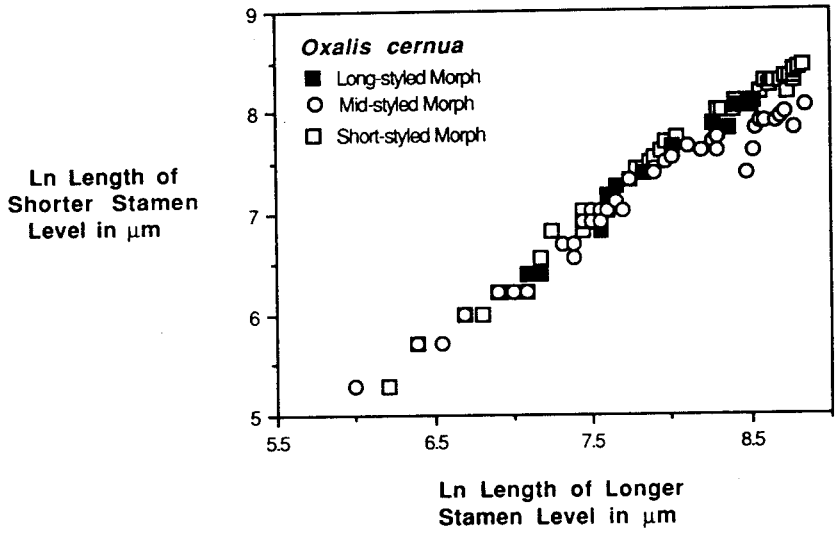


Fig. 34. Ln length of longer stamen level vs. ln length of shorter stamen level for the three morphs of *Oxalis cernua* (= *O. pes-caprae*). The data are consistent with the hypothesis that the three morphs have similar relative growth rates in early stamen development, and the L and S morphs continue this initial rate. The M morph, in contrast, shows a relatively late divergence in growth rate. (Data from Stirling 1936)

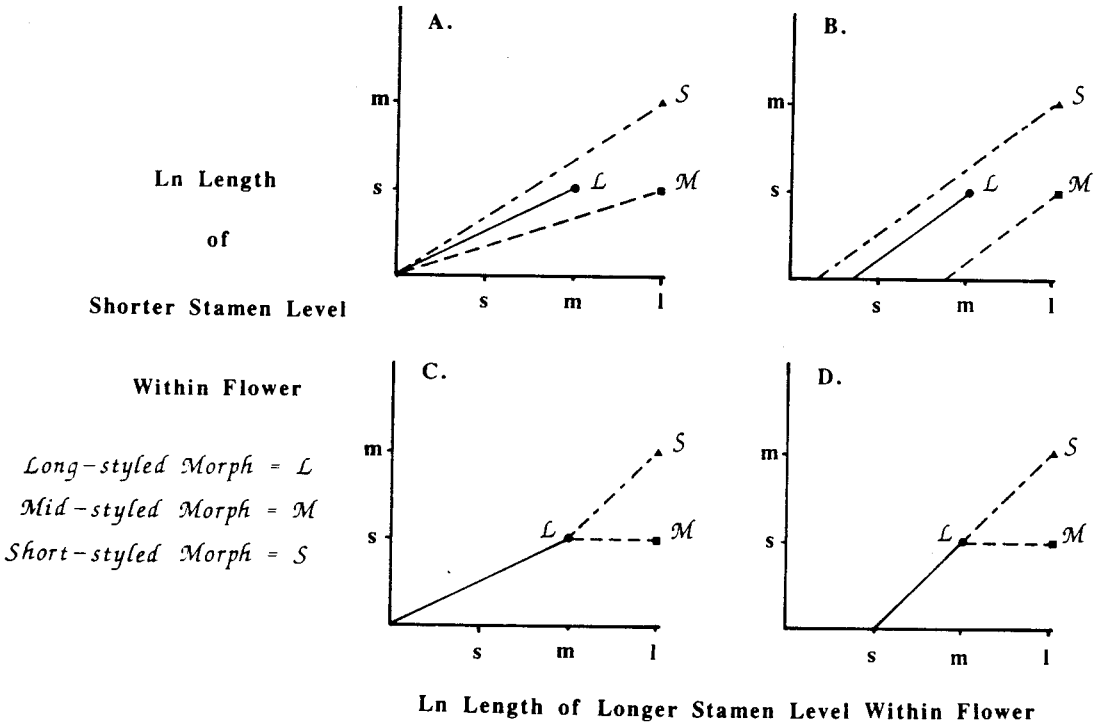


Fig. 35A-D. Four models for relative growth of the longer vs. the shorter stamen level in the three morphs of a tristylous species. **A** Stamen growth in all morphs begins with primordia that are equal in size. The two stamen levels within each flower have different growth rates, however, and the relative difference in growth rates varies among morphs, as does the amount of growth. **B** Stamen primordia from each level within a flower are different in size from initiation, and the degree of difference varies among morphs. Although relative growth rates are similar among morphs, mature organ sizes differ because of the initial differences in primordium size, as well as differences in amount of growth. This model predicts that the primordium size differential is greatest in the M morph. **C** Stamen primordia within a flower are similar sized in all morphs and have the same relative growth rate in early development. Differences among morphs arise because of late-developing changes in relative growth rates, as well as differences in amount of growth. **D** Stamen primordia from each level within a flower differ in size from origin, but the degree of difference is similar among morphs, and early relative growth rates are equal. Differences among morphs in mature stamen height arise because of late-developing changes in relative growth rates. In this case the L and S morphs have similar relative growth rates but differ in the amount of growth, whereas the M morph has a distinctly different relative growth rate in later developmental stages

Although these models are simple and do not include all possibilities, it is clear that none of the tristylous species examined to date follow the pattern found in Fig. 35A. Patterns of primordia initiation and early growth indicate that development of tristyly in the Pontederiaceae is most closely described by some variant of Fig. 35C. The development of tristyly in the Lythraceae and Oxalidaceae, in contrast, is better depicted by Fig. 35B or D. Both figures predict a near or complete overlap of plots for the L and S morphs, as seen in Stirling's data (Fig. 25, 26). These two figures

indicate the importance of acquiring quantitative data on early growth in all three morphs to establish when and how divergence occurs.

Similar models, which compare relative growth rates of bud length to gynoecium length, can be constructed for gynoecium development (Fig. 36). Such models are less complex than stamen models, as gynoecium length varies only between morphs and mature flower length is similar in the three morphs. In Fig. 36A gynoecium primordia are similar in size at origin but subsequently differ in growth rates. Gynoecial primordia have similar lengths at origin and the same relative growth rates in Fig. 36B. In this model differences in style length develop because the primordia arise at different times, and thus the extent of growth varies among morphs. In Fig. 36C the primordia have different sizes at origin but subsequently grow at similar rates. Although there is no evidence for major differences in primordium size or time of gynoecium origin in tristylous species, these models could apply to the size or time of differentiation of the gynoecium into style and ovary. Our studies of the Pontederiaceae show no major differences among morphs in style size or bud length at stylar differentiation, but this possibility should be considered for other tristylous species.

In the last three models (Fig. 36D-F) gynoecium or style lengths are similar at origin, and early relative growth rates are equal among morphs. Divergence occurs later in development as a result of inhibition (Fig. 36D) or differences in relative growth rates (Fig. 36E). Many combinations of these latter two variables are possible in modeling the development of tristyly (e.g., Fig. 36F). Although in our models the initial relative growth rate produces long styles, this initial rate could result in the mid or short styles. The other style lengths could then arise from accelerated, as well as inhibited, relative growth rates. The underlying mechanisms for inhibition vs. growth rate reduction may not differ, since a reduction in growth rate is a type of inhibition. Figure 36D, however, implies a difference among morphs in the onset of inhibition, whereas in Fig. 36E changes in relative growth rate occur at the same point in development, but the morphs differ quantitatively either in amount of an inhibitor or in sensitivity of the style to an inhibitor.

The developmental data on style growth of tristylous species are best described by variants on these last three models. Style differences among morphs in the Pontederiaceae arise from differences in time of inhibition (Fig. 8 vs. Fig. 36D). In the Lythraceae and Oxalidaceae styles appear to diverge in growth rates (Fig. 31, 32 vs. Fig. 36E), although additional data on early development in both families are needed to confirm similarity among morphs in initial relative growth rates.

3.5 Development of Distyly

The data on tristyly show that the developmental processes responsible for tristyly differ among families and developmental patterns leading to different stamen levels can even differ within a species. The models of stamen and style relative growth rates (Figs. 35, 36) present additional possibilities. Because distyly occurs in many unrelated taxa and has diverse structural bases, as reviewed in Section 2.2, a similar diversity of developmental pathways should underlie the heteromorphisms of distylous species. The data to evaluate this hypothesis, however, does not currently exist.

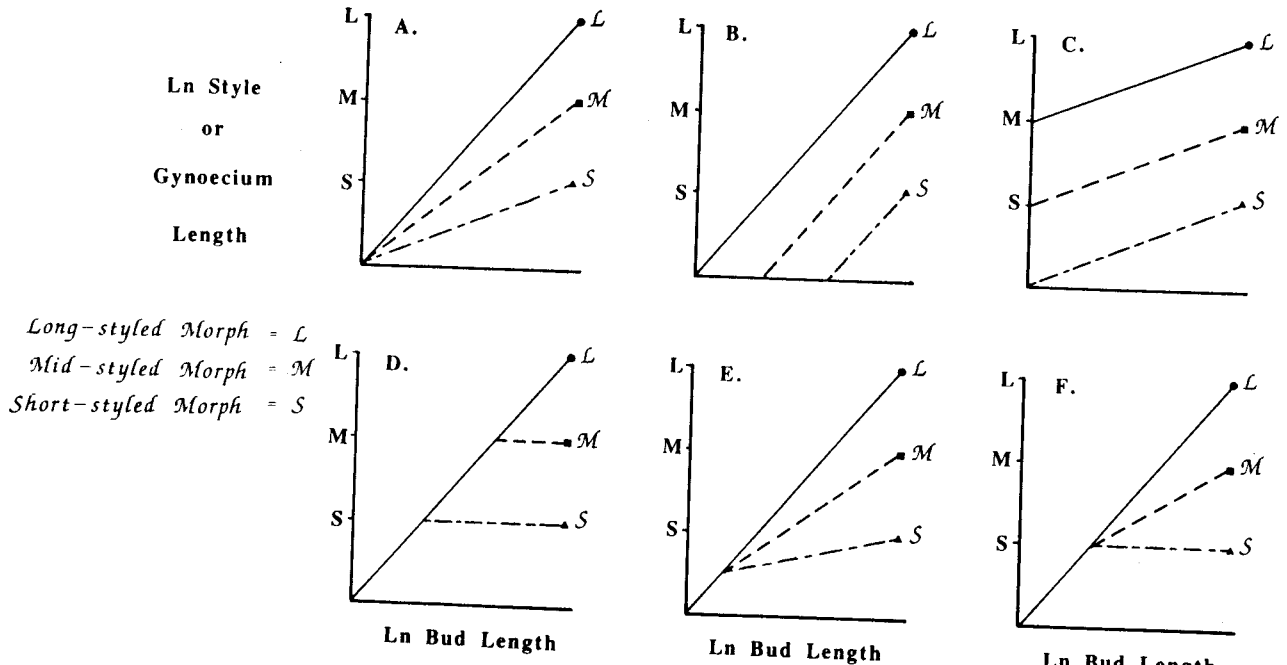


Fig. 36A-F. Six models for relative growth of bud length vs. style or gynoecium length for all morphs of a tristylous species. **A** The style or carpel primordia are equal in size at origin in the three morphs but have different relative growth rates. **B** The style or carpel primordia arise at different times in bud development, first in the L morph, then the M, and finally the S. Although the three morphs have similar gynoecial relative growth rates, the initial timing differences result in different stigma heights. **C** The style or carpel primordia arise at the same time in development in the three morphs, but primordium size at origin differs among morphs. Although gynoecium relative growth rates are the same among morphs, the initial size differences result in different stigma heights in the three morphs. **D** The style or carpel primordia have similar sizes at origin and similar relative growth rates in early development in the three morphs. Stylar variations among morphs result from differences in when style growth stops. **E** The style or carpel primordia are equal sized at initiation and have similar relative growth rates in early development. Relative growth rates diverge subsequently, however, and produce the different stigma heights. **F** This model combines elements of the previous two and provides one example of the many variations on these models that are possible. Style or carpel primordium size are similar among morphs at origin and in early development. Stigma height differences among morphs develop because of early inhibition of growth in the S morph and divergence in relative growth rate in the M morph

Stirling (1932, 1936) studied development of *Hottonia palustris* (Primulaceae), *Menyanthes trifoliata* (Menyanthaceae), and four distylous species of *Primula*. He showed that stamen and pistil growth rates differed between morphs in these taxa, but he did not provide sufficient data to analyze growth patterns. Differences between morphs in stamen or style cell number (Stirling 1932) and cell length (Dulberger 1975, Y. Heslop-Harrison et al. 1981) have been observed in distylous species. In both *Primula sinensis* and *Menyanthes trifoliata* divergence in organ lengths between morphs occurs after meiosis in anthers and ovules (Stirling 1932, 1936), as is found in the development of tristyly in the Pontederiaceae (Richards and Barrett 1984).

We have no evidence to indicate whether stamen and style primordium size or time of initiation differ between distylous morphs (see also the discussion of pollen number dimorphism in Sect. 2.3). If mature flower size is similar in the two morphs, relative growth rates of floral organs can be compared using bud length as a standard. Differences in growth rate, time of origin, size at origin, or time of inhibition (cf. Fig. 36) are possible causes for differences between stamen and style lengths in distylous species. Data on three distylous species of the Rubiaceae show early similarities in style length, followed by subsequent inhibition or reduction in growth rates (Fig. 37, JH Richards unpubl. data). Further comparisons of early development between morphs of distylous species and more complete studies of later stages of growth are needed to understand how the genes that control distyly operate in diverse families. Such data will also help to evaluate evolutionary hypotheses about the origin of the polymorphism (see Sect. 6).

4 Phenotypic Variation in Floral Heteromorphisms

4.1 General Considerations

The flower is usually considered less prone to nongenetic sources of variation than vegetative organs. Many floral traits not only remain relatively constant within individuals but also exhibit a stereotyped plan among populations, species and often families (Stebbins 1951; Berg 1959). Constancy of floral traits is typical of many animal-pollinated plants, where precise positioning of reproductive parts is required for effective pollination. While canalization of floral traits may be especially important in heterostylous species, where the reciprocal arrangement of stamens and styles promotes legitimate pollinations, no explicit comparisons of the patterns of phenotypic variation in heterostylous and nonheterostylous species have been made. We do not know whether floral constancy is, in fact, greater in heterostylous groups. Considerable phenotypic variation in stamen and style length has been reported in distylous *Turnera ulmifolia* (Martin 1965) and *Erythroxylum coca* (Ganders 1979b). How much of this variation is under genetic control and whether it influences legitimate pollination is not known.

Phenotypic variation of floral traits in heterostylous species is often more pronounced where the polymorphism is undergoing evolutionary modification, particularly towards increased self-fertilization through homostyle evolution (see Chap. 10). Two potential sources of phenotypic variation occur in these circumstances.

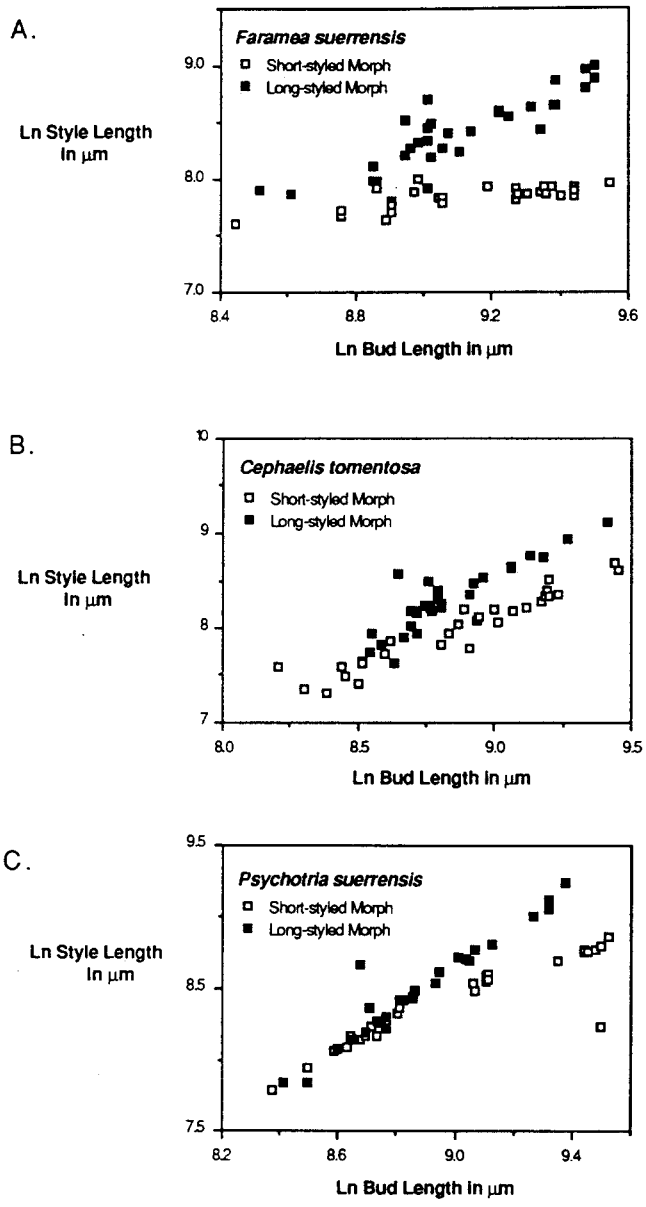


Fig. 37A-C. Ln bud length vs. ln style length for both morphs of three distylous species in the Rubiaceae. Flowers and buds were collected at La Selva, Costa Rica. **A** *Faramea suerrensis;* **B** *Cephaelis tomentosa;* **C** *Psychotria suerrensis.* Growth of the short-morph style of *F. suerrensis* is inhibited when compared to that of the long morph. The morphs of *C. tomentosa* and *P. suerrensis* have similar relative growth rates in early development, but in both species the S morph has a late reduction in stylar growth rate. (JH Richards unpubl. data)

Differences among individuals may result from genetic modifications of floral traits that influence the mating system. In homostylous varieties of *Turnera ulmifolia* variation in stigma-anther separation affects the rate of self-fertilization in natural populations (Barrett and Shore 1987). This variation is polygenically controlled (Shore and Barrett 1990). In contrast, several major genes alter stamen and style length in horticultural varieties of distylous *Primula sinensis*. These genes are nonallelic to the *S* locus and have pleiotropic effects on other aspects of floral phenotype, such as flower color and petal shape (Mather and DeWinton 1941).

A second source of phenotypic variation in floral traits occurs within the individual and is associated with increased inbreeding. Inbred lines of *Primula sinensis*, for example, demonstrate greater intraplant variance in stamen and style length in comparison with their F_1 generations (Mather 1950). A loss of canalization and a reduced ability to buffer against environmental stimuli and accidents of development has been associated with inbreeding and the attendant rise in homozygosity in a wide range of organisms (reviewed in Lerner 1954; Jinks and Mather 1955; Rendel 1959; Levin 1970).

4.2 Floral Variation in *Eichhornia paniculata*

Some of the most striking patterns of floral variation that have been documented in a heterostylous species occur in tristylous *Eichhornia paniculata*, where they are associated with the breakdown of floral trimorphism to monomorphism and the evolution of semi-homostyly. The variation results from both genetic and nongenetic causes and is manifested at a number of levels, including between populations, between genotypes within populations, and between flowers of individual plants. We have attempted to quantify this variation and to determine its genetic, environmental, and developmental basis. The remainder of this section reviews some of our results and discusses their relevance to the ecology and evolution of populations.

4.2.1 Floral Variation Among Populations

The evolutionary breakdown of tristyly in *E. paniculata* involves two important stages: (1) loss of the *S* allele and thus the S morph from populations; and (2) fixation of the *M* allele and hence loss of the L morph from populations. Population structure thus changes from floral trimorphism (L, M, S) through dimorphism (L, M) to monomorphism (M). The decrease in morph diversity is accompanied by the spread and fixation of selfing semi-homostylous variants of the M morph (Barrett 1985a; Barrett et al. 1989). The most significant floral modifications involve alterations in the height of short-level stamens of the M morph, which elongate to the level of the stigma, resulting in automatic self-pollination. Figure 38 illustrates the typical stamen and style configurations of trimorphic, dimorphic, and monomorphic populations of *E. paniculata*. In most dimorphic populations, particularly those in N.E. Brazil, modifications involve elongation of a single outer stamen of the short stamen level. In some monomorphic populations, however, especially in Jamaica, all three stamens of the short-level stamens are elongated to the mid-level position (Fig. 38).

Stamen modifications in the L and S morph of *E. paniculata* have been observed in natural populations. The variants rarely, however, establish successfully in nature to form monomorphic populations, as in the M morph. One exception involves a semi-homostylous L morph in Nicaragua (Barrett 1988). Why the M morph is apparently more susceptible to evolutionary modifications that favor increased self-fertilization is unclear. This situation does not appear to be restricted to *E. paniculata*, since modifications of the M morph are reported in other tristylous species (Stout 1925; Mayura Devi and Hashim 1964; Ornduff 1972; Barrett 1979; Barrett and Anderson 1985). The reason may be that the stigma is located between stamen levels in the M morph. As a result, developmental alterations of stamens or style in either direction will bring anthers and stigmas in proximity with each other. In the L and S morphs reproductively significant alterations can occur in only one direction. The M morph thus has twice the opportunity for such changes. Since alterations of both short-level stamens (*E. paniculata*) and long-level stamens (*E. crassipes*, Barrett 1979) have produced selfing variants of the M morph, both stamen levels are capable of developmental modification.

4.2.2 Floral Variation Within Morphs

An unusual feature of stamen modification in the M morph of *E. paniculata* is the discontinuous nature of the elongation patterns in the short-level stamens. The most common variant observed in N.E. Brazil exhibits a single stamen in the mid-level position (Fig. 39). The change in stamen position results primarily from filament elongation, although the modified stamen is also inserted higher on the floral tube (Fig. 40). The absence of continuous variation in filament length and, instead, the discrete nature of the alteration is consistent with either simple major gene control or polygenic control with a threshold response. Analysis of progeny variation from controlled crosses between modified and unmodified M genotypes from a Brazilian population (B3) are consistent with a model of single recessive gene control for the altered stamen (SCH Barrett unpubl. data). The gene apparently acts relatively late in floral development and appears to have only minimal effects on other facets of floral phenotype. Modification of filament length occurs through cell elongation, not cell division (JH Richards unpubl. data), and is manifested by rapid changes in filament length that occur primarily in the 24 h prior to anthesis (Fig. 41). It seems likely that expansion of filament length in the modified stamen is regulated by hormones, such as gibberellic acid. Hormones have been implicated in the regulation of reproductive organ size in other flowering plants (Greyson and Tepfer 1967; Pharis and King 1985; Koning 1983a,b; Jones and Koning 1986; Koning and Raab 1987).

Further modifications towards semi-homostyly in the M morph of *E. paniculata* involve elongation of the remaining two short-level stamens within a flower to the mid-level position. The genetic basis of these changes is unknown but presumably involves additional modifier genes that regulate positional effects within stamen levels. When a single stamen is modified in *E. paniculata*, it is always the stamen on the side of the flower away from the inflorescence branch (Fig. 39). The modified stamen is inserted on a member of the outer tepal whorl. In addition, although

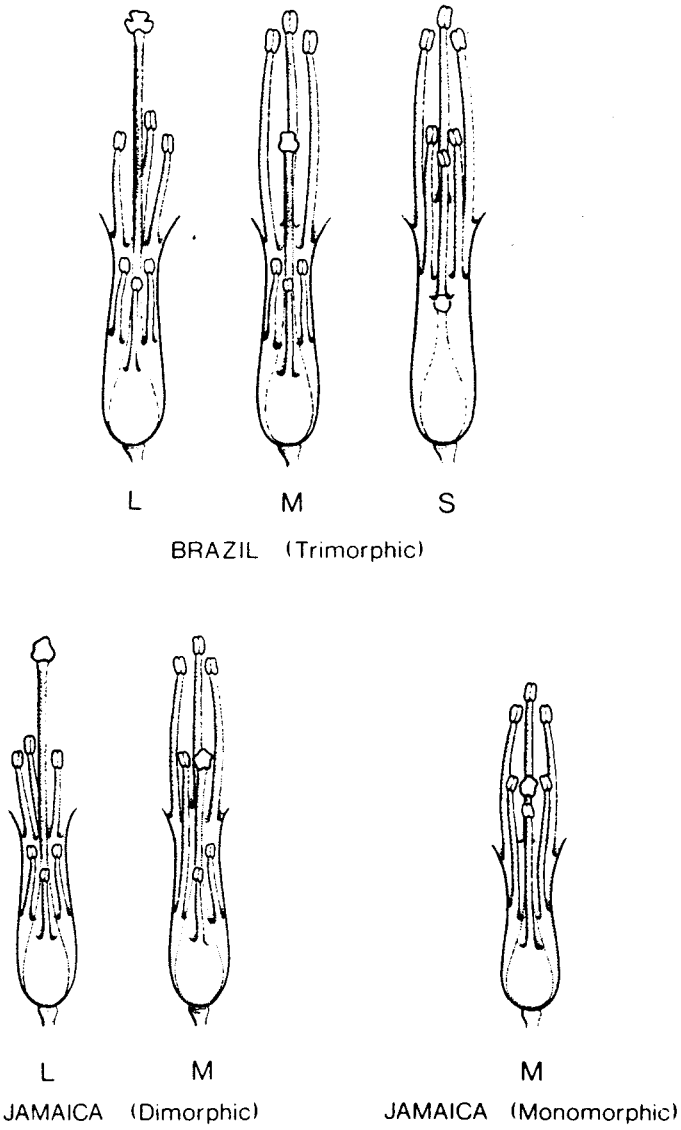


Fig. 38. Stamen and style configurations that accompany the evolutionary breakdown of tristyly to semi-homostyly in *Eichhornia paniculata*. Flowers of the L, M, and S morphs from an outcrossing trimorphic population (B5) are contrasted with two populations from Jamaica (dimorphic J15, monomorphic J3) that have different patterns of stamen modification. In the dimorphic population the L morph is largely unmodified whereas the M morph has a single stamen adjacent to the mid-level stigma. Complete semi-homostyly is evident in the monomorphic population with the three stamens of the "short-level" elongated to the mid position. (For further details see Barrett 1988)

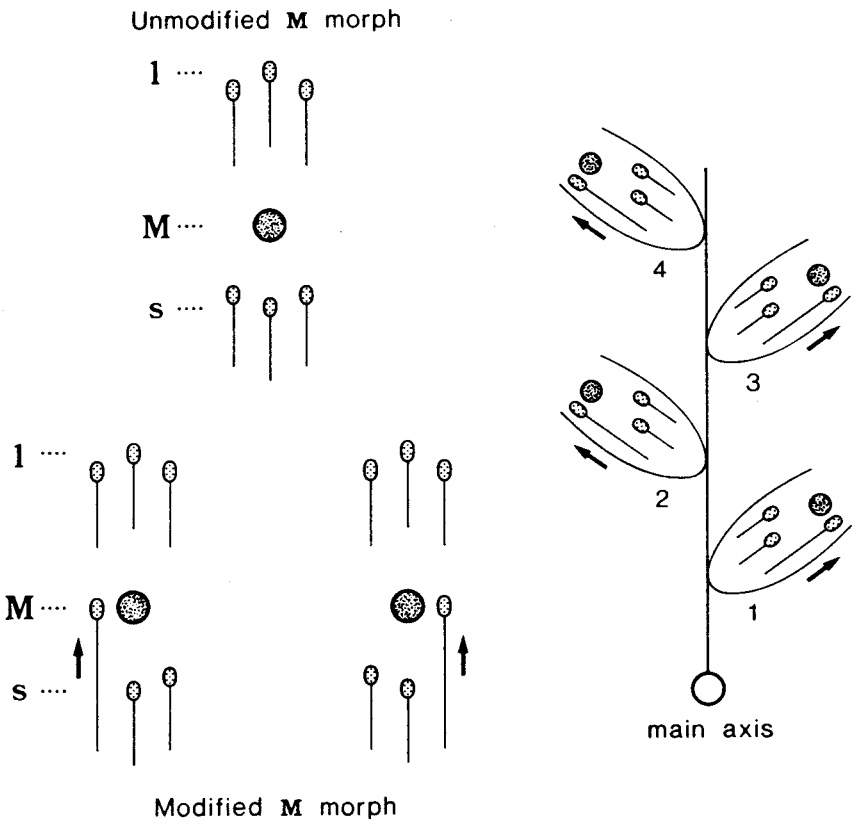


Fig. 39. Patterns of stamen modification in the M morph of *Eichhornia paniculata*. The diagrams illustrate the most common variant observed in Brazilian populations with a single short-level stamen adjacent to the mid-level stigma. Either the right or the left stamen of the short stamen level elongates, depending on the position of the bud on the inflorescence branch

within-series differences in timing of stamen initiation are not apparent in *E. paniculata*, they are present in *Pontederia cordata*, where this outer stamen primordium is the first to develop (Fig. 3). The modifier gene that causes the initial change to the outer stamens acts within this positional/developmental framework. The genes that cause subsequent stamen modification either alter this framework or change the sensitivity of the short-level stamens to these initial positional relationships.

The evolution of complete semi-homostyly in *E. paniculata* (Fig. 38), in which all three stamens are positioned close to the mid-level stigma, is accompanied by manifold changes in other aspects of floral phenotype, including reduction in the size and showiness of perianth parts, weakening of pollen heteromorphism, and decreases in pollen production and ovule number (Barrett 1985b). These changes in floral syndrome are most likely controlled by many additional genes with small effects. The significant point, however, is that the initial change in floral phenotype that precedes the evolution of the selfing syndrome appears to be under relatively

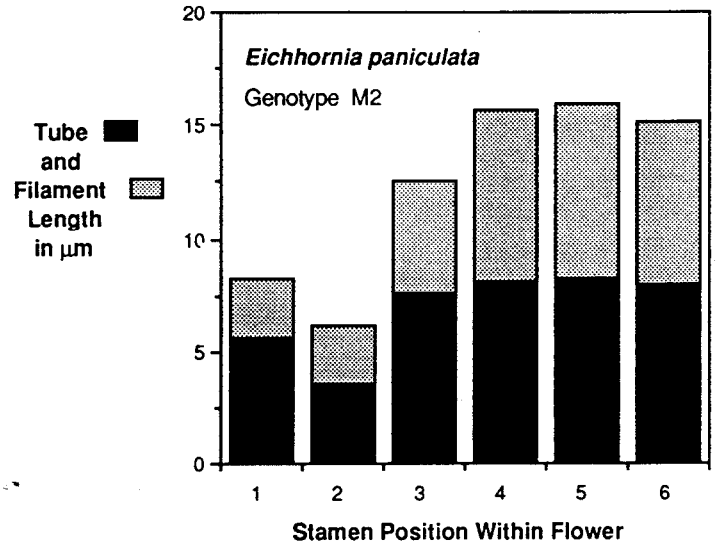


Fig. 40. Length of filament and insertion height (length of floral tube below the filament) in flowers of the M2 genotype (population B3, N.E. Brazil) of *Eichhornia paniculata*. In M2 genotype a single stamen from the short stamen level grows to the level of the mid-length stigma, causing self-pollination. The modified stamen position results primarily from increased free filament length. (JH Richards unpubl. data)

simple genetic control. This illustrates how simple genetic changes that affect floral morphology can alter plant mating systems. Such changes have profound influences on reproductive isolation, character divergence, and speciation (Gottlieb 1984; Barrett 1989).

4.2.3 Intraplant Floral Variation

Until now our discussion of stamen modification in the M morph of *E. paniculata* has involved consideration of phenotypic differences between individuals and populations. A curious feature of the genetic modifications in stamen position in *E. paniculata* is that in some modified plants not all flowers within an inflorescence exhibit the altered phenotype. As a result, inflorescences can be composed of both unmodified and modified flowers. Since the former are incapable but the latter are capable of autonomous self-pollination, genotypes can potentially produce a mixture of selfed and outcrossed seed. Intra-inflorescence variation is particularly evident in populations with both modified and unmodified plants and thus further contributes to a mixed mating system.

A second pattern of intraplant variation is found in primarily selfing populations where all plants possess modified flowers. In this case inflorescences can produce flowers with different numbers (one to three) of modified stamens and, at low

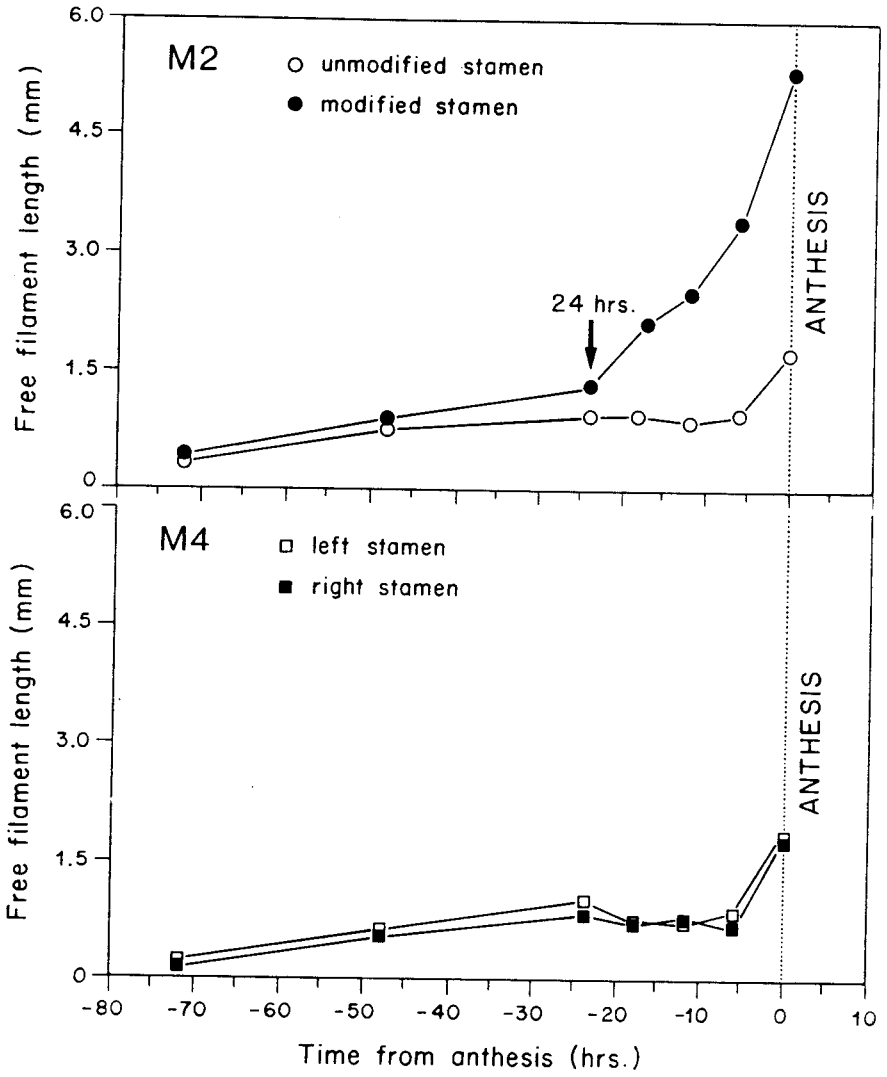


Fig. 41. Growth rate of the free filament of short-level stamens in two genotypes of the M morph of *Eichhornia paniculata* from a population (B3) in N.E. Brazil. Genotype M2 possesses modified stamens that cause autonomous self-pollination of mid-level stigmas. Genotype M4 is unmodified and incapable of autonomous self-pollination. Only one stamen, either the left or right short-level stamen, within each flower of genotype M2 is modified

frequency, unmodified flowers. This pattern of floral instability was first described among Jamaican populations of *E. paniculata* (Barrett 1985b) but is also found in dimorphic and monomorphic populations in N.E. Brazil (Glover and Barrett 1986). The variability in expression of short-stamen modification may result from incomplete penetrance of the modifier gene(s) in different genetic backgrounds, as well as from nongenetic effects associated with development.

Genotypes of *E. paniculata* vary in the frequency of flowers that display stamen modification. In addition, the frequency of modified flowers on successive inflorescences can fluctuate during the blooming period. Figure 42 illustrates these effects for twelve consecutive inflorescences produced by six genotypes of *E. paniculata*. The average frequency of unmodified flowers differs among genotypes (e.g., L9 versus M9) and the degree of instability also varies among genotypes (NA36 versus NA37). No clear pattern is evident with regard to the fluctuations in frequency of modified and unmodified flowers among the six genotypes, suggesting that variation may, in part, result from subtle microenvironmental influences, as well as from random accidents during the course of floral development.

In order to investigate the nature of stamen instability further, Barrett and Harder (1991) used statistical techniques involving logistic regression to examine whether modified flowers are produced at random within inflorescences of *E. paniculata*. The method considers a dichotomous response (unmodified vs. modified flowers) to a group of independent variables (e.g., branch position on an inflorescence, bud position on a branch). The results indicated that modified flowers are more likely to occur on branches positioned towards the distal end of the main inflorescence axis and at bud positions at the proximal end of branches. Flower expansion begins on proximal buds at the base of the inflorescence and proceeds acropetally on the inflorescence and from the base of a branch outwards. Essentially, open flowers occupy a cone that increases in height and then decreases as an inflorescence proceeds through flowering. Flowers most susceptible to modification are thus those that expand at the peak of inflorescence blooming, in the initial phases of fruit development, but when many buds have yet to open. These inflorescence position effects may, therefore, involve complex hormonal and/or nutritional interactions superimposed on a genetic background that allows modification.

The instability of the short stamen position in the M morph of *E. paniculata* does not appear to be the result of genome-wide homozygosity brought about by intense inbreeding. Genotypes NA36 and NA37, which have short-level stamen variation (Fig. 42), result from interpopulation crosses and are heterozygous at a large number of isozyme loci and presumably many other genes. More importantly, the variability in elongation of short-level stamens is not accompanied by increased variation of other floral traits. A multivariate comparison of 14 floral traits in modified and unmodified genotypes from a population in N.E. Brazil failed to detect any increase in intraplant variation associated with short stamen level modification (Seburn et al. 1990). For example, long-level stamens of plants displaying instability in the short-level stamens were as canalized in expression as long-level stamens from unmodified plants. These results suggest that the stamen modifications result from specific changes in the genetic control of short-level stamen variation, as opposed to generalized developmental instability brought about by increased homozygosity.

While developmental instability of the short-level stamens in the M morph of *E. paniculata* does not appear to be correlated with increased variation in other floral

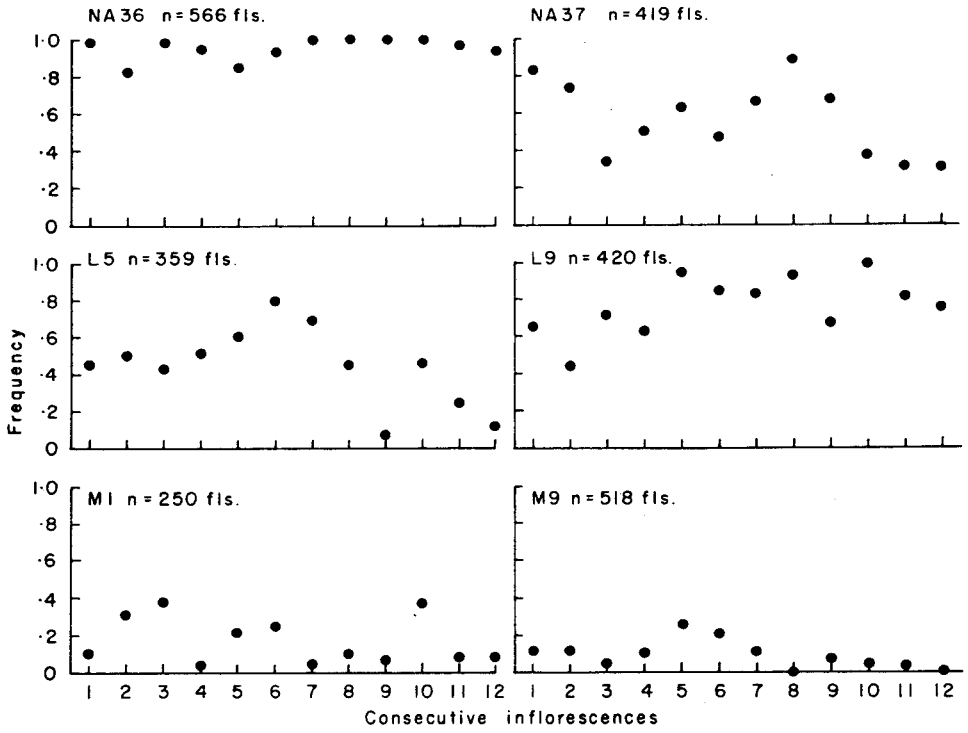


Fig. 42. Comparison of the frequency of unmodified flowers on 12 consecutive inflorescences of six genotypes of the M morph of *Eichhornia paniculata* grown under uniform glasshouse conditions. All flowers on inflorescences were scored as unmodified or modified depending on whether alterations to short-level stamens were evident. Genotypes NA36 and NA37 are F_1 crosses between trimorphic population B1 and monomorphic population J3, genotypes L5 and L9 are from monomorphic population B4, and genotypes M1 and M9 are from dimorphic population B3. See Barrett (1985b) for further details of populations, and Barrett and Harder (1991) for an analysis of developmental instability in the six genotypes

organs, a range of other developmental abnormalities are evident in natural populations with high levels of self-fertilization. These phenodeviants (Lerner 1954) include genotypes with twisted, fasciated, fused, or missing perianth parts, pollen sterility, disturbances in floral pigmentation, and the production of cleistogamous flowers. In most cases the abnormal flowers occur among normally developed flowers within inflorescences and are a relatively stable feature of particular genotypes. More rarely, genotypes are fixed for the abnormality and all flowers are modified. A survey of 16 populations of *E. paniculata* in N.E. Brazil and Jamaica, which had contrasting style morph structure and mating patterns, demonstrated significant differences in occurrence of tepal abnormalities both between regions and between populations within regions (Table 2). In N.E. Brazil populations had relatively few tepal abnormalities, particularly in trimorphic populations. In Jamaica, however, the incidence of tepal instability was significantly higher, and in two populations the majority of flowers

Table 2. A survey of abnormal tepal development in 16 populations of *Eichhornia paniculata* from N.E. Brazil and Jamaica. In each population all flowers open on a single day on one inflorescence of 25 plants were scored for normal or abnormal tepal expression. Abnormalities involved twisted, fused, or missing perianth parts (SCH Barrett, unpubl. data)

Population code	Style morph structure	No. of flowers surveyed	Frequency of abnormality (%)
N.E. Brazil			
B 42	Trimorphic	708	6.2
B 46	Trimorphic	388	20.9
B 56	Trimorphic	204	1.5
B 62	Trimorphic	318	3.8
B 135	Trimorphic	281	3.2
B 65	Dimorphic	169	13.6
B 69	Dimorphic	260	0
B 70	Dimorphic	348	10.0
B 114	Dimorphic	190	5.3
B 63	Monomorphic	200	3.0
			$\bar{x}$ – 6.75
Jamaica			
J 25	Dimorphic	172	17.0
J 17A	Monomorphic	237	72.0
J 17B	Monomorphic	200	82.0
J 18	Monomorphic	96	22.0
J 25	Monomorphic	111	15.0
			$\bar{x}$ – 41.6

exhibited abnormal development. Populations in Jamaica are highly self-fertilizing and abnormal tepal development may be selectively neutral on the island, since pollinators are unlikely to exert much influence on floral form.

5 Genes and the Development of Heterostyly

Heterostyly has evolved repeatedly from an ancestral condition of floral monomorphism (see Chap. 6). Accordingly, the evolution of heterostyly must have occurred through selection of genes that modify development of stamen and style lengths. The reciprocal positioning of stigmas and anthers is hypothesized to be controlled by relatively few loci in both distyly and tristyly. In distyly a single, tightly linked supergene with at least three loci, each with two alleles, segregates as a single gene (Dowrick 1956; Charlesworth and Charlesworth 1979; Muenchow 1981). The supergene controls stigma height, anther height, incompatibility reactions, and various ancillary characters, such as pollen size. In the three-locus supergene model advanced for *Primula* (see Chap. 5) one gene controls stylar morphology and incompatibility, one gene controls pollen size and incompatibility, and a third gene controls anther height (Dowrick 1956). Stamen structure and incompatibility, therefore, are thought to be controlled by separate genes, while style morphology and incompatibility are governed by a single gene.

In tristyly two loci, *S* and *M*, each with two alleles and *S* epistatic to *M*, control the levels of stamens and styles in each flower (Fisher and Mather 1943 for *Lythrum*; Fyfe 1950; Weller 1976b for *Oxalis*; and SCH Barrett unpubl. data for *Eichhornia*). Other loci, however, can affect the expression of the *S* and *M* loci (e.g., Bennett et al. 1986 in *Oxalis*). Charlesworth (1979) proposes that a third locus that creates a gradient of pollen reactions to stigma height is fixed in tristylous species. The existing data, therefore, indicate that relatively simple genetic systems control the inheritance of both distyly and tristyly. While the genetic systems involve a small number of major genes, they are also subject to modifier genes and epigenetic effects that can alter expression of the heterostyly syndrome (cf. Sect. 4).

The structural and developmental data reviewed in this chapter suggest that the genes governing heterostyly control different processes in diverse groups. As we investigate development in heterostylous groups, however, common developmental themes are likely to emerge. Surveys that correlate particular heterostylous developmental patterns with other floral characters, such as presence of an inferior ovary or distinct sepal and petal whorls, would help to clarify the nature of developmental constraints in heterostylous groups. Petal growth in tristylous *Lythrum* and *Oxalis*, for example, is delayed compared to stamen development, whereas in the Pontederiaceae tepal length always exceeds stamen length in early stages of growth. These differences in perianth development may have constrained the organization of tristyly in these families. Comparative studies of heterostylous taxa will provide insights into how ancestral floral structure and developmental patterns have influenced the evolution of distyly and tristyly.

Genetic studies have not yet answered the question of whether the genetic architecture of distyly and tristyly are similar. Although there is evidence for a heterostyly supergene in some distylous species, the genes controlling tristyly may not involve a supergene (Charlesworth 1979; but see Ganders 1979a). Charlesworth (1979) modeled the evolution of tristyly for a species with flowers that already had two anther whorls at different levels. Her model assumes that stigma height automatically determines both anther height and the stigma's incompatibility reaction. Anther height, in turn, determines the incompatibility reaction of the pollen produced. In this floral background tristyly could evolve via a distylous intermediate that has two anther sets. The relation of floral morphology to incompatibility reactions in this model differs from the distyly supergene model, which hypothesizes separate control of anther height and pollen incompatibility.

The contrast between models for the genetic architecture of distyly and tristyly cautions against generalizing from the limited genetic data available. Although the reported inheritance patterns are similar among distylous species, the organization of genes that control the syndrome may differ in these species. Perhaps some distylous species have supergenes, while others have a system that resembles the distylous ancestor in Charlesworth's (1979) tristyly model. Without more data on both distylous and tristylous genetic systems, it is premature to assume that the supergene model describes all distylous species or that the model applies to tristyly.

The available genetic and developmental evidence suggests that distyly is a less complex system than tristyly. The reciprocal herkogamy (Webb and Lloyd 1986) in distylous species can be achieved by quantitative variation of existing processes.

Variation in amount of cell division or elongation or in duration of growth in stamens or styles could lead to the primary differences seen between distylous morphs. The genes that control distyly must regulate these processes. Developmental studies are required to define when and how regulation occurs, as well as to elucidate the diversity of gene action that is likely to occur among the different distylous families.

In contrast to distyly, the development of tristyly requires more than quantitative variation of existing processes in order to develop the three morphs. A dissociation between development of the two stamen levels within a morph had to arise at some point in a tristylous species' evolutionary history. In the radially organized tristylous dicotyledons this dissociation is seen between stamen development in the M morph as compared to the L and S morphs. The relative relation between stamen levels in the S and L morphs is essentially the same, but S morph stamens experience more growth than L morph stamens. The M morph differs from these two in that growth rates of the stamens relative to each other are changed. When considered at the level of the genes controlling tristyly, it can be seen that the *S* locus affects development of both stamen levels, whereas the *M* locus affects growth of a single level.

The dissociation between stamen levels in the dorsiventrally organized Pontederiaceae appears to have occurred prior to the evolution of tristyly. The data on stamen development of the S and L morphs show that the S morph is not a scaled-up version of the L morph but instead has qualitatively different relative growth curves. The *S* locus in the Pontederiaceae primarily affects the upper, shorter stamen level, causing a late-developing elongation, while the *M* locus controls growth of the lower, longer stamen level. Additional evidence for dissociability of growth between stamen levels comes from a study of stamen modification in the mid-styled morph of *Eichhornia paniculata* (Seburn et al. 1990), which shows that variation in length of short-level stamens occurs independently of variation in long-level stamen length.

A precondition for the evolution of tristyly is within-flower stamen dimorphism. This dimorphism can result from the presence of two stamen whorls, as in *Lythrum* and *Oxalis*, or from positional effects related to the horizontal orientation of flowers, as is likely to have characterized the ancestors of tristylous Pontederiaceae. The *S* and *M* loci, which act in this floral background, have distinct effects. The differences between them are most clearly seen in their control of intraflower stamen dimorphism. Our data on tristyly clarify the basic difference between the genes in *Lythrum* and *Oxalis*, which act radially, and those in the Pontederiaceae, which act dorsiventrally. Whether such fundamental differences occur among distylous species remains to be shown.

6 Development and the Evolution of Heterostyly

Models for the evolution of heterostyly differ in (1) what is assumed to be the ancestral condition; and (2) whether the morphological or physiological aspects of the heterostyly syndrome arose first. With the exception of Charlesworth's (1979) model for tristyly, discussed above, these models primarily describe the evolution of distyly. In D Charlesworth and B Charlesworth's (1979) model for the evolution of distyly the ancestral flower was monomorphic with stigma and anthers at the same

level within a flower. The model assumes that diallelic self-incompatibility arose first. The morphological characteristics of distyly evolved subsequently through selection pressures to avoid self-fertilization and inbreeding depression. Baker (1966) proposed that distyly evolved in this sequence in the Plumbaginaceae.

Ganders (1979a) criticized the Charlesworths' model for assuming that the ancestral flower was monomorphic with stigma and style at the same level. He argued that most self-compatible, monomorphic species show some degree of stigma-anther separation. Species with no stigma-anther separation regularly self-pollinate and, thus, be unlikely to experience the levels of inbreeding depression required by their models. Ganders (1979a) agreed, however, with the general sequence of events outlined in the Charlesworths' model.

An alternative hypothesis is that the morphological characteristics of the syndrome evolved first and the physiological self-incompatibility arose secondarily. Darwin (1888) first proposed this sequence. Anderson (1973) hypothesized the same pathway to account for the repeated origin of distyly in the Rubiaceae. Lloyd and Webb in Chaps. 6 and 7 propose a more general version for this sequence. They hypothesize that in most cases the ancestral form was a flower with an exserted style (approach herkogamy (Webb and Lloyd 1986)) and that physiological incompatibility arose after the establishment of reciprocal herkogamy.

Developmental studies can be used to test specific hypotheses concerning the ancestral floral form of different heterostylous groups. For example, a form of protandry in which the anthers dehisce prior to stylar elongation and maturation is common in the Rubiaceae (Verdcourt 1958). Anderson (1973) proposed that distyly in the Rubiaceae evolved after mutations preventing stylar elongation arose in a population. The developmental predictions of this proposal are that (1) development of the long- and short-styled morphs of distylous Rubiaceae resemble two stages in the development of protandrous taxa; (2) the long- and short-styled morphs follow similar pathways in early development; and (3) divergence occurs through inhibition of the short style (cf. Fig. 36D,E). These predictions can be evaluated through comparative studies of floral development within distylous taxa and between distylous and protandrous species. Similar studies of distylous species and relatives with approach herkogamy could be used to examine Lloyd and Webb's hypotheses on the origins of heterostyly (see Chaps. 6 and 7).

The interrelationship of reciprocal herkogamy, physiological incompatibility, and the ancillary characters, such as stigma and pollen heteromorphisms, is central to understanding the sequence of events in the evolution of heterostyly. If any of these characters are expressions of the same developmental process, then they are likely to have had a simultaneous origin. Dulberger (1975a) argued that both the morphological and physiological components of heterostyly result from a single phenomenon, which she hypothesized was a difference in growth rates among morphs. The growth rate difference caused pollen size heteromorphisms that, in turn, resulted in differential growth of stamens and styles (Dulberger 1975a).

Arguments have also been made for associations between (1) the functioning of incompatibility and the ancillary characters (Dulberger 1974, 1975b); (2) stamen and style lengths and some ancillary characters (Dulberger 1975a); and (3) incompatibility and stamen and/or style lengths (Mather and DeWinton 1941; D Charlesworth and B Charlesworth 1979). Lloyd and Webb (Chap. 6) hypothesize that

incompatibility develops as a response to the different stylar environments provided by the previously evolved reciprocal herkogamy. The interrelationships of these features of the heterostyly syndrome are problematic in part because we lack detailed developmental information on which to build hypotheses. The need for additional evidence is especially important for determining the evolutionary sequence in which reciprocal herkogamy and incompatibility are established. Studies of self-compatible heterostylous groups, such as *Amsinckia* and *Eichhornia*, would be particularly useful to determine whether reciprocal herkogamy imposes functional constraints on pollen-pistil interactions, as implied by the Lloyd and Webb model (see Chaps. 6 and 7).

7 Future Research

Heterostyly provides a unique opportunity for plant biologists to study development as an evolutionary process. This breeding system in polyphyletic, controlled by relatively few major genes, and has a direct effect on the evolution of populations through its influence on mating patterns. In addition, variability in expression of the syndrome occurs both within and between taxa, providing systems in which to study the developmental basis of differences in expression of the syndrome.

In this chapter we have reviewed developmental information on heterostyly and drawn attention to the lack of such data in comparison with the wealth of genetic and ecological work on heterostylous species. We have also formalized models that may help to analyze specific developmental problems in both distylous and tristylous species. The utility of detailed structural and developmental studies in understanding genetic and evolutionary change is seen in our studies of the Pontederiaceae. Research on other heterostylous species that analyzes the developmental correlations among reciprocal herkogamy, heteromorphic incompatibility, and the ancillary characters are needed to understand the functions of these characters.

Distylous species offer a wealth of problems for future developmental research. Quantitative studies of stamen and style growth that establish when and how the morphs diverge are needed to evaluate developmental and genetic relations between morphs and between distylous species and nonheterostylous relatives. Developmental studies will help us to understand what the distyly genes control and how the system has evolved. Studies that establish the developmental basis for ancillary characters, such as pollen size and number dimorphism, are needed to understand their relationship to the primary characters and to evaluate hypotheses about their function. For example, do pollen-size dimorphisms and differences in pollen number arise at the same time in development? Finally, comparisons among distylous species will tell us whether the genes controlling development of distyly operate similarly in different species.

Similar developmental comparisons are needed in tristylous species. Tristyly has the additional developmental problem of the relation of intrafloral stamen dimorphism to the evolution of the heterostyly syndrome. Developmental studies of monomorphic taxa that have two stamen series are needed to establish the effect that time of stamen origin has on developmental events, and thus to understand the

development of tristyly in Lythraceae and Oxalidaceae. For example, meiosis occurs at different times in the two anther levels in flowers of *Oxalis* (JH Richards unpubl. data), where intraflower stamen dimorphisms are present at stamen origin. Does timing of meiosis differ between anther series in monomorphic flowers with two stamen series? Does pollen size and/or number vary between anther series of such species in directions that would provide a basis for selection of the particular associations of these characters found in tristylous species? By answering such questions, we will discover the developmental rules (cf. Oster et al. 1988) that underlie evolution of the tristylous syndrome.

Finally, heterostylous groups provide useful experimental tools for integrated studies involving development, genetics and physiology. Quantitative genetic analysis of developmental processes (Cheverud et al. 1983; Atchley 1984) and the determination of phenotypic and genetic correlations of floral traits (Falconer 1981; Shore and Barrett 1990) would help to assess the types of constraints that are involved in the build-up and breakdown of the heterostyly polymorphism. Such data would also shed light on the patterns of developmental integration among components of the syndrome. Work on the effects of growth hormone application on floral organ development, accompanied by determinations of endogenous hormone levels, would help to elucidate the physiological events that lead to differences between mature heterostylous flowers. Comparative studies of the physiology and development of pollen, stamen and style variants, all of which are reported in heterostylous groups, may help to understand the complex processes that underlie the basis for variation in floral form in heterostylous species. Such data may provide more general models for the evolution of floral diversity in the angiosperms.

Acknowledgments. We thank P.K. Diggle for constructive comments on the manuscript, Elizabeth Campolin for drawing several of the figures, and National Science Foundation grant #DCB-8602869 (JHR) and the Natural Sciences & Engineering Research Council of Canada (SCHB) for financial support.

References

Anderson WR (1973) A morphological hypothesis for the origin of heterostyly in the Rubiaceae. Taxon 22:537–542

Atchley WR (1984) Ontogeny, timing of development, and genetic variance-covariance structure. Am Nat 123:519–540

Baker HG (1962) Heterostyly in the Connaraceae with special reference to *Byrsocarpus coccineus*. Bot Gaz 206–211

Baker HG (1966) The evolution, functioning and breakdown of heteromorphic incompatibility systems. I. The Plumbaginaceae. Evolution 20:349–368

Barrett SCH (1978) Floral biology of *Eichhornia azurea* (Swartz) Kunth (Pontederiaceae). Aquat Bot 5:217–228

Barrett SCH (1979) The evolutionary breakdown of tristyly in *Eichhornia crassipes* (Mart.) Solms (water hyacinth). Evolution 33:499–510

Barrett SCH (1985a) Ecological genetics of breakdown in tristyly. In: Haeck J, Woldendorp JW (eds) Structure and Functioning of Plant Populations II: Phenotypic and genotypic variation in plant populations. North-Holland, Amsterdam, The Netherlands, pp 267–275

Barrett SCH (1985b) Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Biol J Linn Soc 25:41–60
Barrett SCH (1988) Evolution of breeding systems in *Eichhornia* (Pontederiaceae): a review. Ann MO Bot Gard 75:741–760
Barrett SCH (1989) Mating system evolution and speciation in heterostylous plants. In: Otte D, Endler J (eds) Speciation and its consequences. Sinauer, Sunderland MA, pp 257–283
Barrett SCH, Anderson JM (1985) Variation in expression of trimorphic incompatibility in *Pontederia cordata* L. (Pontederiaceae). Theor Appl Genet 70:355–362
Barrett SCH, Harder LD (1991) Floral variation in *Eichhornia paniculata* (Spreng.) Solms Pontederiaceae II. Effects of development and environment on the formation of selfing flowers. J Evol Biol (in press)
Barrett SCH, Shore JS (1987) Variation and evolution of breeding systems in the *Turnera ulmifolia* L. complex (Turneraceae). Evolution 41:340–354
Barrett SCH, Morgan MT, Husband B (1989) Dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). Evolution 43:1398–1416
Bawa KS, Beach JH (1983) Self-incompatibility systems in the Rubiaceae of a tropical lowland wet forest. Am J Bot 70:1281–1288
Bennett JH, Leach CR, Goodwins IR (1986) The inheritance of style length in *Oxalis rosea*. Heredity 56:393–396
Berg RL (1959) A general evolutionary principle underlying the origin of developmental homeostasis. Am Nat 93:103–105
Charlesworth D (1979) The evolution and breakdown of tristyly. Evolution 33:486–498
Charlesworth D, Charlesworth B (1979) A model for the evolution of distyly. Am Nat 114:467–498
Cheung M, Sattler R (1967) Early floral development of *Lythrum salicaria*. Can J Bot 45:1609–1618
Cheverud J, Rutledge J, Atchley WR (1983) Quantitative genetics of development: genetic correlations among age-specific trait values and the evolution of ontogeny. Evolution 37:895–905
Darwin C (1888) The different forms of flowers on plants of the same species. Reprinted in 1986 by Univ Chicago Press, Chicago
Dowrick VPJ (1956) Heterostyly and homostyly in *Primula obconica*. Heredity 10:219–236
Dulberger R (1974) Structural dimorphism of stigmatic papillae in distylous *Linum* species. Am J Bot 61:238–243
Dulberger R (1975a) S-gene action and the significance of characters in the heterostylous syndrome. Heredity 35:407–415
Dulberger R (1975b) Intermorph structural differences between stigmatic papillae and pollen grains in relation to incompatibility in Plumbaginaceae. Proc R Soc Lond Ser B 188:257–274
Eckenwalder JE, Barrett SCH (1986) Phylogenetic systematics of Pontederiaceae. Syst Bot 11:373–391
Falconer DS (1981) Introduction to quantitative genetics. Oliver and Boyd, Lond
Feinsinger P, Busby WH (1987) Pollen carryover: experimental comparisons between morphs of *Palicourea lasiorrachis* (Rubiaceae), a distylous, bird-pollinated, tropical treelet. Oecologia 73:231–235
Fisher RA, Mather K (1943) The inheritance of style length in *Lythrum salicaria*. Ann Eugenics 12:1–23
Fyfe VC (1950) The genetics of tristyly in *Oxalis valdiviensis*. Heredity 4:365–371
Ganders FR (1975) Heterostyly, homostyly, and fecundity in *Amsinckia spectabilis* (Boraginaceae). Madroño 23:56–62
Ganders FR (1976) Pollen flow in distylous populations of *Amsinckia* (Boraginaceae). Can J Bot 54:2530–2535
Ganders FR (1979a) The biology of heterostyly. NZ J Bot 17:607–635
Ganders FR (1979b) Heterostyly in *Erythroxylum coca* (Erythroxylaceae). Bot J Linn Soc 78:11–20
Glover DE, Barrett SCH (1986) Variation in the mating system of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Evolution 40:1122–1131
Gottlieb LD (1984) Genetics and morphological evolution in plants. Am Nat 123:681–709
Greyson RI, Tepfer SS (1967) Emasculation effects on the stamen filament of *Nigella hispanica* and their partial reversal by gibberellic acid. Am J Bot 54:971–976

Heslop-Harrison Y, Heslop-Harrison J, Shivanna KR (1981) Heterostyly in *Primula*. 1. Fine-structural and cytochemical features of the stigma and style in *Primula vulgaris* Huds. Protoplasma 107:1851–1871

Hufford LD (1988) Roles of early ontogenetic modifications in the evolution of floral form of *Eucnide* (Loasaceae). Bot Jahrb Syst 109:289–333

Jinks JL, Mather K (1955) Stability in development of heterozygotes and homozygotes. Proc R Soc Lond Ser B 143:561–578

Jones LS, Koning RE (1986) Role of growth substances in the filament growth of *Fuchsia hybrida* cv. "Brilliant". Am J Bot 73:1503–1508

Koning RE (1983a) The roles of auxin, ethylene, and acid growth in filament elongation in *Gaillardia grandiflora* (Asteraceae). Am J Bot 70:602–610

Koning RE(1983b) The roles of plant hormones in style and stigma growth in *Gaillardia grandiflora* (Asteraceae). Am J Bot 70:978–986

Koning RE, Raab MM (1987) Parameters of filament elongation in *Ipomoea nil* (Convolvulaceae). Am J Bot 74:510–516

Lerner IM (1954) Genetic homeostasis. Oliver and Boyd, Lond

Levin DA (1970) Developmental instability and evolution in peripheral isolates. Am Nat 104:343–353

Lewis D (1982) Incompatibility, stamen movement and pollen economy in a heterostyled tropical forest tree, *Cratoxylum formosum* (Guttiferae). Proc R Soc Lond Ser B 214:273–283

Lowden R (1973) Revision of the genus *Pontederia* L. Rhododa 75:426–487

Martin FW (1965) Distyly and incompatibility in *Turnera ulmifolia*. Bull Torrey Bot Club 92:185–192

Martin FW (1966) Distyly, self-incompatibility, and evolution in *Melochia*. J Arnold Arbor Harv. Univ 47:60–74

Mather K (1950) The genetical architecture of heterostyly in *Primula sinensis*. Evolution 4:340–352

Mather K, DeWinton D (1941) Adaptation and counter-adaptation of the breeding system in *Primula*. Ann Bot 5:297–311

Mayura Devi P, Hashim M (1964) Homostyly in heterostyled *Biophytum sensitivum* DC. J Genet 59:245–249

Mayr B (1969) Ontogenetische Studien an Myrtales-Blüten. Bot Jahrb 89:210–271

Muenchow G (1981) An S-locus model for the distyly supergene. Am Nat 118:756–760

Opler PA, Baker HG, Frankie GW (1975) Reproductive biology of some Costa Rican *Cordia* species (Boraginaceae). Biotropica 7:234–247

Ornduff R (1964) The breeding system of *Oxalis suksdorfii*. Am J Bot 51:307–314

Ornduff R (1971) The reproductive system of *Jepsonia heterandra*. Evolution 25:300–311

Ornduff R (1972) The breakdown of trimorphic incompatibility in *Oxalis* section Corniculatae. Evolution 26:52–65

Ornduff R (1975) Heterostyly and pollen flow in *Hypericum aegypticum* (Guttiferae). Bot J Linn Soc 71:51–57

Ornduff R (1979) The morphological nature of distyly in *Lythrum* section Euhyssopifolia. Bull Torrey Bot Club 106:4–8

Ornduff R (1980) Heterostyly, population composition and pollen flow in *Hedyotis caerulea*. Am J Bot 67:95–103

Oster GF, Shubin N, Murray JD, Alberch P (1988) Evolution and morphogenetic rules: The shape of the vertebrate limb in ontogeny and phylogeny. Evolution 42:862–884

Pharis RP, King RW (1985) Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol 36:517–568

Price SD, Barrett SCH (1982) Tristyly in *Pontederia cordata* (Pontederiaceae). Can J Bot 60:897–905

Rendel JM (1959) Canalization of the scute phenotype in *Drosophila*. Evolution 13:425–439

Richards AJ (1986) Plant breeding systems. Allen and Unwin, Lond

Richards JH, Barrett SCH (1984) The developmental basis of tristyly in *Eichhornia paniculata* (Pontederiaceae). Am J Bot 71:1347–1363

Richards JH, Barrett SCH (1987) Development of tristyly in *Pontederia cordata* (Pontederiaceae). I. Mature floral structure and patterns of relative growth of reproductive organs. Am J Bot 74:1831–1841

Sattler R (1973) Organogenesis of flowers. Univ Toronto Press, Toronto

Seburn CNL, Dickinson TD, Barrett SCH (1990) Floral variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae): I. Instability of stamen position in genotypes from northeast Brazil. J Evol Biol 3:1–21

Shore JS, Barrett SCH (1990) Quantitative genetics of floral characters in homostylous *Turnera ulmifolia* var. *angustifolia* Willd. (Turneraceae). Heredity 64:105–112

Stebbins GL (1951) Natural selection and the differentiation of angiosperm families. Evolution 5:299–324

Stirling J (1932) Studies of flowering in heterostyled and allied species. Part I. The Primulaceae. Publ Hartley Bot Lab 8:3–42

Stirling J (1933) Studies of flowering in heterostyled and allied species. Part II. The Lythraceae: *Lythrum salicaria*. Publ Hartley Bot Lab 10:3–24

Stirling J (1936) Studies of flowering in heterostyled and allied species. Part III. Gentianaceae, Lythraceae, Oxalidaceae. Publ Hartley Bot Lab 15:1–24

Stout AB (1925) Studies on *Lythrum salicaria* II. A new form of flower in the species. Bull Torrey Bot Club 52:81–85

Tucker SC (1989) Overlapping organ initiation and common primordia in flowers of *Pisum sativum* (Leguminosae: Papilionoideae). Am J Bot 76:714–729

Verdcourt B (1958) Remarks on the classification of the Rubiaceae. Bull Jard Bot Etat Brux 28:209–281

Vuilleumier B (1967) The origin and evolutionary development of heterostyly in the angiosperms. Evolution 21:210–226

Webb CJ, Lloyd DG (1986) The avoidance of interference between the presentation of pollen and stigmas in angiosperms. II. Herkogamy. NZ J Bot 24:163–178

Weller SG (1976a) Breeding system polymorphism in a heterostylous species. Evolution 30:442–454

Weller SG (1976b) The genetic control of tristyly in *Oxalis* section Ionoxalis. Heredity 37:387–392

Yeo PF (1975) Some aspects of heterostyly. New Phytol 75:147–153