THE EVOLUTION OF SELF-INCOMPATIBILITY IN FLOWERING PLANTS: A PHYLOGENETIC APPROACH[1]

Stephen G. Weller[2], Michael J. Donoghue[3], and Deborah Charlesworth[4]

ABSTRACT. A phylogenetic approach was used to address three questions: (1) Was self-incompatibility (SI) ancestral in angiosperms? (2) Did gametophytic and sporophytic self-incompatibility arise independently? and (3) Is SI in heteromorphic species homologous with SI in homomorphic species? Parsimony analyses using several available hypotheses of phylogenetic relationships and different codings of the incompatibility character imply that self-compatibility (SC) was probably ancestral in angiosperms. It does not appear that the evolution of SI was a major factor in the evolutionary radiation of flowering plants. Sporophytic SI appears to have evolved independently in each family where it is known to occur, and in most cases SC, rather than gametophytic SI, appears to have been the precursor condition. Heteromorphic SI has clearly evolved on numerous occasions, probably from SC precursors, and it is unlikely that heteromorphic SI has evolved from homomorphic, sporophytic SI through loss of alleles. Although phylogenetic approaches provide some valuable insights on the evolution of SI, it is clear that phylogenetic relationships are still poorly understood, as is the distribution of self-incompatibility. Furthermore, detailed genetic information on SI is available for only a small number of species. Additional information from both areas is needed to answer the questions we have outlined more definitively.

A resurgence of interest in phylogeny reconstruction has resulted in the application of phylogenetic analysis to a diversity of evolutionary questions, some of which have previously been addressed primarily by population biologists (e.g., Donoghue, 1989; Brooks and McLennan, 1991; Armbruster, 1992; McDade, 1992; Norton et al., 1993). Our purpose is to examine several questions related to the evolution of self-incompatibility using a phylogenetic approach, as suggested earlier by Olmstead (1989). These questions are: (1) Was self-incompatibility ancestral in angiosperms? (2) Did gametophytic and sporophytic self-incompatibility arise independently, or is there evidence that one system evolved from the other? and (3) Is self-incompatibility in heteromorphic species homologous with self-incompatibility in homomorphic species? The questions raised here are similar to those asked by Charlesworth (1985); the major difference is our more explicit phylogenetic approach.

For our first question, the ancestral condition in angiosperms, it was possible to perform a preliminary phylogenetic analysis, taking into account ambiguity in estimates of phylogeny and in the taxonomic distribution of compatibility systems. Our treatment of the second and third questions is less detailed, mainly because appropriate phylogenies are not yet available.

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[2]Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717, U.S.A.
[3]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, U.S.A.
[4]Department of Ecology and Evolution, University of Chicago, Chicago, IL 60637, U.S.A.

SELF-INCOMPATIBILITY SYSTEMS

In hermaphroditic plant species, self-compatibility (SC) occurs when pollen grains produced by an individual germinate and effect fertilization following self-pollination. Self-incompatibility (SI) represents a complex and widespread collection of breeding systems (de Nettancourt, 1977). Among the flowering plants, SI systems result in rejection of self pollen or outcross pollen possessing the same incompatibility type. How this reaction takes place is variable, and a number of classification schemes are possible based on the genetics underlying SI and variation in associated morphological features. Perhaps the most basic distinction is between homomorphic and heteromorphic SI (de Nettancourt, 1977). In homomorphic systems, the flowers of all incompatibility groups are morphologically similar. Such systems are widely distributed, occurring in as many as 60–90 angiosperm families (Charlesworth, 1985; Gibbs, 1988). The number of incompatibility types in a population may be large, numbering in the dozens. Genetic control of homomorphic systems is variable. In species with gametophytic self-incompatibility (GSI), the incompatibility reaction of the pollen grain is determined by its own genotype (de Nettancourt, 1977). Identity between the allele occurring in the pollen grain and one of the alleles in the pistil is sufficient to prevent fertilization. The number of loci controlling GSI is variable, ranging from 1 to 4, although the number of cases with more than a single locus is small (Lundqvist, 1975). Sporophytic self-incompatibility (SSI) involves determination of the pollen incompatibility reaction by the parental sporophyte (de Nettancourt, 1977). All pollen grains produced by a plant have the same incompatibility reaction, even though they may have different genotypes. Identity between the reaction expressed by the pollen grain and the stigma reaction is sufficient to result in rejection. Nearly all studies have shown that SSI systems are governed by a single locus (de Nettancourt, 1977).

Brewbaker (1957, 1967) demonstrated that in most species GSI is correlated with binucleate pollen, stylar inhibition, wet stigmas, and long-term viability of stored pollen. In contrast, species with SSI usually have trinucleate pollen, stigmatic inhibition, dry stigmas, and short-term viability of stored pollen. Among the 23 flowering plant families with single-locus SI in which crosses have been used to test for the occurrence of GSI versus SSI, there are only two exceptions to the correlation of SSI and trinucleate pollen and GSI and binucleate pollen (Table 1). Pollen cytology (presence of binucleate vs. trinucleate pollen) may thus be useful for predicting the probable genetic basis of self-incompatibility when other information is lacking.

In heteromorphic systems, found in 25–27 plant families, two or three incompatibility groups occur in populations, each type having a different arrangement of stamens and styles in the flowers (Darwin, 1877). Differences among the morphs are reciprocal, such that stigmas and anthers at the same level occur in different morphs. Pollinations between anthers and stigmas at the same level are generally the only ones leading to production of seeds. In dimorphic systems, incompatibility is controlled by a single locus with two alleles. In trimorphic systems, two loci, each with two alleles, control expression of incompatibility types in most cases (Barrett, 1992). Determination of incompatibility types is sporophytic in all cases. As in homomorphic SI, heteromorphic species also show a correlation between pollen cytology and site of pollen tube inhibitions (Brewbaker, 1957).

GENERAL METHODS

Detection of self-incompatibility is often difficult, as emphasized by Charlesworth (1985). The most thorough studies involve a complete genetic analysis for the determination of gametophytic versus sporophytic SI. Such studies are rare, and almost nonexistent for woody plants with long generation intervals. In the absence of such studies, crossing programs involving selfs and outcrosses can be very useful, particularly if genetically distinct individuals can be identified with certainty. Results from such crosses are not always clear-cut, chiefly because it may be difficult to rule out inbreeding depression as the cause of low seed production after self-pollination, unless seed production is near zero (Charlesworth, 1985; Krebs and Hancock, 1990). Studies that rely on observations of low fruit set of isolated plants are generally not adequate for assessment of SI, nor are observations of low fruit set in natural populations. Low pollinator activity may be the cause of reduced seed or fruit production under such conditions. Observations of pollen tube growth can help distinguish between pre-zygotic SI and other causes of low seed set, and the site of inhibition of pollen tube growth provides suggestive information on the occurrence of GSI versus SSI.

Table 1. Pollen cytology (presence of binucleate vs. trinucleate pollen), site of inhibition, and genetic systems controlling homomorphic self-incompatibility. **Boldface** indicates exceptions to association of sporophytic SI and trinucleate pollen for cases of single-locus SI. References provided in Charlesworth (1985).

Family or Genus	Site of Inhibition (Where Known)	SI System	Pollen Cytology
Asteraceae	stigma	S	3
Betulaceae	**stigma**	**S**	**2**
(*Corylus*)			
Brassicaceae	stigma	S	3
Bromeliaceae	--	G	2
(*Ananas*)			
Caryophyllaceae	stigma	S	3
Chenopodiaceae	--	G (multi-locus)	3
(*Beta*)			
Commelinaceae	stigma	G	2
Convolvulaceae	stigma	S	3 (2 in other species)
(*Ipomoea leucantha*)			
Cornaceae	stylar	G	2
Poaceae	stylar	G (2 locus)	3
Fabaceae			
Mimosoideae	stylar	G	2 (3 in *Calliandra*)
Caesalpinoideae	--	--	2
Papilionoideae	stylar	G	2
Liliaceae	stylar	G	2
Nyctaginaceae	stylar	S	3
Onagraceae	stigma	G	2
Papaveraceae	stigma	G	2
Ranunculaceae	--	G (3–4 loci)	2
Rosaceae	style	G	2
Rubiaceae	stigma	--	2
(*Coffea*)			
(*Galium*)	--	S	3
Saxifragaceae	style	G	2
Scrophulariaceae	style	G	2
Solanaceae	style	G (single or multi-locus)	2
Sterculiaceae	**ovary**	**S**	**2**
Theaceae	--	G	2

The reports of SI used in this study are from a database assembled by Charlesworth (1985), augmented with more recent studies. Because the adequacy of the studies varies greatly, we have been conservative in our approach, accepting SI as present in a species only when crosses have been carried out. In a few cases we have used the ancillary features of SI, including site of pollen inhibition and stigma type as an indicator of probable gametophytic versus sporophytic control. Characterization of the type of SI in a family may be based on one or a few reports. Broader sampling within a family has usually revealed identical mechanisms controlling SI (Charlesworth, 1985), although Lewis (1977) has reported multi-locus SSI in the Brassicaceae, where single-locus SI is far more common. Assumptions of uniform genetic mechanisms in a family based on limited sampling represent a possible source of error.

The phylogenies referred to in this study are based on morphological and/or molecular evidence. In cases where the degree of phylogenetic information is limited, we consider alternative hypotheses from a phylogenetic perspective, but have not been able to trace character changes onto trees. In addressing the question of the ancestral condition in angiosperms we were able to use MacClade (vers. 3.0, Maddison and Maddison, 1992), to determine the minimum number of changes along the branches of a particular tree that are needed to account for the distribution of the states of a character found at the tips of the branches (in the terminal taxa). This parsimony optimization (or mapping) procedure establishes the states present at the internal (or ancestral) nodes in the tree and hence the branches along which changes of state are assumed to have occurred.

In order to account for uncertainties about phylogenetic relationships we have considered a variety of different phylogenetic hypotheses. In view of uncertainties concerning the breeding systems present in some taxa, we have also explored the robustness of our conclusions using a range of character codings. Furthermore, we have explored the parsimony of alternate hypotheses of character evolution by "fixing" the state at particular nodes using the "paintbrush" tool in MacClade. This allows us to calculate (and compare) the minimum number of steps that are needed to account for character evolution under the assumption that a particular state was present at given nodes in the tree.

THE ORIGIN OF SELF-INCOMPATIBILITY IN FLOWERING PLANTS

Historical background. In 1950, Whitehouse stated that "...the abrupt rise of the angiosperms from the gymnosperms may have been the result of...the increased efficiency of sexual reproduction made possible by multiple-allelomorph incompatibility." In his view, SI is a retained, ancestral trait (symplesiomorphy) in angiosperms, and self-compatibility (SC) derived. If Whitehouse is correct, then all systems of SI would be homologous.

Crowe (1964) also suggested that incompatibility systems in angiosperms were inherited from more ancient ancestors. She presented a "phylogenetic arrangement" of outbreeding systems in the angiosperms (Figure 1), showing GSI as ancestral and SSI derived from it. In this scheme, heteromorphic systems are suggested to have evolved from homomorphic SSI through loss of alleles.

More recently other authors have suggested the early evolution of SI in angiosperms and the direct derivation of divergent SI systems. Lundqvist (1975) proposed that the presence of complex multi-locus GSI systems in Ranunculaceae and Chenopodiaceae is consistent with a close phylogenetic relationship between these families, as well as a connection to monocots with similar systems (grasses). De Nettancourt (1977) viewed the scattered occurrence of incompatibility systems in the flowering plants as evidence that SI was ancestral. On the basis of pollen wall sculpturing of fossil pollen, Zavada (1984) concluded that the origin of SSI coincided with the appearance of the earliest recognizable angiosperm pollen.

Bernhardt and Thien (1987) suggested that GSI was present in the "protoangiosperms," based on the supposed widespread occurrence of SI in primitive angiosperms.

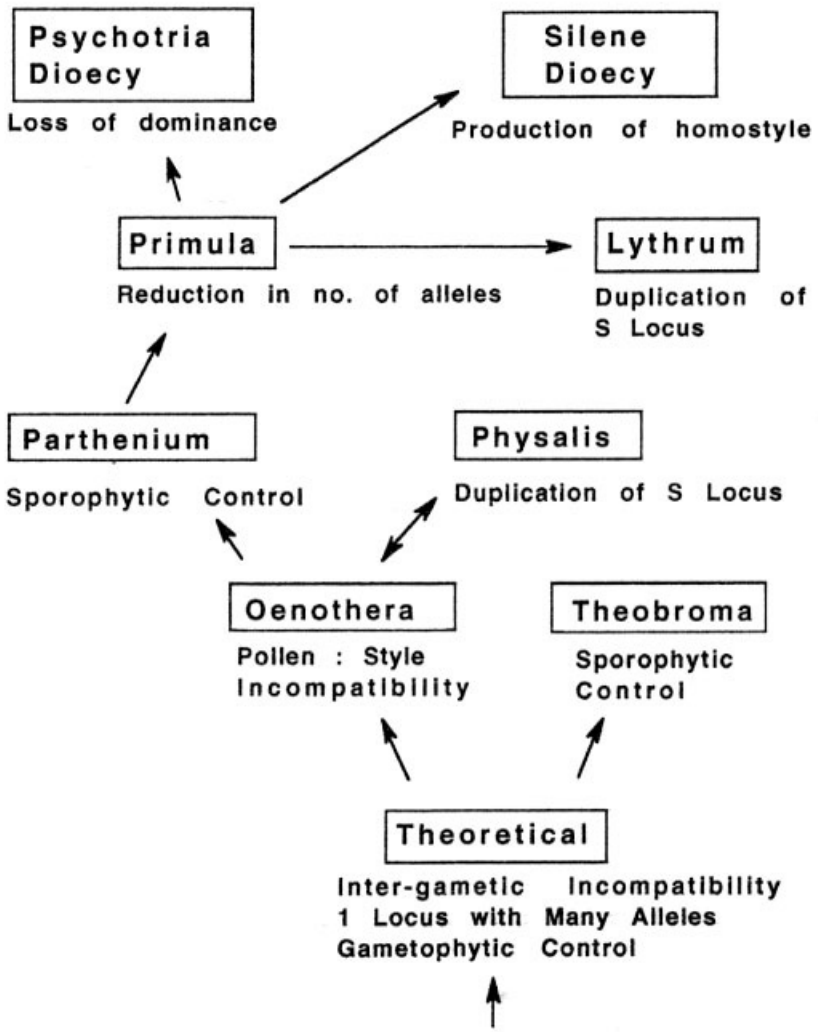

Figure 1. Relationships of self-incompatibility (SI) systems in angiosperms, based on Crowe (1964). Gametophytic self-incompatibility (GSI) is shown as primitive and sporophytic self-incompatibility (SSI) derived from it. Heteromorphic systems are suggested to have evolved from homomorphic SSI through loss of alleles. In this "phylogenetic" arrangement, the first angiosperms were believed to have possessed SI.

In contrast, Bateman (1952) stated that "The highly dispersed incidence of self-incompatibility throughout the Angiosperms is an indication that it has arisen *de novo* a number of times." He further predicted that because they have evolved on many occasions, SI systems should be highly variable. While not rejecting the possibility that SC could be derived, Bateman pointed out that SC could be primitive in many groups. Charlesworth (1985) and Olmstead (1989) suggested the multiple origin of SI on the basis of uniform SI systems within families compared to the variety of systems found among families.

Taxa and trees used in the analysis. In the analyses presented below, we consider a number of alternative phylogenetic hypotheses involving the 27 terminal taxa used by Donoghue and Doyle (1989) in their analysis of basal relationships in angiosperms (see Figure 2). These taxa were selected by Donoghue and Doyle (1989) to represent all potential early lines of angiosperm evolution. In particular, they included many families of Magnoliidae (sensu Cronquist, 1988; see Stebbins, 1974; Takhtajan, 1991), some including only a single genus (and therefore represented by a generic name in Figure 2), as well as some "placeholders," a term used for presumably monophyletic lineages containing several to many families. When enough information was available for the placeholder taxa, we have assigned a code reflecting the inferred ancestral condition in the group, rather than using single exemplar taxa, which might be nested well within the group, and therefore be less likely to represent the ancestral condition. Thus, all monocots were represented by a single taxon in the analysis, on the assumption that these plants form a monophyletic group within angiosperms (i.e., that the root of the angiosperms is not within monocots). Likewise, Monimiaceae(+) represents the so-called "core Laurales," including Hernandiaceae and Lauraceae; Ranunculaceae(+) represents ranunculids, including Berberidaceae, Menispermaceae, Lardizabalaceae, and Papaveraceae; and Hamamelidales(+) represents all of the tricolpate dicot groups (eudicots; Doyle and Hotton, 1991), including rosids, dilleniids, caryophyliids, and asterids.

In view of uncertainties still surrounding the phylogenetic relationships of these taxa, we made use of a number of the alternate trees found by Donoghue and Doyle (1989), as well as trees representing the results of ribosomal RNA sequences (Hamby and Zimmer, 1992), sequences of the chloroplast gene *rbc*L (Chase et al., 1993), and other morphological data sets (Loconte and Stevenson, 1991). For purposes of comparison, the taxa used in all trees are those included in Donoghue and Doyle (1989). To produce trees that could be compared, taxa used in Donoghue and Doyle were rearranged to simulate trees from later studies. Owing to variation in the taxa included in the different analyses, it was not possible to represent exactly trees from the later studies. We were able, however, to capture the basic features of alternative hypotheses for angiosperm evolution.

Although the taxa delimited by Donoghue and Doyle (1989) may be appropriate for examining relationships among major lines of angiosperms, they are not ideal from the standpoint of examining the evolution of SI, because in some taxa (especially larger ones) both SC and SI are known to occur (Table 2). In cases where there was insufficient information to determine whether the basal condition was SC or SI, we coded the taxon as polymorphic (SC/SI). When there are only two character states this amounts to coding the taxon as "unknown." In a few cases discussed below we felt we could safely assign a single code (SC or SI) to a polymorphic taxon based on a previous analysis of phylogenetic relationships within the group in question, which we could use in order to assess which condition was ancestral in the group based on parsimony. Finally, in view of these uncertainties and the possible problems associated with polymorphic terminal taxa (Nixon and Davis, 1991), we also explored the robustness of the results to a variety of alternative codings described below. A

detailed phylogenetic analysis of each of the more inclusive groups in the study would be useful, but beyond the scope of this paper. The use of different coding systems, as well as the analysis of various phylogenetic hypotheses, helps in circumventing the problems associated with inclusion of large placeholders.

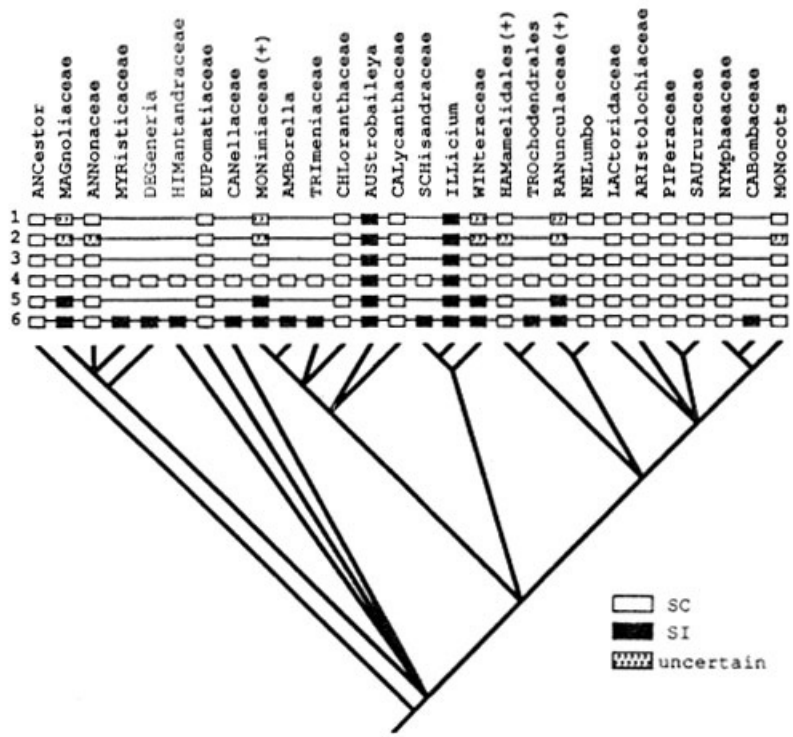

Figure 2. Consensus tree from Donoghue and Doyle (1989) showing the 27 angiosperm taxa and the six character codings discussed in the text. Open boxes are self-compatible (SC), black boxes are self-incompatible (SI), and patterned boxes are polymorphic (SC/SI); the absence of a box represents lack of information or questionable evidence (coded as unknown). Codings 1–6 are used to encompass uncertainties in interpretation of breeding system data. Coding 1 is our preferred coding, based on our assessment of reports in the literature (in coding 1, open boxes are used to represent several groups known to possess SI [Annonaceae, Hamamelidales(+), monocots] because phylogenetic information suggests that SC is basal in each group). In coding 2, the three polymorphic taxa (Annonaceae, Hamamelidales(+), and monocots) are recoded as polymorphic. In coding 3, all polymorphic taxa are recoded as SC, and in coding 4, both polymorphic and unknown taxa are coded as SC. Codings 5 and 6 favor SI: in coding 5, all polymorphic taxa are recoded as SI, and in coding 6, both polymorphic and unknown taxa are recoded as SI. Each of the six character codings was optimized on each of the most parsimonious trees based on Donoghue and Doyle (1989)—not on the consensus—and on a variety of alternative trees (see text and Figure 4), using MacClade. See text for full discussion.

Table 2. Distribution of breeding systems and pollen cytology of taxa used in phylogenetic analysis of early angiosperm lines (Figures 3–6). Pollen cytology is based on Brewbaker (1967). Taxa are listed in order shown in Figure 3. In several polymorphic taxa (Annonaceae, Hamamelidales(+), monocots) references indicating basal SC are listed. For Hamamelidales (+) and monocots, only basal families and their breeding systems are listed; other reports for SC and SI are not listed. Dashes indicate lack of information.

Taxon	Families or Subclasses (for inclusive taxa)	Breeding System	Pollen Cytology	References for Breeding System Information
Magnoliaceae	--	SC/SI	2	Heiser, 1962; McDaniel, 1963; Gottsberger, 1977; Parks et al., 1983
Annonaceae	--	SC/SI (coded as SC)	2	Bawa, 1974; Vithanage, 1984; SC basal: J. Doyle, pers. comm.
Myristicaceae	--	--	2	
Degeneria	--	--	2	Thien, 1980
Himantandraceae	--	--	--	
Eupomatiaceae	--	SC	--	Endress, 1984
Canellaceae	--	--	2	
Monimiaceae(+)	Hernandiaceae	--	2	
	Lauraceae	SC/SI	2	Free, 1970; Kubitzki and Kurz, 1984; Wheelwright, 1985;
	Monimiaceae	SC/SI	2	Lorence, 1985
Amborella	--	--	--	
Trimeniaceae	--	--	--	
Chloranthaceae	--	SC	2	Endress, 1984
Austrobaileya	--	SI	--	Prakash and Alexander, 1984
Calycanthaceae	--	SC	2	L. McDade, pers. comm.
Schisandraceae	--	--	2	
Illicium	--	SI	2	Thien et al., 1983
Winteraceae	--	SC/SI	2	Gottsberger et al., 1980; Godley and Smith, 1981; Lloyd and Wells, 1992
Hamamelidales(+)	Rosidae Dilleniidae Caryophyllidae Asteridae	SC/SI (coded as SC)	2 and 3	Numerous reports of SC and SI, SC basal based on phylogenies of Hufford and Crane, 1989
Trochodendrales	Trochodendraceae	--	2	
Ranunculaceae(+)	Berberidaceae Menispermaceae Lardizabalaceae Papaveraceae Ranunculaceae	SC/SI	2	East, 1940; Fryxell, 1957; Moore, 1968; Humphreys and Gale, 1974; Østerbye, 1977; Lawrence, 1975; Rust and Roth, 1981; Suzuki, 1983
Nelumbo	--	SC	--	Sohmer, 1977
Lactoridaceae	--	SC	--	T. Stuessy, pers. comm.; D. Wiens, pers. comm.
Aristolochiaceae	--	SC	2	Wildman, 1950; Tanaka and Yahara, 1989; M. Donoghue, pers. obser.
Piperaceae	--	SC	2	Semple, 1974
Saururaceae	--	SC	2	S. Tucker, pers. comm.
Nymphaeaceae	--	SC	2	Prance and Anderson, 1976; Schneider and Moore, 1977; Ervik et al., in press
Cabombaceae	--	--	--	Schneider and Jeter, 1982
Monocots	--	SC/SI (coded as SC)	2 and 3	Numerous reports of SC and SI, SC basal based on phylogenies of Dahlgren and Bremer, 1985; G. Bharathan, pers. comm.

Coding of taxa. Reliable data are not available for every group (Table 2). Studies of breeding systems of magnoliid taxa have been difficult because most are woody plants, often found in remote areas, which makes crossing programs impractical. A number of these species also seem to have very low fecundity, regardless of the type of pollination, and this complicates the interpretation of experimental efforts to verify the occurrence of SC versus SI. The absence of a report of SI cannot be equated with SC, and may simply mean that the breeding system has not been investigated. There is also a tendency not to report negative results, in this case the presence of SC.

The best documented cases of SI occur in *Illicium floridanum* (Illiciaceae, Thien et al., 1983), and *Austrobaileya scandens* (Austrobaileyaceae, Prakash and Alexander, 1984). Only a single individual of *Austrobaileya* was used in crosses, although observations of pollen tubes indicated a failure of growth typical of SI (Prakash and Alexander, 1984). We have coded *Illiciaceae* and *Austrobaileya* as SI in the experiments described below (Table 2).

Several other reports of SI seem well founded, but there are also SC species within these terminal taxa and the ancestral condition in the group is uncertain. For this reason, we have coded Monimiaceae(+), Winteraceae, Magnoliaceae, and Ranunculaceae(+) as polymorphic (SC/SI; Table 2). These are discussed in turn below.

With respect to Monimiaceae(+), reports of SI include four genera of Lauraceae (Kubitzki and Kurz, 1984), and several species of *Tambourissa* (Monimiaceae, Lorence, 1985). Bernhardt and Thien (1987) cited *Ocotea* (Lauraceae) as likely to be SI on the basis of a report of a single isolated tree that failed to set fruit (Wheelwright, 1985). *Persea* (Lauraceae), described as SI by Free (1970) and Kubitzki and Kurz (1984), was found to be SC by Wheelwright (1985). Interpretation of data for Lauraceae is complicated by the pronounced synchronized heterodichogamy characteristic of this family, which makes it difficult to carry out self or geitonogamous pollinations because the stigmas are rarely receptive when the anthers are shedding pollen.

In Winteraceae, *Pseudowintera colorata* has been reported as SI (Godley and Smith, 1981; Lloyd and Wells, 1992). In "chase pollination" experiments in *Pseudowintera colorata*, the application of self pollen to stigmas 1–3 days prior to outcrossed pollen resulted in significant reductions in seed production (Lloyd and Wells, 1992), indicating the possibility of SC and ovule abortion due to inbreeding depression. For this reason, and because SC is also well documented in Winteraceae (Gottsberger et al., 1980), this family is coded as SI/SC in our preferred coding. *Belliolum*, *Tasmannia*, and *Zygogynum* (Thien, 1980), and *Exospermum* (Bernhardt and Thien, 1987), are not accepted as SI according to our criteria.

The claim of SI in the Magnoliaceae (McDaniel, 1963) is based primarily on failure of seed production by a few isolated trees of several *Magnolia* spp. grown outside their range. It is difficult to rule out lack of pollinators among other potential factors that could explain these results. Because we lack phylogenetic information within Magnoliaceae we have coded this taxon as SC/SI, a conservative approach given the methods used to detect SI and the predominance of SC in the family (Gottsberger, 1977; Heiser, 1962; McDaniel, 1963, Parks et al., 1983).

SI is well documented within Ranunculaceae and related taxa, but because of uncertainties about phylogenetic relationships within ranunculids they are also coded as SC/SI. *Nelumbo* is coded as SC on the basis of Snigirevskaya's (1964) observations indicating facultative self-pollination, and Sohmer's (1977) crossing study.

In a few cases where SI and SC occur within the same taxon we felt more confident in assessing the ancestral breeding system condition, and these taxa are coded as SC in our preferred coding (see below). Annonaceae is coded as SC because the only report of SI is for *Sapranthus* (Bawa, 1974), which is well nested within the family (J. A. Doyle, pers. comm.).

All other investigated Annonaceae are reported to be SC. Similarly, despite the widespread occurrence of SI in both "placeholder" taxa, SC appears to be basal in the Hamamelidales(+) and related taxa, based on phylogenies of Hufford and Crane (1989), and in the monocots, based on phylogenies of Dahlgren and Bremer (1985). This also appears to hold in the phylogenies of Chase et al. (1993). The monocots were considered polymorphic or SC in various runs, but never SI, because there is no phylogenetic information suggesting that the basal monocots are SI. As information on the phylogeny and breeding systems of placeholder taxa accumulates, these groups can be analyzed in more detail.

Despite possible biases against publishing accounts of SC, there is good evidence for the widespread occurrence of SC among "primitive" angiosperms, especially among paleoherbs (Donoghue and Doyle, 1989; Table 2). In the Aristolochiaceae, two species of *Asarum* (Wildman, 1950; Tanaka and Yahara, 1989) are known to be SC, and *Saruma* also appears to be SC (Donoghue, unpubl.). SI also seems to be absent in Lactoridaceae (D. Wiens and T. Stuessy, pers. comm.), Saururaceae (S. Tucker, pers. comm.), Piperaceae (Semple, 1974), and Nymphaeaceae (Prance and Anderson, 1976). Although Bernhardt and Thien cite Schneider and Moore (1977) as evidence for SI in *Nuphar*, our interpretation of their data suggests little support for SI. Recent studies by Ervik et al. (in press) indicate experimental evidence for self-compatibility in *Nuphar*. Our assessment of SC in the Lactoridaceae and Saururaceae is based on observations of abundant fruit set on isolated individuals. In those magnoliids which have not already been discussed, there is evidence for SC in Eupomatiaceae (Endress, 1984) and Calycanthaceae (L. McDade, pers. comm.; S. Weller, unpubl. obser.). The Chloranthaceae also appear to be SC, although data are restricted to a single species in the family (Endress, 1987).

We coded as "unknown" those taxa for which we lack any information, and those for which we find the available information too difficult to interpret. Information on breeding systems is missing for a number of critical taxa including *Amborella*, Canellaceae, Himantandraceae, Myristicaceae, Schisandraceae, Trimeniaceae, and Trochodendrales. Widespread occurrence of dioecy complicates assessment of incompatibility systems in Myristicaceae and Schisandraceae. Other problematical cases include *Degeneria* (Degeneriaceae), and *Cabomba* (Cabombaceae). Although cited as SI by Bernhardt and Thien (1987), in these cases there is insufficient evidence in the original report to confirm the presence of SI. For example, Schneider and Jeter (1982) used bagging studies to confirm only that pollination is required for seed production in *Cabomba*, and did not attempt to self-pollinate bagged flowers. In Degeneriaceae no information on SI was presented in the original report (Thien, 1980).

Finally, based on outgroup comparison (Maddison et al., 1984), we coded the hypothetical ancestor used to root the tree by Donoghue and Doyle as SC (although coding it as unknown yielded the same basic results discussed below). Gnetales are considered more closely related to angiosperms than are conifers, cycads, or *Ginkgo* (e.g., Doyle and Donoghue, 1986; 1993), but these groups are dioecious, and it is therefore difficult to establish whether they would be (or once were) SC or SI. Although it is possible that an extinct hermaphroditic group more closely related to angiosperms than Gnetales may have been SI, this breeding system is unknown among non-angiosperm seed plants. In contrast, SC has been well documented in conifers, the only extant "gymnosperms" with cosexual species (Sarvas, 1962; Franklin, 1970; Sorensen, 1982). The evolution of SI may be associated with the occurrence of the style in flowering plants.

The result of these deliberations is our preferred coding of the terminal taxa, which we will call coding number 1 (Table 2, Figure 2). In view of the uncertainties considered above, however, and in recognition that some of our decisions might be wrong, we also considered a set of alternative codings (2–6 in Figure 2). In coding 2, the three polymorphic taxa

coded as SC in coding 1 (Annonaceae, Hamamelidales(+), and monocots; Table 2) were recoded as polymorphic. Codings 3 through 6 explore the effect of coding polymorphic and unknown taxa as either SC or SI. Codings 3–6 bracket the possibilities by favoring either SC (codings 3 and 4) or SI (codings 5 and 6). In codings 3 and 5 the taxa coded as SC/SI in coding 1 are coded as SC and SI, respectively. Codings 4 and 6 are even more extreme, in that positive codes are assigned both to the polymorphic taxa in coding 1 and to the taxa coded as unknown. In coding 4 all uncertainties are resolved as SC, while in coding 6 all uncertainties are coded as SI.

Analyses. Using the codings described above, breeding systems were optimized on different phylogenetic trees using three approaches. Under the first hypothesis, no assumptions were made regarding the presence of SI versus SC at the base of the angiosperms. Under the second hypothesis, SI was assumed to have been present at the base of the angiosperms, but could also evolve later from SC ancestors. In the final hypothesis, it was assumed that SI was basal in the angiosperms, and that all cases of SI in terminal taxa are homologous, representing retention of this breeding system from SI ancestors.

Optimizing our preferred coding of breeding systems (coding 1, Figure 2) on all 54 of the most parsimonious trees of Donoghue and Doyle (1989), we obtained the basic results in Figure 3, which shows only one of the most parsimonious trees of Donoghue and Doyle. It is always most parsimonious to assign SC to the base of the angiosperms, which then entails a minimum of six independent origins of SI (Figure 3A; note that four of the origins are at the base of, or within, the polymorphic terminal taxa, and that these are required in every calculation). Assigning SI to the base of the angiosperms entails three extra steps involving loss of SI (Figure 3B). Fixing SI at the base of angiosperms, however, does not ensure that all instances of SI are homologous, or retained from the common ancestor in which it evolved. For example, the occurrence of SI in Magnoliaceae and in Ranunculaceae(+) (Figure 3B) must be seen as separate originations. Insuring that all coded instances of SI are potentially retained from a single origin at the base of angiosperms generally requires an additional six steps (Figure 3C). Based on these analyses, it is more parsimonious to suppose that SC is ancestral in angiosperms and that SI originated several times early in angiosperm evolution.

To account for uncertainty about phylogenetic relationships we optimized character coding 1 on a series of other phylogenetic trees (Figure 4). Using a slightly less parsimonious tree (one additional step is required) from Donoghue and Doyle (1989), which roots the tree among paleoherbs, it is again most parsimonious to assume that SC is ancestral. An additional seven steps are required to ensure that SI is retained from a single origin (Figure 4A). When the taxa used by Donoghue and Doyle (1989) are rearranged to simulate the Hamby and Zimmer (1992) rDNA tree, the root is again among paleoherbs (Figure 4B). As before, SC is ancestral, but nine extra steps are required to make SI a retained condition. When the Donoghue and Doyle taxa are arranged to match the *rbc*L sequence tree of Chase et al. (1993), the tree is rooted between a monosulcate clade and a tricolpate clade (Figure 4C). In this case only five steps are required to place SC at the base of the angiosperms (with SI homologous in *Austrobaileya* and Illiciaceae), while seven extra steps are required if SI is homologous and retained from a common ancestor. The tree presented by Loconte and Stevenson (1991), based on morphological characters, yields the same basic conclusion.

With only a few exceptions (see below), each of the alternative codings discussed above yielded results suggesting that it is most parsimonious to assume that SC is ancestral in angiosperms and that SI evolved several times independently. In the case of codings 2–4 the number of steps separating "SC basal" from "SI basal" and from "SI retained" is generally the same as described above for coding 1. As expected, the number of steps separating these hypotheses is somewhat reduced under codings 5 and 6. In all cases coding 5 favors SC at

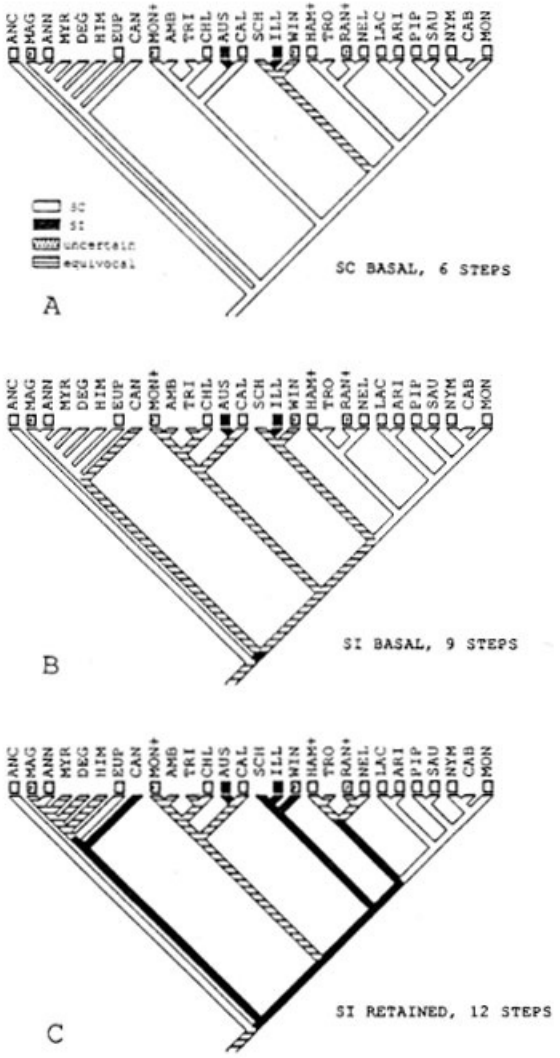

Figure 3. Optimizations of character coding 1 (see Figure 2) on the "preferred" most parsimonious tree of Donoghue and Doyle (1989), using MacClade; abbreviations are the first three letters of the taxon names in Figure 1. In MacClade the states at the tips of the branches and at the internal nodes are indicated by different shading, and a change from one state to another is marked by a change of shading. In some cases it is equally parsimonious to assign either state to a position on a tree, and such instances are indicated by diagonal lines. A. Most parsimonious hypothesis; SC ancestral in angiosperms. B. SI "fixed" at the base of angiosperms (using the "paintbrush" tool in MacClade); some instances of SI are not retained from this origin. C. Most parsimonious optimization, allowing all coded instances of SI to be retained from the common ancestor of angiosperms.

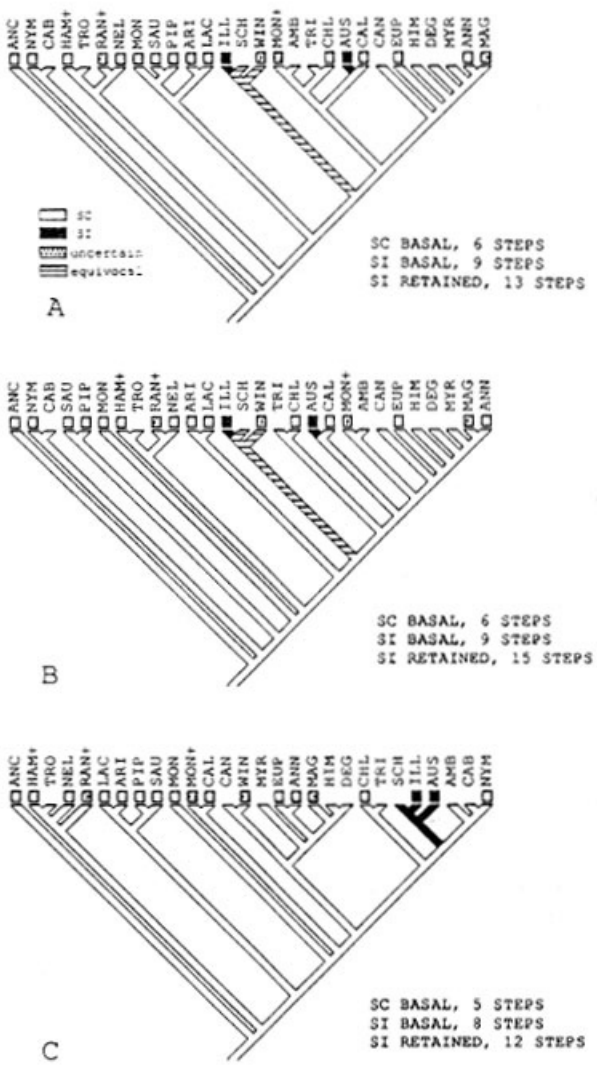

Figure 4. Optimization of character coding 1 (see Figure 2) on alternative phylogenies employing MacClade; SC is ancestral in all cases. Only the most parsimonious optimizations (SC basal in each case) are shown. For purposes of comparison, the taxa used in all trees are those included in Donoghue and Doyle (1989); these taxa were rearranged to simulate trees from later studies. Owing to variation in the taxa included in the different analyses, it was not possible to represent exactly trees from the later studies; see text for details. A. Donoghue and Doyle's (1989) "Nymphaeales-basal" tree. B. Tree representing Hamby and Zimmer's (1992) results based on ribosomal RNA sequences. C. Tree representing Chase et al.'s (1993) results based on sequences of the chloroplast gene *rbc*L.

the base of the angiosperms. Surprisingly, even coding 6, which was designed to strongly favor ancestral SI, slightly favors SC at the base of most of the trees we considered. Several of Donoghue and Doyle's morphological trees, however, yield SI basal with coding 6 (Figure 5A). In these cases, however, trees with SC basal require only one additional step (Figure 5B). Even under these circumstances, it is more parsimonious to assume that SC is ancestral than it is to assume that SI has been retained in all cases from the first angiosperm (Figure 5C).

Conclusions. If transitions between SI and SC are equally weighted, we conclude from these analyses that it is more parsimonious to suppose that SC is ancestral in angiosperms than it is to assume (1) that SI is ancestral, with the possibility of SI also evolving later from SC ancestors, or (2) that SI was ancestral, and all taxa coded as having SI evolved from ancestors also possessing SI. This result holds under a wide variety of codings and for all recent phylogenetic hypotheses. Except under coding 6, the difference in number of steps ranges from 5 to 9. It therefore seems unlikely that SI was a major factor in the success and radiation of angiosperms, as suggested by Whitehouse (1950) and Crowe (1964). If the first angiosperms were colonizing species (Stebbins, 1974; Bond, 1989; Taylor and Hickey, 1992; Doyle and Donoghue, 1993), the occurrence of SC in these species would be consistent with the pattern found in extant colonizing species (Baker, 1965).

Results based on equal weighting of SC and SI imply that transitions between the two breeding systems are similar in both directions, which seems improbable given the evidence for repeated origin of SC from SI within some angiosperm families (e.g., Asteraceae, Brassicaceae). These observations led Stebbins (1957) to suggest that it is easier to lose than to gain SI. In our analyses, weighting against the independent gain of SI would tend to favor placing SI at the base of the angiosperms. How could one determine the appropriate weighting scheme? Although the probability of losing vs. gaining SI is not known, and may vary in different angiosperm lineages, one future approach might be to investigate a variety of weighting schemes, and test the threshold weighting value that would favor placing SI at the base of the angiosperms. If the analysis suggested that only a low probability of SI to SC transitions would prevent placing SI at the base of the angiosperms, the differences in the number of steps found in this study should be interpreted with caution. Despite these caveats, it seems clear based on other evidence that SI systems have evolved independently elsewhere in the angiosperms (see below).

Our conclusion that SC is basal in angiosperms could also be overturned if (1) phylogenetic trees with Magnoliales in the basal position are best supported (this seems unlikely in view of recent molecular data, which tend to support paleoherb rootings); (2) all, or most, of the taxa coded as SC/SI and as unknown are actually SI; (3) SI is discovered among angiosperm outgroups; or (4) extinction of basal lineages possessing SI has occurred.

THE DERIVATION OF SPOROPHYTIC SELF-INCOMPATIBILITY

Few flowering plant families, all of them tricolpate dicots (Donoghue and Doyle, 1989), possess SSI. This form of SI has been claimed on the basis of genetic information in 8 families: Asteraceae, Betulaceae, Brassicaceae, Caryophyllaceae, Convolvulaceae, Nyctaginaceae, Rubiaceae, and Sterculiaceae (Lundqvist, 1990). Our attention will focus on these families. An additional 4 families—Cactaceae (Ganders, 1976), Caprifoliaceae (East, 1940; Pojar, 1974), Lobeliaceae (East, 1940), and Polygalaceae (Zapata and Arroyo, 1978; Sobrevila and Arroyo, 1982)—have SI, verified through crossing programs, and these may be SSI based on their possession of trinucleate pollen. Homomorphic multi-allelic incompatibility

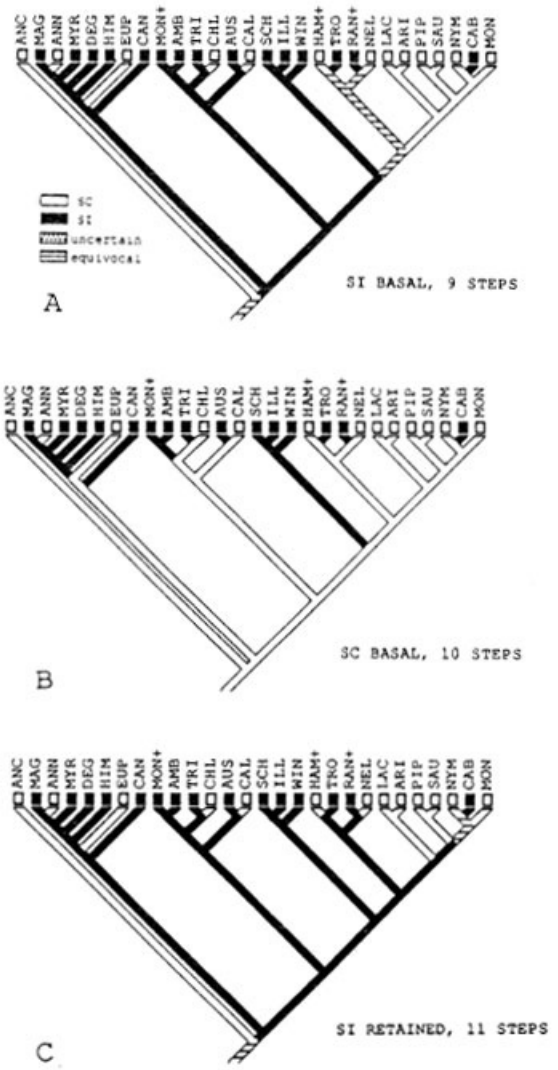

Figure 5. Optimizations of character coding 6 (see Figure 2) on the "preferred" most parsimonious tree of Donoghue and Doyle (1989), using MacClade. A. Most parsimonious hypothesis; SI ancestral in angiosperms. B. An optimization with SC ancestral. C. Most parsimonious optimization allowing all coded instances of SI to be retained from the common ancestor of angiosperms.

is claimed for some Boraginaceae (Dulberger, 1970; Crowe, 1971; Schou and Philipp, 1983), a family typically characterized by SC or heteromorphic incompatibility, although inbreeding depression seems as likely a reason for self-sterility in *Borago officinalis*.

The occurrence of SSI among the more specialized angiosperm families has long been viewed as evidence for the derived nature of this form of SI (Brewbaker, 1957; Crowe, 1964; Brewbaker, 1967). Here we consider two questions from a phylogenetic perspective: first, did SSI evolve independently in the families where it occurs, and second, did SSI evolve from GSI, or from SC?

For those cases with genetic data supporting the occurrence of SSI, as well as those where SSI is likely, it appears that SSI evolved independently in each family where it occurs. Cronquist's (1988) view that different families possessing SSI are not directly related has been supported by morphological and molecular cladistic analyses (e.g., Hufford, 1992; Olmstead et al., 1992; Chase et al., 1993). The likely independent evolution of SSI suggests that the underlying molecular mechanisms could well be different in each case. Unfortunately, only a single family with SSI, Brassicaceae (Nasrallah and Nasrallah, 1986; Haring et al. 1990), has been investigated. Molecular studies of Asteraceae, or other families with SSI, would be very valuable.

What genetic systems preceded the evolution of SSI? Sporophytic self-incompatibility is probably the ancestral condition in Asteraceae, based on the widespread distribution of this form of incompatibility in the different tribes. Phylogenetic analyses of Asteridae (sensu Cronquist, 1988) and Asterales (Olmstead et al., 1992; Olmstead et al., 1993) provide little evidence for precursor conditions to SSI in Asteraceae, again because there is insufficient information on breeding systems for key families (Table 3). Although phylogenetic studies of Asteraceae have provided a good idea of relationships among the major lines (Bremer et al., 1992), we still lack information on the breeding systems of crucial basal groups. Several species of Mutisieae are known to possess SI (Carlquist, 1974; Kalin Arroyo, pers. comm.), although no tests have been carried out to determine the type of SI. Information on breeding systems in the Barnedesinae would be especially useful.

Too little is known of the details of SI in Betulaceae for application of phylogenetic analysis. Aside from *Corylus*, for which detailed crossing studies have demonstrated SSI (Thompson, 1979), self-incompatibility is known in *Alnus* and *Betula* (Hagman, 1975), but no details of genetic control are available. There is no information on breeding systems of related families (Crane, 1989).

All SI species of Brassicaceae that have been investigated have shown sporophytic control, but as in Asteraceae, the breeding systems of the basal, perhaps less-derived genera (e.g., *Stanleya*) have not been investigated (Table 3). The breeding systems of the genera of Capparaceae most closely related to Brassicaceae (Judd et al., 1994; Chase et al., 1993), including *Cleome* and *Polanisia*, have not been studied in detail, although *Cleome spinosa* is SC (Stout, 1923). Based on this limited information (Table 3), SSI probably evolved after the divergence of the Brassicaceae, and the precursor state may have been SC.

In the Caryophyllaceae the only known SI species is *Cerastium arvense* (Lundqvist, 1990). SC is widespread in the family, suggesting that SSI in *Cerastium* evolved independently from SC in the Caryophyllaceae. In related families (e.g., Rodman et al., 1984) SC is the only reported breeding system for hermaphroditic species, further indicating the *de novo* origination of SSI within the Caryophyllaceae. Elsewhere in the Caryophyllidae, SSI is found only in the Nyctaginaceae, where it has been documented in *Bougainvillea* (Zadoo and Khoshoo, 1975). The uniform occurrence of trinucleate pollen in the Nyctaginaceae may indicate sporophytic control where SI is found. The Nyctaginaceae may have evolved within the Phytolaccaceae, where only SC and dioecy have been documented.

Table 3. Breeding systems and pollen cytology (2 vs. 3 nucleate pollen), where known, in relatives of families (either related families or taxa within the family in question) where SSI is claimed. Pollen cytology is taken from Brewbaker (1967). Pollen cytology is given for the family unless there is variation within the family, in which case information is noted for the lowest taxonomic level listed by Brewbaker. SI is homomorphic except where noted. Closely related families with no information are listed to highlight need for more information on these critical families.

Families Possessing SSI and Selected Reports Providing Genetic Evidence	Phylogenetically Related Taxa	Breeding System	Pollen Cytology	References
Asteraceae	Calyceraceae		2	
Parthenium argentatum (Gerstel, 1950)	*Moschopsis insulata*	partially SI		Arroyo and Squeo, 1990
Crepis foetida	Lobeliaceae			
(Hughes and Babcock, 1959)	*Isotoma axillaris*	SI?	3	East, 1940
Cosmos bipinnatus				
(Crowe, 1954)	Goodeniaceae		2	
	Leschenaultia tubiflora	SI	--	Darwin, 1878
	Sellieria radicans	SI	--	Schonland (in Engler & Prantl, cited by East, 1940)
	Menyanthaceae		2?	
	Villarsia spp.	homomorphic and heteromorphic SI	--	Ornduff, 1988
Betulaceae	Fagaceae		2	
Corylus avellana (Thompson, 1979)	*Castanea* spp.	SI	--	Hagman, 1975
Brassicaceae	Capparaceae		2	
Iberis amara	*Capparis pitteri*	SI	--	Bawa et al., 1985
(Bateman, 1955)	*C. verrucosa*	SI	--	Bullock, 1985
	Flacourtiaceae		2	
	Casearia (3 spp.)	SI	--	Bawa et al., 1985; Bullock, 1985
	Moringaceae	no information		
	Tovariaceae	no information		
Caryophyllaceae	Phytolaccaceae		3	
Cerastium arvense	*Phytolacca decandra*	SC	--	East, 1940
(Lunqvist, 1990)	*Rivinia* spp.	SC	--	East, 1940
Convolvulaceae	Solanaceae (many spp.)	GSI	2	East and Manglesdorf, 1925
Ipomoea batatas (Joseph and George, 1983)				
Nyctaginaceae	Phytolaccaceae (see above)			
Bougainvillea (Zadoo and Khoshoo, 1975)				
Rubiaceae	Rubiaceae			
Galium	Cinchoneae			
(Crowe, 1964)	*Exostema caribaeum*	SI	--	Bullock, 1985
	Coussareae			
	Coussarea (2 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Faramea (2 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Rubieae	SI	3	
	Gardenieae		2	
	Gardenia thunbergii	SI	--	East, 1940

Table 3. Continued.

Families Possessing SSI and Selected Reports Providing Genetic Evidence	Phylogenetically Related Taxa	Breeding System	Pollen Cytology	References
	Hamelieae			
	Hamelia patens	SI	--	Bawa and Beach, 1983
	H. versicolor	SC	--	Bullock, 1985
	H. xerocarpa	SI	--	Bawa and Beach, 1983
	Posoqueria grandiflora	SI	--	Bawa and Beach, 1983
	Isertieae		3	
	Mussaenda luteoloa	SI	--	East, 1940
	Psychotrieae			
	Cephaelis elata	heteromorphic SI	--	Bawa and Beach, 1983
	Palicourea angustifolia	heteromorphic SI	--	Sobrevila and Arroyo, 1982
	P. petiolaris	SC	--	Sobrevila and Arroyo, 1982
	Psychotria (3 spp.)	heteromorphic SI	--	Bawa and Beach, 1983
	Psychotria (2 spp.)	SC	--	Sobrevila and Arroyo, 1982; Bawa and Beach, 1983
	Rudgea cornifolia	heteromorphic SI	--	Bawa and Beach, 1983
	R. karstenii	SC	--	Sobrevila and Arroyo, 1982
Sterculiaceae	Sterculiaceae		2	
Cola nitida	*Ayenia micrantha*	SI	--	Bullock, 1985
(Jacob, 1980)	*Guazuma tomentosa*	SI	--	Zapata and Arroyo, 1978
	G. ulmifolia	SI	--	Bullock, 1985
	Physodium corybosum	SI	--	Bullock, 1985
	Sterculia chicha	SI	--	Taroda and Gibbs, 1982
	Melochia sp.	heteromorphic SI	--	Ganders, 1979
	Waltheria sp.	heteromorphic SI	--	Ganders, 1979
	Bombacaceae		2	
	Pachira insignis	SI	--	East, 1940
	Elaeocarpaceae		2	
	Muntingia	SC	--	Bawa and Webb, 1983
	Malvaceae		2	
	Abutilon spp.	SI	--	East, 1940; Sears, 1937
	A. hybridum	SI	--	East, 1940
	Hibiscus syriacus	SI	--	East, 1940
	Tiliaceae		2	
	Goethalsia meiantha	SI	--	Bawa et. al., 1985
	Luhea speciosa	SI	--	Bawa et. al., 1985

In Convolvulaceae, SSI has been documented in *Ipomoea* (Kowyama et al., 1980; Joseph and George, 1983), and is likely in *Argyreia* (Gupta et al., 1981), which is SI and has stigmatic inhibition of pollen tubes. In possibly related families (see Olmstead et al., 1992, 1993), the array of SI systems is complex, and depending on their exact relationships to Convolvulaceae, SSI could have evolved in the common ancestor of several families.

SSI was claimed by Crowe (1964) for *Galium mollugo* (Rubiaceae), although no crossing data were presented to support the claim. The occurrence of trinucleate pollen

in *Galium* is consistent with the presence of SSI (Brewbaker, 1967). Elsewhere in the genus SC and dioecy are known and in the family as a whole homomorphic SI and heterostyly are known (Table 3). The evolutionary relationships among breeding systems in Rubiaceae are unclear. Recent phylogenetic studies based on cpDNA data (Bremer and Jansen, 1991; Bremer and Struwe, 1992), while promising, are still not detailed enough to settle these issues.

Breeding systems in Sterculiaceae appear to be as complex as those in the Rubiaceae (Table 3). Both heteromorphic and homomorphic SI are known (Charlesworth, 1985). Crossing studies using *Cola nitida* indicate that control is sporophytic (Jacob, 1980); in *Theobroma* SSI has been reported, although it is complex (Knight and Rogers, 1955). Because a range of SI systems is present in the Sterculiaceae, and the phylogenetic relationships within the family are so poorly understood, little can be said about precursor conditions to SSI. GSI is likely in related families (Table 3).

To summarize, there is little evidence that GSI preceded SSI in the eight families where SSI is known with certainty, with the possible exception of the Sterculiaceae. If SSI is present in *Galium*, a change from GSI to SSI may have occurred in the Rubiaceae. In Nyctaginaceae, Caryophyllaceae, Betulaceae, and Convolvulaceae, the more likely precursor condition appears to be SC, while in Asteraceae and Brassicaceae information is too preliminary to make an assessment.

THE RELATIONSHIP OF HOMOMORPHIC AND HETEROMORPHIC INCOMPATIBILITY

Phylogenetic analyses (e.g., Chase et al., 1993) support earlier arguments that heteromorphic SI has evolved a number of times (Ganders, 1979; Charlesworth, 1985; Gibbs, 1988; Lloyd and Webb, 1992). In monocots, it occurs in only three families, Amaryllidaceae, Pontederiaceae, and Iridaceae; each of these probably represents a separate origin. Among dicots, heteromorphic SI appears to have evolved independently in every family where it is found (Table 4), with the possible exceptions of Gentianaceae (possibly nested within Loganiaceae) and Santalaceae (perhaps within Olacaceae). This is a minimum number of originations, as heterostyly appears to have evolved several times within some families (see below). Crowe (1964), and later Muenchow (1981) argued that the S locus is homologous in heteromorphic and homomorphic species. Most hypotheses for the evolution of heteromorphic SI suggest a separate origin for heteromorphic and homomorphic systems (Charlesworth, 1982).

Studies of the mechanism of SI in heterostylous species indicate that the underlying controls are different for homomorphic and heteromorphic SI (Stevens and Murray, 1982; Gibbs, 1986; Scribailo and Barrett, 1991; Barrett and Cruzan, 1994). Oppositional systems (incompatibility triggered by like-by-like protein recognition), which are likely to provide the basis for homomorphic SI, seem unlikely to account for incompatibility in tristylous species. In tristylous species, pollen grains from the two sets of anthers produced by a single individual have different incompatibility reactions, leading Charlesworth (1979) and Lloyd and Webb (1992) to argue that developmental factors probably control the different incompatibility reactions rather than incompatibility proteins.

Can phylogenetic methods be applied to this question? Charlesworth (1985) noted that if loss of SI alleles accounted for the evolution of heterostyly from homomorphic ancestors possessing SSI, one might expect the two breeding systems to occur in the same families. There was little evidence for this in her study (Charlesworth, 1985). Here we ask whether in families where both homomorphic SI and heteromorphic SI occur there is any phylogenetic evidence that homomorphic SI directly preceded heteromorphic SI, and if so, whether control of SI was gametophytic or sporophytic.

Table 4. The occurrence of homomorphic self-incompatibility (SI) in families with heteromorphic SI. For families that may possess both heteromorphic and homomorphic SI, cases are listed separately where evidence for presence of heterostyly or homomorphic SI is weak. See text for details.

Families with only heteromorphic SI:	Clusiaceae, Connaraceae, Erythroxylaceae, Gentianaceae, Linaceae, Loganiaceae, Lythraceae, Oleaceae, Oxalidaceae, Plumbaginaceae, Pontederiaceae, Santalaceae, Turneraceae
Families possibly with both heteromorphic and homomorphic SI:	
Strong evidence for both heterostyly and homomorphic SI:	Acanthaceae, Amaryllidaceae, Iridaceae, Menyanthaceae, Olacaceae, Rubiaceae, Saxifragaceae, Sterculiaceae
Weak evidence for heterostyly:	Ericaceae, Fabaceae, Polemoniaceae
Weak evidence for homomorphic SI:	Boraginaceae, Primulaceae

Among families possessing heterostyly, 13 may contain species with homomorphic GSI or SSI, though several of these are weakly documented (Table 4). This list of joint occurrences includes Amaryllidaceae, where tristyly has been widely recognized only recently (Lloyd et al., 1990), Fabaceae, where the occurrence of distyly in *Bauhinia* is in doubt (Lloyd et al., 1990), and the Ericaceae and Polemoniaceae, because of recent reports indicating the possibility of heterostyly (Barrett, 1992). The list does not include Lythraceae, where the claim for homomorphic SI in *Cuphea* (East, 1940) is no longer accepted (S. Graham, pers. comm.). In most cases of joint occurrence, GSI is the most likely mechanism underlying incompatibility in the homomorphic species. Possible exceptions to this generalization are in Boraginaceae and Sterculiaceae, where either trinucleate pollen or genetic data (respectively) suggest the possibility of SSI.

A simple listing of families with heteromorphic and homomorphic SI does not reflect the size of the group in which there is a joint occurrence, the frequency of each breeding system within a family, nor phylogenetic relationships within each clade. As noted by Baker (1984), different breeding systems may have evolved independently in large, diverse families. For example, in the Iridaceae heterostyly occurs in *Nivenia* (Ornduff, 1974), a woody genus that is not likely to bear any close phylogenetic relationship with genera possessing homomorphic SI (*Freesia*, *Gladiolus* [East, 1940]). A report of heterostyly in *Geissorhiza* (Ornduff, 1974) may indicate a closer phylogenetic relationship between heterostylous and homostylous SI, although the nature of the SI system, if any, has not been demonstrated in this genus.

SI is widespread in the Amaryllidaceae, suggesting that tristylous *Narcissus* species may have evolved from an ancestor with homomorphic SI. In *Villarsia* (Menyanthaceae), homomorphic and heteromorphic SI as well as SC are known (Ornduff, 1988). Ornduff suggested that heterostyly may have evolved from a homomorphic SI ancestor, but a phylogenetic analysis of these species is needed to evaluate this hypothesis. Homomorphic and heteromorphic SI are also known in the Olacaceae (Tomlinson, 1974; Zapata and Arroyo, 1978) and possibly the Primulaceae (East, 1940; Pojar, 1974; Ganders, 1979), although again

phylogenetic relationships are uncertain. The pattern of variation in the Rubiaceae is especially complex, although there is some evidence that different types of SI may be partitioned among the tribes. For example, homomorphic SI is found in the Hamelieae (East, 1940; Bawa and Beach, 1983; Bullock, 1985), whereas heterostyly is common in the Psychotrieae (Table 3; Bawa and Beach, 1983). A single genus in the Saxifragaceae (*Jepsonia*) is characterized by heteromorphic SI (Ganders, 1979), whereas some other members of the family have homomorphic SI (Arasu, 1970; Gornall and Bohm, 1984). More information on SI in *Telesonix*, the apparent sister group of *Jepsonia* (Soltis et al., 1993), would help in understanding the origin of distyly in this genus.

Homomorphic SI and heterostyly may occur together in the Boraginaceae, where several monomorphic species are reported as SI (East, 1940; Sobrevila and Arroyo, 1982). The occurrence of trinucleate pollen in the Boraginaceae suggests the possibility that control of SI may be sporophytic in these species. Heterostyly and homomorphic SI are well documented in the Sterculiaceae (Table 3), although phylogenetic relationships of the heterostylous and homomorphic genera are unclear.

When heteromorphic SI has been identified as the only form of SI in a plant family, it may have evolved from SC within the family, or from homomorphic SI in a more inclusive clade. Within the limitations of current phylogenetic information, most evidence suggests that heterostyly in these families evolved from self-compatible ancestors. Distyly is likely to have arisen from SC ancestors in both the Santalaceae and the Turneraceae; in the Clusiaceae it may have evolved from GSI, which is found in the related Theaceae and possibly the Lecythidaceae. For the Erythroxylaceae, Linaceae, and Oxalidaceae, the families that may be most closely related (possibly the Violaceae, Malpighiaceae, and Geraniaceae, respectively; Rodman, 1991; Chase et al., 1993) are basically SC, although there have been reports of SI in *Pelargonium* (Geraniaceae; Darwin, 1878; Sears, 1937).

The families most closely related to the Lythraceae (Punicaceae, Sonneratiaceae) show uniform SC (Graham et al., 1993), and all homomorphic genera of Lythraceae are also SC, indicating that heterostyly has been derived from SC in the Lythraceae. Based on the morphological phylogeny of Graham et al. (1993), it appears that heterostyly evolved 3–5 times within Lythraceae. Heterostyly may have been preceded by GSI in the Oleaceae, which is related to families where SI is widespread; e.g., in Bignoniaceae, where control is likely to be gametophytic on the basis of binucleate pollen and the known occurrence of GSI in the related Scrophulariaceae.

Plumbaginaceae and Polygonaceae also may have evolved heteromorphic SI from SC ancestors, on the basis of the occurrence of SC among potentially related families in the Caryophyllidae (Rodman et al., 1984; Chase et al., 1993). Plumbaginaceae is one of the few families in which the build-up of the heterostylous syndrome from a homomorphic, SC ancestor has been suggested on the basis of a comparative, though not phylogenetic, approach (Baker, 1966). Tristyly and SC are probably basal conditions in the Pontederiaceae, based on morphological and molecular evidence (Eckenwalder and Barrett, 1986; Sean Graham, pers. comm.). Among families closely related to Pontederiaceae (Chase et al., 1993), GSI is present in the Commelinaceae, but evidence for the Commelinaceae as the sister-group to the Pontederiaceae is weak (Sean Graham, pers. comm.), suggesting that GSI in Commelinaceae and heterostylous SSI in Pontederiaceae are unlikely to be evolutionarily linked.

We conclude from these considerations that heterostyly has in most cases evolved from SC precursors, possibly from ancestors with GSI in a few instances, and in all probability not from ancestors with SSI. We therefore concur with Charlesworth (1985) that it is unlikely that heteromorphic SI evolved though loss of alleles from sporophytic SI systems, as suggested by Muenchow (1982).

CONCLUSIONS

Our preliminary phylogenetic analysis favors the hypothesis that the first angiosperms were self-compatible, and implies that the evolution of SI was not a major causal factor in the radiation of flowering plants. Attempts to homologize all self-incompatibility systems in angiosperms seem unwarranted, both on the basis of the phylogenetic considerations discussed here and the evident diversity of self-incompatibility systems. There is little evidence to suggest that sporophytically controlled, homomorphic self-incompatibility evolved in species possessing gametophytic self-incompatibility. In fact, on the basis of phylogenetic reasoning, it seems more likely that both kinds of incompatibility evolved from SC ancestors. Similarly, heteromorphic self-incompatibility evolved independently on many occasions, probably from self-compatible ancestors.

Further information on breeding systems is needed to resolve the many remaining ambiguities. Despite the seven or more decades that have passed since the discovery of the genetic basis of self-incompatibility, genetic data are available for fewer than two dozen species. At an even more basic level, no information on the presence or absence of SI is available for many families. A forum for reporting observations of breeding systems, whether results are negative or positive, would be most useful (e.g., Arroyo and Weller, 1993).

Studies of the molecular basis of self-incompatibility have appeared only recently, and are still limited to only a few families (Nasrallah and Nasrallah, 1986; Haring et al., 1990; Ioerger et al., 1990; Franklin-Tong and Franklin, 1993; Newbigin et al., 1993). The molecular evidence is consistent with the view that sporophytic and gametophytic self-incompatibility evolved independently, since amino acid sequences of SI alleles from the three families for which data are available show little similarity. It may well be that each genetic type of SI includes families that employ different molecular mechanisms. This would be consistent with the results of our phylogenetic analysis. Despite recent progress in understanding the phylogeny of flowering plants, the relationships of major taxonomic groups are still poorly resolved, and few families have been studied in any detail. Better information at both levels will be essential to address evolutionary questions such as the origin of self-incompatibility. In the meantime, it is nevertheless helpful to formulate such questions in phylogenetic terms. This exercise can identify phylogenetically critical taxa whose breeding systems are as yet unknown (e.g., Barnadesinae), as well as taxa with diverse breeding systems, where detailed phylogenetic studies would be especially valuable in elucidating evolutionary pathways (e.g., Boraginaceae, Rubiaceae, Sterculiaceae). Finally, as illustrated by our analysis of the basal condition of angiosperms, it may be possible to reach some conclusions even in the face of significant ambiguity regarding phylogenetic relationships and character coding.

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